

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE FARMACIA

DEPARTAMENTO DE QUÍMICA ORGÁNICA Y FARMACÉUTICA



TESIS DOCTORAL

**New multicomponent reaction for the synthesis of pyridine derivatives as
potential anti-neurodegenerative agents**

**Nuevas reacciones multicomponentes para la síntesis de derivados de piridina
como agentes antineurodegenerativos potenciales**

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

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Madrid, 2015

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como agentes antineurodegenerativos potenciales**

DOCTORAL THESIS

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Madrid, May 2015

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List of publications

The work carried out during my doctoral studies has led so far to the following publications:

1. **Tenti, G.;** Ramos, M. T.; Menéndez, J. C.
One-Pot Access to a Library of Structurally Diverse Nicotinamide Derivatives via a Three-Component Formal Aza [3+3] Cycloaddition
ACS Comb. Sci. **2012**, *14*, 551-557.
2. **Tenti, G.;** Ramos, M. T.; Menéndez, J. C.
A new multicomponent protocol for the synthesis of pyridines and fused pyridines
Curr. Org. Synt. **2013**, *10*, 646-655.
3. **Tenti, G.;** Egea, J.; Villaroya, M.; León, R.; Fernández, J. C.; Padín, J. F.; Sridharan, V.; Ramos, M. T.; Menéndez, J. C.
Identification of 4,6-diaryl-1,4-dihydropyridines as a new class of neuroprotective agents
Med. Chem. Comm. **2013**, *4*, 590-594.
4. Raja, V. P. A.; **Tenti, G.;** Perumal, S.; Menéndez, J. C.
A heavy metal- and oxidant-free, one-pot synthesis of pyridines and fused pyridines based on a Lewis acid- catalyzed multicomponent reaction.
Chem. Comm. **2014**, *50*, 12270-12272.
5. **Tenti, G.;** Parada, E.; León, R.; Egea, J.; Martínez-Revelles, S.; Briones, A.; Sridharan, V.; López, M.; Ramos, M. T.; Menéndez, J. C.
New 5-unsubstituted dihydropyridines with improved Ca_v1.3 selectivity as potential neuroprotective agents against ischemic injury.
J. Med. Chem. **2014**, *57*, 4313-4323.

6. Ma, W.; Mei, R.; **Tenti, G.**; Ackermann, L.
Ruthenium(II)-Catalyzed Oxidative C-H Alkenylations of Sulfonic Acids,
Sulfonyl Chlorides and Sulfonamides
Chem. Eur. J. **2014**, *20*, 15248-15251.

7. **Tenti, G.**; Ramos, M. T.; Menéndez, J. C.
Six-membered heterocycles (Chapter 3, pp 45-85)
Stereoselective Multiple Bond-Forming Transformations in Organic
Synthesis (ISBN: 978-1-118- 67271-6), April **2015**

Summary of the Ph. D. thesis "New multicomponent reactions for the synthesis of pyridine derivatives as potential anti-neurodegenerative agents"

1. Introduction

Pyridine and its partially saturated derivatives are among the most important nitrogen heterocycles found in natural products and show a broad range of applications widespread in so many different fields of chemistry. Above all, these scaffold are of particular interest in medicinal chemistry, being present in many marketed drugs. Thus, the pyridine and, specially, the 1,4-dihydropyridine (1,4-DHP) moieties, due to their pharmacological versatility, can be defined as "privileged scaffolds" (*i.e.* molecular structures which show the ability to interact with different groups of therapeutic targets).

In an attempt to improve synthetic access to this important structural motif (and, more generally, to all heterocycles), in the last decades multicomponent reactions (MCR) have appeared as a powerful alternative to other methods. Multicomponent reactions can be defined as convergent reactions where three or more reagents are combined in such a way that the final product retains significant portions of all starting materials, allowing the creation of several bonds and consequently providing an elevated molecular complexity in a single operation. This methodology has emerged as one of the most promising technologies that allow fulfilling some of the modern requirements of organic chemistry that go beyond the traditional ones such as reactivity and selectivity and that are focused on economic and environmental concerns. Besides their green character associated to the reduction in the number of isolation and purification stages, the use of multicomponent procedures is very attractive for the generation of libraries for drug discovery projects due to their modular nature.

2. Objectives

Within the general framework of the development of multicomponent reactions for the synthesis of pharmacologically relevant dihydropyridines and pyridines,

we have pursued the following specific objectives:

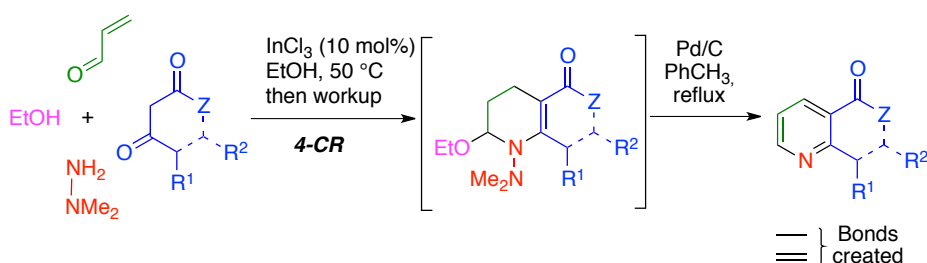
1. Development of a new approach to pyridine derivatives, based on a double elimination process on 1-dimethylamino-6-ethoxytetrahydropyridines generated through a Lewis-acid catalyzed four-component reaction.
2. Development of a new multicomponent route to 4,6-diarylpyridine frameworks and study of the conditions required for the reaction to afford dihydropyridines or pyridines.
3. In view of the fact that some classical 1,4-dihydropyridines have shown good neuroprotective activities that can not be exploited therapeutically because of their cardiovascular effects, we also planned the study of 4,6-diaryl-1,4-dihydropyridines as neuroprotectors, considering that do not satisfy the well-known structure-activity relationships of vasodilator dihydropyridines.
4. Synthesis of fused dihydropyridines and their pyrane oxygen heteroanalogues via a Hantzsch-like three-component reaction and their subsequent study as neuroprotectors via inhibition of GSK3 β .
5. Multicomponent synthesis of hybrid structures related to nimodipine and the benzothiazepine derivative CGP-37157 (a blocker of the mitochondrial Na⁺/Ca²⁺ exchanger) as potential anti-Alzheimer compounds aimed at stabilizing neuronal and mitochondrial calcium cycling by a multitarget approach. Similarly, the synthesis and study of hybrids of a CGP-37157 aza analogue and lipoic acid, a well-known antioxidant able to cross the blood-brain barrier, were planned.
6. Finally, during a 3-month period at Georg-August Universität of Göttingen (Professor Ackermann's group) as part of the requirements for the European Mention to the Ph. D. title, a new Ru(II)-catalyzed oxidative C-H alkenylation of arylsulfonamides was planned.

3. Results and discussion

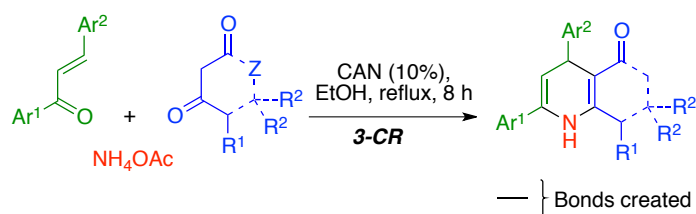
The main results obtained are summarized below:

- We have developed a new indium trichloride-catalyzed four-component reaction between dimethylhydrazine, β -dicarbonyl compounds, acrolein and ethanol. This reaction allowed the formation of 6-ethoxy-1,4,5,6-

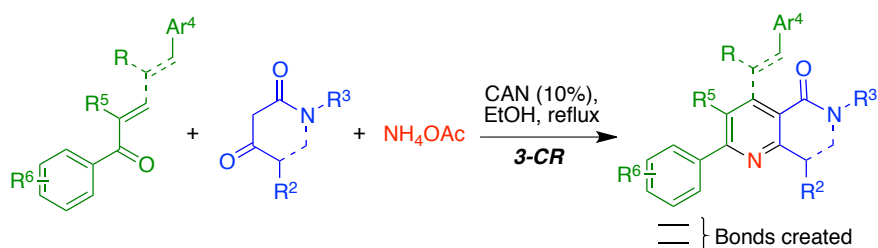
tetrahydropyridine derivatives one C-C, two C-N and one C-O bonds, furnishing the heterocyclic framework from very simple open-chain precursors *via* a formal aza [3+3] cycloaddition. These compounds were subsequently converted into 2,3-disubstituted pyridines by a double elimination reaction and, interestingly, the whole sequence can be performed without the need for purifying any intermediate.



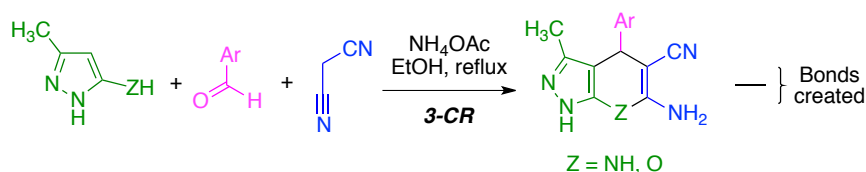
- A library of 4,6-diaryl-1,4-dihydropyridines was generated by a the three-component reaction between β -dicarbonyl compounds, chalcones and ammonium acetate in refluxing ethanol, in the presence of a catalytic amount of cerium(IV) ammonium nitrate. These compounds were shown to provide neuroprotection against calcium overload and oxidative stress models, evaluated on neuroblastoma cells.



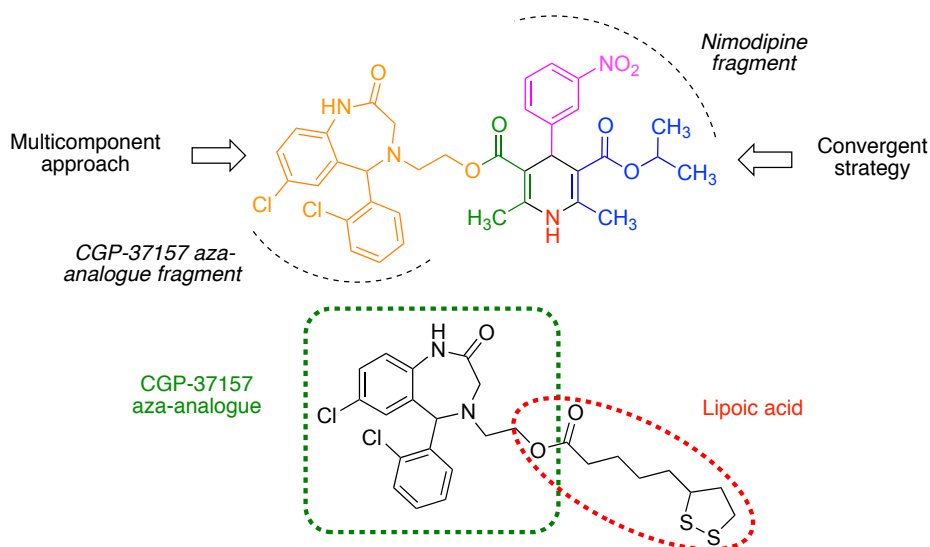
- Interestingly, a small variation of the previous three-component reaction (*i.e.* the use of β -ketoamides as the β -dicarbonyl compound), under similar conditions (CAN as catalyst in refluxing ethanol), directly afforded aromatic compounds that can be regarded as nicotinamide analogues *via* a 3CR-air oxidation sequence. This formal [3+3] aza-annulation leads to the formation of one C-C and two C-N bonds in a single synthetic operation and involves up to five individual steps.



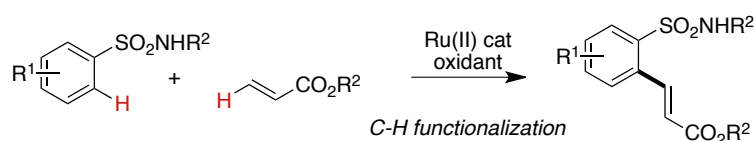
- We also performed the synthesis of fused dihydropyridines and their oxygen heteroanalogues *via* a Hantzsch-like three-component reaction between malononitrile, aromatic aldehydes and C5-functionalized pyrazoles.



- We synthesized a hybrid of an aza analogue of CGP-37157 and nimodipine *via* a multicomponent approach and also *via* a convergent strategy. Moreover, the synthesis of a hybrid of CGP-37157 aza analogue and the antioxidant lipoic acid was performed.



- A new ruthenium-catalyzed oxidative C-H bond alkenylation on aryl sulfonamides was developed.



4. Conclusions

1. We developed three different multicomponent protocols for the synthesis of the pyridine moiety and its fused related derivative. These methodologies allow a rapid and efficient access to densely functionalized pyridines, facilitating the generation of compound libraries of great significance in many areas of medicinal chemistry and drug discovery.
2. We identified a class of 4,6-diaryl-1,4-dihydropyridines showed improved selectivity towards Cav1.3 neuronal calcium channels with regards to previously studied dihydropyridines and behave as neuroprotective agents against ischemic injury.
3. Pyrano[2,3-*c*]pyrazoles and dihydropyrazolo[3,4-*b*]pyridines were rationally designed as inhibitors of GSK3 β , an important anti-Alzheimer target, and synthesized using multicomponent strategies. These compounds are currently under pharmacological evaluation.
4. We developed different strategies for the synthesis of various hybrid compounds containing as pharmacophoric moieties a CGP-37157 aza analogue, nimodipine and lipoic acid. These compounds were designed as multitarget anti-neurodegenerative agents acting on neuronal calcium regulation and as antioxidants, and are being tested against Alzheimer's disease.
5. With the development of a new direct C-H functionalization of sulfonamide derivatives, we have paved the way for the fast synthesis of new types of compounds containing this pharmacophore, which is of critical importance in many areas of medicinal chemistry.

Resumen de la tesis doctoral "Nuevas reacciones multicomponente para la síntesis de derivados de piridina como agentes antineurodegenerativos potenciales"

1. Introducción

La piridina y sus derivados parcialmente saturados figuran entre los heterociclos más habituales en la naturaleza y presentan una amplia gama de aplicaciones en todos los campos de la Química. En concreto, estos esqueletos son de especial interés en Química Farmacéutica, y están presentes en un gran número de fármacos. La piridina y, sobre todo, la 1,4-dihidropiridina (1,4-DHP), presentan una elevada versatilidad farmacológica que permite clasificarlas como "estructuras privilegiadas", es decir, estructuras capaces de interactuar con diferentes familias de dianas terapéuticas.

En un intento de mejorar el acceso sintético a estos motivos estructurales (y, en general, a todo tipo de heterociclos), durante las últimas décadas se han desarrollado métodos basados en el empleo de reacciones multicomponente (MCR) como alternativa a los procedimientos tradicionales. Las reacciones multicomponente se pueden definir como procesos convergentes en los que se combinan tres o más reactivos de tal manera que el producto final contiene fragmentos significativos de todos ellos, de modo que se generan varios enlaces en una sola operación. Esta metodología ha surgido como una de las más prometedoras para satisfacer los exigentes requisitos actuales para los métodos de síntesis orgánica, que van más allá de los tradicionales de reactividad y selectividad para considerar también aspectos económicos y medioambientales. Además de pertenecer al ámbito de la Química Verde por permitir disminuir el número de etapas de aislamiento y purificación, las reacciones multicomponente son muy atractivas para la generación de quimiotecas para el descubrimiento de fármacos a causa de su carácter modular.

2. Objetivos

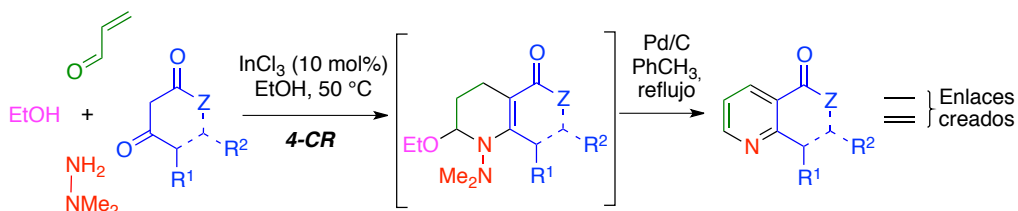
Dentro del marco general del desarrollo de reacciones multicomponente para la síntesis de dihidropiridinas y piridinas de relevancia farmacológica, nuestros objetivos específicos han sido:

1. Desarrollo de un nuevo método para el acceso a derivados de piridina basado en un proceso de doble eliminación a partir de 1-dimetilamino-6-etoxi-1,4,5,6-tetrahidropiridinas, sintetizadas a través de una reacción multicomponente catalizada por ácidos de Lewis.
2. Desarrollo de una nueva ruta multicomponente hacia 4,6-diarilpiridinas o sus 1,4-dihidro derivados, con estudio de las condiciones requeridas para obtener ambos tipos de productos.
3. A la vista de que algunas 1,4-dihidropiridinas clásicas han mostrado Buena actividad neuroprotectora que no puede explotarse a causa de sus efectos cardiovasculares, hemos planeado el estudio de las of 4,6-diaril-1,4-dihidropiridinas como agentes neuroprotectores, ya que estos compuestos no cumplen algunas de las relaciones estructura-actividad de las dihidropiridinas vasodilatadoras.
4. Síntesis de heterociclos fusionados derivados de dihidropiridina y pirano a través de reacciones multicomponente de tipo Hantzsch, así como su estudio como agentes neuroprotectores por inhibición de GSK3 β .
5. Síntesis multicomponente de estructuras híbridas referibles a nimodipino y CGP-37157 (un inhibidor del intercambiador mitocondrial Na⁺/Ca²⁺) como agentes anti-Alzheimer potenciales por estabilización de los niveles neuronales y mitocondriales de calcio a través de una aproximación multidiana. También se planificó la síntesis de estructuras híbridas formadas por un aza análogo de CGP-37157 y el ácido lipoico, un conocido agente antioxidante capaz de atravesar la barrera hematoencefálica
6. Por último, durante una estancia de tres meses en la Georg-August Universität de Göttingen (grupo del professor Ackermann), como parte de los requisitos para la Mención Europea al título de doctor, se planeó una nueva alquienilación C-H oxidativa de arilsulfonamidas catalizada por especies de Ru(II).

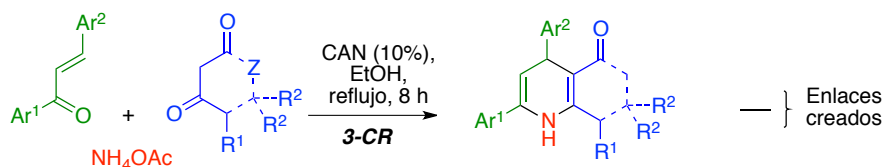
3. Resultados y discusión

Los principales resultados obtenidos pueden resumirse como sigue:

- Hemos desarrollado una nueva reacción en cuatro componentes, catalizada por tricloruro de indio, entre dimetilhidrazina, compuestos β -dicarbonílicos, acroleína y etanol. Esta reacción condujo a la formación de derivados de 1-dimetilamino-6-etoxi-1,4,5,6-tetrahidropiridina mediante la formación de enlaces C-C (1), C-N (2) y C-O (1), proporcionando el heterociclo deseado a partir de precursores acíclicos muy sencillos, a través de una cicloadición formal de tipo aza [3+3]. Estos compuestos se transformaron después en piridinas 2,3-disustituidas a través de una reacción de eliminación doble. Es interesante resaltar que la secuencia completa se llevó a cabo si necesidad de purificar ningún intermedio.

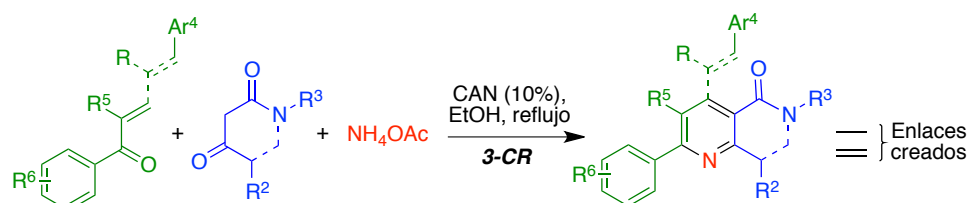


- Se ha sintetizado una quimioteca de 4,6-diaril-1,4-dihidropiridinas mediante una reacción tricomponte entre compuestos β -dicarbonílicos, chalconas y acetato amónico en etanol a reflujo, en presencia de una cantidad catalítica de nitrato cérico amónico. Estos compuestos fueron neuroprotectores en situaciones de sobrecarga de calcio y en modelos de stress oxidativo en células de neuroblastoma.

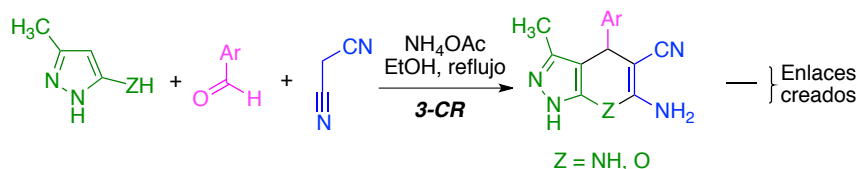


Una pequeña variación de la reacción anterior (usando β -cetoamidas como el componente β -dicarbonílico), en condiciones similares (CAN como catalizador en etanol a reflujo), proporcionó directamente compuestos aromáticos que pueden considerarse análogos de nicotinamida, a través de una secuencia 3CR-oxidación. Esta anelación formal aza [3+3] conduce a la formación de un enlace C-C y dos enlaces C-N en una única operación

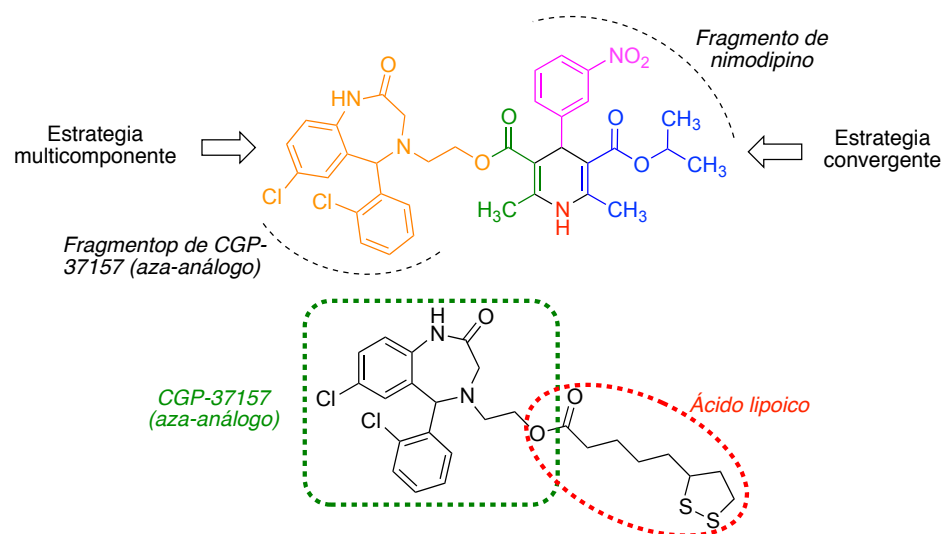
sintética que comprende cinco etapas individuales.



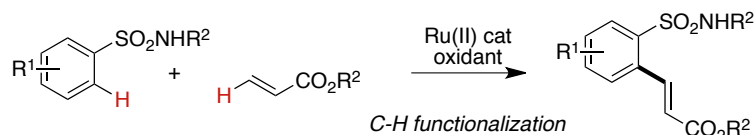
- Hemos llevado a cabo la síntesis de heterociclos fusionados derivados de dihidropiridina y su heteroanálogo oxigenado, el pirano, a través de reacciones tricompente de tipo Hantzsch entre malononitrilo, aldehídos aromáticos y pirazoles funcionalizados en C5.



- Hemos sintetizado un compuesto híbrido formado por un aza análogo de CGP-37157 y por nimodipino, empleando tanto un proceso multicomponente como una estrategia convergente. También se ha logrado la síntesis de un híbrido del aza análogo de CGP-37157 y el ácido lipoico.



- Se ha desarrollado una nueva alquienilación C-H de arilsulfonamidas, catalizada por rutenio.



4. Conclusiones

1. Hemos desarrollado tres protocolos multicomponente para la síntesis de esqueletos de piridina y sus derivados de fusión con pirazol. Estas metodologías permiten un acceso rápido y eficiente a piridinas altamente funcionalizadas, permitiendo la generación de quimiotecas de gran significación en muchos campos de la Química Farmacéutica y el descubrimiento de fármacos.
2. Hemos demostrado que las 4,6-diaril-1,4-dihidropiridinas presentan una selectividad mejorada respecto a canales de calcio neuronal Cav1.3 en comparación con las dihidropiridinas previamente conocidas. Estos compuestos se comportan como agentes neuroprotectores frente a fenómenos isquémicos.
3. Se han diseñado derivados de pirano[2,3-*c*]pirazol y dihidropirazolo[3,4-*b*]piridina como inhibidores de GSK3b, una importante diana anti-Alzheimer, y se han sintetizado empleando estrategias multicomponentes. Estos compuestos están actualmente en evaluación farmacológica.
4. Hemos desarrollado varias estrategias para la síntesis de compuestos híbridos que contienen como farmacóforos un aza análogo de CGP-37157, nimodipino y ácido lipoico. Estos compuestos se habían diseñado como agentes anti-neurodegenerativos multidiana, dirigidos a la regulación del calcio neuronal y como antioxidantes, y están siendo evaluados frente a la enfermedad de Alzheimer.
5. Con el desarrollo de un nuevo método de funcionalización C-H directa de sulfonamidas, hemos preparado el camino para la síntesis de nuevos tipos de compuestos portadores de este farmacóforo, de importancia crucial en Química Farmacéutica.

1. Introduction

1.1 MULTICOMPONENT REACTIONS AND THEIR CURRENT RELEVANCE

In recent years synthetic organic chemistry, in spite of an awesome evolution process, has reached the awareness that only a small part of the extremely vast chemical space has been discovered.¹ The increasingly urgent necessity to extend our knowledge of this chemical space has identified one of the major current challenges of organic synthesis as the aptitude of being able to readily create molecular diversity and complexity.² Thus contemporary organic synthesis, beyond being driven by traditional concepts such as reactivity and selectivity, is also focused on economic and environmental concerns. These groundbreaking requirements are at the basis of the concept of the *ideal synthesis* (Figure 1.1)³ and can be summarized as follows:

- 1.- Ability to generate high molecular diversity and complexity.
- 2.- Use of simple and readily available starting materials.
- 3.- Experimental simplicity, leading to the possibility of automation.
- 4.- Low environmental impact (use of environmentally friendly solvents, atom economy, low use of energy).

¹ (a) Reymond, J.-L.; Ruddigkeit, L.; Blum L. C.; van Deursen R. *WIREs Comput Mol Sci* **2012**, *2*, 717-733; (b) Ruddigkeit, L.; van Deursen R.; Blum L. C.; Reymond, J.-L. *J. Chem. Inf. Model.* **2012**, *52*, 2864–2875.

² Some reviews on diversity-oriented organic synthesis: (a) Schreiber, S. L. *Science* **2000**, *287*, 1964-1969; (b) Spring, D. R. *Org. Biomol. Chem.* **2003**, *1*, 3867-3870; (c) Burke, M. D.; Berger, E. M.; Schreiber, M. L. *Science* **2003**, *302*, 613-618; (d) Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46-58; (e) Tan, D. S. *Nature Chem. Biol.* **2005**, *1*, 74-84; (f) Wessjohann, L. A.; Ruijter, E. *Top. Curr. Chem.* **2005**, *243*, 137-184; (g) Spandl, R. J.; Bender, A.; Spring, D. R. *Org. Biomol. Chem.* **2008**, *6*, 1149-1158; (h) Galloway, W. R. J. D.; Isidro-Llobet, A.; Spring, D. R. *Nat. Commun.* **2010**, *1*, art n°80; (i) O'Connor, C. J.; Beckmann, H. S. G.; Spring, D. R. *Chem. Soc. Rev.* **2012**, *41*, 4444-4456.

³ Wender, P. A.; Miller, B. L. *Nature* **2009**, *460*, 197-201.

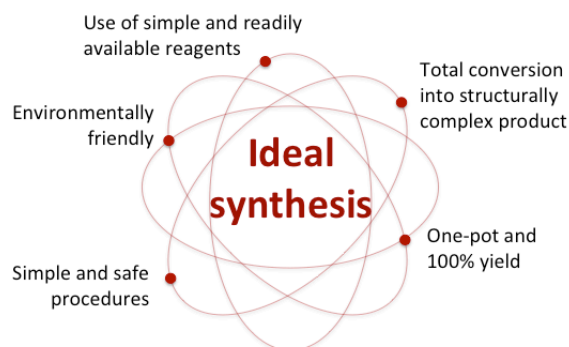


Figure 1.1

In the view of achieving a better knowledge and exploration of the chemical space, the first of these necessities has emerged as particularly important and the development of processes that allow the creation of several bonds, providing consequently an elevated molecular complexity in a single operation, has become one of the more attractive goals of organic synthesis.⁴ In this context, during the last decades, multicomponent reactions (MCRs) have got a renewed attention from the organic chemists, emerging as one of the most promising technologies towards achieving this end.⁵

Multicomponent reactions can be defined as convergent reactions where three or more reagents are combined in such a way that the final product retains significant portions of all starting materials.^{6,7,8} Ideally, they allow the simultaneous addition

⁴ For reviews of multibond forming reactions as a pathway towards eco-compatible chemistry, see: (a) Coquerel, Y.; Boddaert, T.; Pisset, M.; Mailhol, D.; Rodriguez, J. *Ideas in Chemistry and Molecular Sciences*, in *Advances in Synthetic Chemistry*; Pignataro, B., Ed.; Wiley-VCH: Weinheim, Germany, **2010**; Vol. 1, Chapter 9, 187–202; (b) Bonne, D.; Constantieux, T.; Coquerel, Y.; Rodriguez, J. *Chem. Eur. J.* **2013**, *19*, 2218 – 2231. (c) For a Special Issue on this topic, see: Menéndez, J. C. (ed.), *Curr. Org. Chem.* **2013**, *17*, issue 18, 1919–2064.

⁵ Some reviews on multicomponent reactions for the generation of molecular complexity and diversity: (a) Biggs-Houck, J. E.; Younai, A.; Shaw, J. T. *Curr. Opin. Chem. Biol.* **2010**, *14*, 371–382; (b) Ruijter, E.; Scheffelaar, R.; Orru, R. V. A. *Angew. Chem., Int. Ed.* **2011**, *50*, 6234–6246; (c) Eckert, H.; *Molecules* **2012**, *17*, 1074–1102; (d) van der Heijden, G.; Ruijter, E.; Orru, R. V. A. *Synlett* **2013**, *24*, 666–685; (e) Brauch, S.; van Berkel, S. S.; Westermann, B. *Chem. Soc. Rev.* **2013**, *42*, 4948–4962.

⁶ For monographs on multicomponent reactions, see: (a) Zhu, J.; Bienaymé, H. (eds.), *Multicomponent Reactions*. Wiley-VCH, **2005**. (b) Zhu, J.; Wang, Q.; Wang, M. (eds.),

of all reagents, which then combine orderly under the same reaction conditions to afford the final products without isolating intermediates. However, in order to avoid side reactions, very often it is necessary to add the reagents consecutively, and in these cases they are described as sequential multicomponent reactions.

Despite the centuries-old history of MCRs (the firstly known MCRs such as the classical Strecker,⁹ Hantzsch¹⁰ and Biginelli¹¹ reactions were described during the second half of the XIX century), their development and application in medicinal chemistry have experienced an explosive growth only recently because of their perfect adaptability to the creation of libraries with a high degree of structural diversity.¹² As a consequence, the development of new multicomponent reactions is a significant part of the research work currently carried out in and drug discovery programs.¹³

Multicomponent Reactions in Organic Synthesis. Wiley-VCH, 2014. (c) Herrera, R. P.; Marques-López, E. Multicomponent Reactions: Concepts and Applications for Design and Synthesis. Wiley-VCH, 2015.

⁷ Some general reviews on multicomponent reactions, with special emphasis on the use of isonitriles in (a-d, g, m): (a) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168-3210; (b) Ugi, A. *Pure Appl. Chem.* **2001**, *73*, 187-191; (c) Ugi, A. *Molecules* **2003**, *8*, 53-66; (d) Zhu, J. *Eur. J. Org. Chem.* **2003**, 1133-1144; (e) Orru, R. V. A.; de Greef, M. *Synthesis* **2003**, 1471-1499; (f) Tejedor, D.; González-Cruz, D.; Santos-Expósito, A.; Marrero-Tellado, J. J.; de Armas, P.; García-Tellado, F. *Chem. Eur. J.* **2005**, *11*, 3502-3510; (g) Dömling, A. *Chem. Rev.* **2006**, *106*, 17-89; (h) Liéby-Muller, F.; Simon, C.; Constantieux, T.; Rodriguez, J. *QSAR Comb. Sci.* **2006**, *25*, 432-438; (i) Tejedor, D.; García-Tellado, F. *Chem. Soc. Rev.* **2007**, *36*, 484-491; (j) Touré, B. B.; Hall, D. G. *Chem. Rev.* **2009**, *109*, 4439-4486; (k) Sunderhaus, J. D.; Martin, S. F. *Chem. Eur. J.* **2009**, *15*, 1300-1308; (l) Singh, M.S.; Chowdhury, S. *RSC Adv.* **2012**, *2*, 4547-4592; (m) Koopmanschap, G.; Ruijter, E.; Orru, R. V. A. *Beilstein J Org Chem.* **2014**, *10*, 544-598; (n) Cloc, R.; Ruijter, E.; Orru, R. V. A. *Green Chem.* **2014**, *16*, 2958-2975.

⁸ Reviews on asymmetric multicomponent reactions: (a) Ramón, D. J.; Yus, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1602-1634. (b) Guo, H.; Ma, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 354-366; (c) Guillena, G.; Ramón, D. J.; Yus, M. *Tetrahedron: Asymmetry* **2007**, *18*, 693-700; (d) Bonne, D.; Coquerel, Y.; Constantieux, T.; Rodriguez, J. *Tetrahedron: Asymmetry* **2010**, *21*, 1085-1109; (e) Bonne, D.; Coquerel, Y.; Constantieux, T.; Rodriguez, J. en *Targets in Heterocyclic Systems - Chemistry and Properties*, Vol. 15, Eds.: Attanasi, O. A.; Spinelli, D. Società Chimica Italiana, Roma, **2011**, p. 140-163; (f) Yu, J.; Shi, F.; Gong, L.-Z. *Acc. Chem. Res.* **2011**, *44*, 1156-1171; (g) de Graaff, C.; Ruijter, E.; Orru, R. V. A. *Chem. Soc. Rev.* **2012**, *41*, 3969-4009.

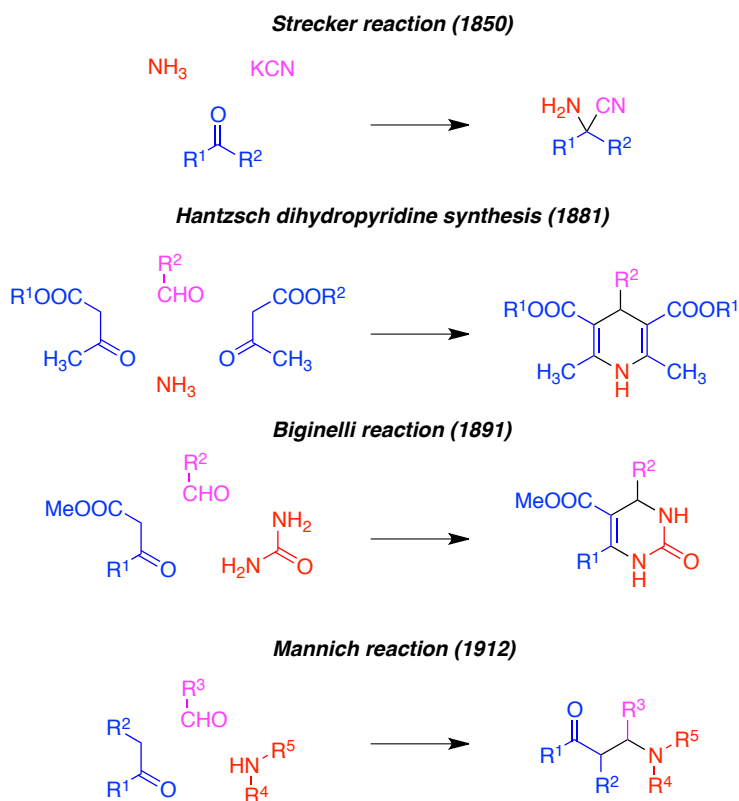
⁹ Strecker, A. *Liebigs Ann.* **1850**, *75*, 27-45.

¹⁰ Hantzsch, A. *Chem. Ber.* **1881**, *14*, 1637-1638.

¹¹ Biginelli, P. *Chem. Ber.* **1891**, *24*, 1317-1319.

¹² Review on the impact of molecular complexity on the discovery of new lead compounds in drug research: Hann, M. M.; Leach, A. R. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 856-864.

¹³ Reviews on the applications of multicomponent reactions in drug discovery: (a) Tietze, L. F.; Modi, A. *Med. Res. Rev.* **2000**, *20*, 304-322; (b) Weber, L. *Curr. Med. Chem.* **2002**, *9*, 2085-2093



Scheme 1.1: The first multicomponent reactions described in the literature

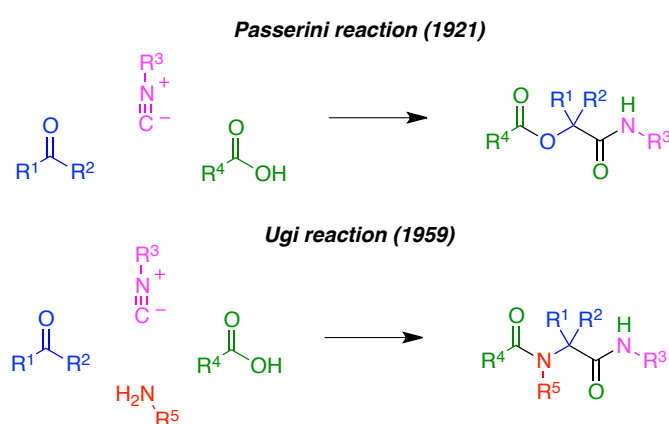
Nowadays the best-studied multicomponent reactions are those involving the use of isocyanides as one of the components, the so-called IMCRs (*isocyanide-based multicomponent reactions*).^{7a-d,g,m} Most IMCRs are focused on the construction of peptide-like structures and can be referred to two classical reactions described firstly by Passerini¹⁴ and Ugi,¹⁵ shown in Scheme 1.2. Their extensive use is owed to different reasons: first of all to chemical ones, because of their particular versatility derived from the presence of the isocyanide group. In fact this group shows a special reactivity bearing a carbon atom that can behave as a nucleophile and an electrophile and also taking part in radical reactions, conferring acidity to

(c) Hulme, C.; Gore, V. *Curr. Med. Chem.* **2003**, *10*, 51-80; (d) Ulaczyk-Lesanko, A.; Hall, D. G. *Curr. Opin. Chem. Biol.* **2005**, *9*, 266-276; (e) Slobbe, P.; Ruijter E.; Orru, R. V. A. *Med. Chem. Commun.* **2012**, *3*, 1189-1218; (f) Dömling, A.; Wang, W.; Wang, K. *Chem. Rev.* **2012**, *112*, 3083-3135; (g) Ruijter E.; Orru, R. V. A. *Drug Discov. Today: Technol.* **2013**, *10*, e15-e20.

¹⁴ Passerini, M. *Gazz. Chim. Ital.* **1921**, *51*, 126-129.

¹⁵ Ugi, I.; Meyr, R.; Fetzer U.; Steinbrückner, C. *Angew. Chem.* **1959**, *71*, 386.

its a protons and having affinity for many metals. Moreover their exceptionally elevated atom economy (a measure of the conversion efficiency of all the atoms involved in the reaction and superior to 90% for both these MCRs) makes these reactions very attractive from an environmental and economical point of view.



Scheme 1.2: The two classical isocyanide-based multicomponent reactions

Bearing in mind that more than 60% of drug molecules are heterocycles, it is surprising that multicomponent reactions leading directly to heterocyclic frameworks have not received closer attention.¹⁶ In this context, the present thesis deals with the application of multicomponent strategies to the synthesis of functionalized polyheterocyclic frameworks (with a special consideration for the pyridine nucleus), in order to identify new small molecules¹⁷ with potential pharmacological activity.¹⁸

¹⁶ For selected reviews of the synthesis of heterocycles using multicomponent reactions as key steps, see: (a) Sapi, J.; Laronze, J.-Y. *Arkivoc* **2004** (vii) 208-222; (b) D'Souza, D. M.; Mueller, T. J. *J. Chem. Soc. Rev.* **2007**, *36*, 1095-1108; (c) Isambert, N.; Lavilla, R. *Chem. Eur. J.* **2008**, *14*, 8444-8454; (d) Sunderhaus, J. D.; Martin, S.-F. *Chem. Eur. J.* **2009**, *15*, 1300-1308; (e) Jiang, B.; Rajale, T.; Wever, W.; Tu, S.-J.; Li, G. *Chem. Asian J.* **2010**, *5*, 2318-2335. (f) Estévez, V.; Villacampa, M.; Menéndez, J. C. *Chem. Soc. Rev.* **2010**, *39*, 4402-4421. (g) Estévez, V.; Villacampa, M.; Menéndez, J. C. *Chem. Soc. Rev.* **2014**, *43*, 4633-4657. (h) For a monograph, see: Ruijter, E.; Orri, R. V. A. *Synthesis of heterocycles via multicomponent reactions*, vols. 1 and 2, Springer Verlag, **2010** (*Topics in Heterocyclic Chemistry* series, volumes 23 and 25).

¹⁷ For some reviews on the importance of small molecules in medicinal chemistry see: (a) Schreiber, S. L. *Chem. Eng. News*. **2003**, *81*, 51-61; (b) Stockwell, B. R. *Nature* **2004**, *432*, 846-854; (c) Spring, D. R. *Chem. Soc. Rev.* **2005**, *34*, 472-482; (d) Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6699-6702.

¹⁸ For some reviews on the biological importance of extending the exploration of chemical

1.2 RELEVANCE OF THE PYRIDINE NUCLEUS AND ITS DERIVATIVES

The pyridine nucleus was described for the first time by the Scottish chemist Anderson in 1846 during some experiments carried out studying the pyrolysis of bones. From this process he could separate “a colorless liquid with unpleasant odor” from which he initially isolated picoline (the first known pyridine base) and at a later stage pyridine itself. Owing to its flammability, Anderson gave to this substance the name we still use, deriving from the Greek πυρ (pyr) that means fire and from the suffix -idine that was used for all aromatic bases.¹⁹ Some years later Körner (1869) and Dewar (1871) independently proposed the correct structure of pyridine, describing it as the mono-aza-analogue of benzene. Since its identification, this nucleus became one of the most investigated aromatic compounds and nowadays it has widespread applications in various chemical fields, from supramolecular²⁰ to coordination chemistry²¹ as well as in polymers science²² and in organic chemistry being part of various organo-²³ and organometallic catalysts.²⁴ However the most attractive aspect of this nucleus is related to its noteworthy biological importance. The first pyridine-based natural compound come to the fore was niacin (already known as vitamin B₃ or nicotinic acid), when, in the 1930s, it was identified as an efficient compound to cure pellagra, one of the most common life-threatening diseases known at that period and characterized by dementia and dermatitis.²⁵ At the turn of 20th century, beyond niacin, other pyridines such as nicotine, nicotinamide and the oxido-reductive NAD-NADH coenzymes had already identified and described. To date

space, see: (a) Dobson, C. M. *Nature* **2004**, *432*, 824-828; (b) Lipinski, C.; Hopkins, A. *Nature* **2004**, *432*, 855-861; (c) Bon, R. S.; Waldmann, H. *Acc. Chem. Res.* **2010**, *43*, 1103-1114; (d) Reymond, J.-L.; Awale, M. *ACS Chem. Neurosci.* **2012**, *3*, 649-657.

¹⁹ Anderson, T. *Liebigs Ann.* **1846**, *60*, 86-103.

²⁰ (a) Kozhevnikov, V. N.; Kozhevnikov, D. N.; Nikitina, T. V.; Rusinov, V. L.; Chupakhin, O. L.; Zabel, M.; König, B. *J. Org. Chem.* **2003**, *68*, 2882-2888; (b) Hofmeier, H.; Schubert, U. S. *Chem. Commun.* **2005**, 2423-2432; (c) Šmejkal, T.; Breit, B. *Angew. Chem., Int. Ed.* **2008**, *47*, 311-315.

²¹ (a) Halcrow, M. A. *Coord. Chem. Rev.* **2005**, *249*, 2880-2908; (b) de Ruiter, G.; Lahav, M.; van der Boom, M. E. *Acc. Chem. Res.* **2014**, *47*, 3407-3416.

²² Raje, V. P.; Bhat, R. P.; Samant, S. D. *Synlett* **2006**, 2676-2678.

²³ (a) Fu, G. C. *Acc. Chem. Res.* **2004**, *37*, 542-547; (b) De Rycke, N.; Couty, F.; David, O. R. P. *Chem. Eur. J.* **2011**, *17*, 12852-12871.

²⁴ (a) Shibatomi, K.; Muto, T.; Sumikawa, Y.; Narayama, A.; Iwasa, S. *Synlett* **2009**, 241-244; (b) Lin, S.; Lu, X. *Org. Lett.* **2010**, *12*, 2536-2539.

²⁵ Elvehjem, C. A.; Madden, R. J.; Strong, F. M.; Woolley, D. W. *J. Biol. Chem.* **1938**, *123*, 137-149.

many other natural compounds containing a pyridine nucleus are known and studied for their interesting biological activity and their potential pharmacological effects (Figure 1.2)²⁶

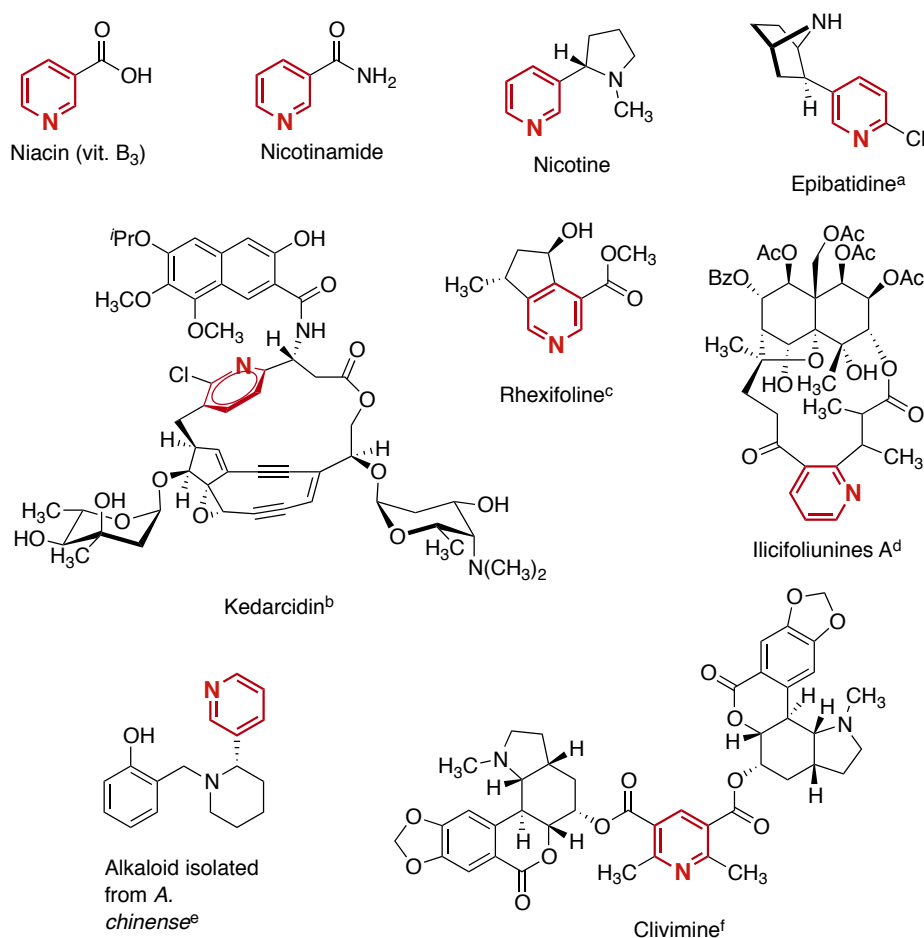


Figure 1.2

²⁶ (a) Epibatidine: Daly, J. W.; Garraffo, H. M.; Spande, T. F.; Decker, M. W.; Sullivan J. P.; Williams, M. *Nat. Prod. Rep.* **2000**, *17*, 131-135; (b) Kedarcidin: Leet, J. E.; Schroeder, D. R.; Langley, D. R.; Colson, K. L.; Huang, S.; Klohr, S. E.; Lee, M. S.; Golik, J.; Hofstead, S. J.; Doyle, T. W.; Matson, J. A. *J. Am. Chem. Soc.* **1993**, *115*, 8432-8443; (c) Rhexifoline: M. R. Roby and F. R. Stermitz, *J. Nat. Prod.* **1984**, *47*, 846-853 (d) Illicifoliunines A: Santos, V. A. F. F. M.; Regasini, L. O.; Nogueira, C. R.; Passerini, G. D.; Martinez, I.; Bolzani, V. S.; Graminha, M. A.; Cicarelli, R. M. B.; Furlan, M. *J. Nat. Prod.* **2012**, *75*, 991-995; (e) Alkaloid isolated from *A. chinense*: Zhang, Y.; Liu, Y.-B.; Li, Y.; Ma, S.-G.; Li, L.; Qu, J.; Zhang, D.; Chen, X.-G.; Jiang, J.-D.; Yu, S.-S. *J. Nat. Prod.* **2013**, *76*, 1058-1063; (f) Clivimine: Ieven, M.; Vlietinck, A. J.; Vanden Berghe, D. A.; Totte, J. *J. Nat. Prod.* **1982**, *45*, 564-573.

Also in the fields of medicinal chemistry and drug discovery various compounds containing the pyridine ring show numerous interesting pharmacological activities such as anti-inflammatory,²⁷ antiasthmatic,²⁸ antiretroviral,²⁹ antihistaminic,³⁰ anticancer,³¹ antiulcer³² and antidiabetic ones.³³

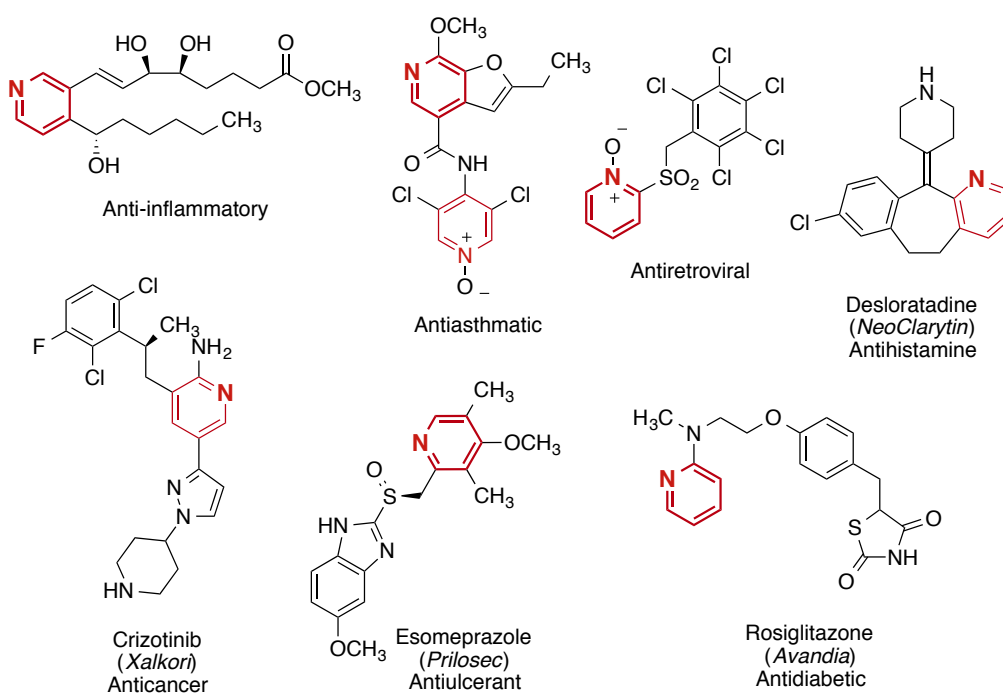


Figure 1.3

Finally it is worth mentioning that pyridine compounds are also important in the agrochemical field, showing different activities such as insecticide³⁴, herbicide³⁵

²⁷ Duffy, C. D.; Maderna, P.; McCarthy, C.; Loscher, C. E.; Godson, C.; Guiry, P. J. *ChemMedChem* **2010**, *5*, 517-522.

²⁸ Buckley, G. M.; Cooper, N.; Davenport, R. J.; Dyke, H. J.; Galleway, F. P.; Gowers, L.; Haughan, A. F.; Kendall, H. J.; Lowe, C.; Montana, J. G.; Oxford, J.; Peake, J. C.; Picken, C. L.; Richard, M. D.; Sabin, V.; Sharpe, A.; Warneck, J. B. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 509-512.

²⁹ Balzarini, J.; Stevens, M.; De Clercq, E.; Schols, D.; Pannecouque, C. *J. Antimicrob. Chemother.* **2005**, *55*, 135-138.

³⁰ Bachert, C. *Clin. Ther.* **2009**, *31*, 921-944.

³¹ Ou, S.-H. I. *Drug Des. Dev. Ther.* **2011**, *5*, 471-485.

³² Saccar, C. L. *Expert. Opin. Drug Metab. Toxicol.* **2009**, *5*, 1113-1124.

³³ Cantello, B. C. C.; Cawthorne, M. A.; Cottam, G. P.; Duff, P. T.; Haigh, D.; Hindley, R. M.; Lister, C. A.; Smith, S. A.; Thurlby, P. L. *J. Med. Chem.* **1994**, *37*, 3977-3985.

³⁴ Zhang, W.; Chen, Y.; Chen, W.; Liu, Z.; Li, Z. *J. Agric. Food Chem.* **2010**, *58*, 6296-6299.

and fungicide³⁶ (Figure 3).

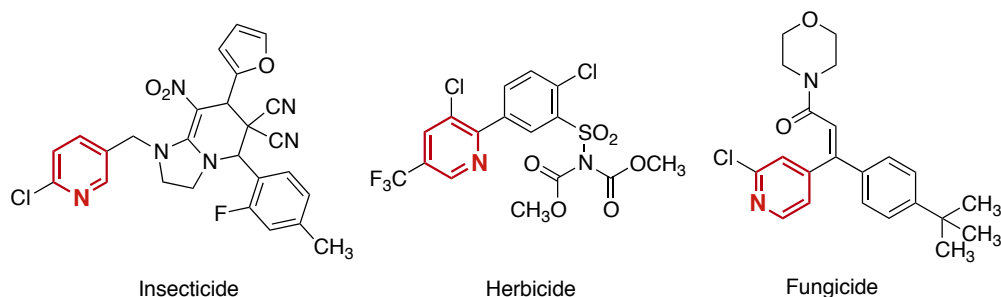


Figure 1.4

The biological interest and the extensive synthetic utility of the pyridine scaffold has led to the development of a large number of methods for its synthesis, which have been reviewed recently.³⁷ Several multicomponent procedures are included among these synthetic methodologies³⁸ and in the next Section we will mention the most frequently employed strategies for building the pyridine ring.

³⁵ Xie, Y.; Chi, H.-W.; Guan, A.-Y.; Liu, C.-L.; Ma, H.-J.; Cui, D.-L. *J. Agric. Food Chem.* **2014**, *62*, 12491-12496.

³⁶ Yan, X.; Qin, W.; Sun, L.; Qi, S.; Yang, D.; Qin, Z.; Yuan, H. *J. Agric. Food Chem.* **2010**, *58*, 2720-2725.

³⁷ (a) Henry, G. D. *Tetrahedron* **2004**, *60*, 6043-6061; (b) Hill, M. D. *Chem. Eur. J.* **2010**, *16*, 12052-12062.

³⁸ Allais, C.; Grassot, J.-M.; Rodriguez, J.; Constantieux, T. *Chem. Rev.* **2014**, *114*, 10829-10868.

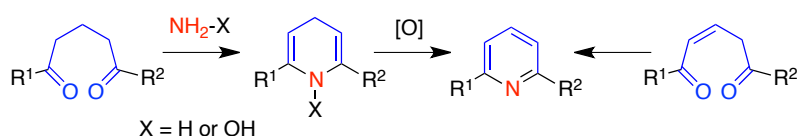
1.3 STRATEGIES FOR THE SYNTHESIS OF PYRIDINE DERIVATIVES: AN OVERVIEW

The present section is a brief overview on the strategies for the synthesis of pyridine derivatives. As in our opinion the best option to describe the various methodologies is organizing them by the typology of the ring-disconnection (as demonstrated by Henry in his excellent review),^{37a} there is no question that the most efficient way to obtain highly functionalized pyridines is to disconnect the ring in the maximum number of fragments. For this reason and having in mind the objective of the present thesis, we will focus especially on multicomponent strategies for pyridine synthesis.

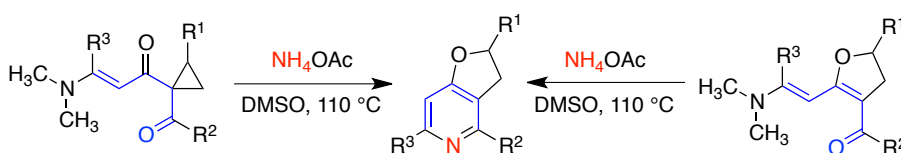
1.3.1 Synthetic methodologies *not* based on multicomponent reactions

The [5+1] approach is the simplest disconnection in the pyridine synthesis. Although the general procedure is represented by the condensation of 1,5-dicarbonyl compounds with ammonia (Scheme 1.3A), there are several variations reported in the literature based on the use of differently conjugated unsaturated compounds (Scheme 1.3B).³⁹

A) General [5+1] approach to pyridine synthesis



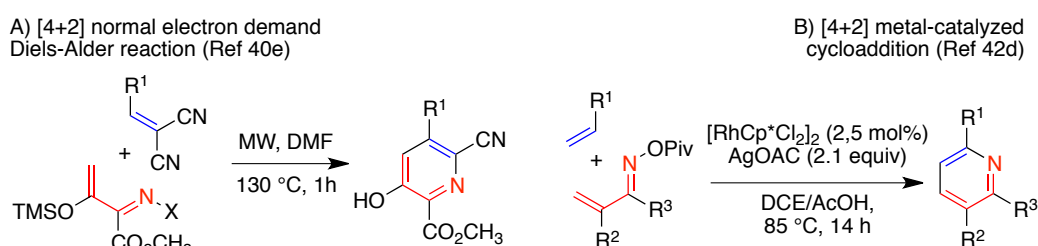
B) Dong's variation (Ref 39c)



Scheme 1.3: The two-component [5+1] approach to pyridine

³⁹ For examples of pyridine synthesis based on the [5+1] approach, see: (a) Perveev, F. Y.; Koshmina, N. V. *Russ. J. Org. Chem.* **1968**, *4*, 167-186; (b) Hu, J.; Zhang, Q.; Yuan, H.; Liu, Q. *J. Org. Chem.* **2008**, *73*, 2442-2445; (c) Huang, P.; Zhang, R.; Liang, Y.; Dong, D. *Org. Lett.* **2012**, *14*, 5196-5199.

The [4+2] disconnection is one of the most widely applied in azaheterocycle synthesis and, differently from the previous strategy, is usually based on a cycloaddition process where the nitrogen could be part of either the 4-atom or the 2-atom reactant. Among the various approaches of this typology, the normal-⁴⁰ and the inverse-⁴¹ electron demand hetero-Diels-Alder cycloadditions represent a powerful strategy for pyridine synthesis (Scheme 1.4A). Transition metal-catalyzed processes are also valuable strategies to the formation of the pyridine ring by coupling azadienes and 2-carbon components (Scheme 1.4B).⁴²



Scheme 1.4: The two-component [4+2] approach to pyridine

The last kind of two-component approach to the pyridine synthesis is based on the [3+3] disconnection. The most popular strategy for this kind of approach is the condensation between 1,3-dicarbonyl compounds and β -enamines (Scheme 1.5A).⁴³ A variation of this kind of strategy is the Bohlmann-Rahtz pyridine

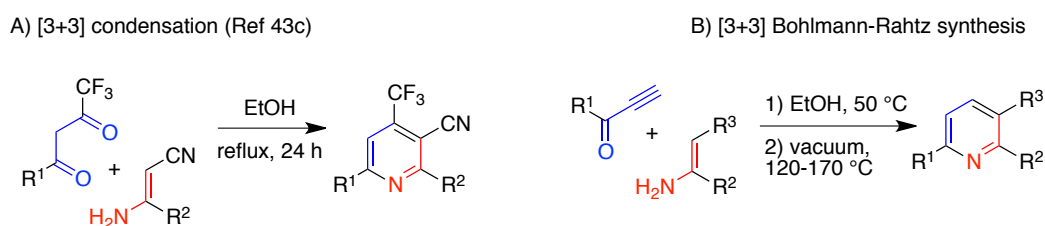
⁴⁰ For examples of pyridine synthesis based on [4+2] normal electron demand Diels-Alder reactions, see: (a) Villacampa, M.; Pérez, J. M.; Avendaño, C.; Menéndez J. C. *Tetrahedron*, **1994**, *50*, 10047-10054; (b) Boger, D. L.; Ichikawa, S.; Jiang, H. *J. Am. Chem. Soc.* **2000**, *122*, 12169-12173; (c) Barluenga, J.; Fernández-Rodríguez, M. A.; García-García, P.; Aguilar, E. *J. Am. Chem. Soc.* **2008**, *130*, 2764-2765; (d) Lu, J.-Y.; Arndt, H.-D. *J. Org. Chem.* **2007**, *72*, 4205-4212; (e) Lu, J.-Y.; Keith, J. A.; Shen, W.-Z.; Schürmann, M.; Preut, H.; Jacob, T.; Arndt, H.-D. *J. Am. Chem. Soc.* **2008**, *130*, 13219-13221.

⁴¹ For examples of pyridine synthesis based on [4+2] inverse electron demand Diels-Alder reaction, see: (a) Pabst, G. R.; Schmid, K.; Sauer, J. *Tetrahedron Lett.* **1998**, *39*, 6691-6694; (b) Linder, I.; Gerhard, M.; Schefzig, L.; Andrä, M.; Bentz, C.; Reissig, H.-U.; Zimmer, R. *Eur. J. Org. Chem.* **2011**, 6070-6077; (c) E. D. Anderson and D. L. Boger, *J. Am. Chem. Soc.* **2011**, *133*, 12285-12292.

⁴² For examples of pyridine synthesis based on [4+2] metal-catalyzed cycloadditions, see: (a) Roesch, K. R.; Larock, R. C. *J. Org. Chem.* **2002**, *67*, 86-94; (b) Too, P. C.; Wang Y.-F.; Chiba, S. *Org. Lett.* **2010**, *12*, 5688-5691; (c) Hyster, T. K.; Rovis, T. *Chem. Commun.* **2011**, *47*, 11846-11848; (d) Neely, J. M.; Rovis, T. *J. Am. Chem. Soc.* **2013**, *135*, 66-69.

⁴³ For examples of pyridine synthesis based on [3+3] condensations, see: (a) Henecke, H. *Chem. Ber.* **1949**, *82*, 36-41; (b) Stork, G.; Ohashi, M.; Kamachi, H.; Kakisawa, H. *J. Org. Chem.* **1971**, *36*,

synthesis⁴⁴ based on the reaction between conjugated ethynylcarbonyl compounds and β -enamines (Scheme 1.5B).⁴⁵



Scheme 1.5: The two-component [3+3] approach to pyridine

Before moving to focus on the multicomponent strategies for the pyridine synthesis, we consider appropriate to make a brief description of the [2+2+2] disconnection that, in our opinion, is borderline between this and the next section. Generally this approach is based on the reaction of cycloaddition between two alkyne components and a nitrile in the presence of various transition metal catalysts.⁴⁶ However, although at first sight this kind of approach could be considered as a multicomponent strategy, it is usually carried on as a linear process employing diynes and nitriles or alkynyl nitriles and alkynes (Scheme 1.6A).⁴⁷ In this manner it is possible to overcome the regioselectivity problems derived from the use of three different two-atom fragments in a one-pot fashion and that could theoretically generate a large number of very similar products (Scheme 1.6B).⁴⁸

2784–2786; (c) Yamaguchi, Y.; Katsuyama, I.; Funabiki, K.; Matsui, M.; Shibata, K. *J. Heterocyclic Chem.* **1998**, *35*, 805-810; (d) Sagitullina, G. P.; Garkushenko, A. K.; Dushek, M. A.; Poendaev, N. V.; Sagitullin, R. S. *Chem. Heterocycl. Compd.* **2011**, *46*, 1250-1254.

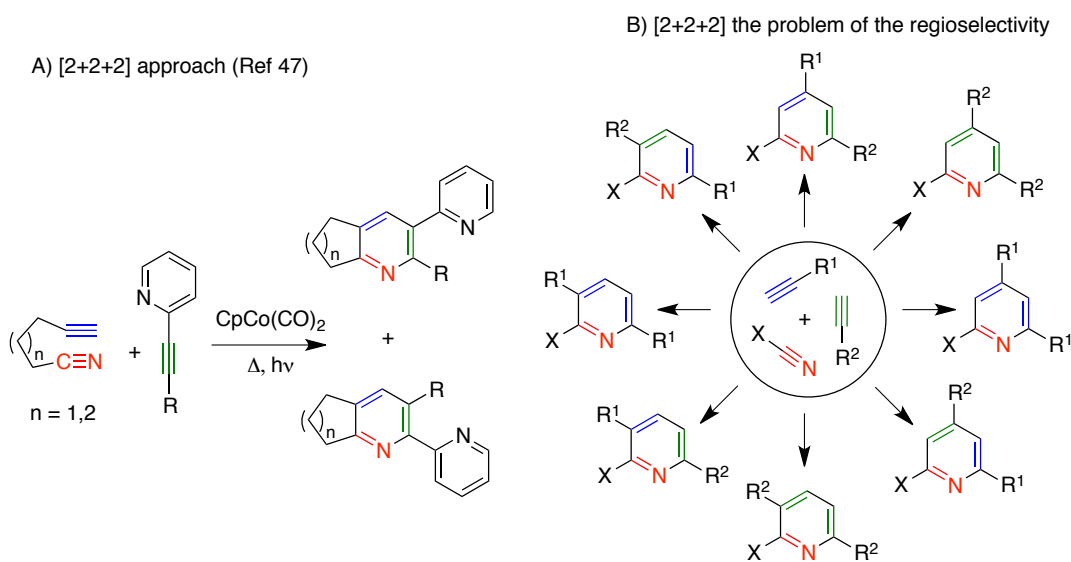
⁴⁴ Bohlmann, F.; Rahtz, D. *Chem. Ber.* **1957**, *90*, 2265-2272.

⁴⁵ The Bohlmann-Rahtz pyridine synthesis has been reviewed: Bagley, M. C.; Glover, C.; Merritt, E. A. *Synlett* **2007**, 2459–2482.

⁴⁶ (a) For a general overview of transition-metal-catalyzed reactions in heterocyclic synthesis including pyridines, see: Nakamura, I.; Yamamoto, Y. *Chem. Rev.* **2004**, *104*, 2127-2198; (b) For a specific review of metal-mediated [2+2+2] cycloaddition reactions, see: Domínguez, G.; Pérez-Castells, J. *Chem. Soc. Rev.* **2011**, *40*, 3430-3444; (c) For a specific review of metal-mediated [2+2+2] pyridine synthesis, see: Varela, J. A.; Saá, C. *Chem. Rev.* **2003**, *103*, 3787-3801.

⁴⁷ Varela, J. A.; Castedo, L.; Saá, C. *J. Org. Chem.* **1997**, *62*, 4189-4192.

⁴⁸ Brandli, C.; Ward, T. R. *J. Comb. Chem.* **2000**, *2*, 1–42.



Scheme 1.6: The [2+2+2] approach to pyridine synthesis

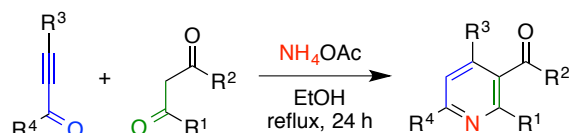
1.3.2 Synthetic methodologies based on multicomponent reactions

As the present thesis deals with multicomponent reaction for the synthesis of pyridine derivatives, we will next focus on this kind of approach, highlighting the various typologies of disconnections described in the literature.

1.3.2.1 Approaches based on the [3+2+1] disconnection

The multicomponent version of the Bohlmann-Rahtz synthesis is one of the simplest approaches based on the [3+2+1] disconnection. Described firstly at the beginning of the millennium, this strategy is based on the formation in situ of the β -enamine component by the condensation between an ammonia analogue and an enolizable carbonyl compound (Scheme 1.7).⁴⁹

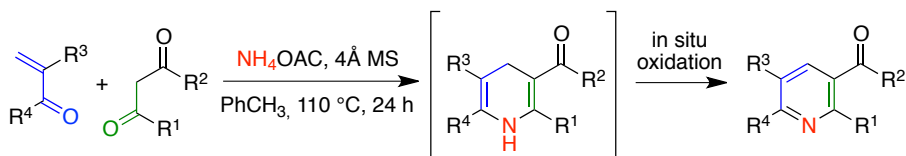
⁴⁹ For examples of pyridine syntheses based on the multicomponent version of the Bohlmann-Rahtz synthesis, see: (a) Bagley, M. C.; Dale, J. W.; Bower, J. *Chem. Commun.* **2002**, 1682-1683; (b) X. Xiong, M. C. Bagley, K. Chapaneri, *Tetrahedron Lett.* **2004**, *45*, 6121-6124; (c) Aulakh, V. S.; Ciufolini, M. A. *J. Am. Chem. Soc.* **2011**, *133*, 5900-5904; (d) Zhang, L.-J.; Wu, Q.; Sun, J.; Yan, C.-G. *Beilstein, J. Org. Chem.* **2013**, *9*, 846-851.



Scheme 1.7: The [3+2+1] multicomponent version of the Bohlmann-Rahtz pyridine synthesis developed by Bagley's group^{49b}

The same *in situ* formation of the β -enamine is used also in the strategy centered on the Michael addition, a comparable methodology based on the reaction of this β -enamine with a α,β -unsaturated carbonyl compound. This methodology generally affords tetrasubstituted pyridines and allows the use of various typologies of Michael acceptors and of enolizable carbonyl compounds.⁵⁰

It is worth reminding that, whereas the Bohlmann-Rahtz reaction affords directly the fully unsaturated pyridine nucleus, the Michael addition-initiated [3+2+1] approach needs a final step of aromatization that usually occurs in a sequential manner, after the formation of the heterocycle, and in the same reactions conditions employed for the multicomponent protocol (Scheme 1.8).



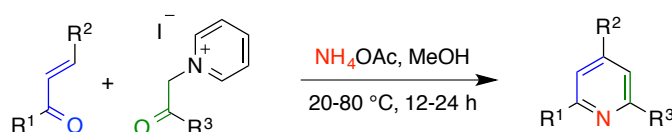
Scheme 1.8: The [3+2+1] Michael addition-based multicomponent strategy for pyridine synthesis developed by Rodriguez's group^{50d}

The major drawbacks of this strategy are represented by the requirement to use β -unsubstituted aldehydes and ketones, probably because of the reversibility of the Michael addition with hindered substrates; the use of activated Michael acceptors

⁵⁰ For examples of multicomponent pyridine syntheses based on the Michael addition strategy, see: (a) Katritzky, A. R.; Abdel-Fattah, A. A. A.; Tymoshenko, D. O.; Essawy, S. A. *Synthesis* **1999**, 2114-2118; (b) Al-Saleh, B.; Abdelkhalik, M. M.; Eltoukhy, A. M.; Elnagdi, M. H. *J. Heterocycl. Chem.* **2002**, *39*, 1035-1038; (c) Kantevari, S.; Chary, M. V.; Vuppapapati, S. V. N.; Lingaiah, N. *J. Heterocycl. Chem.* **2008**, *45*, 1099-1102; (d) Liéby-Muller, F.; Allais, C.; Constantieux, T.; Rodriguez, J. *Chem. Commun.* **2008**, 4207-4209; (e) Allais, C.; Constantieux, T.; Rodriguez, J. *Chem. Eur. J.* **2009**, *15*, 12945-12948; (f) Allais, C.; Liéby-Muller, F.; Constantieux, T.; Rodriguez, J. *Eur. J. Org. Chem.* **2013**, *19*, 4131-4145; (g) Raja, A. V. P.; Tenti, G.; Perumal, S.; Menéndez, J. C. *Chem. Commun.* **2014**, *50*, 12270-12272.

with enhanced electrophilicity represents a way to circumvent the problem.⁵¹

A conceptually comparable approach to the Michael addition-based process is represented by the Kröhnke pyridine synthesis. Described firstly in 1961, this methodology is based on the reaction between a α,β -unsaturated carbonyl compound, a α -pyridinium methyl ketone salt and ammonium acetate as the source of the nitrogen atom.⁵² The reaction usually provides access to pyridines bearing up to four substituents and it is particularly useful for the synthesis of polyaryl derivatives. Initially described as a sequential process, to date different variations in a one-pot multi step fashion have been described (Scheme 1.8),⁵³ including one methodology based on a [2+2+1+1] disconnection characterized by the in situ formation of the α,β -unsaturated carbonyl derivative.⁵⁴



Scheme 1.9: The [3+2+1] one-pot Kröhnke pyridine synthesis described by Lee^{53e}

Finally a more limited approach to pyridine that exploits the [3+2+1] disconnection is the strategy involving the use of Mannich bases as starting materials (Scheme 1.1).⁵⁵ In the most exploited version of this reaction a protonated Mannich base is reacted with an enolizable carbonyl derivative in the presence of ammonium acetate as ammonia source (Scheme 1.9). Although mostly employed in the synthesis of fused pyridine systems, in the last years several approaches to non-polycyclic pyridines based on this method have been

⁵¹ Allais, C.; Constantieux, T.; Rodriguez, J. *Chem. Eur. J.* **2009**, *15*, 12945-12948.

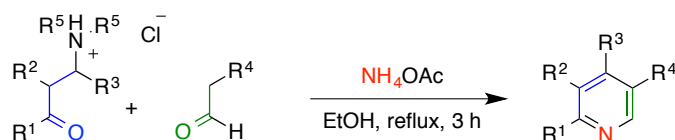
⁵² Zecher, W.; Kröhnke, F. *Chem. Ber.* **1961**, *94*, 690-697.

⁵³ For examples of the Kröhnke pyridine synthesis, see: (a) Constable, E. C.; Lewis, J. *Polyhedron* **1982**, *1*, 303-306; (b) Neve, F.; Crispini, A.; Campagna, S. *Inorg. Chem.* **1997**, *36*, 6150-6156; (c) Katritzky, A. R.; Abdel-Fattah, A. A. A.; Tymoshenko, D. O.; Essawy, S. A. *Synthesis* **1999**, 2114-2118; (d) Zhao, L.-X.; Moon, Y.-S.; Basnet, A.; Kim, E.; Jahng, Y.; Park, J. G.; Jeong, T. C.; Cho, W.-J.; Choi, S.-U.; Lee, C. O.; Lee, S.-Y.; Lee, C.-S.; Lee, E.-S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1333-1337; (e) Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, B.-S.; Jeong, T. C.; Lee, C.-S.; Lee, E.-S. *Bioorg. Med. Chem.* **2007**, *15*, 4351-4359.

⁵⁴ Yan, C.-G.; Cai, X.-M.; Wang, Q.-F.; Wang, T.-Y.; Zheng, M. *Org. Biomol. Chem.* **2007**, *5*, 945-951.

⁵⁵ Mannich, C.; Krösche, W. *Arch. Pharm. (Weinheim)* **1912**, *250*, 647-667.

described.⁵⁶



Scheme 1.10: The [3+2+1] multicomponent strategy for pyridine synthesis from Mannich bases developed by Risch^{56b}

1.3.2.2 Approaches based on the [2+2+1+1] disconnection

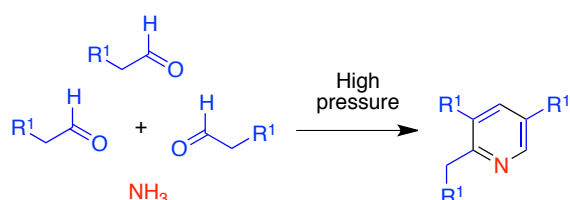
At the beginning of the 20th century the Russian chemist Chichibabin described a pseudo-four-component synthesis of the pyridine ring based on the [2+2+1+1] approach from the reaction between 3 equiv of an enolizable aldehyde and 1 equiv of ammonia (Scheme 1.10A).⁵⁷ The low yield, harsh conditions and numerous byproducts left this protocol in the oblivion till at the end of 1940s Frank and Steven carried out various experiments in an attempt to better understand the reaction. During their mechanistic studies they used plausible intermediates of the reaction, such as α,β -unsaturated carbonyl compounds generated from the condensation of two equiv of the enolizable carbonyl derivative, reaching surprisingly the formation of an unexpected pattern of substitution in the pyridine ring (a 2,4,6-trisubstitution instead of the 2,3,5-trisubstitution depicted by Chichibabin). Pointing at the reversible nature of the aldol condensation, they envisioned a reaction pathway going through the formation of a 1,5-dicarbonyl compound with the final step of ring closure by the intervention of the ammonia source (Figure 1.10B).⁵⁸

⁵⁶ For examples of multicomponent pyridine synthesis based on the Mannich reaction, see: (a) Sielemann, D.; Keuper, R.; Risch, N. *Eur. J. Org. Chem.* **2000**, 543-548; (b) Winter, A.; Risch, N. *Synthesis* **2003**, 2667-2670; (c) Jemmezi, F.; Chtiba, S.; Khiari, J. *J. Heterocycl. Chem.* **2013**, *50*, 206-208.

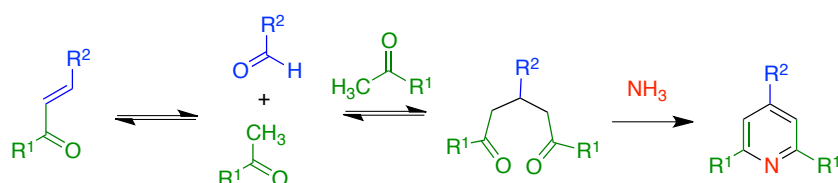
⁵⁷ Chichibabin, A. E. *J. Russ. Phys. Chem. Soc.* **1906**, *37*, 1229-1231.

⁵⁸ Frank, R. L.; Seven, R. P. *J. Am. Chem. Soc.* **1949**, *71*, 2629-2635.

A) The original [2+2+1+1] Chichibabin pyridine synthesis

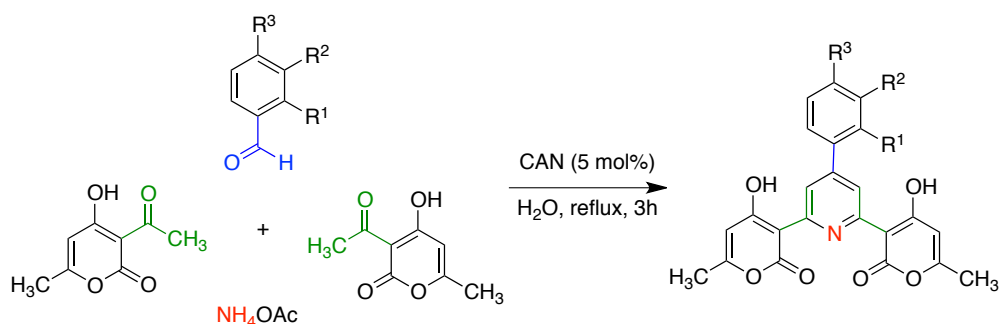


B) The mechanistic studies performed by Frank and Steven



Scheme 1.11

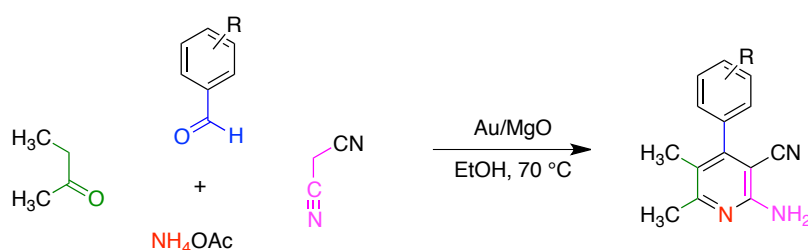
These mechanistic studies allowed to overcome some of the limitations of the original protocol and from that moment most of the variations derived from this pyridine synthesis are directed to the formation of the 2,4,6-trisubstituted pyridine (known as Kröhnke pyridines, as mentioned in the previous section) from the reaction between an ammonia source, a non-enolizable aldehyde and two equivalent of an enolizable ketone (Scheme 1.11).⁵⁹



Scheme 1.12 The [2+2+1+1] Chichibabin reaction-based pseudo-four-component strategy for pyridine synthesis developed by Penta^{59d}

⁵⁹ For examples of pyridine synthesis based on variations to the Chichibabin synthesis, see: (a) Tu, S.; Jia, R.; Jiang, B.; Zhang, J.; Zhang, Y.; Yao, C.; Ji, S. *Tetrahedron* **2007**, *63*, 381-388; (b) Rong, L.; Han, H.; Wang, S.; Zhuang, Q. *Synth. Commun.* **2008**, *38*, 1808-1814; (c) Li, J.; He, P.; Yu, C. *Tetrahedron* **2012**, *68*, 4138-4144; (d) Penta, S.; Vedula, R. R. *J. Heterocycl. Chem.* **2013**, *50*, 859-862.

An interesting variation of this synthetic strategy is represented by the replacement of one of the equivalents of the enolizable ketone by malononitrile; in this case we can properly consider this [2+2+1+1] approach as a real four-component variation of the Chichibabin pyridine synthesis (Scheme 1.12).⁶⁰



Scheme 1.13: The [2+2+1+1] Chichibabin reaction-based four-component strategy for pyridine synthesis developed by Pagadala^{60d}

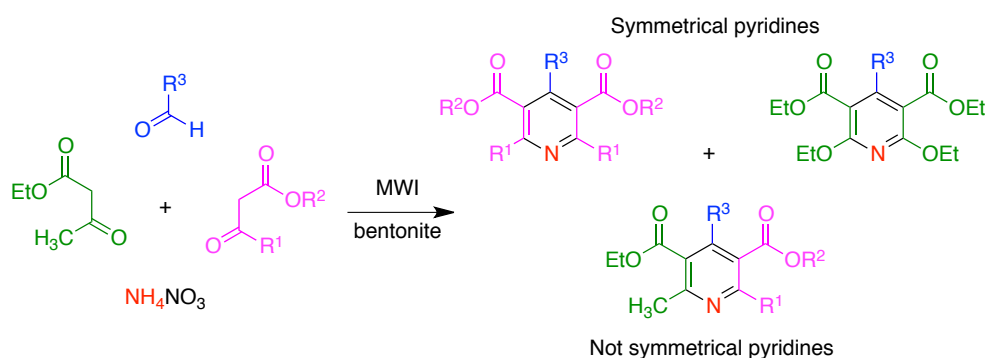
The last [2+2+1+1] approach we are going to describe in this section is probably one of the most common methods for the construction of the pyridine nucleus: the Hantzsch reaction. As already mentioned in the paragraph 1.1, this multicomponent reaction was one of the first described in the literature¹⁰ and allows the formation of a 1,4-dihydropyridine (DHP) derivative by a pseudo-four-component reaction between an aldehyde, a source of ammonia and two equiv of a β -dicarbonyl compound (Scheme 1.1). The oxidation of DHP into pyridine has been thoroughly investigated and it is usually performed with well-known oxidizing agents,⁶¹ including some environmentally friendly protocols that involve O_2 as oxidant.⁶² Although in most cases this oxidation is usually

⁶⁰ For examples of pyridine synthesis based on variations to the Chichibabin synthesis involving malononitrile, see: (a) Kambe, S.; Saito, K.; Sakurai, A.; Midorikawa, H. *Synthesis* **1980**, 366-368; (b) Shi, F.; Tu, S. J.; Fang, F.; Li, T. J. *Arkivoc* **2005**, 137-142; (c) Mungra, D. C.; Patel, M. P.; Patel, R. G. *Arkivoc* **2009**, 64-74; (d) Pagadala, R.; Maddila, S.; Moodley, V.; van Zyl, W. E.; Jonnalagadda, S. B. *Tetrahedron Lett.* **2014**, *55*, 4006-4010.

⁶¹ For examples of dihydropyridine oxidation into pyridine, see: (a) Yadav, J. S.; Reddy, B. V. S.; Basak, A. K.; Baishya, G.; Narsaiah, A.V. *Synthesis*, **2006**, 451-454; (b) Ghorbani-Choghamarani, A.; Nikoorazm, M.; Goudarziafshar, H.; Shiri, L.; Chenani, Z. *Bull. Korean Chem. Soc.* **2009**, *30*, 972-974; (c) Liao, X.; Lin, W.; Lu, J.; Wang, C. *Tetrahedron Lett.* **2010**, *51*, 3859-3861; (d) Jia, X.; Yu, L.; Huo, C.; Wang, Y.; Liu, J.; Wang, X. *Tetrahedron Lett.* **2014**, *55*, 264-266; (e) Sanchez, L. M.; Sathicq, A. G.; Baronetti, G. T.; Thomas, H. J.; Romanelli, G. P. *Catal. Lett.* **2014**, *44*, 172-180; (f) Saikh, F.; De, R.; Ghosh, S. *Tetrahedron Lett.* **2014**, *55*, 6171-6174.

⁶² For examples of dihydropyridine oxidation into pyridine employing O_2 as oxidant, see: (a) Ortiz, M. E.; Nuñez-Vergara, L. J.; Camargo, C.; Squella, J. A. *Pharm. Res.* **2004**, *21*, 428-435; (b)

performed in a sequential manner,⁶³ in the literature we can find also multicomponent procedures focused on affording the pyridine derivative in a one-pot fashion (Scheme 1.13).⁶⁴



Scheme 1.14: The combinatorial study developed by Cotterill employing the [2+2+1+1] approach of the Hantzsch dihydropyridine synthesis^{64a}

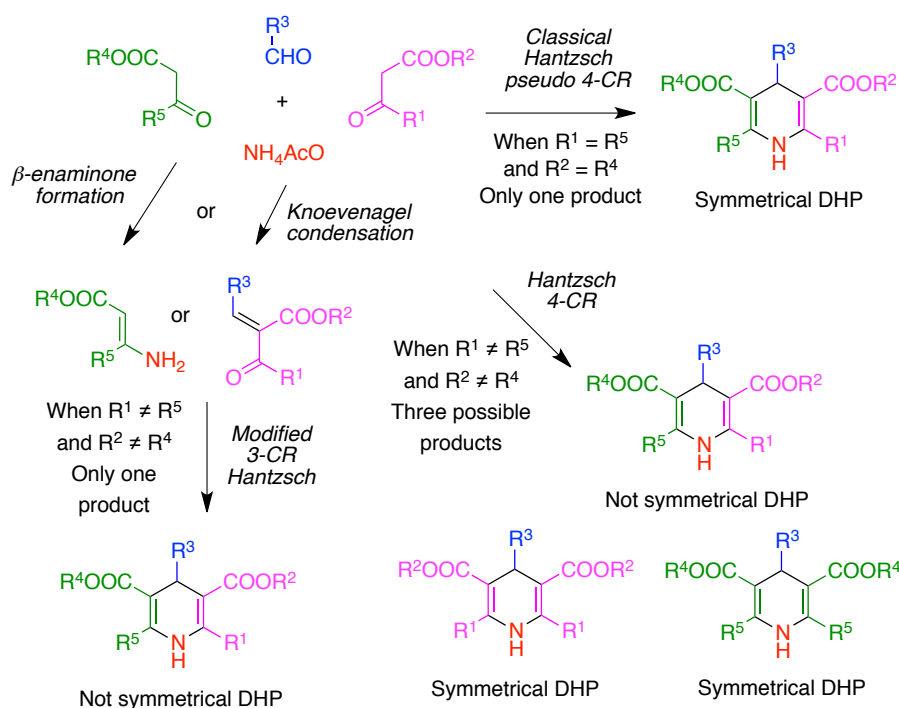
As partially shown in the scheme 1.13, the major limitation of the original protocol is the absence of regioselectivity of the process when two different β-dicarbonyl compounds are employed, namely when we move from a pseudo-four-component reaction to a real four-component reaction. To overcome this drawback, the more efficient strategy is to form previously the intermediates that are supposed to take part in the Hantzsch reaction, such as a Knoevenagel adduct between one of the β-dicarbonyl compound and the aldehyde, or the β-enaminone intermediate derived from the reaction between the ammonia source and one of the β-dicarbonyl compound. This methodology, known as “modified three-component Hantzsch approach”, allows the control of the regioselectivity of the reaction and it usually employed in the generation of non-symmetrical 1,4-DHPs

Han, B.; Kiu, Z.; Liu, Q.; Yang, L.; Liu, Z.-L.; Yu, W. *Tetrahedron* **2006**, *62*, 2492-2496; (c) Shen, L.; Cao, S.; Wu, J.; Zhang, J.; Li, H.; Liu, N.; Qian, X. *Green Chem.* **2009**, *11*, 1414-1420; (d) Abdel-Mohsen, H. T.; Conrad, J.; Beifuss, U. *Green Chem.* **2012**, *14*, 2686-2690; (e) Wei, X.; Wang, L.; Jia, W.; Du, S.; Wu, L.; Liu, Q. *Chin. J. Chem.* **2014**, *32*, 1245-1250.

⁶³ (a) Xia, J. J.; Wang, G. W. *Synthesis* **2005**, 2379-2383; (b) Das Sharma, S.; Hazarika, P.; Konwar, D. *Catal. Commun.* **2008**, *9*, 709-714.

⁶⁴ (a) Cotterill, I. C.; Usyatinsky, A. Y.; Arnold, J. M.; Clark, D. S.; Dordick, J. S.; Michels, P. C.; Khmel'nitsky, Y. L. *Tetrahedron Lett.* **1998**, *39*, 1117-1120; (b) De Paolis, O.; Baffoe, J.; Landge, S.; Török, B. *Synthesis* **2008**, 3423-3428; (c) Shen, L.; Cao, S.; Wu, J.J.; Zhang, J.; Li, H.; Liu, N. J.; Qian, X. H. *Green Chem.* **2009**, *11*, 1414-1420; (d) Mirza-Aghayan, M.; Asadi, F.; Boukherroub, R. *Monatsh. Chem.* **2014**, *145*, 1919-1924.

(where the adjective non-symmetrical indicates the presence of two different ester groups at C-3 and C-5 of the DHP, scheme 1.14).⁶⁵



Scheme 1.15: Regioselectivity in the Hantzsch dihydropyridine synthesis.

Despite its strategic limitations, the Hantzsch synthesis has been and still is one of the most employed strategies, especially in medicinal chemistry because it allows a straightforward access to the 1,4-dihydropyridine nucleus that is present in many marketed drugs.⁶⁶ Indeed, due to their pharmacological versatility, 1,4-DHPs can be defined as “privileged scaffold”, namely, as defined firstly by Evans,⁶⁷ molecular structures which show the ability to interact with different

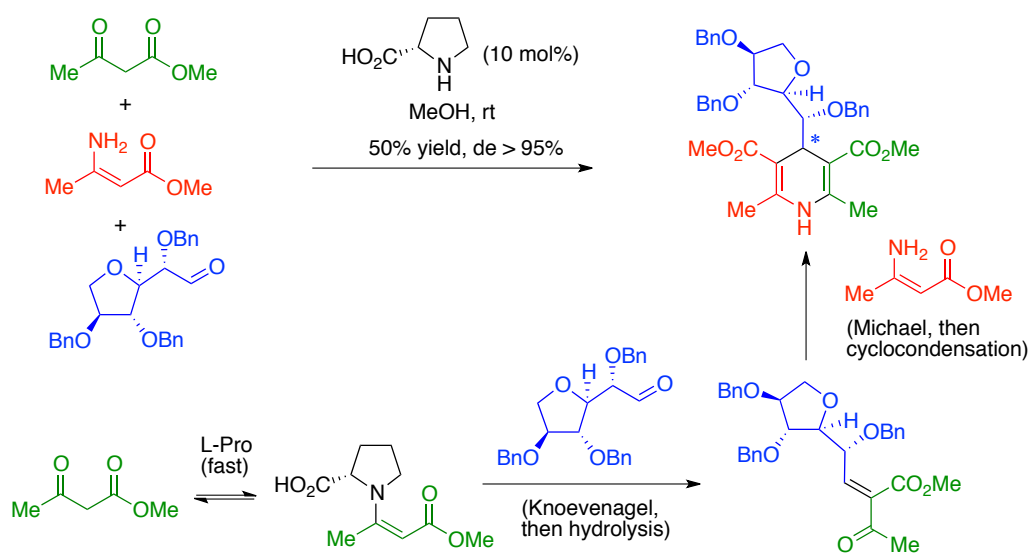
⁶⁵ (a) Andersen, K. H.; Nordlander, M.; Westerlund, R. C. U.S. Patent 5856346, **1999**; (b) Dondoni A, Massi A, Aldhoun M *J Org. Chem.* **2007**, *72*, 7677-7687; (c) Vishnu, N. R.; Puruschottam, J. A.; Kumar, S. S. Eur. Patent EP 2386544, **2011**.

⁶⁶ (a) Ioan, P.; Carosati, E.; Micucci, M.; Cruciani, G.; Broccatelli, F.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. *Curr. Med. Chem.* **2011**, *18*, 4901-4922; (b) Carosati, E.; Ioan, P.; Micucci, M.; Broccatelli, F.; Cruciani, G.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. *Curr. Med. Chem.* **2012**, *19*, 4306-4323.

⁶⁷ Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.;

groups of pharmacological receptor. Owing to this intriguing characteristic, the use of this kind of structures for drug discovery is very attractive.⁶⁸

The therapeutic use of dihydropyridines should be improved by their asymmetric synthesis, considering that the absolute configuration of the stereogenic C-4 atom of chiral DHPs has a significant role on their pharmaceutical profiles. Despite its long history, only in the last decade have diastereoselective and enantioselective versions of the Hantzsch reaction been reported. Thus, in the course of work aimed at the synthesis of C-glycosylated analogues of medically relevant DHPs, Dondoni and co-workers studied conventional Hantzsch reactions having aldoses as substrates, and found that they proceeded only with modest diastereoselectivities (de < 50%). Further research showed that the use of (*S*)- or (*R*)-Pro as organocatalysts led to highly diastereoselective Hantzsch reactions from methyl acetoacetate, its β -enaminone and sugar-derived aldehydes obtained from agarose (Scheme 1.16).



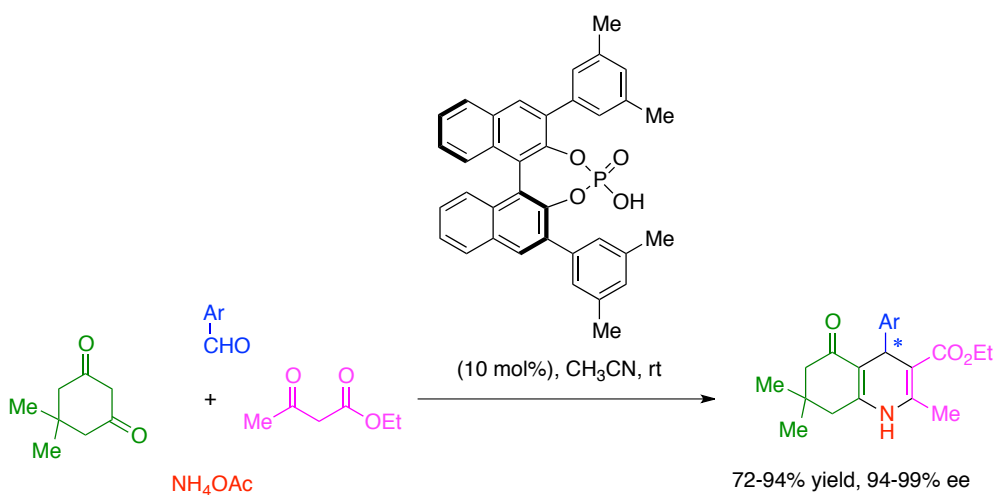
Scheme 1.16. A diastereoselective Hantzsch reaction leading to C-glycosylated 1,4-dihydropyridines

Kunkel, K. A.; Springer, J. P.; Hirshfield, J. J. *Med. Chem.* **1988**, *31*, 2235-2246

⁶⁸ For the importance of privileged scaffolds in medicinal chemistry and drug discovery, see: (a) Costantino, L.; Barlocco, D. *Curr. Med. Chem.* **2006**, *13*, 65-85; (b) Welsch, M. E.; Snyder, S. A.; Stockwell, B. R. *Curr. Opin. Chem. Biol.* **2010**, *14*, 347-361.

Mechanistically, this transformation was proposed to start by the formation of an enaminone from the β -ketoester and L-Pro, followed by a Knoevenagel reaction with the heterocyclic aldehyde and subsequent hydrolysis. A Michael addition of the β -ketoester enaminone to the Knoevenagel product followed by a final cyclocondensation with loss of a molecule of water would explain the isolation of the final dihydropyridine (Scheme 1.16).⁶⁹

Enantioselective versions of the Hantzsch reaction have also been reported. As shown in Scheme 1.17, the reaction between dimedone, ethyl acetoacetate and aromatic aldehydes in the presence of a chiral BINOL-phosphoric acid catalyst gave dihydropyridines in good to excellent yields and in enantiomeric excesses above 94%, although the absolute configuration of the final products was not determined.⁷⁰



Scheme 1.17. Enantioselective Hantzsch reactions in the presence of a chiral Brønsted acid

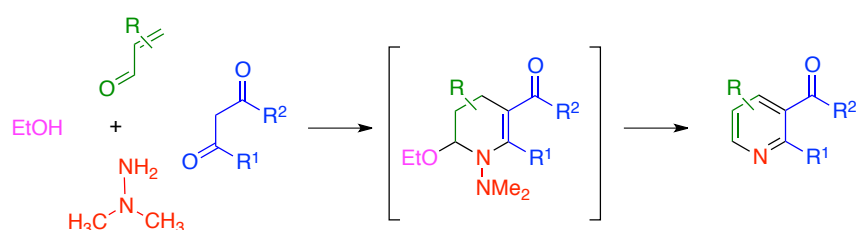
⁶⁹ Ducatti, D. R. B.; Massi, A.; Nosedà, M. D.; Duarte, M. E. R.; Dondoni, A. *Org. Biom. Chem.* **2009**, *7*, 1980-1986.

⁷⁰ Evans, C. G.; Gestwicki, J. E. *Org. Lett.* **2011**, *11*, 2957-2959.

2. Objectives

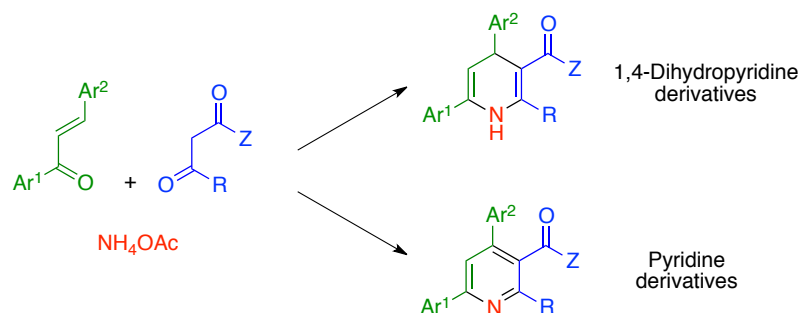
Within the general framework of the development of multicomponent reactions for the synthesis of pharmacologically relevant dihydropyridines and pyridines, we have pursued the following specific objectives:

1. Development of a new approach to pyridine derivatives, based on a double elimination process on a tetrahydropyridine derivative generated through a Lewis-acid catalysed four-component reaction between β -dicarbonyl compounds, dimethylhydrazine, acroleins and ethanol (Scheme 2.1).



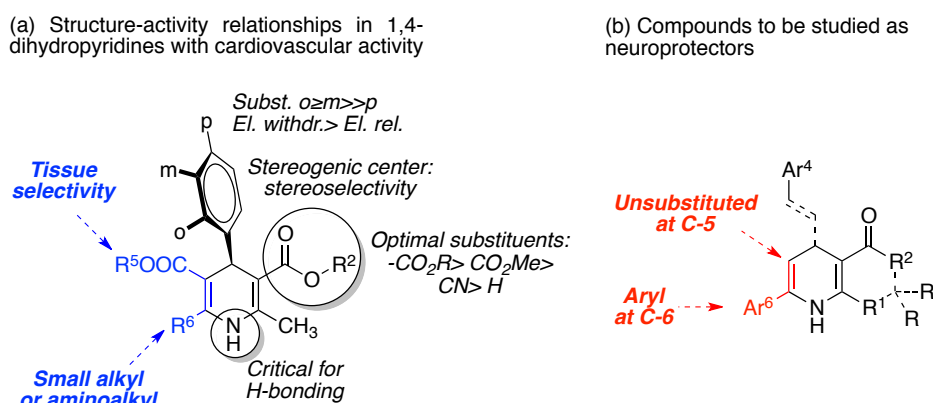
Scheme 2.1: New four-component reaction for the pyridine synthesis

2. Development of a new multicomponent route to 4,6-diarylpyridine frameworks from β -dicarbonyl compounds, chalcones and an ammonia source, and study of the conditions required for the reaction to afford dihydropyridines or pyridines (Scheme 2.2).



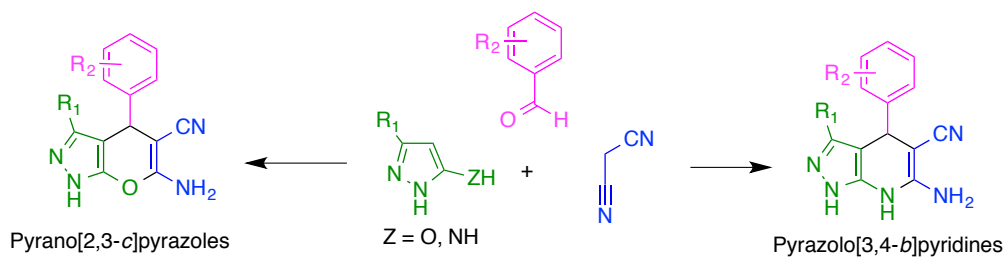
Scheme 2.2: New three-component reaction for the synthesis of DHPs and pyridines

3. Classical 1,4-dihydropyridines have shown good neuroprotective activities, which have not been exploited therapeutically because of their cardiovascular effects. In view of the fact that 4,6-diaryl-1,4-dihydropyridines do not satisfy the well-known structure-activity relationships of vasodilator dihydropyridines, we also planned the study of the compounds mentioned in Objective 2 as neuroprotectors.



Scheme 2.3: Structural variations between classical 1,4-DHPs (a) and our compounds (b)

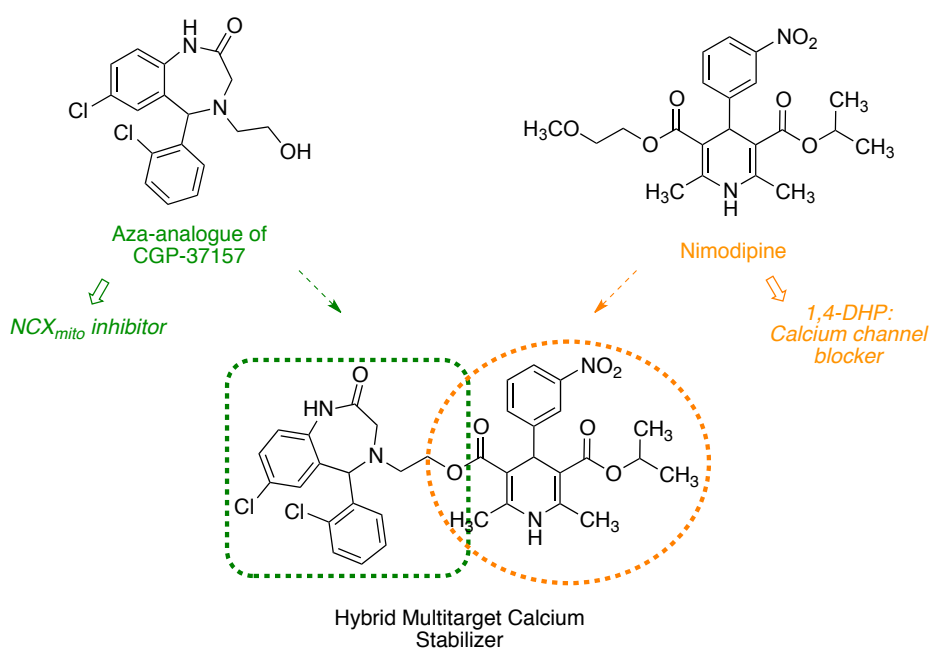
4. Synthesis of fused dihydropyridines and their oxygen heteroanalogues via a Hantzsch-like three-component reaction between malononitrile, aromatic aldehydes and C5-functionalized pyrazoles, and their subsequent study as neuroprotectors via inhibition of GSK3 β .



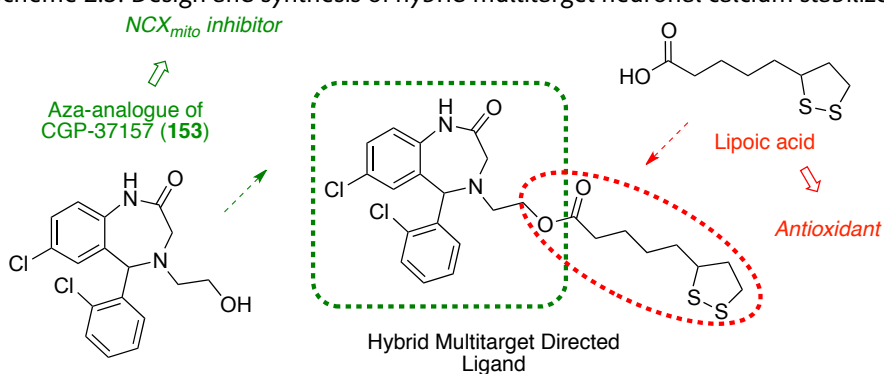
Scheme 2.4: Design of bicyclic GSK3 β inhibitors

5. Multicomponent synthesis of hybrid structures related to nimodipine and CGP-37157 as potential anti-Alzheimer compounds aimed at stabilizing neuronal and mitochondrial calcium cycling by a multitarget approach (Scheme 2.5). Similarly, the synthesis and study of hybrids of a CGP-

37157 aza analogue and lipoic acid, a well-known antioxidant able to cross the blood-brain barrier, were planned (Scheme 2.6).

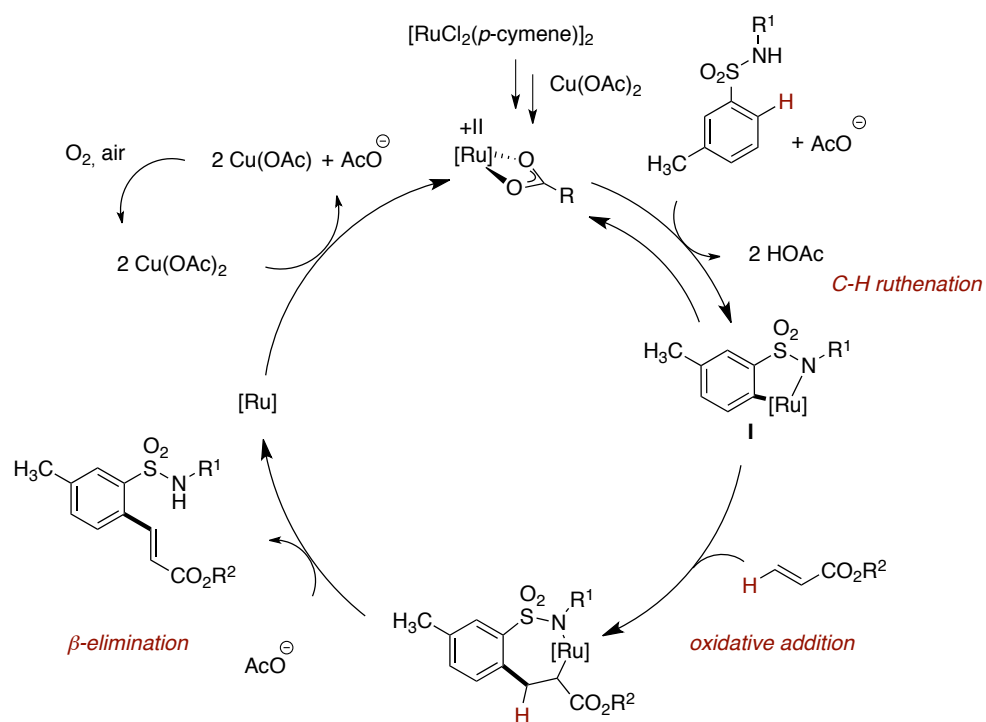


Scheme 2.5: Design and synthesis of hybrid multitarget neuronal calcium stabilizer



Scheme 2.6: Design and synthesis of hybrid multitarget directed ligand with potential antioxidant properties

- During a stay at Georg-August-Universität Göttingen (Professor Ackermann's group) as part of the requirements for the European Mention to the Ph. D. title, a Ru(II)-catalyzed oxidative C-H alkenylation of arylsulfonamides was developed (Scheme 2.7).



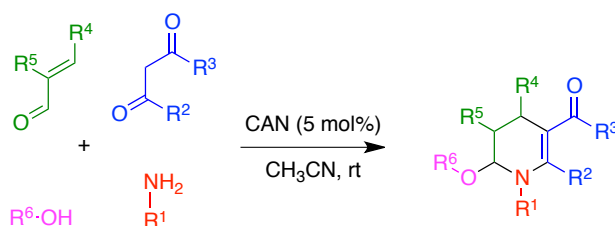
Scheme 2.7: Development of a new Ru(II)-catalyzed oxidative C-H alkenylation of arylsulfonamides

3. A New Multicomponent Protocol For The Synthesis Of Pyridine Derivatives

3.1 SYNTHESIS OF PYRIDINE DERIVATIVES VIA A NEW FOUR-COMPONENT REACTION

3.1.1 Introduction

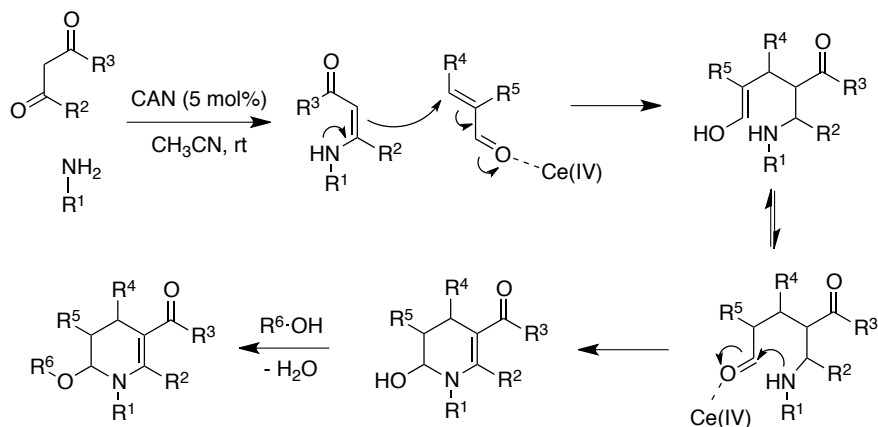
In an attempt to find an innovative pathway for the synthesis of the pyridine motif and related heterocycles, the initial purpose of our work was to extend the range of the applications of a previous four-component protocol developed by our own group and that consisted in a mild, high-yielding synthesis of tetrahydropyridines from α,β -unsaturated aldehydes, amines, 1,3-dicarbonyl compounds and alcohols. The reaction, catalyzed by cerium(IV) ammonium nitrate (CAN) acting as a Lewis acid, takes place under mild reaction conditions and shows a high efficiency, allowing the generation of four bonds (two C-N, one C-O and one C-C) in a single operation (Scheme 3.1).⁷¹



Scheme 3.1: Reaction scheme followed in our previous project.

Previous work also made clear the importance of the use of CAN as a Lewis acid, able to promote the initial reaction between the amine and the 1,3-dicarbonyl components to give the corresponding β -enaminones, and expediting their subsequent Michael addition to the α,β -unsaturated aldehydes (Scheme 3.2).

⁷¹ Sridharan, V.; Maiti, S.; Menéndez, J. C. *Chem. Eur. J.* **2009**, *15*, 4565-4572.



Scheme 3.2: Mechanistic rationale proposed for the four-component reaction leading to tetrahydropyridine compounds.

3.1.2 Preliminary studies

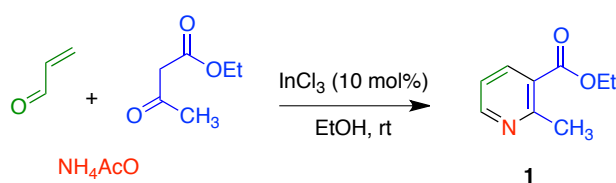
Our initial study was focused on the simplest possible approach to our goal: a multicomponent strategy similar to the one previously studied but using ammonia instead of primary amines as the source of nitrogen. In this way, we expected to obtain a N-unsubstituted tetrahydropyridine derivative that could then aromatize to pyridine *via* a sequential elimination/dehydrogenation process. A literature search revealed a pioneering work from Hoelderich's group based on a similar reaction between β -ketoesters, ammonium acetate and acrolein catalyzed by zeolites, which employed very harsh reaction conditions (reactions carried out in gas phase and at high temperatures) and afforded the corresponding pyridines in moderate yield.⁷² Furthermore, we found in the literature two examples of a similar reaction between β -ketoesters, ammonium acetate and acrolein yielding the corresponding pyridines after an aromatization process in the presence of sulphur, but in very poor yield (17 and 26%).⁷³

Unfortunately, when we employed our usual CAN-catalyzed conditions in the reaction between ammonium acetate, ethyl acetoacetate and acrolein in ethanol, no cyclized derivative was detected. We decided to assay different catalysts,

⁷² Hoelderich, W.; Goetz, N. U.S. Patent US4960894, 1990.

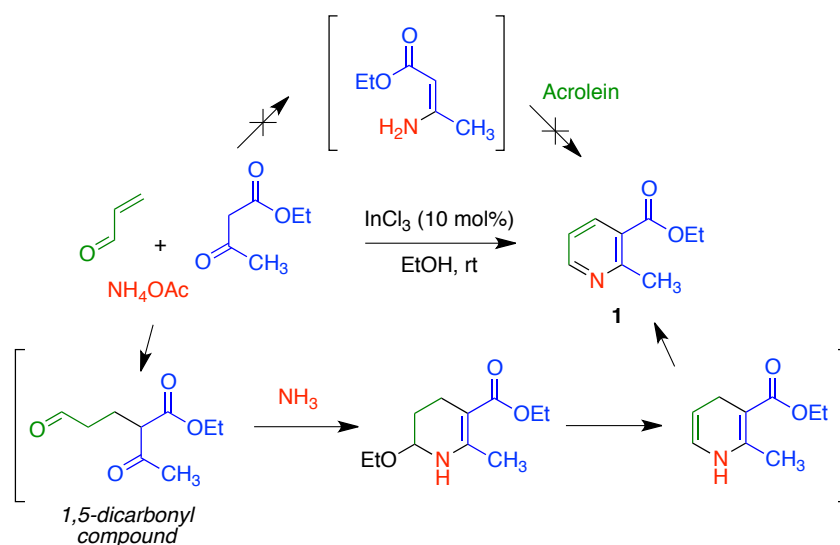
⁷³ Roy, P.J.; Dufresne, C.; Lachance, N.; Leclerc, J.P.; Boisvert, M.; Wang, Z.; Leblanc, Y. *Synthesis* 2005, 2751-2757.

discovering that the reaction carried out in the presence of 10% InCl_3 was strongly exothermic and afforded pyridine derivative **1** as the only product, albeit in a moderate 30% yield, together with recovered ethyl acetoacetate (Scheme 3.3).



Scheme 3.3: Initial study for the multicomponent pyridine synthesis

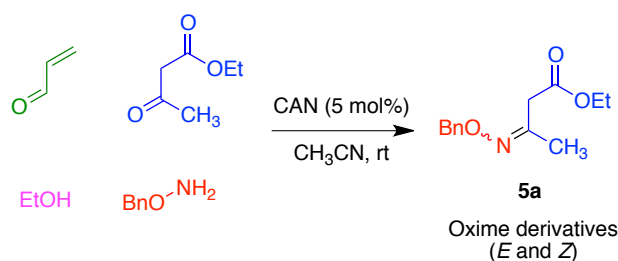
Although we initially postulated that the first step of the process was the formation of the β -enaminone, it is worth emphasizing that this reaction was found not to proceed *via* this intermediate, since it did not occur when ethyl 3-aminocrotonate was employed as the starting material. Therefore, we assumed that our reaction followed a different synthetic pathway that starts with a Michael addition of the ketoester to the unsaturated aldehyde to give a 1,5-dicarbonyl compound, followed by its oxidative cyclodehydration with ammonia (Scheme 3.4).



Scheme 3.4: Supposed synthetic pathway for our initial multicomponent reaction

Since this approach to pyridines was already known in the literature,⁷⁴ we did not further pursue the preparation of pyridines by this route.

With this result in hand, we decided to explore as the next possible approach, the substitution of the primary amine by a compound with a nucleophilic nitrogen linked to a good leaving group in order to achieve the pyridine synthesis by combination of the MCR with a double elimination. *O*-Methyl- and *O*-benzylhydroxylamine were our first choices as the source of the pyridine nitrogen atom but, unfortunately, the sequential four-component reaction between these starting materials, ethyl acetoacetate, acrolein and ethanol in the presence of catalytic amounts of CAN in acetonitrile solution was not successful. Indeed, by employing the previously established conditions for the case of primary amines, we were able to recover only mixtures of the *E* and *Z* oxime derivatives (**5a**), without finding any trace of the expected tetrahydropyridine derivatives (Scheme 3.5).



Scheme 3.5: Use of *O*-benzylhydroxylamine as the source of the pyridine nitrogen atom

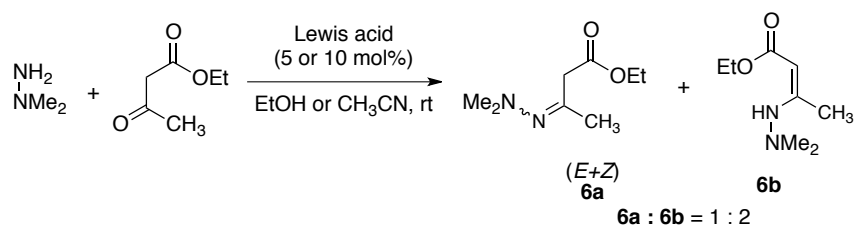
It is important to remark that the NMR spectra of these compounds did not show significant signals that could be ascribed to the corresponding enamine tautomer,⁷⁵ which was assumed to be the reactive species according to the above-mentioned precedent from our group. Even so, we carried on the reaction between *O*-benzylhydroxylamine and ethyl acetoacetate in the presence of various Lewis acids at different concentrations, in the hope that the formation of even only traces of the enamine tautomer could trigger the reaction with acrolein, displacing the

⁷⁴ (a) Liéby-Muller, F.; Allais, C.; Constantieux, T.; Rodriguez, J. *Chem. Commun.* **2008**, 4207-4209. (b) Allais, C.; Constantieux, T.; Rodriguez, J. *Chem. Eur. J.* **2009**, *15*, 12945-12948. (c) Allais, C.; Liéby-Muller, F.; Rodriguez, J.; Constantieux, T. *Eur. J. Org. Chem.* **2013**, 4131-4145.

⁷⁵ Ahlbrecht, H.; Henk, H. *Chem. Ber.* **1976**, *109*, 1516-1529.

equilibria towards the desired products. Despite the different Lewis acids, the various catalyst amounts and the different reaction times employed, our attempts were unsuccessful and this forced us to accept the lack of reactivity of the oxime intermediates, and prompted us to search for alternative reagents as source of the pyridine nitrogen.

Thus we turned our attention to 1,1-dimethylhydrazine, because it combined the nucleophilic character of a free NH₂ group with the presence of a dimethylamino unit as a leaving group. In addition, hydrazones derived from 1,3-dicarbonyl compounds had previously been described to exist as mixtures of imino and enamino tautomers.⁷⁶ Based on our previous experience with the hydroxylamine derivatives, on this occasion we started our study by conducting the reaction between 1,1-dimethylhydrazine and ethyl acetoacetate in acetonitrile in the presence of a catalytic amount of CAN (5 mol%). After stirring at room temperature for 15 minutes the reaction was complete, and afforded the desired product as a mixture of an enamine and a combination of *Z*- and *E*-hydrazone tautomers in 2:1 ratio, which was considered promising for our purpose (Scheme 3.6). Before starting with the four-component process, we focused our attention on evaluating the effect of the catalyst in this model reaction, assaying several different Lewis acids under room temperature conditions. As shown in Table 3.1, all catalysts assayed were found to give excellent results (entries 1-4), specially indium trichloride (entry 2), which proved to be marginally better than our initial choice (CAN) and cheaper than both triflates; for this reason and in view of our previous experience with this reagent, InCl₃ was chosen for subsequent studies.



Scheme 3.6: Use of 1,1-dimethylhydrazine as the source of the pyridine nitrogen atom

As an additional refinement, we successfully replaced the acetonitrile solvent by

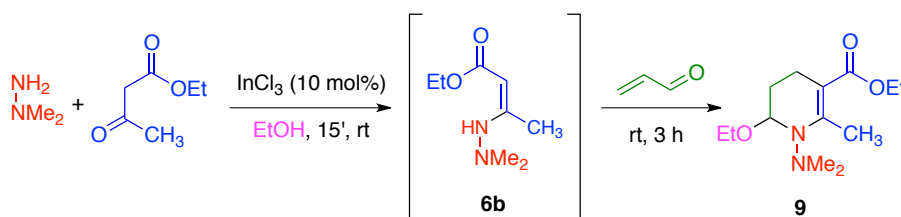
⁷⁶ Ahlbrecht, H.; Henk, H. *Chem. Ber.* **1975**, *108*, 1659-1681.

the less toxic and inexpensive ethanol, which was anyway required as one of the reagents involved in the MCR process (entry 5).

Table 3.1. Lewis acid-catalyzed reaction between 1,1-dimethylhydrazine and ethyl acetoacetate.

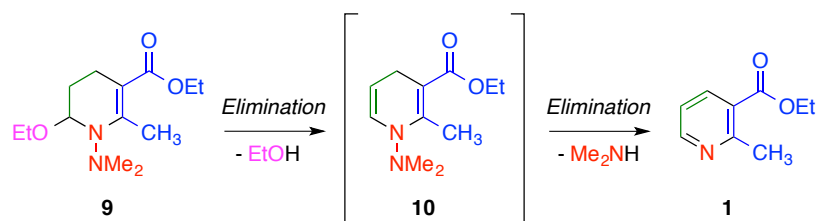
Entry	Lewis acid	Solvent	Yield (%)
1	CAN (5 mol%)	CH ₃ CN	92
2	InCl ₃ (10 mol%)	CH ₃ CN	96
3	Yb(OTf) ₃ (5 mol%)	CH ₃ CN	96
4	Sc(OTf) ₃ (5 mol%)	CH ₃ CN	96
5	InCl ₃ (10 mol%)	EtOH	98

Consequently we applied the optimal conditions to our designed sequential four-component reaction and, since at this stage we were primarily interested in the synthesis of tetrahydropyridine derivatives, we started our study of the multicomponent process by mixing 1,1-dimethylhydrazine and ethyl acetoacetate in ethanol in the presence of a catalytic amount of InCl₃ (10 mol%) followed, after 15 minutes, by the addition of acrolein, the simplest α,β -unsaturated aldehyde. After stirring the reaction mixture at room temperature for 3 hours, we were pleased to discover that the desired 1-dimethylamino-6-ethoxy-1,4,5,6-tetrahydropyridine derivative **9** was obtained in 90% yield (Scheme 3.7).



Scheme 3.7: InCl₃-catalyzed sequential four-component process leading to tetrahydropyridine derivative **9**

We next focused our attention on establishing the most favorable conditions for the two-step aromatization process required to complete the sequence leading to pyridine derivatives (Scheme 3.8).



Scheme 3.8: Study of the two-step aromatization process

For this purpose, we carried out a series of experiments whose results are summarized in [Table 3.2](#). After using initially the isolated and purified compound **9** for these experiments, we subsequently verified that it was possible to start the aromatization from the crude reaction mixture resulting from the workup of the initial four-component reaction, and this became the routine protocol. Our first choice of reaction conditions was based on a previous study carried out in our group,⁷⁷ which showed, on one hand, the possibility to transform 1-alkyl analogues of **9** into 1-alkyl-1,4-dihydropyridines in excellent yields by reflux in a suspension of neutral, grade I alumina in acetonitrile, whereas on the other hand, revealed the instability of the 1-alkyl analogues of **9** in the presence of Brønsted acids.⁷⁸ In our case, the use of neutral, grade I alumina in acetonitrile afforded the desired pyridine **1** as the major product, but its isolated yield was modest owing probably to its adsorption onto the alumina (entry 1). The same conditions were applied to the mixture from the four-component reaction, without any workup in order to minimize the number of purification steps, albeit with a poor result (entry 2). Subsequently, based on the existence of some literature precedent for the use of oxidizing reagents in the aromatization of compounds containing a 1-dimethylamino-1,4-dihydropyridine structural fragment,⁷⁹ we attempted the

⁷⁷ (a) Maiti, S.; Menéndez, J. C. *Synlett* **2009**, 14, 2249-2252; (b) Maiti, S.; Sridharan, V.; Menéndez, J. C. *J. Comb. Chem.* **2010**, 12, 713-722.

⁷⁸ For literature precedent of the acid-promoted elimination of dimethylamine from 1-dimethylamino-1,4-dihydropyridine systems, see: (a) Waldner, A. *Helv. Chim. Acta* **1988**, 71, 486-492; (b) Nebois, P.; Barret, R.; Fillion, H. *Tetrahedron Lett.* **1990**, 31, 2569-2572; (c) Valderrama, J. A.; González, M. F.; Valderrama, C. *Tetrahedron* **1999**, 55, 6039-6050; (d) Manzanaro, S.; Vicent, M. J.; Martín, M. J.; Salvador-Tormo, N.; Pérez, J. M.; Avendaño, C.; Menéndez, J. C.; de la Fuente, J. A. *Bioorg. Med. Chem.* **2004**, 12, 6505-6515.

⁷⁹ (a) Pérez, J. M.; López-Alvarado, P.; Avendaño, C.; Menéndez, J. C. *Tetrahedron Lett.* **1998**, 39, 673-676; (b) Pérez, J. M.; López-Alvarado, P.; Pascual-Alfonso, E.; Avendaño, C.; Menéndez, J. C. *Tetrahedron* **2000**, 56, 4575-4583.

reaction of compound **9** with iron trichloride,⁸⁰ obtaining only complex mixtures (entry 3). Thermal elimination processes carried out under vacuum that led to the desired transformation were also known in the literature,⁸¹ but in our case they were not viable owing to volatility of our materials (entry 4). Finally, it was found that the best conditions to obtain the target compound involved reflux in toluene containing a 20% w/w suspension of Pd/C (10% wt) (entry 5).

Table 3.2. Studies leading to the synthesis of pyridine derivative **1**.

Entry	Conditions	Solvent	T (°C)	Time (h)	Product ratio ^a	Isolated Yield (%) ^b
1	Al ₂ O ₃ ^c Al ₂ O ₃ ^c and	CH ₃ CN	Reflux	48	10:1:0	33
2	InCl ₃ (10 mol%)	EtOH	Reflux	24	0:2:1	0
3	FeCl ₃ ·6 H ₂ O	EtOH	Reflux	24	- ^d	dec. ^d
4	Vacuum (0.1 torr)	-	110	2	-	0
5	Pd/C	PhCH ₃	Reflux	12	1:0:0	85

^a Pyridine (**1**): Dihydropyridine (**10**): Tetrahydropyridine (**9**) ratio calculated on the spectra of the reaction crude

^b Pyridine overall yield for the two-step process, after chromatographic purification

^c Neutral, grade I Al₂O₃

^d Complex mixture, probably due to a decomposition of the intermediates

By interrupting the reaction before completion it was possible to isolate the 1,4-dihydropyridine derivative **10**, which was identified as the intermediate in the transformation of the tetrahydropyridine **9** into pyridine **1** by monitoring the reactions by TLC and ¹H-NMR (Scheme 3.8). As a result of this initial study, we have managed to establish a protocol for the synthesis of pyridine derivatives lacking substituents at positions 4, 5, and 6, which implies the construction of the heterocyclic framework from acrolein, dimethylhydrazine and a 1,3-dicarbonyl compound with 10 mol% InCl₃, in ethanol, and then aromatization by Pd/C in

⁸⁰ Hemanth Kumar, K.; Muralidharan D.; Perumal, P.T. *Tetrahedron Lett.* **2004**, *45*, 7903-7906.

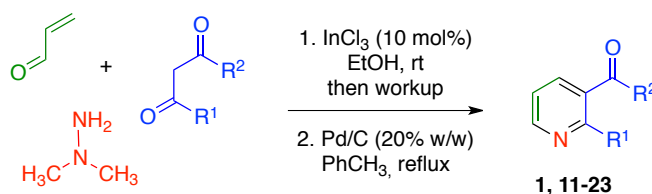
⁸¹ (a) Kitahara, Y.; Kubo, A. *Heterocycles* **1992**, *34*, 1089-1092; (b) Pérez, J. M.; Avendaño, C.; Menéndez, J. C. *Tetrahedron Lett.* **1997**, *38*, 4717-4720; (c) Pérez, J. M.; López-Alvarado, P.; Avendaño, C.; Menéndez, J. C. *Tetrahedron* **2000**, *56*, 1561-1567.

refluxing toluene.

3.1.3 Evaluation of the scope of the new synthesis of pyridine derivatives

3.1.3.1 Variations in the 1,3-dicarbonyl component

The optimized reaction conditions derived from our preliminary studies were then applied to the reactions of acrolein and dimethylhydrazine with a variety of 1,3-dicarbonyl compounds that allowed structural variations of the R¹ and R² substituents (Scheme 3.9).

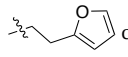


Scheme 3.9: Synthesis of the pyridine derivatives

As shown in Table 3.3, the scope of the reaction included the use of b-ketoesters (entries 1-10), b-ketothioesters (entry 11) and b-diketones, which could be either symmetrical (entry 12) or non-symmetrical (entries 13 and 14). In the latter case, the reaction was found to be completely regioselective in favor of the pyridine derivative obtained from the initial attack onto the less hindered carbonyl group. Regarding the scope of the method in terms of the R¹ substituent, we verified that the reaction could tolerate several types of modifications (including the presence of functional groups), as shown by entries 2-8.

Table 3.3. Scope and yields for the synthesis of the pyridine derivatives

Entry	Cmpd.	R ¹	R ²	Time (h) ^a	Yield (%) ^b
1	1	CH ₃	OEt	3/12	85
2	11	Et	OEt	3/12	74
3	12	Pr	OEt	3/12	76
4	13	^t Pr	OEt	4/9	68

Entry	Cmpd.	R ¹	R ²	Time (h) ^a	Yield (%) ^b
5	14	CH ₂ S ^t Bu ^c	OEt	5/12	58
6	15	(CH ₂) ₂ CH=CH ₂ ^c	OEt	5/12	57
7	16	CH ₂ COOEt	OEt	4/30	82
8	17		OEt	4/36	70
9	18	CH ₃	OtBu	4/15	62
10	19	CH ₃	OAllyl	3/36	67
11	20	CH ₃	StBu	5/15	76
12	21	CH ₃	CH ₃	4/6	55
13	22	CH ₃	Ph	4/9	60
14	23	CH ₃	ⁱ Bu	5/18	47

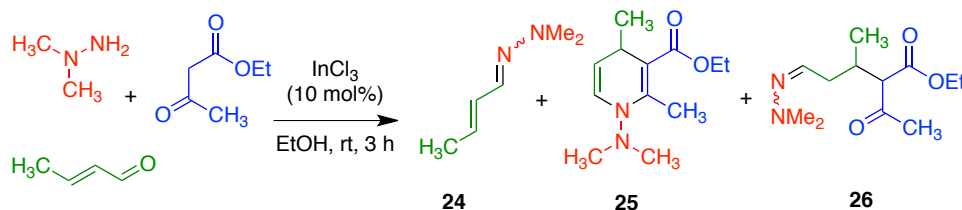
^a Reaction times for the MCR/double elimination sequence.

^b Overall yield after column chromatography.

^c For the preparation of the corresponding 1,3-dicarbonyl compounds (which are not commercially available), see the experimental part.

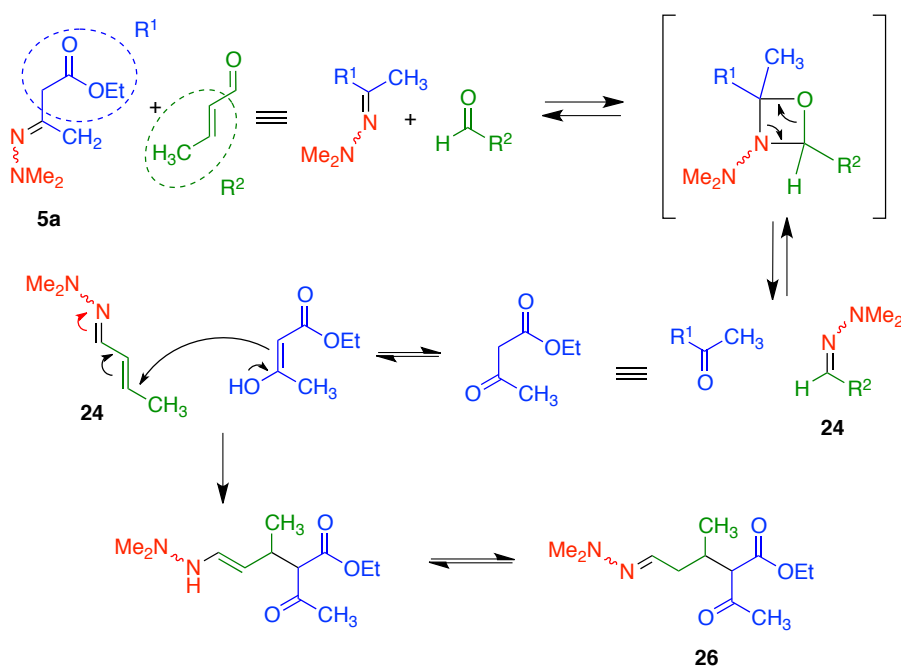
3.1.3.2 Attempted variations in the Michael acceptor

In order to extend the scope of our protocol, we tried as Michael acceptors α,β -unsaturated aldehydes different from acrolein, such as crotonaldehyde and cinnamaldehyde, but all our attempts were unsuccessful, giving very complex mixtures. To understand and address this limitation to our protocol, we decided to resolve by chromatography, the reaction mixture coming from crotonaldehyde (Scheme 3.10) and we verified that its main component was compound **24**. Moreover, we could isolate and identify compound **26** and, finally, we were able to isolate also a little percentage of the expected intermediate, although partially oxidized to the dihydropyridine stage (compound **25**).



Scheme 3.10: Study of the four-component reaction involving crotonaldehyde

Compound **24** was probably derived from a transimination reaction between the dimethylhydrazone of ethyl acetoacetate **6a** (the initial intermediate) and crotonaldehyde and which was proposed to take place via a [2+2] cycloaddition/retro [2+2] cycloaddition process. Compound **26**, probably came from the aldol addition of ethyl acetoacetate to **24** (Scheme 3.11).



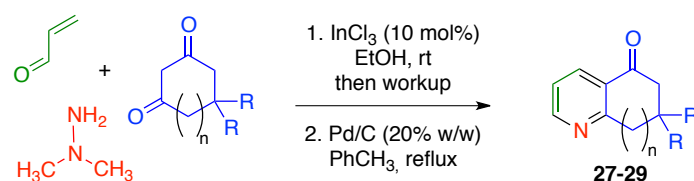
Scheme 3.11: Mechanistic rationale proposed for the formation of side products during the four-component reaction involving crotonaldehyde.

All attempts to modify the outcome of this reaction by using other Lewis acids (CAN, $InCl_3$, $Yb(OTf)_3$ and $Sc(OTf)_3$) at different concentrations (5 and 10 mol%), under various temperature conditions (0°C, room temperature and reflux) and in different solvents (ethanol and acetonitrile) were unsuccessful. Similar products were observed in the crude NMR spectra of the reaction starting from cinnamaldehyde, and methacrolein gave even more complex mixtures.

3.1.3.3 Synthesis of fused pyridines

We also examined briefly the application of our methodology to the synthesis of fused pyridine derivatives by employing cyclic 1,3-diketones as substrates (Scheme 3.12). As shown in Table 3.4, these reactions worked well, although they

were slower at both the MCR and elimination stages, and afforded derivatives of 7,8-dihydroquinolin-5(6*H*)-one (entries 1 and 2) or 6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine (entry 3).



Scheme 3.12: Synthesis of fused pyridine derivatives via a sequence of a four-component 2-ethoxy-1,2,3,4,6,7,8-octahydroquinoline synthesis and a double elimination process.

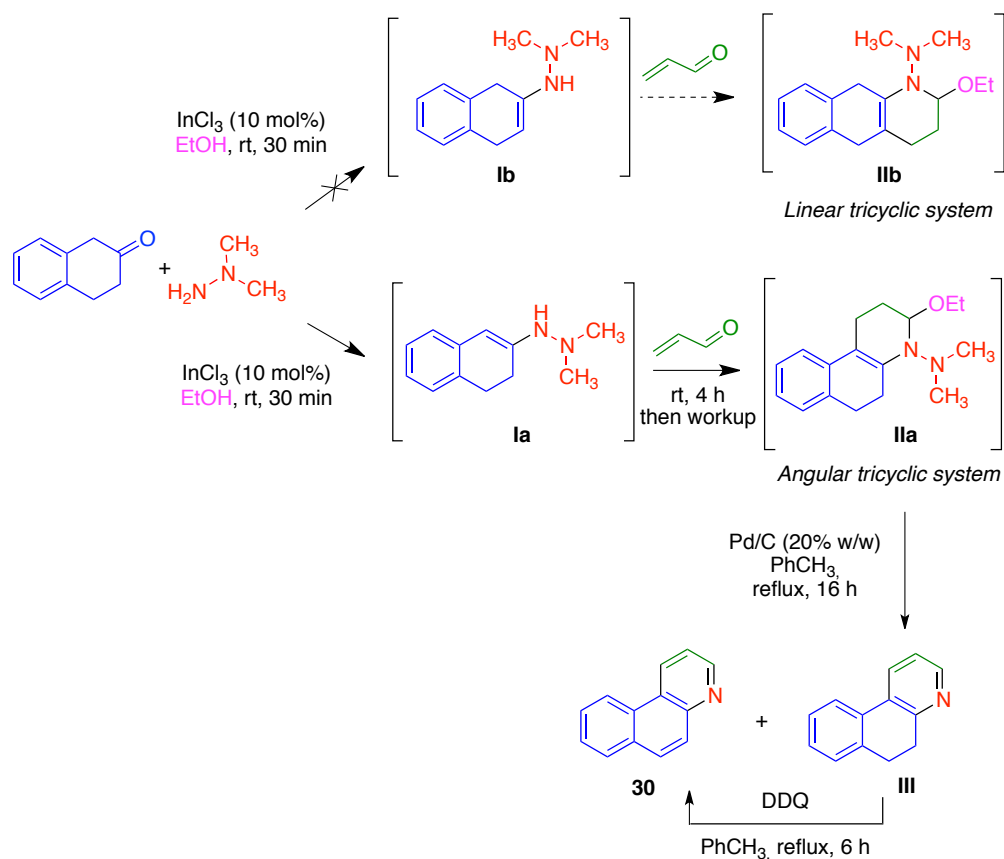
Table 3.4. Scope and yields for the synthesis of the fused pyridine derivatives

Entry	Cmpd.	n	R	Time (h) ^a	Yield (%) ^b
1	27	1	H	15/36	81
2	28	1	CH ₃	15/40	78
3	29	2	H	15/20	76

^a Reaction times for the MCR/double elimination sequence.

^b Overall yield after column chromatography.

Finally, to extend our protocol to the synthesis of tricyclic derivatives, we investigated a reaction starting from β-tetralone *in lieu* of the β-dicarbonyl compound employed in other cases. Interestingly, although the reaction could be expected to afford linear or angular tricyclic systems, we verified that it showed a complete regioselectivity in favor of the angular derivative, since employing the usual protocol we obtained a mixture of the partially (**IIa**) and fully (**30**) oxidized products shown in **Scheme 3.13**. As depicted in this scheme, the observed regioselectivity can be explained by the higher conjugation of the enamine **Ia** that leads to the angular compound.

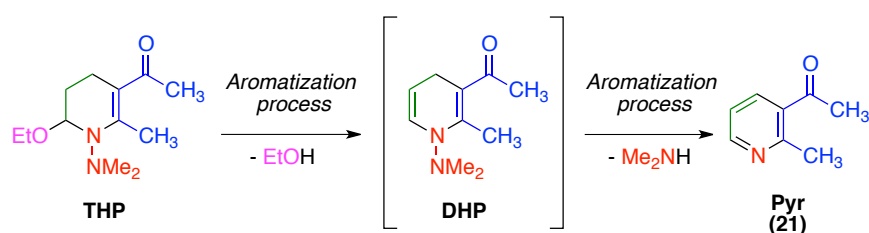


Scheme 3.13: Synthesis of the tricyclic derivative, employing β -tetralone as starting material.

The separation and individual purifications of the components of the above mixture were difficult to achieve. Thus, in order to make the procedure more selective toward the isolation of a single product, we decided to submit the mixture of **II** and **30** to an oxidation with DDQ , which afforded the fully aromatic benzo[*f*]quinoline **30** as a single product in 53% overall yield from acyclic starting materials.

3.2 ADDITIONAL STUDIES ON THE STEP INVOLVING DIMETHYLAMINE ELIMINATION

It is worth emphasizing that whereas palladium is a well-known reagent for dehydrogenation reactions,⁸² to the best of our knowledge, this is the first time that Pd/C has been employed in this type of aromatization, therefore we considered interesting to investigate its role. To this end, we studied the reaction leading to **21** in the absence of catalyst and in the presence of active charcoal (Scheme 3.14).



Scheme 3.14: Study of the double elimination process

Both reactions afforded the expected pyridine derivative without any significant change in the yield, but with a remarkable difference in the reaction time (Table 3.5). These experiments suggest that activated carbon has a role in the aromatization process, probably by increasing the contact surface of the starting materials with oxygen owing to adsorption phenomena. Palladium also has a clear role, which is more difficult to explain at this stage, but can perhaps be linked to its ability to adsorb dimethylamine,⁸³ making our observed transformation mechanistically similar to the palladium-promoted dehydrogenation.

⁸² For an example of its use in a pyridine synthesis related to the ones described here, see: Li, Y., Plesescu, M.; Prakash, S.R. *J. Label. Compd. Radiopharm.* **2006**, *49*, 789-799.

⁸³ For evidence for the adsorption of methylamine on palladium, see: Migone, R. A.; Meitzner, G.; Mykytka W. J. and Sinfelt, J. H. *Catal. Lett.* **1995**, *31*, 27-35.

Table 3.5. Dihydropyridine/pyridine ratios for the synthesis of **21** under several conditions^a

Entry	Time (h)	No catalyst	Activated charcoal	Pd-C
1	6	2.3:1	1.4:1	0:1
2	12	1:1.2	1:6.7	-
3	18	1:2.4	0:1	-
4	24	1:4	-	-
5	30	0:1	-	-

^a Determined by ¹H-NMR of the crude reaction products

4. Identification Of 4,6-Diaryl-1,4-Dihydropyridines As A New Class Of Neuroprotective Agents

4.1 INTRODUCTION

Neurodegenerative diseases (NDDs) constitute a significant public health problem worldwide and, among these, cerebrovascular accidents represent one of the leading causes of death, neurological disability and cognitive impairment.⁸⁴

Thanks to the extensive studies carried out in the last years, a significant progress has been made in the understanding of the physiopathology of stroke, leading to the description of several pathological features that are common with other neurodegenerative disorders. These features include protein misfolding and aggregation, excitotoxicity, oxidative stress, and an ionic imbalance (especially with reference to Ca^{2+}), which is associated with mitochondrial dysfunction.⁸⁵ In the last decades, these concepts prompted the scientific community to consider neuroprotection as a useful instrument to prevent the onset and to combat the progression of neurodegenerative disorders; thus the development of molecules active as neuroprotective agents represents one of the main challenges of modern medicine.⁸⁶

During cerebral ischemia, oxygen and glucose deprivation induces a metabolic cascade that leads to neuronal death. One of the most significant consequences of these changes is the dysregulation of Ca^{2+} homeostasis that seems to play a key role in the molecular mechanism leading to brain damage.⁸⁷ In this context, the

⁸⁴ Jaffer, H.; Morris, V. B.; Stewart, D.; Labhasetwar, V. *Drug Deliv. Transl. Res.* **2011**, *1*, 409-419.

⁸⁵ (a) Jellinger, K. *J. Neural Transm., Suppl.* **2003**, 101-144. (b) Szydłowska, K.; Tymianski, M. *Cell Calcium* **2010**, *47*, 122-129.

⁸⁶ (a) Auriel, E.; Bornstein, N. M. *J. Cell. Mol. Med.* **2010**, *14*, 2200-2202; (b) Yamashita, T.; Deguchi, K.; Sehara, Y.; Lukic-Panin, V.; Zhang, H.; Kamiya, T.; Abe, K. *Neurochem. Res.* **2009**, *34*, 707-710; (c) Ginsberg, M. D. *Neuropharmacol.* **2008**, *55*, 363-389.

⁸⁷ (a) Mattson, M. P. *Aging Cell* **2007**, *6*, 337-350; (b) Zündorf, G.; Reiser, G. *Antioxid. Redox*

stabilization of Ca^{2+} homeostasis by blocking neuronal voltage-gated calcium channels (VGCCs) could represent one important approach for post-ischemic neuroprotection in humans.⁸⁸

VGCCs are multimeric membrane proteins that play a critical role in many physiological functions in a variety of tissues.⁸⁹ These proteins are usually classified into three broad groups (Ca_v1 , Ca_v2 and Ca_v3) and different subtypes, depending on the pore-forming subunit. The members of the Ca_v1 family represent the most intensely studied channels and are classified in four diverse isoforms ($\text{Ca}_v1.1$ - $\text{Ca}_v1.4$) that differ in their tissue distributions and physiological functions.⁹⁰ $\text{Ca}_v1.2$ VGCCs are the major and most targeted isoform,⁹¹ found mainly in the cardiovascular system, where they control vascular tone, although they are also found in neurons. $\text{Ca}_v1.3$ VGCCs have a tissue distribution similar to $\text{Ca}_v1.2$ VGCCs, but they are more neurospecific and are believed to have an important role in neuronal excitability.⁹² Furthermore, recent studies⁹³ have demonstrated the implication of Ca^{2+} entry through $\text{Ca}_v1.3$ VGCCs in the generation of oxidative stress underlying the pathogenesis of Parkinson's disease. Therefore, selective antagonism of $\text{Ca}_v1.3$ VGCCs is considered a potential neuroprotective strategy that could slow down neuronal loss in the early stages of Parkinson's disease.⁹⁴

Among VGCCs blockers, 1,4-dihydropyridines (DHPs) can certainly be considered

Signaling **2011**, *14*, 1275-1287.

⁸⁸ Gribkoff, V. K.; Winkquist, R. J. *Expert Opin. Invest. Drugs* **2005**, *14*, 579-592.

⁸⁹ (a) Lipscombe, D.; Helton, T. D.; Xu, W. *J. Neurophysiol.* **2004**, *92*, 2633-2641; (b) Triggle, D. J. *Curr Pharm Des* **2006**, *12*, 443-457.

⁹⁰ Catterall, W. A.; Pérez-Reyes, E.; Snutch, T. P.; Striessnig, J. *Pharmacol. Rev.* **2005**, *57*, 411-425.

⁹¹ Halling, D. B.; Aracena-Parks, P.; Hamilton, S. L. *Sci STKE* **2005**, *2005*, re15.

⁹² Striessnig, J.; Koschak, A. *Channels (Austin)* **2008**, *2*, 233-251.

⁹³ (a) Chan, C. S.; Guzmán, J. N.; Ilijic, E.; Mercer, J. N.; Rick, C.; Tkatch, T.; Meredith, G. E.; Surmeier, D. J. *Nature* **2007**, *447*, 1081-1086; (b) Hurley, M. J.; Brandon, B.; Gentleman, S. M.; Dexter, D. T. *Brain* **2013**, *136*, 2077-2097.

⁹⁴ Kang, S.; Cooper, G.; Dunne, S. F.; Dusel, B.; Luan, C. H.; Surmeier, D. J.; Silverman, R. B. *Nat. Commun.* **2012**, *3*, 1146.

the most extensively studied due to their pharmacological versatility.⁹⁵ Their broad therapeutic use as antihypertensive drugs has clearly shown positive effects in the prevention of stroke⁹⁶ and in the literature there are several examples of DHPs showing neuroprotective properties.⁹⁷ More interestingly, it is also known that high doses of DHPs, such as nifedipine, induce some beneficial brain effects such as amelioration of age-related working memory deficits among others.⁹⁸ Indeed, nifedipine binds both Ca_v1.2 and Ca_v1.3 isoforms, although it shows high selectivity towards Ca_v1.2, a characteristic that, together with its difficulty in going across the blood–brain barrier, is a key requirement for its use in cardiovascular therapy, but also prevents its use as a neuroprotective agent.⁹⁸ Therefore, a major drawback that needs to be addressed in this area is the prevalence of vascular side effects of currently used DHPs. Thus, a renewed interest exists on the discovery and study of selective Ca_v1.3 L-type VGCCs in the context of efforts to find Ca_v1.3 L-type VGCCs-selective neuroprotective agents for neurodegenerative diseases.^{94,99}

Against this background, we decided to prepare and evaluate a library of 6-aryl-1,4-dihydropyridines that, in contrast to other extensively studied families of DHPs, have received little attention in the medicinal chemistry literature.¹⁰⁰ Thus we have

⁹⁵ (a) Edraki, N.; Mehdipour, A. R.; Khoshneviszadeh, M.; Miri, R. *Drug Discov Today* **2009**, *14*, 1058-1066; (b) Ioan, P.; Carosati, E.; Micucci, M.; Cruciani, G.; Broccatelli, F.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. *Curr Med Chem* **2011**, *18*, 4901-4922.

⁹⁶ Chen, G. J.; Yang, S. *PLoS one* **2013**, *8* (3), e57854.

⁹⁷ (a) Yamagata, K.; Ichinose, S.; Tagami, M. *Hypertens. Res.* **2004**, *27*, 271-282; (b) Yagami, T.; Ueda, K.; Sakaeda, T.; Itoh, N.; Sakaguchi, G.; Okamura, N.; Hori, Y.; Fujimoto, M. *Biochem. Pharmacol.* **2004**, *67*, 1153-1165. (c) León, R.; de los Ríos, C.; Marco-Contelles, J.; López, M. G.; García, A. G.; Villarroya, M. *Eur. J. Med. Chem.* **2008**, *43*, 668-674.

⁹⁸ Striessnig, J.; Koschak, A.; Sinnegger-Brauns, M. J.; Hetzenauer, A.; Nguyen, N. K.; Busquet, P.; Pelster, G.; Singewald, N. *Biochem. Soc. Trans.* **2006**, *34*, 903-909.

⁹⁹ (a) Kang, S.; Cooper, G.; Dunne, S. F.; Luan, C. H.; Surmeier, D. J.; Silverman, R. B. *J Med. Chem.* **2013**, *56*, 4786-4797; (b) Locatelli, A.; Cosconati, S.; Micucci, M.; Leoni, A.; Marinelli, L.; Bedini, A.; Ioan, P.; Spampinato, S. M.; Novellino, E.; Chiarini, A.; Budriesi, R. *J Med. Chem.* **2013**, *56*, 3866-3877; (c) Chang, C. C.; Cao, S.; Kang, S.; Kai, L.; Tian, X.; Pandey, P.; Dunne, S. F.; Luan, C. H.; Surmeier, D. J.; Silverman, R. B. *Bioorg. Med. Chem.* **2010**, *18*, 3147-3158.

¹⁰⁰ (a) Roh, E. J.; Keller, J. M.; Olah, Z.; Iadarola, M. J.; Jacobson, K. A. *Bioorg. Med. Chem.* **2008**, *16*, 9349-9358; (b) Jiang, J. L.; van Rhee, A. M.; Melman, N.; Ji, X.-D.; Jacobson, K. A. *J. Med. Chem.*

designed dihydropyridine derivatives with a substitution pattern that do not satisfy the well-known structure-activity relationships for vascular activity. In particular, the absence of a substituent at C-5 and the presence of an aryl group at C-6, were expected to lead to a reduced vascular activity, since it is known that an ester group at C-5 and a small alkyl or aminoalkyl substituent at C-6 are required for the vascular activity of DHPs.¹⁰¹ It is pertinent to note here that the C-5 substituent is known to have an influence on the tissue selectivity of 1,4-dihydropyridines and also on the antagonistic/agonistic character of the DHPs (Figure 4.1).¹⁰²

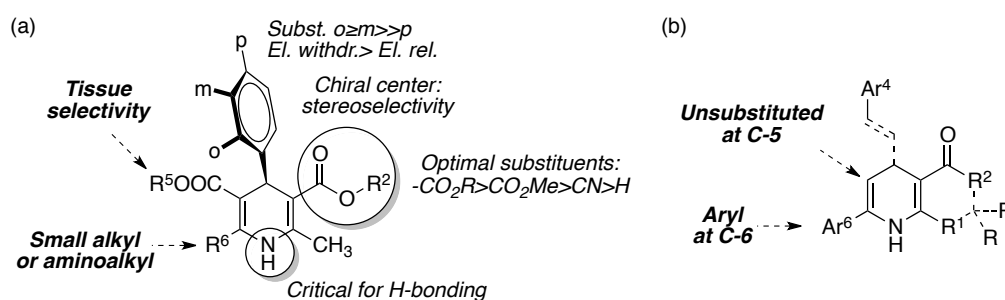


Figure 4.1: Comparison between traditional DHPs with cardiovascular activity (a) and the DHPs studied in this Chapter (b)

In an effort to contribute to the search for new neuroprotective agents for the treatment of neurodegenerative diseases and cerebral ischemia, we decided to evaluate the ability of these DHPs to prevent neuronal $[Ca^{2+}]_c$ overload by blockade of L-type VGCCs, with special focus on any possible selective antagonism of Ca_v1.3 VGCCs. Moreover, we also investigated their neuroprotective effect against oxidative stress in vitro, extending also the investigation of their biological activity by using pharmacological assays that reproduce the pathological conditions of an ischemic episode.

1996, 39, 4667-4675; (c) Jiang, J. L.; van Rhee, A. M.; Chang, L.; Patchornik, A.; Ji, X.-D.; Evans, P.; Melman, N.; Jacobson, K. A. *J. Med. Chem.* **1997**, 40, 2596-2608.

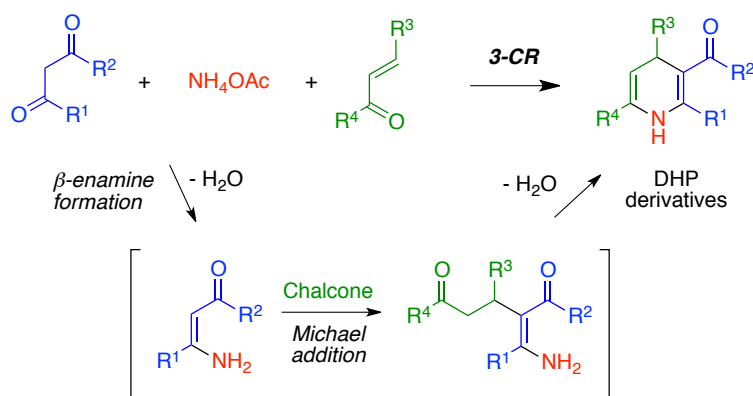
¹⁰¹ van Rhee, A. M.; Jiang, J. L.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. *J. Med. Chem.* **1996**, 39, 2980-2989

¹⁰² Triggle, D. J. *Cell. Mol. Neurobiol.* **2003**, 23, 293-303.

4.2 SYNTHESIS OF A LIBRARY OF 4,6-DIARYL-1,4-DIHYDROPYRIDINES

4.2.1 Proposal and optimization of a reaction to obtain DHPs

On the basis of our previous work, we planned the synthesis of this dihydropyridine library *via* a three-component reaction starting from β -dicarbonyl compounds, chalcone derivatives, and ammonium acetate as a source of ammonia. As this formal [3+3] aza-annulation was supposed to proceed by a Hantzsch-like mechanism involving the initial formation of a β -enaminone from the β -dicarbonyl compound and ammonia, followed by its Michael addition onto the chalcone derivative and a final cyclocondensation with loss of a molecule of water, we needed to find a catalyst able to promote all the steps of the process (Scheme 4.1).



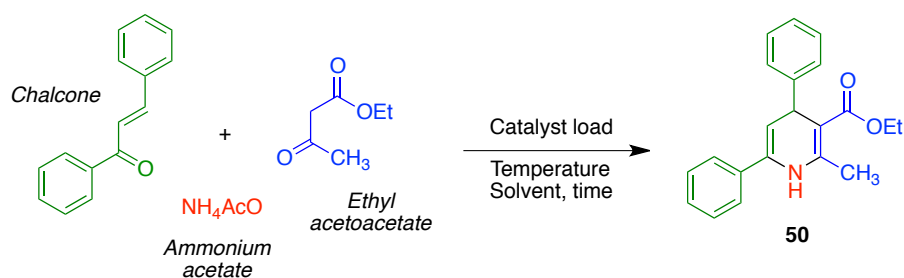
Scheme 4.1: Planned three-component synthesis of DHP derivatives

Thus, we put our attention first in cerium (IV) ammonium nitrate (CAN) as catalyst¹⁰³ because of its low cost and toxicity, tolerance to moisture and, as described in the previous chapter, especially because of the experience in our group with this reagent as a Lewis acid catalyst.¹⁰⁴

¹⁰³ For a review of the use of CAN as a catalyst in organic synthesis, see: Sridharan, V.; Menéndez, J. C. *Chem. Rev.* **2010**, *110*, 3805-3849.

¹⁰⁴ For selected examples, see: (a) Sridharan, V.; Avendaño, C.; Menéndez, J. C. *Org. Lett.* **2008**, *10*,

Thereafter we started a preliminary study of our CAN-protocol employing as a model the reaction between 1,3-diphenylprop-2-en-1-one (the simplest chalcone), ethyl acetoacetate as 1,3-dicarbonyl component and ammonium acetate (Scheme 4.2).



Scheme 4.2: Optimization study for our three-component synthesis of DHP derivatives.

Our first reaction was carried out in *one-pot* fashion employing 1 equiv of chalcone, 1.2 equiv of ethyl acetoacetate and 3 equiv of ammonium acetate in the presence of a catalytic amount of CAN (5 mol%); ethanol was selected as solvent and the reaction was performed at room temperature for 20 h, affording only small amounts of the expected product **50** (Table 4.1, entry 1). When the temperature was increased to reflux conditions, we were pleased to observe that the reaction furnished the expected product albeit in a moderate yield (entry 2). Surprisingly, when the same reaction was carried out in absence of the catalyst, we could notice a slight improvement in the yield (entry 3), therefore we decided to repeat the reaction in presence of CAN in a two-component fashion, but employing the commercially available ethyl 3-aminocrotonate as starting material, since this compound is the expected β -enamine intermediate formed from ethyl acetoacetate and the ammonium acetate (entry 4). In this case, the yield of the reaction was better, which supports the mechanistic proposal shown in the Scheme 4.1. Interestingly, the NMR spectra revealed that only

4303-4306. (b) V. Sridharan, S. Maiti and J. C. Menéndez, *Chem. Eur. J.* **2009**, *15*, 4565-4572. (c) Sridharan, V.; Ribelles, P.; Ramos, M. T.; Menéndez, J. C. *J. Org. Chem.* **2009**, *74*, 5715-5718. (d) Sridharan, V.; Maiti, S.; Menéndez, J. C. *J. Org. Chem.* **2009**, *74*, 9365-9371. (e) Estévez, V.; Villacampa, M.; Menéndez, J. C. *Chem. Commun.* **2013**, *49*, 591-593. (f) Rocchi, D.; González, J. F.; Menéndez, J. C. *Green Chem.* **2013**, *15*, 511-517.

ethyl acetoacetate remained in the crude reaction product, indicating that CAN could promote not only the formation of the enamine, but also the backward reaction of the initial ethyl 3-aminocrotonate to ammonia and ethyl acetoacetate.

By increasing the catalyst load to 10 mol%, it was possible to reduce the reaction time, avoiding the complete reversion of the formed enamine to the starting materials (entry 5). A considerable improvement of the yield was reached when an additional amount of ammonium acetate was added to the reaction mixture to drive forward the equilibrium of enaminone formation. Even after longer reaction times, a little amount of remaining enaminone was detected after the workup (entries 6 and 7).

Table 4.1. Optimization study for our three-component synthesis of DHP derivatives.

Entry	Solvent (conc.) ^{a,b}	Catalyst load	T (°C)	Time (h)	Conversion (%) ^c	EAA:enam. ratio
1	EtOH (0.33 M)	5 mol%	rt	20	Trace	
2	EtOH (0.33 M)	5 mol%	Reflux	20	38	Only EAA
3	EtOH (0.33 M)	-	Reflux	20	51	Only EAA
4	EtOH (0.33 M)	5 mol%	Reflux	20	45 ^d	Only EAAe
5	EtOH (0.33 M)	10 mol%	Reflux	4	22	1:1
6	EtOH (0.33 M)	10 mol%	Reflux	8	58	1:1.45
7	EtOH (0.33 M)	10 mol%	Reflux	24	93 ^f	1:0.75
8	EtOH (1.0 M)	10 mol%	Reflux	4	78	1:0.88
9	EtOH (1.0 M)	-	Reflux	4	58	1:0.55
10	Neat	-	80 °C	4	57	1:4.45
11	EtOH (1.0 M)	10 mol%	Reflux	8	95 ^g	1:1.23

^a referred to chalcone

^b all experiments were carried out with 1:1.2:3 ratio of chalcone:ethyl acetoacetate: ammonium acetate

^c conversion calculated in ¹H-NMR spectra of reaction crudes

^d commercial enaminone was used

^e CAN promotes also the reverse reaction of enamine intermediate to ethyl acetoacetate and ammonia

^f after 5h reflux additional 3 eq. of ammonium acetate were added

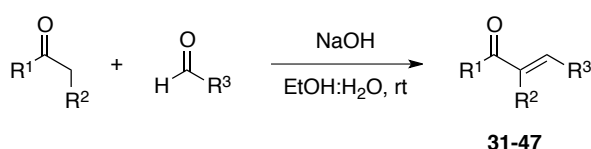
^g after 4h reflux additional 1.5 eq. of ammonium acetate were added

In a further attempt to improve our protocol, we increased the concentration of the

reagents in the reaction mixture, and the reaction times could be reduced without any significant change in the yield, whereas the presence of the catalyst was proved to be essential in these conditions (entries 8 and 9). Because of the improvement obtained by reducing the amount of solvent, we also tried the reaction in the absence of solvent, but the yield was only moderate (entry 10). Finally, by combining the more suitable results obtained in the previous experiments, we could define the optimal conditions to be reflux in ethanol in the presence of CAN (10%), and excess of ammonium acetate added in two portions (entry 11).

4.2.2 Synthesis of the starting chalcones 31-49

To synthesize the library of DHPs, chalcones with different substituents in both rings were required. For the synthesis of different chalcones we employed literature conditions, namely the classical base-catalyzed Claisen–Schmidt condensation reaction between aromatic aldehydes and acetophenone derivatives in alcoholic solution (Scheme 4.3).¹⁰⁵



Scheme 4.3: Synthesis of the starting chalcones 31-49

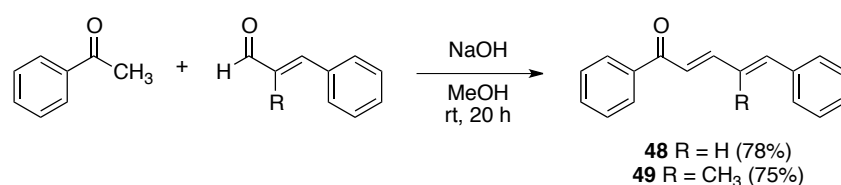
Table 4.2. Scope of the synthesis of chalcones 31-49

Entry	Cmpd.	R ¹	R ²	R ³	Yield (%)
1	31	C ₆ H ₅	H	4-MeC ₆ H ₄	85
2	32	4-MeC ₆ H ₄	H	C ₆ H ₅	83
3	33	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	81
4	34	4-MeC ₆ H ₄	H	4-BrC ₆ H ₄	89

¹⁰⁵ (a) Attar, S.; O'Brien, Z.; Alhaddad, H.; Golden M. L.; Calderón-Urrea A., *Bioorg. Med. Chem.* **2011**, *19*, 2055–2073; (b) Lu, S.-M.; Bolm, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 8920–8923.

Entry	Cmpd.	R ¹	R ²	R ³	Yield (%)
5	35	4-MeC ₆ H ₄	H	2-NO ₂ C ₆ H ₄	74
6	36	C ₆ H ₅	H	4-MeOC ₆ H ₄	86
7	37	4-MeOC ₆ H ₄	H	4-MeOC ₆ H ₄	73
8	38	4-ClC ₆ H ₄	H	C ₆ H ₅	93
9	39	4-ClC ₆ H ₄	H	4-ClC ₆ H ₄	95
10	40	4-ClC ₆ H ₄	H	3-MeOC ₆ H ₄	79
11	41	4-ClC ₆ H ₄	H	2-NO ₂ C ₆ H ₄	61
12	42	C ₆ H ₅	H	4-NO ₂ C ₆ H ₄	53
13	43	2-NO ₂ C ₆ H ₄	H	4-MeC ₆ H ₄	85
14	44	C ₆ H ₅	H	2-thienyl	66
15	45	4-MeC ₆ H ₄	H	2-thienyl	62
16	46	2-furyl	H	4-MeC ₆ H ₄	70
17	47	C ₆ H ₅	CH ₃	C ₆ H ₅	65

The same strategy was applicable to the synthesis of two known Michael acceptors with extended conjugation to the γ and δ positions (Scheme 4.4).¹⁰⁶



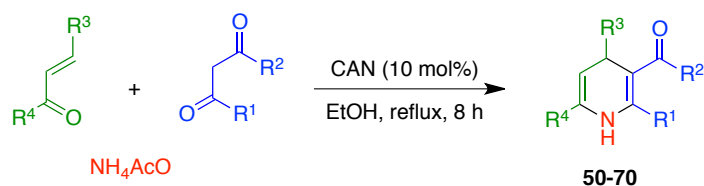
Scheme 4.4: Synthesis of Michael acceptors with extended conjugation

4.2.3 Synthesis of 4,6-diaryl-1,4-dihydropyridine derivatives

With the optimized conditions established in Section 4.2.1, we carried out the synthesis of a library of 6-aryl-1,4-dihydropyridine derivatives, starting with a variety of 1,3-dicarbonyl compounds and chalcones that allowed structural variations at R¹,

¹⁰⁶ Pinto, D. C. G. A.; Silva, A. M. S.; Levai, A.; Cavaleiro, J. A. S.; Patonay, T.; Elguero, J. *Eur. J. Org. Chem.* **2000**, 2593–2599.

R^2 , R^3 and R^4 substituents (Scheme 4.5).

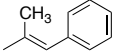


Scheme 4.5: Three-component synthesis of 4,6-diaryl-1,4-DHPs

This method allowed the preparation in good to excellent yields of the compounds summarized in Table 4.3, which bear small alkyl groups at C-2 (R^1), and ester (entries 1-3, 6-8, 10 and 14-20), thioester (entries 4, 9, 11-13 and 21) or ketone group (entry 5) at C-3 from the dicarbonyl reagent. A variety of aryl (entries 1-18 and 20), heteroaryl (entry 19) or styryl (entry 21) substituents at C-4 (R^3), no substitution at C-5, and aryl (entries 1-19 and 21) or heteroaryl (entry 20) groups at C-6 come from the chalcone component, and exhibit electron-releasing and electron-withdrawing substituents in either of the two rings.

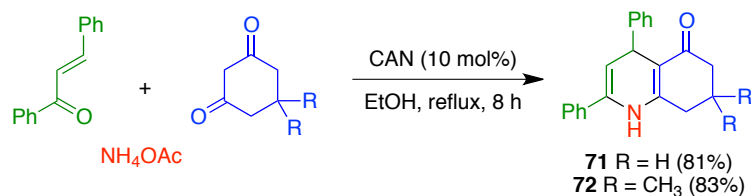
Table 4.3. Scope and yield of the 4,6-diaryl-1,4-DHP multicomponent synthesis

Entry	Cmpd.	R^1	R^2	R^3	R^4	Yield (%)
1	50	CH ₃	OEt	C ₆ H ₅	C ₆ H ₅	95
2	51	C ₂ H ₅	OEt	C ₆ H ₅	C ₆ H ₅	92
3	52	C ₃ H ₇	OEt	C ₆ H ₅	C ₆ H ₅	81
4	53	CH ₃	S ^t Bu	C ₆ H ₅	C ₆ H ₅	99
5	54	CH ₃	CH ₃	C ₆ H ₅	C ₆ H ₅	56
6	55	CH ₃	OEt	C ₆ H ₅	4-ClC ₆ H ₄	71
7	56	CH ₃	OEt	4-ClC ₆ H ₄	4-ClC ₆ H ₄	86
8	57	CH ₃	OEt	4-MeOC ₆ H ₄	C ₆ H ₅	73
9	58	CH ₃	S ^t Bu	4-MeOC ₆ H ₄	C ₆ H ₅	69
10	59	CH ₃	OEt	3-MeOC ₆ H ₄	4-ClC ₆ H ₄	75

Entry	Cmpd.	R ¹	R ²	R ³	R ⁴	Yield (%)
11	60	CH ₃	S ^t Bu	4-BrC ₆ H ₄	4-MeC ₆ H ₄	67
12	61	CH ₃	S ^t Bu	4-MeC ₆ H ₄	C ₆ H ₅	99
13	62	CH ₃	S ^t Bu	4-MeC ₆ H ₄	4-MeC ₆ H ₄	97
14	63	CH ₃	OEt	4-MeC ₆ H ₄	C ₆ H ₅	94
15	64	CH ₃	OAllyl	C ₆ H ₅	4-ClC ₆ H ₄	78
16	65	CH ₃	OEt	4-NO ₂ C ₆ H ₄	C ₆ H ₅	96
17	66	CH ₃	OEt	2-NO ₂ C ₆ H ₄	4-MeC ₆ H ₄	78
18	67	CH ₃	OEt	2-NO ₂ C ₆ H ₄	4-ClC ₆ H ₄	62
19	68	CH ₃	OEt	2-Thienyl	4-MeC ₆ H ₄	57
20	69	CH ₃	OEt	4-MeC ₆ H ₄	2-Furyl	49
21	70	CH ₃	S ^t Bu		C ₆ H ₅	52

Compound **70** (entry 21) was obtained together with the corresponding pyridine derivative resulting from its dehydrogenation, presumably motivated by the formation of a highly conjugated compound. This reaction, which is undesired when dihydropyridines are the goal, was not detected or just traces of the aromatic compounds were observed in some cases.

In order to explore the influence of a higher rigidity on the activity and selectivity of our compounds, we also included in the library the hexahydroquinolines **71** and **72** by application of the CAN-catalyzed multicomponent protocol to 1,3-cyclohexanedione and dimedone (Scheme 4.6).



Scheme 4.6: Three-component synthesis of fused DHP derivatives

4.3 BIOLOGICAL EVALUATION OF 4,6-DIARYL-1,4-DIHYDROPYRIDINES.

The group of Dr. Rafael León in the Instituto Teófilo Hernando and Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, performed the biological assays that follow and the study of molecular modeling of nifedipine and compound **63**.

4.3.1 Neuronal VGCCs blockade in SH-SH5Y neuroblastoma cells

As previously mentioned, DHPs are specific L-type VGCC blockers. Their activity and vascular selectivity require an ester function at C-5 and a small alkyl or aminoalkyl substituent at C-6.¹⁰² To assess the neuroprotective effect of these new compounds against $[Ca^{2+}]$ overload, we first studied whether they had any effect on the Ca^{2+} entry elicited by K^+ promoted depolarization in SH-SY5Y human neuroblastoma cells. IC_{50} values for Ca^{2+} signal blockade data of newly obtained DHPs are collected in [Table 4.4](#), using nifedipine as the reference drug. Most DHPs showed moderate to high potency to block neuronal L-type VGCCs, with IC_{50} values ranging from more than 100 μM (compound **62**) to 2.8 μM (compound **72**).

Regarding SAR studies related to the ester functionality, ethyl ester derivatives were, in general, better VGCCs blockers than *tert*-butyl thioester derivatives. Thus, compound **63** (ethyl ester and C-4 *p*-Me-phenyl) was 2.7 times more potent than **61** (thioester and C-4 *p*-Me-phenyl) and more than 4.8 times better than **62** (thioester and C-6, C-4 *p*-Me-phenyl). The lower Ca^{2+} channel blocking properties of thioester derivatives might be related to an increased steric hindrance originated by the high volume of the *tert*-butyl thioester group. As an exception, compound **58**, a thioester derivative bearing a C-4 *p*-OMe-phenyl substituent, was slightly more potent than its parent ethyl ester derivative **57**. On the other hand, compound **62**, showing two *p*-Me-phenyl groups at C-4 and C-6, showed the lowest potency, with an IC_{50} value above 100 μM . Among ethyl ester derivatives, compound **52** was the most potent with an IC_{50} value of 9.9 μM .

Regarding the effect of the substituents present at the dihydropyridine ring,

replacement of the methyl group at C-2 (**50**) by an ethyl (**51**) or propyl group (**52**) improved their blocking properties from 18.7 μM for compound **50** to 12.8 μM for **51** and 9.9 μM for **52**. Bicyclic derivatives **71** and **72**, which can also be viewed as C-2 modified analogues of **50**, showed good potencies, especially compound **72**, which blocked the Ca^{2+} signal induced by high potassium levels with an IC_{50} of 2.8 μM , being the most potent VGCCs blocker of the series and almost as potent as nifedipine. Its analogue **71**, which shares the same tetrahydroquinolin-5-(1*H*)-one core but lacks the two methyl substituents at position 7, was 8.6 times less potent than **72**.

Table 4.4. IC_{50} Values of compounds **50-72** calculated as blockade of $[\text{Ca}^{2+}]_i$ increase elicited by 70 mM K^+ in SH-SY5Y cells

Entry	Cmpd.	IC_{50} (μM) ^a	Entry	Cmpd.	IC_{50} (μM) ^a
1	Nifedipine	1.35 \pm 0.4	13	61	56.2 \pm 6.4
2	50	18.7 \pm 6.6	14	62	>100
3	51	12.8 \pm 2.1	15	63	20.6 \pm 2.1
4	52	9.9 \pm 2.6	16	64	18.5 \pm 1.1
5	53	95.8 \pm 5.7	17	65	12.2 \pm 1.4
6	54	27.1 \pm 3.4	18	66	44.1 \pm 8.2
7	55	9.3 \pm 1.1	19	67	57.1 \pm 6.6
8	56	28.4 \pm 3.2	20	68	14.9 \pm 3.8
9	57	29.8 \pm 4.8	21	69	55.5 \pm 5.4
10	58	26.8 \pm 3.7	22	70	63.1 \pm 3.0
11	59	15.1 \pm 2.8	23	71	24.0 \pm 6.4
12	60	59.6 \pm 9.1	24	72	2.8 \pm 0.8

^a IC_{50} values were determined from dose-response curves (1–100 μM). Data are expressed as the mean \pm SEM of three to six different cultures in triplicate.

In terms of the substituents present at C-4 of the dihydropyridine ring, compounds bearing unsubstituted phenyl rings were generally better blockers than those having

rings possessing electron-donating or electron-withdrawing groups.

Finally, comparing different substituents at position C-6, electron-withdrawing substituents derivatives were in general more potent VGCCs blockers than the corresponding phenyl derivatives. Also generally speaking, derivatives bearing a *p*-Me-phenyl moiety were poorer blockers than phenyl derivatives.

4.3.2 Functional assay: Ca_v1.2 and Ca_v1.3 IC₅₀ determination and selectivity comparison

As stated above, Ca_v1.2 L-type VGCCs are the major isoform (≈90%)¹⁰⁷ expressed in cardiac myocytes, smooth muscle, and pancreas while Ca_v1.3 channels are more neuron-specific (cerebral cortex, hippocampus, basal ganglia, habenula, and thalamus) and are thought to serve predominantly as modulators in the neuronal system. Ca_v1.3 is connected to mitochondrial oxidative stress and increased vulnerability in SNC dopaminergic neurons.^{93a,108}

Because of our goal of reducing cardiovascular effects in neuroprotective DHPs, it was crucial to study the Ca_v1.3/ Ca_v1.2 L-type VGCCs selectivity of these C-5 unsubstituted DHPs. SH-SY5Y neuroblastoma cells were used to study the blockade of Ca_v1.3 L-type VGCCs.¹⁰⁹ Compounds **63**, **70**, and **72** were selected to calculate their IC₅₀ values for Ca_v1.3 L-type VGCCs because of their Ca²⁺ blockade properties and their overall neuroprotective profile (see [Tables 4.5](#) and [4.6](#)). Compound **72** showed the best IC₅₀ to block Ca²⁺ entry through neuronal L-type VGCCs (IC₅₀ = 2.81 μM) displaying only half potency of that found for nifedipine (IC₅₀ = 1.35 μM), a classical, highly potent L-type blocker of VGCCs. Compounds **63** and **70** were less potent, with IC₅₀ values of 20.6 and 63.1 μM, respectively ([Table 4.4](#)).

To calculate the Ca_v1.3/Ca_v1.2 selectivity ratio, we used the relaxation induced by

¹⁰⁷ Sinnegger-Brauns, M. J.; Huber, I. G.; Koschak, A.; Wild, C.; Obermair, G. J.; Einzinger, U.; Hoda, J. C.; Sartori, S. B.; Striessnig, J. *Mol. Pharmacol.* **2009**, *75*, 407-414.

¹⁰⁸ Guzmán, J. N.; Sánchez-Padilla, J.; Wokosin, D.; Kondapalli, J.; Ilijic, E.; Schumacker, P. T.; Surmeier, D. J. *Nature* **2010**, *468* (7324), 696-700.

¹⁰⁹ Sousa, S.R.; Vetter, I.; Ragnarsson, L.; Lewis, R.J. *PLoS One* **2013**, *8*, e59293.

each compound in 70 mM K⁺ precontracted rat mesenteric resistance arteries. All DHPs induced concentration-dependent relaxation responses. As shown in Table 4.5, the relaxation induced by nifedipine was higher than that induced by **63**, **70**, and **72**. Nifedipine blocked the Ca_v1.2 cardiovascular subtype more potently than neuronal channels by a factor of 950, showing a high selectivity for the Ca_v1.2 VGCC subtype (entry 1). On the contrary, our modifications in the DHP structure have resulted in a drastic decrease in their selectivity toward Ca_v1.2 cardiovascular subtype. Compound **72**, the most potent VGCCs blocker of the new family, showed the highest selectivity toward Ca_v1.2 subtype, since it was only 8.3 times more potent toward this subtype than for neuronal Ca_v1.3 (entry 4), a result that represents a Ca_v1.2/Ca_v1.3 selectivity more than 100 times lower than that of nifedipine. This reduction on selectivity was even higher for compounds **70** and **63**, which were 306- and 500-fold less selective toward Ca_v1.2 subtype than the reference compound (entries 2 and 3). Similar results were obtained when U46619 was used as precontraction stimulus (data not shown). Thus, **63** and **70** have drastically improved their selectivity toward the Ca_v1.3 by lowering their potency toward the cardiovascular subtype while maintaining their activity in the neuronal subtype. The Ca_v1.3/Ca_v1.2 selectivity ratio achieved by these compounds represents a substantial improvement even over isradipine, which so far was the DHP with the highest relative affinity for Ca_v1.3 L-type VGCCs but which is still Ca_v1.2-selective.¹⁰⁸

Table 4.5. IC₅₀ values for VGCC blockade by nifedipine and compounds **63**, **70**, and **72**

Entry	Cmpd.	Experimental IC ₅₀ (μM)		Ca _v 1.3/Ca _v 1.2
		Neuronal (SH-SY5Y)	Cardiovascular (70 mM K ⁺)	
1	nifedipine	1.35	0.00142	950.7
2	63	20.6	10.6	1.9
3	70	63.1	20.3	3.1
4	72	2.81	0.34	8.3

4.3.3 Molecular modeling

Docking calculations were performed to acquire deeper insights into the molecular basis for the interaction of these new C-5-unsubstituted DHPs with Ca_v1.2 L-type channels. Although the X-ray structure of the L-type VGCCs is not available, several models have been described in recent years, among which we have used the one developed by Zhorov *et al.*¹¹⁰ The nomenclature used by these authors was maintained for a better understanding. Experimental mutagenesis results¹¹¹ and predicted interactions^{99b,110,112} have located the DHP binding site in the interface of domains III and IV of the pore-forming α 1 subunit, in transmembrane segments IIS5, IIIS6, IVS6, and IIIP.

It is known that although potent DHP antagonists have H-bond acceptors at both the port and starboard sides and H-bond donors at the stern, elimination of the port side ester group does not abolish the channel-blocking effect of DHPs.¹¹³ We performed our docking calculations with nifedipine as a reference compound. As shown in **Figure 4.2A**, nifedipine shares a similar position as that found for (S)-nimodipine, in which the NH group (stern) H-bonds to Y³¹⁰. Nifedipine also has a polar *o*-NO₂ group at the bowsprit that H-bonds to Y⁴¹¹ and this residue, in turn, H-bonds to the carboxymethyl ester moiety present at port side.

Then we docked compound **63** using the previously optimized settings, constraining the calculations to the DHP binding region where, presumably, these new C-5-

¹¹⁰ Tikhonov, D. B.; Zhorov, B. S. *J. Biol. Chem.* **2009**, *284*, 19006–19017.

¹¹¹ (a) Wappl, E.; Mitterdorfer, J.; Glossmann, H.; Striessnig, J. *J. Biol. Chem.* **2001**, *276*, 12730–12735. (b) Yamaguchi, S.; Okamura, Y.; Nagao, T.; Adachi-Akahane, S. *J. Biol. Chem.* **2000**, *275*, 41504–41511. (c) Schuster, A.; Lacinova, L.; Klugbauer, N.; Ito, H.; Birnbaumer, L.; Hofmann, F. *EMBO J.* **1996**, *15*, 2365–2370. (d) Peterson, B. Z.; Johnson, B. D.; Hockerman, G. H.; Acheson, M.; Scheuer, T.; Catterall, W. A. *J. Biol. Chem.* **1997**, *272*, 18752–18758.

¹¹² (a) Zhorov, B. S.; Folkman, E. V.; Ananthanarayanan, V. S. *Arch. Biochem. Biophys.* **2001**, *393*, 22–41. (b) Cosconati, S.; Marinelli, L.; Lavecchia, A.; Novellino, E. *J. Med. Chem.* **2007**, *50*, 1504–1513.

¹¹³ Goldmann, S.; Stoltefuß, J. *1,4-Dihydropyridines: Effects of Chirality and Conformation on Their Activity*. In *Dihydropyridines*; Busse, W.-D., Garthoff, B., Seuter, F., Eds.; Springer: Berlin and Heidelberg, Germany, **1993**; pp 24–35.

unsubstituted DHPs may interact. We docked the (*S*)-**63** enantiomer because it leaves the unsubstituted configuration at the port side position, and as we mentioned before, this deletion does not abolish the antagonistic activity by itself. The (*S*)-**63** enantiomer presents a 2-methyl and ethyl 3-carboxylate groups at the starboard side and a bulky phenyl moiety at the port side, and more importantly, it lacks the second carboxylic ester functionality at the port side. Docking results of (*S*)-**63** (Figure 4.2B) predict an orientation of the DHP core similar to that found for nifedipine but show a noticeably different interaction pattern. The inclusion of a bulky phenyl group increases the steric hindrance with F^{3p49}, and therefore (*S*)-**63** appears displaced toward the side entry of the DHP binding site formed by Met³ⁱ¹⁸, Met⁴ⁱ¹², Thr^{3o16}, and Ser⁴ⁱ⁹ (not shown). Thus, the phenyl moiety at the port side is partially accommodated in a hydrophobic pocket formed by Met³ⁱ¹⁹ and Phe^{3p49} and the core of DHP is slightly displaced away from the selectivity pore of the channel. The NH at the stern H-binds to Tyr³ⁱ¹⁰ and the carbonyl group at the starboard establish an H-bond to Gln^{3o18}.

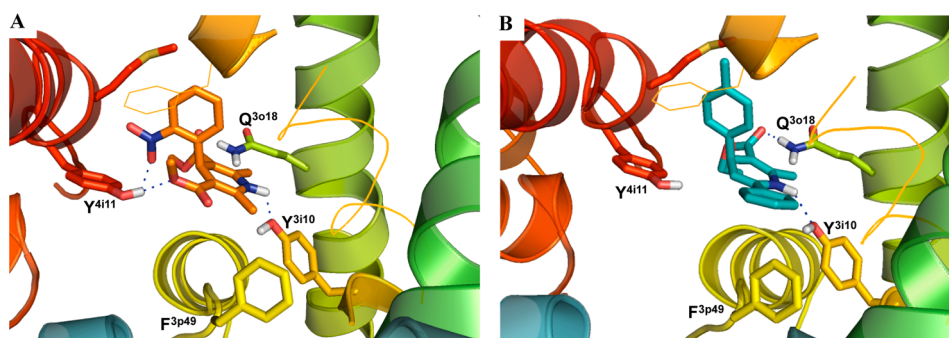


Figure 4.2: Docking studies for (*S*)-nimodipine (A) and (*S*)-**63** (B)

Comparing both complexes, we can conclude that the absence of several H-bond interactions and the inclusion of a bulky group at port side are destabilizing the interaction between our C-5-unsubstituted DHPs and the Ca_v1.2 L-type VGCCs. These results, together with our experimental data, give some hints of the reason for the observed decrease in selectivity toward Ca_v1.2.

4.3.4 Evaluation of neuroprotective properties

4.3.4.1 Neuroprotection in $[Ca^{2+}]_c$ overload model

A tight control of Ca^{2+} homeostasis by neurons is crucial for cell survival. In several NDDs, Ca^{2+} dysregulation is thought to be one of the main causes of neurodegeneration.¹¹⁴ Thus, drugs restoring the Ca^{2+} balance should indeed be a therapeutic alternative for the disease.¹¹⁵

In line with these findings, a study of the neuroprotective profile of the C-5-unsubstituted DHPs against $[Ca^{2+}]_c$ overload and oxidative stress was performed. In this study we discovered an interesting potential neuroprotective activity of the novel compounds **50-72** on SH-SY5Y cells exposed for 24 h to a depolarizing concentration of K^+ , which induced $[Ca^{2+}]_c$ overload and consequently cell death. Cells were co-incubated with drugs at 5 μ M and high K^+ (70 mM, hypertonic). Thereafter, MTT reduction was measured as a parameter of cell death.¹¹⁶ As shown in **Table 4.6**, all the synthesized DHPs have a similar protective activities ranged from 22.1% (**66**, entry 19) to 40.1% (**59**, entry 12), and the best neuroprotection result was obtained with compound **68**, which was able to prevent the death of 51% of cells (entry 21). In general, compounds bearing an ester functionality showed a higher neuroprotective profile compared to those bearing a thioester moiety or fused DHPs derivatives **71** and **72** (entries 24 and 25), the latter of which had a low neuroprotective activity.

Taking into account compounds of monocyclic type studied, **68**, bearing a 2-thienyl substituent at C-4, showed the best neuroprotective capability against $[Ca^{2+}]_c$ overload (entry 21) and **66** showed the poorest profile (entry 19). Generally,

¹¹⁴ (a) Marx, J. *Science* **2007**, *318* (5849), 384-385; (b) Green, K. N.; LaFerla, F. M. *Neuron* **2008**, *59*, 190-194.

¹¹⁵ Rosini, M.; Simoni, E.; Bartolini, M.; Cavalli, A.; Ceccarini, L.; Pascu, N.; McClymont, D. W.; Tarozzi, A.; Bolognesi, M. L.; Minarini, A.; Tumiatti, V.; Andrisano, V.; Mellor, I. R.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 4381-4384.

¹¹⁶ Maroto, R.; De la Fuente, M. T.; Artalejo, A. R.; Abad, F.; Lopez, M. G.; Garcia-Sancho, J.; Garcia, A. G. *Eur. J. Pharmacol.* **1994**, *270*, 331-339.

compounds bearing an electron-donating group were better neuroprotectants than those bearing an electron-withdrawing group.

VGCCs blockers have been previously studied as neuroprotectant agents, and as a common feature, it has been widely reported that their potency as $[Ca^{2+}]_c$ elevation blockers and the neuroprotective effect against Ca^{2+} overload are not usually well correlated.¹¹⁷ This characteristic is also present in our family of DHPs; i.e., compound **72** showed the best Ca^{2+} signal blockade (79% blockade), but it showed a moderate activity as neuroprotectant in the $[Ca^{2+}]_c$ overload model (entry 25). However, **68** blocked 36% of the Ca^{2+} signal and protected 40% of cells (entry 21). Similar results were obtained for compounds **63** and **59**, with 39% and 40% protection (entries 16 and 12), respectively, which decreased only 43% and 48% the calcium increase elicited by 70 mM K^+ (Table 4.4). These results led us to focus our attention on the possibility of an additional mechanism of action for our compounds, possibly related to their antioxidant action (see below). The correlation between Ca^{2+} influx through L-type VGCCs and increased mitochondrial oxidative stress has been recently reported.¹¹⁸ Furthermore, L-type antagonists reverted the oxidative stress measured in the cells.¹¹⁹ Therefore, the neuroprotective effect of our compounds against Ca^{2+} overload might be composed of VGCC blocker potency and antioxidant effect. The protective capabilities of these DHPs in an oxidative stress model are summarized in Table 4.6.

¹¹⁷ (a) Marco-Contelles, J.; León, R.; de Los Ríos, C.; Guglietta, A.; Terencio, J.; López, M. G.; García, A. G.; Villarroya, M. *J. Med. Chem.* **2006**, *49*, 7607-7610; (b) Marco-Contelles, J.; León, R.; López, M. G.; García, A. G.; Villarroya, M. *Eur. J. Med. Chem.* **2006**, *41*, 1464-9; (c) León, R.; Marco-Contelles, J. *Curr. Med. Chem.* **2011**, *18*, 552-576.

¹¹⁸ Khaliq, Z. M.; Bean, B. P. *J. Neurosci.* **2010**, *30*, 7401-7413.

¹¹⁹ Dryanovski, D. I.; Guzmán, J. N.; Xie, Z.; Galteri, D. J.; Volpicelli-Daley, L. A.; Lee, V. M.; Miller, R. J.; Schumacker, P. T.; Surmeier, D. J. *J. Neurosci.* **2013**, *33*, 10154-10164.

Table 4.6. Neuroprotection Exerted by Compounds 3a–u and 5a,b against Ca²⁺ Overload Induced by 70 mM K⁺ in SH- SY5Y Human Neuroblastoma Cells^a

Entry	Cmpd.	K ⁺ (70 mM co-incubation)	
		% survival	% protection
	basal	100	
	70 mM K ⁺	52.1 ± 3.5 ^{###}	
1	Nifedipine (5 μM)	62.9 ± 3.4 [*]	20.0
2	Nifedipine (0.3 μM)	58.7 ± 2.2 [*]	15.8
3	50	67.6 ± 3.1 ^{***}	34.6
4	51	67.3 ± 2.3 ^{***}	31.4
5	52	65.4 ± 3.3 [*]	23.3
6	53	68.0 ± 2.2 ^{***}	34.1
7	54	67.4 ± 1.4 ^{***}	32.5
8	55	69.4 ± 2.4 ^{***}	36.6
9	56	65.1 ± 4.2 [*]	24.8
10	57	69.4 ± 3.6 ^{***}	34.1
11	58	66.7 ± 2.9 ^{***}	31.3
12	59	72.7 ± 2.9 ^{***}	40.1
13	60	67.2 ± 3.3 ^{***}	34.5
14	61	67.4 ± 1.9 ^{***}	30.4
15	62	69.9 ± 2.4 ^{***}	36.2
16	63	72.3 ± 3.6 ^{***}	39.8
17	64	68.7 ± 2.5 ^{***}	29.8
18	65	64.6 ± 4.3 [*]	23.5
19	66	62.9 ± 5.7 [*]	22.1
20	67	59.3 ± 5.5 ^{ns}	13.7
21	68	75.6 ± 2.1 ^{***}	50.7
22	69	68.5 ± 3.7 ^{**}	32.0

Entry	Cmpd.	K ⁺ (70 mM co-incubation)	
		% survival	% protection
23	70	69.0 ± 2.6 ^{***}	35.3
24	71	66.4 ± 1.3 ^{***}	28.2
25	72	64.0 ± 4.1 [*]	21.4

^a Data are the mean ± SEM of at least five different cultures in triplicate. % protection was calculated considering MTT reduction by nontreated cells (basal) as 100% survival. % of toxicity was normalized for 70 mM K⁺ seen for each treatment and subtracted to 100. (***) $p < 0.001$. (**) $p < 0.01$. (*) $p < 0.05$. ns = not significant with respect to 70 mM K⁺ treated cells. (###) $p < 0.001$ compared to basal conditions. All compounds were assayed at 5 μM.

4.3.4.2 Neuroprotection in an oxidative stress model

Oxidative stress has been widely regarded as a key mechanism of neuronal death in several NDDs. As a common feature, high concentrations of reactive oxygen species (ROS) are produced by abnormal pathological mitochondria.¹²⁰ ROS oxidize lipids, causing membrane impairment and inducing cell death.¹²¹ Oxidation would also affect ribosomal functioning and reduce protein synthesis.¹²² Free radicals may also oxidize DNA and RNA, as shown by the detection of high levels of oxidized products in vulnerable neurons.

Rotenone and oligomycin A block complexes I and V, respectively, of the mitochondrial electron transport chain, thus disrupting ATP synthesis.¹²⁰ The mixture of rotenone plus oligomycin A (rot/olig) constitutes a good model of oxidative stress having its origin in mitochondria. Exposure to this combination induces neurotoxicity, and it has been widely used to evaluate potential protective drugs for

¹²⁰ Egea, J.; Rosa, A. O.; Cuadrado, A.; García, A. G.; López, M. G. *J. Neurochem.* **2007**, *102*, 1842-1852.

¹²¹ Butterfield, D. A.; Drake, J.; Pocernich, C.; Castegna, A. *Trends Mol. Med.* **2001**, *7*, 548-554.

¹²² Honda, K.; Smith, M. A.; Zhu, X.; Baus, D.; Merrick, W. C.; Tartakoff, A. M.; Hattier, T.; Harris, P. L.; Siedlak, S. L.; Fujioka, H.; Liu, Q.; Moreira, P. I.; Miller, F. P.; Nunomura, A.; Shimohama, S.; Perry, G. *J. Biol. Chem.* **2005**, *280*, 20978-20986.

neurodegenerative diseases.¹²³

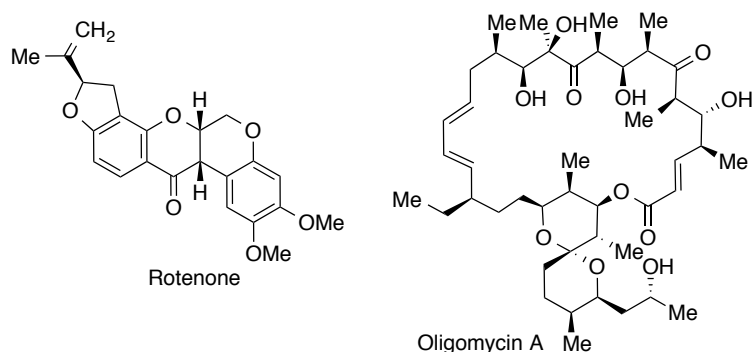


Figure 4.3: Molecular structure of rotenone and oligomycin A

First, we were interested in assessing the potential antioxidant effect of compounds **50-72** with a co-incubation protocol. C-5-unsubstituted DHPs were co-incubated for 8 h at a concentration of 5 μM with the stressor, followed by a 16 h postincubation of drugs at the same concentration without the toxic stimuli. These experimental conditions were designed to study the potential neuroprotective effect of the tested compounds based on their antioxidant capabilities. Besides, as described in the [Section 4.1](#), oxidative stress may be increased by $[\text{Ca}^{2+}]_c$ overload;¹⁰⁸ thus, in the co-incubation model of oxidative stress, neuroprotection afforded by C-5-unsubstituted DHPs could be dependent on their VGCC antagonism properties. As summarized in [Table 4.7](#), the tested compounds showed, in general, interesting neuroprotective effects, ranging from 32.5% of derivative **50** (entry 3) to 61.9% for **54** (entry 7), bearing an acetyl group instead of an ester or thioester substitution. Results from our compounds are compared with melatonin (0.3 μM) and nifedipine (5 μM), which were used as positive control and reference compound, respectively.

In general, in a comparison of DHPs possessing the same substituents at positions 2,

¹²³ (a) Romero, A.; Egea, J.; García, A. G.; López, M. G. *J. Pineal Res.* **2010**, *49*, 141-148; (b) González-Muñoz, G. C.; Arce, M. P.; López, B.; Pérez, C.; Romero, A.; del Barrio, L.; Martín de Saavedra, M. D.; Egea, J.; León, R.; Villarroya, M.; López, M. G.; García, A. G.; Conde, S.; Rodríguez-Franco, M. I. *Eur. J. Med. Chem.* **2011**, *46*, 2224-2235.

3, and 6, ethyl ester and ketone derivatives showed similar protection profiles indicating that both groups have similar participation on their antioxidant effect. For the ethyl ester family, derivatives bearing electron-withdrawing groups were less potent neuroprotectants than those with electron-donating groups. Thus, compounds **65** (entry 18) and **66-67** (entries 19 and 20), having respectively 4-NO₂ or 2-NO₂ groups, were the less active neuroprotectants in this model. On the other hand, methyl- and methoxyphenyl derivatives showed a protective effect over 40% in most cases, compound **63** (entry 16) being the best with a 53.3% protection. Compounds bearing a *p*-chlorophenyl moiety at the C-6 position showed similar protection values as those compounds lacking this substituent, i.e., compounds **57** (*p*-methoxyphenyl at C-4 and phenyl at C-6, entry 10) and **59** (*m*-methoxyphenyl at C-4 and *p*-chlorophenyl at C-6, entry 12) with protection values of 46.2% and 47.8%, respectively. Regarding the C-2-substituent, an increase of its steric volume was associated with an improvement of their neuroprotective profile, with protection values of 32.5% (methyl, **50**, entry 3), 32.8% (ethyl, **51**, entry 4), and 42.9% (propyl, **52**, entry 5). A similar correlation was also observed for their antioxidant effect. Regarding thioester derivatives, all tested compounds showed statistically significant neuroprotection with values ranging from 39.6% (**53**, entry 6) to 57.9% (**60**, entry 13). Compounds **70** (entry 23) and **60** (entry 13) afforded neuroprotective effects over 50%. Finally, polycyclic derivatives **71** and **72** (entries 24 and 25) were also able to protect SH-SY5Y cells in a statistically significant manner.

Table 4.7 Neuroprotective effect of C-5-unsubstituted DHPs **50-72** derivatives (5 μ M) on SH-SY5Y cells viability against toxicity induced by: (a) 8 h co-incubation of rot/olig mixture (30/10 μ M) in presence of drugs, followed by 16 h post-incubation of drugs, or (b) 8 h incubation of rot/olig mixture without any drug, followed by 16 h post-incubation of drugs.^a

Entry	Cmpd.	8 h rot/olig co-incubation + 16 h postincubation		8 h rot/olig + 16 h postincubation	
		% survival	% protection	% survival	% protection
	basal	100	-	100	-
	RO	65.6 \pm 1.3 ^{###}	-	70.6 \pm 1.8 ^{###}	-
1	Melatonin	82.5 \pm 2.7 ^{***}	44.7	87.8 \pm 3.9 ^{**}	58.3
2	Nifedipine	78.4 \pm 2.9 ^{**}	29.3	73.8 \pm 2.4 ^{ns}	11.7
3	50	78.4 \pm 3.4 ^{**}	32.5	-	-
4	51	77.4 \pm 5.9 ^{**}	32.8	-	-
5	52	80.5 \pm 2.9 ^{**}	42.9	80.6 \pm 3.3 ^{ns}	34.8
6	53	79.5 \pm 6.0 ^{**}	39.6	-	-
7	54	87.3 \pm 3.1 ^{***}	61.9	76.7 \pm 4.1 ^{ns}	21.7
8	55	82.7 \pm 3.2 ^{***}	46.2	-	-
9	56	69.9 \pm 3.9 ^{ns}	11.9	-	-
10	57	81.3 \pm 7.0 ^{**}	46.2	83.5 \pm 2.2 [*]	42.0
11	58	82.3 \pm 3.9 ^{***}	45.7	81.8 \pm 3.1 ^{ns}	38.6
12	59	81.9 \pm 3.4 ^{**}	47.8	76.6 \pm 3.9 ^{ns}	20.5
13	60	85.7 \pm 4.3 ^{***}	57.9	74.2 \pm 5.1 ^{ns}	12.3
14	61	83.3 \pm 4.2 ^{***}	49.4	83.4 \pm 3.1 [*]	43.3
15	62	79.9 \pm 2.9 ^{**}	36.2	-	-
16	63	83.5 \pm 5.3 ^{**}	53.3	88.0 \pm 2.6 ^{**}	60.1
17	64	79.7 \pm 3.2 [*]	41.5	84.3 \pm 3.1 [*]	44.1
18	65	64.6 \pm 1.5 ^{n.s}	-	-	-
19	66	69.9 \pm 2.2 ^{ns}	11.7	-	-
20	67	60.0 \pm 3.1 ^{ns}	-	-	-
21	68	81.9 \pm 4.0 ^{***}	45.3	77.5 \pm 4.7 ^{ns}	25.6
22	69	78.0 \pm 3.6 [*]	36.6	79.9 \pm 3.8 ^{ns}	33.2
23	70	83.7 \pm 1.7 ^{***}	52.4	86.2 \pm 1.2 ^{**}	52.2
24	71	74.6 \pm 4.3 ^{n.s}	26.9	-	-

Entry	Cmpd.	8 h rot/olig co-incubation +		8 h rot/olig +	
		16 h postincubation		16 h postincubation	
		% survival	% protection	% survival	% protection
25	72	80.3 ± 3.9**	39.8	-	-

^aData are means ± SEM of between five and 7 different cultures in triplicate. % Protection was calculated considering MTT reduction by nontreated cells (basal) as 100 % survival, % of toxicity was normalized for rot/olig seen for each treatment and subtracted to 100. (***) $p < 0.001$ (**) $p < 0.01$ and (*) $p < 0.05$, ns = not significant, with respect to rot/olig treated cells; (###) $p < 0.001$ compared to basal conditions. All compounds were assayed at 5 μM concentration.

These encouraging results prompted us to study the compounds with the best overall neuroprotective profile on a second model of oxidative stress. A neuroprotective compound with potential therapeutic interest will be used clinically after neurons already have become vulnerable; for instance, diseases are diagnosed after neuronal damage has already been established.¹²⁴ Because we wanted to simulate experimental conditions closer to the clinical situation, in search for compounds able to protect cells already exposed to oxidative stress, we employed a protocol that consisted of an 8 h incubation period with the stressor, followed by a 16 h incubation period with the drug alone. We selected 11 DHPs to be tested in this postincubation oxidative stress model, and data are collected in Table 4.7. Melatonin (0.3 μM) and nifedipine (5 μM) were also tested as positive and reference compounds, respectively. Among all tested compounds, derivatives **57**, **61**, **63**, **64**, and **70** (respectively entries 10, 14, 16, 17 and 23) demonstrated a neuroprotective effect after a 16 h postincubation in a statistically significant manner. Protection values were, in all cases, over 40%, ranging from 43.3% of derivative **61** to the 60.1% protection afforded by compound **63**. It is interesting to emphasize that nifedipine, which showed significant neuroprotection in the co-incubation stress model, lost its ability to protect in the postincubation model. This result may indicate that neuroprotection is not fully dependent on the Ca^{2+} signal blockade properties.

¹²⁴ Parada, E.; Egea, J.; Romero, A.; del Barrio, L.; García, A. G.; López, M. G. *Free Radic. Biol. Med.* **2010**, *49*, 1815-1821.

4.3.4.3 Neuroprotection: oxygen-glucose deprivation of hippocampal slices. An acute model of ischemia/reperfusion

Oxygen and glucose deprivation (OGD) is an acute model of the lesion produced by $[Ca^{2+}]_c$ overload during the OGD period followed by free radical generation during the reoxygenation phase.¹²⁵ In neurons, the OGD period depolarizes the membrane after mitochondrial failure. Depolarization induces substantial $[Ca^{2+}]_c$ elevation and a massive glutamate liberation, leading to increased cytotoxicity. Among all the experimental models of neurotoxicity elicited by Ca^{2+} overload, glutamate-induced Ca^{2+} overload seems to be the most relevant from a pathogenic point of view and has been related to several neurodegenerative diseases and stroke.¹²⁶ Additionally, recent observations have confirmed the influence of mitochondria-mediated cell Ca^{2+} regulation on glutamate-induced excitotoxicity.¹²⁷ On the other hand, the functional impairment of mitochondria, promoted by the lack of oxygen, is increased when reoxygenation triggers a massive production of reactive oxygen species raised by the OGD-induced overwork of the NADPH oxidase (NOX) enzyme.¹²⁸

To further characterize the neuroprotective profile of compounds **63** and **70**, we used this model, where toxicity depends on $[Ca^{2+}]_c$ overload and oxidative stress. Rat hippocampal slices were subjected to 15 min OGD followed by 120 min reoxygenation (see protocol in [Figure 4.4A](#)), and cell viability was assessed by MTT reduction. Under these experimental conditions, slices were treated with **63** or **70** at increasing concentrations (1, 3, and 10 μ M) and with nifedipine (10 μ M) as a control. Considering cell viability in basal slices as 100%, OGD reduced cell viability by 40%.

Compounds **63** and **70** afforded maximum protection at 10 μ M (45%, [Figure 4.4B](#)). Nifedipine (10 μ M) treatment produced no significant protection, corroborating the

¹²⁵ Egea, J.; Rosa, A. O.; Sobrado, M.; Gandía, L.; López, M. G.; García, A. G. *Neuroscience* **2007**, *145*, 866-872.

¹²⁶ Lau, A.; Tymianski, M. *Pflugers Arch.* **2010**, *460*, 525-542.

¹²⁷ Abramov, A. Y.; Duchen, M. R. *Biochim. Biophys. Acta* **2010**, *1800*, 297-304.

¹²⁸ Chen, H.; Yoshioka, H.; Kim, G. S.; Jung, J. E.; Okami, N.; Sakata, H.; Maier, C. M.; Narasimhan, P.; Goeters, C. E.; Chan, P. H. *Antioxidants & redox signaling* **2011**, *14*, 1505-1517.

results obtained in SH-SY5Y cells subjected to rot/oligo stress postincubation.

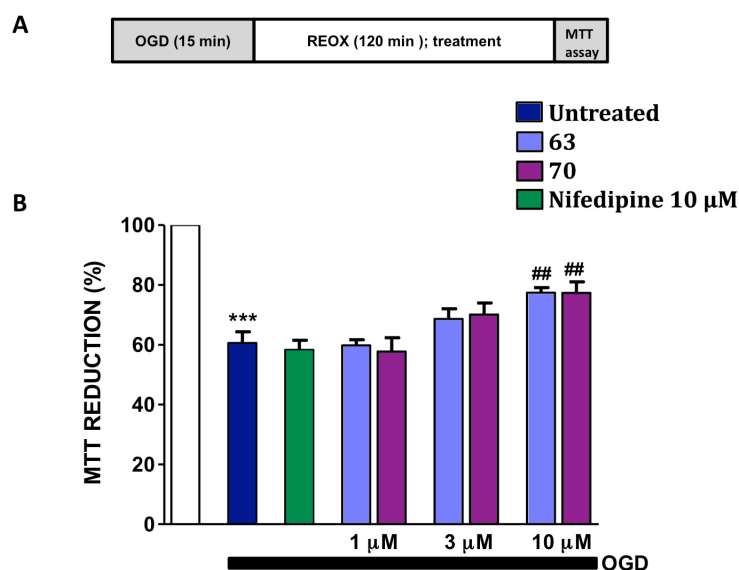


Figure 4.4: Post-OGD treatment with **63** and **70** protects hippocampal slices against oxygen and glucose deprivation followed by reoxygenation. (A) Protocol used to elicit toxicity. Hippocampal slices were exposed for 15 min to OGD followed by 2 h in control solution (Reox). **63**, **70** and nifedipine, when used, were present during the 2 h reox period. (B) Cell viability was measured by the MTT reduction activity. Values are expressed as the mean \pm SEM of five independent experiments: (***) $p < 0.001$, compared to the basal; (##) $p < 0.01$ with respect to OGD-treated slices.

In summary our new class of C5-unsubstituted-C6-aryl-1,4-dihydropyridines has shown the ability to block Ca^{2+} entry after a depolarizing stimulus and, more interestingly, showed an improved $\text{Ca}_v1.3/\text{Ca}_v1.2$ selectivity when compared to classical dihydropyridines. Considering that the different functions of $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ L-type VGCCs seem to be involved in different pathological conditions, the improved selectivity of our DHPs, could be promising for the future development of new generations of more selective C5-unsubstituted 1,4-dihydropyridines.

Moreover, since our DHPs protected neuroblastoma cells against $[\text{Ca}^{2+}]_i$ overload and oxidative stress-induced toxicity, their selectivity ratio makes them highly

interesting for the treatment of neurological disorders where Ca^{2+} dyshomeostasis and high levels of oxidative stress have been demonstrated. Indeed their low potency toward the cardiovascular channel subtype makes them potentially safer than classical 1,4-dihydropyridines as far as cardiovascular side effects are concerned. Interestingly, some compounds afforded good protection in a postincubation model, which better simulates the clinical condition, offering a therapeutic window of opportunity of great interest for patient recovery after a brain ischemic episode. Good activities were also found in acute ischemia/reperfusion (oxygen and glucose deprivation) models. Taken together, these compounds deserve further investigation on neurological disease animal models to confirm their good in vitro neuroprotective profile described here.

**5. One-Pot Access To a Library of Densely Substituted
Nicotinamides Via a Three-Component Formal Aza
[3+3] Cycloaddition**

5.1 INTRODUCTION: BIOLOGICAL RELEVANCE OF NICOTINAMIDE DERIVATIVES

Nicotinic acid derivatives and, especially, nicotinamides constitute one of the most important families of biologically relevant compounds containing a pyridine ring. They are members of the B-vitamin group and play a key role in many essential metabolic processes. The NAD/NADP coenzymes are nicotinamide derivatives, leading to many potential targets for interference with drugs (Figure 5.1).¹²⁹

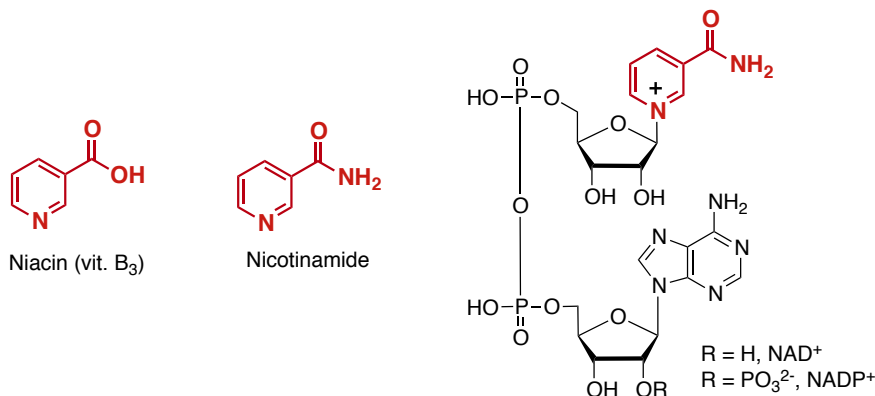


Figure 5.1: Biologically relevant nicotinamide derivatives

Besides, a number of derivatives of nicotinamide have demonstrated pharmacological activity at other types of targets. Thus, nicorandil is an established drug acting as a selective activator of ATP-dependent potassium channel that is employed in the treatment of cardiac ischemia.¹³⁰

¹²⁹ (a) Ávalos, J. L.; Bever, K. M.; Wolberger, C. *Mol. Cell* **2005**, *17*, 855-868. (b) Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G. *Cell. Mol. Life Sci.* **2008**, *65*, 4000-4018.

¹³⁰ Eeckhout, E. *Eur. Heart J.* **2003**, *24*, 1282-1284.

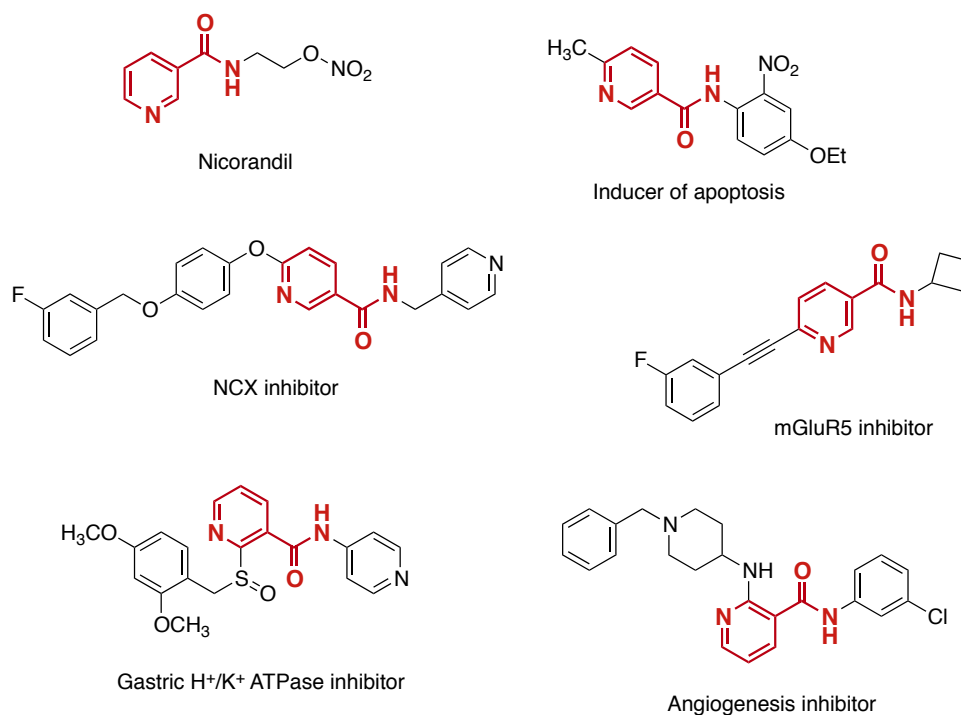


Figure 5.2: Examples of pharmacologically relevant nicotinamides

Many other pharmacologically relevant nicotinamides have been described, including antiarrhythmic compounds acting by inhibition of the sodium–calcium exchanger (NCX),¹³¹ anti-cancer compounds acting by inducing apoptosis¹³² or by inhibiting vascular endothelial growth factor (VEGF)-induced angiogenesis,¹³³ anxiolytic, and antidepressant activity associated to the inhibition of Type 5 metabotropic glutamate receptors (mGluR5)¹³⁴ and inhibitors of the gastric H⁺/K⁺ ATPase acting as antiulcer agents (Figure 5.2).¹³⁵ Nicotinamide itself, in high doses, is a neuroprotective agent in *in vitro* models of cytotoxicity and *in vivo* in

¹³¹ Kuramochi, T.; Kakefuda, A.; Yamada, H.; Tsukamoto, I.; Taguchi, T.; Sakamoto, S. *Bioorg. Med. Chem.* **2005**, *13*, 4022–4036.

¹³² Cai, S. X.; Nguyen, B.; Jia, S.; Guastella, J.; Reddy, S.; Tseng, B.; Drewe, J.; Kasibhatla, S. *J. Med. Chem.* **2003**, *46*, 2474–2481.

¹³³ Choi, H. E.; Yoo, M. S.; Lee, J. H.; Kim, J. H.; Kim, J. H.; Lee, J. K.; Kim, G. I.; Park, Y.; Chi, Y. H.; Paik, S. H.; Lee, J. H. *Bioorg. Med. Chem.* **2011**, *21*, 6236–6241.

¹³⁴ Cleva, R. M.; Foster Olive, M. *Molecules* **2011**, *16*, 2097–2106.

¹³⁵ Terauchi, H.; Tanitame, A.; Tada, K.; Nakamura, K.; Seto, Y.; Nishikawa, Y. *J. Med. Chem.* **1997**, *40*, 313–321.

brain ischaemia,¹³⁶ the mechanisms of this neuroprotection being complex and not well known.¹³⁷ Furthermore, nicotinamide is involved in the aging process¹³⁸ and is the physiological inhibitor of the sirtuins,¹³⁹ a family of NAD⁺ dependent histone deacetylases that have emerged as potential therapeutic targets for treatment of pathologies including metabolic, cardiovascular and neurodegenerative diseases, and also cancer.¹⁴⁰

Considering the biological significance of these derivatives, we thought that it was important to have efficient and versatile synthetic routes to obtain these compounds. There are not many precedents in the literature, particularly as it relates to multicomponent reactions, thus we assumed that we could adapt our previous multicomponent protocol carried out for the synthesis of 1,4-dihydropyridines to the synthesis of nicotinamide derivatives, simply by employing β -ketoamides as the 1,3-dicarbonyl components of the reaction.

¹³⁶ Słomka, M.; Ziemińska, E.; Salińska, E.; Lazarewicz, J. W. *Folia Neuropathol.* **2008**; *46*, 69-80.

¹³⁷ Maiese, K.; Chong, Z.Z. *Trends Pharmacol. Sci.* **2003**, *24*, 228-232.

¹³⁸ Xu, P.; Sauve, A.A. *Mech. Ageing Dev.* **2010**, *131*, 287-298.

¹³⁹ (a) Jackson, M. D.; Schmidt, M. T.; Oppenheimer, N. J.; Denu, J. M. *J. Biol. Chem.* **2003**, *278*, 50985-50998; (b) Avalos, J. L.; Bever, K. M.; Wolberger, C. *Mol. Cell* **2005**, *17*, 855-868.

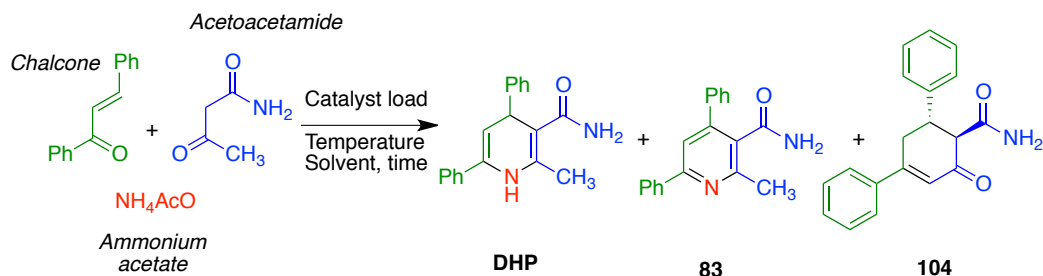
¹⁴⁰ (a) Michan, S.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1-13; (b) Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G. *Cell. Mol. Life Sci.* **2008**, *65*, 4000-4018; (c) Horio, Y.; Hayashi, T.; Kuno, A.; Kunimoto, R. *Clin. Sci.* **2011**, *121*, 191-203; (d) Carafa, V.; Nebbioso, A.; Altucci, L. *Front. Pharmacol.* **2012**, *3*, 1-6.

5.2 SYNTHESIS OF NICOTINAMIDES BY A NEW MULTICOMPONENT REACTION

5.2.1 Proposal and optimization of a multicomponent reaction to obtain nicotinamide derivatives

Thereafter we started the study of the multicomponent process that would afford nicotinamide derivatives by examining the reaction between chalcone, acetoacetamide and ammonium acetate (Scheme 5.1 and Table 5.1).

According to our previous experience, the first reaction was performed in refluxing ethanol and in the presence of ceric ammonium nitrate (entry 1). It provided a rather complex mixture from which we could isolate the desired compound **83**, the expected dihydropyridine (**DHP**) and a small amount of another compound identified as the cyclohexenone derivative **104** by its spectroscopic data. The *trans* disposition of substituents was assigned taking into account the value of the coupling constant between the corresponding protons ($J=8.9$ Hz). The latter compound arises from the Robinson annulation between the chalcone and acetoacetamide without incorporation of ammonia, and its identity was unambiguously confirmed by its independent synthesis from chalcone and acetoacetamide in the presence of piperidine.¹⁴¹



Scheme 5.1: Initial study for the three-component synthesis of nicotinamides

As shown in Table 5.1, subsequent experiments showed that the use of a higher catalyst load (entry 2) or of a more concentrated solution (entry 3) did not greatly

¹⁴¹ Rajanarendar, E.; Kalyan Rao, E.; Raju, S. *Indian J. Chem.* **2009**, *48B*, 749-753.

improve the results, whereas a longer reaction time (15 h) was found to be highly beneficial, leading almost exclusively to the target compound **83** (entry 4). A control experiment carried out under the same conditions but in the absence of catalyst also led to consumption of the starting materials and to the formation of **83**, but in this case a higher amount of the side product **104** was observed (entry 5). Therefore, subsequent experiments were performed in the presence of 10 mol% of CAN in the conditions of entry 4 which corresponds to the best yield of **83**.

Table 5.1. Optimization study of the multicomponent reaction

Entry	Catalyst load ^a	Solvent	Temper.	Time (h)	DHP: 83 : 104 ratio ^b
1	10 mol%	EtOH	Reflux	4	41:45:14
2	20 mol%	EtOH	Reflux	4	30:55:15
3	10 mol%	EtOH ^c	Reflux	4	40:48:12
4	10 mol%	EtOH	Reflux	15	0:95:5
5	-	EtOH	Reflux	15	0:76:24

^a all the experiments were carried out with 1:1:3 ratio of chalcone: β -ketoamides:ammonium acetate and the catalyst load is referred to the limiting reagent.

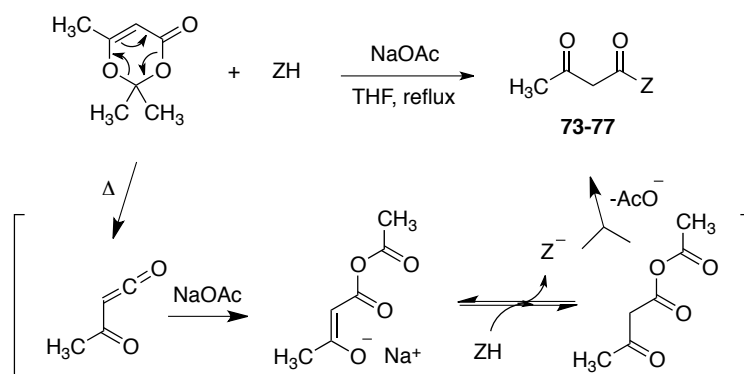
^b conversion in crude ¹H-NMR spectra

^c 2 M solution of the reagents (in all the other experiments, reagent concentration was 1 M).

5.2.2 Synthesis of the starting β -ketoamides 73-80

Once established the optimal condition for our protocol, different chalcones and ketoamides were required to explore the scope of this methodology. We had some commercial chalcones, and others were available by aldol condensation as described in Section 4.2.2, so we proceeded to the preparation of an ample variety of β -ketoamides. To this end, we initially prepared the N-mono- and disubstituted acetoacetamides **73-77** by a procedure developed in our group based on the treatment of primary and secondary amines with 2,2,6-trimethyl-1,3-dioxin-4-one in the presence of sodium acetate, which leads to the formation of an intermediate mixed anhydride, and allows the use of milder temperature conditions than

alternative protocols (Scheme 5.2 and Table 5.2).¹⁴²



Scheme 5.2: Synthesis of the starting β -ketoamides **73-77**

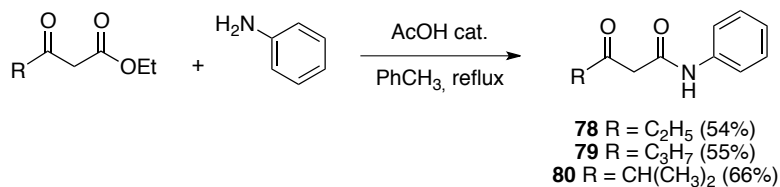
Table 5.2. Scope and yield of the synthesis of β -ketoamides **73-77**

Entry	Cmpd.	Z	Yield (%)
1	73		95
2	74		95
3	75		90
4	76		71
5	77		96

In addition, β -oxoanilides **78-80** were also prepared by a literature method,¹⁴³ based on the reaction between the corresponding β -ketoesters and the appropriate aniline in refluxing toluene. The reflux condenser was connected to a Dean-Stark trap to continuously remove the alcohol generated during the reaction (Scheme 5.3).

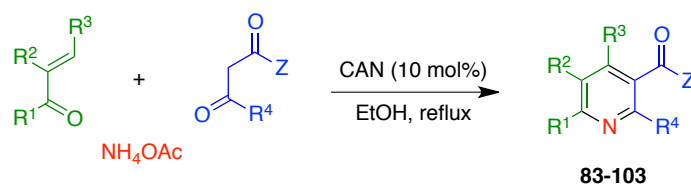
¹⁴² Sridharan, V.; Ruiz, M.; Menéndez, J.C. *Synthesis* **2010**, 6, 1053-1057.

¹⁴³ Jendralla, H.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Kerekjarto, B. V.; Kessler, K.; Krause, R.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, 33, 61-70.

Scheme 5.3: Synthesis of the starting β -oxoanilides **78-80**

5.2.3 Synthesis of nicotinamide derivatives




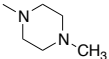
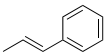
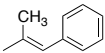
With the optimal reaction conditions in hand, we explored the scope of the method (Scheme 5.4 and Table 5.3).

Scheme 5.4: Three-component synthesis of nicotinamides **83-103**

As shown in the Table 5.3 the reaction normally proceeded in good to excellent yields, and tolerated well the presence of either electron-withdrawing or electron-releasing groups at the chalcone aromatic rings. Ortho-substitution at the R¹ substituent (entry 8, compound **90**) led to a decrease in yield due to steric hindrance, but it had little effect on the other aromatic ring (substituent R³), as shown by the 85% yield obtained for compound **89** (entry 7).

Table 5.3. Scope and yield of the synthesis of nicotinamides **83-103**

Entry	Cmpd.	R ¹	R ²	R ³	R ⁴	Z	Yield (%) ^a
1	83	C ₆ H ₅	H	C ₆ H ₅	CH ₃	NH ₂	92
2	84	4-MeC ₆ H ₄	H	C ₆ H ₅	CH ₃	NH ₂	95
3	85	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃	NH ₂	95
4	86	C ₆ H ₅	H	4-MeOC ₆ H ₄	CH ₃	NH ₂	95
5	87	4-MeOC ₆ H ₄	H	4-MeOC ₆ H ₄	CH ₃	NH ₂	73

Entry	Cmpd.	R ¹	R ²	R ³	R ⁴	Z	Yield (%) ^a
6	88	4-ClC ₆ H ₄	H	4-ClC ₆ H ₄	CH ₃	NH ₂	95
7	89	4-ClC ₆ H ₄	H	2-NO ₂ C ₆ H ₄	CH ₃	NH ₂	85
8	90	2-NO ₂ C ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃	NH ₂	66
9	91^b	C ₆ H ₅	CH ₃	C ₆ H ₅	CH ₃	NH ₂	20
10	92	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃		83
11	93	C ₆ H ₅	H	C ₆ H ₅	CH ₃	NHPh	84
12	94^{b,c}	C ₆ H ₅	H	C ₆ H ₅	C ₂ H ₅	NHPh	74
13	95	C ₆ H ₅	H	C ₆ H ₅	C ₃ H ₇	NHPh	82
14	96^c	C ₆ H ₅	H	C ₆ H ₅	CH(CH ₃) ₂	NHPh	74
15	97	C ₆ H ₅	H	C ₆ H ₅	CH ₃		64
16	98	C ₆ H ₅	H	C ₆ H ₅	CH ₃		80
17	99	C ₆ H ₅	H	C ₆ H ₅	CH ₃		67
18	100	C ₆ H ₅	H	2-thienyl	CH ₃	NH ₂	70
19	101	4-MeC ₆ H ₄	H	2-thienyl	CH ₃	NH ₂	72
20	102	C ₆ H ₅	H		CH ₃	NH ₂	74
21	103	C ₆ H ₅	H		CH ₃	NH ₂	68

^a isolated yield after crystallization or chromatography

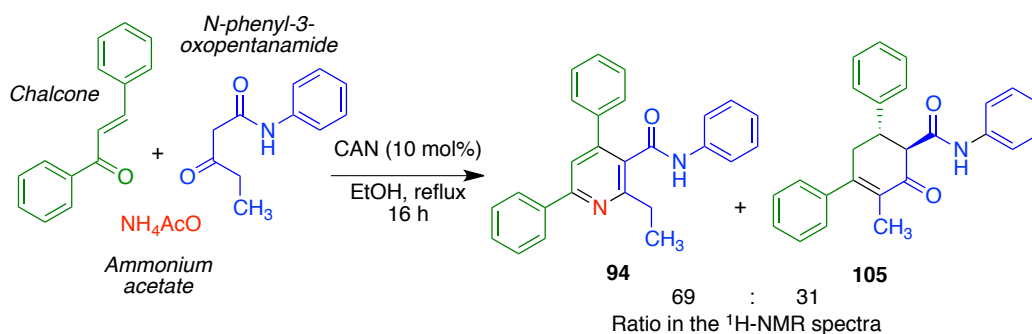
^b experiments carried out with 1:2:3 ratio of chalcone:β-ketoamides:ammonium acetate (+1 additional eq of ammonium acetate after 24 h)

^c experiments carried out at 50°C

To prove the ability of the method to introduce further structural diversity in the nicotinamide derivatives, we examined also the preparation of compounds bearing substituents other than phenyl at the pyridine C-4 position by use of suitably modified chalcones. This way, we prepared compounds **100** and **101** (entries 18 and 19), with a heterocyclic moiety at C-4, and **102** and **103** (entries 20 and 21), with a styryl chain.

The main limitation of the method came from the presence of substituents

different from hydrogen at R² (entry 9, compound **91**), which was clearly detrimental for yield and which could not be overcome in spite of having attempted many different conditions (finally we had to modify slightly the experimental procedure for this derivative). In particular, it was interesting to note that not even by raising the temperature nor by extending the reaction time, we were able to increase the conversion of the starting chalcone into the expected product. Instead, we could observe only its progressive degradation to the parent compounds propiophenone and benzaldehyde. On the other hand, while R⁴ was methyl in most cases due to a better accessibility of the starting materials, we verified that the presence of other substituents was also possible (entries 12-14, compounds **94-96**). In some of these cases, it was necessary to change slightly the experimental conditions in order to avoid the formation of the side products coming from the Robinson annulation, as confirmed by the identification of the compound **105** obtained from the reaction between chalcone, ammonium acetate and *N*-phenyl-3-oxopentanamide in the standard conditions (Scheme 5.5).



Scheme 5.5: Competition between the three-component nicotinamides synthesis and Robinson annulation

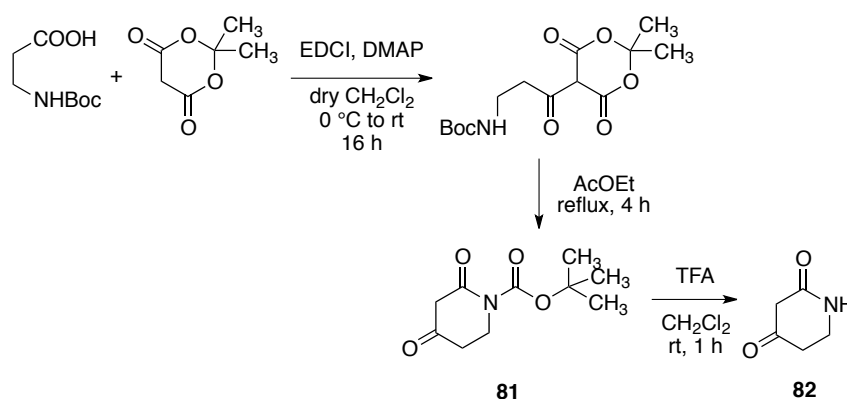
Finally, the amide nitrogen could be unsubstituted (entries 1-9 and 18-21, compounds **83-91** and **100-103**), monosubstituted with alkyl (entry 10, compound **92**) or aryl (entries 11-14, compounds **93-96**) groups or disubstituted (entries 15-17, compounds **97-99**).

It is worth underlining that the synthesis of compound **83** was already known in the literature and it was based on a related two-component reaction starting from

chalcone and 2-methylcrotonamide, giving however just a modest 36% yield.¹⁴⁴

5.2.4 Use of cyclic β -ketoamides for the synthesis of fused pyridine systems

Finally, we also examined briefly the preparation of fused pyridines by employing, as the starting material, the β -ketolactams **81** and **82**. These compounds were prepared following a literature procedure that involves the initial acylation of Meldrum's acid with *N*-Boc- β -alanine in the presence of EDCI and DMAP, and subsequent cyclization to compound **81** in refluxing AcOEt.¹⁴⁵ Finally, removal of the *N*-Boc protecting group of **81** in acid conditions, afforded the β -ketolactam **82** (Scheme 5.6).

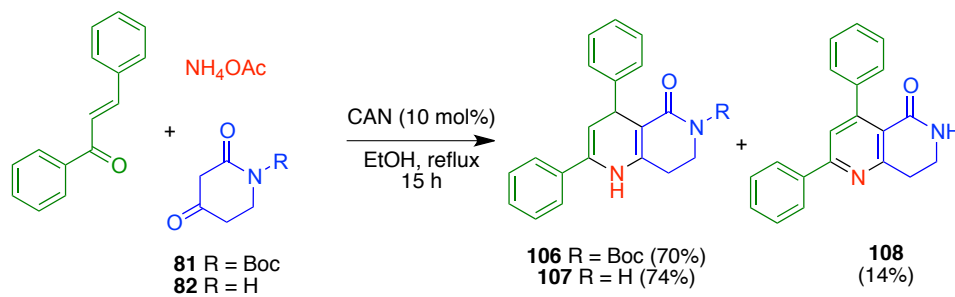


Scheme 5.6: Synthesis of the starting β -ketolactams **81** and **82**

With the desired β -ketolactams in hand, we studied their reaction with chalcone and ammonium acetate in refluxing ethanol. As shown in Scheme 5.7, in this case the main products were the 1,6-naphthyridine derivatives **106-107**, containing a fused dihydropyridine substructure, together with a small amount of the corresponding pyridine **108**, only when R = H.

¹⁴⁴ Kato, T.; Noda, M. *Chem. Pharm. Bull.* **1976**, *24*, 1408–1410.

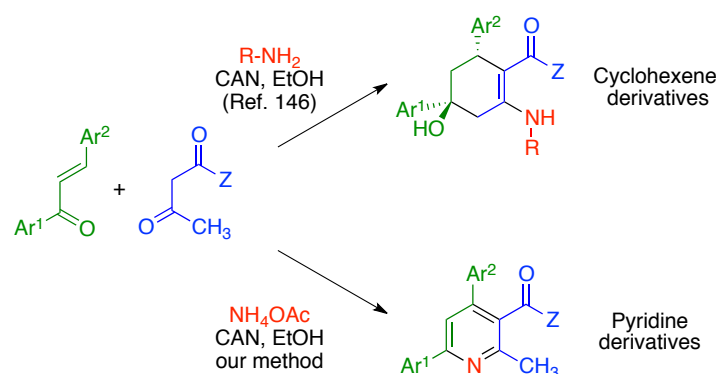
¹⁴⁵ Vanotti, E.; Amici, R.; Bargiotti, A.; Berthelsen, J.; Bosotti, R.; Ciavolella, A.; Cirila, A.; Cristiani, C.; D'Alessio, R.; Forte, B.; Isacchi, A.; Martina, K.; Menichincheri, M.; Molinari, A.; Montagnoli, A.; Orsini, P.; Pillan, A.; Roletto, F.; Scolaro, A.; Tibolla, M.; Valsasina, B.; Varasi, M.; Volpi, D.; Santocanale, C. *J. Med. Chem.* **2008**, *51*, 487–501.



Scheme 5.7: Three-component reaction between chalcones, ammonium acetate and β -ketolactams

5.3 MECHANISTIC PROPOSAL

Before focusing the attention on the mechanistic proposal, it is interesting to note that a similar reaction involving the use of primary amines instead of ammonia has been found to give cyclohexene derivatives rather than the pyridines obtained in the present work.¹⁴⁶ Thus, the exquisite balance between the two chemodivergent pathways involving [3+3] and aza-[3+3] formal cycloadditions, respectively, seems to depend on the steric hindrance of the nitrogen of the N-nucleophile (Scheme 5.8).



Scheme 5.8: Chemodivergent pathways of slightly different three-component reactions

Mechanistically, both reactions are proposed to start by the generation of an intermediate β -enaminone **I** via the CAN-catalyzed reaction between the starting primary amine or ammonia and the β -dicarbonyl compound. This assumption is based on two experimental facts:

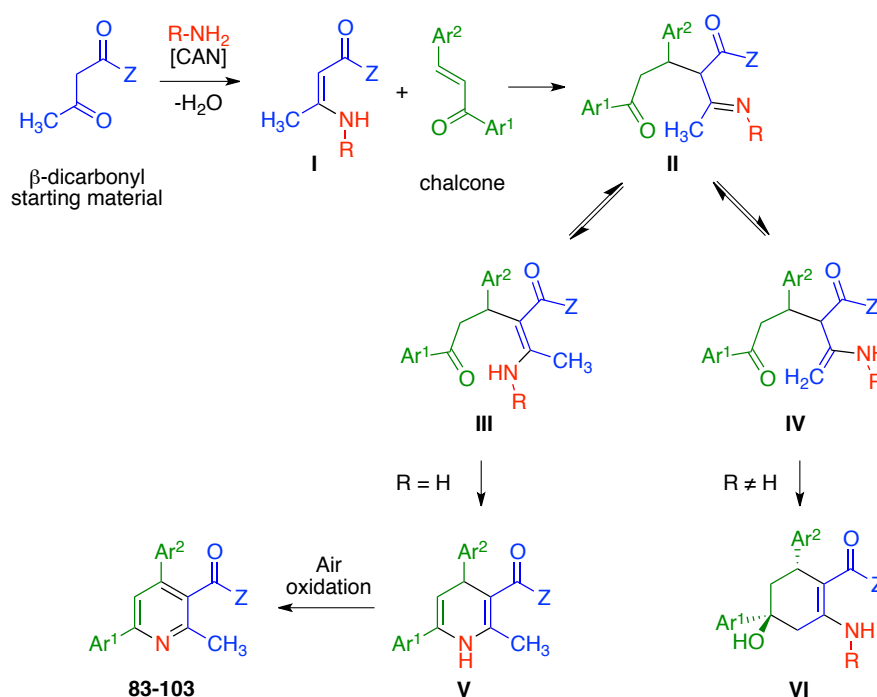
- CAN catalysis is known to lead to the very fast formation of β -enaminones;¹⁴⁷
- control experiments carried out from an isolated enaminone (3-aminocrotonamide) led to a result that was identical to that of our three-component reactions.

A Michael addition of **I** onto the enone fragment of chalcone should lead to intermediate **II**, which would be in tautomeric equilibrium with two enamine

¹⁴⁶ Sridharan, V.; Menéndez, J.C. *Org. Lett.* **2008**, *10*, 4303–4306.

¹⁴⁷ Sridharan, V.; Avendaño, C.; Menéndez, J. C. *Synlett* **2007**, 881–884.

species **III** and **IV**. In the case $R = H$, the unhindered nitrogen atom is able to attack the opposing carbonyl as a nucleophile, leading to dihydropyridine **V** after loss of a molecule of water, in a Hantzsch-like process. On the other hand, for the more hindered cases where R is different from hydrogen, the reaction with the side chain carbonyl is slower because of steric hindrance and the system tends to evolve via the less stable, but also more reactive, intermediate **IV**, which affords the cyclohexene derivatives **VI** (Scheme 5.9).



Scheme 5.9: Mechanistic proposal for our three-component reaction

While dihydropyridines are normally reasonably stable, during the optimization studies we never observed compounds **V** as the only reaction products but as part of mixtures with pyridines **83-103**, indicating that they are particularly prone to oxidation. Furthermore, as already demonstrated in the previous chapter, when an ester ($Z = \text{OAlk}$) or ketone ($Z = \text{Alk}$) derivative was employed as starting material, the same protocol generated a stable dihydropyridine, which shows that this ease of oxidation has to be attributed to the presence of the amide substituent. This behavior can be attributed to the very strong conjugation between the amide carbonyl and its nitrogen, which makes the carbonyl less prone to accept electron

density from the dihydropyridine nitrogen and hence makes the latter more electron-rich and the corresponding heterocycle more readily oxidizable. In the case of compounds **106** and **107**, the more rigid structure must hamper dehydrogenation by facilitating conjugation of the dihydropyridine nitrogen with the lactam carbonyl.

6. Design And Synthesis Of GSK-3 β Inhibitors For The Treatment Of Alzheimer's Disease

6.1 ALZHEIMER'S DISEASE: AN OVERVIEW

6.1.1 Etiology and pathophysiology of Alzheimer's disease

First described in 1906, Alzheimer's disease (AD) is the most common neurodegenerative disease,¹⁴⁸ with an estimated prevalence of more than 44 million people worldwide in 2013, a number that is expected to reach 75 million in 2030 and 135 million in 2050.¹⁴⁹ After more than a century and despite of an extensive research, the causes of AD are still unknown and consequently the discovery of effective therapies remains a long-standing and crucial objective of modern medicine.¹⁵⁰

This devastating neurological disorder is characterized by the progressive impairment of cognitive functions, memory loss, altered behavior and neuronal death. The pathological hallmarks of AD are known and at morphological levels are characterized by the presence and accumulation of senile plaques formed by a β -amyloid peptide ($A\beta$), derived from the amyloid protein precursor (APP), and of intracellular neurofibrillary tangles (NFTs) composed of a hyperphosphorylated form of the microtubular protein tau.

During the last thirty years various etiological hypothesis for AD have been formulated by studying the different histopathological lesions discovered in the brain of AD patients. The first remarkable pharmacological results in the treatment of AD have been achieved by the use of acetylcholinesterase inhibitors, based on the evident deficit of acetylcholine in the cholinergic system typical of the AD patients. Indeed, despite of its limitations, the *cholinergic hypothesis* has allowed the development of four of the five drugs currently approved for the

¹⁴⁸ Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777-781.

¹⁴⁹ Alzheimer's Disease International. Global impact of dementia 2013; **2013**; <http://www.alz.co.uk/research/GlobalImpactDementia2013.pdf>.

¹⁵⁰ Holtzman, D. M.; Morris John, C.; Goate, A. *Sci. Trans. Medl.* **2011**, *77*, 77sr1.

treatment of AD. Although the therapy with acetylcholinesterase inhibitors produces improvement in the patients' cognitive abilities and in their quality of lives, this kind of treatment is just a palliative remedy considering that the neurodegenerative process does not stop.¹⁵¹ Some years later, the *amyloid hypothesis* emerged as a new encouraging theory to explore, considering that the accumulation of amyloid plaques is characteristic in all AD patients. Since the eighties, the majority of the investigations carried on AD have been focused on drug development in the context of this hypothesis; nevertheless, the extended efforts made in searching for effective therapies focused on this target have repeatedly failed.¹⁵² The difficulty in finding effective therapies based on this hypothesis, together with other controversial aspects such as the unsolved dilemma of whether A β is one of the causes or one of the risk factors for AD, and the not so strong correlation between the presence of A β deposits and some clinical manifestations in AD patients, prompted the scientific community to investigate additional pharmacological targets. Thus, at the end of the past century, a new theory for AD etiology was postulated: the *tau hypothesis*, based on the aberration of a microtubule-associated protein (MAP) called tau that is particularly abundant in the CNS and whose main physiological function is the stabilization of the neuronal cytoskeleton.¹⁵³

6.1.2 Tau protein, NFTs and glycogen synthase kinase-3 β (GSK-3 β)

In physiological conditions tau helps to regulate microtubule dynamics and to promote their stability; conversely, in the pathological state tau undergoes post-translational modifications that result in its dissociation from microtubules. Among these modifications, the most important one is certainly the hyperphosphorylation of the protein that elicits the formation of filamentous inclusion and tau aggregation in NFTs that, together with the senile plaques, constitute the classical aberrant structures present in AD patients.¹⁵⁴

¹⁵¹ Birks J. *Cochrane Database of Systematic Reviews* **2006**, Issue 1. Art. No.: CD005593. DOI: 10.1002/14651858.CD005593.

¹⁵² Hardy, J.; Selkoe, D. J. *Science*, **2002**, 297, 353-356.

¹⁵³ Giacobini, E.; Gold, G. *Nat. Rev. Neurol.* **2013**; 9, 677-686.

¹⁵⁴ (a) Johnson, G. V. W.; Hartigan, J. A. *J. Alzheimers Dis.* **1999**, 1, 329-351; (b) Avila, J. *FEBS Lett.* **2006**, 580, 2922-2927.

In recent years different studies reported a tight connection between the two characteristic pathological features present in AD patients,¹⁵⁵ but if on one hand it seems clear that A β requires the presence of tau to induce neurodegeneration, on the other hand the existence of several taupathies (different from AD) that lack the A β component, suggested tau as a new tangible therapeutic target in neurodegenerative diseases.¹⁵⁶

Tau phosphorylation is regulated by a fine equilibrium between the activities of tau kinases and phosphatases; a disruption of the physiological stability toward the former ones, leads to a hyperphosphorylation of tau that decreases its flexibility and its affinity for microtubules determining the formation of NTFs and subsequently the neurodegeneration. Among the numerous kinases implicated in this post-translational control of tau, one of the most important and relevant is the glycogen synthase kinase-3 β (GSK-3 β).

GSK-3 β , together with GSK-3 α , represents one of the two GSK-3 isoforms existing in mammals; they are encoded by two different genes and are ubiquitously expressed, however they are not similar in terms of functionality, considering that neither can compensate the deficiency of the other one. Although the exact functional distinction has to be still exactly established, it is clear that their activities are modulated by insulin and Wnt signaling and that they regulate many cellular processes by controlling different signaling pathways; interestingly their dysregulations are involved in the development of cancer, diabetes, AD and bipolar disorders.¹⁵⁷

Recently, several data suggest that these kinases, and especially GSK-3 β , play a pivotal role in the etiopathogenesis of AD, leading to the postulation of the *GSK-3 hypothesis* of AD.¹⁵⁸ More in detail, besides being directly involved in the hyperphosphorylation of tau, GSK-3 β has been proposed also as a molecular linker between A β and tau,¹⁵⁹ and as a player in choline metabolism, having therefore a

¹⁵⁵ Ittner, L. M.; Götz, J. *Nat. Rev. Neurosci.* **2011**, *12*, 67-72.

¹⁵⁶ Himmelstein, D. S.; Ward, S. M.; Lancia, J. K.; Patterson, K. R.; Binder, L. I. *Pharmacol. Ther.* **2012**, *136*, 8-22.

¹⁵⁷ For an overview see the review and the references inside: Beurel, E.; Grieco, S. F.; Jope, R. S. *Pharmacol. Ther.* **2015**, *148*, 114-131.

¹⁵⁸ (a) Hooper, C.; Killick, R.; Lovestone, S. *J. Neurochem.* **2008**, *104*, 1433-1439; (b) Medina, M.; Ávila, J. *Expert Opin. Ther. Targets* **2014**, *18*, 69-77.

¹⁵⁹ Llorens-Martin, M.; Jurado, J.; Hernández, F.; Ávila, J. *Front. Mol. Neurosci.* **2014**, *7*, 46.

role also in the mechanism leading to the alteration of the cholinergic system characteristic of AD.¹⁶⁰ Moreover there is no doubt about an upregulation of GSK-3 β in the brains of AD patients, highlighting the undoubted participation of this kinase in the progression of this neurodegenerative process.¹⁶¹ In addition, it has been also shown that GSK-3 β is a key mediator of apoptosis, taking part therefore in the mechanism involved in the neuronal loss in AD.¹⁶² For these reasons, in the last years, GSK-3 β has emerged as a promising therapeutic target against AD and various taupathies.¹⁶³

6.1.3 Oxidative damage in AD and the Nrf2-ARE pathway

In recent years an increasing body of evidence indicates that oxidative stress, besides increasing with the age, is clearly involved in the pathogenesis and evolution of a number of neurological disorders.¹⁶⁴ This phenomenon arises from an imbalance between an excessive production of reactive oxygen and nitrogen species (ROS and RNS respectively) and antioxidant defenses. Although ROS generation is a physiological event that takes part in the normal functioning of the cytosolic signaling system, it has to be strictly controlled because an abnormally increased ROS production leads to irreparable cellular damages such as lipid peroxidation and to macromolecular oxidation processes involving proteins and nucleic acids. These phenomena are even more harmful in the brain, where the elevated energy demand, the high oxygen consumption, the high content of redox-active transition metal ions such as Fe²⁺ and Cu⁺ (that exacerbate ROS production via the Fenton reaction)¹⁶⁵ and the relative lack of antioxidant enzymes, make its tissues highly susceptible to the oxidative imbalances and extremely prone to

¹⁶⁰ Samadi, A.; Valderas, C.; de los Ríos, C.; Bastida, A.; Chioua, M.; González-Lafuente, L.; Colmena, I.; Gandía, L.; Romero, A.; del Barrio, L.; Martín-de-Saavedra, M. D.; López, M. G.; Villarroya, M.; Marco-Contelles, J. *Bioorg. Med. Chem.* **2011**, *19*, 122-133.

¹⁶¹ Leroy, K.; Yilmaz, S.; Brion, J. P. *Neuropathol. Appl. Neurobiol.* **2007**, *33*, 43-55.

¹⁶² Mines, M. A.; Beurel, E.; Jope, R. S. *Int. J. Alzheimers Dis.* **2011**, *2011*, 861072.

¹⁶³ Martín, L.; Latypova, X.; Wilson, C. M.; Magnaudeix, A.; Perrin, M.-L.; Yardina, C.; Terro, F. *Ageing Res. Rev.* **2013**, *12*, 289-309.

¹⁶⁴ (a) Kume, T.; Katsuki, H.; Akaike, A. *Biol. Pharm. Bull.* **2004**, *27*, 964-967; (b) Chinopoulos, C.; Adam-Vizi, V. *FEBS J.* **2006**, *273*, 433-450; (c) Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787-795; (d) Yan, M. H.; Wang, X.; Zhu, X. *Free Radic. Biol. Med.* **2013**, *62*, 90-101.

¹⁶⁵ Carin, T.; Mackey, M. M.; Diaz, A. A.; Cox, D. P. *Redox Report* **2009**, *14*, 102-108.

permanent damages.¹⁶⁶ In particular, it has been demonstrated that the oxidative stress derived from the initiation and the abnormal propagation of ROS generation plays a major role in the pathogenesis and the progression of AD.¹⁶⁷ Moreover, in recent years, some studies suggest that the oxidative stress is not only a consequence of the primary AD cascade of events, but could be also involved in the initial onset of AD,¹⁶⁸ even in the preliminary phase known as mild cognitive impairment (MCI), when the senile plaques and the neurofibrillary tangles are not yet so evident.¹⁶⁹ Another interesting evidence is that oxidative stress and A β seem to be linked in a downward spiral, with oxidative stress exacerbating the A β toxicity while A β increases oxidative stress.¹⁷⁰

To counteract the harmful effects of ROS/RNS and stabilize the redox imbalance generated in the oxidative stress, cells employ an endogenous antioxidant defense system composed of numerous detoxifying enzymes. The nuclear factor erythroid 2-related factor (Nrf2)/antioxidant response element (ARE) transcriptional pathway represents the primary sensor to oxidative stress and the most important mechanism to reduce the detrimental effects of oxidant species.¹⁷¹ Nrf2 is a cap'n'collar (CNC) basic-region leucine zipper transcription factor that, by

¹⁶⁶ (a) Chong, Z. Z.; Li, F.; Maiese, K. *Prog. Neurobiol.* **2005**, *75*, 207-246; (b) Dasuri, K.; Zhang, L., Keller, J. N. *Free Radic. Biol. Med.* **2012**, *62*, 170-185.

¹⁶⁷ (a) Smith C. D.; Carney J. M.; Starke-Reed P. E.; Oliver, C. N.; Stadtman, E. R.; Floyd, R. A.; Markesbery, W. R. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10540-10543; (b) Smith, M. A.; Perry, G.; Richey, P. L.; Sayrec, L. M.; Anderson, V. E.; Beal, M. F.; Kowall, N. *Nature* **1996**, *382*, 120-121; (b) Christen, Y. *Am. J. Clin. Nutr.* **2000**, *71*(suppl), 621S-629S; (b) Sultana, R.; Butterfield D. A. *J. Alzheimers Dis.* **2010**, *19*, 341-353;

¹⁶⁸ (a) Nonomura, A.; Perry, G.; Aliev, G.; Hirai, K.; Takeda, A.; Balraj, E. K.; Jones, P. K.; Smith, H. *J. Neuropathol. Exp. Neurol.* **2001**, *60*, 759-767; (b) Pratico, D.; Sung, S. *J. Alzheimers Dis.* **2004**, *6*, 171-175; (c) Zhu, X.; Su, B.; Wang, X.; Smith, M. A.; Perry, G. *Cell. Mol. Life Sci.* **2007**, *64*, 2202-2210.

¹⁶⁹ Pratico, D.; Clark, C. M.; Liun, F.; Rokach, J.; Lee, V. Y.; Trojanowski, J. Q. *Arch. Neurol.* **2002**, *59*, 972-976.

¹⁷⁰ (a) Varadarajan, S.; Yatin, S.; Aksenova, M.; Butterfield, D. A. *J. Struct. Biol.* **2000**, *130*, 184-208; (b) Butterfield, D. A.; Drake, J.; Pocernich, C.; Castegna, A. *Trends Mol. Med.* **2001**, *7*, 548-554; (c) Coma, M.; Guix, F. X.; Ill-Raga, G.; Uribealago, I.; Alameda, F.; Valverde, M. A.; Munoz, F. *J. Neurobiol. Aging* **2008**, *29*, 969-980; (d) Kanamaru, T.; Kamimura, N.; Yokota, T.; Iuchi, K.; Nishimaki, K.; Takami, S.; Akashiba, H.; Shitaka, Y.; Katsura, K.-I.; Kimura, K.; Ohta, S. *Neurosci. Lett.* **2015**, *587*, 126-131.

¹⁷¹ (a) Itoh, K.; Chiba, T.; Takahashi, S.; Ishii, T.; Igarashi, K.; Katoh, Y.; Oyake, T.; Hayashi, N.; Satoh, K.; Hatayama, I.; Yamamoto, M.; Nabeshima, Y. *Biochem. Biophys. Res. Comm.* **1997**, *236*, 313-322; (b) Motohashi, H.; Yamamoto, M. *Trends Mol. Med.* **2004**, *10*, 549-557.

binding to ARE (an enhancer element) in the nucleus, promotes the transcription of detoxifying phase II enzymes such as NADPH quinone oxidoreductase 1 (NQO-1), glutathione peroxidase (GPX), glutathione transferase (GST), catalase, superoxide dismutase (SOD), N-acyltransferase (NAT) and heme oxygenase 1 (HO-1).¹⁷²

Under basal condition, Nrf2 is located in the cytoplasm where it is negatively controlled by the kelch-like ECH-associated protein 1 (Keap1) that prevents Nrf2 translocation to the nucleus and promotes its rapid degradation (half life \approx 20 min) by the ubiquitin proteasome system (UPS).¹⁷³ Under conditions of oxidative stress such as the presence of ROS, reactive electrophiles or ARE inducers, some highly reactive cysteine residues of Keap1 are alkylated or oxidized to cystine, generating a conformational change in Keap1 leading to the release of Nrf2 and to its rapid translocation to the nucleus. Once in the nucleus, Nrf2 forms a heterodimer with its partner small Maf protein and then binds to ARE in the promoter region of antioxidant genes, initiating their transcription (Figure 6.1).¹⁷⁴

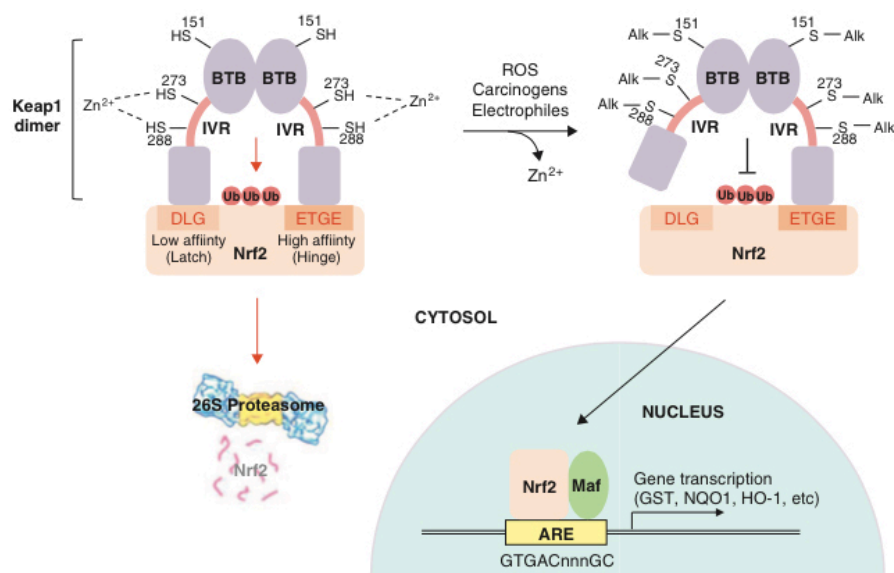


Figure 6.1: Activation mechanism of Nrf2/ARE pathway

¹⁷² Zhang, M.; An, C.; Gao, Y.; Leak, R. K.; Chen, J.; Zhang, F. *Prog. Neurobiol.* **2013**, *100*, 30-47.

¹⁷³ Itoh, K.; Wakabayashi, N.; Katoh, Y.; Ishii, T.; O'Connor, T.; Yamamoto, M. *Genes Cells* **2003**, *8*, 379-391.

¹⁷⁴ Hur, W.; Gray, N. *Curr. Opin. Chem. Biol.* **2011**, *15*, 162-173.

6.1.4 Interference with the Nrf2-ARE pathway: A new potential therapeutic approach to AD treatment?

In view of the tight connection between oxidative stress and the pathogenesis of AD, in recent years the Nrf2-ARE pathway has been extensively studied to evaluate if its eventual dysregulation could be involved in the evolution of this neurodegenerative disease.

An interesting study published by Ramsey *et al* in 2007 revealed that, differently from normal age-matched controls, in hippocampal neurons of AD patients, Nrf2 is predominantly cytoplasmic without being translocated into the nucleus. Interestingly, it was also demonstrated that there was no difference between the cytoplasmic levels of Nrf2 in AD patients and the age-matched control cases, suggesting that the nuclear deficit in AD was not a result of a generalized loss of Nrf2, but just a disruption of the mechanism of nuclear localization.¹⁷⁵ Thus this study suggests that the Nrf2/ARE pathway is impaired in AD and that could be involved in the progression of the pathology.

In this context, the role of Nrf2/ARE pathway as a valuable pharmacological target for the treatment of AD has been investigated.¹⁷⁶ Recent *in vitro* studies have shown that the activation of Nrf2 has a protective effect against the toxicity induced by A β ;¹⁷⁷ furthermore, an *in vivo* experiment of an intrahippocampal release of the gene expressing Nrf2 has shown cognitive improvement in a mouse model of AD.¹⁷⁸ In another recent and very interesting study it was also shown that the Nrf2 pathway takes part also in the reduction of levels of phosphorylated tau by inducing a mechanism of autophagy in neurons, suggesting therefore an

¹⁷⁵ Ramsey, C. P.; Glass, C. A.; Montgomery, M. B.; Lindl, K. A.; Ritson, G. P.; Chia, L. A.; Hamilton, R. L.; Chu, C. T.; Jordan-Sciutto, K. L. *J. Neuropathol. Exp. Neurol.* **2007**, *66*, 75-85.

¹⁷⁶ Gan, L.; Johnson, J. A. *Biochim. Biophys. Acta* **2014**, *1842*, 1208-1218.

¹⁷⁷ (a) Kanninen, K.; Malm, T. M.; Jyrkkanen, H. K.; Goldsteins, G.; Keksa-Goldsteine, V.; Tanila, H.; Yamamoto, M.; Yla-Herttuala, S.; Levonen, A. L.; Koistinaho, J. *Mol. Cell Neurosci.* **2008**, *39*, 302-313; (b) Lee, C.; Park, G. H.; Lee, S. R.; Jang, J. H. *Oxid. Med. Cell. Longev.* **2013**, *2013*, 313510; (c) Karkkainen, V.; Pomeschchik, Y.; Savchenko, E.; Dhungana, H.; Kurronen, A.; Lehtonen, S.; Naumenko, N.; Tavi, P.; Levonen, A.-L.; Yamamoto, M.; Malm, T.; Magga, J.; Kanninen, K. M.; Koistinaho, J. *Stem cells* **2014**, *32*, 1904-1916.

¹⁷⁸ Kanninen, K.; Heikkinen, R.; Malm, T.; Rolova, T.; Kuhmonen, S.; Leinonen, H.; Yla-Herttuala, S.; Tanila, H.; Levonen, A.-L.; Koistinaho, M.; Koistinaho, J. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16505-16510

additional protective role against tau-induced toxicity.¹⁷⁹

6.1.5 Linking the Nrf2-ARE pathway to the activity of glycogen synthase kinase-3β in AD

GSK-3β is a ubiquitous enzyme with prevalent brain distribution that has a critical central role in various signaling pathways¹⁸⁰ and, as already mentioned in [Paragraph 6.1.2](#), its dysregulation is associated with multiple neuropathological mechanisms involved in AD. Interestingly, in the last decade it has been demonstrated that GSK-3β is also implicated in the regulation of Nrf2, thereby underscoring another possible connection with the pathological pathways leading to the AD.

The activity of GSK-3β regulates in a negative manner the subcellular distribution of Nrf2, by promoting its cytosolic distribution through two different mechanism of control: (a) by fostering Nrf2 exclusion from the nucleus or (b) by preventing the translocation of Nrf2 to the nucleus. In a model of prolonged oxidative stress, that can simulate the pathological conditions in AD, the activation of GSK-3β results in the extrusion of Nrf2 from the nucleus; conversely, the inhibition of GSK-3β reverts the situation and promotes the nucleus accumulation of Nrf2 and the activation of the ARE transcription.¹⁸¹ Although the exclusion of Nrf2 from the nucleus was initially supposed to depend on its direct phosphorylation by GSK-3β,¹⁸² it was later demonstrated that the Nrf2 phosphorylation is actually mediated by the tyrosine kinase Fyn that is, in turn, activated by GSK-3β.¹⁸³ A very recent study has revealed that GSK-3β prevents the Nrf2 translocation to the nucleus also by phosphorylating Nrf2 when is located in the cytoplasm; in this

¹⁷⁹ Jo, C.; Gundemir, S.; Pritchard, S.; Jin, Y. N.; Rahman, I.; Johnson, G. V. W. *Nat. Commun.* **2014**, *5*, 3496.

¹⁸⁰ Grimes, C. A.; Jope, R. S. *Prog. Neurobiol.* **2001**, *65*, 391-426.

¹⁸¹ (a) Rojo, A. I.; de Sagarra, M. R.; Cuadrado, A. *J. Neurochem.* **2008**, *105*, 192-202; (b) Rojo, A. I.; Rada, P.; Egea, J.; Rosa, A. O.; López, M. G.; Cuadrado, A. *Mol. Cell. Neurosci.* **2008**, *39*, 125-132.

¹⁸² Salazar, M.; Rojo, A. I.; Velasco, D.; De Sagarra, R. M.; Cuadrado, A. *J. Biol. Chem.* **2006**, *281*, 14841-14851.

¹⁸³ (a) Jain, A. K.; Jaiswal, A. K. *J. Biol. Chem.* **2006**, *281*, 12132-12142; (b) Jain, A. K.; Jaiswal, A. K. *J. Biol. Chem.* **2007**, *282*, 16502-16510.

case the phosphorylation takes place on a degron of Nrf2, promoting its degradation by the proteasome.¹⁸⁴

The analysis of these data suggests, therefore, that an increased activation of GSK-3 β results in a drop of the protective effects promoted by the Nrf2- ARE pathway; consequently the inhibition of GSK-3 β could potentially represent a useful therapeutic strategy to restore the protective cellular response to the oxidative damage.

6.2. GLYCOGEN SYNTHASE KINASE-3 β INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

The increasing evidence that an overactivation of GSK-3 β has a role in various pathological mechanisms involved in the progression of the AD, together with the consideration that our organism is not able to downregulate this kinase, has generated a lot of interest in the search for molecules that could have a therapeutic effect by downregulating GSK-3 β and thus restoring its functionality to the physiological one.¹⁸⁵ The validation of this theory seems to be supported by the promising results obtained in various animal AD models where the use of GSK-3 β inhibitors or the specific genetic knockdown of this kinase lead to an improvement of the cognitive impairments and to an attenuation of the neuropathology in the animal models. Moreover, these auspicious data in mouse models were supported by the evidence that epidemiological studies in human patients with bipolar disorders treated with lithium (the first GSK-3 β inhibitor discovered),¹⁸⁶ showed a reduced tendency to develop AD and a reduced rate of dementia if compared with bipolar patients treated with different drugs. Nevertheless, to date, apart from lithium, only a limited number of GSK-3 β inhibitors has reached the clinical phase, and thus the development of new GSK-3 β inhibitors represents a very attractive goal in the treatment of AD.¹⁸⁷

¹⁸⁴ Chowdhry, S.; Zhang, Y.; McMahon, M.; Sutherland, C.; Cuadrado, A.; Hayes, J. D. *Oncogene* **2013**, *32*, 3765-3781.

¹⁸⁵ Martínez, A.; Gil, C.; Pérez, D. I. *Int. J. Alzheimers Dis.* **2011**, *2011*, 280502.

¹⁸⁶ Ryves, W. J.; Harwood A. J. *Biochem. Biophys. Res. Comm.* **2001**, *280*, 720-725.

¹⁸⁷ (a) Arfeen, M.; Bharatam, P. V. *Curr. Pharm. Des.* **2013**, *19*, 4755-4775; (b) King, M. K.; Pardo, M.; Cheng, Y.; Downey, K.; Jope, R. S.; Beurel, E. *Pharmacol. Ther.* **2014**, *141*, 1-12.

We can classify the various typologies of GSK-3 β inhibitors in different categories, depending on the inhibition mechanism: (a) inhibition by metal (b) inhibition at the ATP binding site, (c) inhibition at the non-ATP binding site, (d) peptide like inhibitors. The inhibition by metal is strictly related to the competition between the metal and the Mg²⁺ ions that in many kinases are responsible for the binding of ATP in its pocket. Lithium represents the best-known inhibitor of this category and its activity depends also on an indirect increase in the phosphorylation at Ser9.

The ATP competitive inhibitors are surely the most extensively studied and are usually organized in two generations: the first one is composed prevalently by natural compounds like paullone¹⁸⁸ and staurosporine¹⁸⁹ (among others); on the other hand, the second generation is prevalently formed by small synthetic molecules resulting from numerous studies addressed to find, beyond inhibitory activity, also high selectivity for GSK-3 β over other kinases. Many inhibitors belonging to the second generation show common structural features such as aryl-substituted heterocyclic cores like heteroaryl-pyrazolo[3,4-*b*]pyridine,¹⁹⁰ 3-(7-azaindoly)-4-arylmaleimides¹⁹¹, [1,3,4]-triazole,¹⁹² heteroarylpyrazine,¹⁹³ dipyrrolo-fuopyrrolopyrazinones¹⁹⁴ derivatives (Figure 6.2).

¹⁸⁸ Leost, M.; Schultz, C.; Link, A.; Wu, Y.-Z.; Biernat, J.; Mandelkow, E.-M.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Zaharevitz, D. W.; Gussio, R.; Senderowicz, A. M.; Sausville, E. A.; Kunick, C.; Meijer, L. *Eur. J. Biochem.* **2000**, *267*, 5983-5994.

¹⁸⁹ Toledo, L. M.; Lydon, N. B. *Structure* **1997**, *5*, 1551-1556.

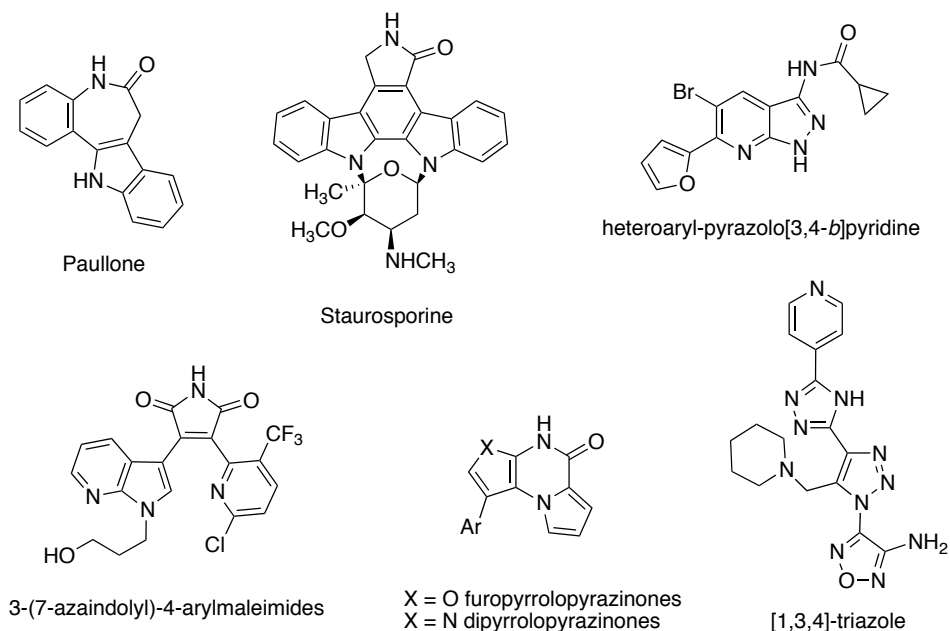
¹⁹⁰ Witherington, J.; Bordas, V.; Gaiba, A.; Naylor, A.; Rawlings, A. D.; Slingsby, B. P.; Smith, D. G.; Takle, A. K.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3059-3062.

¹⁹¹ Zhang, H.-C.; Ye, H.; Conway, B. R.; Derian, C. K.; Addo, M. F.; Kuo, G.-H.; Hecker, L. R.; Croll, D. R.; Li, J.; Westover, L.; Xu, J. Z.; Look, R.; Demarest, K. T.; Andrade-Gordon, P.; Damiano, B. P.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3245-3250.

¹⁹² Olesen, P. H.; Sorensen, A. R.; Urso, B.; Kurtzhals, P.; Bowler, A. N.; Ehrbar, U.; Hansen, B. F. *J. Med. Chem.* **2003**, *46*, 3333-3341.

¹⁹³ Berg, S.; Bergh, M.; Hellberg, S.; Högdin, K.; Lo-Alfredsson, Y.; Söderman, P.; von Berg, S.; Weigelt, T.; Ormö, M.; Xue, Y.; Tucker, J.; Neelissen, J.; Jerning, E.; Nilsson, Y.; Bhat, R. *J. Med. Chem.* **2012**, *55*, 9107-9119

¹⁹⁴ Rochais, C.; Duc, N. V.; Lescot, E.; Sopkova-de Oliveira Santos, J.; Bureau, R.; Meijer, L.; Dallemagne, P.; Rault, S. *Eur. J. Med. Chem.* **2009**, *44*, 708-716.

Figure 6.2: Examples of ATP competitive inhibitors of GSK-3 β

The catalytic triad (see later) seems to be another valuable target for the inhibition of GSK-3 β ; inhibitors acting at this site are not ATP-competitive and prevent the proper orientation of the substrate in the substrate-binding site. To date a set of thiadiazole derivatives are the only reported inhibitors (Figure 6.3A),¹⁹⁵ but the postulation of this kind of inhibition still needs to be verified.

Peptide-like inhibitors also compete with substrate preventing its binding in catalytic site of the GSK-3 β (Figure 6.3B). In the last decade some small peptides have shown interesting preclinical result, although their poor pharmacokinetic characteristics make their pharmacological development problematic.¹⁹⁶

¹⁹⁵ (a) Martínez, A.; Alonso, M.; Castro, A.; Pérez, C.; Moreno, F. J. *J. Med. Chem.* **2002**, *45*, 1292-1299; (b) Palomo, V.; Pérez, D. I.; Pérez, C.; Morales-García, J. A.; Soteras, I.; Alonso-Gil, S.; Encians, A.; Castro, A.; Campillo, N. E.; Pérez-Castillo, A.; Gil, C.; Martínez, A. *J. Med. Chem.* **2012**, *55*, 1645-1661.

¹⁹⁶ (a) Plotkin, B.; Kaidanovich, O.; Talior, I.; Eldar-Finkelman, H. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 974-980; (b) Eldar-Finkelman, H.; Eisenstein, M. *Curr. Pharm. Des.* **2009**, *15*, 2463-2470.

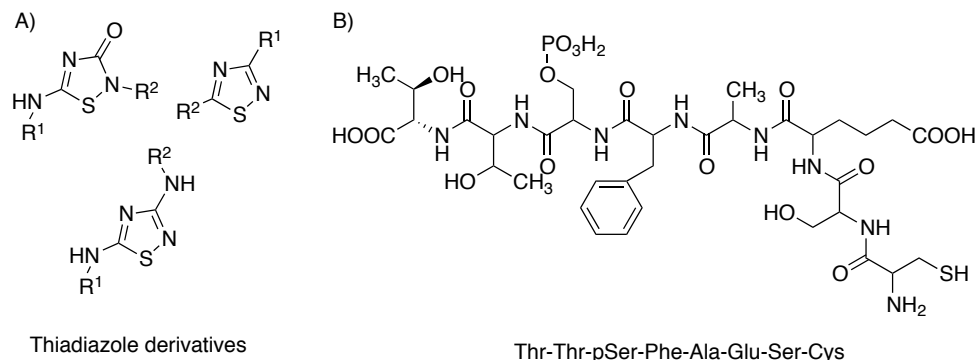


Figure 6.3: Thiadiazoles not ATP-competitive inhibitors of GSK-3 β (A) and peptide-like inhibitors of GSK-3 β (B)

Thus we decided to design a new family of compounds that shares some structural features with some known GSK-3 β inhibitors such as PP1-PP2¹⁹⁷ or PP3¹⁹⁸ and whose synthesis could be achieved via a multicomponent process (Figure 6.4).

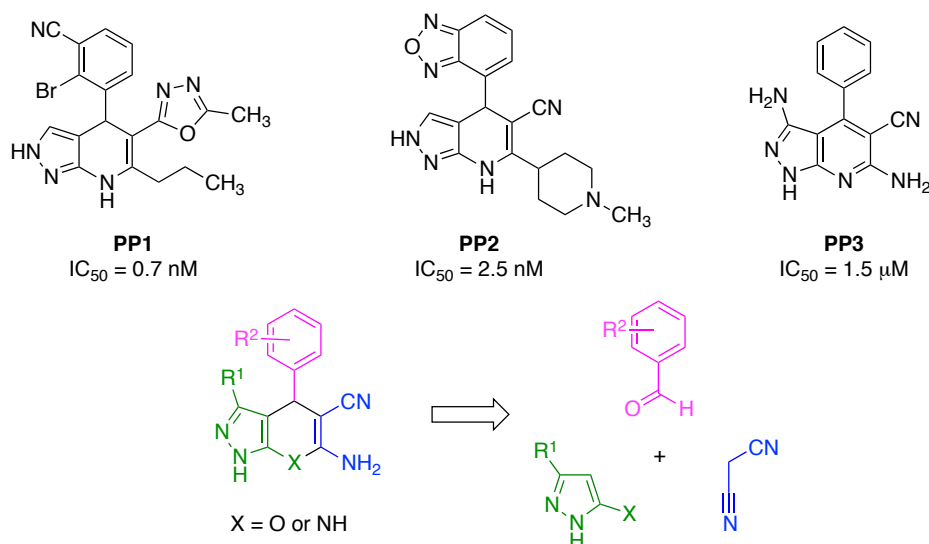


Figure 6.4: Design of compounds sharing structural features with some known GSK-3 β inhibitors

¹⁹⁷ (a) Kim, H.-J.; Choo, H.; Cho, Y. S.; No, K. T.; Pae, A. N. *Bioorg. Med. Chem.* **2008**, *16*, 636-643; (b) Kohara, T.; Fukunaga, K.; Masatake, F.; Hanano, T.; Okabe, H. Patent U.S. 6977262B2.

¹⁹⁸ Chioua, M.; Samadi, A.; Soriano, E.; Lozach, O.; Meijer, L.; Marco-Contolles, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4566-4569.

In an attempt to achieve a better understanding of the possible interactions between our compounds and the residues located at the catalytic site of GSK-3 β , we carried out also some molecular modeling studies. Before going into the details of the computationally-driven approach employed for the calculation of the affinity between our compounds and the binding site of GSK-3 β , hereunder is reported a brief description of the structure of GSK-3 β and of its regulation mechanism.

In the last decade, the elucidation of the crystal structure of GSK-3 β has allowed the use of structure-based drug design strategies for predicting the behavior of newly designed inhibitors of the kinase. GSK-3 β is a homodimer and each monomer is composed of a small N-terminal lobe, formed prevalently of β -sheets, and of a C-terminal lobe constituted of α -helices and loops. Regarding the possible interaction with ligands, there are two interesting pockets: the catalytic domain (the ATP binding site), located between the two lobes and bordered by the glycine-rich loop, and a pocket formed by a catalytic triad (Arg96, Arg180 and Lys205), situated on the interface of the two lobes, in the activation loop of the kinase (residues 200-226) (Figure 6.5).¹⁹⁹

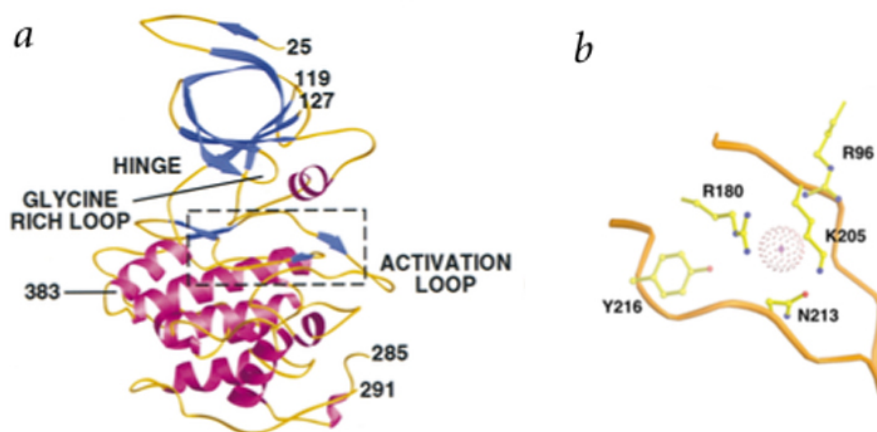


Figure 6.5: Ribbon representation of GSK-3 β (a) and the pocket formed by the catalytic triad (b)

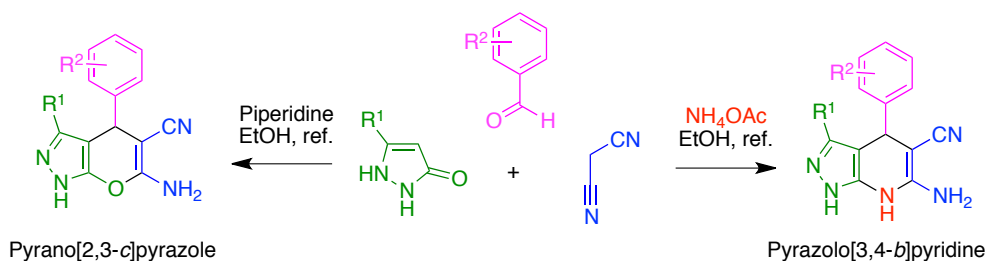
¹⁹⁹ (a) Dajani, R.; Frase, E.; Roe, S. M.; Young, N.; Good, V.; Dale, T. C.; Pearl, L. H. *Cell* **2001**, *105*, 721-732; (b) ter Haar, E.; Coll, J. T.; Austen, D. A.; Hsiao, H.-M.; Swenson, L.; Jain, J. *Nat. Struct. Biol.* **2001**, *8*, 593-596.

Regarding the functionality of GSK-3 β , there are two phosphorylation sites that regulate its activity: (a) Ser9, located at the N-terminal domain; when this residue is phosphorylated by Akt/PKB, the protein becomes inactivated due to blocked access of the substrate to the catalytic domain. (b) Tyr216, located in the activation loop, that moves out the binding site when phosphorylated, allowing the entrance of the substrate and increasing the activity of GSK-3 β by >200-fold.

6.3 SYNTHESIS OF A LIBRARY OF 1,4-DIHYDROPYRANO[2,3-*c*]PYRAZOLE DERIVATIVES

6.3.1 Initial study of the reaction

The proposed retrosynthetic approach relied on the reaction between an aromatic aldehyde, malononitrile and a pyrazolone to build a pyridine ring. Interestingly, an article published in 2010 by Mohammed and coworkers reported a multicomponent approach to our target pyrazolopyridine derivatives based on a protocol that, depending on the reaction conditions, led either to the pyranopyrazole or to the pyrazolopyridine nucleus in chemodivergent fashion (Scheme 6.1).²⁰⁰ This approach was attractive for our purposes, since it allowed generating greater molecular diversity in an easy way.

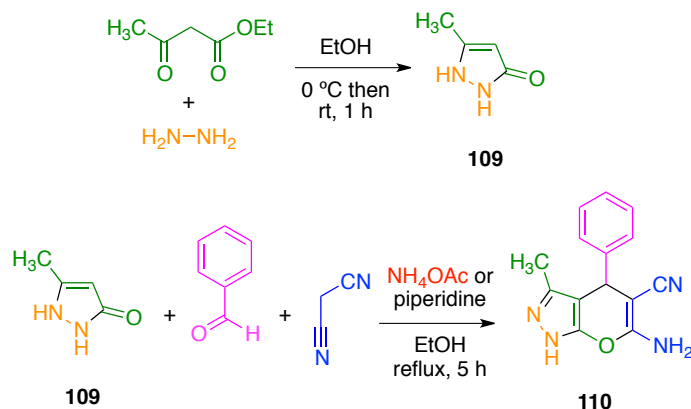


Scheme 6.1: The multicomponent approach to pyrazolopyridine reported by Mohammed²⁰⁰

Thus we started the study of the reaction employing 5-methylpyrazol-3-one (previously synthesized by condensation between hydrazine and ethyl acetoacetate), benzaldehyde and malononitrile as starting materials. We carried out the reaction under both conditions described in the published procedure in order to get the two target families of compounds, but unexpectedly, in both cases the reaction afforded the same product. After a spectroscopic analysis and a comparison with data described in literature,²⁰¹ we could confirm that the product obtained was the pyranopyrazole **110** (Scheme 6.2).

²⁰⁰ Mohamed, N. R.; Khairaldin, N. Y.; Fahmy, A. F.; El-Sayed, A. A. *Der Pharma Chem.* **2010**, *2*, 400-417.

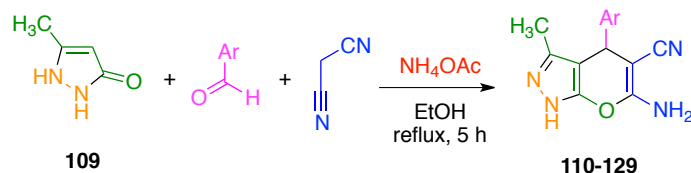
²⁰¹ Lehmann, F.; Holm, M.; Laufer, S. J. *Comb. Chem.* **2008**, *10*, 364-367.



Scheme 6.2: Initial study of the three-component reaction

6.3.2 Scope of the reaction

As this kind of product represented one of our target molecules, we decided anyway to explore the scope of this reaction employing a variety of aromatic aldehydes that allowed structural variations at C-4. As in the initial model experiments, the use of ammonium acetate allowed a good yield (86%), better than the one obtained by using piperidine (64%) and, considering that this multicomponent protocol was already known in the literature, we decided to explore the scope of the reaction without any further optimization studies (Scheme 6.3 and Table 6.1).

Scheme 6.3: Scope of the three-component synthesis of pyrano[2,3-c]pyrazoles **110-129**

As shown in the Table 6.1, the reaction normally proceeded in good to excellent yields, tolerating well the presence of either electron-withdrawing or electron-releasing groups at the aldehyde aromatic ring.

Table 6.1. Yield and scope for the synthesis of pyrano[2,3-c]pyrazoles **110-129**

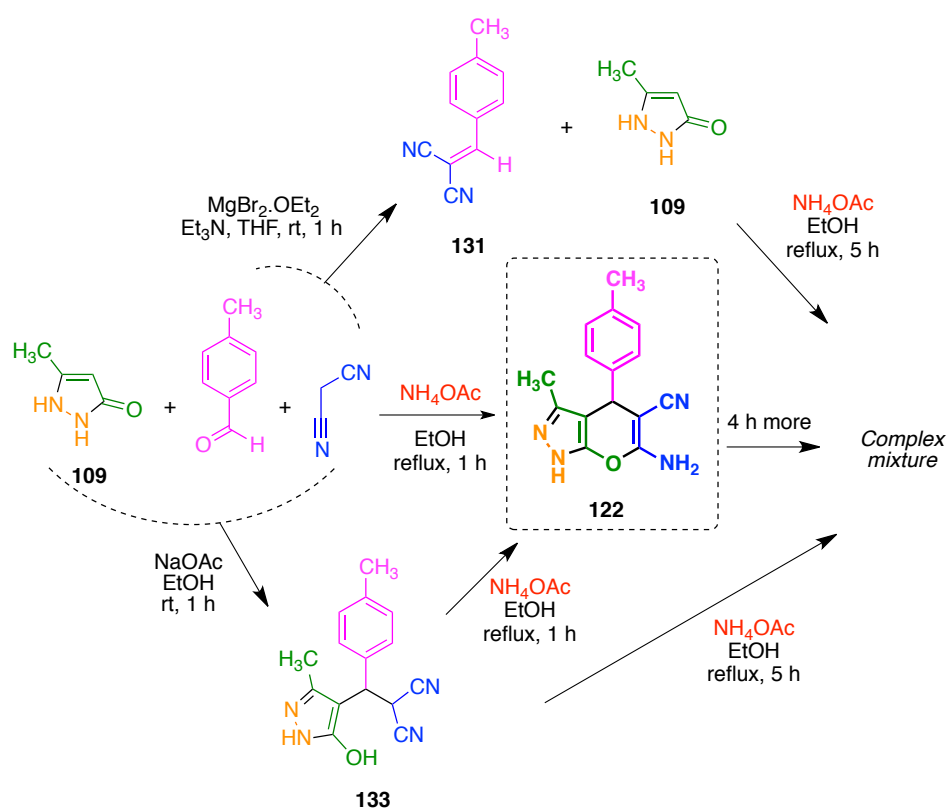
Entry	Cmpd.	Ar	Yield (%)	Entry	Cmpd.	Ar	Yield (%)
1	110	C ₆ H ₅	86	11	120	2-MeC ₆ H ₄	88
2	111	2-ClC ₆ H ₄	85	12	121	3-MeC ₆ H ₄	84
3	112	3-ClC ₆ H ₄	77	13	122	4-MeC ₆ H ₄	81 ^a
4	113	4-ClC ₆ H ₄	88	14	123	2-MeOC ₆ H ₄	87
5	114	2-BrC ₆ H ₄	82	15	124	3-MeOC ₆ H ₄	83
6	115	4-BrC ₆ H ₄	92	16	125	4-MeOC ₆ H ₄	71
7	116	4-FC ₆ H ₄	73	17	126	3-pyridyl	85
8	117	2-NO ₂ C ₆ H ₄	94	18	127	4-pyridyl	84
9	118	3-NO ₂ C ₆ H ₄	91	19	128	2-furyl	83
10	119	4-NO ₂ C ₆ H ₄	93	20	129	2-thienyl	78

^a Reaction time: 1h

As shown in the **Table 6.1**, the reaction normally proceeded in good to excellent yields, tolerating well the presence of either electron-withdrawing or electron-releasing groups at the aldehyde aromatic ring. Under the standard reaction conditions, the reaction with 4-methylbenzaldehyde initially did not afford the expected product **122**, but only a complex mixture of compounds that we were not able to purify. Thus, in an attempt to better understand the reason for this different conduct, we decided to investigate the reaction involving 4-methylbenzaldehyde in more detail (**Scheme 6.4**). In particular, we initially simplified the protocol by forming the previously the Knoevenagel adduct **131** between the aldehyde and malononitrile and submitting this compound to the subsequent reaction with pyrazolone **109**. Also in this case, our standard conditions led to the same complex mixture obtained in the multicomponent process. We decided therefore to go ahead in this study by resorting to a further simplification of the protocol and we decided therefore to examine the cyclization process of the proposed intermediate **133**, synthesized according to a literature method.²⁰² Interestingly, when we studied the ring closure of this intermediate under our standard conditions, we realized that the formation of the expected product was very fast,

²⁰² Elinson, M. N.; Nasybullin, R. F.; Nikishin, G. I. *C. R. Chim.* **2013**, *16*, 789-794.

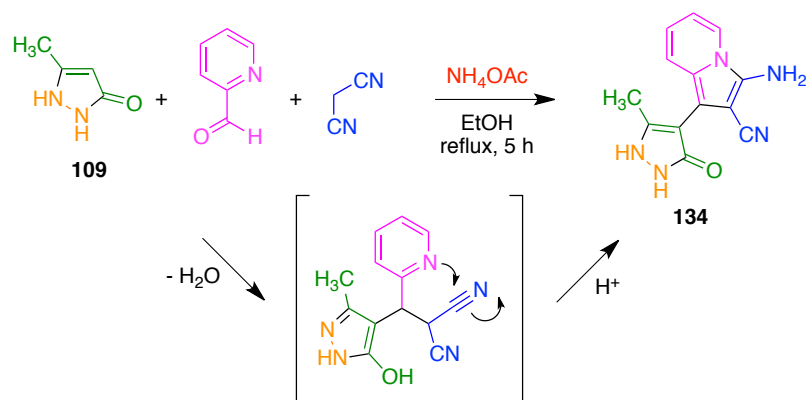
being complete in 1 hour, while a complex mixture of compounds was obtained when the cyclization process of **133** was performed under our conditions for 5 hours. In view of this outcome, we went back to repeat our three-component reaction employing a shorter time and we were pleased to notice that, by this simple modification, our protocol allowed the formation of the expected product **122** (entry 13).



Scheme 6.4: Study of the reaction involving 4-methylbenzaldehyde

To introduce further structural diversity in the 1,4-dihydropyrano[2,3-*c*]pyrazole derivatives, we synthesized also compounds bearing heterocyclic moieties at the C-4 position (compounds **126-129**, entries 17-20). Interestingly, the reaction involving pyridinecarboxaldehydes afforded two different kinds of products depending on the nitrogen position in the aromatic ring of the aldehyde. Thus, when 3- and 4-pyridinecarboxaldehydes were used as starting materials, the reaction afforded the corresponding expected 1,4-dihydropyrano[2,3-*c*]pyrazole

(compounds **126** and **127**, entries 17-18); on the other hand, when the reaction was performed employing 2-pyridinecarboxaldehyde, we were isolated a different. After its spectroscopic analysis, we identified its structure as **134**, which ensued from a competitive nucleophilic addition of the pyridine nitrogen onto one of the cyano groups of the dinitrile fragment (Scheme 6.5). We confirmed that this kind of reactivity is congruent with one described in a recent paper published by Li's group where a one-pot multistep synthesis of 3-aminoindolizines is described.²⁰³



Scheme 6.5: Study of the reaction involving 2-pyridinecarboxaldehyde

²⁰³ Li, L.; Chua, W. K. S. *Tetrahedron Lett.* **2011**, 52, 1392-1394.

6.4 MECHANISTIC PROPOSAL

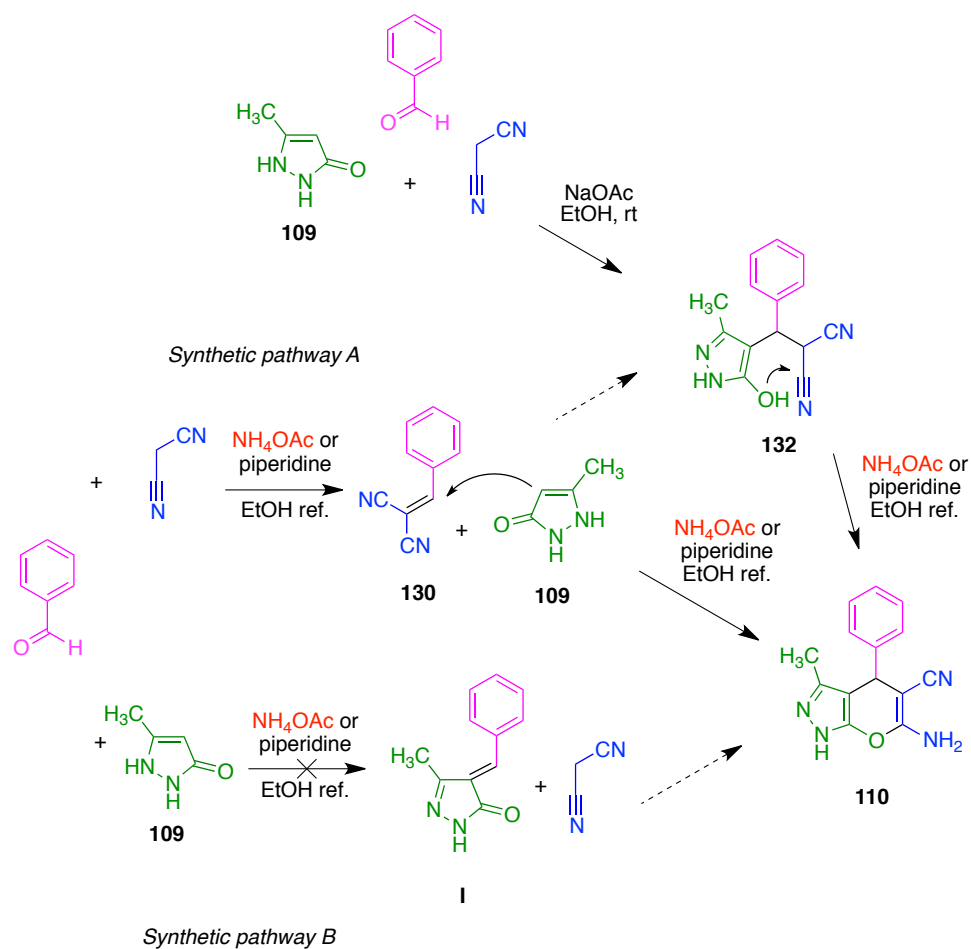
Before undertaking the synthesis of the alternative 4,7-dihydropyrazolo[3,4-*b*]pyridine derivatives, we decide to focus our attention on the reaction mechanism, in an attempt to elucidate the overall synthetic pathway leading to both the fused bicyclic structures, and how to manage it toward the synthesis of the desired compound.

Our starting point in the mechanistic investigation was to consider compound **132** as the immediate precursor for the final ring closure, and therefore we chose to explore two possible sequential synthetic pathways leading to this key intermediate. Our first proposal (Scheme 6.6, pathway A) was based on the sequence of reactions already planned for the study of the synthesis of **122**. Thus, we proceeded to the formation of the Knoevenagel adduct **130** starting from benzaldehyde and malononitrile under two different reaction conditions: in the presence of ammonium acetate in refluxing ethanol (our established conditions) and with piperidine in refluxing ethanol (the other conditions published by Mohammed).²⁰⁰ Both reactions led to the expected intermediate **130** that subsequently was treated with the pyrazolone **109**, under the two above-described conditions. In both cases, the desired product **110** was formed, validating the results obtained in the initial multicomponent study of our protocol (Paragraph 6.3.1, Scheme 6.2). In order to corroborate the viability of this synthetic pathway, we also synthesized intermediate **132**, which was subsequently submitted to the cyclization process under the two experimental conditions previously investigated. Also in this case, both conditions afforded the expected product **110**, confirming the feasibility of the proposed synthetic sequence.

Considering the nucleophilic character of C-4 of pyrazolone **109**, we decided to investigate also its addition to the carbonyl group of the benzaldehyde as the primary event of the multicomponent process (Scheme 6.6, pathway B). Also in this case the reaction between **109** and benzaldehyde was carried out under both the experimental procedures employed in our mechanistic study; however, the formation of the Michael-like compound **I** did not occur in none of our experimental conditions, probably because this kind of reaction requires harsher

conditions (higher temperatures and longer reaction times).²⁰⁴

In view of these results we are prone to consider pathway A as the most probable one for the formation of the 1,4-dihydropyrano[2,3-c]pyrazole nucleus.



Scheme 6.6: Mechanistic study of the three-component reaction

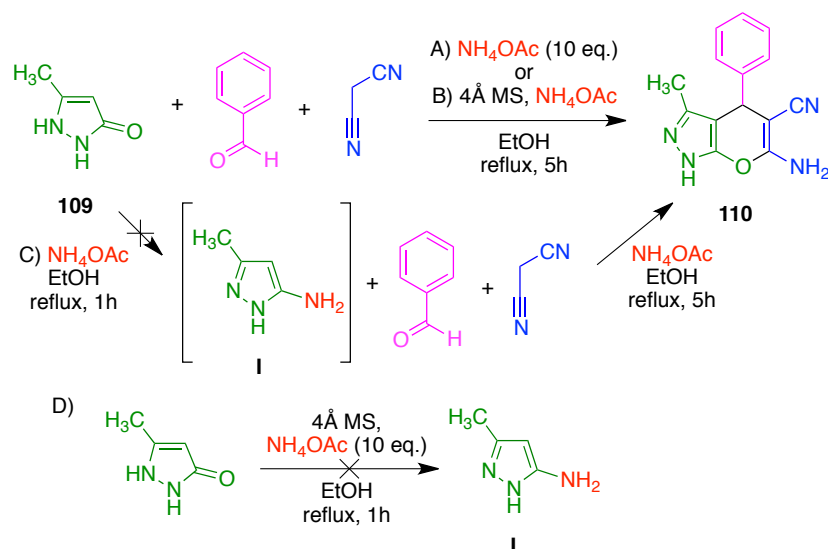
²⁰⁴ Ahmed, M; Sharma, R, Nagda, D. P.; Salvi, V. K.; Talesara, G. L. *E-J. Chem.* **2006**, 3, 28-34.

6.5 SYNTHESIS OF A LIBRARY OF 4,7-DIHYDRO-1H-PYRAZOLO[3,4-*b*]PYRIDINE DERIVATIVES

6.5.1 Initial study of the reaction and optimization

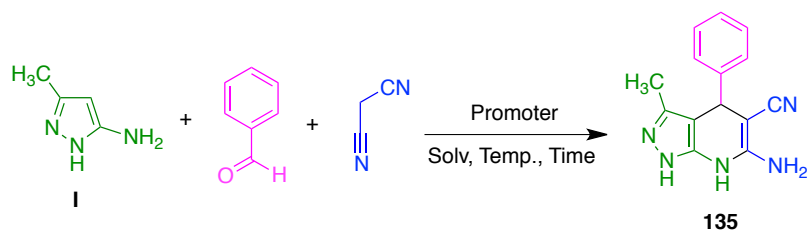
Our next goal was to achieve the multicomponent synthesis of 4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine compounds. We reasoned that the easiest way to construct this bicyclic nucleus, was to convert the pyrazolone **109** into the corresponding pyrazolamine **I**, incorporating in this way the nitrogen required for the formation of the pyridine nucleus. It should be possible to achieve this goal simply by converting the carbonyl group into an enamine by reaction with an ammonia source (Scheme 6.7). This conversion, the same published in the above-mentioned literature precedent,²⁰⁰ would have allowed reaching the attractive goal of chemodivergence, i.e., obtaining different products from the same starting materials under different experimental conditions.

Considering ammonium salts to be the most practical ammonia sources for the conversion of a carbonyl group into an enamine, we decided to explore once again the reaction initially investigated, trying to force the conditions in order to get the desired transformation. Thus, to drive the reaction towards the formation of the nitrogen nucleus, we attempted the following modifications: (a) carrying out the multicomponent process in the presence of a large excess of ammonium acetate (10 equiv., Scheme 6.7, A); (b) performing the reaction in the presence of 4Å molecular sieves to assist the removal of water arising from the reaction between the carbonyl group and the ammonia (Scheme 6.7, B); (c) performing the reaction in a sequential manner (Scheme 6.7, C). Unfortunately all these attempts led again to the formation of the 1,4-dihydropyrano[2,3-*c*]pyrazole derivative **110**, demonstrating how difficult was this apparently easy conversion. Finally, in a last effort to reach the required transformation, we tried to perform a reaction just between the pyrazolone **109** and a large excess of ammonium acetate (10 equiv.), again in the presence of 4Å molecular sieves and in refluxing ethanol (the experimental conditions employed in our multicomponent process); disappointingly, not even in this case we were able to get the target pyrazolamine **I** (Scheme 6.7, D).



Scheme 6.7: Attempts for the conversion of pyrazolone **109** in its corresponding pyrazolamine **I**

In view of these experimental evidences, we decided to move our attention to the direct use of the pyrazolamine **I** as starting material in the multicomponent process with benzaldehyde and malononitrile (Scheme 6.8).



Scheme 6.8: Initial study of the three-component reaction involving the pyrazolamine **I**

As shown in Table 6.2, the first attempts were performed simply by mixing the three starting materials in ethanol at different temperatures; as shown in entries 1-4, heating was necessary to obtain compound **135**, the best option being the use of refluxing ethanol for 16 hours (yield 27%, entry 4). In an attempt to improve the yield of the reaction we began to investigate the effect of different promoters. Initially, as we supposed that the formation of the dihydropyridine ring could be facilitated by a basic medium, we examined the action of different organic bases (entries 5-8) without getting any traces of the desired product. Mindful of the role of ammonium acetate in our precedent protocol, we decided to try the effect of

this salt as promoter. We were pleased to notice that the use of 1.0 equivalent of ammonium acetate could foster the formation of product **135**, even though in a modest yield (18%, entry 9), and interestingly, when the reaction time was increased, a better yield could be achieved (entries 10-11). These results prompted us to study in more depth if the effect of this salt was related to any of its two ions. Thus we performed two reactions employing two salts, each one containing one of the ions of ammonium acetate: ammonium chloride and sodium acetate (entries 12-13). Remarkably these two experiments did not afford the expected product, revealing that the effect of the ammonium acetate was not depending on the nature of its singular ions, but on some other aspect. We thought therefore that the accelerating effect of this salt could be attributed to the in situ formation of acetic acid from partial hydrolysis of the salt. As shown in entries 14 and 15, investigating directly the effect of the acetic acid in the reaction, we were gratified to observe that we could get the formation of the desired product in similar yields to the ones obtained in the same conditions with ammonium acetate (16% and 38% against 18% and 44%). Reducing the amount of acetic acid from 1.0 to 0.25 equivalents was detrimental for the yield, denoting a better result when the promoter was used in stoichiometric quantity (entry 16). The complementary effect of the ammonium and the acetate was confirmed later in the experiment performed mixing two salts containing individually each one of both ions composing ammonium acetate (ammonium chloride and sodium acetate): in this case it was possible to detect the formation of the product **135**, although it was impossible to calculate exactly the yield because of the co-precipitation of the NaCl formed during the purification process, which was not easy to remove owing to the high polarity of our compound (entry 17). Our next interest was focused on investigating other Brønsted acids as promoters: as shown by the entries 18-20, only benzoic acid was able to promote efficiently the reaction leading to the desired product **135** (entry 18). As benzoic acid appeared to afford a better yield than ammonium acetate in a comparable reaction time (5 hours), we tried to use the same promoter for longer times, resulting however in an overall reduction of the yield (compare entries 11 and 21). Also, a reduction in the amount of benzoic acid (from 1.0 to 0.25 equivalent) resulted in a corresponding drop in the yield (entry 22). As ammonium acetate emerged as the best promoter for our protocol, at a later stage we decided to investigate also the effect of the

solvent in the reaction, and ethanol revealed to be the best option (entries 9 and 23-27).

Table 6.2. Optimization study for synthesis of 4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridines

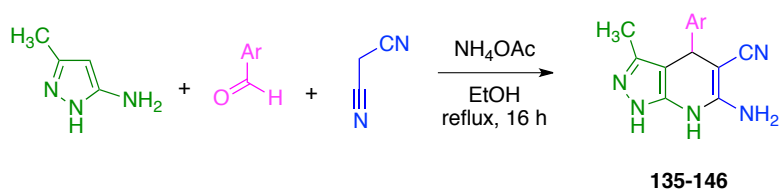
Entry	Solv.	Temp.	Time (h)	Promoter (equiv.)	Yield (%)
1	EtOH	rt	16	-	No
2	EtOH	50 °C	5	-	Trace
3	EtOH	Reflux	5	-	Trace
4	EtOH	Reflux	16	-	27
5	EtOH	Reflux	5	Piper. (1.0)	No
6	EtOH	Reflux	5	Pyr. (1.0)	No
7	EtOH	Reflux	5	Et ₃ N (1.0)	No
8	EtOH	Reflux	5	Et ₂ NH (1.0)	No
9	EtOH	Reflux	5	NH ₄ AcO (1.0)	18
10	EtOH	Reflux	8	NH ₄ AcO (1.0)	24
11	EtOH	Reflux	16	NH ₄ AcO (1.0)	44
12	EtOH	Reflux	5	NH ₄ Cl (1.0)	No
13	EtOH	Reflux	5	NaAcO (1.0)	No
14	EtOH	Reflux	5	AcOH (1.0)	16
15	EtOH	Reflux	16	AcOH (1.0)	38
16	EtOH	Reflux	5	AcOH (0.25)	12
17	EtOH	Reflux	5	NH ₄ Cl (1.0) + NaAcO (1.0)	60 ^a
18	EtOH	Reflux	5	PhCOOH (1.0)	30
19	EtOH	Reflux	5	PTSA (1.0)	No
20	EtOH	Reflux	5	HCl conc. (1.0)	No
21	EtOH	Reflux	16	PhCOOH (1.0)	35
22	EtOH	Reflux	16	PhCOOH (0.25)	21
23	DMF	100 °C	5	NH ₄ AcO (1.0)	No
24	Dioxane	100 °C	5	NH ₄ AcO (1.0)	5
25	CH ₃ CN	Reflux	5	NH ₄ AcO (1.0)	19
26	MeOH	Reflux	5	NH ₄ AcO (1.0)	No
27	THF	Reflux	5	NH ₄ AcO (1.0)	8
28	EtOH	Reflux	40	NH ₄ AcO (1.0)	64
29	EtOH	Reflux	40	AcOH (1.0)	58

^a Contaminated with NaCl

Finally, two last experiments focused on the influence of the reaction time (entries 28 and 29) showed that longer times resulted in improvement of the yields. After this exhaustive study, we decided to choose the reaction conditions reported in the entry 11 as the optimal ones, considering that the excessive extension in the reaction time was not compensated by the slight improvement in the yield.

6.5.2 Scope of the reaction

With the optimal reaction conditions in hand, we explored the scope of the reaction using different aromatic aldehydes (Scheme 6.9). As shown in Table 6.3, the reaction proceeded in moderate yields with aromatic aldehydes bearing electron-withdrawing substituents, and with pyridinecarbaldehydes.



Scheme 6.9: Three-component synthesis of 4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridines

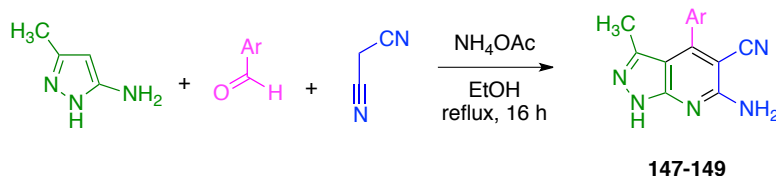
Table 6.3. Scope and yield of the synthesis of 4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridines

Entry	Cmpd.	Ar	Yield (%)	Entry	Cmpd.	Ar	Yield (%)
1	135	C ₆ H ₅	44	7	141	4-FC ₆ H ₄	36
2	136	2-ClC ₆ H ₄	34	8	142	3-NO ₂ C ₆ H ₄	40
3	137	3-ClC ₆ H ₄	39	9	143	4-NO ₂ C ₆ H ₄	35
4	138	4-ClC ₆ H ₄	48	10	144	3-pyridyl	49
5	139	2-BrC ₆ H ₄	32	11	145	4-pyridyl	52
6	140	4-BrC ₆ H ₄	51	12	146	2-thienyl	24 ^a

a: In this case, a 14% yield of the corresponding oxidized product **147** was also recovered

When aromatic aldehydes with electron-rich rings were employed, we were able to isolate only low quantities of the fully oxidized pyrazolo[3,4-*b*]pyridine derivatives, showing a clear relationship between the electron density of the aldehyde aromatic ring and the ease of oxidation of the formed dihydropyridine

(Scheme 6.10 and Table 6.4). As previously mentioned, the reaction with thiophene-2-carbaldehyde affords a mixture of dihydro (**146**) and oxidized (**147**) derivatives.



Scheme 6.10: Three-component reaction involving electron-rich aromatic aldehydes

Table 6.4. Yield of some representative three-component reaction involving electron-rich aromatic aldehydes

Entry	Cmpd.	Ar	Yield (%)
1	147	2-thienyl	14 ^a
2	148	2-furyl	8
3	149	4-MeOC ₆ H ₄	23

^a Together with 24% of the corresponding dihydro derivative

Additional experiments were carried out in an effort to avoid the final dehydrogenation step. Unfortunately, when the reaction with *p*-methoxybenzaldehyde was carried out at low temperature, no signals of the products in the crude NMR spectra were detected. Moreover, shortening the reaction time did not allow finding the dihydropyridine derivative in the reaction mixture, but only smaller amounts of the oxidized analogous, demonstrating that the higher electron density, apparently, facilitates the oxidation of the pyridine ring.

All compounds obtained in this chapter are currently under study as GSK-3 β inhibitors by the group of Prof. R. León (Instituto Teófilo Hernando and Pharmacology Department, Facultad de Medicina, Universidad Autónoma, Madrid).

**7. New Benzodiazepine-Dihydropyridine Hybrid
Compounds As Potential Multi-Target-Directed
Ligands For The Treatment Of Alzheimer's Disease**

7.1. CALCIUM, MITOCHONDRIA AND NEURODEGENERATION: AN OVERVIEW.

As already mentioned in the introduction to Chapter 4, the increasingly alarming situation related to neurodegenerative disorders has stimulated considerable research efforts aimed at achieving a better understanding of these diseases. The extensive studies carried out in the last decades on the most common neurodegenerative disorders, namely Alzheimer's, Parkinson's and Huntington's diseases (AD, PD, HD, respectively) and multiple and amyotrophic lateral sclerosis (MS and ALS), have pointed out the multifactorial pathogenic character of these disorders that are caused by genetic, endogenous and environmental factors. Although each disease is determined by its etiology and characterized by its own molecular mechanism and different clinical manifestations, there are some general traits that can be considered to be common in these pathologies, including protein misfolding and aggregation, an increase of the free radical formation and of oxidative stress and a dysregulation of ionic homeostasis (especially of Ca^{2+}), associated with a mitochondrial dysfunction.⁸⁵

In this context, continuing our research on neuroprotective agents able to combat the onset and the progression of such kinds of diseases, we focused again our attention on the alteration of Ca^{2+} homeostasis and on its relationship with mitochondrial dysfunctions. These two issues indeed seemed to be clearly implicated in the pathogenesis of different neurodegenerative illness²⁰⁵, from AD^{87a} to PD²⁰⁶ including ALS²⁰⁷ and especially HD.²⁰⁸

²⁰⁵ (a) Nicholls, D. G. *Biochim. Biophys. Acta* **2009**, *1787*, 1416-1424; (b) Cali, T.; Ottolini, D.; Brini, M. *Cell Calcium* **2012**, *52*, 73-85; (c) Duchen, M. R. *Pflugers Arch. - Eur. J. Physiol.* **2012**, *464*, 111-121.

²⁰⁶ (a) Schapira, A. H. V. *Lancet Neurol.* **2008**, *7*, 97-109; (b) Yao, Z.; Wood, N. W. *Antiox. Redox Signal.* **2009**, *11*, 2135-2149.

²⁰⁷ (a) Von Lewinski, F.; Keller, B. U. *Trends Neurosci.* **2005**, *28*, 494-500; (b) Grosskreutz, J.; Van

Before going into detail, it is worth highlighting the importance of calcium as an universal second messenger that is able to control fundamental biosynthetic pathways implicated in the normal life of all eukaryotic cells and particularly in neurons, where it has a pivotal importance taking part in the transmission of depolarizing signals and contributing to the synaptic activity. To maintain their integrity and their functions, neurons have developed a fine-tuned control system of Ca^{2+} homeostasis based on structures that regulate its flux across the plasma membrane and that control its intracellular distribution. This system, ingeniously described as “calcium signaling toolkit” by Brini *et al.*,²⁰⁹ is fundamental for maintaining the neuronal functions, and it is clearly involved in neuronal survival. It is composed of different ion channels, exchangers and pumps variously distributed among the cellular and subcellular structures. (Figure 7.1)

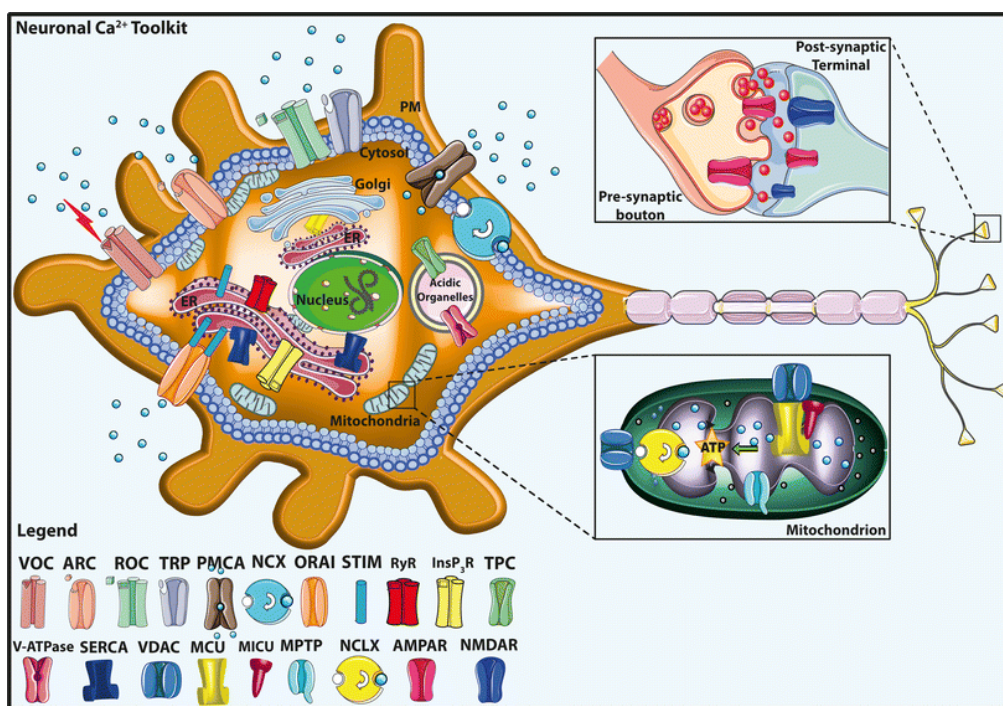


Figure 7.1: Neuronal calcium signaling toolkit.²⁰⁹

Den Bosch, L.; Keller, B. U. *Cell Calcium* **2010**, *47*, 165-174.

²⁰⁸ (a) Quintanilla, R.A.; Johnson, G. W. W. *Brain Res. Bull.* **2009**, *80*, 242-247; (b) Oliveira, J. M. A. *J. Neurochem.* **2010**, *114*, 1-12.

²⁰⁹ Brini, M.; Cali, T.; Ottolini, D.; Carafoli, E. *Cell. Mol. Life Sci.* **2014**, *71*, 2787-2814.

Among the organelles and intracellular compartments that constitute one part of this “neuronal Ca^{2+} signaling toolkit”, mitochondria (together with endoplasmic reticulum) are certainly ones of the most important players. Although their active role in the cellular homeostasis of Ca^{2+} was supposed since the early 60s, with the proposal and later the demonstration of the chemiosmotic theory,²¹⁰ only in recent years it was established how close is the connection between these two issues.²¹¹ Thanks to their extended Ca^{2+} transport system, mitochondria can reproduce rapid changes of $[\text{Ca}^{2+}]_m$ in response to the typical oscillations in $[\text{Ca}^{2+}]_c$, generating in this way specific metabolic responses such as the activation of the enzymes implicated in the cellular respiration and the related ATP production. Thus, mitochondria show a central role in controlling cytosolic Ca^{2+} fluctuations, in part by executing their physiological activity, but also acting as adjustable buffers of Ca^{2+} inside cells and tuning cytosolic Ca^{2+} signals in two principal ways: by acting, in a tight communication with the vicinal endoplasmic reticulum, as sinks able to shape the cytosolic Ca^{2+} transient and by removing Ca^{2+} from specific regions, known as Ca^{2+} microdomains, in the proximity of calcium channels and transporters.²¹²

In recent years the sophisticated mitochondrial transport system that allows this dynamic interaction between mitochondria and calcium, has received a pronounced attention and it has started to be extensively studied; although this system is still only partially known, it can be briefly described. As shown in **Figure 7.2**, to enter the mitochondrial matrix, Ca^{2+} has to cross two different phospholipid bilayers that constitute the outer and the inner mitochondrial membranes (OMM and IMM, respectively). Since the OMM does not represent an obstacle to ionic permeation because of the presence of abundant large

²¹⁰ For an interesting historical overview of the relationship between mitochondria and calcium see: Carafoli, E. *Trends Biochem. Sci.* **2003**, *28*, 175-181.

²¹¹ (a) Duchen, M. R. *J. Physiol.* **2000**, *529*, 57-68; (b) Vandecasteele, G.; Szabadkai, G.; Rizzuto, R. *IUBMB Life* **2001**, *52*, 213-219; (c) Paul S. Brookes, P. S.; Yoon, Y.; Robotham, J. L.; Anders, M. W.; Sheu, S.-S. *Am. J. Physiol. Cell Physiol.* **2004**, *287*, C817-C833; (d) Szabadkai, G.; Simoni, A. M.; Bianchi, K.; De Stefani, D.; Leo, S.; Wieckowski, M. R.; Rizzuto, R. *Biochim. Biophys. Acta* **2006**, *1763*, 442-449; (e) Bernardi, P.; Rasola, A. *Subcell. Biochem.* **2007**, *45*, 481-506; (f) Rizzuto, R.; De Stefani, D.; Raffaello, A.; Mammucari, C. *Nature Rev. Mol. Cell Biol.* **2012**, *13*, 566-578.

²¹² Rizzuto, R.; Brini, M.; Murgia, M.; Pozzan, T. *Science* **1993**, *262*, 744-747.

conductance channels called voltage dependent anion channels (VDACs), the Ca^{2+} diffusion through the OMM is not considered as the limiting factor in the mitochondrial Ca^{2+} uptake. On the other hand, the ion impermeability of the IMM (which, owing to its greater extension than OMM, shows a compartmentalization into numerous internal foldings called cristae) hampers the entrance of Ca^{2+} in the mitochondrial matrix. In this case, the transmembrane flow of this cation takes place through two different systems: a mitochondrial uniporter (MCU) that allows an accumulation of Ca^{2+} down its electrochemical gradient (generated by the activity of the electron transport chain components localized in the IMM), and two exchangers with H^+ and Na^+ (called HCX_{mito} and NCX_{mito} , mostly expressed in nonexcitable and excitable cells, respectively) whose driving forces are the electrochemical gradients of the monovalent cations, allowing the extrusion of Ca^{2+} from the matrix.²¹³

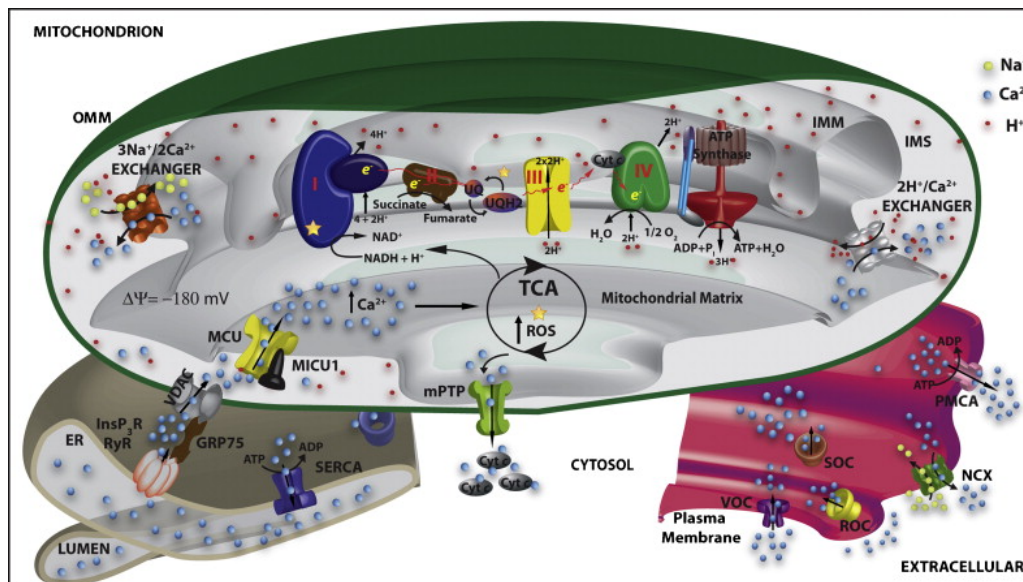


Figure 7.2: The calcium mitochondrial transport system.^{205b}

²¹³ Celsi, F.; Pizzo, P.; Brini, M.; Leo, S.; Fotino, C.; Pinton, P.; Rizzuto, R. *Biochim. Biophys. Acta* **2009**, *1787*,335-344.

7.1.1 The mitochondrial sodium/calcium exchanger (NCX_{mito})

The existence of a transporter able to extrude Ca²⁺ from the mitochondria was reported for the first time in the 1970s, when Carafoli's group discovered that in heart mitochondria a massive Ca²⁺ uptake was counterbalanced by a subsequent extrusion of the same cation, through an exchanger triggered by the presence of Na⁺ in the extramitochondrial solution.²¹⁴ In the same study and in further experiments it was demonstrated that this exchanger was rather selective toward Ca²⁺ over other bivalent cations as Mg²⁺ and Mn²⁺, which were not transported, and slightly selective for Na⁺ (in this case Li⁺ can substitute this ion, whereas other monovalent cation as Cs⁺, K⁺ and Rb⁺ failed to activate the exchanger).²¹⁵ Since the beginning of its discovery, owing to the capacity to transport differently charged ions, various hypothesis about the electronic balance of this kind of transport were advanced, and only in recent years some studies finally provided the evidence for a stoichiometry of 3 Na⁺/ 1 Ca²⁺ per transport mediated by the NCX_{mito}.²¹⁶ Furthermore, it seems that the mystery concerning the molecular identity of this exchanger has been solved very recently, when it was reported that a member of the Na⁺/Ca²⁺ exchanger family (namely a Na⁺/Ca²⁺/Li⁺ exchanger called NCLX), localized in the IMM, shows an activity that matches the one of the putative NCX_{mito}, suggesting that this could be the structure of the long-sought mitochondrial exchanger.²¹⁷

Thus, the awareness that NCX_{mito} can influence Ca²⁺-dependent processes in neurons by taking part in the modulation of [Ca²⁺]_m and [Ca²⁺]_c, generated a rising interest in reaching a deeper understanding about the role of this exchanger in the neuronal physiology. Although our knowledge about the correlation between the NCX_{mito} activity and the neuronal functionality is still limited, in recent years some studies have revealed that the NCX_{mito}, besides being involved in the cytosolic oscillation of Ca²⁺ and Na⁺, takes part in the regulation of Ca²⁺ recycling

²¹⁴ Carafoli, E.; Tiozzo, R.; Lugli, G.; Crovetto, F.; Kratzing, C. *J. Mol. Cell. Cardiol.* **1974**, *6*, 361-371.

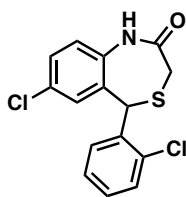
²¹⁵ Crompton, M.; Capano, M.; Carafoli, E. *Eur. J. Biochem.* **1976**, *69*, 453-462.

²¹⁶ Dash, R.K.; Beard, D.A. *J. Physiol.* **2008**, *586*, 3267-3285.

²¹⁷ Palty, R.; Silverman, W. F.; Hershinkel, M.; Caporale, T.; Sensi, S. L.; Parnis, J.; Nolte, C.; Fishman, D.; Shoshan-Barmatz, V.; Herrmann, S.; Khananshvil, D.; Sekler, I. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 436-441.

between mitochondria and endoplasmic reticulum, in the neurotransmitter release and in the synaptic plasticity.²¹⁸

The novelty of NCX_{mito} and its physiological importance since its discovery, prompted the scientific community to search for a selective inhibitor to be used in physiological and pharmacological studies. Some benzothiazepine analogues, already known as Ca²⁺ channel blockers, were the first compounds described as NCX_{mito} inhibitors; however, their multiple interactions with various receptors and proteins complicated the interpretation of these physiological studies.²¹⁹ Following a rational design strategy, the benzothiazepine derivative CGP-37157, namely 7-chloro-3,5-dihydro-5-phenyl-1H-4,1-benzothiazepin-2-one, turned out as a promising inhibitor of NCX_{mito} (Figure 7.3).²²⁰



CGP-37157

Figure 7.3: Molecular structure of the NCX_{mito} inhibitor CGP-37157

A subsequent full pharmacological characterization of this drug showed that in rat cardiomyocytes it could inhibit the Na⁺/Ca²⁺ exchanger in submicromolar concentration, without showing significantly relevant effects on other receptors as L-type Ca²⁺ channels, the plasma membrane Na⁺/Ca²⁺ exchanger (NCX_{plm}) or the Na⁺/K⁺ ATPase.²²¹ However, in recent years various studies questioned the initially hypothesized high selectivity of CGP-37157 for NCX_{mito}; in particular it was shown that this benzothiazepine could also inhibit VGCCs,²²² the NCX_{plm}²²³

²¹⁸ Palty, R.; Hershfinkel, M.; Sekler, I. *J. Biol. Chem.* **2012**, *287*, 31650-31657.

²¹⁹ Palty, R.; Sekler, I. *Cell Calcium* **2012**, *52*, 9-15.

²²⁰ Chiesi, M.; Schwaller, R.; Eichenberger, K. *Biochem. Pharmacol.* **1988**, *37*, 4399-4403.

²²¹ Cox, D. A.; Conforti, L.; Sperelakis, N.; Matlib, M. A. *J. Cardiovasc. Pharmacol.* **1993**, *21*, 595-599.

²²² Baron, K.T.; Thayer, S.A. *Eur. J. Pharmacol.* **1997**, *340*, 295-300.

²²³ Czyz, A.; Kiedrowski, L. *Biochem. Pharmacol.* **2003**, *66*, 2409-2411.

or the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA),²²⁴ even at higher concentrations than the ones needed for NCX_{mito} inhibition.

7.1.2 The NCX_{mito} and neurological diseases

As in recent years an increasing body of evidence indicates that calcium dysregulation and mitochondrial alterations play a key role in neurodegenerative diseases, the question of whether NCX_{mito} takes part in these mitochondrial impairments has become more and more relevant and pressing. Nevertheless, the role of NCX_{mito} in neurodegenerative processes is still largely unexplored or controversial, and further investigations are needed to establish whether this exchanger could be considered as a new interesting pharmacological target in the research and development of new drugs to treat these diseases.

There are few published studies focused on the investigation of the role of NCX_{mito} and its eventual connection with neurological disorders. For example, as regards the brain ischemia, the modalities of participation of NCX_{mito} in the genesis of the neuronal damage are unclear, and this is why the kind of necessary pharmacological intervention to combat this illness condition is still under debate. Based on different physiological theories found in literature, there are some evidences indicating that a greater beneficial effect is expected by blocking pharmacologically the exchanger, rather than by activating it.²²⁵

The relationship among Alzheimer's and Parkinson's diseases and NCX_{mito} is intriguing and still under debate. Thus, the manifestation of a concomitant mitochondrial dysfunction and calcium dysregulation in this disease is evident, but the role of NCX_{mito} remains essentially uncharted.²²⁵ We will extend this discussion in the following Section.

²²⁴ Nerumann, J. T.; Diaz-Sylvester, P. L.; Fleischer, S.; Copello, J. A. *Mol. Pharmacol.* **2011**, *79*, 141-147.

²²⁵ Castaldo, P.; Cataldi, M.; Magi, S.; Lariccia, V.; Arcangeli, S.; Amoroso, S. *Prog. Neurobiol.* **2009**, *87*, 58-79.

7.1.3 Calcium dysregulation and Alzheimer's disease

Alzheimer's disease is a devastating neurological disorder characterized by the progressive impairment of cognitive functions, memory loss, altered behavior and neuronal death. As mentioned in previous chapters, the pathological hallmarks of this disease are known and at morphological levels are characterized by the presence and accumulation of senile plaques formed by a peptide β -amyloid ($A\beta$), derived from the amyloid protein precursor (APP), and of intracellular neurofibrillary tangles composed of a hyperphosphorylated form of the microtubular protein tau. The majority of the investigations carried on AD have been focused on drug development in the context of the amyloid hypothesis, considering that the accumulation of amyloid plaques is characteristic in all AD patients. However, the extended efforts made in searching for effective therapies focused on this target have repeatedly failed; thus it has been necessary to investigate additional pharmacological targets to treat AD. In recent years a number of studies have increasingly pointed out the connection between Ca^{2+} and AD pathogenesis, leading to the formulation of a "calcium hypothesis" of AD.²²⁶ Among the findings that generated this hypothesis we can enumerate the evidence that experiments performed with APP transgenic mice revealed abnormal high basal Ca^{2+} levels in neuritis located close to amyloid deposits.²²⁷ Very recently it has been also demonstrated that $A\beta$ can modify the interconnections between ER and mitochondria, altering the Ca^{2+} regulation process mediated by these organelles.²²⁸ Moreover, $A\beta$ oligomers may enhance Ca^{2+} entry in the cell by altering the permeability of the membrane or the sensitive of some ion channels.²²⁹ Recent studies showed that also presenilins play a role in the dysregulation of Ca^{2+} homeostasis by altering the release of Ca^{2+} from the internal

²²⁶ (a) LaFerla, F. M. *Nat. Rev. Neurosci.* **2002**, *3*, 862-872; (b) Bezprozvanny, I.; Mattson, M. P. *Trends Neurosci.* **2008**, *31*, 454-463; (c) Berridge, M. J. *Pflugers Arch.* **2010**, *459*, 441-449.

²²⁷ Kuchibhotla, K. V.; Goldman, S. T.; Lattarulo, C. R.; Wu, H. Y.; Hyman, B. T.; Bacsikai, B. J. *Neuron* **2008**, *59*, 214-225.

²²⁸ Hedskog, L.; Pinho, C. M.; Filadi, R.; Ronnback, A.; Hertwig, L.; Wiehager, B.; Larssen, P.; Gellhaar, S.; Sandebring, A.; Westerlund, M.; Graff, C.; Winblad, B.; Galter, D.; Behbahani, H.; Pizzo, P.; Glaser, E.; Ankarcróna, M. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 7916-7921.

²²⁹ (a) Kawahara, M.; Kuroda, Y. *Brain. Res. Bull.* **2000**, *53*, 389-397. (b) Kagan B. L.; Hirakura, Y.; Azimov, R.; Azimova, R.; Lin, M. C. *Peptides* **2002**, *23*, 1311-1315.

deposits of ER, by forming new channels or by modifying the activity of structural ER channels.²³⁰ Recently, a novel voltage-gate ion channel CALHM1 that promotes the Ca^{2+} intracellular influx has emerged as another ionic checkpoint altered in AD, leading to modification in Ca^{2+} homeostasis.²³¹

7.1.4 Multitarget calcium stabilizers: a new approach for Alzheimer's disease?

Considering that neurons are excitable cells in an unceasing state of potential triggering action, we decided to focus our attention on the fluctuations of intracellular and mitochondrial Ca^{2+} fluxes. Indeed, in neurons, the continuous Ca^{2+} influx from the extracellular space has to be adequately counterbalanced by a set of cellular responses based on a Ca^{2+} efflux through the plasma membrane, on the Ca^{2+} uptake mediated by intracellular organelles and on the buffering contribution provided by some cytoplasmic calcium binding proteins (CBP). The equilibrium between the physiological condition of neurons (i.e. the conversion of the Ca^{2+} signaling in the vital coupling between the mitochondrial respiration and ATP production or in the release of neurotransmitters) and the pathological situation (with a deficit of the mitochondrial bioenergetic contribution leading to a condition of susceptibility for neurons to enter in apoptosis and die) depends on the harmonious coordination of these Ca^{2+} circuits.

The multifactorial nature of the aforementioned neurodegenerative diseases describes a complex physiopathological framework that is difficult to challenge with the classical pharmacological strategy based on the concept “a single molecule for a single target”. Thus, in recent years, the hypothesis of a compound

²³⁰ (a) Stutzmann, G. E.; Caccamo, A.; LaFerla, F. M.; Parker, I. *J. Neurosci.* **2004**, *24*, 508-513; (b) Tu, H.; Nelson, O.; Bezprozvanny, A.; Wang, Z.; Lee, S. F.; Hao, Y. H.; Serneels, L.; De Strooper, B.; Yu, G.; Bezprozvanny, I. *Cell* **2006**, *126*, 981-993; (c) Nelson, O.; Tu, H.; Lei, T.; Bentahir, M.; de Strooper, B.; Bezprozvanny, I. *J. Clin. Invest.* **2007**, *117*, 1230-1239; (d) Green, K. N.; Demuro, A.; Akbari, Y.; Hitt, B. D.; Smith, I. F.; Parker, I.; LaFerla, F. M. *J. Cell Biol.* **2008**, *181*, 1107-1116.

²³¹ Dreses-Werringloer, U.; Lambert, J. C.; Vingtdeux, V.; Zhao, H.; Vais, H.; Siebert, A.; Jain, A.; Koppel, J.; Rovelet-Lecrux, A.; Hannequin, D.; Pasquier, F.; Galimberti, D.; Scarpini, E.; Mann, D.; Lendon, C.; Campion, D.; Amouyel, P.; Davies, P.; Foskett, J. K.; Campagne, F.; Marambaud, P. *Cell* **2008**, *133*, 1149-1161.

capable to interact with two or more targets assumed as responsible for the pathogenic mechanisms of a disease, has been receiving a growing attention. This strategy, conceptually similar to the multiple-medication therapy which has been successfully employed in the treatment of chronic and complex diseases such as cancer and AIDS, is known as “Multi-Target-Directed Ligands” (MTDLs) and is thought to be potentially effective in the treatment of neurodegenerative illnesses.²³² Thus, we supposed that designing compounds that could act as multitarget calcium stabilizers of the neuronal Ca^{2+} fluxes could represent an interesting approach to the development of new molecules to be employed as neuroprotective agents.²³³

In this context we decided to focus our attention on NCX_{mito} to investigate how to maintain a functional and physiological mitochondrial calcium cycle and its fine equilibrium within the complex cellular calcium organization in neurons. To reach this goal it is necessary to know inside which limits we can modify the NCX_{mito} activity in order to let the related $[\text{Ca}^{2+}]_c$ changes remain within a critical set point, avoiding that a cytoprotective signal might turn into a cytotoxic one.²³⁴ In fact, mitigation of the efflux rate via the NCX_{mito} could slightly increase the $[\text{Ca}^{2+}]_m$ and possibly stimulate ATP synthesis, but a mitochondrial Ca^{2+} overload could be the signaling to produce the mitochondrial permeability transition pore (mPTP) that excessively increase the permeability of the mitochondrial membrane, leading the cell to the apoptotic process. In spite of this apparent paradoxical situation, the hypothesis that a controlled Ca^{2+} accumulation in the mitochondria could convert these organelles into useful Ca^{2+} sinks, promoting a possible neuroprotective role by removing Ca^{2+} excess from the cytosol, prompted us to look for a therapeutic application of the NCX_{mito} inhibition in combination with a pharmacological intervention on another cellular Ca^{2+} checkpoint.²³³

In the literature, the studies regarding the neuroprotective effect of CGP-37157 in

²³² For some selected reviews of the Multi-Target-Directed Ligands approach for the treatment of neurodegenerative disease, see: (a) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 347-372; (b) León, R.; García, A. G.; Marco-Contelles, J. *Med. Res. Rev.* **2013**, *33*, 139-189; (c) Trinchês Dias, K. S.; Viegas, C. Jr. *Curr. Neuropharmacol.* **2014**, *12*, 239-255.

²³³ Fernández-Morales, J. C.; Arranz-Tangarro, J. A.; Calvo-Gallardo, E.; Maroto, M.; Padín, J. F.; García, A. G. *ACS Chem. Neurosci.* **2012**, *3*, 873-883.

²³⁴ Koike, T.; Martin, D. P.; Johnson, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6421-6425.

a model of neuronal death, showed that this NCX_{mito} inhibitor can slow down the mitochondrial futile Ca²⁺ ion recycling through the MCU and the NCX_{mito}, leading to a partially delay or prevention of cell death and exerting a neuroprotective effect.²³⁵ It must be taken into account that CGP-37157 is able to block also other ionic channels and this aptitude could take part in eliciting neuroprotection in the studied model,²³⁵ also by modifying other cellular calcium circuits beyond the mitochondrial one. In view of these evidences, we think that the synthesis and the biological evaluation of a multitarget calcium stabilizer could lead to a better comprehension of the physiopathological mechanisms involved in the generation of neurotoxicity elicited by a disruption of the Ca²⁺ homeostasis.²³⁶

²³⁵ (a) Nicolau, S. M.; de Diego, A. M.; Cortés, L.; Egea, J.; González, J. C.; Mosquera, M.; López, M. G.; Hernández-Guijo, J. M.; García, A. G. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 844-854; (b) Nicolau, S. M.; Egea, J.; López, M. G.; García, A. G. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 140-144.

²³⁶ We thank Dr. Antonio García (Instituto Teófilo Hernando and Departamento de Farmacología, Facultad de Medicina Universidad Autónoma de Madrid) for the ideas that led into this project and his valuable input.

7.2 RATIONAL DESIGN OF A MULTITARGET CALCIUM STABILIZER

Against the aforementioned background, we wanted to design a multitarget calcium stabilizer that, combining different bioactive moieties, were capable to interact with different targets involved in the regulation of the cellular and mitochondrial calcium cycles and could potentially provide neuroprotective effects in vulnerable neurons. Our initial choice was based on the combination of a benzothiazepine system analogous to CGP-37157 to interact with the NCX_{mito}, and a dihydropyridine derivative to target the L-type VGCCs: in this manner we planned to intervene directly in the modulation of both the cellular and mitochondrial calcium cycles. The resulting molecule should contain the pharmacophores for both activities, and we envisioned a hybrid structure by linking two structurally active fragments. The CGP-37157 molecule did not offer many options to establish a link with another molecule without affecting its activity. In this regard, it was very useful a study published in 2003, about the synthesis of a large number of benzothiazepines analogous to CGP-37157, as well as other heterocyclic analogues, evaluating their inhibitory activity of NCX_{mito} in search of a potential therapeutic activity in diabetes treatment.²³⁷ The authors accomplished a detailed structure-activity relationship (SAR) analysis that is summarized in Figure 7.4.

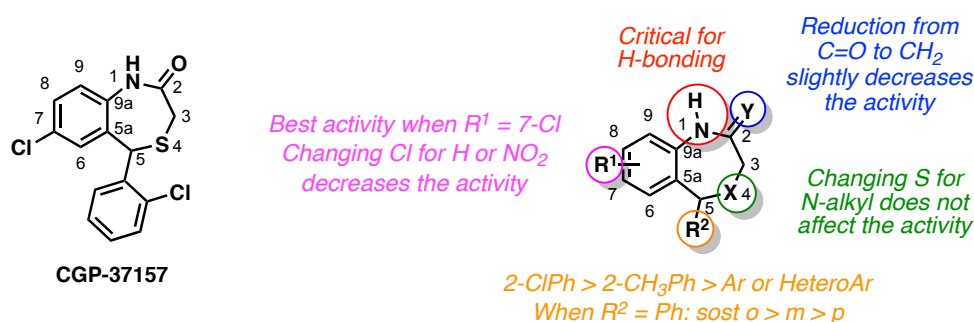


Figure 7.4: SAR analysis of the CGP-37157 analogous.

The SAR analysis provides information to make various considerations about the

²³⁷ Pei, Y.; Lilly, M. J.; Owen, D. J.; D'Souza, L. J.; Tang, X. Q.; Yu, J.; Nazarboghi, R.; Hunter, A.; Anderson, C. M.; Glasco, S.; Ede, N. J.; James, I. W.; Maitra, U.; Chandrasekaran, S.; Moos, W. H.; Ghosh, S. S. *J. Org. Chem.* **2003**, *68*, 92-103.

critical moieties of the benzothiazepine structure with respect to the NCX_{mito}:

- Regarding the fused phenyl ring, the peak activity is obtained when there is a chlorine atom in the position 7. Changing chlorine for hydrogen or nitro groups leads to decreased activity.
- The NH group in position 1 is required for the activity, probably because it is involved as a H-bond donor; changing H for alkyl or acyl substituents leads to a loss of activity.
- The carbonyl group in position 2 is not so critical and its reduction to methylene group leads only to a slight reduction of the activity.
- The presence of a phenyl ring in position 5 is generally required for the activity on the NCX_{mito}; moreover the nature of the substituents on the ring seems to be relevant, with the 2-Cl substitution showing the best results. When the chlorine atom is changed for a methyl group, the activity of the molecule slightly decreases and, considering the substitution pattern of a same substituent, it was clear that the activity decreases progressively when the substituent is moved from the *ortho* to the *para* position. Finally the switch from a phenyl ring to a heteroaromatic aryl group leads to a reduction of the activity.
- The last critical point of the molecule is represented by the position 4: the substitution of the sulphur atom of CGP-37157 was investigated and interestingly, some derivatives bearing aminoalkyl chains exhibited a similar potency in blocking the NCX_{mito} to that of the parent compound. This finding was very interesting for two reasons: on one hand this new substitution could increase the water solubility of the molecule (improving therefore one of the major weaknesses of CGP-37157) and, on the other hand, this kind of modification offered a possible connection point between the benzothiazepine structure and the dihydropyridine moiety.

With this information, we chose as NCX_{mito} blocker partner, a benzodiazepinone derivative having in the position 4 a nitrogen atom instead of the sulphur, and bearing on this nitrogen a 2-hydroxyethyl substituent (this derivative, hereafter called **153**, shows an IC₅₀ value of 7.9 μM compared to the IC₅₀ value of 1.4 μM

of CGP-37157). In this manner we conceived that the two methylenic groups could act as the linker between the benzodiazepine moiety and the dihydropyridine group, whereas the OH could be used for the formation of an ester group with one of the carbonyl functions located at C-3 or C-5 of the DHP. It is worth emphasizing that the derivative similar to **153** with a OEt instead of OH showed a stronger inhibition of NCX_{mito} (with an IC_{50} value of $3.2 \mu\text{M}$), which was promising for our purpose to convert this same free OH in an ester group.

Regarding the other component of our hybrid, we decided to choose nimodipine as the dihydropyridinic element due to its well-known behaviour as calcium channel blocker, and also because one of its ester groups has a structural similarity with the linker (Figure 7.5).

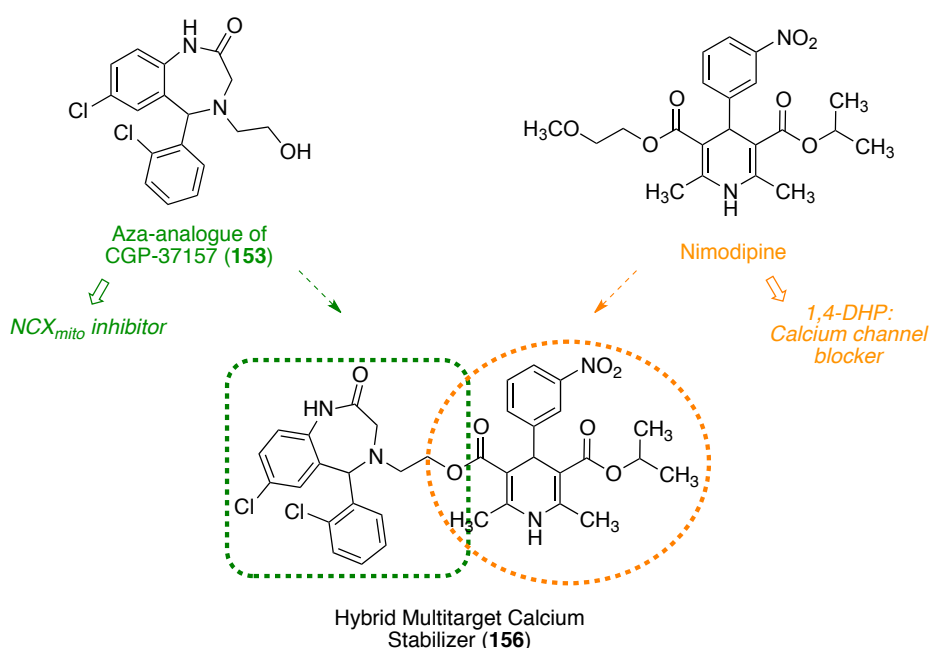
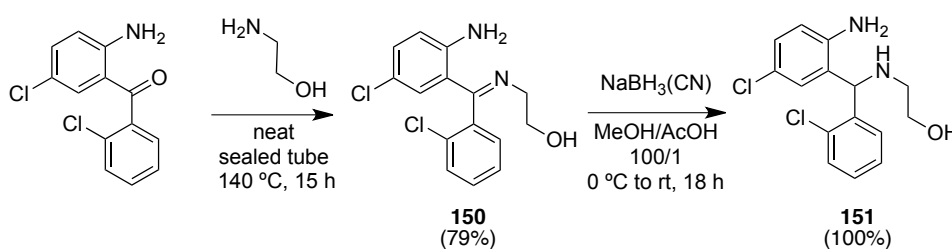


Figure 7.5: Rational approach to the synthesis of the multitarget calcium stabilizer **156**.

7.3 SYNTHESIS OF THE HYBRID BETWEEN THE AZA-ANALOGUE OF CGP-37157 AND NIMODIPINE

7.3.1 Synthesis of the aza-analogue of CGP-37157 (153)

Compound **153** was synthesized modifying the methodology described in the above-mentioned work about the benzothiazepine analogues of CGP-37157.²³⁷ As illustrated in the **Scheme 7.1**, 2-amino-2',5-dichlorobenzophenone was condensed with a large excess of ethanolamine in absence of solvent at 140 °C, in a sealed tube. The obtained imine **150** was subsequently reduced in quantitative yield using NaBH₃(CN) in a mixture of methanol and acetic acid (100:1) at room temperature, to afford the β-hydroxyamine **151**. Although CGP-37157 is chiral, it is only available in racemic form and therefore every published study regarding this compound is based on the use of racemic material²³⁸ and there is no information on whether the two enantiomers show different inhibition properties of NCX_{mito}. Thus, at this stage we decided to carry out this reduction without stereochemical control and employ from this point the racemic form of the synthesized compounds.

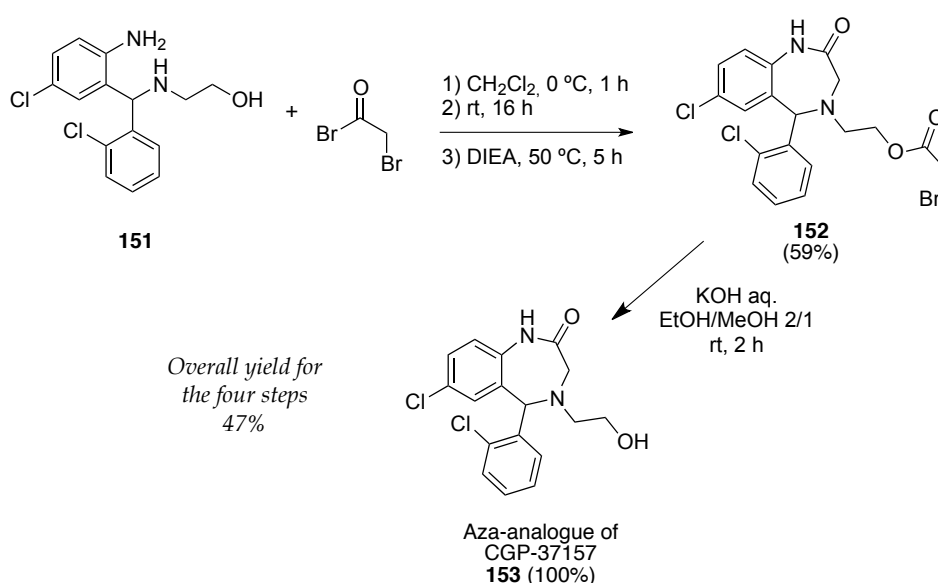


Scheme 7.1: Synthesis of compounds **150** and **151**

With diamine **151** in hand, we proceeded to the formation of the diazepinic ring by reacting compound **151** with an excess of bromoacetyl bromide in a one-pot multi step fashion, with the initial formation of the amidic bond between the primary amine and the acyl group at room temperature and the subsequent ring closure achieved by the addition of DIPEA and by increasing the temperature to 50 °C. Against literature precedent,²³⁷ this experiment did not afford the expected

²³⁸ Fitosa, I.; Visya, J.; Simonyi, M.; Hermansson, J. *J. Chromat. A* **1995**, 709, 265-273.

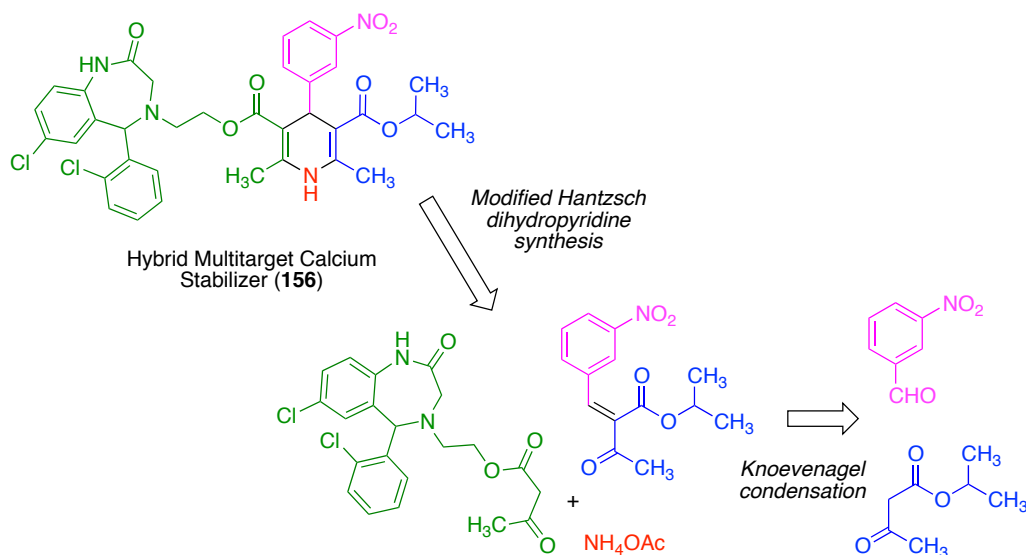
aza-analogue of CGP-37157 **153** with the free OH at the end of the alkyl chain because of its further reaction with another molecule of bromoacetyl bromide. Thus, we had subsequently to hydrolyze the initially obtained compound **152** by classical base-promoted saponification in alcoholic solution at room temperature to finally get the expected aza-analogue of CGP-37157 **153** in an overall 47% yield (Scheme 7.2).



Scheme 7.2: Synthesis of compounds **152** and **153**

7.3.2 Synthesis of the 153-nimodipine hybrid *via* a multicomponent process

With the aza-analogue of CGP-37157 (compound **153**) in hand, our next step was to obtain a hybrid with nimodipine. Based on our previous experience and considering that, as mentioned in Section 1.1, the dihydropyridine represents one of the first heterocycles synthesized through a multicomponent reaction,¹⁰ we thought that an elegant approach to reach our goal would be to synthesize the expected hybrid in a multicomponent fashion, according to the reaction planning depicted in Scheme 7.3.



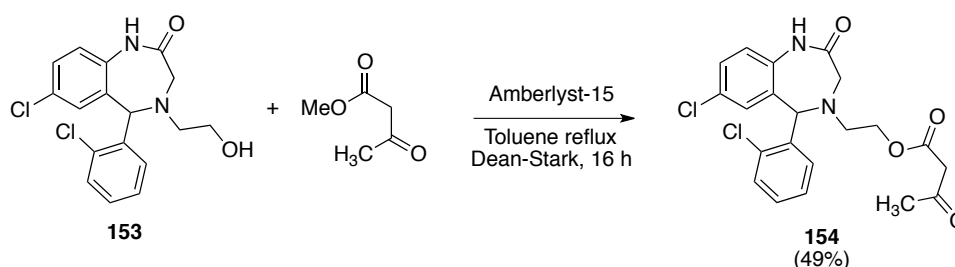
Scheme 7.3: Retrosynthetic approach to the hybrid **156** via a multicomponent process

As reported in [Section 1.3.2.1](#), Hantzsch's original protocol is a pseudo four-component reaction based on a one-pot cyclocondensation of 1 equiv of an aldehyde, 2 equiv of a β -ketoester, and 1 equiv of ammonia or a synthetic equivalent to afford symmetrical 1,4-dihydropyridines. If non-symmetrical 1,4-dihydropyridines (where the adjective non-symmetrical indicates the presence of two different ester groups at C-3 and C-5 of the DHP) are desired, as in our case, the classical Hantzsch four-component reaction has to be adapted in methodology, known as "modified three-component Hantzsch approach" ([Scheme 1.14](#)). Therefore, as depicted in [Scheme 7.3](#), we prepared on one hand, the β -ketoester derived from acetoacetic acid and alcohol **153** and, on the other hand, we prepared the conjugated enone derived from the condensation between methanitrobenzaldehyde and isopropyl acetoacetate. In this manner, by forming previously the bond between the aldehyde and one of the β -ketoesters and employing a modified three-component Hantzsch reaction, we could avoid the possible generation of symmetrical DHPs deriving from the Hantzsch (pseudo) four-component reaction.

The synthesis of the β -ketoester derived from acetoacetic acid and alcohol **153** was achieved by a known protocol in the literature,²³⁹ based on the

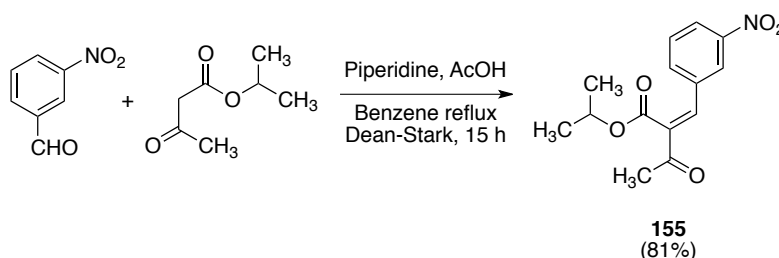
²³⁹ Chavan, S. P.; Subbarao, Y. T.; Dantale, S. W.; Sivappa, R. *Synth. Commun.* **2001**, *31*, 289-294.

transesterification of methyl acetoacetate with compound **153**, in presence of the acidic ion exchange resin Amberlyst® 15 in refluxing toluene, affording compound **154** in a moderate yield. The use of a Dean-Stark trap facilitated the progress of the reaction by removing the methanol produced during the transesterification reaction (Scheme 7.4).



Scheme 7.4: Synthesis of the β -ketoester **154**

The synthesis of the other component of our Hantzsch-like protocol was achieved by the classical conditions for the Knoevenagel condensation,²⁴⁰ employing 3-nitrobenzaldehyde and isopropyl acetoacetate as reagents, in refluxing benzene and in the presence of catalytic amounts of piperidine and acetic acid. The corresponding conjugated enone was obtained in 81% yield. Also in this case, the use of a Dean-Stark trap facilitated the progress of the reaction by removing the water generated during the condensation (Scheme 7.5).

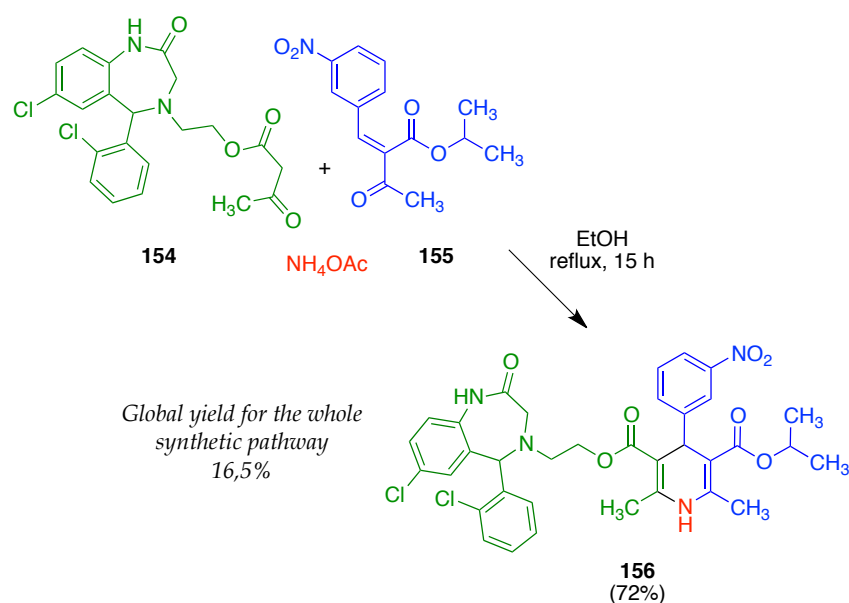


Scheme 7.5: Synthesis of **155** via Knoevenagel condensation

To obtain the desired hybrid **156** we performed the final three-component reaction between compounds **154**, **155** and ammonium acetate, in refluxing ethanol. We were pleased to find out that our protocol afforded the desired compound in good

²⁴⁰ Poindexter, G. S.; Temple, D. L. Patent GB 2158065 A, 1985.

yield (71%) and that the global yield for the entire synthetic pathway, from the initial acetophenone to the final hybrid, was satisfactory (16,5%) (Scheme 7.6).



Scheme 7.6: Multicomponent synthesis of the hybrid **156**

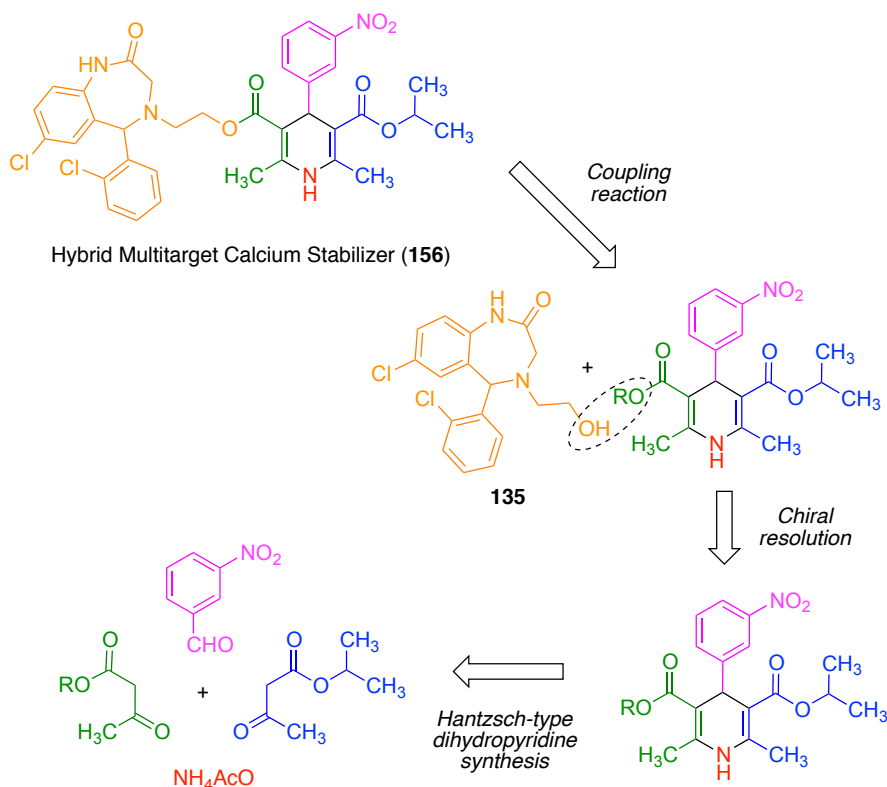
We obtained compound **156** as a mixture of diastereomers that were impossible to separate using standard chromatography methodologies. Nevertheless, the pharmacological study of compound **156** will provide useful information on the feasibility of our multitarget approach.

7.3.3 Convergent synthesis of the hybrid **156**

A future synthesis of **156** or related compounds in enantiomerically pure form could be initiated by an enantioselective Hantzsch reaction, but the methods known for this transformation are very scarce.²⁴¹ Alternatively, a chiral resolution, which is the usual strategy employed for the preparation of optically active

²⁴¹ For a review of the stereoselective synthesis of the dihydropyridine ring see: Tenti, G; Ramos, M. T.; Menéndez, J. C., in Rodriguez, J.; Bonne, D. (eds.) *Stereoselective Multiple Bond-Forming Transformations in Organic Synthesis* Chapter 3: "Six-membered heterocycles" Wiley-VCH: Weinheim, Germany, 2015.

DHPs,²⁴² could be planned. In preparatory work towards this goal, we also designed an alternative synthetic pathway that involved obtaining separately the benzodiazepine and the dihydropyridine rings and linking them in the last step. Although at this preliminary stage we have not proceeded to the separation of the enantiomers, this linear synthetic pathway could allow the resolution of the racemic dihydropyridine using well-established methods before the last coupling reaction with benzodiazepine **153** (Scheme 7.7).

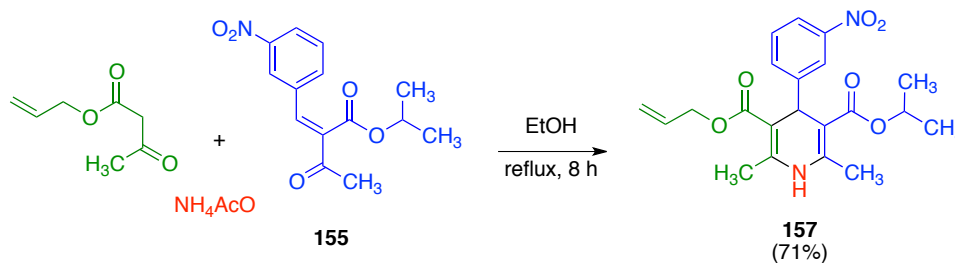


Scheme 7.7: Retrosynthetic approach to the linear synthesis of the hybrid **156**

The key step in the designed synthetic pathway is clearly the final coupling reaction and, bearing in mind the presence of a hydroxy group in benzodiazepine **153**, we thought that this step could be easily achieved through an esterification

²⁴² (a) Shibamura, T.; Iwami, M.; Okuda, K.; Takenaka, T.; Murakami, M. *Chem. Pharm. Bull.* **1980**, *28*, 2809-2812; (b) Arhancet, G. B.; Woodard, S. S.; Dietz, J. D.; Garland, D. J.; Wagner, G. M.; Iyanar, K.; Collins, J. T.; Blinn, J. R.; Numann, R. E.; Hu, X.; Huang, H.-C. *J. Med. Chem.* **2010**, *53*, 4300-4304.

reaction involving the carboxylic acid of the adequate dihydropyridine. Thus, our first goal was the synthesis of a non-symmetrical 1,4-dihydropyridine dicarboxylate in which just one of its ester groups could be univocally hydrolyzed to carboxylic acid. Considering that nimodipine has an isopropyl ester group on one side of the molecule, we looked for another ester group that, after the formation of the DHP, could be easily converted into a carboxylic acid. After a literature search, our first choice was an allylic ester, which is known for the possibility to be selectively hydrolyzed even in the presence of other ester groups.²⁴³ Also in this case, the need to get a non-symmetrical dihydropyridine with different ester groups at C-3 and C-5 prompted us to carry out our first reaction by the Hantzsch-like three-component reaction similar to that one performed in the last step of our previous multicomponent protocol. Thus we obtained dihydropyridine **157** in good yield by reacting enone **155**, allyl acetoacetate and ammonium acetate in refluxing ethanol (Scheme 7.8).

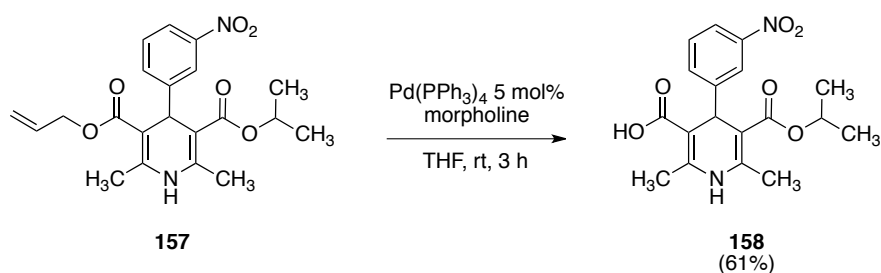


Scheme 7.8: Synthesis of the 1,4-DHP **157**

With the DHP **157** in hand, we proceeded to the conversion of the allyl ester into a carboxylic acid employing the literature methodology for the deprotection of the allyloxycarbonyl (Aloc) group. After an initial unsuccessful attempt with Pd(OAc)₂ and triphenylphosphine in the presence of triethylamine and formic acid, the conversion into carboxylic acid was achieved in acceptable yield employing morpholine and a catalytic amount of palladium *tetrakis* triphenylphosphine in anhydrous THF at room temperature (Scheme 7.9).²⁴⁴

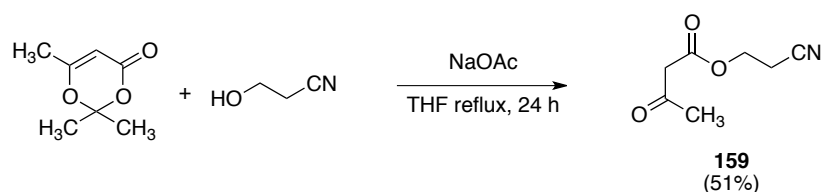
²⁴³ (a) Kosugi, Y.; Hori, M.; Nagasaka, T. *Heterocycles* **1994**, *39*, 591-602; (b) Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. *Angew. Chem. Int. Ed.* **2000**, *39*, 2290-2294; (c) Sarabia, F.; Chammaa, S. *J. Org. Chem.* **2005**, *70*, 7846-7857.

²⁴⁴ Schmidt, D. R.; Kwon, O.; Schreiber, S. L. *J. Comb. Chem.* **2004**, *6*, 286-292.



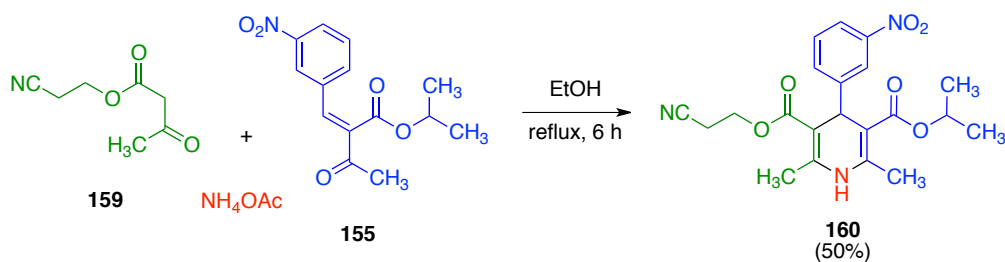
Scheme 7.9: Palladium-catalyzed conversion of the allyl ester into carboxylic acid

To avoid the use of costly palladium catalysts we decided to investigate also other possibilities to obtain compound **158**. We found that also the 2-cyanoethyl ester could be selectively converted into carboxylic acid, employing mild conditions and without using expensive catalysts.²⁴⁵ Thus, the first step in this new synthetic pathway was the synthesis of the 2-cyanoethyl acetoacetate, that was carried out employing the procedure shown in Section 5.2.1 and developed in our group, based on the treatment of alcohols with 2,2,6-trimethyl-1,3-dioxin-4-one in the presence of sodium acetate. The expected β -ketoester was obtained in refluxing THF in 51% yield (Scheme 7.10).

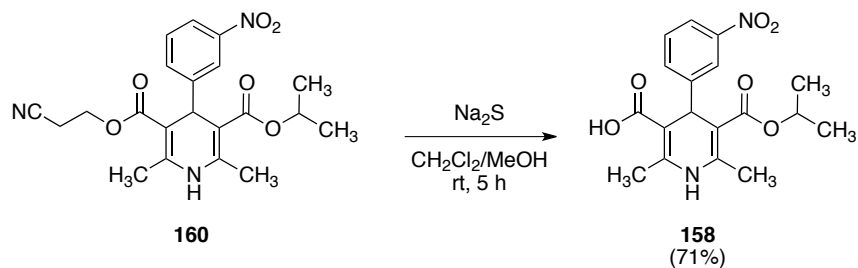
Scheme 7.10: synthesis of the 2-cyanoethyl acetoacetate **159**

We then proceeded to the synthesis of the corresponding dihydropyridine by the usual Hantzsch-like three-component reaction between the enone **155**, 2-cyanoethyl acetoacetate **159** and ammonium acetate in refluxing ethanol (Scheme 7.11).

²⁴⁵ (a) Ogawa, T.; Nakazato, A.; Tsuchida, K.; Hatayama, K. *Chem. Pharm. Bull.* **1993**, *41*, 108-116; (b) Ogawa, T.; Hatayama, K.; Maeda, H.; Kita, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1579-1589; (c) Bäracker, L.; Kuhl, A.; Hillisch, A.; Grosser, R.; Figueroa-Pérez, S. Heckroth, H.; Nitsche, A.; Ergüden, J.-K.; Gielen-Haertwig, H.; Schlemmer, K.-H.; Mittendorf, J.; Paulsen, H.; Platzek, J.; Kolkhof P. *Chem. Med. Chem.* **2012**, *7*, 1385-1403.

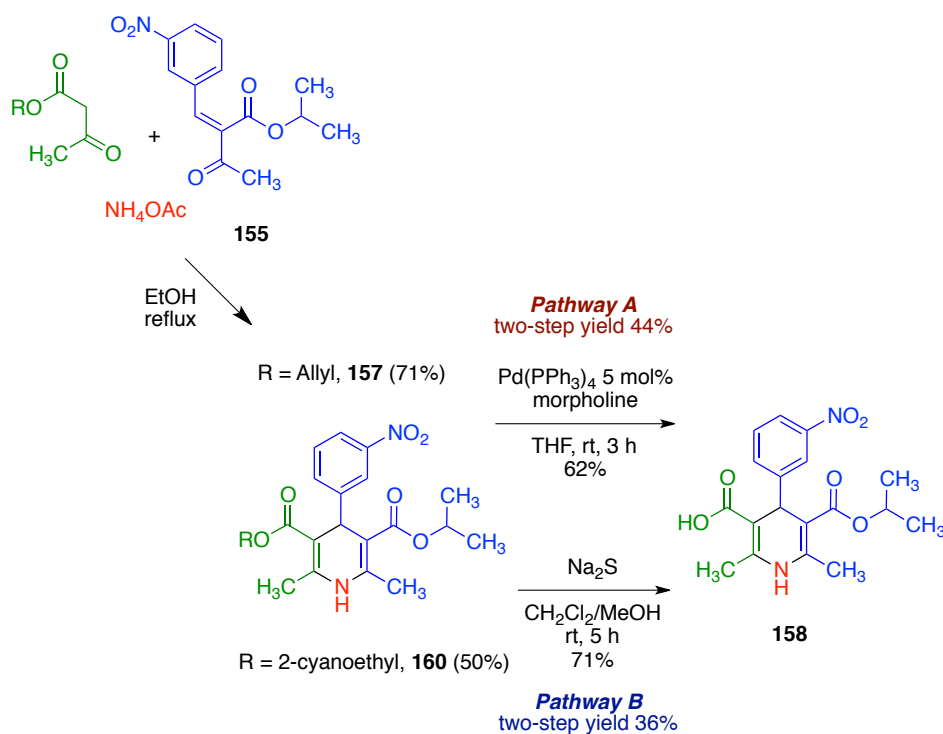
Scheme 7.11: Synthesis of the 1,4-DHP **160**

Once we had access to the DHP **160** bearing the 2-cyanoethyl ester group, we could perform its selective conversion into the monocarboxylic acid **158** by a literature methodology,^{245b} consisting in a reaction at room temperature in a mixture of dichloromethane and methanol and in the presence of sodium sulfide hydrate; the DHP **158** was obtained in good yield (Scheme 7.12).



Scheme 7.12: Conversion of the 2-cyanoethyl ester into carboxylic acid

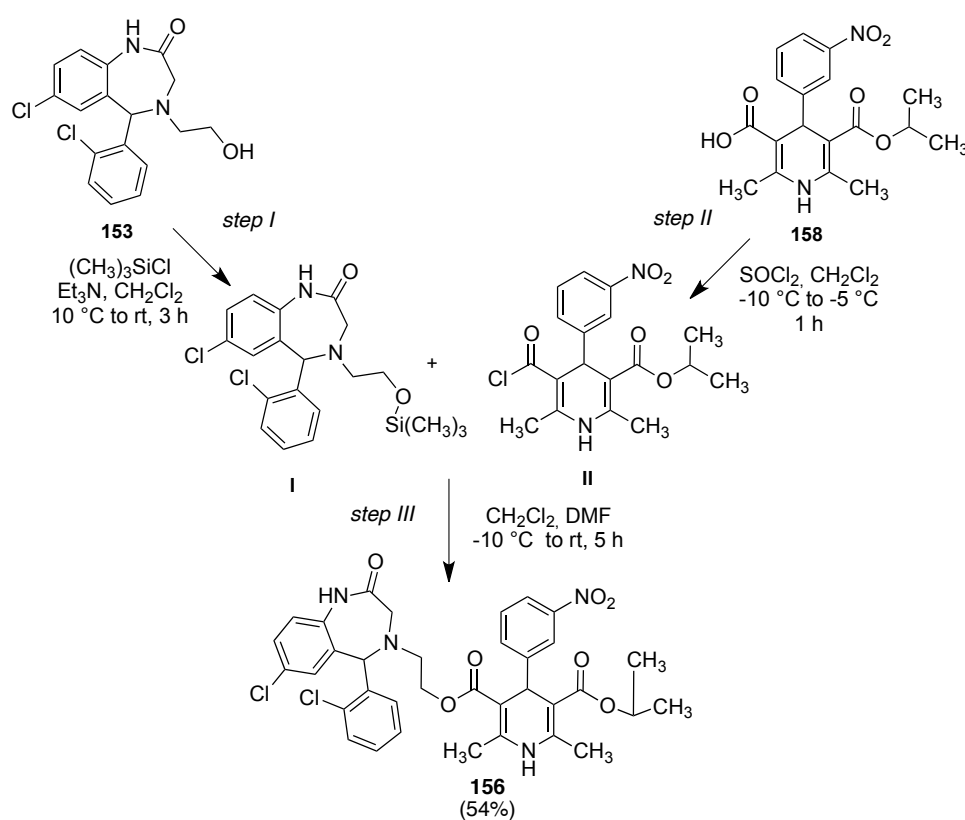
Comparing similar steps (namely the DHP formation and its subsequent deprotection to carboxylic acid) of the two linear synthetic pathways (the first one through the intermediate **157**, that we call A and the second one through the intermediate **160**) to the common intermediate **158**, we can see how similar is the two-step yield for both routes (44% and 36% respectively); we can affirm therefore, that the pathway B is an efficient and cheaper alternative to the more expensive route A (Scheme 7.13).

Scheme 7.13: Comparison between route A and B to **158**

With the mono acidic dihydropyridine derivative **158** in hand we could finally proceed to the study of the coupling step with the benzodiazepine **153** making use of an esterification reaction. To increase the reactivity of the carboxylic group of **158** to facilitate its coupling with the hydroxy group of **153** we firstly attempted its conversion into an acyl chloride^{243a} or a mixed anhydride derivative,²⁴⁶ but unfortunately, the coupling reactions with **153** did not afford the expected hybrid **156**, probably due to the steric hindrance exerted by the benzodiazepine moiety that could reduce the reactivity of the hydroxyl. Hence we decided to convert not only the carboxylic acid, but also the hydroxyl group into more reactive species. In particular, we transformed the OH group of **153** into a silylated alcohol by reaction with trimethylsilyl chloride in dichloromethane in the presence of triethylamine (step I), and on the other hand, we transformed the carboxylic acid of **158** into the corresponding acyl chloride (step II) by reaction with thionyl

²⁴⁶ Chang, C.-C.; Cao, S.; Kang, S.; Kai, L.; Tian, X.; Pandey, P.; Fernandez Dunne, S.; Luan, C.-H.; Surmeier, D. J.; Silverman, R. B. *Bioorg. Med. Chem.* **2010**, *18*, 3147-3158.

chloride in a mixture of dichloromethane and N,N'-dimethylformamide. The critical coupling reaction was carried out simply adding the intermediate obtained in step II into the reaction mixture coming from the step I, affording finally the expected hybrid **156** in good yield (54% overall three steps) (Scheme 7.14).²⁴⁷



Scheme 7.14: Final coupling step between **153** and **158** for the synthesis of **156**

The hybrid obtained in this chapter is currently under study as calcium stabilizers in SH-SY5Y human neuroblastoma cells to assess its potential neuroprotective activity, by the group of Prof. R. León (Instituto Teófilo Hernando and Pharmacology Department, Facultad de Medicina, Universidad Autónoma, Madrid).

²⁴⁷ Bandi, P. R.; Kura, R. R.; Rapolu, R. R.; Dasari, M. R.; Dandamudi, S. K. US Patent US 2010/0087653 A1 2010.

7.4 SYNTHESIS OF THE HYBRID BETWEEN COMPOUND 153 AND LIPOIC ACID

As mentioned in the [Paragraph 6.1.3](#), in recent years large experimental evidence has demonstrated that oxidative stress is another very important factor in tight connection with calcium homeostasis, pathological conditions of mitochondria and neurodegenerative diseases.²⁴⁸ As the oxidative stress arises from an imbalance between an excessive production of reactive oxygen species (ROS) and antioxidant defenses, it is interesting to underline that among the intracellular organelles that can generate ROS, mitochondria are the most prolific, being responsible for more than 90% of ROS production. If on one hand the ROS generation is fundamental for the cell life, taking part in the cytosolic signaling system, on the other hand it has to be strictly controlled to avoid an excessive ROS production that leads to irreparable cellular damages such as those already mentioned in the [Section 6.1.3](#).

The high susceptibility of the brain to oxidative damage and the evidence that the abnormal propagation of ROS generation play a major role in the pathogenesis and the progression of AD (insertar referencia cruzada), it seems clear that molecules with antioxidant properties could represent an interesting tool to combat this kind of diseases.²⁴⁹ Thus, considering that calcium dysregulation, mitochondrial impairment and an oxidative stress increase are tightly connected in the pathogenesis of AD, we decided to extend the concept of multitarget neuroprotective agent, involving in our project also a molecule with antioxidant properties. A suitable candidate was lipoic acid (LA), a natural precursor for some mitochondrial enzymes, that has been suggested to have multiple anti-AD and, more in general, anti-dementia properties derived from its capability to increase acetylcholine production, to chelate transition metals, to scavenge ROS increasing GSH levels and down-regulating the inflammatory process, and to induce the

²⁴⁸ (a) Kume, T.; Katsuki, H.; Akaike, A. *Biol. Pharm. Bull.* **2004**, *27*, 964-967; (b) Chinopoulos, C.; Adam-Vizi, V. *FEBS J.* **2006**, *273*, 433-450; (c) Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787-795; (d) Yan, M. H.; Wang, X.; Zhu, X. *Free Radic. Biol. Med.* **2013**, *62*, 90-101.

²⁴⁹ (a) Grundman, M.; Delaney, P. *Proc. Nutr. Soc.* **2002**, *61*, 191-202; (b) Casetta, I.; Govoni, V.; Granieri, E. *Curr. Pharm. Des.* **2005**, *11*, 2033-2052.

synthesis of antioxidant protective enzymes.²⁵⁰

So, we proceeded to the rational design of a new potential multitarget-directed ligand by combining the benzodiazepine **153** and lipoic acid in order to act on the mitochondrial calcium homeostasis (by targeting the NCX_{mito}) and on the harmful oxidative processes. Also in this case, the hydroxyl moiety of **153** and the carboxylic group of LA were the more evident option for the junction between the two molecules (Figure 7.6).

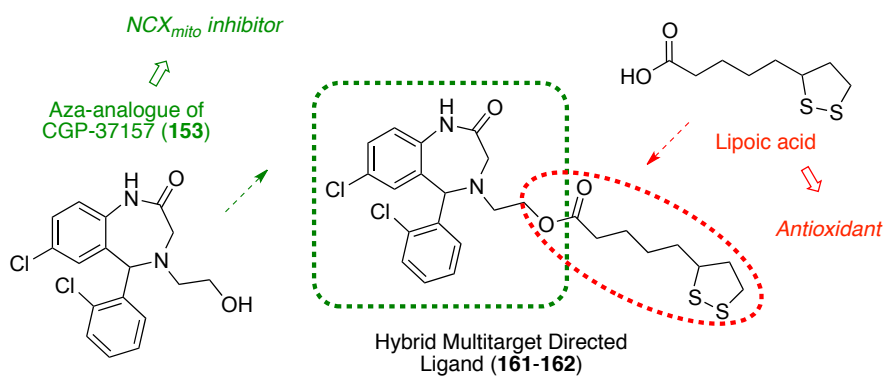
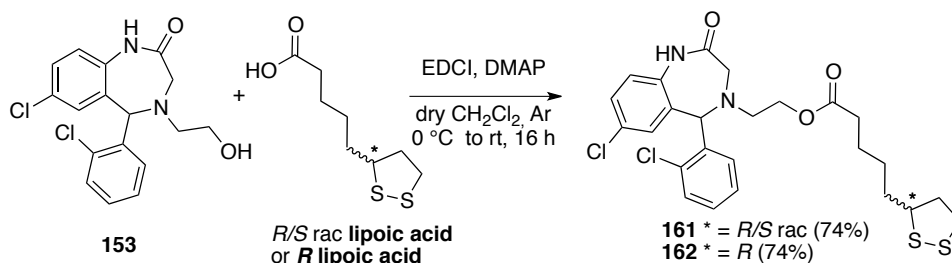


Figure 7.6: Rational approach to the synthesis of the hybrids **161** and **162**

The conditions used to obtain **156** from **153** and **158** were not successful in this case, but the synthesis of the new hybrid **161** was achieved in good yield, using EDCI as coupling agent in the presence of DMAP, in dichloromethane at room temperature (Scheme 7.15).²⁵¹



Scheme 7.15: Synthesis of the hybrids **161** and **162**

²⁵⁰ Maczurek, A.; Hager, K.; Kenklies, M.; Sharman, M.; Martins, R.; Engel, J.; Carlson, D. A.; Münch, G. *Adv. Drug Deliv. Rev.* **2008**, *60*, 1463-1470.

²⁵¹ Bernini, R.; Crisante, F.; Merendino, N.; Molinari, R.; Soldatelli, M. C.; Velotti, F. *Eur. J. Med. Chem.* **2011**, *46*, 439-446.

Also in this case the final product was a mixture of diastereomers that was impossible to separate using the basic chromatography methodologies. To simplify the product mixture we also prepared the hybrid using the pure R-(+)-enantiomer of the lipoic acid (hybrid **162**), so that, **162** was obtained as a mixture of only two diastereomers.

Also these two hybrids are currently under study as calcium stabilizers and antioxidant agents in SH-SY5Y human neuroblastoma cells to evaluate their potential neuroprotective activity, by the group of Prof. R. León (Instituto Teófilo Hernando and Pharmacology Department, Facultad de Medicina, Universidad Autónoma, Madrid).

8. Ruthenium(II)-Catalyzed Oxidative C-H Alkenylations of Sulfonamides

8.1 INTRODUCTION

8.1.1 The C-H functionalization strategy

The increasingly urgent need for green and sustainable chemistry has led organic chemists to search new efficient strategies for the creation of carbon-carbon bonds; in the last years the possibility to get this kind of connection from the activation and functionalization of simple carbon-hydrogen bond has emerged, more and more, as a very interesting and challenging target in organic synthesis,²⁵² with interesting applications in medicinal chemistry and drug discovery.²⁵³ Coupling the oxidations of two unreactive C-H bonds to build a new C-C bond allows the use of simple reagents, a reduction in the number of steps of a synthetic pathway leading consequently to a general reduction of the waste products and to more economic procedures. Being thermodynamically unfavorable the simultaneous formation of a C-C bond and the loss of a H-H molecule, these transformations, known as dehydrogenative cross-coupling reaction, require the intervention of a sacrificial oxidant as the driving force of the process (Figure 8.1).²⁵⁴

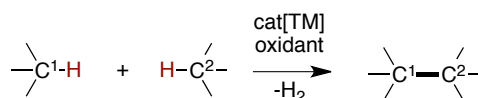


Figure 8.1: Dehydrogenative cross-coupling reaction

²⁵² (a) Bergman, R. G. *Nature* **2007**, *446*, 391-393; (b) Ackermann, L. *Modern Arylation Methods*, Wiley-VCH, Weinheim, **2009**.

²⁵³ (a) Wencel-Delord, J.; Glorius, F. *Nat. Chem.* **2013**, *5*, 369-375; (b) Ackermann, L.; Vicente, R.; Kapdi, A. *Angew. Chem. Int. Ed.* **2009**, *48*, 9792 - 9826 .

²⁵⁴ For a review of the catalytic dehydrogenative cross-coupling reaction, see: Yeung, C. S.; Dong, V. M. *Chem. Rev.* **2011**, *111*, 1215-1292.

Although palladium remains the best known metal for this kind of transformations, other transition metal-catalyzed carbon-carbon bond formations have received great attention from synthetic chemists.²⁵⁵ In particular ruthenium(II) complexes have emerged as an efficient, less expensive²⁵⁶ and equally effective alternative to palladium in the field of the C-H activation.²⁵⁷

8.1.2 Carboxylated-assisted ruthenium-catalyzed C-H functionalization

At the turn of 1990s, the innovative works of Lewis²⁵⁸ and Murai²⁵⁹ described for the first time the potential efficiency of ruthenium catalysts in the conversion of C-H bonds into C-C or C-Het bonds. Subsequently great efforts were made to discover more stable and more efficient ruthenium catalysts to be employed in new metal-catalyzed reactions,²⁶⁰ though the major progresses in ruthenium-catalyzed C-H activation were achieved by the use of ruthenium complexes generated in situ in catalytic systems with modest robustness.²⁶¹

In recent years, in order to find more efficient and practical ruthenium catalytic systems, the Ackermann group deeply investigated various procedures based on this transition metal, for C-H bond functionalization. After an extensive investigation, they could achieve the formation of a robust and efficient ruthenium catalytic system that involves the use of carboxylated ligands as cocatalytic additives. The carboxylates are supposed to form the ruthenium catalytic complex in situ and are expected to act as basic bifunctional ligands generating a six-membered transition state (Figure 8.2).

²⁵⁵ Ackermann, L.; Vicente, R.; Kapdi, A. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 9792-9826.

²⁵⁶ April 2015, <http://www.infomine.com/investment/precious-metals/>: Prices of gold, platinum, rhodium, palladium, iridium and ruthenium: €1110, €1073, €1058, €709, €530 and €46 per troy ounce, respectively.

²⁵⁷ For recent reviews on ruthenium-catalyzed C-H bond functionalization, see: (a) Ackermann, L. *Pure Appl. Chem.* **2010**, *82*, 1403-1413; (b) Arockiam, P. B.; Bruneau, C.; Dixneuf, P. H. *Chem. Rev.* **2012**, *112*, 5879-5918.

²⁵⁸ Lewis, L. N.; Smith, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 2728-2735.

²⁵⁹ Murai, S.; Kakiuchi, F.; Sekine, S.; Tanaka, Y.; Kamatani, A.; Sonoda, M.; Chatani, N. *Nature* **1993**, *366*, 529-531.

²⁶⁰ Oi, S.; Fukita, S.; Hirata, N.; Watanuki, N.; Miyano, S.; Inoue, Y. *Org. Lett.* **2001**, *3*, 2579-2581.

²⁶¹ Ackermann, L.; Vicente, R. *Top. Curr. Chem.* **2010**, *292*, 211-229.

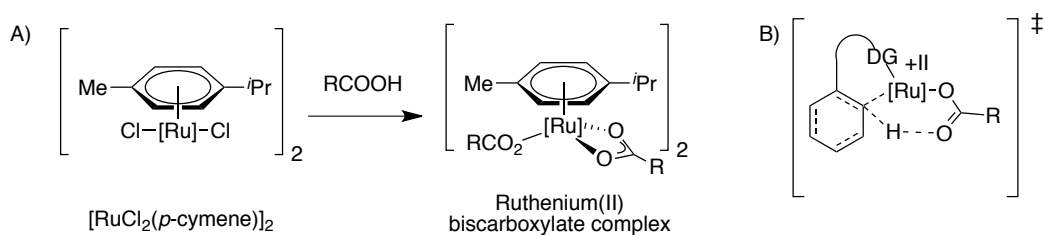
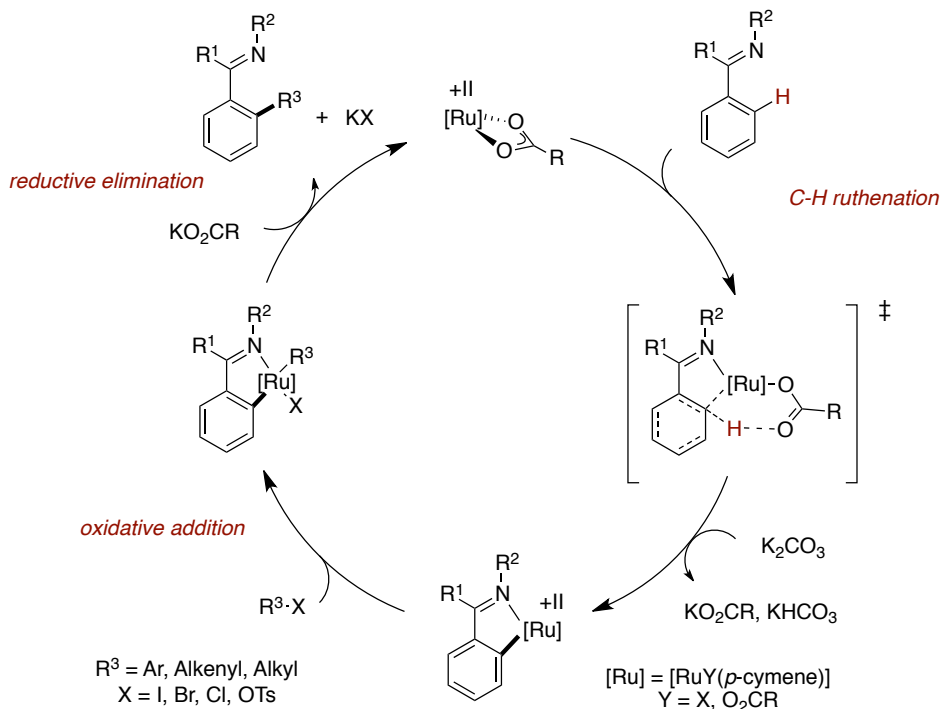


Figure 8.2: (A) Formation of the bis-carboxylated ruthenium complex;
(B) Proposed transition state for base-assisted ruthenations.

The subsequent exhaustive mechanistic studies of this catalytic system carried out in ruthenium-catalyzed directed arylations and alkylations, effectively highlighted the key role of the carboxylated additives in promoting the C-H functionalization (Scheme 8.1).²⁶²



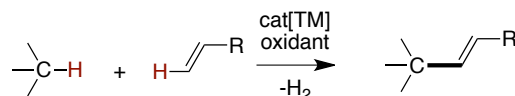
Scheme 8.1: Proposed mechanism for direct arylations and alkylations by carboxylate assistance

²⁶² Ackermann, L. *Acc. Chem. Res.* **2014**, *47*, 281–295.

8.1.3 Ruthenium-catalyzed directed oxidative alkenylation of arenes

Among the various transformations that can be classified as dehydrogenative cross-coupling reactions, the direct oxidative alkenylation of arenes via a double C-H cleavage is an appealing tool for the synthesis of highly functionalized aromatic rings (Figure 8.3A). This process (firstly proposed by Fujiwara and Moritani)²⁶³ based on a direct two-fold C-H activation allows to overcome the formation of pre-functionalized starting materials, emerging as a more atom- and step-economical strategy than the classical Heck (also known as the Mizoroki–Heck) reaction: the palladium-catalyzed alkenylation of aryl halides with alkenes (Figure 8.3B).²⁶⁴

A) Heck-type Dehydrogenative Cross-Coupling Reaction



B) Mizoroki–Heck Reaction

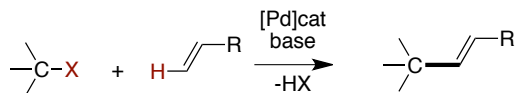


Figure 8.3: Strategies for alkenylation of arenes

Also for this kind of transformation, various synthetic protocols catalyzed by palladium (and also by rhodium) are known,²⁶⁵ however the use of ruthenium catalysts for the C-H alkenylation of arenes emerged for the first time only at the beginning of millennium.²⁶⁶ The initial limitations observed in this pioneering work were successively overcome in recent years by the investigations carried out by different research groups, including those of Ackermann and Miura & Satoh, that have led to the identification of different ruthenium complexes, to the introduction and to the use of Lewis-basic directing groups in the arene, and to the

²⁶³ Fujiwara, Y.; Moritani, I.; Matsuda, M. *Tetrahedron* **1968**, *24*, 4819-4824.

²⁶⁴ (a) Mizoroki, T.; Mori, K.; Ozaki, A. *Bull. Chem. Soc. Jap.* **1971**, *44*, 581; (b) Heck, R. F.; Nolley, J. P. *J. Org. Chem.*, **1972**, *37*, 2320-2322; c) Oestreich, M. *The Mizoroki–Heck Reaction*, Wiley, Weinheim, **2009**.

²⁶⁵ Zhou, L.; Lu, W. *Chem. Eur. J.* **2014**, *20*, 634-642.

²⁶⁶ Weissman, H.; Song, X.; Milstein, D. *J. Am. Chem. Soc.* **2001**, *123*, 337-338.

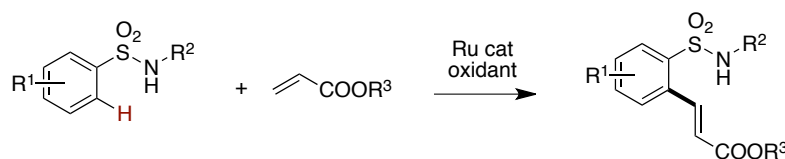
employment of external oxidants and additives. This extensive study has allowed the direct oxidative alkenylation of arenes bearing whether electron-withdrawing or electron-donating groups and, notably, also of heteroaromatic rings.²⁶⁷

²⁶⁷ Kozhushkov, S. I.; Ackermann, L. *Chem. Sci.* **2013**, *4*, 886-896.

8.2 RUTHENIUM(II)-CATALYZED OXIDATIVE C-H ALKENYLATIONS OF ARYL SULFONAMIDES

This section of the present doctoral thesis was developed in the group of Prof. Ackermann at the Institut für Organische und Biomolekulare Chemie of the Georg-August Universität of Göttingen during a 3-month period in September-December 2013.

Despite the large variety of experiments focused on exploring the aptitudes the role of numerous directing groups for the ruthenium-catalyzed oxidative C-H functionalization of arenes, the investigation of C-H activation on aryl sulfonamides has remained almost unexplored. Thus, a literature search revealed that only some cases of C-H functionalization of sulfonamides based had been reported and were based on the use of very expensive transition-metal catalyst such as palladium²⁶⁸ and rhodium.²⁶⁹ The great interest toward this kind of derivatives stems from their pronounced pharmaceutical properties and their cardinal importance in medicinal chemistry and makes the possibility to directly functionalize the aryl sulfonamide moiety really attractive. This prompted Ackermann and his group to investigate the efficiency of its ruthenium catalytic system in the oxidative C-H olefination of aryl sulfonamides (Scheme 8.2).



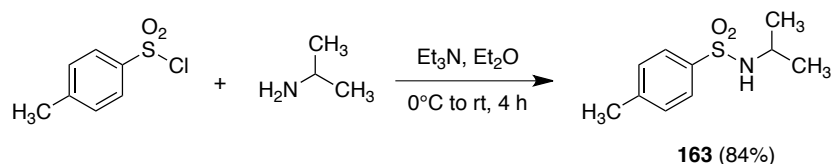
Scheme 8.2: The project proposal for the ruthenium-catalyzed oxidative C-H alkenylation of aryl sulfonamides

An initial study of the reaction was conducted employing *N*-isopropyl-4-methyl benzenesulfonamide as starting material that was synthesized according to a known procedure, simply reacting the *p*-toluenesulfonyl chloride with isopropylamine in diethyl ether in the presence of triethylamine at room

²⁶⁸ Dai, H.-X.; Stepan, A. F.; Plummer, M. S.; Zhang, Y.-H.; Yu, J.-Q. *J. Am. Chem. Soc.* **2011**, *133*, 7222-7228.

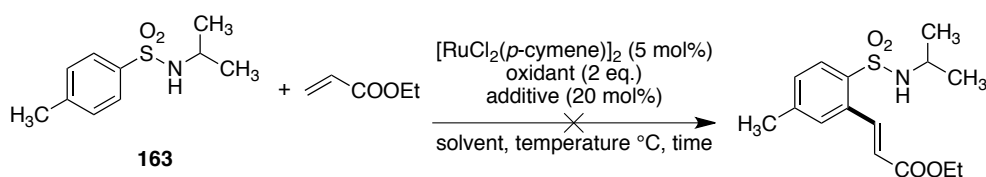
²⁶⁹ Pham, M. V.; Ye, B.; Cramer, N. *Angew. Chem. Int. Ed.* **2012**, *51*, 10610-10614.

temperature (Scheme 8.3).²⁷⁰



Scheme 8.3: Synthesis of the starting material **163**

Our initial screening was carried out employing **163** and an excess of ethyl acrylate (3 equiv) as starting materials (Scheme 8.4). The catalytic system developed by the Ackermann group consists of the ruthenium catalyst $[\text{RuCl}_2(\textit{p}\text{-cymene})]_2$, the sacrificial oxidant and an additive. The effect of different solvents at various temperatures tried for this initial study, are shown in Table 8.1.



Scheme 8.4: Initial screening of the reaction conditions for the oxidative C-H alkenylation employing *N*-isopropyl-4-methyl benzenesulfonamide as starting material

Unfortunately, as shown in the Table 8.1, despite the numerous reaction conditions tested, this initial screening did not afford the desired product, and only the starting material (in some experiments together with the dimer of the acrylate) was recovered in all the reactions.

Table 8.1. Study of the catalytic reaction involving **163** as starting material

Entry	Solvent	Oxidant	Additive	Temp.	Time	Product (Yield %) ^a
1	DMA	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	AgSbF_6	120 °C, under N_2	18 h	sm
2	<i>t</i> AmOH	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	AgSbF_6	120 °C, under N_2	18 h	sm (86%)

²⁷⁰ Hamura, S.; Oda, T.; Shimizu, Y.; Matsubara, K.; Nagashima, H. *J. Chem. Soc., Dalton Trans.* **2002**, 1521-1527.

Entry	Solvent	Oxidant	Additive	Temp.	Time	Product (Yield %) ^a
3	PhCH ₃	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	120 °C, under N ₂	18 h	sm
4	H ₂ O	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	18 h	sm (91%)
5	DCE	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	18 h	sm (93%) + da
6	DME	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	16 h	sm (83%) + da
7	1,4-dioxane	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	16 h	sm (81%) + da
8	<i>o</i> -xylene	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	16 h	sm
9	NMP	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	100 °C, under N ₂	16 h	sm
10	MeOH	Cu(OAc) ₂ ·H ₂ O	AgSbF ₆	80 °C, under N ₂	16 h	sm
11	DCE	AgOAc	^t BuLeu- OH	100 °C, under air	16 h	sm
12	DCE	AgOAc	No additive	100 °C, under air	16 h	sm
13	DCE	Cu(OAc) ₂ ·H ₂ O	No additive	100 °C, under air	16 h	sm
14	DCM ^b	AgOAc	^t BuLeu- OH	80 °C, under air	16 h	sm (86%)

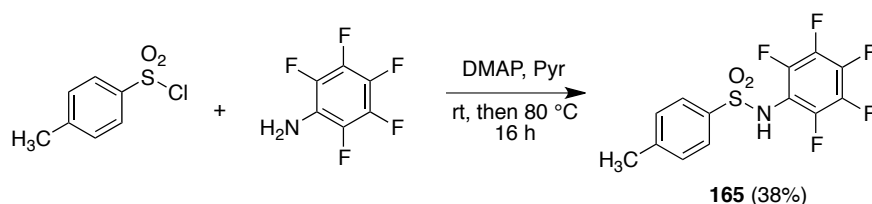
^a: sm = starting material (**163**); da = dimer of acrylate;

^b: Reaction conditions employed in C-H olefination Pd-catalyzed²⁷¹

Owing to the lack of reactivity of our benzenesulfonamide **163**, we decided to move toward the use of a more acidic benzenesulfoanilide derived from pentafluoroaniline. Also in this case, the formation of the desired starting material **165** was achieved according to a known literature procedure²⁷¹ based on the reaction between the *p*-toluenesulfonyl chloride and pentafluoroaniline in

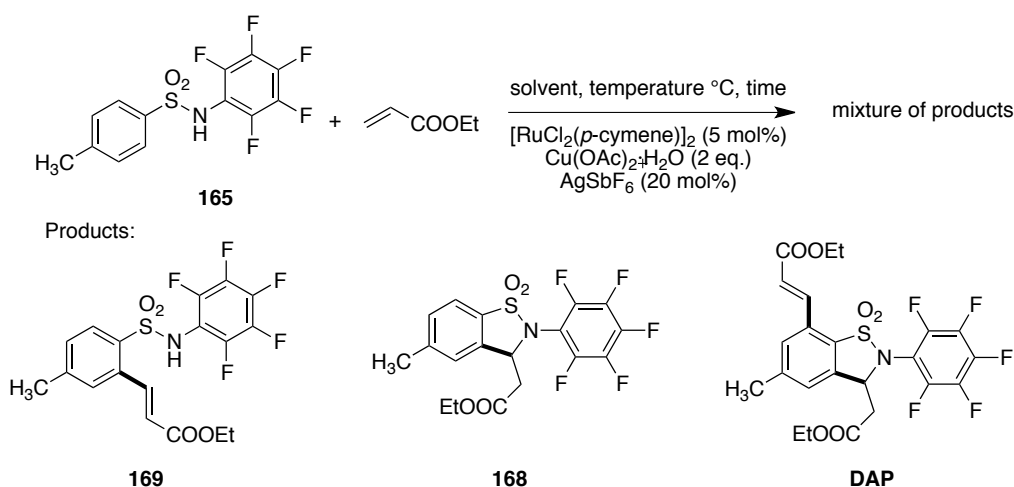
²⁷¹ Dai, H.-X.; Stepan, A. F.; Plummer, M. S.; Zhang, Y.-H.; Yu, J.-Q. *J. Am. Chem. Soc.* **2011**, *133*, 7222-7228

refluxing pyridine in the presence of DMAP for 16 hours (Scheme 8.5).



Scheme 8.5: Synthesis of the new starting material

With this new benzenesulfoanilide in hand, we started again the study of the reaction conditions for the C-H alkenylation; we were pleased to realize that the new *N*-arylbenzenesulfonamide **165** did show a higher reactivity than **163**, affording an encouraging mixture of new products (Scheme 8.6).



Scheme 8.6: Screening of the reaction conditions for the oxidative C-H alkenylation using **165** as starting material

As shown in the Scheme 8.6 and in the Table 8.2, purifying the crude mixtures obtained in these reactions, we could isolate and identify two interesting products (**168** and **169**) obtained by the effective direct C-H *ortho*-alkenylation of the aryl sulfonamide **165**. Notably these two products were both derived from the mono-alkenylation of **165** but, interestingly, in some cases the alkenylated product **169** underwent a chemoselective intramolecular aza-Michael reaction affording the cyclic compound **168** (entries 6-13). Moreover in some experiments it was also

possible to detect (even if it was impossible to achieve its purification due to an identical polarity with **168**) the presence of a small amount of compound **DAP** resulting from a double *ortho*-alkenylation reaction (entries 7-13).

Table 8.2. Study of the catalytic reaction involving **165** as starting material

Entry	Solvent	Conditions	Time (h)	Product (Yield %)			
				sm ^a	169	168	DAP
1	DCE	100 °C, under N ₂	16	77	-	-	-
2	DME	100 °C, under N ₂	16	72	12	-	-
3	1,4-dioxane	100 °C, under N ₂	16	68	9	-	-
4	^t AmOH	120 °C, under N ₂	16	68	10	-	-
5	PhCH ₃	120 °C, under N ₂	16	85	-	-	-
6	DMA	120 °C, under N ₂	16	50	19	5	-
7	DMF	120 °C, under N ₂	16	9	31	6	9
8	H ₂ O	120 °C, under N ₂	16	15	26	15	5
9	DMF/H ₂ O (1/5)	120 °C, under N ₂	20	22	37	15	11
10	DMF/H ₂ O (1/10)	120 °C, under N ₂	20	23	38	17	9
11	DMF/H ₂ O (1/10)	120 °C, under air	16	38	4	38	3
12 ^b	DMF/H ₂ O (1/10)	120 °C, under N ₂	16	12	46	12	23
13 ^{b,c}	DMF/H ₂ O (1/10) ^a	120 °C, under N ₂	16	13	37	12	7

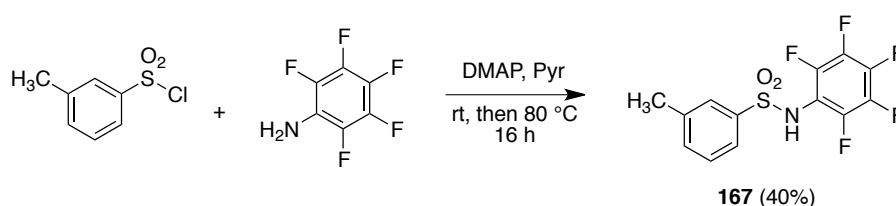
^a: sm = **165**

^b: Reactions carried out in the absence of any additive (AgSbF₆);

^c: In this reaction 1,5 eq. of ethyl acrylate was employed

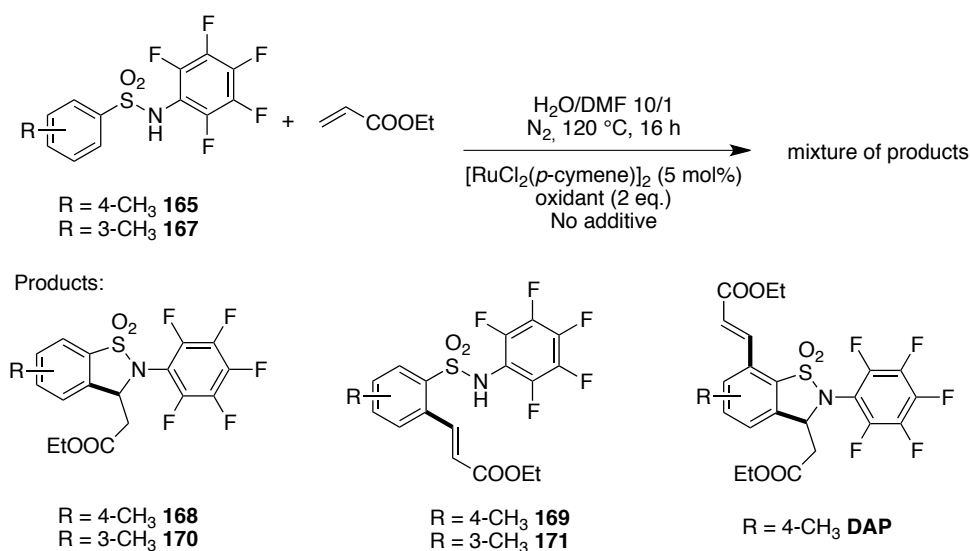
Among the numerous solvents tested (entries 1-8), DMF and water seemed to be the most suitable ones for a better conversion of the starting material **165** (entries 7 and 8); therefore we decided to study the effect of the combination in different ratio of these two solvents. Commendably the two examined different combinations of DMF and H₂O (entries 9 and 10) allowed the formation of the products in interesting yields; we decided to choose the mixture with ratio 1:10 because of its easier handling during the workup. Considering the oxidative character of our reaction, we investigated also the effect of the air atmosphere instead of the usual nitrogen atmosphere and surprisingly the latter conditions gave better results. Finally, it is worth highlighting that, initially, based on our previous laboratory experience, the reactions were performed in presence of AgSbF₆; nevertheless a better conversion of our starting material **165** was achieved in the absence of any additive (entries 12 and 13). After this screening, we decided therefore to choose the conditions reported in the entry 12 as our optimal ones.

Nevertheless, the lack of selectivity of our reaction, with the formation of a mixture of the three products **168**, **169** and **DAP**, represented a relevant problem that remained unsolved in these experimental conditions. We tried to overcome this obstacle by decreasing the amount of the alkenylating agent, but unfortunately, we could not avoid the formation of the di-olefinated product **DAP** (entry 13). Thus, to prevent the formation of this undesired mixture of products, we decided again to change our starting material, employing this time a *meta*-substituted benzene sulfonamide in order to direct the alkenylation process toward the less hindered *ortho*-C-H bond of the aryl sulfonamide. We proceeded therefore to the synthesis of the 3-methyl-*N*-(pentafluorophenyl) benzenesulfonamide **167** employing the same reaction conditions used in the preparation of **165** (Scheme 8.7).



Scheme 8.7: Synthesis of the new *meta*-substituted starting material **167**

With the optimal conditions determined in the previous experiments, we started studying the effects of different oxidizing agents employing starting materials **165** and **167**. As shown in **Scheme 8.8**, the new compound **167** afforded a less complex mixture of products containing only the open **171** and the cyclized mono-olefinated compounds **170**.



Scheme 8.8: Screening of different oxidants for the oxidative C-H alkenylation

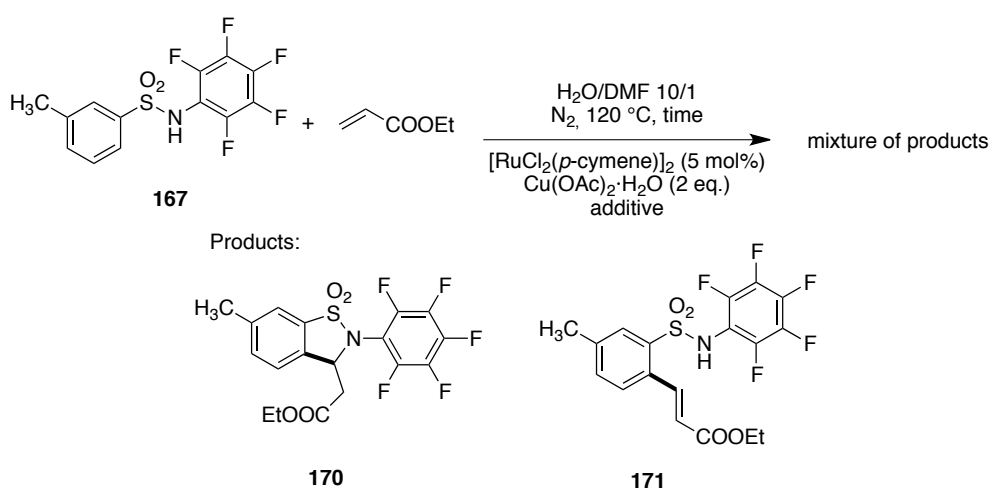
Table 8.3. Screening of different oxidants for the oxidative C-H alkenylation

Entry	Oxidant	Product (Yield %)	
		Sm: 165	Sm: 167
1	$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$	sm (12%), 168 (12%) 169 (46%), DAP (23%)	sm (14%), 170 (21%) 171 (52%)
2	CuBr_2	sm (74%) 168 (5%)	sm (67%), 170 (7%)
3	$\text{CuBr}_2/\text{NaOAc}$	sm (74%), 168 (5%)	sm (48%), 170 (14%) 171 (22%)
4	AgOAc	sm (48%), 168 (18%) 169 (5%)	sm (62%), 170 (6%) 171 (7%)
5	AgX^a	sm (72%), 168 (5%)	sm (61%), 170 (12%)
6	Benzoquinone	No identified products	No identified products
7	$\text{PhI}(\text{OAc})_2$	sm (84%)	sm (89%)

a: AgNO_3 was employed with sm = **165**, whereas AgCl was used with sm = **167**.

As expected, $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ proved to be effective among various sacrificial oxidants. Interestingly, also CuBr_2 and AgNO_3 or AgCl could be employed as oxidants, affording small amounts of the desired products, which could be increased if additional quantities of an acetate salt were present (entries 2-5), thus indicating the importance of the carboxylate assistance. Finally, whereas $\text{PhI}(\text{OAc})_2$ proved to be ineffective as terminal oxidant (entry 7), benzoquinone evidenced some role as a reagent, generating a complex reaction mixture that we could not purify but that did not contain the expected products (entry 8).

In an attempt to improve the conversion of the starting material and to achieve the exclusive formation of the cyclized product **170** derived from the intramolecular *aza*-Michael addition of **171** in a one-pot fashion, we started a screening of different additives (Scheme 8.9).



Scheme 8.9: Screening of different additives for the oxidative C-H alkenylation

As shown in Table 8.4, different additives were tested (entries 2-6) and only AgSbF_6 and KPF_6 gave promising results (entries 5 and 6) with the latter one showing a slightly better conversion of **167** into the desired product **170**. Unfortunately neither increasing the reaction time (entry 7), nor the additive load (entry 8), afforded the expected improvement in the yield of **170**. As the key step for this conversion was the intramolecular *aza*-Michael cyclization of **171**, we decided to examine also the reactivity of two Lewis acids that are usually employed in this kind of reactions; unfortunately, neither CAN nor FeCl_3 could

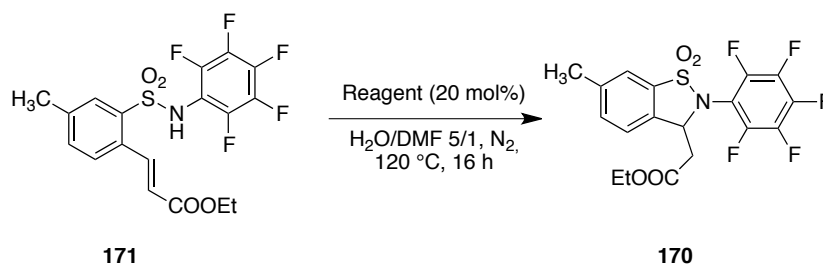
promote the desired cyclization of **171** (entries 9 and 10). Finally we also investigated the effect of the AgSbF_6 and KPF_6 in pure DMF, but once again the reaction afforded the known mixture of **170** and **171**.

Table 8.4. Screening of different additives for the oxidative C-H alkenylation

Entry	Additive	Add. load (mol %)	Time (h)	Products yield (%)		
				sm (167)	170	171
1	-	-	16	14	21	52
2	MesCOOH	20	16	29	25	40
3	MesCOOK	20	16	19	25	29
4	N-Piv-LeuOH	20	16	19	35	30
5	AgSbF_6	20	16	18	28	39
6	KPF_6	20	16	20	30	31
7	KPF_6	20	20	12	30	43
8	KPF_6	30	16	15	22	49
9	CAN	20	16	37	27	13
10	FeCl_3	20	16	20	20	56
11	KPF_6^a	20	16	62	-	12
12	AgSbF_6^a	20	16	62	-	19

^a: reaction carried out in DMF

To finish, we decided to study the conversion of the isolated **171** into **170** by employing different reagents in reaction conditions similar to the ones used for the catalytic reaction (due to the small amount of the reactive employed and its low solubility in water, the ratio between H_2O and DMF was slightly increased from 10/1 to 5/1 in favor of the second one) (Scheme 8.10).

Scheme 8.10: Study of the conditions for the intramolecular α -Michael cyclization of **171**

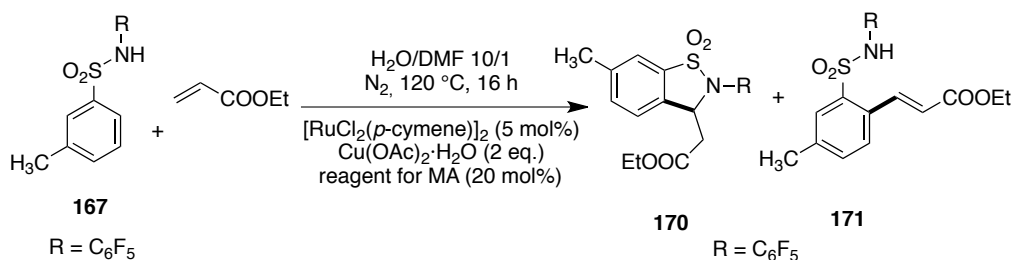
As shown in the [Table 8.5](#) all the examined reagents such as Brønsted acids (entry 1) or bases (entry 2) and Lewis acids (entries 3-5) afforded, in these reaction conditions, a good to excellent conversion of **171** into **170**, even though the reaction seems to proceed slowly (after 4 hours at 120 °C, the reaction was monitored by TLC and only a partial conversion of **171** into **170** was appreciable).

Table 8.5. Use of different reagents for the intramolecular *aza*-Michael cyclization of **171**

Entry	Reagent	Conversion ^a
1	PTSA	88%
2	DBU	81%
3	CAN	88%
4	InCl ₃	93%
5	BF ₃ ·OEt ₂	89%

^a: calculated by H-NMR

With these promising results in hand, we tried again to carry out the reaction in a one-pot fashion using as additives these reagents that had shown to promote the intramolecular *aza*-Michael reaction. The use of PTSA was avoided since, according to our laboratory experience in similar reaction conditions, it underwent the same kind of C-H activation as **167** ([Scheme 8.11](#)).



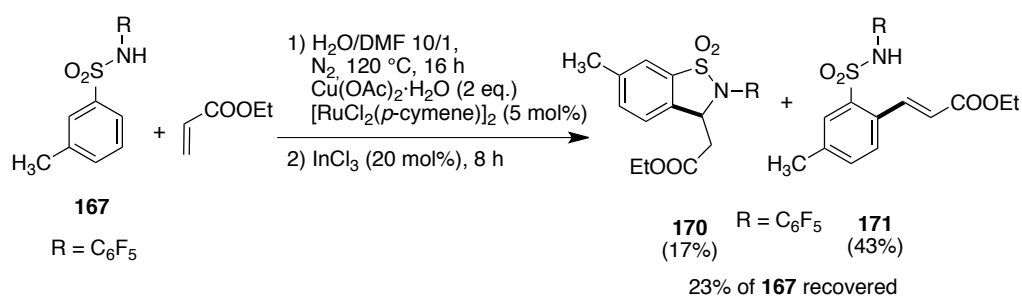
Scheme 8.11: Attempts for a one-pot domino oxidative C-H alkenylation/*aza*-Michael reaction

As shown in the [Table 8.6](#), in the examined conditions none of the reagents employed seemed to promote efficiently the one-pot cyclization, being the major product the open monoolefinated **171**.

Table 8.6 Study for a one-pot domino oxidative C-H alkenylation/aza-Michael reaction

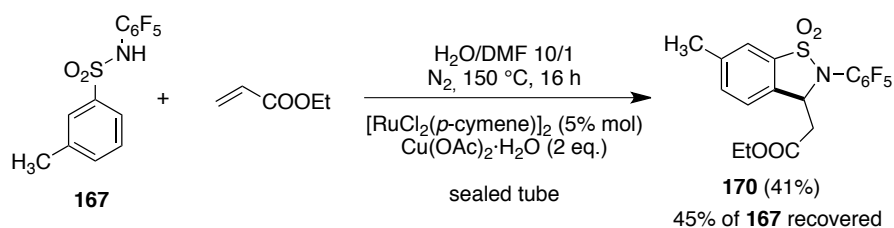
Entry	Additive	Products yield (%)		
		sm (167)	170	171
1	DBU	16	16	51
2	CAN	37	27	13
3	InCl ₃	26	26	48
4	BF ₃ ·OEt ₂	25	20	45

In a further attempt to get exclusively the bicyclic product **170**, we tried to do the reaction in a sequential manner, adding InCl₃ to the mixture after the first 16 hours of reaction, and leaving the whole at the same conditions for additional 8 hours. Unfortunately, as shown in Scheme 8.12, once again, the reaction afforded the usual mixture of compounds **170** and **171**.



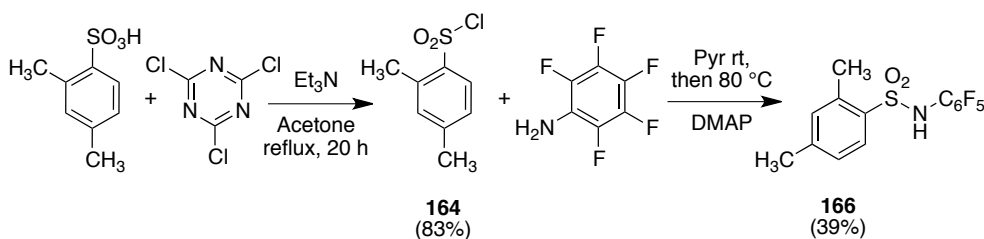
Scheme 8.12: Sequential catalytic reaction

In a further attempt to develop a synthetic methodology that could afford only the cyclized product **170**, we ran the reaction under the standard conditions without any additive and at 150 °C in a sealed tube. In this case, only **170** was isolated after column chromatography in a moderate yield (41% isolated), although with recovery of 45% of the starting material (Scheme 8.13).



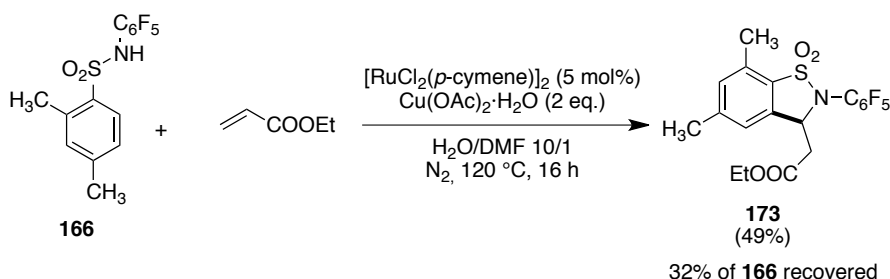
Scheme 8.13: Catalytic reaction performed at 150 °C in a sealed tube.

To reduce molecular complexity and facilitate the C-H activation on the ortho-position of the sulfonamide group we decided to synthesize also the 2,4-dimethylbenzenesulfonamide **166**. According to a literature procedure,²⁷² we initially prepared the sulfonylchloride **164** by reacting 2,4-dimethylbenzenesulfonic acid with 2,4,6-trichloro-1,3,5-triazine in refluxing acetone in the presence of triethylamine; the obtained sulfonyl chloride **164** was then employed in the preparation of the corresponding pentafluoroanilide according to the previously described procedure (Scheme 8.14).



Scheme 8.14: Synthesis of 2,4-dimethylbenzenesulfonamide **166** as starting material.

The behavior of the 2,4-dimethylbenzenesulfonamide **166** was similar to that of **167**, and when it was employed as starting material in our standard condition, we were able to recover, also in this case in a moderate yield, only the cyclized product **173** together with recovered starting material (Scheme 8.15). Unfortunately, due to the identical polarity of the starting material and the final product, it was impossible to isolate and characterize the latter one.

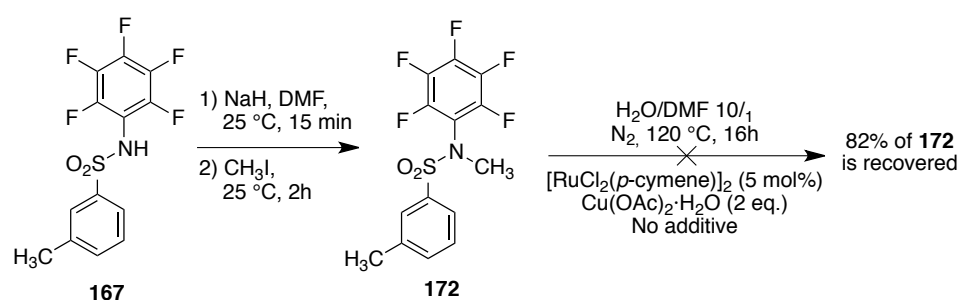


Scheme 8.15: Oxidative C-H alkenylation using **166** as starting material.

²⁷² Blotny, G. *Tetrahedron Lett.* **2003**, *44*, 1499-1501

8.3 MECHANISTIC PROPOSAL

Finally, to prove the importance of the free-NH of the benzenesulfonamides for directing the alkenylation on its *ortho*-position, the N-Me derived of **167** was prepared by a two-step procedure employing sodium hydride in anhydrous DMF to deprotonate the sulfonamide NH followed by the alkylation with methyl iodide, to afford compound **172**.²⁷³

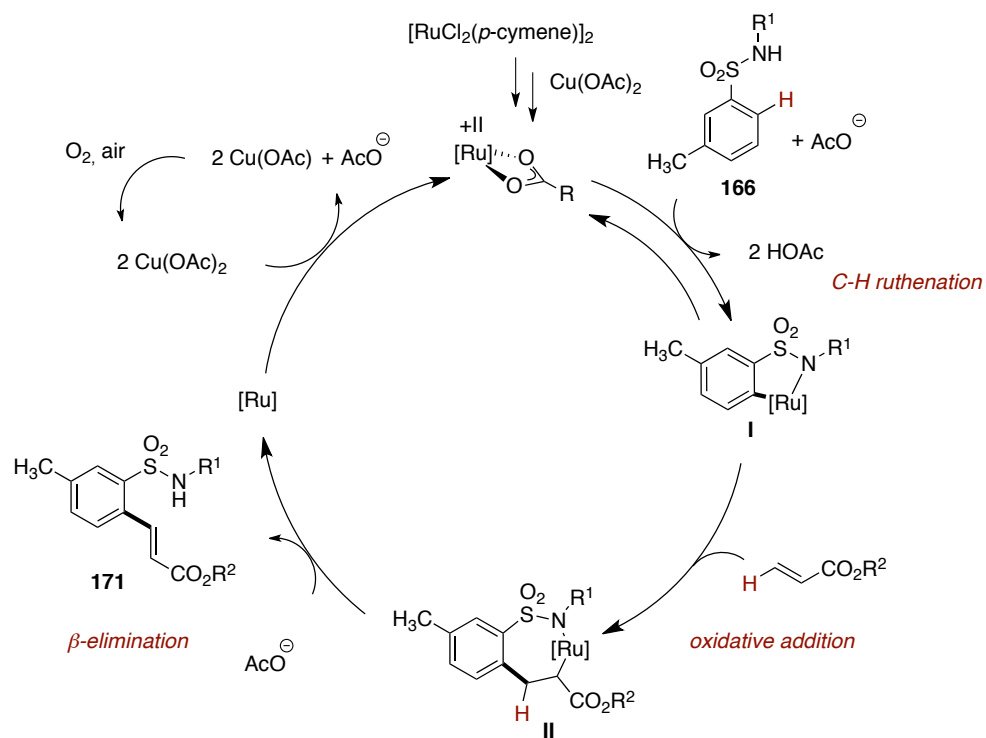


Scheme 8.16: Use of a *N*-methylated starting material **172** for the ruthenium-catalyzed alkenylation.

As expected, in the optimal reaction conditions, this new starting material did not undergo the C-H activation process and the starting material could be recovered after the reaction, confirming the pivotal role of the free NH in the mechanism of the ruthenium-catalyzed alkenylation (Scheme 8.16).

Based on previous mechanistic studies, as shown in the Scheme 8.17, the Ackermann group proposed a plausible catalytic cycle that initiates with a reversible cyclometalation by a cationic ruthenium(II) complex to deliver complex **I**. Then, the subsequent insertion of the alkene generates the ruthenacycle **II**, which by β -hydride elimination furnishes the desired product **171**. Reductive elimination gives rise to a ruthenium(0) species, which is finally re-oxidized by Cu(OAc)₂.

²⁷³ X. Zheng, H. Oda, K. Takamatsu, Y. Sugimoto, A. Tai, E. Akaho, H. I. Ali, T. Oshiki, H. Kakuta, K. Sasaki *Bioorg. Med. Chem.* **2007**, *15*, 1014-1021.



Scheme 8.17: Mechanistic rationale for the oxidative alkenylation of aryl sulfonamide

9. Experimental section

9.1 GENERAL EXPERIMENTAL INFORMATION

All air- and/or moisture-sensitive reactions were carried out under an argon atmosphere in oven-dried glassware. Solvents and reagents were transferred by syringe or via cannula through rubber septa.

Solvents

Solvents were dried and purified by standard procedures. "Petroleum ether" refers to the fraction of light petroleum ether boiling in the range 40-60 °C.

Reagents

All commercially available reagents employed (Panreac, Probus, Scharlau, Fluka, Aldrich, Alfa-Aesar) were used without further purification.

Chromatography

Analytical thin layer chromatography (TLC) was performed using commercially available aluminium-backed plates coated with silica gel Scharlau Cf 530 with fluorescent indicator and visualized under ultra-violet light lamp Camag UV-II (at 254 and 366 nm).

Flash column chromatography was carried out using silica gel SDS 60 ACC or Scharlau Ge 048 and the eluent indicated in each case.

Automatic flash chromatography was performed in a Teledyne ISCO COMBI *Flash* Rf instrument using RediSep Rf silica columns (24 g and 40 g) or self packed silica cartridges.

Melting Point

Melting points were determined either using a Kofler-type heating platine microscope from Reicher, 723 Model, or in a Stuart Scientific apparatus, SMP3 Model, and are uncorrected.

IR Spectroscopy

Infrared spectra were recorded using two different kind of equipments:

- a Perkin-Elmer FTIR Paragon-1000 spectrometer; samples were prepared in a film form, by evaporation of a few drops of sample solution over a sodium chloride or potassium bromide window.
- an Agilent Cary630 FTIR spectrometer with a diamond ATR accessory for solid and liquid samples, requiring no sample preparation.

NMR Spectroscopy

^1H and ^{13}C nuclear magnetic resonance spectra were recorded at 250 and 500 MHz in the following spectrometers: Bruker AV-250 (^1H , 250 MHz; ^{13}C , 63 MHz) and Bruker AV-500 (^1H , 500 MHz; ^{13}C , 125 MHz). CDCl_3 and MeOD were used as deuterated solvents and all chemical shifts are quoted in parts per million and reported as follows: chemical shift δ (ppm) (multiplicity, number of protons, coupling constant J (Hz), assignment). Coupling constants are given in Hertz and multiplicity of NMR signals indicated as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), sext (sextet), hept (heptet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets) and td (triplet of doublets). NMR chemical shifts pointed as * show that the assignment can be interchanged between two or more magnetic nuclei with similar chemical shifts. Spectral assignments were carried out using DEPT, COSY, NOESY, HMQC, and HMBC experiments. The numeration shown in the structures of every single product doesn't correspond to the official IUPAC numeration; its only purpose is to make the NMR assignment easier to understand.

Mass Spectrometry

Gas chromatography mass spectra (GC-MS) were taken with different ionization methods, as Electronic Impact (EI) or Electrospray Ionization (ESI) in both the positive and negative ion mode were carried out by Unidad de Espectrometría de Masas from Universidad Complutense Madrid (Spain).

Elemental Analysis

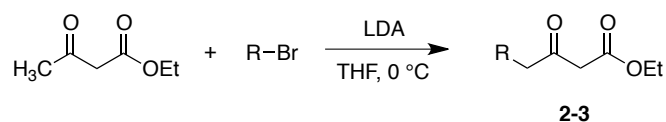
Quantitative elemental analysis by combustion of carbon, hydrogen, nitrogen and

sulfur were carried out in Servicio de Microanálisis Elemental from Universidad Complutense (Madrid, Spain), using a Leco CHNS 932 Elemental Analyzer.

9.2 A NEW MULTICOMPONENT PROTOCOL FOR THE SYNTHESIS OF PYRIDINE DERIVATIVES

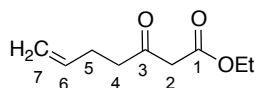
9.2.1 Synthesis of starting β -ketoesters 1-3.

9.2.1.1 Synthesis of β -ketoesters 1 and 2.



Compound	R
2	Allyl
3	

To a solution of LDA (2.3 equiv, 23 mmol), generated *in situ* from diisopropylamine and *n*-butyllithium in THF anhydrous at 0 °C for 30 min, ethyl acetoacetate (1 equiv, 10 mmol) was added. The reaction mixture was stirred for 45 min, then the corresponding bromide derivative (1.3 equiv, 13 mmol) was added and the mixture was stirred for 2 hours at 0 °C. The mixture was quenched with saturated aqueous NH_4Cl solution and allowed to warm to room temperature. The two phases were separated, and the aqueous phase was repeatedly extracted with ethyl acetate. The combined organic layers were washed with brine, dried with Na_2SO_4 and the solvent was evaporated under reduced pressure to give compounds **2** and **3**. The crude product thus obtained was used in subsequent reactions with no further purification.

Ethyl 3-oxohept-6-enoate (2)

Prepared from ethyl acetoacetate (1.301 g, 10 mmol) and allyl bromide (1.573 g, 13 mmol).

Yield: 1.545 g (91%). Pale yellow liquid.

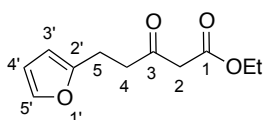
Data of 2:

IR (NaCl) ν 1744 (C-3=O), 1718 (C-1=O), 1317 (C-O), 916 (C=CH₂) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) 1.28 (t, *J* = 7.12 Hz, 3H, OCH₂CH₃), 2.31-2.39 (m, 2H, CH₂-5), 2.60-2.75 (m, 2H, CH₂-4), 3.47 (s, 2H, CH₂-2), 4.20 (q, *J* = 7.12 Hz, 2H, OCH₂CH₃), 4.97-5.08 (m, 2H, CH₂-7), 5.72-5.84 (m, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 202.5 (C-3=O), 167.6 (C-1=O), 137.0 (CH-6), 115.9 (CH₂-7), 61.8 (OCH₂CH₃), 49.8 (CH₂-2), 42.4 (CH₂-4), 27.8 (CH₂-5), 14.5 (OCH₂CH₃) ppm.

These data are consistent with those described in the literature.²⁷⁴

Ethyl 5-(furan-2-yl)-3-oxopentanoate (3)

Prepared from ethyl acetoacetate (1.301 g, 10 mmol) and 2-(bromomethyl)furan (2.079 g, 13 mmol).

Yield: 1.535 g (73%). Brown liquid.

Data of 3:

IR (NaCl) ν 1744 (C-3=O), 1717 (C-1=O), 1317 (C-O) cm⁻¹;

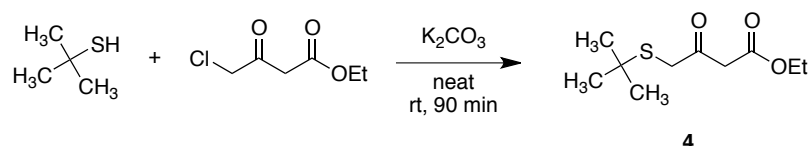
¹H-NMR (CDCl₃, 250 MHz) δ 1.30 (t, *J* = 7.12 Hz, 3H, OCH₂CH₃), 2.91-2.94 (m, 4H, CH₂-4 and CH₂-5), 3.47 (s, 2H, CH₂-2), 4.21 (q, *J* = 7.12 Hz, 2H, OCH₂CH₃), 6.03 (dd, *J* = 0.75, 3.13 Hz, 1H, CH-3'), 6.29 (dd, *J* = 1.85, 3.13 Hz, 1H, CH-4'), 7.31 (dd, *J* = 0.75, 1.85 Hz, 1H, CH-5') ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 201.8 (C-3=O), 167.4 (C-1=O), 154.4 (C-2'), 141.5 (CH-5'), 110.6 (CH-4'), 105.7 (CH-3'), 61.7 (OCH₂CH₃), 49.5 (CH₂-2), 41.3 (CH₂-4), 22.2 (CH₂-5), 14.4 (OCH₂CH₃) ppm.

These data are consistent with those described in the literature.²⁷⁵

²⁷⁴ Huckin, S. N.; Weiler, L. J. *Am. Chem. Soc.* **1974**, *96*, 1082-1087.

²⁷⁵ Matsui, M.; LaForge, F. B.; Green, N.; Schechter, M. S. *J. Am. Chem. Soc.* **1952**, *74*, 2181-2182.

9.2.1.2 Synthesis of ethyl 4-(*tert*-butylthio)-3-oxobutanoate (**4**)

A mixture of 2-methylpropane-2-thiol (1 equiv, 10 mmol), ethyl 4-chloro-3-oxobutanoate (1 equiv, 10 mmol) and potassium carbonate (1.1 equiv, 11 mmol) was stirred, in the absence of any solvents, for 90 min. After completion of the reaction (checked by TLC), the mixture was diluted with CH₂Cl₂ (40 mL), washed with water (10 mL) and brine, and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure, and the residue mixture was then purified by silica gel column chromatography using petroleum ether:ethyl acetate mixture (from 10:1 to 5:1, v/v) as eluent to give 1.725 g (79% yield) of **4** as a brown liquid.

Data of 4:

Elemental analysis calcd (%) for C₁₀H₈O₃S: C 55.02, H 8.31, S 14.69; found: C 54.78, H 8.30, S 14.85.

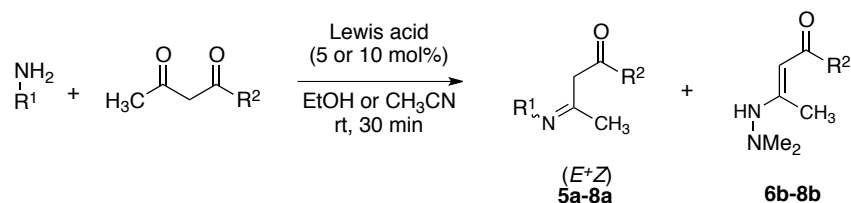
IR (NaCl) ν 1735 (C-1=O), 1366 (C-1-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.24-1.34 (m, 12H, OCH₂CH₃ and SC(CH₃)₃), 3.44 (s, 2H, CH₂-2), 3.69 (s, 2H, CH₂-4), 4.19 (q, *J* = 7.12 Hz, 2H, OCH₂CH₃) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 200.7 (C-3=O), 167.6 (C-1=O), 61.7 (OCH₂CH₃), 46.8 (CH₂-2), 44.0 (SC(CH₃)₃), 40.0 (CH₂-4), 31.1 (SC(CH₃)₃), 14.4 (OCH₂CH₃) ppm.

These data are consistent with those described in the literature.²⁷⁶

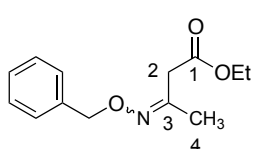
²⁷⁶ Hutchinson, J. H.; Riendeau, D.; Brideau, C.; Chan, C.; Delorme, D.; Denis, D.; Falguyret, J. P.; Fortin, R.; Guay, J. *J. Med. Chem.* **1993**, *36*, 2771-2787.

9.2.2 Synthesis of the oxime and the β -enaminones 5-8.

Compound	R ¹	R ²
5	OBn	OEt
6	NMe ₂	OEt
7	NMe ₂	SC(CH ₃) ₃
8	NMe ₂	Ph

To a stirred solution of 1,1-dimethylhydrazine or the suitable *O*-substituted hydroxylamine (1 equiv, 1 mmol) and 1,3-dicarbonyl compounds (1 equiv, 1 mmol) in ethanol or acetonitrile (1 mL) was added a Lewis acid (5 or 10 mol%) and stirring was continued for 30 min at room temperature. After completion of the reaction (checked by TLC), the mixture was diluted with CH₂Cl₂ (30 mL), washed with water followed by brine, dried over anhydrous Na₂SO₄, and the solvent was evaporated under reduced pressure.

Data of representative compounds **5-8**, and the experimental conditions employed for their synthesis are indicated below.

(Z and E)-Ethyl 3-(benzyloxyimino)butanoate (5a)

Prepared from *O*-benzylhydroxylamine (123 mg, 1 mmol), ethyl acetoacetate (130 mg, 1 mmol) and CAN (27 mg, 5 mol%).

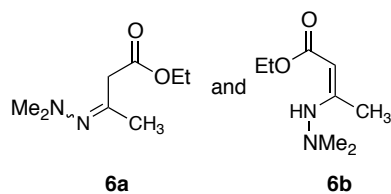
Reaction time: 30 minutes.

Yield: 222 mg (95%). Colorless viscous liquid.

Data of 5a:

¹H-NMR (CDCl₃, 250 MHz) two isomers δ 1.23-1.32 (m, 3H, OCH₂CH₃), 2.00 and 2.01 (s, 3H, CH₃-4), 3.24 and 3.40 (s, 2H, CH₂-2), 4.12-4.24 (m, 2H, OCH₂CH₃), 5.12 and 5.15 (s, 2H, OCH₂Ph), 7.29-7.40 (m, 5H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) two isomers δ 170.0 and 169.2 (C-1=O), 152.4 and 151.3 (C-3=N), 138.4 and 138.3 (CAr), 128.75 and 128.72 (2xCHAR), 128.3 and 128.2 (2xCHAR), 128.13 and 128.10 (CHAR), 76.0 and 75.9 (OCH₂Ph), 61.4 and 61.3 (OCH₂CH₃), 41.8 and 36.0 (CH₂-2), 21.0 and 15.1 (CH₃-4), 14.57 and 14.52 (OCH₂CH₃) ppm.

(Z and E)-Ethyl 3-(2,2-dimethylhydrazono)butanoate (6a) and ethyl 3-(2,2-dimethylhydrazinyl)but-2-enoate (6b)

Prepared from 1,1-dimethylhydrazine (60 mg, 1 mmol), ethyl acetoacetate (130 mg, 1 mmol) and InCl₃ (22 mg, 10 mol%).

Reaction time: 30 minutes.

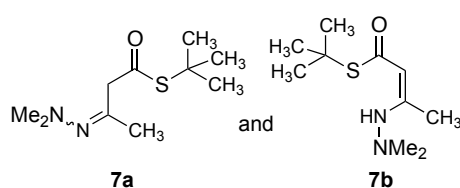
Yield: 169 mg (98%). Pale yellow viscous liquid.

Data of 6a and 6b:

¹H-NMR (CDCl₃, 250 MHz) three isomers δ 1.19-1.28 (m, 3H, OCH₂CH₃ for **6a** and **6b**), 1.97 (s, 3H, CH₃-4 for **6b**), 1.99 and 2.01 (s, 3H, CH₃-4 for **6a**), 2.37 and 2.45 (s, 6H, N(CH₃)₂ for **6a**), 2.49 (s, 6H, N(CH₃)₂ for **6b**), 3.22 and 3.48 (s, 2H, CH₂-2 for **6a**), 4.01-4.15 (m, 2H, OCH₂CH₃ for **6a** and **6b**), 4.32 (s, 1H, CH-2 for **6b**), 8.88 (bs, 1H, NH for **6b**) ppm.

These data are consistent with those described in the literature.²⁷⁷

(Z and E)-S-tert-butyl 3-(2,2-dimethylhydrazono)butanethioate (7a) and S-tert-Butyl 3-(2,2-dimethylhydrazinyl)but-2-enethioate (7b)



Prepared from 1,1-dimethylhydrazine (60 mg, 1 mmol), *S*-tert-butyl 3-oxobutane thioate (174 mg, 1 mmol) and InCl_3 (22 mg, 10 mol%).

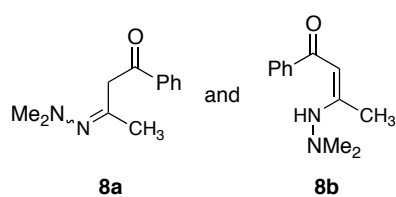
Reaction time: 20 minutes.

Yield: 210 mg (97%). Orange viscous liquid.

Data of 7a and 7b:

¹H-NMR (CDCl_3 , 250 MHz) three isomers δ 1.47, 1.48 and 1.50 (s, 9H, $\text{S}(\text{CH}_3)_3$ for 7a and 7b), 1.96 (s, 3H, CH_3 -4 for 7b), 2.02 and 2.08 (s, 3H, CH_3 -4 for 7a), 2.42 and 2.50 (s, 6H, $\text{N}(\text{CH}_3)_2$ for 7a), 2.53 (s, 6H, $\text{N}(\text{CH}_3)_2$ for 7b), 3.40 and 3.72 (s, 2H, CH_2 -2 for 7a), 4.77 (s, 1H, CH -2 for 7b), 9.65 (bs, 1H, NH for 7b) ppm.

(Z and E)-3-(2,2-dimethylhydrazono)-1-phenylbutan-1-one (8a) and 3-(2,2-Dimethylhydrazinyl)-1-phenylbut-2-en-1-one (8b)



Prepared from 1,1-dimethylhydrazine (60 mg, 1 mmol), 1-phenylbutane-1,3-dione (162 mg, 1 mmol) and InCl_3 (22 mg, 10 mol%).

Reaction time: 30 minutes.

Yield: 195 mg (96%). Pale yellow viscous liquid.

Data of 8a and 8b:

¹H-NMR (CDCl_3 , 250 MHz) three isomers δ 2.20 (s, 3H, CH_3 -4 for 8b), 2.23 and 2.33 (s, 3H, CH_3 -4 for 8a), 2.63 (s, 6H, $\text{N}(\text{CH}_3)_2$ for 8a and 8b), 3.85 (s, 2H, CH_2 -2 for 8a), 5.59 (s, 1H, CH -2 for 8b), 7.29-7.47 (m, 3H, ArH for 8a and 8b), 7.85-7.91 (m, 2H, ArH for 8a and 8b), 11.76 (bs, 1H, NH for 8b) ppm.

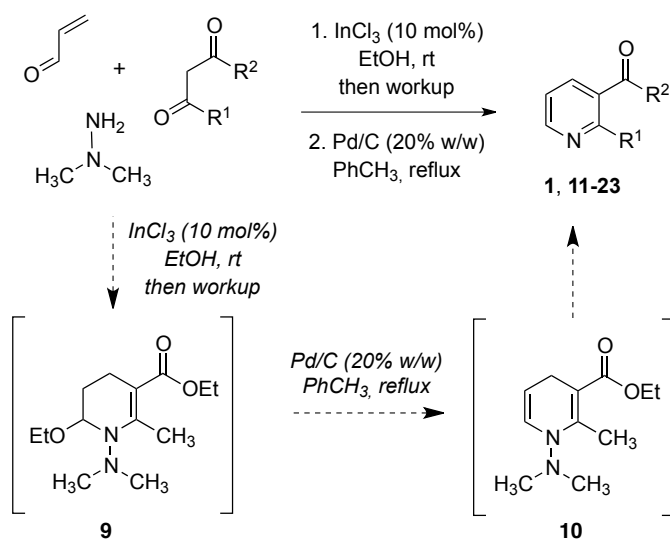
²⁷⁷ Coqueret, X.; Bourelle-Wargnier, F.; Chucho, J. *Tetrahedron* **1986**, *42*, 2263-2273.

¹³C-NMR (CDCl₃, 63 MHz) δ 188.0 (C-1=O), 165.1 (C-3), 140.4 (CAr), 130.9 (CHAR), 128.5 (2xCHAR), 127.3 (2xCHAR), 90.4 (CH-2), 48.8 (N(CH₃)₂), 19.0 (CH₃-4) ppm.

These data are consistent with those described in the literature.²⁷⁸

²⁷⁸ Jones, W. D.; Schnettler, R. A.; Huber, E. W. *J. Heterocyclic Chem.* **1990**, *27*, 511-518.

9.2.3 Synthesis of pyridine derivatives 1, 11-23. General procedure



Cmpd.	R ¹	R ²	Cmpd.	R ¹	R ²
1	CH ₃	OEt	17		OEt
11	Et	OEt	18	CH ₃	O ^t Bu
12	Pr	OEt	19	CH ₃	OAllyl
13	ⁱ Pr	OEt	20	CH ₃	S ^t Bu
14	CH ₂ S ^t Bu	OEt	21	CH ₃	CH ₃
15	(CH ₂) ₂ CH=CH ₂	OEt	22	CH ₃	Ph
16	CH ₂ COOEt	OEt	23	CH ₃	ⁱ Bu

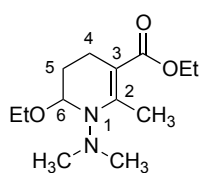
To a stirred solution of 1,1-dimethylhydrazine (1 equiv, 3 mmol) and the appropriate 1,3-dicarbonyl compound (1 equiv, 3 mmol) in ethanol (3 mL) was added InCl_3 (10 mol%) and stirring was continued for 30 min at room temperature. Acrolein (1.2 equiv, 3.6 mmol) was then added and stirring was continued at the same conditions for the time period specified in the compound data sheet. After completion of the reaction (checked by NMR), the mixture was diluted with CH_2Cl_2 (20 mL), washed with water followed by brine, dried over anhydrous Na_2SO_4 , and the solvent was evaporated under reduced pressure.

The residue containing the tetrahydropyridine derivative was then dissolved in

toluene (10 mL), Palladium on Carbon 10 wt % loading (20% wt/crude) was added and the resulting mixture was heated under reflux for the time periods specified in the compound data sheet. After completion of the reaction (checked by TLC), the mixture was diluted with CH₂Cl₂ (20 mL) and filtered through Celite, which was then washed repeatedly with additional CH₂Cl₂ in 5 mL portions. The combined organic layers were concentrated to dryness and the residue was purified by silica gel column chromatography using petroleum ether:ethyl acetate mixtures as eluent to give pure compounds (**1**, **11-23**).

Data of compounds **1**, **9**, **10** and **11-23**, and the experimental conditions employed for their synthesis are indicated below.

Ethyl 1-(dimethylamino)-6-ethoxy-2-methyl-1,4,5,6-tetrahydropyridine-3-carboxylate (**9**)



Isolated from the reaction among 1,1-dimethylhydrazine, ethyl acetoacetate and acrolein, without performing any further step of oxidation.

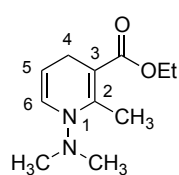
Pale yellow viscous liquid. In the spectra there is also a low percentage of the dihydropyridine derivative **10** due to the oxidation of **9** during the chromatographic purification.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 10:1, v/v)

Data of **9**:

¹H-NMR (CDCl₃, 250 MHz) δ 1.12-1.28 (m, 6H, 2xOCH₂CH₃), 1.99-2.95 (m, 2H, CH₂-5), 2.20-2.28 (m, 2H, CH₂-4), 2.37 (s, 3H, C-2CH₃), 2.39 (s, 3H, NCH₃), 2.55 (s, 3H, NCH₃), 3.38-3.49 (m, 2H, C-6OCH₂CH₃), 4.00-4.07 (m, 2H, COOCH₂CH₃), 4.67 (bs 1H, CH-6) ppm.

Ethyl 1-(dimethylamino)-2-methyl-1,4-dihydropyridine-3-carboxylate (**10**)



Generated from the partial oxidation of **9**.

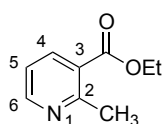
Pale yellow viscous liquid.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 10:1, v/v)

Data of **10**:

¹H-NMR (CDCl₃, 250 MHz) δ 1.28 (t, *J* = 7.1, 3H, OCH₂CH₃), 2.38 (s, 3H, C-2CH₃), 2.53 (s, 6H, N(CH₃)₂), 3.11-3.13 (m, 2H, CH₂-4), 4.15 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.92 (dt, *J* = 3.75, 8.2 Hz, 1H, CH-5), 6.05 (td, *J* = 1.5, 8.2 Hz, 1H, CH-6) ppm.

Ethyl 2-methylnicotinate (**1**)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl acetoacetate (390 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 3 h for the first step and 12 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v)

Yield: 420 mg (85%). Pale yellow viscous liquid.

Data of 1:

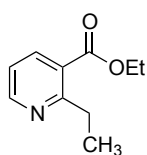
Elemental analysis calcd (%) for C₉H₁₁NO₂: C 65.44, H 6.71, N 8.48; found: C 65.18, H 6.81, N 8.29.

IR (NaCl) ν : 1724 (C=O), 1585 (C=N), 1278 (C-O) cm⁻¹

¹H-NMR (CDCl₃, 250 MHz) δ 1.41 (t, *J* = 7.3 Hz, 3H, OCH₂CH₃), 2.86 (s, 3H, C-2CH₃), 4.39 (q, *J* = 7.3 Hz, 2H, OCH₂CH₃), 7.22 (dd, *J* = 4.8, 7.85 Hz, 1H, CH-5), 8.20 (dd, *J* = 1.8, 7.85 Hz, 1H, CH-4), 8.62 (dd, *J* = 1.8, 4.8 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 167.0 (C=O), 160.2 (C-2), 152.1 (CH-6), 138.8 (CH-4), 126.1 (C-3), 121.3 (CH-5), 61.7 (OCH₂CH₃), 25.2 (C-2CH₃), 14.7 (OCH₂CH₃) ppm.

Ethyl 2-ethylnicotinate (**11**)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl 3-oxopentanoate (433 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 3 h for the first step and 12 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 397 mg (74%). Pale yellow viscous liquid.

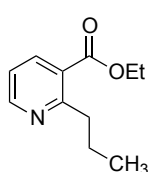
Data of 11:

Elemental analysis calcd (%) for C₁₀H₁₃NO₂: C 67.02, H 7.31, N 7.82; found: C 66.89, H 7.29, N 7.69.

IR (NaCl) ν 1724 (C=O), 1584 (C=N), 1269 (C-O) cm⁻¹

¹H-NMR (CDCl₃, 250 MHz) δ 1.33 (t, *J* = 7.5 Hz, 3H, OCH₂CH₃), 1.42 (t, *J* = 7.15 Hz, 3H, C-2CH₂CH₃), 3.19 (q, *J* = 7.5 Hz, 2H, C-2CH₂CH₃), 4.40 (q, *J* = 7.15 Hz, 2H, OCH₂CH₃), 7.22 (dd, *J* = 4.8, 7.87 Hz, 1H, CH-5), 8.16 (dd, *J* = 1.8, 7.87 Hz, 1H, CH-4), 8.66 (dd, *J* = 1.8, 4.8 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 167.1 (C=O), 164.7 (C-2), 152.1 (CH-6), 138.7 (CH-4), 125.9 (C-3), 121.1 (CH-5), 61.7 (OCH₂CH₃), 30.7 (C-2CH₂CH₃), 14.6 (OCH₂CH₃), 14.3 (C-2CH₂CH₃) ppm.

Ethyl 2-propylnicotinate (12)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl butyrylacetate (475 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 3 h for the first step and 12 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 440 mg (76%). Pale yellow viscous liquid.

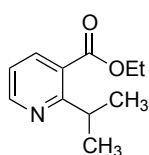
Data of 12:

Elemental analysis calcd (%) for C₁₁H₁₅NO₂: C 68.37, H 7.82, N 7.25; found: C 68.19, H 7.77, N 7.53.

IR (NaCl) ν 1725 (C=O), 1584 (C=N), 1261 (C-O) cm⁻¹

¹H-NMR (CDCl₃, 250 MHz) δ 1.00 (t, *J* = 7.35 Hz, 3H, C-2CH₂CH₂CH₃), 1.40 (t, *J* = 7.15 Hz, 3H, OCH₂CH₃), 1.70-1.79 (m, 2H, C-2CH₂CH₂CH₃), 3.10-3.16 (m, 2H, C-2CH₂CH₂CH₃), 4.38 (q, *J* = 7.15 Hz, 2H, OCH₂CH₃), 7.19 (dd, *J* = 4.8, 7.87 Hz, 1H, CH-5), 8.14 (dd, *J* = 1.8, 7.87 Hz, 1H, CH-4), 8.64 (dd, *J* = 1.8, 4.8 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 167.2 (C=O), 163.6 (C-2), 152.0 (CH-6), 138.7 (CH-4), 126.2 (C-3), 121.1 (CH-5), 61.7 (OCH₂CH₃), 39.4 (C-2CH₂CH₂CH₃), 23.7 (C-2CH₂CH₂CH₃), 14.58 (OCH₂CH₃*), 14.54 (C-2CH₂CH₂CH₃*) ppm.

Ethyl 2-isopropylnicotinate (13)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl isobutyrylacetate (475 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 9 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 395 mg (68%). Yellow viscous liquid.

Data of 13:

Elemental analysis calcd (%) for C₁₁H₁₅NO₂: C 68.37, H 7.82, N 7.25; found: C

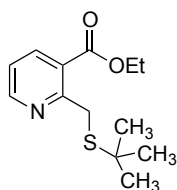
68.05, H 7.82, N 7.39.

IR (NaCl) ν 1724 (C=O), 1585 (C=N), 1278 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.34 (d, $J = 6.75$ Hz, 6H, C-2CH(CH₃)₂), 1.43 (t, $J = 7.15$ Hz, 3H, OCH₂CH₃), 3.85 (sep, $J = 6.75$ Hz, 1H, C-2CH(CH₃)₂), 4.42 (q, $J = 7.15$ Hz, 2H, OCH₂CH₃), 7.20 (dd, $J = 4.78, 7.88$ Hz, 1H, CH-5), 8.06 (dd, $J = 1.88, 7.88$ Hz, 1H, CH-4), 8.71 (dd, $J = 1.88, 4.78$ Hz, 1H, CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 167.7 (C=O*), 167.5 (C-2*), 152.0 (CH-6), 138.2 (CH-4), 126.1 (C-3), 120.9 (CH-5), 61.8 (OCH₂CH₃), 32.7 (C-2CH(CH₃)₂), 22.7 (C-2CH(CH₃)₂), 14.6 (OCH₂CH₃) ppm.

Ethyl 2-(*tert*-butylthiomethyl)nicotinate (**14**)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl 4-(*tert*-butylthio)-3-oxobutanoate **3** (654 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 5 h for the first step and 12 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 12:1 to 3:1, v/v).

Yield: 440 mg (58%). Yellow viscous liquid.

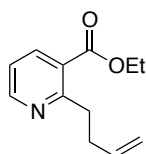
Data of **14**:

Elemental analysis calcd (%) for C₁₃H₁₉NO₂S: C 61.63, H 7.56, N 5.53, S 12.66; found: C 61.78, H 7.29, N 5.75, S 12.85.

IR (NaCl) ν 1724 (C=O), 1570 (C=N), 1266 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.35 (s, 9H, SC(CH₃)₃), 1.43 (t, $J = 7.15$ Hz, 3H, OCH₂CH₃), 4.38 (s, 2H, C-2CH₂S), 4.42 (q, $J = 7.15$ Hz, 2H, OCH₂CH₃), 7.26 (dd, $J = 4.85, 7.95$ Hz, 1H, CH-5), 8.20 (dd, $J = 1.78, 7.95$ Hz, 1H, CH-4), 8.64 (dd, $J = 1.78, 4.85$ Hz, 1H, CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 166.7 (C=O), 161.0 (C-2), 151.9 (CH-6), 139.2 (CH-4), 126.4 (C-3), 122.1 (CH-5), 62.0 (OCH₂CH₃), 43.4 (SC(CH₃)₃), 34.9 (C-2CH₂S), 31.3 (SC(CH₃)₃), 14.6 (OCH₂CH₃) ppm.

Ethyl 2-(but-3-enyl)nicotinate (15)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), ethyl 3-oxohept-6-enoate **1** (510 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 5 h for the first step and 12 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 350 mg (57%). Yellow viscous liquid.

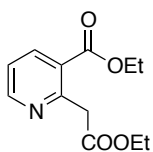
Data of 15:

Elemental analysis calcd (%) for C₁₂H₁₅NO₂: C 70.22, H 7.37, N 6.82; found: C 69.93, H 7.44, N 6.72.

IR (NaCl) ν 1725 (C=O), 1584 (C=N), 1253 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) 1.43 (t, *J* = 7.15 Hz, 3H, OCH₂CH₃), 2.47-2.56 (m, 2H, C-2CH₂CH₂), 3.25-3.31 (m, 2H, C-2CH₂CH₂), 4.41 (q, *J* = 7.15 Hz, 2H, OCH₂CH₃), 4.96-5.12 (m, 2H, CH₂CH=CH₂), 5.89-6.00 (m, 1H, CH₂CH=CH₂), 7.24 (dd, *J* = 4.78, 7.88 Hz, 1H, CH-5), 8.18 (dd, *J* = 1.85, 7.88 Hz, 1H, CH-4), 8.68 (dd, *J* = 1.85, 4.78 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 167.1 (C=O), 162.8 (C-2), 152.2 (CH-6), 138.9 (CH-4), 138.4 (CH₂CH=CH₂), 126.3 (C-3), 121.3 (CH-5), 115.3 (CH₂CH=CH₂), 61.8 (OCH₂CH₃), 36.8 (C-2CH₂CH₂), 34.3 (C-2CH₂CH₂), 14.7 (OCH₂CH₃) ppm.

Ethyl 2-(2-ethoxy-2-oxoethyl)nicotinate (16)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), diethyl 3-oxopentanedioate (606 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 30 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).

Yield: 584 mg (82%). Yellow viscous liquid.

Data of 16:

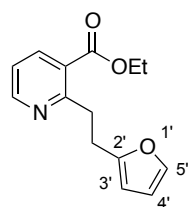
Elemental analysis calcd (%) for C₁₂H₁₅NO₄: C 60.75, H 6.37, N 5.90; found: C 60.60, H 6.44, N 6.03.

IR (NaCl) ν 1738 (C=O), 1722 (C=O), 1573 (C=N), 1270 (C-O), 1246 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.28 (t, $J = 7.13$ Hz, 3H, C-2 $\text{CH}_2\text{CO}_2\text{CH}_2\text{CH}_3$), 1.41 (t, $J = 7.13$ Hz, 3H, C-3 $\text{CO}_2\text{CH}_2\text{CH}_3$), 4.21 (q, $J = 7.13$ Hz, 2H, C-2 $\text{CH}_2\text{CO}_2\text{CH}_2\text{CH}_3$), 4.31 (s, 2H, C-2 $\text{CH}_2\text{CO}_2\text{CH}_2\text{CH}_3$), 4.39 (q, $J = 7.13$ Hz, 2H, C-3 $\text{CO}_2\text{CH}_2\text{CH}_3$), 7.36 (dd, $J = 4.83, 7.9$ Hz, 1H, CH-5), 8.34 (dd, $J = 1.75, 7.9$ Hz, 1H, CH-4), 8.71 (dd, $J = 1.75, 4.83$ Hz, 1H, CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 170.7 (C-2 $\text{CH}_2\text{C=O}$), 166.0 (C-3 C=O), 155.8 (C-2), 152.0 (CH-6), 138.7 (CH-4), 126.2 (C-3), 122.2 (CH-5), 61.5 ($\text{CH}_2\text{CO}_2\text{CH}_2\text{CH}_3$), 60.9 (C-3 $\text{CO}_2\text{CH}_2\text{CH}_3$), 43.9 (C-2 CH_2COOR), 14.2 (2 $\times\text{OCH}_2\text{CH}_3$) ppm.

Ethyl 2-[2-(furan-2-yl)ethyl]nicotinate (17)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), 5-(furan-2-yl)-3-oxopentanoate **2** (630 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 36 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).

Yield: 515 mg (70%). Yellow viscous liquid.

Data of **17**:

Elemental analysis calcd (%) for $\text{C}_{14}\text{H}_{15}\text{NO}_3$: C 68.56, H 6.16, N 5.71; found: C 68.38, H 5.93, N 5.68.

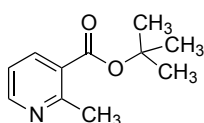
IR (NaCl) ν 1722 (C=O), 1569 (C=N), 1258 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.43 (t, $J = 7.15$ Hz, 3H, OCH_2CH_3), 3.09-3.16 (m, 2H, C-2 CH_2CH_2), 3.51-3.57 (m, 2H, C-2 CH_2CH_2), 4.42 (q, $J = 7.15$ Hz, 2H, OCH_2CH_3), 6.04 (dd, $J = 0.75, 3.13$ Hz, 1H, CH-3'), 6.30 (dd, $J = 1.85, 3.13$ Hz, 1H, CH-4'), 7.27 (dd, $J = 4.8, 7.9$ Hz, 1H, CH-5), 7.34 (dd, $J = 0.75, 1.85$ Hz, 1H, CH-5'), 8.21 (dd, $J = 1.83, 7.9$ Hz, 1H, CH-4), 8.70 (dd, $J = 1.83, 4.8$ Hz, 1H, CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 167.0 (C=O), 162.0 (C-2), 155.9 (C-2'), 152.2 (CH-6), 141.3 (CH-5'), 138.9 (CH-4), 126.4 (C-3), 121.5 (CH-5), 110.5 (CH-4'), 105.5 (CH-3'), 61.9 (OCH_2CH_3), 35.9 (C-2 CH_2CH_2), 28.3 (C-2 CH_2CH_2), 14.6

(OCH₂CH₃) ppm.

***tert*-Butyl 2-methylnicotinate (18)**



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), *tert*-butyl acetoacetate (475 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 15 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 359 mg (62%). Yellow viscous liquid.

Data of 18:

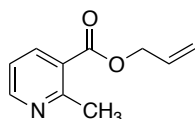
Elemental analysis calcd (%) for C₁₁H₁₅NO₂: C 68.37, H 7.82, N 7.25; found: C 68.12, H 7.72, N 7.36.

IR (NaCl) ν 1721 (C=O), 1584 (C=N), 1287 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.62 (s, 9H, OC(CH₃)₃), 2.82 (s, 3H, C-2CH₃), 7.20 (dd, *J* = 4.8, 7.85 Hz, 1H, CH-5), 8.11 (dd, *J* = 1.8, 7.85 Hz, 1H, CH-4), 8.59 (dd, *J* = 1.8, 4.8 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 166.5 (C=O), 159.5 (C-2), 151.6 (CH-6), 138.6 (CH-4), 127.8 (C-3), 121.2 (CH-5), 82.4 (C(CH₃)₃), 28.6 (C(CH₃)₃), 25.2 (C-2CH₃) ppm.

Allyl 2-methylnicotinate (19)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), allyl acetoacetate (426 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 3 h for the first step and 36 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 355 mg (67%). Yellow viscous liquid.

Data of 19:

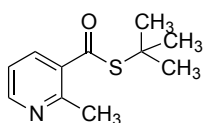
Elemental analysis calcd (%) for C₁₀H₁₁NO₂: C 67.78, H 6.26, N 7.90; found: C 67.56, H 6.63, N 8.10.

IR (NaCl) ν 1726 (C=O), 1585 (C=N), 1275 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 2.88 (s, 3H, C-2CH₃), 4.86 (dt, *J* = 1.28-5.75 Hz, 2H, OCH₂CH=CH₂), 5.31-5.49 (m, 2H, OCH₂CH=CH₂), 5.99-6.12 (m, 1H, OCH₂CH=CH₂), 7.26 (dd, *J* = 4.85, 7.88 Hz, 1H, CH-5), 8.25 (dd, *J* = 1.78, 7.88 Hz, 1H, CH-4), 8.65 (dd, *J* = 1.78, 4.85 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 166.6 (C=O), 160.4 (C-2), 152.3 (CH-6), 138.9 (CH-4), 132.3 (OCH₂CH=CH₂), 125.8 (C-3), 121.3 (CH-5), 119.2 (OCH₂CH=CH₂), 66.3 (OCH₂CH=CH₂), 25.3 (C-2CH₃) ppm.

***S*-tert-Butyl 2-methylpyridine-3-carbothioate (20)**



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), *S*-tert-butyl 3-oxobutanethioate (523 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 5 h for the first step and 15 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 5:1, v/v).

Yield: 477 mg (76%). Pale brown viscous liquid.

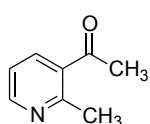
Data of 20:

Elemental analysis calcd (%) for C₁₁H₁₅NOS: C 63.12, H 7.22, N 6.69, S 15.32; found: C 62.95, H 5.65, N 7.51, S 15.29.

IR (NaCl) ν 1662 (C=O), 1563 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.59 (s, 9H, SC(CH₃)₃), 2.69 (s, 3H, C-2CH₃), 7.18 (dd, *J* = 4.9, 7.75 Hz, 1H, CH-5), 7.91 (dd, *J* = 1.6, 7.75 Hz, 1H, CH-4), 8.57 (dd, *J* = 1.6, 4.9 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 195.1 (C=O), 156.3 (C-2), 151.3 (CH-6), 135.8 (CH-4), 134.6 (C-3), 121.0 (CH-5), 49.6 (SC(CH₃)₃), 30.1(SC(CH₃)₃), 23.7 (C-2CH₃) ppm.

1-(2-Methylpyridin-3-yl)ethanone (21)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), acetylacetone (300 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 6 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v)

Yield: 223 mg (55%). Pale yellow viscous liquid.

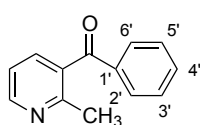
Data of 21:

Elemental analysis calcd (%) for C₈H₉NO: C 71.09, H 6.71, N 10.36; found: C 70.86, H 6.97, N 10.49.

IR (NaCl) ν 1689 (C=O), 1563 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 2.60 (s, 3H, C-3COCH₃), 2.76 (s, 3H, C-2CH₃), 7.25 (dd, *J* = 4.85, 7.85 Hz, 1H, CH-5), 7.98 (dd, *J* = 1.4, 7.85 Hz, 1H, CH-4), 8.60 (dd, *J* = 1.4, 4.85 Hz, 1H, CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 200.8 (C=O), 158.4 (C-2), 151.6 (CH-6), 137.1 (CH-4), 133.2 (C-3), 121.2 (CH-5), 29.7 (C(O)CH₃), 25.0 (C-2CH₃) ppm.

(2-Methylpyridin-3-yl)(phenyl)methanone (22)

Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), benzoylacetone (487 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 4 h for the first step and 9 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 355 mg (60%). Pale yellow viscous liquid.

Data of 22:

Elemental analysis calcd (%) for C₁₃H₁₁NO: C 79.16, H 5.62, N 7.10; found: C 78.98, H 5.84, N 7.10.

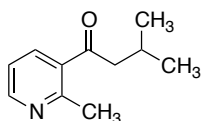
IR (NaCl) ν 1666 (C=O), 1580 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 2.56 (s, 3H, C-2CH₃), 7.25 (dd, *J* = 4.9, 7.65 Hz, 1H, CH-5), 7.47-7.53 (m, 2H, CH-3' and CH-5'), 7.60-7.67 (m, 2H, CH-2' and CH-6'), 7.78-7.82 (m, 2H, CH-4 and CH-4'), 8.66 (dd, *J* = 1.4, 4.9 Hz, 1H, CH-6)

ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 197.4 (C=O), 157.0 (C-2), 150.9 (CH-6), 137.3 (CPh), 136.5 (CH-4), 134.4 (C-3), 134.2 (CH-4'), 130.4 (CH-2' and CH-6'), 129.2 (CH-3' and CH-5'), 120.8 (CH-5), 23.7 (C-2CH₃) ppm.

3-Methyl-1-(2-methylpyridin-3-yl)butan-1-one (23)



Prepared from 1,1-dimethylhydrazine (180 mg, 3 mmol), 6-methylheptane-2,4-dione (426 mg, 3 mmol) and acrolein (202 mg, 3.6 mmol).

Reaction time: 5 h for the first step and 18 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 250 mg (47%). Pale yellow viscous liquid.

Data of 23:

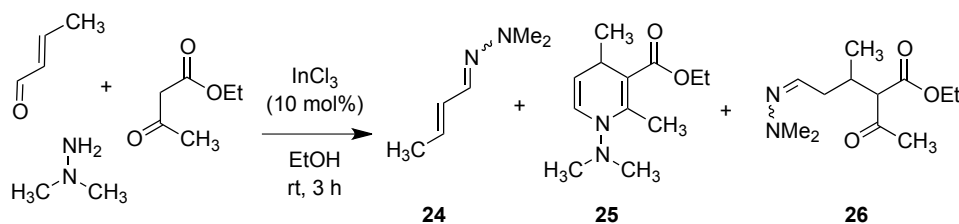
Elemental analysis calcd (%) for C₁₁H₁₅NO: C 74.54, H 8.53, N 7.90; found: C 74.35, H 8.48, N 7.76.

IR (NaCl) ν 1689 (C=O), 1564 (C=N) cm⁻¹.

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.01 (d, J = 6.9 Hz, 6H, CH₂CH(CH₃)₂), 2.27 (sep, J = 6.9 Hz, 1H, CH(CH₃)₂), 2.73 (s, 3H, C-2CH₃), 2.79 (d, J = 6.9 Hz, 2H, CH₂CH(CH₃)₂), 7.24 (dd, J = 4.85, 7.8 Hz, 1H, CH-5), 7.89 (dd, J = 1.7, 7.8 Hz, 1H, CH-4), 8.61 (dd, J = 1.7, 4.85 Hz, 1H, CH-6) ppm.

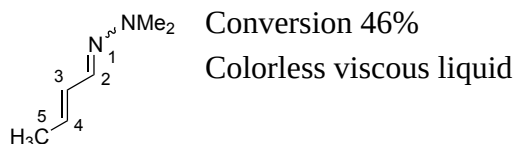
$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 203.9 (C=O), 157.9 (C-2), 151.2 (CH-6), 136.2 (CH-4), 134.2 (C-3), 121.2 (CH-5), 51.0 (CH₂CH(CH₃)₂), 25.5 (CH₂CH(CH₃)₂), 24.6 (C-2CH₃), 23.0 (CH₂CH(CH₃)₂) ppm.

9.2.4 Study of the reaction involving crotonaldehyde



To a stirred solution of 1,1-dimethylhydrazine (1 equiv, 3 mmol) and ethyl acetoacetate (1 equiv, 3 mmol) in ethanol (3 mL) was added InCl_3 (10 mol%) and stirring was continued for 30 min at room temperature. Crotonaldehyde (1.2 equiv, 3.6 mmol) was then added and stirring was continued under the same conditions for 3 hours. After completion of the reaction (checked by NMR), the mixture was diluted with CH_2Cl_2 (20 mL), washed with water followed by brine, dried over anhydrous Na_2SO_4 and then, the solvent was evaporated under reduced pressure.

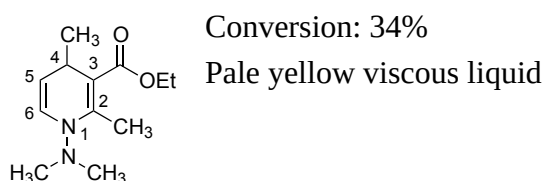
After workup, the residue was purified by silica gel column chromatography using a petroleum ether:ethyl acetate mixture (7:1, v/v) as eluent to give pure compounds **24-26**. Characterization data for all compounds of this reaction follow.

2-(But-2-enylidene)-1,1-dimethylhydrazine (24)*Data of 24:*

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.77-1.80 (dd, $J = 1.5, 6.75$ Hz, 3H, CH_3 -5), 2.79 (s, 6H, $\text{N}(\text{CH}_3)_2$), 5.72-5.86 (m, 1H, CH-3), 6.11-6.23 (m, 1H, CH-4), 6.97-7.01 (d, $J = 8.85$ Hz, 1H, CH-2) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 137.0 (CH-2), 130.6 (CH-4*), 130.3 (CH-2*), 43.1 and 43.0 ($\text{N}(\text{CH}_3)_2$), 18.5 (CH_3 -5) ppm.

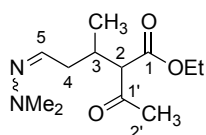
These data are consistent with those described in the literature.²⁷⁹

Ethyl 1-(dimethylamino)-2,4-dimethyl-1,4-dihydropyridine-3-carboxylate (25)*Data of 25:*

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 0.96-0.99 (d, $J = 6.5$ Hz, 3H, C-4 CH_3) 1.25-1.30 (t, $J = 7.2$, 3H, OCH_2CH_3), 2.40 (s, 3H, C-2 CH_3), 2.51 (s, 3H, NCH_3), 2.56 (s, 3H, NCH_3), 3.30-3.40 (m, 1H, CH-4), 4.06-4.24 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.98-5.03 (dd, $J = 5.75, 7.8$ Hz, 1H, CH-5), 6.14-6.17 (d, $J = 7.8$ Hz, 1H, CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 169.4 (C=O), 151.5 (C-2), 121.0 (CH-6), 110.58 (CH-5), 99.9 (C-3), 59.4 (OCH_2CH_3), 45.1 (NCH_3), 44.0 (NCH_3), 28.5 (CH-4), 25.0 (C-2 CH_3), 15.2 (C-4 CH_3^*), 14.8 ($\text{OCH}_2\text{CH}_3^*$) ppm.

²⁷⁹ Waldner, A. *Helv. Chim. Acta* **1988**, *71*, 486-492.

Ethyl 2-acetyl-5-(2,2-dimethylhydrazono)-3-methylpentanoate (26)

Conversion 20%

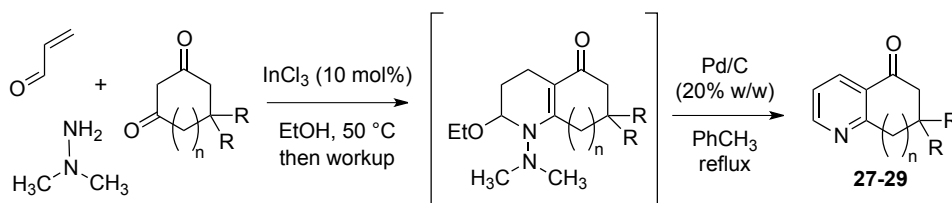
Pale yellow viscous liquid

Data of 26:

¹H-NMR (CDCl₃, 250 MHz) (two diastereoisomer) δ 0.94-1.01 (d, *J* = 6.8 Hz, 3H, C-3CH₃) 1.22-1.31 (t, *J* = 7.2, 3H, OCH₂CH₃), 2.01-2.19 (m, 1H, CH-3), 2.25 (s, 3H, CH₃-2'), 2.31-2.61 (m, 2H, CH₂-4), 2.74 (s, 6H, N(CH₃)₂), 3.34-3.44 (d, *J* = 8.2 Hz, 1H, CH-2), 4.12-4.24 (q, *J* = 7.2 Hz, 2H, OCH₂CH₃), 6.53-6.59 (m, 1H, CH-5) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 203.5 and 203.4 (C-1'=O), 169.4 and 169.2 (C-1=O), 136.1 and 136.0 (CH-5), 65.8 and 65.4 (CH-2), 61.7 and 61.6 (OCH₂CH₃), 43.59 and 43.55 (N(CH₃)₂), 38.2 and 38.1 (CH₂-4), 32.4 and 32.3 (CH₃-2'), 30.6 and 30.0 (CH-3), 17.6 and 17.4 (C-3CH₃), 14.5 and 14.4 (OCH₂CH₃) ppm.

9.2.5 Synthesis of fused pyridine derivatives 27-29. General procedure

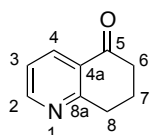


Compound	n	R
27	1	H
28	1	CH ₃
29	2	H

To a stirred solution of 1,1-dimethylhydrazine (1 equiv, 2 mmol) and the corresponding cyclic 1,3-dicarbonyl compound (1 equiv, 2 mmol) in ethanol (2 mL) was added InCl₃ (10 mol%), and stirring was continued for 30 min at 50 °C. Acrolein (1.2 equiv, 2.4 mmol) was then added and stirring was continued under the same conditions overnight. After completion of the reaction, the mixture was diluted with CH₂Cl₂ (15 mL), washed with water followed by brine, dried over anhydrous Na₂SO₄, and the solvent was evaporated under reduced pressure.

The residue containing the tetrahydropyridine derivatives was then dissolved in toluene (7 mL), Palladium on Carbon 10 wt % loading (20% wt/crude) was added, and the resulting mixture was heated under reflux for the time periods specified in the compound data sheet. After completion of the reaction (checked by TLC), the mixture was diluted with CH₂Cl₂ (20 mL) and filtered through Celite, which was then washed repeatedly with additional CH₂Cl₂ in 5 mL portions. The combined organic layers were concentrated to dryness and the residue was purified by silica gel column chromatography using petroleum ether:ethyl acetate mixtures as eluent to give pure compounds (27-29).

Data of compounds 27-29, and the experimental conditions employed for their synthesis are indicated below.

7,8-Dihydroquinolin-5(6H)-one (27)

Prepared from 1,1-dimethylhydrazine (120 mg, 2 mmol), 1,3-cyclohexanedione (224 mg, 2 mmol) and acrolein (134 mg, 2.4 mmol).

Reaction time: 15 h for the first step and 36 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 7:1, v/v)

Yield: 238 mg (81%). Yellow viscous liquid.

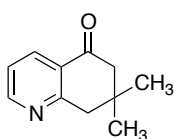
Data of 27:

Elemental analysis calcd (%) for C₉H₉NO: C 73.45, H 6.16, N 9.52; found: C 73.18, H 6.32, N 9.44.

IR (NaCl) ν 1688 (C=O), 1583 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 2.20-2.28 (m, 2H, CH₂-7), 2.70-2.75 (m, 2H, CH₂-6), 3.19 (t, *J* = 6.2 Hz, 2H, CH₂-8), 7.31 (dd, *J* = 4.78, 7.88 Hz, 1H, CH-3), 8.30 (dd, *J* = 1.88, 7.88 Hz, 1H, CH-4), 8.70 (dd, *J* = 1.88, 4.78 Hz, 1H, CH-2) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 198.4 (C=O), 164.1 (C-8a), 153.9 (CH-2), 135.4 (CH-4), 128.6 (C-4a), 122.7 (CH-3), 39.0 (CH₂-6), 32.9 (CH₂-8), 22.3 (CH₂-7) ppm.

7,7-Dimethyl-7,8-dihydroquinolin-5(6H)-one (28)

Prepared from 1,1-dimethylhydrazine (120 mg, 2 mmol), 5,5-dimethyl-1,3-cyclohexanedione (280 mg, 2 mmol) and acrolein (134 mg, 2.4 mmol).

Reaction time: 15 h for the first step and 40 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 7:1, v/v).

Yield: 273 mg (78%). Yellow viscous liquid.

Data of 28:

Elemental analysis calcd (%) for C₁₁H₁₃NO: C 75.40, H 7.48, N 7.99; found: C 75.43, H 7.59, N 8.07.

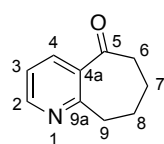
IR (NaCl) ν 1692 (C=O), 1584 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.14 (s, 6H, 2xCH₃), 2.58 (s, 2H, CH₂-6), 3.07 (s,

2H, CH₂-8), 7.32 (dd, *J* = 4.8, 7.85 Hz, 1H, CH-3), 8.29 (dd, *J* = 1.7, 7.85 Hz, 1H, CH-4), 8.73 (dd, *J* = 1.7, 4.8 Hz, 1H, CH-2) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 198.5 (C=O), 162.7 (C-8a), 154.3 (CH-2), 135.0 (CH-4), 127.6 (C-4a), 122.6 (CH-3), 52.5 (CH₂-6), 46.8 (CH₂-8), 33.4 (C-7), 28.7 (2xCH₃) ppm.

6,7,8,9-Tetrahydrocyclohepta[*b*]pyridin-5-one (29)



Prepared from 1,1-dimethylhydrazine (120 mg, 2 mmol), 1,3-cycloheptanedione (252 mg, 2 mmol) and acrolein (134 mg, 2.4 mmol).

Reaction time: 15 h for the first step and 20 h for the second step.

Purification: *n*-hexane:ethyl acetate (from 15:1 to 7:1, v/v).

Yield: 245 mg (76%). Yellow viscous liquid.

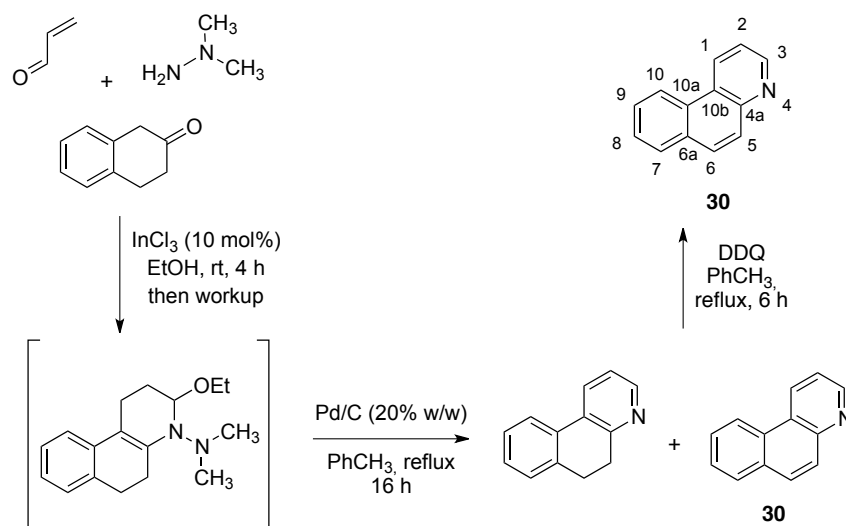
Data of 29:

Elemental analysis calcd (%) for C₁₀H₁₁NO: C 74.51, H 6.88, N 8.69; found: C 74.34, H 7.01, N 8.57.

IR (NaCl) ν 1680 (C=O), 1580 (C=N) cm⁻¹;

¹H-NMR (CDCl₃, 250 MHz) δ 1.87-2.04 (m, 4H, CH₂-7 and CH₂-8), 2.79-2.84 (m, 2H, CH₂-6), 3.20 (t, *J* = 5.8 Hz, 2H, CH₂-9), 7.28 (dd, *J* = 4.83, 7.75 Hz, 1H, CH-3), 8.03 (dd, *J* = 1.8, 7.75 Hz, 1H, CH-4), 8.62 (dd, *J* = 1.8, 4.83 Hz, 1H, CH-2) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 204.9 (C=O), 161.5 (C-9a), 152.2 (CH-2), 137.1 (CH-4), 134.3 (C-4a), 122.3 (CH-3), 41.2 (CH₂-6), 36.2 (CH₂-9), 24.4 (CH₂-8), 21.8 (CH₂-7) ppm.

9.2.6 Synthesis of benzo[*f*]quinolone (30)

To a stirred solution of 1,1-dimethylhydrazine (1 equiv, 3 mmol) and β -tetralone (1 equiv, 3 mmol) in ethanol (3 mL) was added InCl_3 (10 mol%), and stirring was continued for 30 min at room temperature. Acrolein (1.2 equiv, 3.6 mmol) was then added and stirring was continued under the same conditions for 4 hours. After completion of the reaction (checked by NMR), the mixture was diluted with CH_2Cl_2 (20 mL), washed with water followed by brine, dried over anhydrous Na_2SO_4 , and the solvent was evaporated under reduced pressure.

The residue containing the tetrahydropyridine derivative was then dissolved in toluene (10 mL), Palladium on Carbon 10 wt % loading (20% wt/crude) was added and the resulting mixture was heated under reflux for 16 hours. After this time, the mixture was diluted with CH_2Cl_2 (20 mL) and filtered through Celite, which was then washed repeatedly with additional CH_2Cl_2 in 5 mL portions. The combined organic layers were concentrated to dryness and the residue was purified by silica gel column chromatography using a petroleum ether-ethyl acetate mixture (7:1, v/v) as eluent to give a mixture of 5,6-dihydrobenzo[*f*]quinoline and benzo[*f*]quinoline in a ratio of 2:1 (2,15mmol, 72% yield).

The mixture (1 eq, 2.15 mmol) was then dissolved in toluene (7 mL), DDQ (1.2 eq, 2.6 mmol) was added and the solution was refluxed for 6 hours. The resulting

dark brown colored mixture was then poured into hexanes (20 mL) to precipitate out the hydroquinone by-product. The residue was then chromatographed on silica gel, eluting with a 5:1 petroleum ether-ethyl acetate mixture to yield 280 mg of **30** as a white solid (73% yield for this step; the overall yield for the whole process is 53%).

Data of 30

Mp: 92-93 °C.

Elemental analysis calcd (%) for C₁₃H₉N: C 87.12, H 5.06, N 7.82; found: C 86.91, H 5.30, N 7.81.

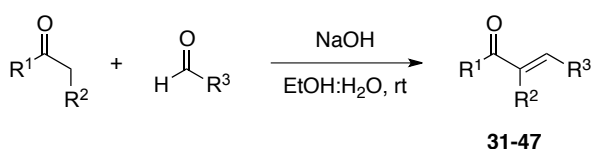
IR (NaCl) ν 1571 (C=N) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 7.59-7.64 (m, 1H, ArH), 7.67-7.79 (m, 2H, ArH), 7.99 (dd, *J* = 1.75, 7.6 Hz, 1H, ArH), 8.04 (s, 2H, ArH), 8.69 (dd, *J* = 1.65, 9.1 Hz, 1H, ArH), 9.00 (s, 1H, ArH), 9.01-9.03 (m, 1H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 148.7 (CH-3), 147.2 (C-4a), 130.7 (C-6a*), 129.9 (CHAr), 129.7 (CHAr), 128.6 (C-10a*), 127.7 (CHAr), 127.2 (CHAr), 126.3 (CHAr), 126.1 (CHAr), 124.4 (C-10b), 121.6 (CHAr), 120.3 (CH) ppm.

9.3 MULTICOMPONENT SYNTHESIS OF 4,6-DIARYL-1,4-DIHYDROPYRIDINE DERIVATIVES

9.3.1 Synthesis of chalcones 31-47. General procedure



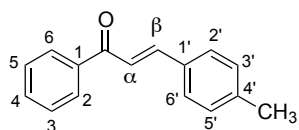
Cpd.	R ¹	R ²	R ³	Cpd.	R ¹	R ²	R ³
31	C ₆ H ₅	H	4-MeC ₆ H ₄	40	4-ClC ₆ H ₄	H	3-MeOC ₆ H ₄
32	4-MeC ₆ H ₄	H	C ₆ H ₅	41	4-ClC ₆ H ₄	H	2-NO ₂ C ₆ H ₄
33	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	42	C ₆ H ₅	H	4-NO ₂ C ₆ H ₄
34	4-MeC ₆ H ₄	H	4-BrC ₆ H ₄	43	2-NO ₂ C ₆ H ₄	H	4-MeC ₆ H ₄
35	4-MeC ₆ H ₄	H	2-NO ₂ C ₆ H ₄	44	C ₆ H ₅	H	2-thienyl
36	C ₆ H ₅	H	4-MeOC ₆ H ₄	45	4-MeC ₆ H ₄	H	2-thienyl
37	4-MeOC ₆ H ₄	H	4-MeOC ₆ H ₄	46	2-furyl	H	4-MeC ₆ H ₄
38	4-ClC ₆ H ₄	H	C ₆ H ₅	47	C ₆ H ₅	CH ₃	C ₆ H ₅
39	4-ClC ₆ H ₄	H	4-ClC ₆ H ₄				

To a stirred solution of the appropriate aromatic ketone (1 equiv, 10 mmol) and aromatic aldehyde (1.05 equiv, 10.5 mmol) derivatives in ethanol (20 mL) at room temperature, is slowly added a solution of NaOH (1.2 equiv, 12 mmol) in a mixture of ethanol:water (1:1, 18 mL + 18 mL). The resulting mixture is stirred vigorously at the same temperature for the time period specified in the compound data sheet. After completion of the reaction (checked by TLC), the mixture was poured into ice-water (50 mL) and the precipitate formed was collected by filtration and washed with cold water. In case of an oily product, the reaction mixture was extracted with CH₂Cl₂ (3x25 mL); the organic phase was collected, washed with brine and dried over anhydrous Na₂SO₄, and then, the solvent was evaporated under reduced pressure.

The crude residue was crystallized from EtOH or purified by silica gel column chromatography using *n*-hexane:ethyl acetate mixtures as eluents to give pure

compounds (**31-47**).

Data of compounds **31-47**, and the experimental conditions employed for their synthesis are indicated below.

1-Phenyl-3-(4-methylphenyl)prop-2-en-1-one (31)

Prepared from acetophenone (1.201 g, 10 mmol), 4-methylbenzaldehyde (1.261 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 1.890 g (85%). Pale yellow solid.

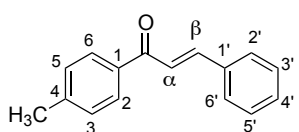
Data of 31:

IR (NaCl) ν 1657 (C=O), 983 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.43 (s, 3H, C-4' CH_3), 7.27 (d, $J = 8$ Hz, 2H, CH-3' and CH-5'), 7.50-7.65 (m, 6H, CH- α , CH-3, CH-4, CH-5, CH-2' and CH-6'), 7.83 (d, $J = 15.7$ Hz, 1H, CH- β), 8.05 (dt, $J = 1.6, 7.8$ Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 191.1 (C=O), 145.4 (CH- β), 141.5 (C-1*), 138.8 (C-4'*), 133.1 (CHAr), 132.5 (C-1'), 130.1 (2xCHAr), 129.0 (2xCHAr), 128.92 (2xCHAr), 128.90 (2xCHAr), 121.5 (CH- α), 22.0 (C-4' CH_3) ppm.

These data are consistent with those described in the literature.²⁸⁰

3-Phenyl-1-(4-methylphenyl)prop-2-en-1-one (32)

Prepared from 4'-methylacetophenone (1.342 g, 10 mmol), benzaldehyde (1.114 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 1.845 g (83%). Yellow solid.

Data of 32:

IR (NaCl) ν 1662 (C=O), 979 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.47 (s, 3H, C-4' CH_3), 7.35 (d, $J = 8$ Hz, 2H, CH-3

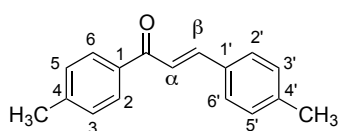
²⁸⁰ Rocchi, D.; González, J. F.; Menéndez, J. C. *Molecules* **2014**, *19*, 7317-7326.

and CH-5), 7.42-7.46 (m, 3H, CH-3', CH-4' and CH-5'), 7.57 (d, $J = 15.7$ Hz, 1H, CH- α), 7.66-7.70 (m, 2H, CH-2' and CH-6'), 7.85 (d, $J = 15.7$ Hz, 1H, CH- β), 7.97 (d, $J = 8$ Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.5 (C=O), 144.8 (CH- β), 144.1 (C-4), 136.0 (C-1'*), 135.4 (C-1*), 130.9 (CH-4'), 129.8 (2xCHAR), 129.4 (2xCHAR), 129.1 (2xCHAR), 128.8 (2xCHAR), 122.5 (CH- α), 22.1 (C-4CH₃) ppm.

These data are consistent with those described in the literature.²⁸⁰

1,3-Bis(4-methylphenyl)prop-2-en-1-one (33)



Prepared from 4'-methylacetophenone (1.342 g, 10 mmol), 4-methylbenzaldehyde (1.261 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 1.915 g (81%). Yellow solid.

Data of 33:

IR (NaCl) ν 1654 (C=O), 992 (CH- α =CH- β) cm^{-1}

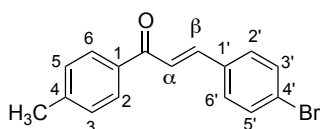
$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.43 (s, 3H, C-4'CH₃*), 2.47 (s, 3H, C-4CH₃*), 7.25 (d, $J = 8$ Hz, 2H, CH-3' and CH-5'), 7.34 (d, $J = 8$ Hz, 2H, CH-3 and CH-5), 7.50-7.59 (m, 3H, CH- α , CH-2' and CH-6'), 7.83 (d, $J = 15.7$ Hz, 1H, CH- β), 7.97 (d, $J = 8$ Hz, 2H, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.5 (C=O), 144.9 (CH- β), 143.9 (C-4*), 141.4 (C-4'*), 136.2 (C-1*), 132.7 (C-1'*), 130.1 (2xCHAR), 129.7 (2xCHAR), 129.0 (2xCHAR), 128.9 (2xCHAR), 121.5 (CH- α), 22.1 (C-4CH₃*), 22.0 (C-4'CH₃*) ppm.

These data are consistent with those described in the literature.²⁸⁰

3-(4-Bromophenyl)-1-(4-methylphenyl)prop-2-en-1-one (34)

Prepared from 4'-methylacetophenone (1.342 g, 10 mmol), 4-bromobenzaldehyde (1.943 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).



Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 2.680 g (89%). Yellow solid.

Data of 34:

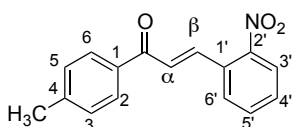
IR (NaCl) ν 1659 (C=O), 1072 (C-Br), 984 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.47 (s, 3H, C-4 CH_3), 7.34 (d, $J = 8$ Hz, 2H, CH-3 and CH-5), 7.52-7.60 (m, 5H, CH- α , CH-2', CH-3', CH-5' and CH-6'), 7.77 (d, $J = 15.7$ Hz, 1H, CH- β), 7.97 (d, $J = 8$ Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.1 (C=O), 144.3 (C-4), 143.3 (CH- β), 135.8 (C-1*), 134.3 (C-1'*), 132.6 (2xCHAR), 130.2 (2xCHAR), 129.8 (2xCHAR), 129.1 (2xCHAR), 125.1 (C-4'), 122.9 (CH- α), 22.1 (C-4 CH_3) ppm.

These data are consistent with those described in the literature.²⁸⁰

3-(2-Nitrophenyl)-1-(4-methylphenyl)prop-2-en-1-one (35)



Prepared from 4'-methylacetophenone (1.342 g, 10 mmol), 2-nitrobenzaldehyde (1.587 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 24 h.

Purification: Crystallization from EtOH.

Yield: 1.975 g (74%). Yellow solid.

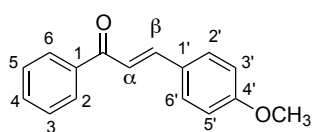
Data of 35:

IR (NaCl) ν 1663 (C=O), 1521 (NO_2), 1342 (NO_2), 972 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.48 (s, 3H, C-4 CH_3), 7.35 (d, $J = 15.7$ Hz, 1H, CH- α), 7.35 (d, $J = 8.3$ Hz, 2H, CH-3 and CH-5), 7.56-7.63 (m, 1H, CH-5'), 7.69-7.79 (m, 2H, CH-4' and CH-6'), 7.97 (d, $J = 8.3$ Hz, 2H, CH-2 and CH-6), 8.09 (d, $J = 1.1$ Hz, 1H, CH-3'), 8.15 (d, $J = 15.7$ Hz, 1H, CH- β) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.4 (C=O), 149.1 (C-2'), 144.5 (C-4), 140.1 (CH- β), 135.3 (C-1), 133.9 (CH-5'), 131.8 (C-1'), 130.7 (CH-4'*), 129.8 (2xCHAR), 129.7 (CH-6'*), 129.4 (2xCHAR), 127.8 (CH-3'), 125.4 (CH- α), 22.1 (C-4 CH_3) ppm.

These data are consistent with those described in the literature.²⁸⁰

3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (36)

Prepared from acetophenone (1.201 g, 10 mmol), 4-methoxybenzaldehyde (1.430 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 2.050 g (86%). Yellow solid.

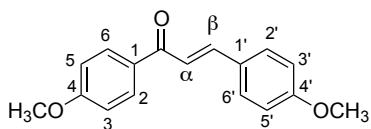
Data of 36:

IR (NaCl) ν 1656 (C=O), 983 (CH- α =CH- β) cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 3.89 (s, 3H, C-4'OCH₃), 6.98 (d, J = 8.7 Hz, 2H, CH-3' and CH-5'), 7.45 (d, J = 15.7 Hz, 1H, CH- α), 7.50-7.66 (m, 5H, CH-3, CH-4, CH-5, CH-2' and CH-6'), 7.83 (d, J = 15.7 Hz, 1H, CH- β), 8.03-8.06 (m, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 191.0 (C=O), 162.1 (C-4'), 145.2 (CH- β), 138.9 (C-1), 133.0 (C-1'), 130.7 (2xCHAR), 129.0 (2xCHAR), 128.8 (2xCHAR), 128.0 (CHAR), 120.2 (CH- α), 114.8 (2xCHAR), 55.8 (C-4'OCH₃) ppm.

These data are consistent with those described in the literature.²⁸⁰

1,3-Bis(4-methoxyphenyl)prop-2-en-1-one (37)

Prepared from 4'-methoxyacetophenone (1.502 g, 10 mmol), 4-methoxybenzaldehyde (1.430 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 1.960 g (73%). solid.

Data of 37:

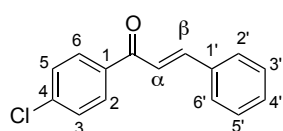
IR (NaCl) ν 1654 (C=O), 980 (CH- α =CH- β) cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 3.83 (s, 3H, C-4OCH₃*), 3.87 (s, 3H, C-

4'OCH₃*), 6.87-7.03 (m, 4H, CH-3, CH-5, CH-3' and CH-5'), 7.42 (d, $J = 15.6$ Hz, 1H, CH- α), 7.59 (d, $J = 8.8$ Hz, 2H, CH-2' and CH-6'), 7.78 (d, $J = 15.6$ Hz, 1H, CH- β), 8.03 (d, $J = 8.9$ Hz, 2H, CH-2 and CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 188.8 (C=O), 163.6 (C-4'), 161.6 (C-4), 143.9 (CH- β), 131.4 (C-1), 130.8 (2xCHAR), 130.1 (2xCHAR), 127.9 (C-1'), 119.6 (CH- α), 114.4 (2xCHAR), 113.9 (2xCHAR), 55.6 (C-4OCH₃*), 55.5 (C-4'OCH₃*) ppm. These data are consistent with those described in the literature.²⁸⁰

1-(4-Chlorophenyl)-3-phenylprop-2-en-1-one (38)



Prepared from 4'-chloroacetophenone (1.546 g, 10 mmol), benzaldehyde (1.114 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 2.255 g (93%). Yellow solid.

Data of 38:

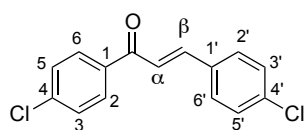
IR (NaCl) ν 1661 (C=O), 1090 (C-Cl), 983 (CH- α =CH- β) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 7.42-7.55 (m, 6H, CH- α , CH-2', CH-3', CH-4', CH-5' and CH-6'), 7.64-7.69 (m, 2H, CH-3 and CH-5), 7.85 (d, $J = 15.7$ Hz, 1H, CH- β), 8.00 (dt, $J = 2.35, 8.7$ Hz, 2H, CH-2 and CH-6) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 189.6 (C=O), 145.8 (CH- β), 139.6 (C-4), 136.9 (C-1*), 135.1 (C-1'*), 131.2 (CHAR), 130.3 (2xCHAR), 129.44 (2xCHAR), 129.37(2xCHAR), 128.9 (2xCHAR), 121.9 (CH- α) ppm.

These data are consistent with those described in the literature.²⁸⁰

1,3-Bis(4-chlorophenyl)prop-2-en-1-one (39)



Prepared from 4'-chloroacetophenone (1.546 g, 10 mmol), 4-chlorobenzaldehyde (1.476 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 2.630 g (95%). Yellow solid.

Data of 39:

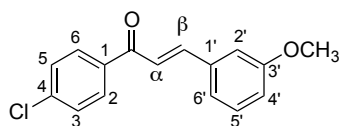
IR (NaCl) ν 1653 (C=O), 1090 (C-Cl), 982 (CH- α =CH- β) cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 7.40-7.52 (m, 5H, CH- α , CH-2', CH-3', CH-5' and CH-6'), 7.61 (d, J = 8.4 Hz, 2H, CH-3 and CH-5), 7.79 (d, J = 15.7 Hz, 1H, CH- β), 7.99 (d, J = 8.4 Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 189.3 (C=O), 144.2 (CH- β), 143.3 (C-4), 137.1 (C-1*), 136.7 (C-4'*), 133.6 (C-1'), 130.3 (2xCHAR), 130.1 (2xCHAR), 129.7 (2xCHAR), 129.4 (2xCHAR), 122.2 (CH- α) ppm.

These data are consistent with those described in the literature.²⁸¹

1-(4-Chlorophenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (40)



Prepared from 4'-chloroacetophenone (1.546 g, 10 mmol), 3-methoxybenzaldehyde (1.430 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 2.155 g (79%). Pale yellow solid.

Data of 40:

IR (NaCl) ν 1663 (C=O), 1091 (C-Cl), 982 (CH- α =CH- β) cm^{-1} .

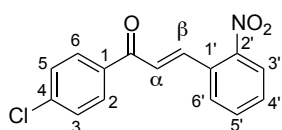
$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 3.89 (s, 3H, C-3'OCH₃), 7.01 (dd, J = 1.7, 8 Hz, 1H, CH-4'), 7.17-7.26 (m, 2H, CH-2' and CH-6'), 7.35-7.54 (m, 4H, CH- α , CH-3, CH-5 and CH-5'), 7.81 (d, J = 15.7 Hz, 1H, CH- β), 8.00 (dt, J = 1.85, 8 Hz, 2H, CH-2 and CH-6);

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 189.6 (C=O), 160.4 (C-3'), 145.7 (CH- β), 139.7 (C-4), 136.9 (C-1*), 136.4 (C-1'*), 130.4 (CH-5'), 130.3 (2xCHAR), 129.4 (2xCHAR), 122.2 (CH-6'*), 121.6 (CH- α *), 116.9 (CH-4'), 113.9 (CH-2'), 55.8 (C-3'OCH₃) ppm.

²⁸¹ Miura, Y.; Kitagishi, Y.; Ueno, S. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3282-3288.

These data are consistent with those described in the literature.²⁸⁰

1-(4-Chlorophenyl)-3-(2-nitrophenyl)prop-2-en-1-one (41)



Prepared from 4'-chloroacetophenone (1.546 g, 10 mmol), 2-nitrobenzaldehyde (1.587 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 24 h.

Purification: Crystallization from EtOH.

Yield: 1.755 g (61%). Grey solid.

Data of 41:

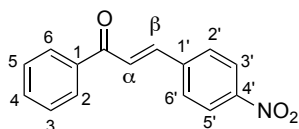
IR (NaCl) ν 1667 (C=O), 1517 (NO₂), 1340 (NO₂), 1090 (C-Cl) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 7.30 (d, J = 15.7 Hz, 1H, CH- α), 7.52 (dt, J = 1.75, 8.5 Hz, 2H, CH-3 and CH-5), 7.58-7.65 (m, 1H, CH-5'), 7.69-7.79 (m, 2H, CH-4' and CH-6'), 8.00 (dt, J = 1.75, 8.5 Hz, 2H, CH-2 and CH-6), 8.11 (d, J = 1.1 Hz, 1H, CH-3'), 8.17 (d, J = 15.7 Hz, 1H, CH- β) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 189.7 (C=O), 148.9 (C-2'), 141.1 (CH- β), 140.1 (C-4), 136.1 (C-1), 134.1 (CH-5'), 131.6 (C-1'), 130.9 (CH-4'*), 130.6 (2xCHAr), 129.7(CH-6'*), 129.5 (2xCHAr), 127.2 (CH-3'), 125.5 (CH- α) ppm.

These data are consistent with those described in the literature.²⁸⁰

3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (42)



Prepared from acetophenone (1.201 g, 10 mmol), 4-nitrobenzaldehyde (1.587 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Purification: Crystallization from EtOH.

Yield: 1.340 g (53%). Pale yellow solid.

Data of 42:

IR (NaCl) ν 1665 (C=O), 1518 (NO₂), 1338 (NO₂) cm⁻¹.

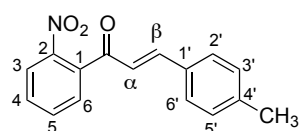
¹H-NMR (CDCl₃, 250 MHz) δ , 7.48-7.57 (m, 2H, CH-3 and CH-5), 7.59-7.70 (m,

2H, CH- α and CH-4) 7.75-7.88 (m, 3H, CH- β , CH-2' and CH-6'), 8.04 (dd, J = 1.3, 8.3 Hz, 2H, CH-2 and CH-6), 8.28 (d, J = 8.9 Hz, 2H, CH-3' and CH-5') ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 189.7 (C=O), 141.6 (CH- β), 141.1 (C-4'), 137.6 (C-1'), 133.5 (CHAr), 129.0 (2xCHAr), 128.9 (2xCHAr), 128.7 (2xCHAr), 125.8 (CH- α), 128.7 (2xCHAr) ppm. C-1 is missing

These data are consistent with those described in the literature.²⁸⁰

3-(4-Methylphenyl)-1-(2-nitrophenyl)prop-2-en-1-one (43)



Prepared from 2'-nitroacetophenone (1.615 g, 10 mmol), 4-methylbenzaldehyde (1.261 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 24 h.

Purification: Crystallization from EtOH.

Yield: 2.272 g (85%). Pale yellow solid.

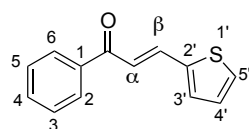
Data of 43:

IR (NaCl) ν 1663 (C=O), 1522 (NO_2), 1360 (NO_2), 972 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.40 (s, 3H, C-4' CH_3), 7.00 (d, J = 16.2 Hz, 1H, CH- α), 7.21-7.27 (m, 3H, CH- β , CH-3' and CH-5'), 7.43 (d, J = 8.1 Hz, 2H, CH-2' and CH-6'), 7.54 (dd, J = 1.5, 7.5 Hz, 1H, CH-6), 7.69 (dt, J = 1.5, 7.5 Hz, 1H, CH-5), 7.80 (dt, J = 1.1, 7.5 Hz, 1H, CH-4), 8.22 (dd, J = 1.1, 8.1 Hz, 1H, CH-3) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 193.5 (C=O), 147.0 (CH- β), 142.2 (C-2), 136.8 (C-4'), 134.5 (CH-5), 131.6 (C-1), 131.0 (CH-4*), 130.2 (2xCHAr), 129.3 (CH-6*), 129.0 (2xCHAr), 125.7 (CH-3), 125.0 (CH- α), 22.1 (C-4' CH_3) ppm.

1-Phenyl-3-(thiophen-2-yl)prop-2-en-1-one (44)



Prepared from acetophenone (1.201 g, 10 mmol), thiophene-2-carbaldehyde (1.178 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 10:1, v/v).

Yield: 1.415 g (66%). Pale brown solid.

Data of 44:

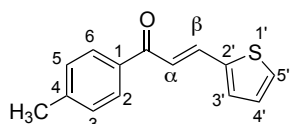
IR (NaCl) ν 1652 (C=O), 975 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 7.13 (dd, $J = 3.7, 5.0$ Hz, 1H, CH-4'), 7.34–7.40 (m, 2H, CH- α and CH-3'), 7.46 (d, $J = 5.0$ Hz, 1H, CH-5'), 7.50–7.65 (m, 3H, CH-3, CH-4 and CH-5), 7.95–8.07 (m, 3H, CH- β , CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.4 (C=O), 140.8 (C-2'), 138.5 (C-1), 137.7 (CH- β), 133.2 (CH-4*), 132.6 (CH-5'*), 129.3 (CH-3'), 129.1 (2xCHAr), 128.8 (2xCHAr), 128.8 (CH-4'), 120.5 (CH- α) ppm.

These data are consistent with those described in the literature.²⁸⁰

1-(4-Methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (45)



Prepared from 4'-methylacetophenone (1.342 g, 10 mmol), thiophene-2- carbaldehyde (1.178 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 15 h.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 10:1, v/v).

Yield: 1.415 g (62%). Yellow solid.

Data of 45:

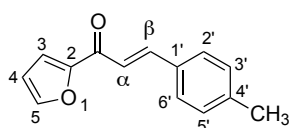
IR (NaCl) ν 1655 (C=O), 961 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.47 (s, 3H, C-4CH₃), 7.12 (dd, $J = 3.7, 5.0$ Hz, 1H, CH-4'), 7.32–7.40 (m, 4H, CH- α , CH-3', CH-3 and CH-5), 7.45 (d, $J = 5.0$ Hz, 1H, CH-5'), 7.94–8.00 (m, 3H, CH- β , CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 189.8 (C=O), 144.1 (C-4), 140.9 (C-2'), 137.2 (CH- β), 135.9 (C-1), 132.4 (CH-5'), 129.8 (2xCHAr), 129.1 (CH-3'), 129.0 (2xCHAr), 128.8 (CH-4'), 121.2 (CH- α), 22.1 (C-4CH₃)ppm.

These data are consistent with those described in the literature.²⁸²

1-(Furan-2-yl)-3-(4-methylphenyl)prop-2-en-1-one (46)



Prepared from 2-acetylfuran (1.101 g, 10 mmol), 4-methylbenzaldehyde (1.261 g, 10.5 mmol) and sodium hydroxide (0.480 g, 12 mmol).

Reaction time: 24 h.

Purification: Crystallization from EtOH.

Yield: 1.485 g (70%). Pale yellow solid.

Data of 46:

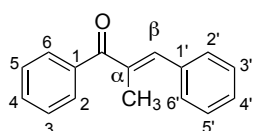
IR (NaCl) ν 1649 (C=O), 979 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.43 (s, 3H, C-4' CH_3), 6.62 (dd, J = 1.6, 3.6 Hz, 1H, CH-4), 7.26 (d, J = 8.1 Hz, 2H, CH-3' and CH-5'), 7.35 (dd, J = 0.7, 3.6 Hz, 1H, CH-3), 7.44 (d, J = 15.7 Hz, 1H, CH- α), 7.59 (d, J = 8.1 Hz, 2H, CH-2' and CH-6'), 7.68 (dd, J = 0.7, 1.6 Hz, 1H, CH-5), 7.90 (d, J = 15.7 Hz, 1H, CH- β) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 178.6 (C=O), 154.2 (C-2), 146.9 (CH-5*), 144.5 (CH- β *), 141.6 (C-4'), 132.4 (C-1'), 130.1 (2xCHAr), 129.0 (2xCHAr), 120.5 (CH- α), 117.8 (CH-3), 112.9 (CH-4), 22.0 (C-4' CH_3) ppm.

These data are consistent with those described in the literature.²⁸²

2-Methyl-1,3-diphenylprop-2-en-1-one (47)



Prepared from propiophenone (1.342 g, 10 mmol), benzaldehyde (1.592 g, 15 mmol) and sodium hydroxide (0.600 g, 15 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 30:1 to 20:1, v/v).

Yield: 1.445 g (65%). Pale yellow liquid.

²⁸² Musumarra, G.; Ballistreri, F. P. *Organic Magnetic Resonance* **1980**, *14*, 384-391.

Data of 47:

IR (NaCl) ν 1641 (C=O) cm^{-1} .

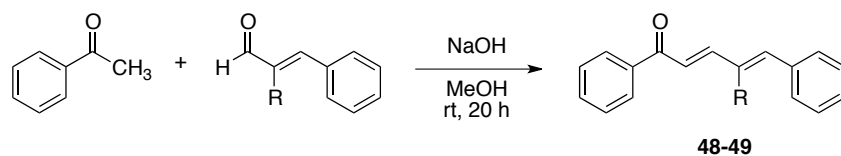
$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.30 (d, $J = 1.4$ Hz, 3H, C- αCH_3), 7.21-7.22 (m, 1H, CH- β), 7.33-7.61 (m, 8H, CH- β , CH-3, CH-4, CH-5 and C β ArH), 7.76-7.81 (m, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 200.0 (C=O), 142.7 (CH- β), 138.9 (C-1*), 137.3 (C-1'*), 136.2 (C- α^*), 132.1 (CH-4), 130.1 (2xCHAr), 129.9 (2xCHAr), 129.0 (CH-4'), 128.9 (2xCHAr), 128.6 (2xCHAr), 14.9 (C- αCH_3) ppm.

These data are consistent with those described in the literature.²⁸³

²⁸³ Lu, S.-M.; Bolm, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 8920-8923.

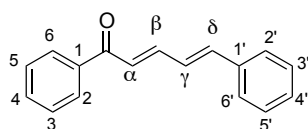
9.3.2 Synthesis of chalcones **48** and **49**. General procedure



Compound	R
48	H
49	CH_3

To a stirred solution of acetophenone (1 equiv, 5.0 mmol) in methanol (30 mL) was slowly added an aqueous solution of sodium hydroxide (60%, 25 mL). After cooling the solution to room temperature, cinnamaldehyde, or its methyl derivative, (1.1 equiv, 5.5 mmol) was added and the mixture was stirred at the same temperature for 20 h. After completion of the reaction (checked by TLC), the mixture was poured into ice-water (50 mL) and 2N hydrochloric acid was added to adjust pH to ca. 5. The obtained solid was removed by filtration, dissolved in dichloromethane (100 mL) and washed with an aqueous solution of sodium hydrogen carbonate (5%, 2x50 mL). The organic layer was collected, dried over anhydrous sodium sulfate and the solution evaporated to dryness. The residue was purified by silica gel column chromatography with dichloromethane as eluent.

Data of compounds **48** and **49** and the experimental conditions employed for their synthesis are indicated below.

1,5-Diphenylpenta-2,4-dien-1-one (48)

Prepared from acetophenone (600 mg, 5 mmol) and cinnamaldehyde (727 mg, 5.5 mmol).

Reaction time: 20 h.

Purification: dichloromethane.

Yield: 915 mg (78%). Yellow solid.

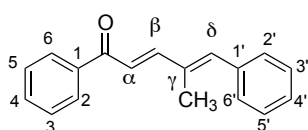
Data of 48:

IR (NaCl) ν 1650 (C=O), 994 (CH- α =CH- β) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 7.05-7.16 (m, 3H, CH- α , CH- γ and CH- δ), 7.29-7.44 (m, 3H, CH-3', CH-4' and CH-5'), 7.49-7.70 (m, 6H, CH- β , CH-3, CH-4, CH-5, CH-2' and CH-6'), 8.02 (d, $J = 7.65$ Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 190.9 (C=O), 145.3 (CH- β), 142.4 (CH- δ), 138.6 (C-1), 136.5 (C-1'), 133.1 (CH-4), 129.7 (CH-4'), 129.3 (2xCHAR), 129.0 (2xCHAR), 128.9 (2xCHAR), 127.7 (2xCHAR), 127.4 (CH- γ), 125.8 (CH- α) ppm.

These data are consistent with those described in the literature.²⁸⁴

4-Methyl-1,5-diphenylpenta-2,4-dien-1-one (49)

Prepared from acetophenone (600 mg, 5 mmol) and 2-methyl-3-phenylacrylaldehyde (804 mg, 5.5 mmol).

Reaction time: 20 h.

Purification: dichloromethane.

Yield: 931 mg (75%). Yellow solid.

Data of 49:

IR (NaCl) ν 1653 (C=O), 978 (CH- α =CH- β) cm^{-1} .

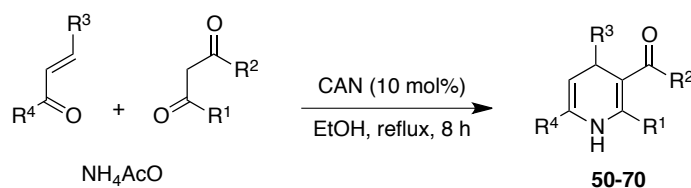
$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 2.47 (d, $J = 1.1$ Hz, 3H, C- γCH_3), 7.01 (bs, 1H, CH- δ), 7.09 (d, $J = 15.3$ Hz, 1H, CH- α), 7.31-7.36 (m, 1H, CH-4'), 7.39-7.43 (m, 4H, CH-2', CH-3', CH-5' and CH-6'), 7.47-7.63 (m, 3H, CH-3, CH-4 and CH-5), 7.68 (d, $J = 15.4$ Hz, 1H, CH- β), 8.01-8.04 (m, 2H, CH-2 and CH-6) ppm.

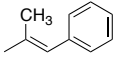
²⁸⁴ Pinto, D. C. G. A.; Silva, A. M. S.; Levai, A.; Cavaleiro, J. A. S.; Patonay, T.; Elguero, J. *Eur. J. Org. Chem.* **2000**, 2593–2599.

¹³C-NMR (CDCl₃, 63 MHz) δ 191.3 (C=O), 150.7 (CH-β), 141.0 (CH-δ), 138.9 (C-1), 137.2 (C-1'), 135.1 (C-γ), 133.0 (CH-4), 130.0 (2xCHAR), 129.0 (2xCHAR), 128.9 (2xCHAR), 128.8 (2xCHAR), 128.3 (CH-4'), 121.9 (CH-α) 14.4 (C-γCH₃) ppm.

These data are consistent with those described in the literature.²⁸⁴

9.3.3 Synthesis of 1,4-dihydropyridine derivatives 50-70. General procedure



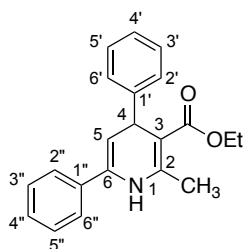
Cmpd.	R ¹	R ²	R ³	R ⁴
50	CH ₃	OEt	C ₆ H ₅	C ₆ H ₅
51	C ₂ H ₅	OEt	C ₆ H ₅	C ₆ H ₅
52	C ₃ H ₇	OEt	C ₆ H ₅	C ₆ H ₅
53	CH ₃	S ^t Bu	C ₆ H ₅	C ₆ H ₅
54	CH ₃	CH ₃	C ₆ H ₅	C ₆ H ₅
55	CH ₃	OEt	C ₆ H ₅	4-ClC ₆ H ₄
56	CH ₃	OEt	4-ClC ₆ H ₄	4-ClC ₆ H ₄
57	CH ₃	OEt	4-MeOC ₆ H ₄	C ₆ H ₅
58	CH ₃	S ^t Bu	4-MeOC ₆ H ₄	C ₆ H ₅
59	CH ₃	OEt	3-MeOC ₆ H ₄	4-ClC ₆ H ₄
60	CH ₃	S ^t Bu	4-BrC ₆ H ₄	4-MeC ₆ H ₄
61	CH ₃	S ^t Bu	4-MeC ₆ H ₄	C ₆ H ₅
62	CH ₃	S ^t Bu	4-MeC ₆ H ₄	4-MeC ₆ H ₄
63	CH ₃	OEt	4-MeC ₆ H ₄	C ₆ H ₅
64	CH ₃	OAllyl	C ₆ H ₅	4-ClC ₆ H ₄
65	CH ₃	OEt	4-NO ₂ C ₆ H ₄	C ₆ H ₅
66	CH ₃	OEt	2-NO ₂ C ₆ H ₄	4-MeC ₆ H ₄
67	CH ₃	OEt	2-NO ₂ C ₆ H ₄	4-ClC ₆ H ₄
68	CH ₃	OEt	2-Thienyl	4-MeC ₆ H ₄
69	CH ₃	OEt	4-MeC ₆ H ₄	2-Furyl
70	CH ₃	S ^t Bu		C ₆ H ₅

To a stirred solution of 1,3-diphenyl-2-propen-1-one derivatives (1 equiv, 2 mmol), 1,3-dicarbonyl compounds (1.1 equiv, 2.2 mmol) and ammonium acetate (3 equiv, 6 mmol) in ethanol (2 mL) was added ceric ammonium nitrate (CAN, 10

mol%) and the resulting mixture was heated under reflux for 4 hours. After this time, ammonium acetate (1.5 equiv, 3 mmol) was again added and stirring was continued under the same conditions for additional 4 hours. After this time, the mixture was allowed to cool to room temperature, diluted with CH₂Cl₂ (20 mL) and washed with water to remove CAN and the excess of ammonium acetate. The organic layer is then washed with brine and dried over anhydrous Na₂SO₄, and the solvent was evaporated under reduced pressure.

The crude residue was crystallized from EtOH or purified by silica gel column chromatography using *n*-hexane: ethyl acetate mixtures as eluent to give pure compounds (**50-70**).

Data of compounds **50-70**, and the experimental conditions employed for their syntheses are indicated below.

Ethyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (50)

Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: Crystallization from EtOH.

Yield: 574 mg (95%). Yellow solid.

Data of 50:

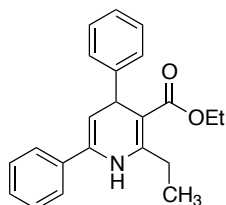
Mp: 244-246 °C

Elemental analysis calcd (%) for C₂₁H₂₁NO₂: C 78.97, H 6.63, N 4.39; found: C 78.87, H 6.71, N 4.42.

IR (NaCl) ν 1717 (C=O), 1220 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.28 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.56 (s, 3H, C-2CH₃), 4.17 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.84 (d, *J* = 5.5 Hz, 1H, CH-4), 5.34 (dd, *J* = 1.8, 5.5 Hz, 1H, CH-5), 5.73 (bs, 1H, NH), 7.30-7.33 (m, 1H, ArH), 7.38-7.52 (m, 9H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.84 (C=O), 149.28 (C-2*), 147.29 (C-1'*), 136.34 (C-6*), 134.73 (C-1''*), 129.20 (2xCHAR), 128.91 (CHAR), 128.64 (2xCHAR), 128.20 (2xCHAR), 126.47 (CHAR), 125.50 (2xCHAR), 105.52 (CH-5), 99.50 (C-3), 59.69 (OCH₂CH₃), 41.36 (CH-4), 21.12 (C-2CH₃), 14.68 (OCH₂CH₃) ppm.

Ethyl 2-ethyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (51)

Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), ethyl 3-oxopentanoate (317 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 613 mg (92%). White solid.

Data of 51:

Mp: 143-145 °C

Elemental analysis calcd (%) for C₂₂H₂₃NO₂: C 79.25, H 6.95, N 4.20; found: C

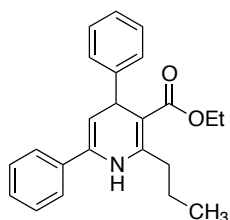
78.94, H 6.88, N 4.40.

IR (NaCl) ν 1666 (C=O), 1217 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.28 (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 1.44 (t, $J = 7.5$ Hz, 3H, OCH_2CH_3), 2.97 (dq, $J = 4.3, 7.2$ Hz, 2H, CH_2CH_3), 4.17 (dq, $J = 0.6, 7.5$ Hz, 2H, OCH_2CH_3), 4.84 (d, $J = 5.6$ Hz, 1H, CH-4), 5.33 (dd, $J = 1.9, 5.6$ Hz, 1H, CH-5), 5.79 (bs, 1H, NH), 7.23-7.33 (m, 1H, ArH), 7.38-7.57 (m, 9H, ArH) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 168.34 (C=O), 152.73 (C-2*), 149.37 (C-1'**), 136.43 (C-6*), 134.63 (C-1''*), 129.22 (2xCHAR), 128.91 (CHAR), 128.63 (2xCHAR), 128.12 (2xCHAR), 126.43 (CHAR), 125.47 (2xCHAR), 105.40 (CH-5), 98.59 (C-3), 59.65 (OCH_2CH_3), 41.36 (CH-4), 27.43 (CH_2CH_3), 14.61 (OCH_2CH_3), 13.51 (CH_2CH_3) ppm.

Ethyl 4,6-diphenyl-2-propyl-1,4-dihydropyridine-3-carboxylate (52)



Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), ethyl butyrylacetate (345 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 562 mg (81%). Yellow paste.

Data of 52:

Elemental analysis calcd (%) for $\text{C}_{23}\text{H}_{25}\text{NO}_2$: C 79.51, H 7.25, N 4.03; found: C 79.21, H 6.93, N 3.94.

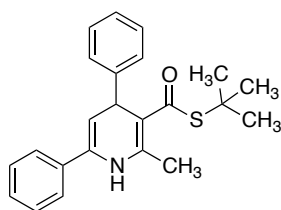
IR (NaCl) ν 3363 (N-H), 1724 (C=O), 1216 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.08 (t, $J = 7.3$ Hz, 3H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.17 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.69-1.84 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.69-2.90 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 4.06 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.72-4.75 (d, $J = 5.6$ Hz, 1H, CH-4), 5.20-5.23 (dd, $J = 1.9, 5.6$ Hz, 1H, CH-5), 5.68 (bs, 1H, NH), 7.15-7.43 (m, 10H, ArH) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 168.47 (C=O), 151.61 (C-2*), 149.43 (C-1'**), 136.43 (C-6*), 134.63 (C-1''*), 129.21 (2xCHAR), 128.90 (CHAR), 128.65 (2xCHAR), 128.15 (2xCHAR), 126.44 (CHAR), 125.52 (2xCHAR), 105.37 (CH-5), 98.98 (C-3), 59.68 (OCH_2CH_3), 41.43 (CH-4), 36.11 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 22.70

(CH₂CH₂CH₃), 14.64 (OCH₂CH₃*), 14.60 (CH₂CH₂CH₃*) ppm.

***S*-tert-butyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carbothioate (53)**



Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), *S*-tert-butyl 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: crystallization from EtOH.

Yield: 718 mg (99%). Yellow solid.

Data of 53:

Mp: 158-160 °C

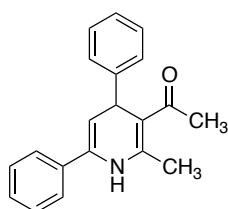
Elemental analysis calcd (%) for C₂₃H₂₅NOS: C 75.99, H 6.93, N 3.85, S 8.82; found: C 75.86, H 6.74, N 4.05, S 8.57.

IR (NaCl) ν 1630 (C=O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.44 (s, 9H, SC(CH₃)₃), 2.45 (s, 3H, C-2CH₃), 4.87 (d, *J* = 6 Hz, 1H, CH-4), 5.30 (dd, *J* = 1.9, 6 Hz, 1H, CH-5), 5.68 (bs, 1H, NH), 7.20-7.24 (m, 1H, ArH), 7.31-7.40 (m, 9H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 193.12 (C=O), 147.94 (C-2*), 144.72 (C-1'*), 136.06 (C-6*), 134.53 (C-1''*), 129.20 (2xCHAR), 128.96 (CHAR), 128.79 (2xCHAR), 127.87 (2xCHAR), 126.59 (CHAR), 125.51 (2xCHAR), 108.04 (C-3), 105.63 (CH-5), 47.59 (SC(CH₃)₃), 41.44 (CH-4), 30.53 (SC(CH₃)₃), 21.66 (C-2CH₃) ppm.

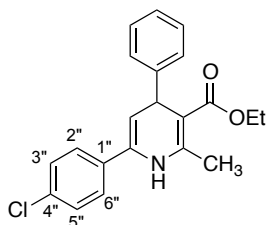
1-(2-Methyl-4,6-diphenyl-1,4-dihydropyridin-3-yl)ethanone (54)



Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), acetylacetone (220 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 10:1, v/v).

Yield: 324 mg (56%). Yellow solid.

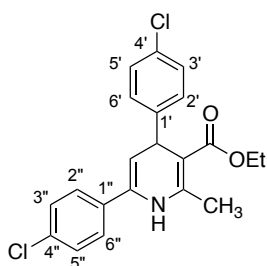
Data of 54:**Mp:** 153-155 °C**Elemental analysis** calcd (%) for C₂₀H₁₉NO: C 83.01, H 6.62, N 4.84; found: C 82.83, H 6.45, N 4.98.**IR** (NaCl) ν 1637 (C=O) cm⁻¹.**¹H-NMR** (CDCl₃, 250 MHz) δ 2.07 (s, 3H, COCH₃), 2.48 (s, 3H, C-2CH₃), 4.74 (d, *J* = 5.6 Hz, 1H, CH-4), 5.30 (dd, *J* = 1.9, 5.6 Hz, 1H, CH-5), 5.70 (bs, 1H, NH), 7.19-7.25 (m, 1H, ArH), 7.30-7.40 (m, 9H, ArH) ppm.**¹³C-NMR** (CDCl₃, 63 MHz) δ 199.25 (C=O), 148.33 (C-2*), 147.26 (C-1'*), 135.99 (C-6*), 134.08 (C-1''*), 129.21 (2xCHAr), 129.17 (2xCHAr), 129.01 (CHAr), 127.74 (2xCHAr), 126.81 (CHAr), 125.44 (2xCHAr), 107.83 (C-3), 106.36 (CH-5), 42.52 (CH-4), 29.94 (COCH₃), 22.21 (C-2CH₃) ppm.**Ethyl 6-(4-chlorophenyl)-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (55)**

Prepared from 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (487 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 7:1, v/v).
Yield: 500 mg (71%). Yellow solid.**Data of 55:****Mp:** > 250 °C**Elemental analysis** calcd (%) for C₂₁H₂₀ClNO₂: C 71.28, H 5.70, N 3.96; found: C 70.91, H 5.68, N 4.00.**IR** (NaCl) ν 1722 (C=O), 1223 (C-O), 1097 (C-Cl) cm⁻¹.**¹H-NMR** (CDCl₃, 250 MHz) δ 1.16 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.44 (s, 3H, C-2CH₃), 4.05 (dq, *J* = 1.6, 7.1 Hz, 2H, OCH₂CH₃), 4.71 (d, *J* = 5.5 Hz, 1H, CH-4), 5.19 (dd, *J* = 1.8, 5.5 Hz, 1H, CH-5), 5.52 (bs, 1H, NH), 7.16-7.22 (m, 1H, ArH), 7.27-7.36 (m, 8H, ArH) ppm.**¹³C-NMR** (CDCl₃, 63 MHz) δ 168.69 (C=O), 149.01 (C-2*), 147.03 (C-1'*),

134.79 (C-6*), 134.66 (C-1''*), 133.75 (C-4''*), 129.36 (2xCHAr), 128.67 (2xCHAr), 128.14 (2xCHAr), 126.81 (2xCHAr), 126.54 (CHAr), 106.03 (CH-5), 99.73 (C-3), 59.74 (OCH₂CH₃), 41.33 (CH-4), 21.09 (C-2CH₃), 14.63 (OCH₂CH₃) ppm.

Ethyl 4,6-bis(4-chlorophenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (56)



Prepared from 1,3-bis(4-chlorophenyl)prop-2-en-1-one (554 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 7:1, v/v).

Yield: 666 mg (86%). Yellow solid.

Data of 56:

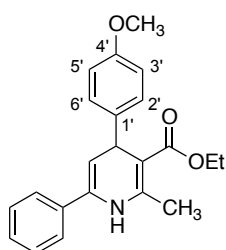
Mp: > 250 °C

Elemental analysis calcd (%) for C₂₁H₁₉Cl₂NO₂: C 64.96, H 4.93, N 3.61; found: C 64.78, H 4.84, N 3.43.

IR (NaCl) ν 3348 (N-H), 1724 (C=O), 1231 (C-O), 1091 (C-Cl) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.17 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.44 (s, 3H, C-2CH₃), 4.05 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.69 (d, *J* = 5.5 Hz, 1H, CH-4), 5.15 (dd, *J* = 1.88, 5.5 Hz, 1H, CH-5), 5.57 (bs, 1H, NH), 7.26 (s, 4H, ArH), 7.35 (s, 4H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.51 (C=O), 147.55 (C-2*), 147.27 (C-1'*), 134.83 (C-6*), 134.59 (C-1''*), 134.02 (C-4''*), 132.15 (C-4'*), 129.49 (2xCHAr), 129.41 (2xCHAr), 128.77 (2xCHAr), 126.85 (2xCHAr), 105.48 (CH-5), 99.39 (C-3), 59.85 (OCH₂CH₃), 40.85 (CH-4), 21.11 (C-2CH₃), 14.67 (OCH₂CH₃) ppm.

Ethyl 4-(4-methoxyphenyl)-2-methyl-6-phenyl-1,4-dihydropyridine-3-carboxylate (57)

Prepared from 3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (476 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).

Yield: 510 mg (73%). Yellow solid.

Data of 57:

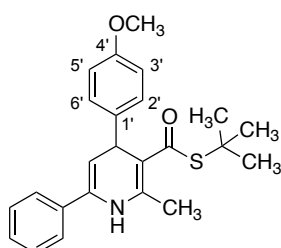
Mp: 220-222 °C

Elemental analysis calcd (%) for C₂₂H₂₃NO₃: C 75.62, H 6.63, N 4.01; found: C 75.42, H 6.48, N 4.00.

IR (NaCl) ν 3375 (N-H), 1722 (C=O), 1250 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.20 (t, *J* = 7.12 Hz, 3H, OCH₂CH₃), 2.43 (s, 3H, C-2CH₃), 3.80 (s, 3H, OCH₃), 4.08 (q, *J* = 7.12 Hz, 2H, OCH₂CH₃), 4.67 (d, *J* = 5.5 Hz, 1H, CH-4), 5.21 (dd, *J* = 1.62, 5.5 Hz, 1H, CH-5), 5.60 (bs, 1H, NH), 6.84 (d, *J* = 8.7 Hz, 2H, CH-3' and CH-5'), 7.27 (d, *J* = 8.7 Hz, 2H, CH-2' and CH-6'), 7.35-7.46 (m, 5H, C-6ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.91 (C=O), 158.31 (C-4'), 146.93 (C-2*), 141.80 (C-1''*), 136.37 (C-1'*), 134.59 (C-6*), 129.20 (4xCHAR), 128.87 (CHAR), 125.47 (2xCHAR), 113.96 (2xCHAR), 105.62 (CH-5), 99.82 (C-3), 59.69 (OCH₂CH₃), 55.64 (C-4'OCH₃), 40.35 (CH-4), 21.15 (C-2CH₃), 14.74 (OCH₂CH₃) ppm.

S-tert-Butyl 4-(4-methoxyphenyl)-2-methyl-6-phenyl-1,4-dihydropyridine-3-carbothioate (58)

Prepared from 3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (476 mg, 2 mmol), *S*-tert-butyl 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).

Yield: 542 mg (69%). Yellow solid.

Data of 58:

Mp: 138-140 °C

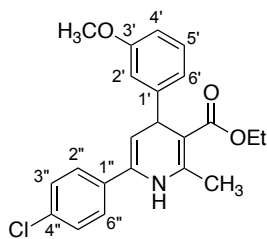
Elemental analysis calcd (%) for C₂₄H₂₇NO₂S: C 73.25, H 6.92, N 3.56, S 8.15; found: C 73.11, H 6.75, N 3.73, S 7.91.

IR (NaCl) ν 1634 (C=O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.56 (s, 9H, SC(CH₃)₃), 2.55 (s, 3H, C-2CH₃), 3.92 (s, 3H, OCH₃), 4.92 (d, *J* = 6.0 Hz, 1H, CH-4), 5.40 (dd, *J* = 1.6, 6.0 Hz, 1H, CH-5), 5.78 (bs, 1H, NH), 6.97 (d, *J* = 8.6 Hz, 2H, CH-3' and CH-5'), 7.38 (d, *J* = 8.6 Hz, 2H, CH-2' and CH-6'), 7.49-7.51 (m, 5H, C-6ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 193.19 (C=O), 158.40 (C-4'), 144.44 (C-2*), 140.52 (C-1''), 136.12 (C-6*), 134.32 (C-1'*), 129.20 (2xCHAR), 128.91 (CHAR), 128.88 (2xCHAR), 125.50 (2xCHAR), 114.14 (2xCHAR), 108.39 (C-3), 105.83 (CH-5), 55.60 (C-4'OCH₃), 47.56 (SC(CH₃)₃), 40.51 (CH-4), 30.56 (SC(CH₃)₃), 21.65 (C-2CH₃) ppm.

Ethyl 6-(4-chlorophenyl)-4-(3-methoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylate (59)



Prepared from 1-(4-chlorophenyl)-3-(3-methoxyphenyl) prop-2-en-1-one (545 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).
Yield: 575 mg (75%). Yellow syrup.

Data of 59:

Elemental analysis calcd (%) for C₂₂H₂₂ClNO₃: C 68.83, H 5.78, N 3.65; found: C 68.59, H 5.67, N 3.72.

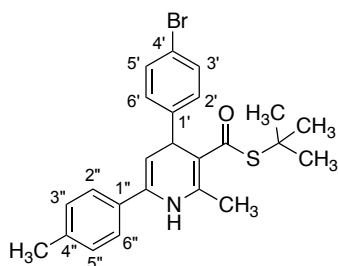
IR (NaCl) ν 3356 (N-H), 1724 (C=O), 1224 (C-O), 1093 (C-Cl) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.17 (t, *J* = 7.12 Hz, 3H, OCH₂CH₃), 2.44 (s, 3H, C-2CH₃), 3.81 (s, 3H, OCH₃), 4.08 (q, *J* = 7.12 Hz, 2H, OCH₂CH₃), 4.69 (d, *J* =

5.5 Hz, 1H, CH-4), 5.19 (dd, $J = 1.2, 5.5$ Hz, 1H, CH-5), 5.54 (bs, 1H, NH), 6.72-6.77 (dd, $J = 2.5, 8.1$ Hz, 1H, CH-6'), 6.89-6.96 (m, 2H, CH-2' and CH-4') 7.23 (t, $J = 7.8$ Hz, 1H, CH-5'), 7.35 (s, 4H, C-6ArH) ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 168.65 (C=O), 160.05 (C-3'), 150.70 (C-1'*), 147.06 (C-2*), 134.77 (C-6*), 134.68 (C-1''*), 133.81 (C-4''*), 129.58 (CHAR), 129.36 (2xCHAR), 126.82 (2xCHAR), 120.61 (CHAR), 114.26 (CHAR), 111.39 (CHAR), 105.89 (CH-5), 99.56 (C-3), 59.76 (OCH_2CH_3), 55.54 (C-3'OCH₃), 41.32 (CH-4), 21.12 (C-2CH₃), 14.68 (OCH_2CH_3) ppm.

S-tert-Butyl 4-(4-bromophenyl)-2-methyl-6-(4-methylphenyl)-1,4-dihydropyridine-3-carbothioate (60)



Prepared from 3-(4-bromophenyl)-1-(4-methylphenyl)prop-2-en-1-one (602 mg, 2 mmol), *S-tert-butyl* 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 610 mg (67%). Light yellow solid.

Data of 60:

Mp: 164-166 °C

Elemental analysis calcd (%) for $\text{C}_{24}\text{H}_{26}\text{BrNOS}$: C 63.15, H 5.74, N 3.07, S 7.03; found: C 62.98, H 5.78, N 3.36, S 6.99.

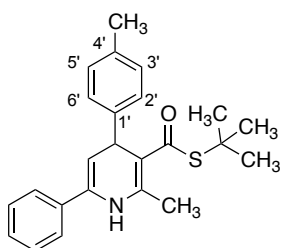
IR (NaCl) ν 3299 (N-H), 1612 (C=O), 1088 (C-Br) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.56 (s, 9H, $\text{SC}(\text{CH}_3)_3$), 2.49 (s, 3H, ArCH_3^*), 2.55 (s, 3H, C-2CH₃*), 4.93 (d, $J = 5.9$ Hz, 1H, CH-4), 5.32 (dd, $J = 1.6, 5.9$ Hz, 1H, CH-5), 5.79 (bs, 1H, NH), 7.28-7.40 (m, 6H, ArH), 7.54 (d, $J = 8.3$ Hz, 2H, CH-3' and CH-5') ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 192.50 (C=O), 146.77 (C-2*), 144.71 (C-1'*), 138.82 (C-6*), 134.41 (C-4''*), 132.68 (C-1''*), 131.55 (2xCHAR), 129.61 (2xCHAR), 129.32 (2xCHAR), 125.10 (2xCHAR), 120.03 (C-4'), 107.28 (C-3), 104.03 (CH-5), 47.45 ($\text{SC}(\text{CH}_3)_3$), 40.65 (CH-4), 30.22 ($\text{SC}(\text{CH}_3)_3$), 21.42 (C-

4''CH₃*), 21.31 (C-2CH₃*) ppm.

S-tert-Butyl 2-methyl-4-(4-methylphenyl)-6-phenyl-1,4-dihydropyridine-3-carbothioate (61)



Prepared from 3-(4-methylphenyl)-1-phenylprop-2-en-1-one (444 mg, 2 mmol), *S*-tert-butyl 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: crystallization from EtOH.

Yield: 745 mg (99%). Yellow solid.

Data of 61:

Mp: 172-174 °C

Elemental analysis calcd (%) for C₂₄H₂₇NOS: C 76.35, H 7.21, N 3.71, S 8.49; found: C 75.92, H 7.10, N 4.01, S 8.11.

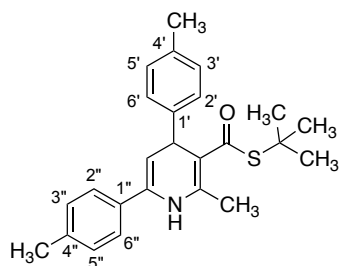
IR (NaCl) ν 3279 (N-H), 1612 (C=O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.45 (s, 9H, SC(CH₃)₃), 2.34 (s, 3H, ArCH₃*), 2.44 (s, 3H, C-2CH₃*), 4.82 (d, *J* = 6.1 Hz, 1H, CH-4), 5.31 (dd, *J* = 1.8, 6.1 Hz, 1H, CH-5), 5.68 (bs, 1H, NH), 7.12 (d, *J* = 8.0 Hz, 2H, CH-3' and CH-5'), 7.25 (d, *J* = 8.0 Hz, 2H, CH-2' and CH-6'), 7.33-7.39 (m, 5H C-6ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 193.13 (C=O), 145.10 (C-2*), 144.76 (C-1'*), 136.10 (C-6*), 136.07 (C-1''*), 134.45 (C-4'*), 129.54 (2xCHAR), 129.19 (2xCHAR), 128.92 (CHAR), 127.73 (2xCHAR), 125.52 (2xCHAR), 108.12 (C-3), 105.82 (CH-5), 47.58 (SC(CH₃)₃), 40.90 (CH-4), 30.57 (SC(CH₃)₃), 21.71 (C-4'CH₃*), 21.54 (C-2CH₃*) ppm.

S-tert-Butyl 2-methyl-4,6-bis(4-methylphenyl)-1,4-dihydropyridine-3-carbothioate (62)

Prepared from 1,3-bis(4-methylphenyl)prop-2-en-1-one (472 mg, 2 mmol), *S*-tert-butyl 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).



Purification: crystallization from EtOH.

Yield: 760 mg (97%). Yellow solid.

Data of 62:

Mp: 169-171 °C

Elemental analysis calcd (%) for C₂₅H₂₉NOS: C 76.68, H 7.46, N 3.58, S 8.19; found: C 76.57, H

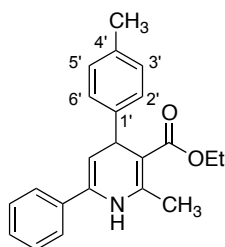
7.36, N 3.79, S 8.13.

IR (NaCl) ν 1614 (C=O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.46 (s, 9H, SC(CH₃)₃), 2.35 (s, 3H, C-4'CH₃*), 2.38 (s, 3H, C-4''CH₃*), 2.44 (s, 3H, C-2CH₃*), 4.82 (d, *J* = 6.1 Hz, 1H, CH-4), 5.27 (dd, *J* = 1.7, 6.1 Hz, 1H, CH-5), 5.67 (bs, 1H, NH), 7.04-7.38 (m, 8H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 193.11 (C=O), 145.21 (C-2*), 144.79 (C-1'*), 138.85 (C-6*), 136.01 (C-4'*), 134.33 (C-4''*), 133.24 (C-1''*), 129.83 (2xCHAR), 129.51 (2xCHAR), 127.73 (2xCHAR), 125.39 (2xCHAR), 108.08 (C-3), 105.15 (CH-5), 47.54 (SC(CH₃)₃), 40.85 (CH-4), 30.58 (SC(CH₃)₃), 21.74 (C-4'CH₃*), 21.60 (C-4''CH₃*), 21.54 (C-2CH₃*) ppm.

Ethyl 2-methyl-4-(4-methylphenyl)-6-phenyl-1,4-dihydropyridine-3-carboxylate (63)



Prepared from 3-(4-methylphenyl)-1-phenylprop-2-en-1-one (444 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 625 mg (94%). Yellow solid.

Data of 63:

Mp: 233-235 °C

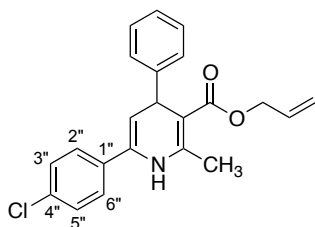
Elemental analysis calcd (%) for C₂₂H₂₃NO₂: C 79.25, H 6.95, N 4.20; found: C 79.01, H 6.89, N 4.14.

IR (NaCl) ν 1723 (C=O), 1235 (C-O) cm⁻¹;

¹H-NMR (CDCl₃, 250 MHz) δ 1.31 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.45 (s, 3H, C-4'CH₃*), 2.55 (s, 3H, C-2CH₃*), 4.19 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.81 (d, *J* = 5.5 Hz, 1H, CH-4), 5.34 (dd, *J* = 1.9-5.5 Hz, 1H, CH-5), 5.75 (bs, 1H, NH), 7.24 (d, *J* = 8.0 Hz, 2H, CH-3' and CH-5'), 7.38 (d, *J* = 8.0 Hz, 2H, CH-2' and CH-6'), 7.45-7.57 (m, 5H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.84 (C=O), 147.01 (C-2*), 146.36 (C-1'*), 136.38 (C-6*), 135.91 (C-1''*), 134.64 (C-4'*), 129.32 (2xCHAR), 129.17 (2xCHAR), 128.84 (CHAR), 128.08 (2xCHAR), 125.46 (2xCHAR), 105.61 (CH-5), 99.72 (C-3), 59.67 (OCH₂CH₃), 40.80 (CH-4), 21.47 (C-4'CH₃*), 21.15 (C-2CH₃*), 14.70 (OCH₂CH₃) ppm.

Allyl 6-(4-chlorophenyl)-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (64)



Prepared from 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (487 mg, 2 mmol), allyl acetoacetate (313 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 568 mg (78%). Orange syrup.

Data of 64:

Elemental analysis calcd (%) for C₂₂H₂₀ClNO₂: C 72.22, H 5.51, N 3.83; found: C 71.97, H 5.43, N 4.00.

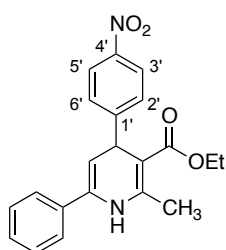
IR (NaCl) ν 1675 (C=O), 1219 (C-O), 1091 (C-Cl) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 2.45 (s, 3H, C-2CH₃), 4.52 (dd, *J* = 0.85, 5.3 Hz, 2H, OCH₂CH=CH₂), 4.74 (d, *J* = 5.58 Hz, 1H, CH-4), 5.10-5.23 (m, 3H, CH-5, OCH₂CH=CH₂), 5.60 (bs, 1H, NH) 5.76-5.93 (m, 1H, OCH₂CH=CH₂), 7.17-7.24 (m, 1H, ArH), 7.28-7.38 (m, 8H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 165.86 (C=O), 146.44 (C-2*), 145.26 (C-1'*), 132.28 (C-6*), 132.25 (C-1''*), 131.26 (C-4''*), 130.95 (OCH₂CH=CH₂), 126.92 (2xCHAR), 126.32 (2xCHAR), 125.66 (2xCHAR), 124.41 (2xCHAR), 124.18 (CHAR), 114.96 (OCH₂CH=CH₂), 103.75 (CH-5), 96.85 (C-3), 62.12

(OCH₂CH=CH₂), 38.81 (CH-4), 18.76 (C-2CH₃) ppm.

Ethyl 2-methyl-4-(4-nitrophenyl)-6-phenyl-1,4-dihydropyridine-3-carboxylate (65)



Prepared from 3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (507 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: Crystallization from EtOH.

Yield: 700 mg (96%). Orange solid.

Data of 65:

Mp: 108-110 °C

Elemental analysis calcd (%) for C₂₁H₂₀N₂O₄: C 69.22, H 5.53, N 7.69; found: C 69.41, H 5.27, N 7.50.

IR (NaCl) ν 3386 (N-H), 1723 (C=O), 1518 (NO₂), 1220 (C-O) cm⁻¹.

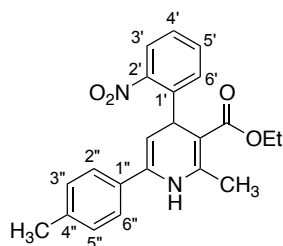
¹H-NMR (CDCl₃, 250 MHz) δ 1.17 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.48 (s, 3H, C-2CH₃), 4.07 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.86 (d, *J* = 5.5 Hz, 1H CH-4), 5.14 (dd, *J* = 1.88, 5.5 Hz, 1H, CH-5), 5.75 (bs, 1H, NH), 7.38-7.44 (m, 5H, C-6ArH), 7.51 (d, *J* = 8.75 Hz, 2H, CH-2' and CH-6'), 8.18 (d, *J* = 8.75 Hz, 2H, CH-3' and CH-5') ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.25 (C=O), 156.40 (C-1'), 148.13 (C-2*), 146.73 (C-4'*), 135.79 (C-6*), 135.68 (C-1''*), 129.36 (3xCHAR), 128.84 (2xCHAR), 125.53 (2xCHAR), 124.16 (2xCHAR), 103.87 (CH-5), 98.46 (C-3), 59.96 (OCH₂CH₃), 41.68 (C-4), 21.22 (C-2CH₃), 14.69 (OCH₂CH₃) ppm.

Ethyl 2-methyl-6-(4-methylphenyl)-4-(2-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (66)

Prepared from 1-(4-methylphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (534 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).



Yield: 590 mg (78%). Yellow syrup.

Data of 66:

Elemental analysis calcd (%) for $C_{22}H_{22}N_2O_4$: C 69.83, H 5.86, N 7.40; found: C 69.86, H 5.69, N 7.64.

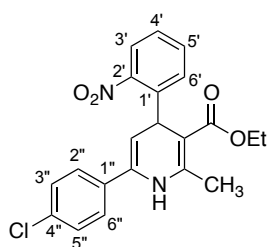
IR (NaCl) ν 3332 (N-H), 1674 (C=O), 1524 (NO_2), 1221

(C-O) cm^{-1} .

1H -NMR ($CDCl_3$, 250 MHz) δ 0.98 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.38 (s, 3H, C-4'' CH_3^*), 2.49 (s, 3H, C-2 CH_3^*), 3.92 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 5.21 (d, $J = 5.05$ Hz, 1H, CH-4), 5.38 (dd, $J = 1.8, 5.05$ Hz, 1H, CH-5), 5.67 (bs, 1H, NH), 7.19 (d, $J = 7.95$ Hz, 2H, CH-3'' and CH-5''), 7.25-7.33 (m, 3H, CH-2'', CH-6'' and CH-6'), 7.55 (dt, $J = 1.0, 7.42$ Hz, 1H, CH-4'), 7.66 (dd, $J = 1.22, 7.87$ Hz, 1H, CH-5'), 7.74 (dd, $J = 1.0, 8.08$ Hz, 1H, CH-3') ppm.

^{13}C -NMR ($CDCl_3$, 63 MHz) δ 167.97 (C=O), 148.92 (C-2*), 148.22 (C-1'*), 144.08 (C-2'*), 139.15 (C-4''*), 135.09 (C-1''*), 133.50 (CHAR), 133.06 (C-6*), 131.94 (CHAR), 129.89 (2xCHAR), 126.90 (CHAR), 125.28 (2xCHAR), 123.47 (CHAR), 103.57 (CH-5), 98.47 (C-3), 59.70 (OCH_2CH_3), 37.14 (CH-4), 21.59 (C-4'' CH_3^*), 20.86 (C-2 CH_3^*), 14.30 (OCH_2CH_3) ppm.

Ethyl 6-(4-chlorophenyl)-2-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (67)



Prepared from 1-(4-chlorophenyl)-3-(2-nitrophenyl) prop-2-en-1-one (575 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 12:1 to 5:1, v/v).

Yield: 495 mg (62%). Yellow syrup.

Data of 67:

Elemental analysis calcd (%) for $C_{21}H_{19}ClN_2O_4$: C 69.22, H 5.53, N 7.69; found: C 69.11, H 5.37, N 7.66.

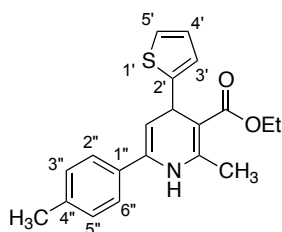
IR (NaCl) ν 3188 (N-H), 1721 (C=O), 1528 (NO_2), 1269 (C-O), 1094 (C-Cl) cm^{-1}

1.

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 0.97 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.49 (s, 3H, C-2 CH_3), 3.91 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 5.21 (d, $J = 5.1$ Hz, 1H, CH-4), 5.40 (dd, $J = 1.7, 5.1$ Hz, 1H, CH-5), 5.57 (bs, 1H, NH), 7.27-7.34 (m, 1H, CH-6'), 7.36 (s, 4H, C-6ArH), 7.53-7.66 (m, 2H, CH-4' and CH-5'), 7.76 (dd, $J = 1, 8.2$ Hz, 1H, CH-3') ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 167.80 (C=O), 148.73 (C-2*), 148.23 (C-2'*), 143.68 (C-1'*), 134.96 (C-6*), 134.41 (C-1''*), 134.29 (C-4'*), 133.57 (CHAr), 131.84 (CHAr), 129.42 (2xCHAr), 127.06 (CHAr), 126.81 (2xCHAr), 123.57 (CHAr), 104.73 (CH-5), 98.74 (C-3), 59.81 (OCH_2CH_3), 37.16 (C-4), 20.83 (C-2 CH_3), 14.27 (OCH_2CH_3) ppm.

Ethyl 2-methyl-4-(thiophen-2-yl)-6-(4-methylphenyl)-1,4-dihydropyridine-3-carboxylate (68)



Prepared from 1-(4-methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (456 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 387 mg (57%). Orange syrup.

Data of 68:

Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{21}\text{NO}_2\text{S}$: C 70.77, H 6.24, N 4.13, S 9.45; found: C 70.76, H 6.06, N 4.25, S 9.46.

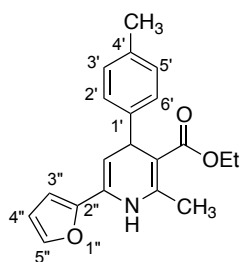
IR (NaCl) ν 1718 (C=O), 1221 (C-O) cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 1.30 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.40 (s, 3H, C-4' CH_3^*), 2.41 (s, 3H, C-2 CH_3^*), 4.18 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 5.04 (d, $J = 5.8$ Hz, 1H, CH-4), 5.28 (dd, $J = 1.8, 5.8$ Hz, 1H, CH-5), 5.76 (bs, 1H, NH), 6.92-6.95 (m, 2H, CH-3' and CH-4'), 7.14 (dd, $J = 1.6, 4.7$ Hz, 1H, CH-5'), 7.22 (d, $J = 8.0$ Hz, 2H, CH-3'' and CH-5''), 7.36 (d, $J = 8.0$ Hz, 2H, CH-2'' and CH-6'') ppm.

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 168.53 (C=O) 153.85 (C-2*), 147.11 (C-2'*),

139.10 (C-6*), 135.68 (C-4''*), 133.29 (C-1''*), 129.93 (2xCHAR), 126.95 (CH-thienyl), 125.60 (2xCHAR), 123.92 (CH-thienyl), 123.29 (CH-thienyl), 103.30 (CH-5), 99.56 (C-3), 59.87 (OCH₂CH₃), 35.47 (CH-4), 21.62 (C-4CH₃*), 21.16 (C-2CH₃*), 14.81 (OCH₂CH₃) ppm.

Ethyl 6-(furan-2-yl)-2-methyl-4-(4-methylphenyl)-1,4-dihydropyridine-3-carboxylate (69)



Prepared from 1-(furan-2-yl)-3-(4-methylphenyl)prop-2-en-1-one (424 mg, 2 mmol), ethyl acetoacetate (286 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 8:1, v/v).

Yield: 315 mg (49%). Yellow syrup.

Data of 69:

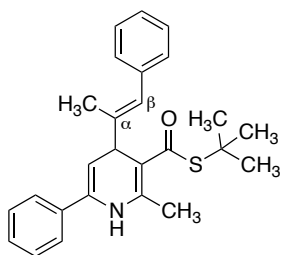
Elemental analysis calcd (%) for C₂₀H₂₁NO₃: C 74.28, H 6.55, N 4.33; found: C 73.99, H 6.37, N 4.22.

IR (NaCl) ν 3348 (N-H), 1724 (C=O), 1268 (C-O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.18 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.32 (s, 3H, C-4'CH₃*), 2.44 (s, 3H, C-2CH₃*), 4.05 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 4.64 (d, *J* = 5.65 Hz, 1H, CH-4), 5.37 (dd, *J* = 1.58, 5.65 Hz, 1H, CH-5), 5.90 (bs, 1H, NH), 6.40-6.44 (m, 2H, CH-3'' and CH-4''), 7.10 (d, *J* = 8.02 Hz, 2H, CH-3' and CH-5'), 7.23 (d, *J* = 8.02 Hz, 2H, CH-2' and CH-6'), 7.39-7.40 (m, 1H, CH-5'') ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 168.71 (C=O), 149.10 (C-2''*), 146.63 (C-2*), 146.03 (C-1'*), 141.88 (CH-furanyl), 136.04 (C-4'), 129.36 (2xCHAR), 128.07 (2xCHAR), 126.30 (C-6), 111.96 (CH-furanyl), 105.09 (CH-furanyl), 103.41 (CH-5), 99.66 (C-3), 59.70 (OCH₂CH₃), 40.12 (CH-4), 21.48 (C-4'CH₃*), 21.08 (C-2CH₃*), 14.67 (OCH₂CH₃) ppm.

S-tert-Butyl 2-methyl-6-phenyl-4-(1-phenylprop-1-en-2-yl)-1,4-dihydropyridine-3-carbothioate (70)



Prepared from 4-methyl-1,5-diphenylpenta-2,4-dien-1-one (497 mg, 2 mmol), *S*-tert-butyl 3-oxobutanethioate (383 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: *n*-hexane:ethyl acetate (from 15:1 to 10:1, v/v).

Yield: 420 mg (52%). Yellow solid.

Data of 70:

Mp: 113-115 °C

Elemental analysis calcd (%) for C₂₆H₂₉NOS: C 77.38, H 7.24, N 3.47, S 7.95; found: C 77.21, H 7.04, N 3.61, S 7.85.

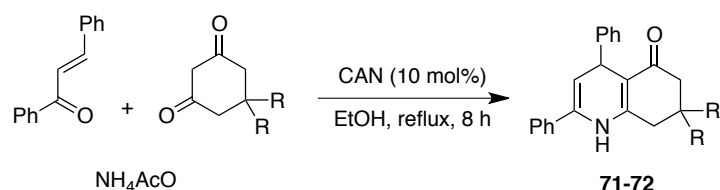
IR (NaCl) ν 3357 (N-H), 1633 (C=O) cm⁻¹.

¹H-NMR (CDCl₃, 250 MHz) δ 1.5 (s, 9H, SC(CH₃)₃), 1.95 (d, *J* = 1.3 Hz, 3H, C- α CH₃), 2.39 (s, 3H, C-2CH₃) 4.41 (d, *J* = 5.8 Hz, 1H, CH-4), 5.19 (dd, *J* = 1.9, 5.8 Hz, 1H, CH-5), 5.55 (bs, 1H, NH), 6.40 (s, 1H, CH- β), 7.20-7.24 (m, 1H, ArH), 7.27-7.45 (m, 9H, ArH) ppm.

¹³C-NMR (CDCl₃, 63 MHz) δ 193.74 (C=O), 144.68 (C-2*), 142.62 (C-1'**), 139.23 (C-6*), 136.28 (C-1'**), 135.75 (C- α *), 129.37 (2xCHAR), 129.25 (2xCHAR), 128.96 (CHAR), 128.34 (2xCHAR), 126.25 (CHAR), 125.68 (CH- β), 125.50 (2xCHAR), 106.88 (C-3), 103.78 (CH-5), 47.40 (SC(CH₃)₃), 45.76 (CH-4), 30.64 (SC(CH₃)₃), 21.35 (C-2CH₃), 15.67 (C- α CH₃) ppm.

9.3.4 Synthesis of 1,4-dihydropyridine bicyclic derivatives **71** and **72**.

General procedure

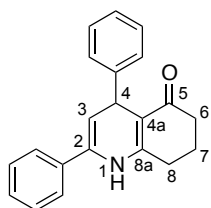


Compound	R
71	H
72	CH ₃

To a stirred solution of 1,3-diphenyl-2-propen-1-one (1 equiv, 2 mmol), cyclohexane-1,3-dione or its derivatives, (1.1 equiv, 2.2 mmol) and ammonium acetate (3 equiv, 6 mmol) in ethanol (2 mL) was added ceric ammonium nitrate (CAN, 10 mol%) and the resulting mixture was heated under reflux for 4 hours. After this time, ammonium acetate (1.5 equiv, 3 mmol) was again added and stirring was continued at the same conditions for additional 4 hours. After this time, the mixture was allowed to cool to room temperature, diluted with CH₂Cl₂ (20 mL), and washed with water to remove CAN and the excess of ammonium acetate. The organic layer was then washed with brine and dried over anhydrous Na₂SO₄, and the solvent was evaporated under reduced pressure.

The crude residue was crystallized from EtOH or purified by silica gel column chromatography using petroleum ether: ethyl acetate mixtures as eluent to give pure compounds **71** and **72**.

Data of compounds **71** and **72**, and the experimental conditions employed for their synthesis are indicated below.

2,4-Diphenyl-4,6,7,8-tetrahydroquinolin-5(1H)-one (71)

Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), 1,3-cyclohexanedione (246 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: petroleum ether:ethyl acetate (from 8:1 to 3:1, v/v).

Yield: 488 mg (81%). White solid.

Data of 71:

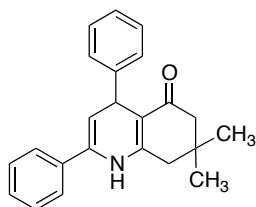
Mp: 208-210 °C

Elemental analysis calcd (%) for C₂₁H₁₉NO: C 83.69 H 6.35, N 4.65; found: C 83.45, H 6.01, N 4.51.

IR (NaCl) ν 1584 (C=O) cm⁻¹.

¹H-NMR (DMSO-*d*₆, 250 MHz) δ 1.75-1.99 (m, 2H, CH₂-7), 2.19-2.26 (m, 2H, CH₂-8), 2.55-2.71 (m, 2H, CH₂-6), 4.61 (d, *J* = 5.4 Hz, 1H, CH-4), 5.24 (dd, *J* = 1.6, 5.4 Hz, 1H, CH-3), 7.08-7.51 (m, 10H, ArH), 8.71 (bs, 1H, NH) ppm.

¹³C-NMR (DMSO-*d*₆, 63 MHz) δ 194.66 (C=O), 154.65 (C-8a*), 148.66 (C-1'*), 135.55 (C-2*), 134.85 (C-1''*), 128.81 (2xCHAR), 128.68 (CHAR), 128.46 (2xCHAR), 127.67 (2xCHAR), 125.96 (CHAR), 125.91 (2xCHAR), 107.38 (C-4a), 106.20 (CH-3), 37.28 (CH₂-6), 37.24 (CH-4), 27.25 (CH₂-8), 21.30 (CH₂-7) ppm.

7,7-Dimethyl-2,4-diphenyl-4,6,7,8-tetrahydroquinolin-5(1H)-one (72)

Prepared from 1,3-diphenyl-2-propen-1-one (416 mg, 2 mmol), 5,5-dimethyl-1,3-cyclohexanedione (308 mg, 2.2 mmol) and ammonium acetate (462 mg, 6 mmol + 231 mg, 3 mmol).

Purification: petroleum ether:ethyl acetate (from 8:1 to 3:1, v/v).

Yield: 545 mg (83%). Pale yellow solid.

Data of 72:

Mp: 204-206 °C

Elemental analysis calcd (%) for $C_{23}H_{23}NO$: C 83.85; H 7.04; N 4.25; found: C 83.90, H 7.07, N 4.34.

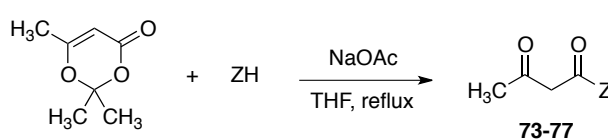
IR (NaCl) ν 1587 (C=O) cm^{-1} .



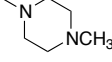
1H -NMR ($CDCl_3$, 250 MHz) δ 1.05 (s, 3H, C-7 CH_3), 1.14 (s, 3H, C-7 CH_3), 2.16-2.47 (m, 4H, CH_2 -6 and CH_2 -8), 4.78 (d, J = 5.2 Hz, 1H, CH-4), 5.33 (dd, J = 1.8, 5.2 Hz, 1H, CH-3), 5.98 (bs, 1H, NH), 7.14-7.45 (m, 10H, ArH) ppm.

^{13}C -NMR ($CDCl_3$, 63 MHz) δ 195.99 (C=O), 151.15 (C-8a*), 148.03 (C-1'*), 136.19 (C-2*), 134.44 (C-1''*), 129.90 (CHAr), 129.27 (2xCHAr), 128.69 (2xCHAr), 128.29 (2xCHAr), 126.47 (CHAr), 125.53 (2xCHAr), 108.86 (C-4a), 107.55 (CH-3), 51.14 (CH_2 -6), 42.50 (CH_2 -8), 38.15 (CH-4), 32.94 (C-7), 29.83 (C-7 CH_3^*), 27.83 (C-7 CH_3^*) ppm.

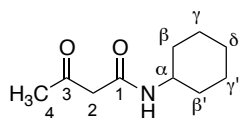
9.4 NICOTINAMIDE DERIVATIVES

9.4.1) Synthesis of β -ketoamides 73-77. General procedure



Compound	Z
73	NHc-Hex
74	
75	
76	
77	NHC ₆ H ₅

A mixture of the appropriate amine (1 equiv, 5 mmol), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.3 equiv, 6.5 mmol) and anhydrous NaOAc (1 equiv, 5 mmol) in THF (1 mL) was heated under reflux for the time period specified in the compound data sheet. After completion of the reaction (checked by TLC), the mixture was allowed to cool to room temperature and diluted with Et₂O (10 mL). The organic layer was washed with water and brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel column chromatography using petroleum ether:ethyl acetate mixtures as eluent to give pure compounds **73-77**.

N-Cyclohexyl-3-oxobutanamide (73)

Prepared from cyclohexanamine (496 mg, 5 mmol), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (924 mg, 6.5 mmol) and sodium acetate (410 mg, 5 mmol).

Reaction time: 24 h.

Purification: petroleum ether:ethyl acetate (from 10:1 to 4:1, v/v).

Yield: 870 mg (95%). Pale yellow solid.

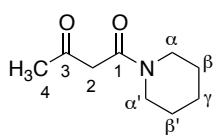
Data of 73:

IR (KBr) ν 3296 (N-H), 1722 (C-3=O), 1644 (C-1=O), 1556 (N-C=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 1.14–1.48 (m, 5H, CH_2 - γ , CH_2 - γ' and CH- δ_{ax}), 1.61–1.78 (m, 3H, CH- β_{ax} , CH- β'_{ax} and CH- δ_{eq} *), 1.90–1.94 (m, 2H, CH- β_{eq} and CH- β'_{eq}), 2.30 (s, 3H, CH_3 -4), 3.43 (s, 2H, CH_2 -2), 3.74–3.89 (m, 1H, CH- α), 6.86 (bs, 1H, CONH) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 205.39 (C-3=O), 164.82 (C-1=O), 50.21 (CH_2 -2), 48.62 (CH- α), 33.25 (CH_2 - β and CH_2 - β'), 31.50 (CH_3 -4), 25.90 (CH_2 - δ), 25.12 (CH_2 - γ and CH_2 - γ') ppm.

These data are consistent with those described in the literature.²⁸⁵

1-(Piperidin-1-yl)butane-1,3-dione (74)

Prepared from piperidine (426 mg, 5 mmol), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (924 mg, 6.5 mmol) and sodium acetate (410 mg, 5 mmol).

Reaction time: 24 h.

Purification: petroleum ether:ethyl acetate (from 12:1 to 7:1, v/v).

Yield: 805 mg (95%). Yellow oil.

Data of 74:

IR (KBr) ν 1721 (C-3=O), 1639 (C-1=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 1.54–1.65 (m, 6H, CH_2 - β , CH_2 - β' and CH_2 - γ),

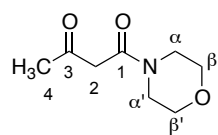
²⁸⁵ Sridharan, V.; Ruiz, M.; Menéndez, J.C. *Synthesis* **2010**, 6, 1053-1057.

2.28 (s, 3H, CH₃-4), 3.32–3.37 (m, 2H, CH- α ax and CH- α' ax), 3.55 (s, 2H, CH₂-2), 3.55–3.59 (m, 2H, CH- α eq and CH- α' eq) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 203.00 (C-3=O), 165.12 (C-1=O), 50.48 (CH₂-2), 47.82 (CH₂- α^*), 43.17 (CH₂- α'^*), 30.46 (CH₃-4), 26.62 (CH₂- β^*), 25.79 (CH₂- β'^*), 24.65 (CH₂- γ) ppm.

These data are consistent with those described in the literature.²⁸⁵

1-Morpholinobutane-1,3-dione (75)



Prepared from morpholine (436 mg, 5 mmol), 2,2,6-trimethyl-4H-1,3-dioxin-4-one (924 mg, 6.5 mmol) and sodium acetate (410 mg, 5 mmol).

Reaction time: 24 h.

Purification: petroleum ether:ethyl acetate (from 10:1 to 7:1, v/v).

Yield: 770 mg (90%). Pale yellow oil.

Data of 75:

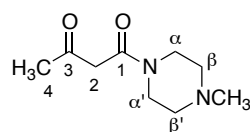
IR (KBr) ν 1716 (C-3=O), 1629 (C-1=O), 1111 (C-O-C) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.26 (s, 3H, CH₃-4), 3.38–3.42 (m, 2H, CH- α ax and CH- α' ax), 3.56 (s, 2H, CH₂-2), 3.62–3.68 (m, 6H, CH- α eq, CH- α' eq, CH₂- β and CH₂- β') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 202.61 (C-3=O), 165.45 (C-1=O), 67.08 (CH₂- β^*), 66.96 (CH₂- β'^*), 50.17 (CH₂-2), 47.17 (CH₂- α^*), 42.54 (CH₂- α'^*), 30.75 (CH₃-4) ppm.

These data are consistent with those described in the literature.²⁸⁶

1-(4-Methylpiperazin-1-yl)butane-1,3-dione (76)



Prepared from 1-methylpiperazine (501 mg, 5 mmol), 2,2,6-trimethyl-4H-1,3-dioxin-4-one (924 mg, 6.5 mmol) and sodium acetate (410 mg, 5 mmol).

²⁸⁶ Bose, D. S.; Idrees, M.; Jakka, N. M.; Rao, J. V. *J. Comb. Chem.* **2010**, *12*, 100-110.

Purification: petroleum ether:ethyl acetate (from 10:1 to 4:1, v/v).

Reaction time: 24 h.

Yield: 653 mg (71%). Yellow oil.

Data of 76:

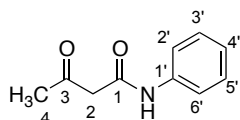
IR (KBr) ν 1719 (C-3=O), 1630 (C-1=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 2.18 (s, 3H, CH_3 -4), 2.22 (s, 3H, NCH_3), 2.29–2.33 (m, 4H, CH_2 - β and CH_2 - β'), 3.32–3.36 (m, 2H, CH - α ax and CH - α' ax), 3.48 (s, 2H, CH_2 -2), 3.54–3.58 (m, 2H, CH - α eq and CH - α' eq) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 202.69 (C-3=O), 165.24 (C-1=O), 55.24 (CH_2 - β^*), 54.82 (CH_2 - β'^*), 50.29 (CH_2 -2), 46.60 (CH_2 - α^*), 46.27 (NCH_3), 42.01 (CH_2 - α'^*), 30.58 (CH_3 -4) ppm.

These data are consistent with those described in the literature.²⁸⁷

3-Oxo-*N*-phenylbutanamide (77)



Prepared from aniline (465 mg, 5 mmol), 2,2,6-trimethyl-4H-1,3-dioxin-4-one (924 mg, 6.5 mmol) and sodium acetate (410 mg, 5 mmol).

Reaction time: 24 h.

Purification: petroleum ether:ethyl acetate (from 8:1 to 3:1, v/v).

Yield: 850 mg (96%). White solid.

Data of 77:

IR (KBr) ν 3302 (N-H), 1710 (C-3=O), 1663 (C-1=O), 1549 (N-C=O) cm^{-1} .

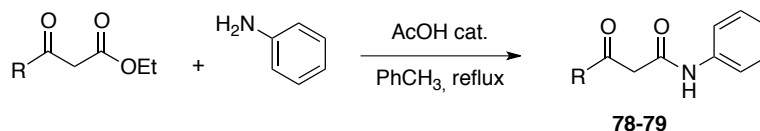
^1H NMR (250 MHz, CDCl_3) δ 2.36 (s, 3H, CH_3 -4), 3.60 (s, 2H, CH_2 -2), 7.15 (t, $J=7.4$ Hz, 1H, CH -4'), 7.36 (t, $J=8.2$ Hz, 2H, CH -3' and CH -5'), 7.58 (d, $J=7.7$ Hz, 2H, CH -2' and CH -6'), 9.16 (bs, 1H, CONH) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 205.80 (C-3=O), 163.90 (C-1=O), 137.87 (C-1'), 129.43 (2xCHAr), 125.02 (CH -4'), 120.59 (2xCHAr), 50.07 (CH_2 -2), 31.75 (CH_3 -4) ppm.

²⁸⁷ Boido, A.; Novelli, F.; Savelli, F.; Sparatore, F.; Russo, S.; Filippelli, W.; Susanna, V.; Marmo, E. *Farmaco* **1989**, *44*, 279-301.

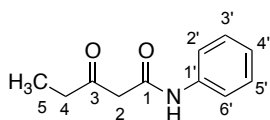
These data are consistent with those described in the literature.²⁸⁵

9.4.2 Synthesis of β -ketoamides 78-80. General procedure



Compound	R
78	C ₂ H ₅
79	C ₃ H ₇
80	CH(CH ₃) ₂

A solution of the appropriate β -ketoester (1 equiv, 5 mmol), aniline (1.1 equiv, 5.5 mmol) and glacial acetic acid (0.1 mL) in toluene (15 mL) was heated under reflux with a Dean-Stark trap for the time period specified in the compound data sheet. After completion of the reaction (checked by TLC), the solvent was evaporated under reduced pressure and the crude residue was purified by silica gel column chromatography using petroleum ether:ethyl acetate mixtures as eluent to give pure compounds **78-80**.

3-Oxo-N-phenylpentanamide (78)

Prepared from ethyl 3-oxopentanoate (720 mg, 5 mmol) and aniline (512 mg, 5.5 mmol).

Reaction time: 7 h.

Purification: petroleum ether:ethyl acetate (from 10:1 to 5:1, v/v).

Yield: 515 mg (54%). Pale yellow solid.

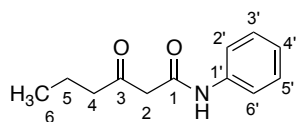
Data of 78:

IR (KBr) ν 3253 (N-H), 1712 (C-3=O), 1655 (C-1=O), 1594 (N-C=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 1.15 (t, $J=7.2$, 3H, CH_3 -5), 2.65 (q, $J=7.2$, 2H, CH_2 -4), 3.60 (s, 2H, CH_2 -2), 7.14 (t, $J=7.4$ Hz, 1H, CH-4'), 7.35 (t, $J=8.2$ Hz, 2H, CH-3' and CH-5'), 7.57 (d, $J=7.7$ Hz, 2H, CH-2' and CH-6'), 9.21 (bs, 1H, CONH) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 208.64 (C-3=O), 163.90 (C-1=O), 137.93 (C-1'), 129.42 (2xCHAR), 124.94 (CH-4'), 120.51 (2xCHAR), 49.06 (CH_2 -2), 42.70 (CH_2 -4), 7.80 (CH_3 -5) ppm.

These data are consistent with those described in the literature.²⁸⁸

3-Oxo-N-phenylhexanamide (79)

Prepared from ethyl 3-oxohexanoate (791 mg, 5 mmol) and aniline (512 mg, 5.5 mmol).

Reaction time: 10 h.

Purification: petroleum ether:ethyl acetate (from 10:1 to 5:1, v/v).

Yield: 565 mg (55%). White solid.

Data of 79:

IR (KBr) ν 3285 (N-H), 1711 (C-3=O), 1655 (C-1=O), 1595 (N-C=O) cm^{-1} .

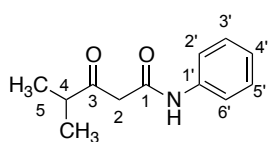
^1H NMR (250 MHz, CDCl_3) δ 0.99 (t, $J=7.4$, 3H, CH_3 -6), 1.71 (sext, $J=7.4$, 2H,

²⁸⁸ Steinhuebel, D.; Sun, Y.; Matsumura, K.; Sayo, N.; Saito T. *J. Am. Chem. Soc.* **2009**, *131*, 11316-11317.

CH₂-5), 2.61 (t, $J=7.2$, 2H, CH₂-4), 3.60 (s, 2H, CH₂-2), 7.15 (t, $J=7.4$ Hz, 1H, CH-4'), 7.36 (t, $J=8.3$ Hz, 2H, CH-3' and CH-5'), 7.59 (d, $J=8.2$ Hz, 2H, CH-2' and CH-6'), 9.21 (bs, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 208.26 (C-3=O), 163.92 (C-1=O), 137.93 (C-1'), 129.42 (2xCHAR), 124.94 (CH-4'), 120.53 (2xCHAR), 49.29 (CH₂-2), 46.47 (CH₂-4), 17.25 (CH₂-5), 13.95 (CH₃-6) ppm.

4-Methyl-3-oxo-*N*-phenylpentanamide (80)



Prepared from ethyl 4-methyl-3-oxopentanoate (791 mg, 5 mmol) and aniline (512 mg, 5.5 mmol).

Reaction time: 7 h.

Purification: petroleum ether:ethyl acetate (from 10:1 to 5:1, v/v).

Yield: 675 mg (66%). White solid.

Data of 80:

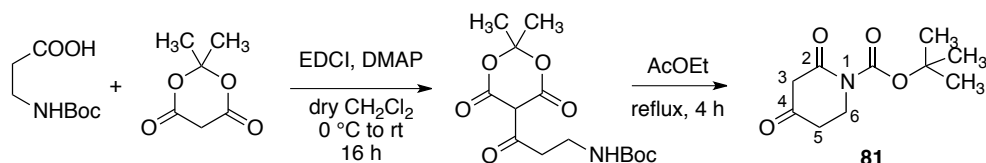
IR (KBr) ν 3297 (N-H), 1712 (C-3=O), 1656 (C-1=O), 1597 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 1.20 (d, $J=6.9$, 6H, CH(CH₃)₂), 2.77 (hept, 1H, CH(CH₃)₂), 3.64 (s, 2H, CH₂-2), 7.13 (t, $J=7.5$ Hz, 1H, CH-4'), 7.35 (t, $J=7.6$ Hz, 2H, CH-3' and CH-5'), 7.58 (d, $J=8.0$ Hz, 2H, CH-2' and CH-6'), 9.28 (bs, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 212.00 (C-3=O), 164.08 (C-1=O), 137.95 (C-1'), 129.41 (2xCHAR), 124.91 (CH-4'), 120.50 (2xCHAR), 47.29 (CH₂-2), 42.70 (CH(CH₃)₂), 18.15 (CH(CH₃)₂) ppm.

These data are consistent with those described in the literature.²⁸⁸

9.4.3) Synthesis of *tert*-butyl 2,4-dioxopiperidine-1-carboxylate (**81**).



To a solution of *N*-Boc- β -alanine (1 equiv, 20 mmol, 3.780 g), 2,2-dimethyl-1,3-dioxane-4,6-dione (1.1 equiv, 22 mmol, 4.268 g), and DMAP (1.9 equiv, 38 mmol, 4.636 g) in anhydrous CH_2Cl_2 (110 mL) at 0 °C was added EDCI (1.5 equiv, 30 mmol, 5.751 g), and the resulting solution was stirred overnight at room temperature. The reaction mixture was washed (100 mL x 4) with 5% potassium bisulfate aqueous solution, the organic layer was dried over anhydrous sodium sulfate, filtered and concentrated, thereby affording crude *tert*-butyl [3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-3-oxopropyl]carbamate that was dissolved in 100 mL of ethyl acetate and refluxed for 4 h. At the end of the reaction, the solvent was removed in vacuo and the residue was purified by chromatography on silica gel (eluent: chloroform:ethyl acetate 30:1), yielding 2.640 g (62%) of the desired product **81** as a pale yellow solid.

Data of **81**:

IR (KBr) ν 1767 (C-4=O), 1732 (NC(O)O), 1671 (C-2=O) cm^{-1} .

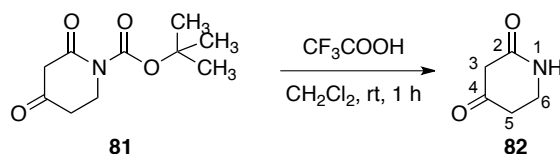
^1H NMR (250 MHz, CDCl_3) δ 1.59 (s, 9H, C(CH₃)₃), 2.67 (t, J =6.1 Hz, 2H, CH₂-5), 3.55 (s, 2H, CH₂-3), 4.15 (t, J =7 Hz, 2H, CH₂-6) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 202.73 (C-4=O), 165.88 (C-2=O), 151.58 (NC(O)O), 84.61 (C(CH₃)₃), 52.67 (CH₂-3), 41.11 (CH₂-5), 38.62 (CH₂-6), 28.34 (C(CH₃)₃) ppm.

These data are consistent with those described in the literature.²⁸⁹

²⁸⁹ Vanotti, E.; Amici, R.; Bargiotti, A.; Berthelsen, J.; Bosotti, R.; Ciavolella, A.; Cirila, A.; Cristiani, C.; D'Alessio, R.; Forte, B.; Isacchi, A.; Martina, K.; Menichincheri, M.; Molinari, A.; Montagnoli, A.; Orsini, P.; Pillan, A.; Roletto, F.; Scolaro, A.; Tibolla, M.; Valsasina, B.; Varasi, M.; Volpi, D.; Santocanale, C. *J. Med. Chem.* **2008**, *51*, 487-501.

9.4.4 Synthesis of piperidine-2,4-dione (**82**).



To a solution of *tert*-butyl 2,4-dioxopiperidine-1-carboxylate **81** (1 equiv, 6 mmol, 1.278 g) in anhydrous CH₂Cl₂ (10 mL) at room temperature was added trifluoroacetic acid (10 mL), and the resulting mixture was stirred for 1 h at the same temperature. At the end of the reaction (as checked by TLC), the solvent and the trifluoroacetic acid were removed under reduced pressure and the residue was purified by column chromatography on silica gel (eluent: chloroform:ethyl acetate 10:1), yielding 525 mg (93%) of the desired product **82** as a white solid.

Data of **82**:

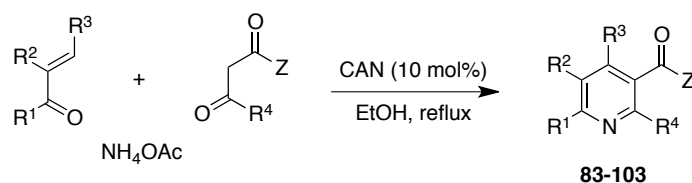
IR (KBr) ν 3185 (N-H), 1718 (C-4=O), 1648 (C-2=O) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.67 (t, *J*=6.2 Hz, 2H, CH₂-5), 3.37 (s, 2H, CH₂-3), 3.58-3.64 (m, 2H, CH₂-6), 6.85 (bs, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 203.74 (C-4=O), 170.45 (C-2=O), 48.66 (CH₂-3), 38.52 (CH₂-5), 37.52 (CH₂-6) ppm.

These data are consistent with those described in the literature.²⁸⁹

9.4.5 Synthesis of nicotinamide derivatives 83-103. General procedure



Cmpd.	R ¹	R ²	R ³	R ⁴	Z
83	C ₆ H ₅	H	C ₆ H ₅	CH ₃	NH ₂
84	4-MeC ₆ H ₄	H	C ₆ H ₅	CH ₃	NH ₂
85	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃	NH ₂
86	C ₆ H ₅	H	4-MeOC ₆ H ₄	CH ₃	NH ₂
87	4-MeOC ₆ H ₄	H	4-MeOC ₆ H ₄	CH ₃	NH ₂
88	4-ClC ₆ H ₄	H	4-ClC ₆ H ₄	CH ₃	NH ₂
89	4-ClC ₆ H ₄	H	2-NO ₂ C ₆ H ₄	CH ₃	NH ₂
90	2-NO ₂ C ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃	NH ₂
91^a	C ₆ H ₅	CH ₃	C ₆ H ₅	CH ₃	NH ₂
92	4-MeC ₆ H ₄	H	4-MeC ₆ H ₄	CH ₃	NHc-Hex
93	C ₆ H ₅	H	C ₆ H ₅	CH ₃	NHPh
94^{a,b}	C ₆ H ₅	H	C ₆ H ₅	C ₂ H ₅	NHPh
95	C ₆ H ₅	H	C ₆ H ₅	C ₃ H ₇	NHPh
96^b	C ₆ H ₅	H	C ₆ H ₅	CH(CH ₃) ₂	NHPh
97	C ₆ H ₅	H	C ₆ H ₅	CH ₃	
98	C ₆ H ₅	H	C ₆ H ₅	CH ₃	
99	C ₆ H ₅	H	C ₆ H ₅	CH ₃	
100	C ₆ H ₅	H	2-thienyl	CH ₃	NH ₂
101	4-MeC ₆ H ₄	H	2-thienyl	CH ₃	NH ₂
102	C ₆ H ₅	H		CH ₃	NH ₂
103	C ₆ H ₅	H		CH ₃	NH ₂

^a experiments carried out with 1:2:3(+1 eq additional after 24 h) ratio of chalcone:β-ketoamides:ammonium acetate

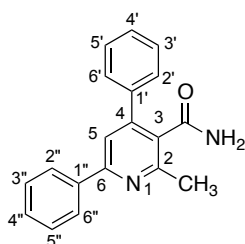
^b experiments carried out at 50°C

To a stirred solution of the suitable 1,3-diphenyl-2-propen-1-one derivatives (1 equiv, 1 mmol), the appropriate primary or secondary β -ketoamide derivatives (1.1 equiv, 1.1 mmol) and ammonium acetate (3 equiv, 3 mmol) in ethanol (1 mL) was added ceric ammonium nitrate (CAN, 10 mol%), and the resulting mixture was heated under reflux for the time period specified in the compound data sheet. After completion of the reaction (checked by TLC), the mixture was poured into ice-water (15 mL) and the precipitate formed was collected by filtration and washed with cold water.

For the derivatives **97-102** a different workup was carried on: after completion of the reaction (checked by TLC), the mixture was allowed to cool to room temperature, diluted with CH_2Cl_2 (15 mL) and washed with water to remove CAN and the excess of ammonium acetate. The organic layer was then washed with brine and dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure.

The crude residue was crystallized from EtOH or purified by silica gel column chromatography using chloroform:ethyl acetate or *n*-hexane:ethyl acetate mixtures as eluents to give pure compounds (**83-103**).

Data of compounds **83-103** and the experimental conditions employed for their synthesis are indicated below.

2-Methyl-4,6-diphenylnicotinamide (83)

Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 265 mg (92%). White solid.

Data of 83:

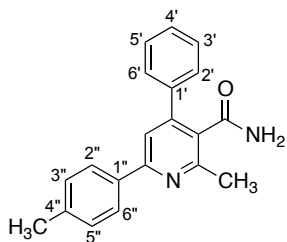
Mp: 219-221 °C

Elemental analysis calcd (%) for C₁₉H₁₆N₂O: C 79.14, H 5.59, N 9.72; found: C 78.93, H 5.53, N 9.51.

IR (KBr) ν 3378 (N-H), 3204 (N-H), 1694 (C=O), 1651 (C=N), 1621 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.80 (s, 3H, C-2CH₃), 5.40 (bs, 1H, CONH), 5.63 (bs, 1H, CONH), 7.41-7.64 (m, 9H, ArH and CH-5), 8.05 (dd, *J* = 7.9, 1.6 Hz, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 170.84 (C=O), 157.40 (C-2*), 155.90 (C-6*), 147.67 (C-4), 138.89 (C-1'*), 138.27 (C-1''*), 129.48 (CHAR), 129.01 (3xCHAR), 128.96 (2xCHAR), 128.90 (C-3), 128.38 (2xCHAR), 127.28 (2xCHAR), 118.74 (CH-5), 23.12 (C-2CH₃) ppm.

2-Methyl-6-(4-methylphenyl)-4-phenylnicotinamide (84)

Prepared from 1-(4-methylphenyl)-3-phenylprop-2-en-1-one (222 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 286 mg (95%). White solid.

Data of 84:

Mp: 248-250 °C

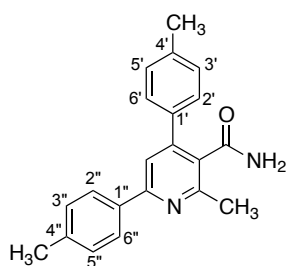
Elemental analysis calcd (%) for $C_{20}H_{18}N_2O$: C 79.44, H 6.00, N 9.26; found: C 79.24, H 5.95, N 9.30.

IR (KBr) ν 3380 (N-H), 3204 (N-H), 1692 (C=O), 1651 (C=N), 1621 (N-C=O) cm^{-1} .

1H NMR (250 MHz, $DMSO-d_6$) δ = 2.38 (s, 3H, C-4''CH₃), 2.60 (s, 3H, C-2CH₃), 7.31 (d, J = 7.8 Hz, 2H, CH-3'' and CH-5''), 7.38-7.67 (m, 6H, ArH' and CH-5), 7.71 (s, 1H, CONH), 7.87 (s, 1H, CONH), 8.06 (d, J = 7.8 Hz, 2H, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, $DMSO-d_6$) δ 169.82 (C=O), 154.89 (C-2*), 153.98 (C-6*), 146.76 (C-4), 138.77 (C-1'*), 138.47 (C-1''*), 135.53 (C-4''), 130.79 (C-3), 129.37 (2xCHAR), 128.39 (5xCHAR), 126.70 (2xCHAR), 117.70 (CH-5), 22.62 (C-2CH₃), 20.89 (C-4''CH₃) ppm.

4,6-Bis(4-methylphenyl)-2-methylnicotinamide (85)



Prepared from 1,3-di-(4-methylphenyl)prop-2-en-1-one (236 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 300 mg (95%). White solid.

Data of 85:

Mp: 247-249 °C

Elemental analysis calcd (%) for $C_{21}H_{20}N_2O$: C 79.72, H 6.37, N 8.85; found: C 79.81, H 6.26, N 8.62.

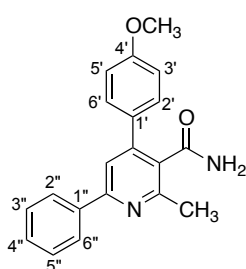
IR (KBr) ν 3289 (N-H), 3180 (N-H), 1652 (C=O), 1606 (N-C=O) cm^{-1} .

1H NMR (250 MHz, $CDCl_3$) δ = 2.44 (s, 6H, 2xArCH₃), 2.76 (s, 3H, C-2CH₃), 5.44 (bs, 1H, CONH), 5.69 (bs, 1H, CONH), 7.19-7.37 (m, 4H, CH-3', CH-5', CH-3'' and CH-5''), 7.47 (d, J = 8.1 Hz, 2H, CH-2' and CH-6'), 7.55 (s, 1H, CH-5), 7.94 (d, J = 8.2 Hz, 2H, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, $CDCl_3$) δ 171.19 (C=O), 157.28 (C-2*), 155.67 (C-6*), 147.57 (C-4), 139.48 (C-1'*), 138.99 (C-1''*), 136.13 (C-4'*), 135.43 (C-4''*), 129.66 (2xCHAR), 129.64 (2xCHAR), 128.61 (C-3), 128.25 (2xCHAR), 127.11

(2xCHAR), 118.35 (CH-5), 23.06 (C-2CH₃), 21.45 (C-4'CH₃*), 21.41 (C-4''CH₃*) ppm.

4-(4-Methoxyphenyl)-2-methyl-6-phenylnicotinamide (86)



Prepared from 3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (238 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 302 mg (95%). White solid.

Data of 86:

Mp: 233-235 °C

Elemental analysis calcd (%) for C₂₀H₁₈N₂O₂: C 75.45, H 5.70, N 8.80; found: C 75.11, H 5.64, N 8.71.

IR (KBr) ν 3373 (N-H), 3195 (N-H), 1682 (C=O), 1651 (C=N), 1608 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 2.59 (s, 3H, C-2CH₃), 3.82 (s, 3H, C-4'OCH₃), 7.05 (d, *J* = 8.7 Hz, 2H, CH-3' and CH-5'), 7.40-7.63 (m, 6H, CH-5, CH-2', CH-6', CH-3'', CH-4'' and CH-5''), 7.72 (s, 1H, CONH), 7.88 (s, 1H, CONH), 8.14 (dd, *J* = 7.8, 1.5 Hz, 2H, CH-2'' and CH-6'') ppm.

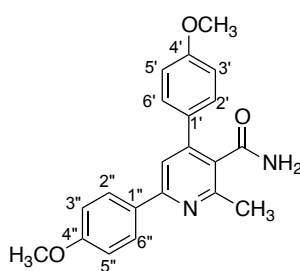
¹³C NMR (63 MHz, DMSO-*d*₆) δ 170.05 (C=O), 159.57 (C-4'), 154.86 (C-2*), 154.06 (C-6*), 146.38 (C-4), 138.42 (C-1''), 130.92 (C-1'*), 130.58 (C-3*), 129.74 (2xCHAR), 129.13 (CHAR), 128.74 (2xCHAR), 126.80 (2xCHAR), 117.98 (CH-5), 113.89 (2xCHAR), 55.26 (C-4'OCH₃), 22.60 (C-2CH₃) ppm.

4,6-Bis(4-methoxyphenyl)-2-methylnicotinamide (87)

Prepared from 1,3-di-(4-methoxyphenyl)prop-2-en-1-one (268 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: chloroform:ethyl acetate (5:1, v/v).



Yield: 254 mg (73%). Pale yellow solid.

Data of 87:

Mp: 239-241 °C

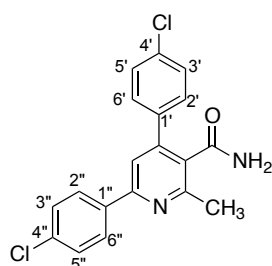
Elemental analysis calcd (%) for $C_{21}H_{20}N_2O_3$: C 72.40, H 5.79, N 8.04; found: C 72.11, H 5.74, N 7.90.

IR (KBr) ν 3363 (N-H), 3188 (N-H), 1683 (C=O), 1644 (C=N), 1607 (N-C=O) cm^{-1} .

1H NMR (250 MHz, $DMSO-d_6$) δ = 2.57 (s, 3H, C-2CH₃), 3.82 (s, 3H, C-4'OCH₃), 3.83 (s, 3H, C-4''OCH₃), 7.04 (d, $J=7.4$ Hz, 4H, CH-3', CH-5', CH-3'' and CH-5''), 7.53 (s, 1H, CH-5), 7.58 (d, $J=8.6$ Hz, 2H, CH-2' and CH-6'), 7.65 (s, 1H, CONH), 7.84 (s, 1H, CONH), 8.11 (d, $J=8.6$ Hz, 2H, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, $DMSO-d_6$) δ 170.17 (C=O), 160.22 (C-4'*), 159.51 (C-4''*), 154.58 (C-2*), 153.82 (C-6*), 146.32 (C-4), 130.87 (C-1'*), 130.73 (C-1''*), 130.22 (C-3*), 129.70 (2xCHAR), 128.15 (2xCHAR), 117.04 (CH-5), 114.08 (2xCHAR), 113.86 (2xCHAR), 55.27 (C-4'OCH₃*), 55.25 (C-4''OCH₃*), 22.62 (C-2CH₃) ppm.

4,6-Bis(4-chlorophenyl)-2-methylnicotinamide (88)



Prepared from 1,3-di-(4-chlorophenyl)prop-2-en-1-one (277 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: Crystallization from EtOH.

Yield: 338 mg (95%). White solid.

Data of 88:

Mp: 259-261 °C

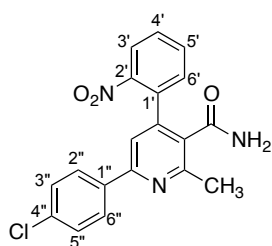
Elemental analysis calcd (%) for $C_{19}H_{14}Cl_2N_2O$: C 63.88, H 3.95, N 7.84; found: C 63.54, H 3.94, N 7.88.

IR (KBr) ν 3314 (N-H), 3157 (N-H), 1668 (C=O), 1600 (N-C=O), 1091 (C-Cl) cm^{-1} .

¹H NMR (250 MHz, DMSO-*d*₆) δ = 2.61 (s, 3H, C-2CH₃), 7.51-7.68 (m, 7H, CH-5, CH-2', CH-3', CH-5', CH-6', CH-3'' and CH-5''), 7.82 (s, 1H, CONH), 7.93 (s, 1H, CONH), 8.21 (d, *J*=8.6 Hz, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 169.48 (C=O), 154.32 (C-2*), 153.72 (C-6*), 145.73 (C-4), 137.03 (C-1'*), 136.95 (C-1''*), 134.16 (C-4'*), 133.53 (C-4''*), 131.20 (C-3*), 130.30 (2xCHAR), 128.79 (2xCHAR), 128.62 (2xCHAR), 128.43 (2xCHAR), 118.03 (CH-5), 22.57 (C-2CH₃) ppm.

6-(4-Chlorophenyl)-2-methyl-4-(2-nitrophenyl)nicotinamide (89)



Prepared from 1-(4-chlorophenyl)-3-(2-nitrophenyl) prop-2-en-1-one (277 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: chloroform:ethyl acetate (from 15:1 to 10:1, v/v).

Yield: 312 mg (85%). Pale yellow solid.

Data of 89:

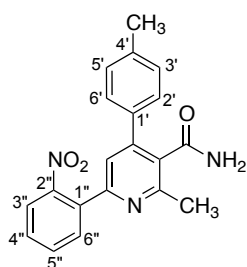
Mp: 228-230 °C

Elemental analysis calcd (%) for C₁₉H₁₄ClN₃O₃: C 62.05, H 3.84, N 11.43; found: C 61.95, H 3.87, N 11.24.

IR (KBr) ν 3443 (N-H), 3122 (N-H), 1682 (C=O), 1652 (C=N), 1584 (N-C=O), 1520 (NO₂), 1094 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ = 2.78 (s, 3H, C-2CH₃), 5.76 (bs, 1H, CONH), 6.08 (bs, 1H, CONH), 7.29 (s, 1H, CH-5), 7.40-7.50 (m, 3H, CH-4', CH-3'' and CH-5''), 7.58-7.78 (m, 2H, CH-2' and CH-5'), 7.94 (d, *J*=8.5 Hz, 2H, CH-2'' and CH-6''), 8.07 (d, *J*=7.3 Hz, 1H, CH-3' and CH-5') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 169.60 (C=O), 156.09 (C-2*), 155.93 (C-6*), 148.22 (C-4*), 144.54 (C-2'*), 136.74 (C-1''*), 135.86 (C-1'*), 133.58 (CHAR), 133.11 (C-4''), 131.73 (CHAR), 129.90 (CHAR), 129.10 (2xCHAR), 128.94 (C-3), 128.55 (2xCHAR), 124.57 (CHAR), 116.59 (CH-5), 23.04 (C-2CH₃) ppm.

2-Methyl-4-(4-methylphenyl)-6-(2-nitrophenyl)nicotinamide (90)

Prepared from 3-(4-methylphenyl)-1-(2-nitrophenyl)prop-2-en-1-one (267 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 30 h.

Purification: chloroform:ethyl acetate (5:1, v/v).

Yield: 230 mg (66%). White solid.

Data of 90:

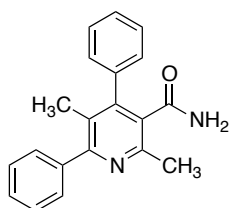
Mp: 214-216 °C

Elemental analysis calcd (%) for $C_{20}H_{17}N_3O_3$: C 69.15, H 4.93, N 12.10; found: C 68.95, H 4.95, N 12.11.

IR (KBr) ν 3370 (N-H), 3188 (N-H), 1667 (C=O), 1585 (N-C=O), 1538 (NO_2) cm^{-1} .

1H NMR (250 MHz, $DMSO-d_6$) δ = 2.39 (s, 3H, C-4' CH_3), 2.49 (s, 3H, C-2 CH_3), 7.31 (d, $J=8.1$ Hz, 2H, CH-3' and CH-5'), 7.55 (d, $J=8.1$ Hz, 2H, CH-2' and CH-6'), 7.59 (s, 1H, CH-5), 7.64 (s, 1H, CONH), 7.66-7.75 (m, 1H, CH-4''), 7.76-7.85 (m, 1H, CH-5''), 7.88-8.07 (m, 3H, CONH, CH-3'' and CH-6'') ppm.

^{13}C NMR (63 MHz, $DMSO-d_6$) δ 169.49 (C=O), 154.03 (C-2*), 153.31 (C-6*), 149.27 (C-4*), 146.82 (C-2''*), 138.19 (C-1'), 134.98 (C-4'*), 133.45 (C-1''*), 132.67 (CHAR), 131.44 (C-3), 131.28 (CHAR), 129.91 (CHAR), 129.10 (2xCHAR), 128.31 (2xCHAR), 124.29 (CHAR), 120.35 (CH-5), 22.20 (C-2 CH_3), 20.82 (C-4' CH_3) ppm.

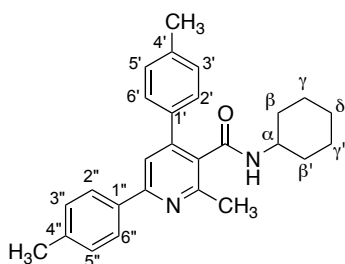
2,5-Dimethyl-4,6-diphenylnicotinamide (91)

Prepared from 2-methyl-1,3-diphenylprop-2-en-1-one (222 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 30 h.

Purification: chloroform:ethyl acetate (5:1, v/v).

Yield: 60 mg (20%). White solid.

Data of 91:**Mp:** 228-230 °C**Elemental analysis** calcd (%) for C₂₀H₁₈N₂O: C 79.44, H 6.00, N 9.26; found: C 79.25, H 6.07, N 9.16.**IR** (KBr) ν 3346 (N-H), 3175 (N-H), 1668 (C=O), 1652 (C=N), 1614 (N-C=O) cm⁻¹.**¹H NMR** (250 MHz, CDCl₃) δ = 2.04 (s, 3H, C-5CH₃), 2.69 (s, 3H, C-2CH₃), 5.37 (bs, 1H, CONH), 5.53 (bs, 1H, CONH), 7.38-7.58 (m, 8H, ArH), 7.32 (dd, $J=7.5, 1.8$ Hz, 2H, CH-2'' and CH-6''), ppm.**¹³C NMR** (63 MHz, CDCl₃) δ 170.45 (C=O), 159.43 (C-2*), 151.63 (C-6*), 147.86 (C-4), 140.74 (C-1'*), 137.41 (C-1''*), 130.37 (C-5), 129.12 (2xCHAR), 128.77 (2xCHAR), 128.64 (2xCHAR), 128.44 (2xCHAR), 128.34 (CHAR), 128.25 (CHAR), 126.43 (C-3), 22.59 (C-2CH₃), 17.84 (C-5CH₃) ppm.***N*-Cyclohexyl-4,6-bis(4-methylphenyl)-2-methylnicotinamide (92)**Prepared from 1,3-di-(4-methylphenyl)prop-2-en-1-one (236 mg, 1 mmol), *N*-cyclohexyl-3-oxobutanamide (202 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 5:1, v/v)

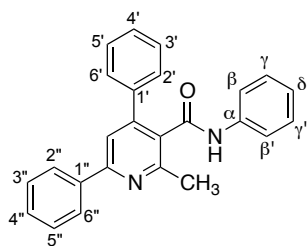
Yield: 330 mg (83%). Pale yellow solid.

Data of 92:**Mp:** 214-216 °C**Elemental analysis** calcd (%) for C₂₇H₃₀N₂O: C 81.37, H 7.59, N 7.03; found: C 81.10, H 7.55, N 6.91.**IR** (KBr) ν 3230 (N-H), 1620 (C=O) cm⁻¹.**¹H NMR** (250 MHz, CDCl₃) δ = 0.78-0.97 (m, 2H, CH- γ ax and CH- γ' ax), 1.03-1.17 (m, 1H, CH- γ eq*), 1.21-1.41 (m, 2H, CH- γ' eq* and CH- δ ax*), 1.47-1.74 (m, 5H, CH- δ eq*, CH₂- β and CH₂- β'), 2.43 (s, 6H, 2xArCH₃), 2.73 (s, 3H, C-2CH₃), 3.74-3.94 (m, 1H, CH- α), 5.25 (d, $J=8.5$ Hz, 1H, CONH), 7.18-7.37 (m, 4H, CH-

3', CH-5', CH-3'' and CH-5''), 7.44 (d, $J=8.1$ Hz, 2H, CH-2' and CH-6'), 7.54 (s, 1H, CH-5), 7.93 (d, $J=8.2$ Hz, 2H, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 167.98 (C=O), 157.07 (C-2*), 155.91 (C-6*), 147.64 (C-4), 139.30 (C-1'*), 138.72 (C-1''*), 136.39 (C-4'*), 135.57 (C-4''*), 129.78 (C-3), 129.62 (2xCHAR), 129.45 (2xCHAR), 128.27 (2xCHAR), 127.10 (2xCHAR), 118.27 (CH-5), 48.24 (CH- α), 32.59 (CH₂- β and CH₂- β'), 25.49 (CH₂- δ), 24.67 (CH₂- γ , CH₂- γ'), 23.01 (C-2CH₃), 21.45 (C-4'CH₃*), 21.38 (C-4''CH₃*) ppm.

2-Methyl-*N*,4,6-triphenylnicotinamide (93)



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 3-oxo-*N*-phenylbutanamide (195 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 305 mg (84%). Pale yellow solid.

Data of 93:

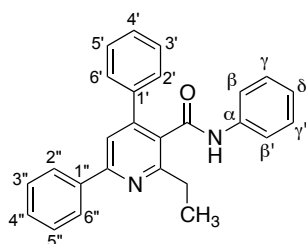
Mp: 226-228 °C

Elemental analysis calcd (%) for $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}$: C 82.39, H 5.53, N 7.69; found: C 82.14, H 5.61, N 7.57.

IR (KBr) ν 3270 (N-H), 1649 (C=O), 1621 (C=N), 1546 (N-C=O) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO}-d_6$) δ = 2.64 (s, 3H, C-2CH₃), 7.08 (t, $J=7.2$ Hz, 1H, CH- δ), 7.30 (t, $J=7.8$ Hz, 2H, CH- γ and CH- γ'), 7.38-7.59 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.64 (d, $J=6.5$ Hz, 2H, CH- β and CH- β'), 7.86 (s, 1H, CH-5), 8.20 (d, $J=6.5$ Hz, 2H, CH-2'' and CH-6''), 10.50 (s, 1H, CONH) ppm.

^{13}C NMR (63 MHz, $\text{DMSO}-d_6$) δ 166.38 (C=O), 155.63 (C-2*), 154.54 (C-6*), 147.47 (C-4), 138.63 (C-1'*), 138.21 (C-1''*), 138.05 (C- α *), 130.58 (C-3), 129.40 (CHAR), 128.83 (2xCHAR), 128.79 (2xCHAR), 128.61 (CHAR), 128.55 (2xCHAR), 128.27 (2xCHAR), 126.96 (2xCHAR), 123.94 (CHAR), 119.70 (2xCHAR), 118.28 (CH-5), 22.63 (C-2CH₃) ppm.

2-Ethyl-*N*,4,6-triphenylnicotinamide (94)

Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 3-oxo-*N*-phenylpentanamide (210 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 32 h.

Purification: Crystallization from EtOH.

Yield: 280 mg (74%). White solid.

Data of 94:

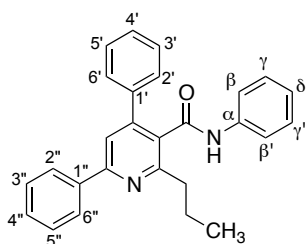
Mp: 233-235 °C

Elemental analysis calcd (%) for C₂₆H₂₂N₂O: C 82.51, H 5.86, N 7.40; found: C 82.23, H 5.89, N 7.56.

IR (KBr) ν 3265 (N-H), 1652 (C=O), 1538 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 1.36 (t, *J*=7.5 Hz, 3H, C-2CH₂CH₃), 2.91 (q, *J*=7.5 Hz, 2H, C-2CH₂CH₃), 7.06 (t, *J*=7.3, 1H, CH- δ), 7.28 (t, *J*=7.8, 2H, CH- γ and CH- γ'), 7.36-7.58 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.63 (dd, *J*=7.9, 1.5 Hz, 2H, CH- β and CH- β'), 7.85 (s, 1H, CH-5), 8.21 (dd, *J*=7.9, 1.5 Hz, 2H, CH-2'' and CH-6''), 10.46 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 166.34 (C=O), 159.00 (C-2*), 155.68 (C-6*), 147.46 (C-4), 138.61 (C-1'*), 138.39 (C-1''*), 138.12 (C- α *), 130.20 (C-3), 129.37 (CHAr), 128.84 (2xCHAr), 128.78 (2xCHAr), 128.56 (CHAr), 128.52 (2xCHAr), 128.31 (2xCHAr), 126.95 (2xCHAr), 123.91 (CHAr), 119.69 (2xCHAr), 118.26 (CH-5), 28.66 (C-2CH₂CH₃), 13.74 (C-2CH₂CH₃) ppm.

***N*,4,6-Triphenyl-2-propylnicotinamide (95)**

Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 3-oxo-*N*-phenylhexanamide (226 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 320 mg (82%). White solid.

Data of 95:

Mp: 231-233 °C

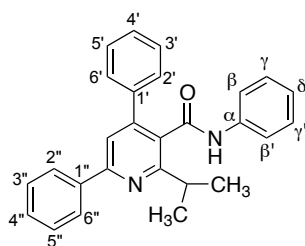
Elemental analysis calcd (%) for C₂₇H₂₄N₂O: C 82.62, H 6.16, N 7.14; found: C 82.36, H 6.09, N 7.00.

IR (KBr) ν 3230 (N-H), 1645 (C=O), 1621 (C=N), 1538 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 0.97 (t, *J*=7.4 Hz, 3H, C-2CH₂CH₂CH₃), 1.76-1.96 (m, 2H, C-2CH₂CH₂CH₃), 2.80-2.90 (m, 2H, C-2CH₂CH₂CH₃), 7.06 (t, *J*=7.3 Hz, 1H, CH- δ), 7.28 (t, *J*=7.8 Hz, 2H, CH- γ and CH- γ'), 7.36-7.56 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.63 (dd, *J*=7.9, 1.5 Hz, 2H, CH- β and CH- β'), 7.84 (s, 1H, CH-5), 8.20 (dd, *J*=7.9, 1.4 Hz, 2H, CH-2'' and CH-6''), 10.42 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 166.33 (C=O), 157.95 (C-2*), 155.56 (C-6*), 147.46 (C-4), 138.59 (C-1'*), 138.38 (C-1''*), 138.15 (C- α *), 130.45 (C-3), 129.36 (CHAr), 128.83 (2xCHAr), 128.78 (2xCHAr), 128.54 (CHAr), 128.50(2xCHAr), 128.31 (2xCHAr), 126.94 (2xCHAr), 123.92 (CHAr), 119.74 (2xCHAr), 118.22 (CH-5), 37.41 (CH-2CH₂CH₂CH₃), 22.22 (CH-2CH₂CH₂CH₃), 14.18 (CH-2CH₂CH₂CH₃) ppm.

2-Isopropyl-*N*,4,6-triphenylnicotinamide (96)



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 4-methyl-3-oxo-*N*-phenylpentanamide (226 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 20:1 to 5:1, v/v).

Yield: 290 mg (74%). White solid.

Data of 96:

Mp: 252-254 °C

Elemental analysis calcd (%) for C₂₇H₂₄N₂O: C 82.62, H 6.16, N 7.14; found: C 82.41, H 6.22, N 7.05.

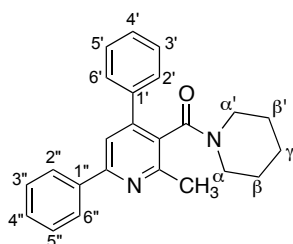
IR (KBr) ν 3230 (N-H), 1646 (C=O), 1588 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 1.36 (d, *J*=6.5 Hz, 6H, C-2CH(CH₃)₂), 3.11-

3.36 (m, 1H, C-2CH(CH₃)₂), 7.05 (t, *J*=7.1 Hz, 1H, CH-δ), 7.27 (t, *J*=7.7 Hz, 2H, CH-γ and CH-γ'), 7.34-7.57 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.64 (d, *J*=6.7 Hz, 2H, CH-β and CH-β'), 7.83 (s, 1H, CH-5), 8.22 (d, *J*=6.8 Hz, 2H, CH-2'' and CH-6''), 10.44 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 166.38 (C=O), 162.47 (C-2*), 155.60 (C-6*), 147.37 (C-4), 138.58 (C-1'*), 138.51 (C-1''*), 138.20 (C-α*), 129.71 (C-3), 129.32 (CHAr), 128.81 (2xCHAr), 128.72 (2xCHAr), 128.45 (3xCHAr), 128.31 (2xCHAr), 126.85 (2xCHAr), 123.87 (CHAr), 119.67 (2xCHAr), 118.21 (CH-5), 32.79 (CH-2CH(CH₃)₂), 22.66 (CH-2CH(CH₃)₂) ppm.

(2-Methyl-4,6-diphenylpyridin-3-yl)(piperidin-1-yl)methanone (97)



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 1-(piperidin-1-yl)butane-1,3-dione (186 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: chloroform:ethyl acetate (from 20:1 to 15:1, v/v).

Yield: 228 mg (64%). Pale yellow solid.

Data of 97:

Mp: 137-139 °C

Elemental analysis calcd (%) for C₂₄H₂₄N₂O: C 80.87, H 6.79, N 7.86; found: C 80.58, H 6.70, N 7.59.

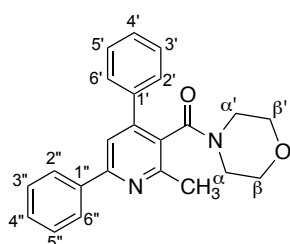
IR (KBr) ν 1628 (C=O) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ = 0.58-0.80 (m, 1H, CH-γax*), 1.14-1.35 (m, 2H, CH-βax*, CH-β'ax*), 1.35-1.60 (m, 3H, CH-βeq*, CH-β'eq* and CH-γeq*), 2.68 (s, 3H, C-2CH₃), 2.72-2.86 (m, 1H, CH-αax*), 2.92-3.07 (m, 1H, CH-α'ax*), 3.42-3.58 (m, 1H, CH-αeq*), 3.58-3.74 (m, 1H, CH-α'eq*), 7.41-7.55 (m, 6H, C-4ArH and CH-4''), 7.55-7.71 (m, 3H, CH-5, CH-3'' and CH-5''), 7.96-8.15 (m, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 167.70 (C=O), 156.89 (C-2*), 155.32 (C-6*), 146.90 (C-4), 139.09 (C-1'*), 138.19 (C-1''*), 129.18 (CHAr), 128.85 (3xCHAr), 128.71 (2xCHAr), 128.67 (2xCHAr), 127.11 (2xCHAr), 118.20 (CH-5), 47.27

(CH₂-α*), 42.10 (CH₂-α'*), 25.85 (CH₂-β*), 25.19 (CH₂-β'*), 24.25 (CH₂-γ), 22.94 (C-2CH₃) ppm.

(2-Methyl-4,6-diphenylpyridin-3-yl)(morpholino)methanone (98)



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 1-morpholinobutane-1,3-dione (188 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 8:1 to 2:1, v/v).

Yield: 285 mg (80%). Pale yellow solid.

Data of 98:

Mp: 132-134 °C

Elemental analysis calcd (%) for C₂₃H₂₂N₂O₂: C 77.07, H 6.19, N 7.82; found: C 76.91, H 6.24, N 7.80.

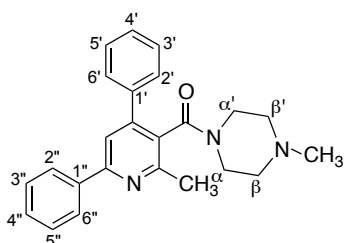
IR (KBr) ν 1634 (C=O), 1113 (C-O-C) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ = 2.56-2.68 (m, 1H, CH-α'ax*), 2.70 (s, 3H, C-2CH₃), 2.75-2.91 (m, 1H, CH-αax*), 3.01-3.17 (m, 1H, CH-α'eq*), 3.22-3.41 (m, 2H, CH-αeq* and CH-βax*), 3.49-3.80 (m, 3H, CH-βeq* and CH₂-β'*), 7.45-7.62 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.64 (s, 1H, CH-5), 8.00-8.11 (m, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 168.09 (C=O), 157.41 (C-2*), 155.60 (C-6*), 147.22 (C-4), 138.88 (C-1'*), 138.03 (C-1''*), 129.44 (CHAr), 129.25 (CHAr), 129.00 (2xCHAr), 128.96 (2xCHAr), 128.65 (2xCHAr), 127.82 (C-3), 127.21 (2xCHAr), 118.17 (CH-5), 66.39 (CH₂-α*), 66.36 (CH₂-α'*), 46.63 (CH₂-β*), 41.72 (CH₂-β'*), 23.01 (C-2CH₃) ppm.

(2-Methyl-4,6-diphenylpyridin-3-yl)(4-methylpiperazin-1-yl)methanone (99)

Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), 1-(4-



methylpiperazin-1-yl)butane-1,3-dione (202 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 8:1 to 2:1, v/v).

Yield: 248 mg (67%). Yellow solid.

Data of **99**:

Mp: 132-134 °C

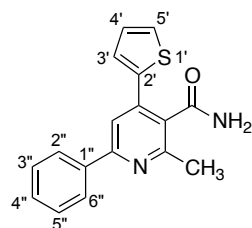
Elemental analysis calcd (%) for C₂₄H₂₅N₃O: C 77.60, H 6.78, N 11.31; found: C 77.44, H 6.67, N 11.02.

IR (KBr) ν 1620 (C=O) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ = 1.37-1.52 (m, 1H, CH- β ax*), 1.99-2.19 (m, 2H, CH- β eq* and CH- β' ax*), 2.15 (s, 3H, NCH₃), 2.32-2.49 (m, 1H, CH- β' eq*), 2.69 (s, 3H, C-2CH₃), 2.75-2.91 (m, 1H, CH- α ax*), 3.01-3.22 (m, 1H, CH- α' ax*), 3.52-3.81 (m, 2H, CH- α eq* and CH- α' eq*), 7.45-7.61 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.63 (s, 1H, CH-5), 8.06 (dd, *J*=8.0 Hz, 1.6, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 167.99 (C=O), 157.27 (C-2*), 155.54 (C-6*), 147.11 (C-4), 139.04 (C-1'*), 138.14 (C-1''*), 129.35 (CHAr), 129.01 (CHAr), 128.94 (2xCHAr), 128.92 (2xCHAr), 128.71 (2xCHAr), 128.23 (C-3), 127.19 (2xCHAr), 118.19 (CH-5), 54.56 (CH₂- β *), 54.20 (CH₂- β' *), 46.04 (CH₂- α *), 45.91 (N-CH₃), 41.12 (CH₂- α' *), 23.05 (C-2CH₃) ppm.

2-Methyl-6-phenyl-4-(thiophen-2-yl)nicotinamide (**100**)

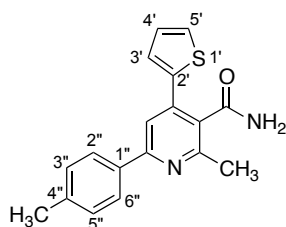


Prepared from 1-phenyl-3-(thiophen-2-yl)prop-2-en-1-one (214 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: chloroform:ethyl acetate (from 20:1 to 15:1, v/v).

Yield: 205 mg (70%). Pale yellow solid.

Data of 100:**Mp:** 199-201 °C**Elemental analysis** calcd (%) for C₁₇H₁₄N₂OS: C 69.36, H 4.79, N 9.52, S 10.89; found: C 68.99, H 4.92, N 9.42, S 10.85.**IR** (KBr) ν 3310 (N-H), 3140 (N-H), 1652 (C=O), 1591 (N-C=O) cm⁻¹.**¹H NMR** (250 MHz, CDCl₃) δ = 2.77 (s, 3H, C-2CH₃), 5.71 (bs, 1H, CONH), 5.86 (bs, 1H, CONH), 7.16 (dd, J =5.1, 3.7 Hz, 1H, CH-4'), 7.42-7.58 (m, 5H, CH-3', CH-5', CH-3'', CH-4'' and CH-5''), 7.68 (s, 1H, CH-5), 8.03 (dd, J =8.0, 1.7 Hz, 2H, CH-2'' and CH-6'') ppm.**¹³C NMR** (63 MHz, CDCl₃) δ 171.12 (C=O), 157.55 (C-2*), 155.83 (C-6*), 139.95 (C-4), 138.92 (C-1''*), 138.69 (C-2''*), 129.55 (CHAr), 128.96 (2xCHAr), 128.53 (CHAr), 128.33 (CHAr), 128.00 (C-3), 127.82 (CHAr), 127.25 (2xCHAr), 118.15 (CH-5), 22.94 (C-2CH₃) ppm.**2-Methyl-6-(4-methylphenyl)-4-(thiophen-2-yl)nicotinamide (101)**

Prepared from 1-(4-methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (228 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

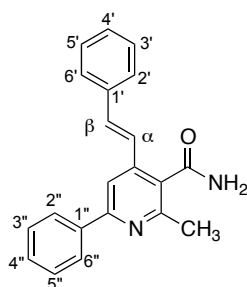
Purification: chloroform:ethyl acetate (from 20:1 to 15:1, v/v).

Yield: 220 mg (72%). Pale yellow solid.

Data of 101:**Mp:** 206-208 °C**Elemental analysis** calcd (%) for C₁₈H₁₆N₂OS: C 70.10, H 5.23, N 9.08, S 10.40; found: C 69.88, H 5.30, N 8.98, S 10.33.**IR** (KBr) ν 3438 (N-H), 3278 (N-H), 1651 (C=O), 1585 (N-C=O) cm⁻¹.**¹H NMR** (250 MHz, CDCl₃) δ = 2.45 (s, 3H, C-4''CH₃), 2.76 (s, 3H, C-2CH₃), 5.73 (s, 1H, CONH), 5.79 (s, 1H, CONH), 7.16 (dd, J =5.1, 3.6 Hz, 1H, CH-4'), 7.32 (d, J =8.2 Hz, 2H, CH-3'' and CH-5''), 7.48 (dd, J =5.1, 0.9 Hz, 1H, CH-5'), 7.53 (dd, J =3.6, 0.9 Hz, 1H, CH-3'), 7.65 (s, 1H, CH-5), 7.94 (d, J =8.2 Hz, 2H, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 171.12 (C=O), 157.51 (C-2*), 155.71 (C-6*), 139.93 (C-4*), 139.70 (C-1''*), 139.02 (C-2'*), 135.83 (C-4''), 129.68 (2xCHAR), 128.52 (CHAR), 128.29 (CHAR), 127.75 (CHAR), 127.13 (2xCHAR), 117.82 (CH-5), 22.93 (C-2CH₃), 21.48 (C-4''CH₃) ppm.

2-Methyl-6-phenyl-4-styrylnicotinamide (102)



Prepared from 1,5-diphenylpenta-2,4-dien-1-one (234 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 8:1 to 1:1, v/v).

Yield: 231 mg (74%). Pale yellow solid.

Data of 102:

Mp: 199-201 °C

Elemental analysis calcd (%) for $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}$: C 80.23, H 5.77, N 8.91; found: C 79.90, H 5.79, N 8.82.

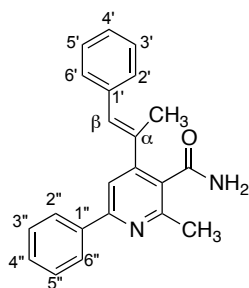
IR (KBr) ν 3380 (N-H), 3180 (N-H), 1640 (C=O), 958 (CH- α =CH- β) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO-}d_6$) δ = 2.56 (s, 3H, C-2CH₃), 7.15 (d, $J=16.3$ Hz, 1H, CH- α), 7.32-7.66 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.72-7.91 (m, 2H, CH-5 and CH- β), 8.09-8.27 (m, 4H, 2xCONH, CH-2'' and CH-6'') ppm.

^{13}C NMR (63 MHz, $\text{DMSO-}d_6$) δ 169.71 (C=O), 154.84 (C-2*), 154.08 (C-6*), 141.66 (C-4), 138.50 (C-1''*), 136.31 (C-1'*), 134.25 (CH- β), 130.90 (C-3), 129.14 (CHAR), 129.07 (2xCHAR), 128.86 (CHAR), 128.71 (2xCHAR), 126.93 (2xCHAR), 126.78 (2xCHAR), 123.41 (CH- α), 112.63 (CH-5), 22.55 (C-2CH₃) ppm.

2-Methyl-6-phenyl-4-(1-phenylprop-1-en-2-yl)nicotinamide (103)

Prepared from 4-methyl-1,5-diphenylpenta-2,4-dien-1-one (248 mg, 1 mmol), acetoacetamide (111 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).



Reaction time: 24 h.

Purification: *n*-hexane:ethyl acetate (from 8:1 to 1:1, v/v).

Yield: 222 mg (68%). White solid.

Data of 103:

Mp: 186-188 °C

Elemental analysis calcd (%) for C₂₂H₂₀N₂O: C 80.46, H 6.14, N 8.53; found: C 80.14, H 6.07, N 8.65.

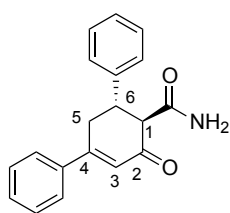
IR (KBr) ν 3398 (N-H), 3201 (N-H), 1689 (C=O), 1651 (C=N), 1620 (N-C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 2.25 (d, *J*=1.2 Hz, 3H, C- α CH₃), 2.59 (s, 3H, C-2CH₃), 6.65 (s, 1H, CH- β), 7.23-7.57 (m, 8H, C-4ArH, CH-3'', CH-4'' and CH-5''), 7.66 (s, 1H, CONH), 7.77 (s, 1H, CH-5), 7.92 (s, 1H, CONH), 8.14 (dd, *J*=8.0, 1.5 Hz, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 169.99 (C=O), 154.66 (C-2*), 154.06 (C-6*), 150.97 (C-4), 138.36 (C-1''*), 137.02 (C-1'**), 135.46 (C- α), 130.57 (C-3), 129.87 (CH- β), 129.13 (CHAr), 128.92 (2xCHAr), 128.74 (2xCHAr), 128.38 (2xCHAr), 127.04 (CHAr), 126.73 (2xCHAr), 116.44 (CH-5), 22.72 (C-2CH₃), 18.79 (C- α CH₃) ppm.

9.4.6 Robinson annulation products 104 and 105

2-Oxo-4,6-diphenylcyclohex-3-enecarboxamide (104)



Isolated from the reaction among 1,3-diphenyl-2-propen-1-one, acetoacetamide and ammonium acetate.

Pale yellow solid

Data of 104:

Mp: 176-177 °C

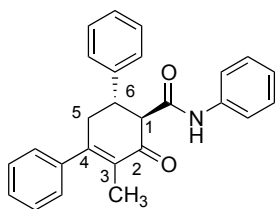
IR (KBr) ν 3049 (N-H), 1679 (C-2=O), 1650 (C=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 3.06 (dd, $J=18.0, 9.0$, 1H, one H of CH_2 -5), 3.33 (dd, $J=18.0, 5.0$, 1H, one H of CH_2 -5), 3.62 (d, $J=8.9$, 1H, CH-1), 4.10 (td, $J=8.8, 5.2$, 1H, CH-6), 5.43 (bs, 1H, CONH), 5.97 (bs, 1H, CONH), 6.60 (s, 1H, CH-3), 7.41-7.32 (m, 5H, ArH), 7.52-7.42 (m, 3H, ArH), 7.59 (dd, $J=6.7, 3.0$, 2H, ArH) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 196.08 (C-2=O), 170.89 (CONH_2), 159.94 (C-4), 142.38 (CAr), 138.21 (CAr), 130.99 (CHAR), 129.30 (2xCHAR), 129.21 (2xCHAR), 127.87 (2xCHAR), 127.60 (CHAR), 126.71 (2xCHAR), 124.68 (CH-3), 59.14 (CH-1), 42.82 (CH-6), 35.34 (CH_2 -5) ppm.

These data are consistent with those described in the literature.²⁹⁰

3-Methyl-2-oxo-*N*,4,6-triphenylcyclohex-3-enecarboxamide (105)



Isolated from the reaction among 1,3-diphenyl-2-propen-1-one, 3-oxo-*N*-phenylpentanamide and ammonium acetate.

White solid

Data of 105:

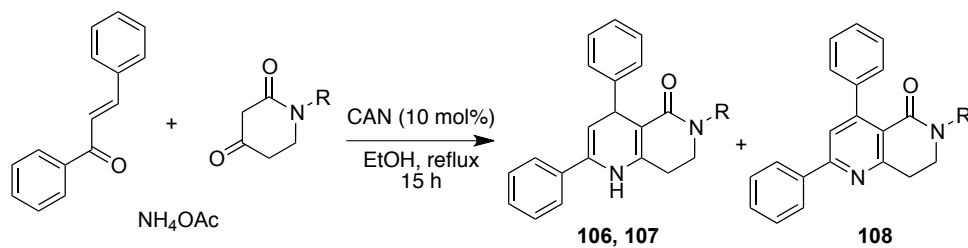
Mp: 212-214 °C

IR (KBr) ν 3380 (N-H), 3180 (N-H), 1640 (C=O) cm^{-1} .

²⁹⁰ Kato, T.; Noda, M. *Chem. Pharm. Bull.* **1975**, *23*, 2193-2200.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.73 (s, 3H, C-3CH₃) 2.76 (dd, *J*=18.3, 3.6 Hz, 1H, one H of CH₂-5), 2.97-3.18 (m, 1H, one H of CH₂-5), 3.78-3.96 (m, 1H, CH-6), 4.02 (d, *J*=13.2 Hz, 1H, CH-1), 7.01 (t, *J*=7.2 Hz, 1H, ArH), 7.14-7.35 (m, 5H, ArH), 7.35-7.53 (m, 9H, ArH), 10.04 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 196.12 (C-2=O), 167.90 (CONH), 156.34 (C-4), 142.56 (CAr), 140.62 (CAr), 139.18 (CAr), 130.22 (C-3), 129.01 (2xCHAR), 128.77 (2xCHAR), 128.69 (3xCHAR), 127.78 (2xCHAR), 127.73 (2xCHAR), 127.15 (CHAR), 123.63 (CHAR), 119.37 (2xCHAR), 60.05 (CH-1), 42.99 (CH-6), 40.25 (CH₂-5), 13.29 (C-3CH₃) ppm.

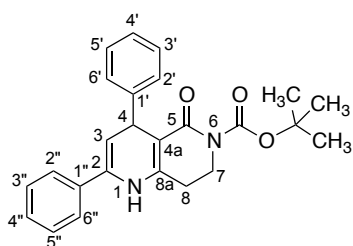
9.4.7 Synthesis of 1,4-dihydropyridine bicyclic derivatives 106-108.

Compound	R
106	Boc
107	H
108	H

The same procedure described above in section **x.4.5** was employed for the synthesis of derivatives **106-108**.

Data of compounds **106-108**, and the experimental conditions employed for their synthesis are indicated below.

***tert*-Butyl 5-oxo-2,4-diphenyl-1,4,5,6,7,8-hexahydro-1,6-naphthyridine-6-carboxylate (106)**



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), *tert*-butyl 2,4-dioxopiperidine-1-carboxylate (234 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: Crystallization from EtOH.

Yield: 280 mg (70%). White solid.

Data of 106:

Mp: 253-254 °C (dec.)

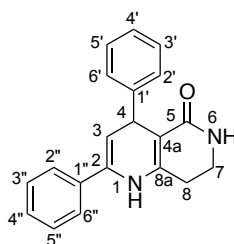
Elemental analysis calcd (%) for C₂₅H₂₆N₂O₃: C 74.60, H 6.51, N 6.96; found: C 74.70, H 6.42, N 7.08.

IR (KBr) ν 3324 (N-H), 1748 (NC(O)O), 1656 (C-5=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.42 (s, 9H, OC(CH₃)₃), 2.57-2.82 (m, 2H, CH₂-8), 3.39-3.56 (m, 1H, CH-7), 3.94-4.08 (m, 1H, CH-7), 4.63 (d, *J*=5.4 Hz, 1H, CH-4), 5.21 (dd, *J*=5.3, 1.6 Hz, 1H, CH-3), 7.09-7.21 (m, 1H, CH-4'), 7.26-7.31 (m, 4H, CH-2', CH-3', CH-5' and CH-6'), 7.34-7.45 (m, 3H, CH-3'', CH-4'' and CH-5''), 7.45-7.53 (m, 2H, CH-2'' and CH-6''), 8.79 (s, 1H, NH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 164.56 (C-5=O), 152.59 (C-2*), 149.58 (C-8a*), 148.25 (NC(O)O*), 134.99 (C-1'*), 134.20 (C-1''*), 128.51 (2xCHAR), 128.42 (CHAR), 128.27 (2xCHAR), 127.41 (2xCHAR), 125.83 (CHAR), 125.51 (2xCHAR), 105.17 (CH-3), 99.50 (C-4a), 80.85 (OC(CH₃)₃), 42.03 (CH₂-7), 37.94 (CH-4), 27.82 (OC(CH₃)₃), 26.35 (CH₂-8) ppm.

2,4-Diphenyl-4,6,7,8-tetrahydro-1,6-naphthyridin-5(1H)-one (107)



Prepared from 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), piperidine-2,4-dione (124 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: chloroform:ethyl acetate (from 10:1 to 5:1, v/v).

Yield: 222 mg (74%). Pale yellow solid.

Data of 107:

Mp: 267-269 °C

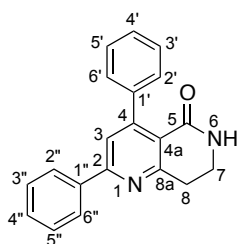
Elemental analysis calcd (%) for C₂₀H₁₈N₂O C 79.44, H 6.00, N 9.26; found: C 79.54, H 5.80, N 8.99.

IR (KBr) ν 3395 (N-H), 3227 (N-H), 1652 (C=O) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 2.50-2.59 (m, 2H, CH₂-8), 3.09-3.29 (m, 2H, CH₂-7), 4.61 (d, *J*=5.3 Hz, 1H, CH-4), 5.09 (dd, *J*=5.3, 1.6 Hz, 1H, CH-3), 6.77 (s, 1H, CONH), 7.04-7.18 (m, 1H, CH-4'), 7.24-7.26 (m, 4H, CH-2', CH-3', CH-5' and CH-6'), 7.31-7.42 (m, 3H, CH-3'', CH-4'' and CH-5''), 7.48 (dd, *J*=8.0, 1.7 Hz, 2H, CH-2'' and CH-6''), 8.27 (s, 1H, NH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 168.11 (C=O), 148.97 (C-2*), 145.57 (C-8a*), 135.58 (C-1'*), 135.01 (C-1''*), 128.46 (2xCHAR), 128.21 (CHAR), 128.13 (2xCHAR), 127.45 (2xCHAR), 125.55 (CHAR), 125.46 (2xCHAR), 103.66 (CH-3), 99.42 (C-4a), 37.83 (CH₂-7), 37.45 (CH-4), 26.38 (CH₂-8) ppm.

2,4-Diphenyl-7,8-dihydro-1,6-naphthyridin-5(6H)-one (108)



Isolated from the reaction between 1,3-diphenyl-2-propen-1-one (208 mg, 1 mmol), piperidine-2,4-dione (124 mg, 1.1 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 15 h.

Purification: chloroform:ethyl acetate (from 10:1 to 5:1, v/v).

Yield: 42 mg (14%). Pale yellow solid.

Data of 108:

Mp: 236-238 °C

Elemental analysis calcd (%) for C₂₀H₁₆N₂O: C 79.98, H 5.37, N 9.33; found: C 79.95, H 5.54, N 9.14.

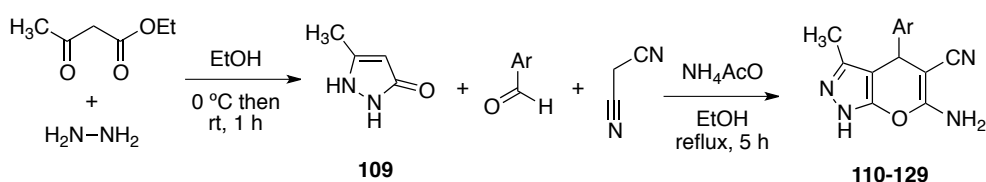
IR (KBr) ν 1668 (C=O), 1652 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ = 3.13 (t, *J*=6.0, 2H, CH₂-8), 3.43-3.57 (m, 2H, CH₂-7), 7.40 (s, 5H, C-4ArH), 7.45-7.55 (m, 3H, CH-3'', CH-4'' and CH-5''), 7.72 (s, 1H, CH-3), 8.05 (s, 1H, CONH), 8.11-8.23 (m, 2H, CH-2'' and CH-6'') ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 163.54 (C=O), 160.78 (C-2*), 156.65 (C-8a*), 151.33 (C-4*), 139.91 (C-1'), 137.67 (C-1''), 129.82 (CHAr), 128.84 (2xCHAr), 128.50 (2xCHAr), 127.65 (3xCHAr), 127.14 (2xCHAr), 121.94 (C-4a), 121.17 (CH-3), 37.90 (CH₂-8), 32.72 (CH₂-7) ppm.

9.5 FUSED PYRAZOLE DERIVATIVES

9.5.1 Synthesis of 1,4-dihydropyrano[2,3-c]pyrazole derivatives 110-129. General procedure



Cmpd.	Ar	Cmpd.	Ar
110	C ₆ H ₅	120	2-MeC ₆ H ₄
111	2-ClC ₆ H ₄	121	3-MeC ₆ H ₄
112	3-ClC ₆ H ₄	122	4-MeC ₆ H ₄
113	4-ClC ₆ H ₄	123	2-MeOC ₆ H ₄
114	2-BrC ₆ H ₄	124	3-MeOC ₆ H ₄
115	4-BrC ₆ H ₄	125	4-MeOC ₆ H ₄
116	4-FC ₆ H ₄	126	3-pyridyl
117	2-NO ₂ C ₆ H ₄	127	4-pyridyl
118	3-NO ₂ C ₆ H ₄	128	2-furyl
119	4-NO ₂ C ₆ H ₄	129	2-thienyl

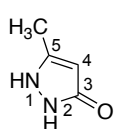
9.5.1.1 Synthesis of 5-methyl-1H-pyrazol-3(2H)-one (109)

To a solution of ethyl acetoacetate (1.05 equiv, 10.5 mmol) in ethanol (15 mL) at 0 °C was added dropwise hydrazine (1 equiv, 10 mmol). The mixture was allowed to warm to room temperature and then stirring was continued for 1 hour. After this time the solvent was removed in vacuum and the solid residue purified by crystallization and employed in the next step.

9.5.1.2 Synthesis of 1,4-dihydropyrano[2,3-c]pyrazole derivatives 110-129.

A solution of pyrazolone derivative **109** (1 equiv, 1 mmol), malononitrile (1 equiv, 1 mmol), the corresponding arylaldehyde (1 equiv, 1 mmol) and ammonium acetate (1 equiv, 1 mmol) in ethanol (2 mL) was heated under reflux for 5 hours. After this time the solvent was evaporated under reduced pressure and the crude residues were crystallized from EtOH or purified by silica gel column chromatography using dichloromethane:methanol as eluent to give pure compounds (**110-129**).

Data of compounds **109-129** and the experimental conditions employed for their synthesis are indicated below.

5-Methyl-1H-pyrazol-3(2H)-one (109)

Prepared from ethyl acetoacetate (1.366 g, 10.5 mmol) and hydrazine (0.501 g, 10 mmol).

Reaction time: 1 h.

Purification: Crystallization from EtOH.

Yield: 0.970 g (99%). White solid.

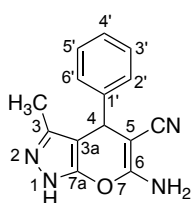
Data of 109:

IR (neat) ν 1607 (C=O) cm^{-1} .

^1H NMR (250 MHz, DMSO- d_6) δ 2.08 (s, 3H, C-5CH₃), 5.29 (s, 1H, CH-4), 10.45 (bs, 2H, NHx2) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 161.81 (C=O), 139.35 (C-5), 88.91 (CH-4), 11.22 (C-5CH₃) ppm.

These data are consistent with those described in the literature.²⁹¹

6-Amino-3-methyl-4-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (110)

Prepared from 5-methyl-1H-pyrazol-3(2H)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), benzaldehyde (106 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 217 mg (86%). White solid.

Data of 110:

Mp: 249-250 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₂N₄O: C 66.65, H 4.79, N 22.21; found: C 66.43, H 4.82, N 22.08.

HR-MS (ESI-Neg) m/z calcd for C₁₄H₁₁N₄O [M-H]⁻ 251.09383, found 251.09420.

²⁹¹ Lehmann, F.; Holm, M.; Laufer, S. *J. Comb. Chem.*, **2008**, *10*, 364-367.

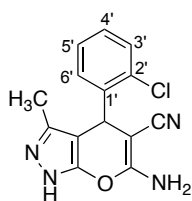
IR (neat) ν 3368 (N-H), 3164 (N-H), 2191 (CN), 1647 (C=N) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO-}d_6$) δ 1.78 (s, 3H, C-3 CH_3), 4.59 (s, 1H, CH-4), 6.90 (s, 2H, C-6 NH_2), 7.11-7.27 (m, 3H, CH-3', CH-4' and CH-5'), 7.27-7.38 (m, 2H, CH-2' and CH-6'), 12.11 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, $\text{DMSO-}d_6$) δ 160.91 (C-6), 154.80 (C-7a), 144.50 (C-1'), 135.61 (C-3), 128.49 (2xCHAR), 127.52 (2xCHAR), 126.79 (CHAR), 120.88 (CN), 97.68 (C-3a), 57.15 (C-5), 36.25 (CH-4), 9.79 (C-3 CH_3) ppm.

These data are consistent with those described in the literature.²⁹¹

6-Amino-4-(2-chlorophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**111**)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-chlorobenzaldehyde (141 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 243 mg (85%). White solid.

Data of 111:

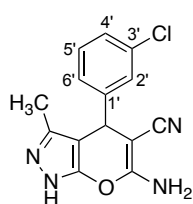
Mp: 253-254 °C (dec.)

Elemental analysis calcd (%) for $\text{C}_{14}\text{H}_{11}\text{ClN}_4\text{O}$: C 58.65, H 3.87, N 19.54; found: C 58.26, H 3.91, N 19.44.

IR (neat) ν 3385 (N-H), 3156 (N-H), 2187 (CN), 1650 (C=N), 1045 (C-Cl) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO-}d_6$) δ 1.76 (s, 3H, C-3 CH_3), 5.06 (s, 1H, CH-4), 6.96 (s, 2H, C-6 NH_2), 7.11-7.37 (m, 3H, CH-4', CH-5' and CH-6'), 7.41 (d, $J = 7.3$ Hz, 1H, CH-3'), 12.13 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, $\text{DMSO-}d_6$) δ 161.32 (C-6), 154.97 (C-7a), 140.96 (C-1'), 135.39 (C-3), 131.97 (C-2'), 130.76 (CHAR), 129.52 (CHAR), 128.64 (CHAR), 127.81 (CHAR), 120.47 (CN), 96.87 (C-3a), 55.68 (C-5), 33.48 (CH-4), 9.57 (C-3 CH_3) ppm.

6-Amino-4-(3-chlorophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (112)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 3-chlorobenzaldehyde (141 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 220 mg (77%). Pale yellow solid.

Data of 112:

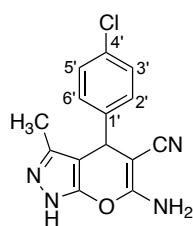
Mp: 205-206 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₁ClN₄O: C 58.65, H 3.87, N 19.54; found: C 58.42, H 3.86, N 19.45.

IR (neat) ν 3366 (N-H), 3175 (N-H), 2191 (CN), 1646 (C=N), 1070 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.80 (s, 3H, C-3CH₃), 4.66 (s, 1H, CH-4), 6.99 (s, 2H, C-6NH₂), 7.14 (d, *J* = 7.1 Hz, 1H, CH-4'), 7.20 (s, 1H, CH-2'), 7.25-7.45 (m, 2H, CH-5' and CH-6'), 12.18 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.06 (C-6), 154.73 (C-7a), 147.14 (C-1'), 135.78 (C-3), 133.09 (C-3'), 130.55 (CHAr), 127.29 (CHAr), 126.90 (CHAr), 126.37 (CHAr), 120.75 (CN), 97.07 (C-3a), 56.47 (C-5), 35.82 (CH-4), 9.83 (C-3CH₃) ppm.

6-Amino-4-(4-chlorophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (113)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-chlorobenzaldehyde (141 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 252 mg (88%). White solid.

Data of 113:

Mp: 229-230 °C (dec.)

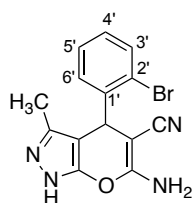
Elemental analysis calcd (%) for C₁₄H₁₁ClN₄O: C 58.65, H 3.87, N 19.54; found: C 58.55, H 3.88, N 19.55.

IR (neat) ν 3404 (N-H), 3166 (N-H), 2185 (CN), 1649 (C=N), 1075 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.79 (s, 3H, C-3CH₃), 4.63 (s, 1H, CH-4), 6.94 (s, 2H, C-6NH₂), 7.19 (d, *J* = 8.4 Hz, 2H, CH-2' and CH-6'), 7.38 (d, *J* = 8.4 Hz, 2H, CH-3' and CH-5'), 12.14 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 160.69 (C-6), 154.48 (C-7a), 143.29 (C-1'), 135.46 (C-3), 131.01 (C-4'), 129.16 (2xCHAR), 128.25 (2xCHAR), 120.46 (CN), 96.98 (C-3a), 56.50 (C-5), 35.33 (CH-4), 9.53 (C-3CH₃) ppm.

6-Amino-4-(2-bromophenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (**114**)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-bromobenzaldehyde (185 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 271 mg (82%). White solid.

Data of 114:

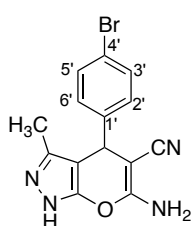
Mp: 248-249 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₁BrN₄O: C 50.77, H 3.35, N 16.92; found: C 50.61, H 3.34, N 16.81.

IR (neat) ν 3386 (N-H), 3142 (N-H), 2187 (CN), 1651 (C=N), 1047 (C-Br) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.76 (s, 3H, C-3CH₃), 5.07 (s, 1H, CH-4), 6.97 (s, 2H, C-6NH₂), 7.07-7.27 (m, 2H, CH-5' and CH-6'), 7.35 (t, *J* = 7.4 Hz, 1H, CH-4'), 7.58 (d, *J* = 7.7 Hz, 1H, CH-3'), 12.15 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.24 (C-6), 154.91 (C-7a), 142.60 (C-1'), 135.42 (C-3), 132.66 (CHAR), 130.99 (CHAR), 128.95 (CHAR), 128.45 (CHAR), 122.44 (C-2'), 120.38 (CN), 97.06 (C-3a), 55.92 (C-5), 35.86 (CH-4), 9.71 (C-3CH₃) ppm.

6-Amino-4-(4-bromophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (115)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-bromobenzaldehyde (185 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 305 mg (92%). Pale yellow solid.

Data of 115:

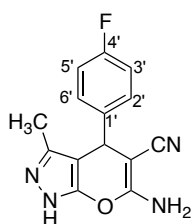
Mp: 232-233 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₁BrN₄O: C 50.77, H 3.35, N 16.92; found: C 50.78, H 3.46, N 17.03.

IR (neat) ν 3392 (N-H), 3177 (N-H), 2188 (CN), 1638 (C=N), 1073 (C-Br) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.79 (s, 3H, C-3CH₃), 4.62 (s, 1H, CH-4), 6.95 (s, 2H, C-6NH₂), 7.14 (d, *J* = 8.4 Hz, 2H, CH-2' and CH-6'), 7.51 (d, *J* = 8.4 Hz, 2H, CH-3' and CH-5'), 12.15 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 160.94 (C-6), 154.72 (C-7a), 143.95 (C-1'), 135.70 (C-3), 131.41 (2xCHAr), 129.78 (2xCHAr), 120.70 (C-4'*), 119.78 (CN*), 97.15 (C-3a), 56.64 (C-5), 35.63 (CH-4), 9.78 (C-3CH₃) ppm.

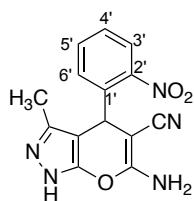
6-Amino-4-(4-fluorophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (116)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-fluorobenzaldehyde (124 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: dichloromethane:methanol (from 100:0 to 96:4, v/v).

Yield: 197 mg (73%). Pale yellow solid.

Data of 116:**Mp:** 223-224 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₁FN₄O: C 62.22, H 4.10, N 20.73; found: C 62.26, H 4.15, N 20.62.**IR** (neat) ν 3220 (N-H), 3122 (N-H), 2193 (CN), 1642 (C=N) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 4.63 (s, 1H, CH-4), 6.91 (s, 2H, C-6NH₂), 7.09-7.25 (m, 4H, CH-2', CH-3', CH-5' and CH-6'), 12.13 (s, 1H, NH-1) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 160.97 (d, *J* = 242.4 Hz, C-4'), 160.86 (C-6), 154.73 (C-7a), 140.71 (d, *J* = 3.0 Hz, C-1'), 135.66 (C-3), 129.38 (d, *J* = 8.2 Hz, 2xCHAr), 120.77 (CN), 115.21 (d, *J* = 21.4 Hz, 2xCHAr), 97.52 (C-3a), 57.04 (C-5), 35.44 (CH-4), 9.77 (C-3CH₃) ppm.**¹⁹F NMR** (235 MHz, DMSO-*d*₆) δ -116.64 (m) ppm.**6-Amino-3-methyl-4-(2-nitrophenyl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (117)**Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-nitrobenzaldehyde (124 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

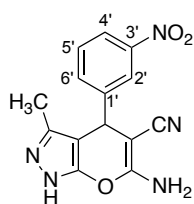
Yield: 280 mg (94%). Pale yellow solid.

Data of 117:**Mp:** 228-229 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₁N₅O₃: C 56.56, H 3.73, N 23.56; found: C 56.37, H 3.79, N 23.39.**IR** (neat) ν 3409 (N-H), 3159 (N-H), 2184 (CN), 1650 (C=N), 1593 (NO₂), 1345 (NO₂) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.76 (s, 3H, C-3CH₃), 5.09 (s, 1H, CH-4), 7.04 (s, 2H, C-6NH₂), 7.32 (dd, *J* = 7.8, 1.0 Hz, 1H, CH-6'), 7.49 (dt, *J* = 8.0, 1.0 Hz,

1H, CH-4'), 7.66 (dt, $J = 7.6, 1.0$ Hz, 1H, CH-5'), 7.85 (dd, $J = 8.1, 1.0$ Hz, 1H, CH-3'), 12.21 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 161.18 (C-6), 154.96 (C-7a), 149.15 (C-2'), 137.62 (C-1'*), 135.72 (C-3*), 133.40 (CHAr), 131.29 (CHAr), 128.35 (CHAr), 123.60 (CHAr), 120.26 (CN), 96.39 (C-3a), 55.99 (C-5), 31.39 (CH-4), 9.51 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(3-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (118)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 3-nitrobenzaldehyde (124 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 270 mg (91%). Pale yellow solid.

Data of 118:

Mp: 226-227 °C (dec.)

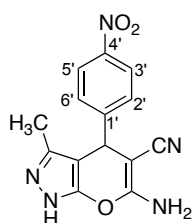
Elemental analysis calcd (%) for C₁₄H₁₁N₅O₃: C 56.56, H 3.73, N 23.56; found: C 56.15, H 3.80, N 23.27.

IR (neat) ν 3412 (N-H), 3162 (N-H), 2193 (CN), 1644 (C=N), 1593 (NO₂), 1345 (NO₂) cm⁻¹.

^1H NMR (250 MHz, DMSO- d_6) δ 1.80 (s, 3H, C-3CH₃), 4.88 (s, 1H, CH-4), 7.06 (s, 2H, C-6NH₂), 7.59-7.72 (m, 2H, CH-5' and CH-6'), 8.03 (s, 1H, CH-2'), 8.12 (dt, $J = 6.5, 2.4$ Hz, 1H, CH-4'), 12.21 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 161.51 (C-6), 155.05 (C-7a), 148.23 (C-3'*), 147.20 (C-1'*), 136.27 (C-3), 134.78 (CHAr), 130.64 (CHAr), 122.37 (CHAr), 122.22 (CHAr), 120.91 (CN), 97.03 (C-3a), 56.44 (C-5), 35.98 (CH-4), 10.13 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(4-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (119)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-nitrobenzaldehyde (124 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 275 mg (93%). Pale yellow solid.

Data of 119:

Mp: 279-280 °C (dec.)

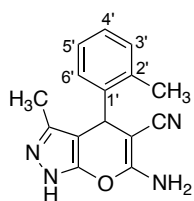
Elemental analysis calcd (%) for C₁₄H₁₁N₅O₃: C 56.56, H 3.73, N 23.56; found: C 56.40, H 3.79, N 23.21.

IR (neat) ν 3410 (N-H), 3219 (N-H), 2194 (CN), 1640 (C=N), 1511 (NO₂), 1347 (NO₂) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.80 (s, 3H, C-3CH₃), 4.83 (s, 1H, CH-4), 7.06 (s, 2H, C-6NH₂), 7.46 (d, *J* = 8.7 Hz, 2H, CH-2' and CH-6'), 8.21 (d, *J* = 8.7 Hz, 2H, CH-3' and CH-5'), 12.21 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.18 (C-6), 154.70 (C-7a), 152.15 (C-4'), 146.41 (C-1'), 135.92 (C-3), 128.88 (2xCHAR), 123.95 (2xCHAR), 120.55 (CN), 96.59 (C-3a), 55.90 (C-5), 35.90 (CH-4), 9.78 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(2-methylphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (120)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-methylbenzaldehyde (120 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 235 mg (88%). Grey solid.

Data of 120:

Mp: 241-242 °C (dec.)

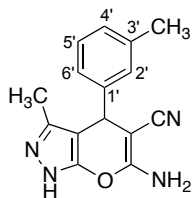
Elemental analysis calcd (%) for C₁₅H₁₄N₄O: C 67.65, H 5.30, N 21.04; found: C 67.51, H 5.31, N 20.99.

IR (neat) ν 3362 (N-H), 3152 (N-H), 2191 (CN), 1650 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.68 (s, 3H, C-3CH₃), 2.28 (s, 3H, C-2'CH₃), 4.84 (s, 1H, CH-4), 6.86 (s, 2H, C-6NH₂), 6.96-7.01 (m, 1H, CH-6'), 7.09-7.18 (m, 3H, CH-3', CH-4' and CH-5'), 12.09 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 160.80 (C-6), 155.11 (C-7a), 141.95 (C-1'), 135.39 (C-2'*), 135.01 (C-3*), 130.54 (CHAr), 128.95 (CHAr), 126.64 (CHAr), 126.39 (CHAr), 120.81 (CN), 97.58 (C-3a), 56.69 (C-5), 33.02 (CH-4), 18.99 (C-2'CH₃), 9.60 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(3-methylphenyl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5- carbonitrile (**121**)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 3-methylbenzaldehyde (120 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 223 mg (84%). Pale yellow solid.

Data of 121:

Mp: 205-206 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₁ClN₄O: C 58.65, H 3.87, N 19.54; found: C 58.42, H 3.86, N 19.45.

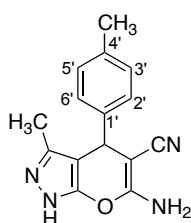
IR (neat) ν 3366 (N-H), 3175 (N-H), 2191 (CN), 1646 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 2.27 (s, 3H, C-3'CH₃), 4.54 (s, 1H, CH-4), 6.87 (s, 2H, C-6NH₂), 6.92-7.00 (m, 2H, CH-2' and CH-6'), 7.03 (d, *J* = 7.6 Hz, 1H, CH-4'), 7.20 (t, *J* = 7.8 Hz, 1H, CH-5'), 12.09 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 160.88 (C-6), 154.76 (C-7a), 144.47 (C-1'), 137.53 (C-3'*), 135.58 (C-3*), 128.32 (CHAr), 127.92 (CHAr), 127.49 (CHAr),

124.72 (CHAR), 120.89 (CN), 97.70 (C-3a), 57.18 (C-5), 36.20 (CH-4), 21.12 (C-3'CH₃), 9.81 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(4-methylphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (122)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-methylbenzaldehyde (120 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 1 h.

Purification: Crystallization from EtOH.

Yield: 215 mg (81%). White solid.

Data of 122:

Mp: 205-206 °C (dec.)

Elemental analysis calcd (%) for C₁₅H₁₄N₄O: C 67.65, H 5.30, N 21.04; found: C 67.55, H 5.26, N 21.03.

IR (neat) ν 3372 (N-H), 3184 (N-H), 2191 (CN), 1645 (C=N) cm⁻¹.

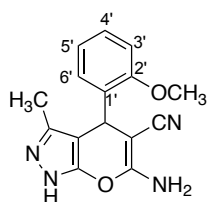
¹H NMR (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 2.27 (s, 3H, C-4'CH₃), 4.54 (s, 1H, CH-4), 6.84 (s, 2H, C-6NH₂), 7.04 (d, *J* = 7.8 Hz, 2H, CH-2' and CH-6'), 7.12 (d, *J* = 7.8 Hz, 2H, CH-3' and CH-5'), 12.08 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 160.78 (C-6), 154.77 (C-7a), 141.52 (C-1'), 135.73 (C-4'*), 135.54 (C-3'*), 129.02 (2xCHAR), 127.38 (2xCHAR), 120.85 (CN), 97.74 (C-3a), 57.34 (C-5), 35.85 (CH-4), 20.66 (C-4'CH₃), 9.78 (C-3CH₃) ppm.

6-Amino-4-(2-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (123)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-methoxybenzaldehyde (136 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.



Purification: Crystallization from EtOH.

Yield: 245 mg (87%). Grey solid.

Data of

Mp: 235-236 °C (dec.)

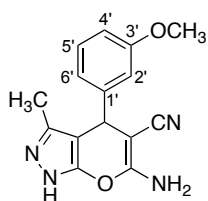
Elemental analysis calcd (%) for $C_{15}H_{14}N_4O_2$: C 63.82, H 5.00, N 19.85; found: C 63.52, H 5.08, N 19.59.

IR (neat) ν 3371 (N-H), 3151 (N-H), 2192 (CN), 1654 (C=N), 1256 (C-O-C) cm^{-1} .

1H NMR (250 MHz, DMSO- d_6) δ 1.78 (s, 3H, C-3CH₃), 3.78 (s, 3H, OCH₃), 4.96 (s, 1H, CH-4), 6.80 (s, 2H, C-6NH₂), 6.89 (t, $J = 7.3$ Hz, 1H, CH-4'), 6.94-7.04 (m, 2H, CH-3' and CH-6'), 7.19 (t, $J = 7.6$ Hz, 1H, CH-5'), 12.00 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 161.51 (C-6), 156.36 (C-7a*), 155.09 (C-2'*), 135.08 (C-3), 132.13 (C-1'), 128.65 (CHAR), 127.95 (CHAR), 120.96 (CN), 120.84 (CHAR), 111.30 (CHAR), 97.85 (C-3a), 56.33 (C-2'OCH₃), 55.59 (C-5), 29.15 (CH-4), 9.55 (C-3CH₃) ppm.

6-Amino-4-(3-methoxyphenyl)-3-methyl-1,4-dihydropyranopyrazole-5-carbonitrile (124)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 3-methoxybenzaldehyde (136 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: dichloromethane:methanol (from 100:0 to 95:5, v/v).

Yield: 235 mg (83%). Pale yellow solid.

Data of **124**:

Mp: 201-202 °C (dec.)

Elemental analysis calcd (%) for $C_{15}H_{14}N_4O_2$: C 63.82, H 5.00, N 19.85; found: C 63.60, H 5.07, N 19.51.

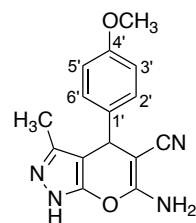
IR (neat) ν 3383 (N-H), 3161 (N-H), 2184 (CN), 1641 (C=N), 1263 (C-O-C) cm^{-1} .

^1H NMR (250 MHz, DMSO- d_6) δ 1.81 (s, 3H, C-3CH₃), 3.72 (s, 3H, OCH₃), 4.57 (s, 1H, CH-4), 6.69-6.75 (m, 2H, CH-2' and CH-6'), 6.80 (dd, $J = 8.0, 1.8$ Hz, 1H, CH-4'), 6.88 (s, 2H, C-6NH₂), 7.24 (t, $J = 8.0$ Hz, 1H, CH-5'), 12.10 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 160.93 (C-6), 159.24 (C-3'*), 154.76 (C-7a*), 146.16 (C-1'), 135.62 (C-3), 129.62 (CHAr), 120.85 (CN), 119.70 (CHAr), 113.51 (CHAr), 111.67 (CHAr), 97.50 (C-3a), 56.97 (C-5), 54.99 (C-3'OCH₃), 36.18 (CH-4), 9.84 (C-3CH₃) ppm.

6-Amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (125)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-methoxybenzaldehyde (136 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).



Reaction time: 5 h.

Purification: dichloromethane:methanol (from 100:0 to 95:5, v/v).

Yield: 200 mg (71%). Yellow solid.

Data of 125:

Mp: 202-203 °C (dec.)

Elemental analysis calcd (%) for C₁₅H₁₄N₄O₂: C 63.82, H 5.00, N 19.85; found: C 63.73, H 4.98, N 19.62.

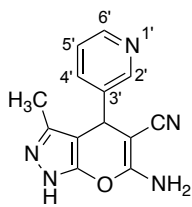
IR (neat) ν 3231 (N-H), 3122 (N-H), 2190 (CN), 1639 (C=N), 1258 (C-O-C) cm^{-1} .

^1H NMR (250 MHz, DMSO- d_6) δ 1.78 (s, 3H, C-3CH₃), 3.73 (s, 3H, OCH₃), 4.53 (s, 1H, CH-4), 6.83 (s, 2H, C-6NH₂), 6.87 (d, $J = 8.7$ Hz, 2H, CH-3' and CH-5'), 7.07 (d, $J = 8.7$ Hz, 2H, CH-2' and CH-6'), 12.07 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 161.04 (C-6), 158.31 (C-4'*), 155.10 (C-7a*), 136.85 (C-1'), 135.90 (C-3), 128.86 (2xCHAr), 121.22 (CN), 114.11 (2xCHAr),

98.24 (C-3a), 57.90 (C-5), 55.35 (C-4'OCH₃), 35.78 (CH-4), 10.12 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(pyridin-3-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (126)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 3-pyridinecarboxaldehyde (107 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 215 mg (85%). Grey solid.

Data of 126:

Mp: 223-224 °C (dec.)

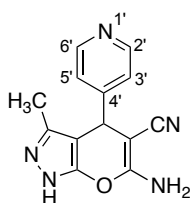
Elemental analysis calcd (%) for C₁₃H₁₁N₅O: C 61.65, H 4.38, N 27.65; found: C 61.63, H 4.28, N 27.48.

IR (neat) ν 3388 (N-H), 3165 (N-H), 2190 (CN), 1644 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 4.69 (s, 1H, CH-4), 7.01 (s, 2H, C-6NH₂), 7.35 (dd, *J* = 7.9, 4.8 Hz, 1H, CH-5'), 7.52 (dd, *J* = 7.9, 1.7 Hz, 1H, CH-4'), 8.45 (s, 2H, CH-2' and CH-6'), 12.19 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.11 (C-6), 154.78 (C-7a), 148.82 (CHAr), 148.32 (CHAr), 139.78 (C-3'), 135.74 (C-3), 135.23 (CHAr), 123.95 (CHAr), 120.71 (CN), 96.82 (C-3a), 56.23 (C-5), 33.68 (CH-4), 9.77 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (127)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 4-pyridinecarboxaldehyde (107 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 212 mg (84%). White solid.

Data of 127:

Mp: 224-225 °C (dec.)

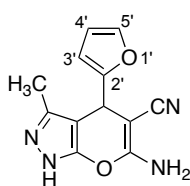
Elemental analysis calcd (%) for C₁₃H₁₁N₅O: C 61.65, H 4.38, N 27.65; found: C 61.29, H 4.40, N 27.34.

IR (neat) ν 3315 (N-H), 3025 (N-H), 2169 (CN), 1657 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.81 (s, 3H, C-3CH₃), 4.66 (s, 1H, CH-4), 7.04 (s, 2H, C-6NH₂), 7.19 (d, *J* = 5.8 Hz, 2H, CH-3' and CH-5'), 8.51 (d, *J* = 5.8 Hz, 2H, CH-2' and CH-6'), 12.20 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.26 (C-6), 154.76 (C-7a), 152.86 (C-4'), 149.95 (2xCHAR), 135.82 (C-3), 122.79 (2xCHAR), 120.55 (CN), 96.26 (C-3a), 55.56 (C-5), 35.54 (CH-4), 9.76 (C-3CH₃) ppm.

6-Amino-4-(furan-2-yl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (128)



Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-furancarboxaldehyde (96 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).

Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 200 mg (83%). Grey solid.

Data of 128:

Mp: > 300 °C (dec.)

Elemental analysis calcd (%) for C₁₂H₁₀N₄O₂: C 59.50, H 4.16, N 23.13; found: C 59.31, H 4.20, N 22.67.

IR (neat) ν 3345 (N-H), 3162 (N-H), 2185 (CN), 1647 (C=N) cm⁻¹.

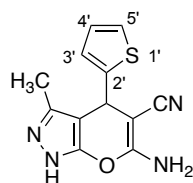
¹H NMR (250 MHz, DMSO-*d*₆) δ 1.97 (s, 3H, C-3CH₃), 4.77 (s, 1H, CH-4), 6.17 (d, *J* = 3.0 Hz, 1H, CH-3'), 6.37 (dd, *J* = 3.0, 1.9 Hz, 1H, CH-4'), 6.96 (s, 2H, C-

6NH₂), 7.53 (s, 1H, CH-5'), 12.16 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.51 (C-6), 155.74 (C-7a*), 154.84 (C-2'*), 142.32 (CHAr), 135.87 (C-3), 120.65 (CN), 110.28 (CHAr), 105.68 (CHAr), 95.14 (C-3a), 53.97 (C-5), 29.83 (CH-4), 9.62 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(thiophen-2-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (129)

Prepared from 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-thiophencarboxaldehyde (112 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).



Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 201 mg (78%). Pale yellow solid.

Data of 129:

Mp: 243-244 °C (dec.)

Elemental analysis calcd (%) for C₁₂H₁₀N₄OS: C 55.80, H 3.90, N 21.69, S 12.41; found: C 55.45, H 3.82, N 21.46, S 12.27.

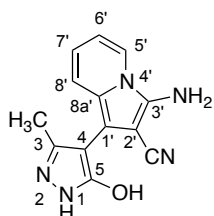
IR (neat) ν 3339 (N-H), 3160 (N-H), 2189 (CN), 1646 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.91 (s, 3H, C-3CH₃), 4.99 (s, 1H, CH-4), 6.73-7.11 (m, 4H, CH-3', CH-4' and C-6NH₂), 7.38 (d, *J* = 4.9 Hz, 1H, CH-5'), 12.18 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 161.01 (C-6), 154.65 (C-7a), 150.15 (C-2'), 136.39 (C-3), 126.88 (CHAr), 125.37 (CHAr), 124.74 (CHAr), 121.00 (CN), 97.92 (C-3a), 57.89 (C-5), 31.75 (CH-4), 10.11 (C-3CH₃) ppm.

3-amino-1-(5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)indolizine-2-carbonitrile (134)

Isolated from the reaction between 5-methyl-1*H*-pyrazol-3(2*H*)-one (98 mg, 1 mmol), malononitrile (66 mg, 1 mmol), 2-pyridinecarboxaldehyde (107 mg, 1 mmol) and ammonium acetate (77 mg, 1 mmol).



Reaction time: 5 h.

Purification: Crystallization from EtOH.

Yield: 178 mg (70%). Yellow solid.

Data of 134:

Mp: °C (dec.)

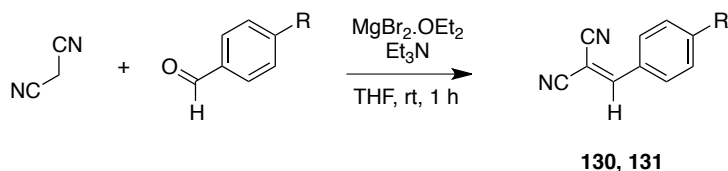
Elemental analysis calcd (%) for $C_{13}H_{11}N_5O$: C 61.65, H 4.38, N 27.65; found: C 61.58, H 4.50, N 27.48.

IR (neat) ν 3340 (N-H), 3260 (N-H), 2205 (CN) cm^{-1} .

1H NMR (250 MHz, DMSO) δ 2.09 (s, 3H, C-3CH₃), 6.16 (s, 2H, NH₂), 6.36 – 6.55 (m, 2H, CH-6' and CH-7'), 6.94 (d, J = 8.9 Hz, 1H, CH-8'), 7.78 (d, J = 7.1 Hz, 1H, CH-5'), 10.15 (bs, 1H, OH), 10.89 (bs, 1H, NH) ppm.

^{13}C NMR (63 MHz, DMSO) δ 159.56 (C-5), 138.05 (C-3*), 137.86 (C-8a'*), 122.56 (C-3'), 120.98 (CH-5'*), 119.88 (CH-8'*), 117.80 (CN), 115.04 (CH-7'*), 111.42 (CH-6'*), 103.15 (C-4), 94.88 (C-1'), 79.22 (C-2'), 11.18 (C-3CH₃).

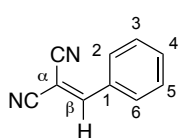
9.5.2 Synthesis of Knoevenagel adducts **130**-**131**. General procedure



Compound	R
130	H
131	CH ₃

To a mixture of malononitrile (1 equiv, 3 mmol), triethylamine (1 equiv, 3 mmol) and magnesium bromide diethyl etherate (0.2 equiv, 0.6 mmol) in THF (5 mL), was added the appropriate benzaldehyde (1 equiv, 3 mmol) and the mixture was stirred at room temperature for 1 h, until the complete disappearance of the aldehyde (checked by TLC). The mixture was diluted with 20 mL of diethyl ether, the organic phase washed with water and filtered through a short Na₂SO₄ pad. The solvent was removed under reduced pressure and the products were recrystallized using hexane.

Data of compounds **130** and **131** and the experimental conditions employed for their synthesis are indicated below.

2-Benzylidenemalononitrile (130)

Prepared from malononitrile (198 mg, 3 mmol), benzaldehyde (318 mg, 3 mmol) and magnesium bromide diethyl etherate (155 mg, 0.6 mmol).

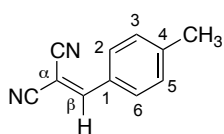
Yield: 440 mg (95%). Pale yellow solid.

Data of 130:

$^1\text{H NMR}$ (250 MHz, CDCl_3) δ 7.49 – 7.71 (m, 3H, CH-3, CH-4 and CH-5), 7.79 (s, 1H, CH- β), 7.86 – 7.97 (m, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C NMR}$ (63 MHz, CDCl_3) δ 160.40 (CH- β), 135.09 (CH-4), 131.33 (2xCHAR), 131.18 (C-1), 130.07 (2xCHAR), 114.14 (CN), 112.97 (CN), 83.27 (C- α) ppm.

These data are consistent with those described in the literature.²⁹²

2-(4-Methylbenzylidene)malononitrile (131)

Prepared from malononitrile (198 mg, 3 mmol), 4-methylbenzaldehyde (360 mg, 3 mmol) and magnesium bromide diethyl etherate (155 mg, 0.6 mmol).

Yield: 470 mg (93%). Yellow solid.

Data of 131:

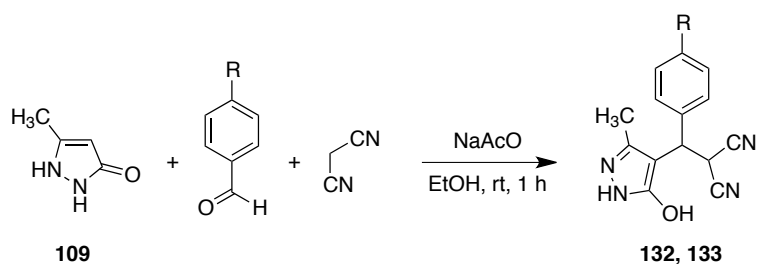
$^1\text{H NMR}$ (250 MHz, CDCl_3) δ 2.46 (s, 3H, C-4 CH_3), 7.34 (d, $J = 8.2$ Hz, 2H, CH-3 and CH-5), 7.72 (s, 1H, CH- β), 7.81 (d, $J = 8.3$ Hz, 2H, CH-2 and CH-6) ppm.

$^{13}\text{C NMR}$ (63 MHz, CDCl_3) δ 160.23 (CH- β), 146.84 (C-4), 131.36 (2xCHAR), 130.81 (2xCHAR), 128.87 (C-1), 114.46 (CN), 113.30 (CN), 81.59 (C- α), 22.46 (C-4 CH_3) ppm.

These data are consistent with those described in the literature.²⁹²

²⁹² Abaee, M. S.; Mojtahedi, M. M.; Zahedi, M. M.; Khanalizadeh, G. *Arkivoc* 2006, xv, 48-52.

9.5.3 Synthesis of the acyclic precursor of 1,4-dihydropyrano[2,3-c]pyrazole **132-133**. General procedure

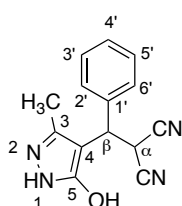


Compound	R
132	H
133	CH ₃

A solution of the suitable aromatic aldehyde (1 equiv, 3 mmol), 2-pyrazol in-5-one (1 equiv, 3 mmol), malononitrile (1 equiv, 3 mmol) and sodium acetate (0.1 equiv, 0.3 mmol) in EtOH (5 mL) was stirred at room temperature for 1 h. After the reaction was finished (as checked by TLC), the solution was filtered out to isolate the solid product, which was then rinsed with an ice-cold ethanol/water solution (9:1) and dried.

Data of compounds **132** and **133** and the experimental conditions employed for their synthesis are indicated below.

2-((5-Hydroxy-3-methyl-1H-pyrazol-4-yl)(phenyl)methyl)malononitrile (132)



Prepared from 5-methyl-1H-pyrazol-3(2H)-one (294 mg, 3 mmol), malononitrile (198 mg, 3 mmol), benzaldehyde (318 mg, 3 mmol) and sodium acetate (25 mg, 0.3 mmol).

Yield: 200 mg (80%). White solid.

Data of 132:

Mp: 243-244 °C (dec.)

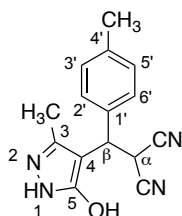
IR (neat) ν 3360 (N-H), 2191 (CN) cm^{-1} .

^1H NMR (250 MHz, DMSO- d_6) δ 2.08 (s, 3H, C-3CH₃), 4.64 (d, J = 11.5 Hz, 1H, CH- α), 5.53 (d, J = 11.5 Hz, 1H, CH- β), 7.22 – 7.41 (m, 3H, CH-3', CH-4' and CH-5'), 7.48 (d, J = 7.0 Hz, 2H, CH-2' and CH-6'), 10.81 (bs, 2H, NH and OH) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 159.36 (C-5), 140.22 (C-1'*), 138.20 (C-3*), 129.00 (2xCHAR), 128.02 (2xCHAR), 127.93 (CH-4'), 114.49 (CN), 98.94 (CN), 41.66 (CH- β), 27.75 (CH- α), 10.10 (C-3CH₃) ppm.

These data are consistent with those described in the literature.²⁹³

2-((5-Hydroxy-3-methyl-1H-pyrazol-4-yl)(4-methylphenyl)methyl)malononitrile (133)



Prepared from 5-methyl-1H-pyrazol-3(2H)-one (294 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-methyl benzaldehyde (360 mg, 3 mmol) and sodium acetate (25 mg, 0.3 mmol).

Yield: 240 mg (90%). White solid.

Data of 133:

Mp: 198-199 °C (dec.)

IR (neat) ν 3354 (N-H), 2187 (CN) cm^{-1} .

^1H NMR (250 MHz, DMSO- d_6) δ 2.06 (s, 3H, C-3CH₃), 2.26 (s, 3H, C-4'CH₃),

²⁹³ Elinson, M. N.; Naysbullin, R. F.; Nikishin, G. I. R. *Chim.* **2013**, *16*, 789-794.

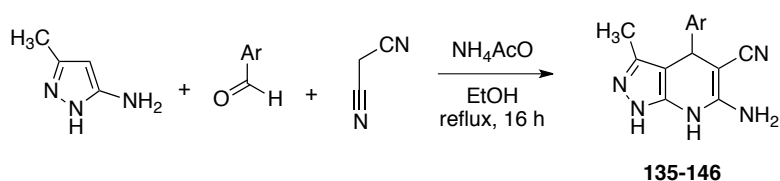
4.58 (d, $J = 11.4$ Hz, 1H, CH- α), 5.48 (d, $J = 11.4$ Hz, 1H, CH- β), 7.14 (d, $J = 7.9$ Hz, 2H, CH-3' and CH-5'), 7.35 (d, $J = 8.0$ Hz, 2H, CH-2' and CH-6'), 10.77 (bs, 2H, NH and OH) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 159.35 (C-5), 138.13 (C-1'*), 137.22 (C-3*), 137.12 (C-4'*), 129.52 (2xCHAR), 127.91 (2xCHAR), 114.51 (CN), 99.07 (CN), 41.41 (CH- β), 27.81 (CH- α), 21.00 (C-4'CH₃), 10.11 (C-3CH₃) ppm.

These data are consistent with those described in the literature.²⁹³

9.5.4 Synthesis of 4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine derivatives

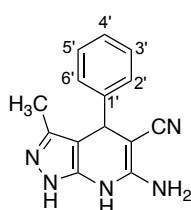
135-146. General procedure



Cmpd.	Ar	Cmpd.	Ar
135	C ₆ H ₅	141	4-FC ₆ H ₄
136	2-ClC ₆ H ₄	142	3-NO ₂ C ₆ H ₄
137	3-ClC ₆ H ₄	143	4-NO ₂ C ₆ H ₄
138	4-ClC ₆ H ₄	144	3-pyridyl
139	2-BrC ₆ H ₄	145	4-pyridyl
140	4-BrC ₆ H ₄	146	2-thienyl

A solution of 3-methyl-1*H*-pyrazol-5-amine (1 equiv, 3 mmol), malononitrile (1 equiv, 3 mmol), the corresponding arylaldehyde (1 equiv, 3 mmol) and ammonium acetate (1 equiv, 3 mmol) in ethanol (5 mL) was heated under reflux for 16 hours. After this time the solvent was evaporated under reduced pressure and the crude residues were crystallized from EtOH or purified by silica gel column chromatography using dichloromethane:methanol mixtures as eluent to give pure compounds **135-146**.

Data of compounds **135-146** and the experimental conditions employed for their synthesis are indicated below.

6-Amino-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (135)

Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), benzaldehyde (318 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 330 mg (44%). White solid.

Data of 135:

Mp: > 300 °C (dec.)

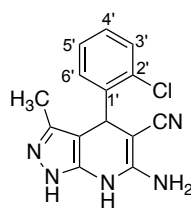
Elemental analysis calcd (%) for C₁₄H₁₃N₅: C 66.92, H 5.21, N 27.87; found: C 66.83, H 5.21, N 27.69.

HR-MS (ESI-Neg) *m/z* calcd for C₁₄H₁₂N₅ [M-H]⁻ 250.10982, found 250.11047.

IR (neat) ν 3401 (N-H), 3324 (N-H), 3225 (N-H), 2163 (CN), 1628 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.75 (s, 3H, C-3CH₃), 4.61 (s, 1H, CH-4), 5.44 (s, 2H, C-6NH₂), 7.03-7.21 (m, 3H, CH-3', CH-4' and CH-5'), 7.21-7.39 (m, 2H, CH-2' and CH-6'), 8.87 (s, 1H, NH-7), 11.69 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 153.38 (C-6), 146.87 (C-7a*), 146.17 (C-1'*), 134.87 (C-3), 128.25 (2xCHAR), 127.32 (2xCHAR), 126.20 (CHAR), 123.30 (CN), 100.53 (C-3a), 55.35 (C-5), 37.79 (CH-4), 9.51 (C-3CH₃) ppm.

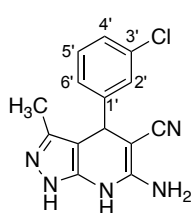
6-Amino-4-(2-chlorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (136)

Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 2-chlorobenzaldehyde (422 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 90:10, v/v).

Yield: 290 mg (34%). White solid.

Data of 136:**Mp:** 267-268 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₂ClN₅: C 58.85, H 4.23, N 24.51; found: C 58.76, H 4.13, N 24.38.**IR** (neat) ν 3406 (N-H), 3313 (N-H), 3212 (N-H), 2175 (CN), 1636 (C=N), 1045 (C-Cl) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.73 (s, 3H, C-3CH₃), 5.14 (s, 1H, CH-4), 5.48 (s, 2H, C-6NH₂), 7.11-7.32 (m, 3H, CH-4', CH-5' and CH-6'), 7.37 (dd, *J* = 7.7, 1.2 Hz, 1H, CH-3'), 8.95 (s, 1H, NH-7), 11.72 (s, 1H, NH-1) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 153.70 (C-6), 146.38 (C-7a*), 143.60 (C-1'*), 134.65 (C-3*), 131.19 (C-2'*), 130.92 (CHAr), 129.02 (CHAr), 127.96 (CHAr), 127.68 (CHAr), 122.78 (CN), 99.92 (C-3a), 54.17 (C-5), 34.40 (CH-4), 9.27 (C-3CH₃) ppm.**6-Amino-4-(3-chlorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (137)**Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 3-chlorobenzaldehyde (422 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

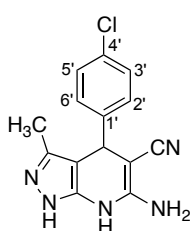
Purification: dichloromethane:methanol (from 100:0 to 90:10, v/v).

Yield: 335 mg (39%). White solid.

Data of 137:**Mp:** 281-282 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₂ClN₅: C 58.85, H 4.23, N 24.51; found: C 58.62, H 4.24, N 24.39.**IR** (neat) ν 3398 (N-H), 3272 (N-H), 3194 (N-H), 2164 (CN), 1625 (C=N), 1074 (C-Cl) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 4.67 (s, 1H, CH-4), 5.51 (s, 2H, C-6NH₂), 7.09-7.16 (m, 2H, CH-2' and CH-6'), 7.21-7.27 (m, 1H, CH-4'),

7.32 (t, $J = 7.9$ Hz, 1H, CH-5'), 8.94 (s, 1H, NH-7), 11.76 (s, 1H, NH-1) ppm.
 ^{13}C NMR (63 MHz, DMSO- d_6) δ 153.51 (C-6), 149.48 (C-7a*), 146.15 (C-1'*), 134.93 (C-3*), 132.90 (C-3'*), 130.24 (CHAR), 126.99 (CHAR), 126.26 (CHAR), 126.05 (CHAR), 123.07 (CN), 99.92 (C-3a), 54.75 (C-5), 37.39 (CH-4), 9.46 (C-3CH₃) ppm.

6-Amino-4-(4-chlorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (138)



Prepared from 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-chlorobenzaldehyde (422 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 410 mg (48%). White solid.

Data of 138:

Mp: 297-298 °C (dec.)

Elemental analysis calcd (%) for C₁₄H₁₂ClN₅: C 58.85, H 4.23, N 24.51; found: C 58.81, H 4.12, N 24.58.

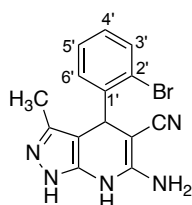
IR (neat) ν 3211 (N-H), 3074 (N-H), 2167 (CN), 1627 (C=N), 1087 (C-Cl) cm⁻¹.

^1H NMR (250 MHz, DMSO- d_6) δ 1.76 (s, 3H, C-3CH₃), 4.65 (s, 1H, CH-4), 5.48 (s, 2H, C-6NH₂), 7.15 (d, $J = 8.4$ Hz, 2H, CH-2' and CH-6'), 7.34 (d, $J = 8.4$ Hz, 2H, CH-3' and CH-5'), 8.92 (s, 1H, NH-7), 11.73 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, DMSO- d_6) δ 153.39 (C-6), 146.15 (C-7a*), 145.86 (C-1'*), 134.94 (C-3*), 130.66 (C-4'*), 129.16 (2xCHAR), 128.26 (2xCHAR), 123.12 (CN), 100.08 (C-3a), 54.99 (C-5), 37.14 (CH-4), 9.49 (C-3CH₃) ppm.

6-Amino-4-(2-bromophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (139)

Prepared from 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 2-bromobenzaldehyde (555 mg, 3 mmol) and ammonium



acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 315 mg (32%). White solid.

Data of 139:

Mp: 236-237 °C (dec.)

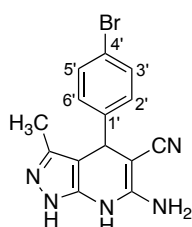
Elemental analysis calcd (%) for C₁₄H₁₂BrN₅: C 50.93, H 3.66, N 21.21; found: C 50.77, H 3.66, N 21.08.

IR (neat) ν 3339 (N-H), 3216 (N-H), 3055 (N-H), 2153 (CN), 1626 (C=N), 1023 (C-Br) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.73 (s, 3H, C-3CH₃), 5.13 (s, 1H, CH-4), 5.48 (s, 2H, C-6NH₂), 6.94-7.17 (m, 2H, CH-5' and CH-6'), 7.32 (t, *J* = 7.0 Hz, 1H, CH-4'), 7.53 (d, *J* = 7.9 Hz, 1H, CH-3'), 8.95 (s, 1H, NH-7), 11.73 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 153.60 (C-6), 146.31 (C-7a*), 145.33 (C-1'*), 134.71 (C-3), 132.14 (CHAR), 131.18 (CHAR), 128.31 (2xCHAR), 122.67 (CN*), 121.76 (C-2'*), 100.14 (C-3a), 54.47 (C-5), 36.98 (CH-4), 9.43 (C-3CH₃) ppm.

6-Amino-4-(4-bromophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (140)



Prepared from 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-bromobenzaldehyde (555 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 505 mg (51%). White solid.

Data of 140:

Mp: > 300 °C (dec.)

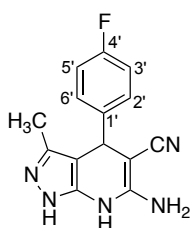
Elemental analysis calcd (%) for C₁₄H₁₂BrN₅: C 50.93, H 3.66, N 21.21; found: C 50.78, H 3.46, N 21.22.

IR (neat) ν 3430 (N-H), 3214 (N-H), 3070 (N-H), 2161 (CN), 1626 (C=N), 1069 (C-Br) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO-}d_6$) δ 1.76 (s, 3H, C-3 CH_3), 4.63 (s, 1H, CH-4), 5.48 (s, 2H, C-6 NH_2), 7.09 (d, $J = 8.2$ Hz, 2H, CH-2' and CH-6'), 7.47 (d, $J = 8.2$ Hz, 2H, CH-3' and CH-5'), 8.91 (s, 1H, NH-7), 11.73 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, $\text{DMSO-}d_6$) δ 153.40 (C-6), 146.28 (C-7a*), 146.16 (C-1'*), 134.91 (C-3), 131.17 (2xCHAR), 129.56 (2xCHAR), 123.10 (CN*), 119.16 (C-4'*), 100.02 (C-3a), 54.90 (C-5), 37.20 (CH-4), 9.47 (C-3 CH_3) ppm.

6-Amino-4-(4-fluorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (141)



Prepared from 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-fluorobenzaldehyde (372 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 290 mg (36%). White solid.

Data of 141:

Mp: > 300 °C (dec.)

Elemental analysis calcd (%) for $\text{C}_{14}\text{H}_{12}\text{FN}_5$: C 62.44, H 4.49, N 26.01; found: C 62.31, H 4.48, N 25.94.

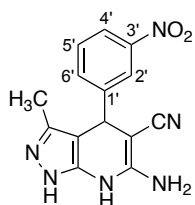
IR (neat) ν 3464 (N-H), 3191 (N-H), 3140 (N-H), 2177 (CN), 1599 (C=N) cm^{-1} .

^1H NMR (250 MHz, $\text{DMSO-}d_6$) δ 1.75 (s, 3H, C-3 CH_3), 4.65 (s, 1H, CH-4), 5.45 (s, 2H, C-6 NH_2), 6.94-7.27 (m, 4H, CH-2', CH-3', CH-5' and CH-6'), 8.89 (s, 1H, NH-7), 11.71 (s, 1H, NH-1) ppm.

^{13}C NMR (63 MHz, $\text{DMSO-}d_6$) δ 160.68 (d, $J = 241.5$ Hz, C-4'), 153.30 (C-6), 146.15 (C-7a), 143.08 (d, $J = 2.9$ Hz, C-1'), 134.85 (C-3), 129.05 (d, $J = 8.1$ Hz, 2xCHAR), 123.17 (CN), 114.93 (d, $J = 21.2$ Hz, 2xCHAR), 100.39 (C-3a), 55.28 (C-5), 36.99 (CH-4), 9.46 (C-3 CH_3) ppm.

^{19}F NMR (235 MHz, $\text{DMSO-}d_6$) δ -117.52 (m) ppm.

6-Amino-3-methyl-4-(3-nitrophenyl)-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (142)



Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 3-nitrobenzaldehyde (453 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 355 mg (40%). Yellow solid.

Data of 142:

Mp: 249-250 °C (dec.)

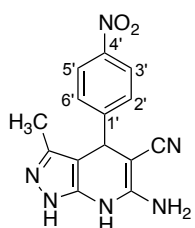
Elemental analysis calcd (%) for C₁₄H₁₂N₆O₂: C 56.75, H 4.08, N 28.36; found: C 56.77, H 4.01, N 28.17.

IR (neat) ν 3357 (N-H), 3162 (N-H), 2161 (CN), 1644 (C=N), 1594 (NO₂), 1340 (NO₂) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.77 (s, 3H, C-3CH₃), 4.87 (s, 1H, CH-4), 5.58 (s, 2H, C-6NH₂), 7.56-7.67 (m, 2H, CH-5' and CH-6'), 7.96 (s, 1H, CH-2'), 8.07 (dt, *J* = 6.7, 2.2 Hz, 1H, CH-4'), 9.02 (s, 1H, NH-7), 11.80 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 153.67 (C-6*), 149.23 (C-3'*), 147.89 (C-7a*), 146.17 (C-1'*), 135.16 (C-3), 134.17 (CHAR), 130.01 (CHAR), 122.98 (CN), 121.58 (CHAR), 121.46 (CHAR), 99.56 (C-3a), 54.44 (C-5), 37.29 (CH-4), 9.48 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(4-nitrophenyl)-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (143)

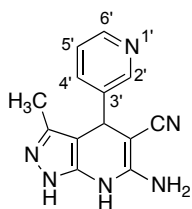


Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-nitrobenzaldehyde (453 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: Crystallization from EtOH.

Yield: 310 mg (35%). Yellow solid.

Data of 143:**Mp:** > 300 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₂N₆O₂: C 56.75, H 4.08, N 28.36; found: C 56.74, H 4.01, N 28.17.**IR** (neat) ν 3440 (N-H), 3358 (N-H), 3220 (N-H), 2165 (CN), 1625 (C=N), 1597 (NO₂), 1350 (NO₂) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.77 (s, 3H, C-3CH₃), 4.83 (s, 1H, CH-4), 5.58 (s, 2H, C-6NH₂), 7.41 (d, *J* = 7.8 Hz, 2H, CH-2' and CH-6'), 8.18 (d, *J* = 7.8 Hz, 2H, CH-3' and CH-5'), 9.02 (s, 1H, NH-7), 11.80 (s, 1H, NH-1) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 154.42 (C-6*), 153.66 (C-4'*), 146.16 (C-7a*), 146.01 (C-1'*), 135.14 (C-3), 128.52 (2xCHAR), 123.82 (2xCHAR), 122.92 (CN), 99.42 (C-3a), 54.18 (C-5), 37.52 (CH-4), 9.45 (C-3CH₃) ppm.**6-Amino-3-methyl-4-(pyridin-3-yl)-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (144)**

Prepared from 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 3-pyridinecarboxaldehyde (321 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

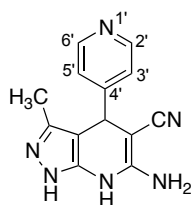
Purification: Crystallization from EtOH.

Yield: 370 mg (49%). White solid.

Data of 144:**Mp:** 290-291 °C (dec.)**Elemental analysis** calcd (%) for C₁₄H₁₂N₆: C 61.89, H 4.79, N 33.31; found: C 61.72, H 4.81, N 32.97.**IR** (neat) ν 3389 (N-H), 3325 (N-H), 3191 (N-H), 2158 (CN), 1625 (C=N) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.75 (s, 3H, C-3CH₃), 4.70 (s, 1H, CH-4), 5.52 (s, 2H, C-6NH₂), 7.32 (dd, *J* = 7.8, 4.7 Hz, 1H, CH-2'), 7.48 (dt, *J* = 7.8, 1.8 Hz, 1H, CH-6'), 8.40 (dd, *J* = 4.7, 1.8 Hz, 2H, CH-4' and CH-5'), 8.95 (s, 1H, NH-7), 11.76 (s, 1H, NH-1) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 153.56 (C-6), 148.50 (CHAR), 147.70 (CHAR),

146.17 (C-7a), 141.94 (C-3'), 134.97 (CHAR + C-3), 123.76 (CHAR), 123.05 (CN), 99.65 (C-3a), 54.50 (C-5), 35.18 (CH-4), 9.44 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(pyridin-4-yl)-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (145)



Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-pyridinecarboxaldehyde (321 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 85:15, v/v).

Yield: 395 mg (52%). White solid.

Data of 145:

Mp: > 300 °C (dec.)

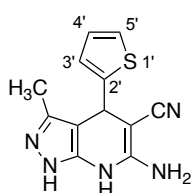
Elemental analysis calcd (%) for C₁₄H₁₂N₆: C 61.89, H 4.79, N 33.31; found: C 61.83, H 4.71, N 33.10.

IR (neat) ν 3456, (N-H), 3365 (N-H), 3189 (N-H), 2166 (CN), 1628 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.78 (s, 3H, C-3CH₃), 4.66 (s, 1H, CH-4), 5.55 (s, 2H, C-6NH₂), 7.13 (dd, *J* = 4.5, 1.5 Hz, 2H, CH-3' and CH-5'), 8.47 (dd, *J* = 4.5, 1.5 Hz, 2H, CH-2' and CH-6'), 8.97 (s, 1H, NH-7), 11.78 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 154.93 (C-6*), 153.75 (C-4'*), 149.73 (2xCHAR), 146.16 (C-7a), 135.08 (C-3), 122.92 (CN), 122.57 (2xCHAR), 99.08 (C-3a), 53.81 (C-5), 37.10 (CH-4), 9.45 (C-3CH₃) ppm.

6-Amino-3-methyl-4-(thiophen-2-yl)-4,7-dihydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (146)



Prepared from 3-methyl-1*H*-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 2-thiophenecarboxaldehyde (336 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 90:10, v/v).

Yield: 185 mg (24%). White solid.

Data of 146:

Mp: 275 -276 °C (dec.)

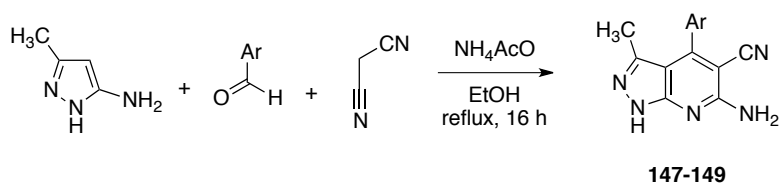
Elemental analysis calcd (%) for C₁₂H₁₁N₅S: C 56.01, H 4.31, N 27.22, S 12.46; found: C 56.06, H 4.24, N 27.04, S 12.35.

IR (neat) ν 3390 (N-H), 3330 (N-H), 3061 (N-H), 2164 (CN), 1627 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.89 (s, 3H, C-3CH₃), 5.00 (s, 1H, CH-4), 5.48 (s, 2H, C-6NH₂), 6.73-7.01 (m, 2H, CH-3' and CH-4'), 7.31 (dd, *J* = 4.5, 1.7 Hz, 1H, CH-5'), 8.93 (s, 1H, NH-7), 11.76 (s, 1H, NH-1) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 153.21 (C-6*), 152.56 (C-2'*), 145.70 (C-7a), 135.29 (C-3), 126.30 (CHAr), 124.39 (CHAr), 123.06 (CHAr + CN), 100.34 (C-3a), 55.61 (C-5), 32.83 (CH-4), 9.50 (C-3CH₃) ppm.

9.5.5 Formation of oxidized pyrazolo[3,4-*b*]pyridine derivatives **147-149**.

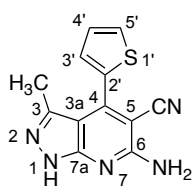


Cmpd.	Ar
147	2-thienyl
148	2-furyl
149	4-MeOC ₆ H ₄

The same procedure described above in section **x.5.4** was employed for the achievement of derivatives **147-149**.

Data of compounds **147-149**, and the experimental conditions employed for their synthesis are indicated below.

6-Amino-3-methyl-4-(thiophen-2-yl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (147)



Isolated from the reaction between 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 2-thiophenecarboxaldehyde (336 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 90:10, v/v).

Yield: 105 mg (14%). Pale yellow solid.

Data of 147:

Mp: 250 -251 °C (dec.)

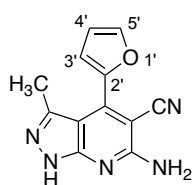
Elemental analysis calcd (%) for C₁₂H₉N₅S: C 56.45, H 3.55, N 27.43, S 12.56; found: C 56.31, H 3.58, N 27.19, S 12.32.

IR (neat) ν 3388 (N-H), 3290 (N-H), 3060 (N-H), 2200 (CN), 1604 (C=N) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.95 (s, 3H, C-3CH₃), 6.96 (s, 2H, NH₂), 7.27 (dd, *J* = 5.0, 3.6 Hz, 1H, CH-4'), 7.40 (dd, *J* = 3.5, 1.1 Hz, 1H, CH-3'), 7.89 (dd, *J* = 5.0, 1.2 Hz, 1H, CH-5'), 12.90 (s, 1H, NH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 158.96 (C-7a*), 153.19 (C-6*), 144.56 (C-4*), 142.18 (C-3*), 133.42 (C-2'), 129.91 (CHAr), 128.97 (CHAr), 127.58 (CHAr), 116.83 (CN), 106.72 (C-3a), 87.88 (C-5), 14.30 (C-3CH₃) ppm.

6-Amino-4-(furan-2-yl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (148)

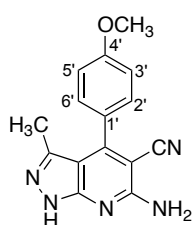


Isolated from the reaction between 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 2-furancarboxaldehyde (288 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 95:5, v/v).

Yield: 60 mg (8%). Yellow solid.

Data of 148:**Mp:** 293 -294 °C (dec.)**Elemental analysis** calcd (%) for C₁₂H₉N₅O: C 60.25, H 3.79, N 29.27; found: C 60.02, H 3.90, N 29.09.**IR** (neat) ν 3374 (N-H), 3301 (N-H), 2201 (CN), 1600 (C=N) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 2.23 (s, 3H, C-3CH₃), 6.82 (dd, *J* = 3.3, 1.7 Hz, 1H, CH-4'), 6.94 (s, 2H, NH₂), 7.21 (d, *J* = 3.4 Hz, 1H, CH-3'), 8.07 (s, 1H, CH-5'), 12.89 (s, 1H, NH) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 159.12 (C-7a*), 153.92 (C-6*), 146.40 (C-4*), 145.48 (CHAr), 142.27 (C-3*), 138.60 (C-2'), 117.47 (CN), 114.60 (CHAr), 112.38 (CHAr), 104.27 (C-3a), 83.94 (C-5), 15.34 (C-3CH₃) ppm.**6-amino-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (149)**

Isolated from the reaction between 3-methyl-1H-pyrazol-5-amine (291 mg, 3 mmol), malononitrile (198 mg, 3 mmol), 4-methoxybenzaldehyde (408 mg, 3 mmol) and ammonium acetate (231 mg, 3 mmol).

Reaction time: 16 h.

Purification: dichloromethane:methanol (from 100:0 to 95:5, v/v).

Yield: 190 mg (23%). White solid.

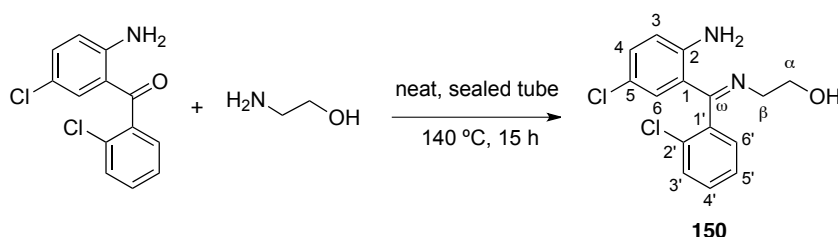
Data of 149:**Mp:** 267 -2698 °C (dec.)**Elemental analysis** calcd (%) for C₁₅H₁₃N₅O: C 64.51, H 4.69, N 25.07; found: C 64.34, H 4.77, N 24.81.**IR** (neat) ν 3368 (N-H), 3304 (N-H), 2203 (CN), 1602 (C=N) cm⁻¹.**¹H NMR** (250 MHz, DMSO-*d*₆) δ 1.86 (s, 3H, C-3CH₃), 3.85 (s, 3H, C-4'CH₃), 6.86 (s, 2H, NH₂), 7.11 (d, *J* = 8.1 Hz, 2H, CH-3' and CH-5'), 7.44 (d, *J* = 8.0 Hz, 2H, CH-2' and CH-6'), 12.81 (s, 1H, NH) ppm.**¹³C NMR** (63 MHz, DMSO-*d*₆) δ 160.09 (C-4'*), 159.05 (C-7a*), 153.28 (C-6*),

151.95 (C-4*), 142.30 (C-3), 130.28 (2xCHAR), 126.54 (C-1'), 117.30 (CN), 113.73 (2xCHAR), 106.29 (C-3a), 86.88 (C-5), 55.26 (C-4'OCH₃), 14.61 (C-3CH₃).

9.6 AZA-ANALOGUE OF CGP-37157 AND HYBRYDS WITH NIMODIPINE AND LIPOIC ACID

9.6.1 Synthesis of the aza-analogue of CGP-37157

9.6.1.1) Synthesis of 2-(((2-amino-5-chlorophenyl)(2-chlorophenyl)methylene)amino) ethanol (**150**)



In a sealed reaction tube were stirred 2-amino-2',5-dichlorobenzophenone (2.661 g, 10 mmol) and ethanolamine (7.5 mL, 125 mmol) at 140 °C for 20 hours. The mixture was cooled to room temperature and diluted with CH₂Cl₂. The CH₂Cl₂ solution was washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The crude product was purified using flash chromatography on silica gel (chloroform:ethyl acetate 5:1) to give **150** as a yellow solid (2.440 g, 79%).

Data of 150:

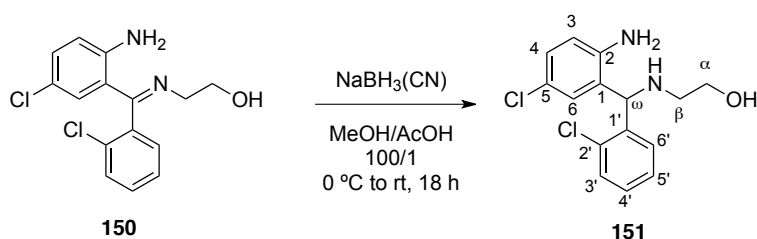
Mp 121-122 °C

IR (neat) ν 3212 (N-H), 1612 (C=N), 1054 (C-Cl) cm⁻¹.

¹H-NMR (250 MHz, CDCl₃) δ 3.29-3.49 (m, 2H, CH₂- β), 3.82-3.99 (m, 2H, CH₂- α), 6.57-6.81 (m, 2H, CH-3 and CH-6), 7.04-7.18 (m, 2H, CH-4 and CH-5'), 7.36-7.45 (m, 2H, CH-3' and CH-4'), 7.47-7.56 (m, 1H, CH-6') ppm.

¹³C NMR (63 MHz, CDCl₃) δ 170.00 (C=N), 148.05 (C-2), 135.79 (C-1'*), 131.77 (C-2'*), 131.58 (CHAr), 131.23 (CHAr), 130.55 (CHAr), 130.36 (CHAr), 129.25 (CHAr), 127.77 (CHAr), 120.56 (C-5*), 119.82 (C-1*), 118.31 (CHAr), 63.49 (CH₂- α), 56.13 (CH₂- β) ppm.

9.6.1.2 Synthesis of 2-(((2-amino-5-chlorophenyl)(2-chlorophenyl)methyl)amino) ethanol (**151**)



To a stirred solution of **150** (2.169 g 7.0 mmol) in 55 mL of MeOH/AcOH (100/1) at 0 °C was added NaBH₃(CN) (2.199 g, 35.0 mmol). The mixture was stirred for 18 hours and allowed to warm to room temperature. The reaction mixture was diluted with CH₂Cl₂, washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated to give pure **151** as a light yellow solid (2.178 g, 100%).

Data of 151:

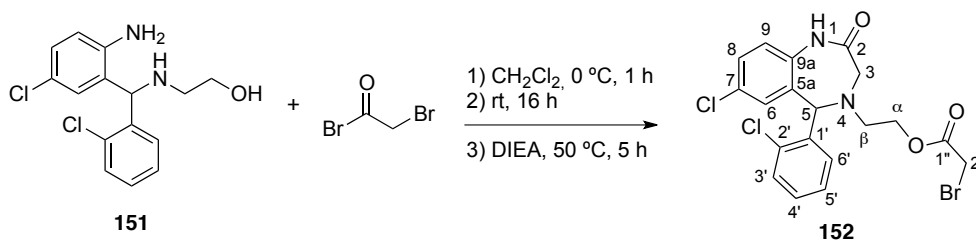
Mp: 99-100 °C

IR (neat) ν 3233 (N-H), 1034 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.58-2.98 (m, 2H, CH₂- β), 3.30 (bs, 4H, C-2NH₂, C- ω NH, CH₂- α OH), 3.72 (t, J = 5.1 Hz, 2H, CH₂- α), 5.26 (s, 1H, CH- ω), 6.60 (d, J = 8.4 Hz, 1H, CH-3), 6.98 (d, J = 2.3 Hz, 1H, CH-6), 7.04 (dd, J = 8.4, 2.3 Hz, 1H, CH-4), 7.19-7.32 (m, 2H, CH-4' and CH-5'), 7.34-7.46 (m, 2H, CH-3' and CH-6')

¹³C NMR (63 MHz, CDCl₃) δ 144.71 (C-2), 138.36 (C-1'*), 134.34 (C-2'*), 130.38 (CHAr), 129.68 (CHAr), 129.35 (CHAr), 128.84 (CHAr), 128.51 (CHAr), 127.73 (CHAr), 127.28 (C-5'*), 123.30 (C-1'*), 118.13 (CHAr), 62.14 (CH₂- α), 60.80 (CH- ω), 50.05 (CH₂- β) ppm.

9.6.1.3 Synthesis of 2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-4-yl)ethyl 2-bromoacetate (**152**)



To a stirred solution of **151** (2.116 g, 6.8 mmol) in 35 mL of CH₂Cl₂ at 0 °C was added bromoacetyl bromide (1.48 mL, 17.0 mmol), and stirring was continued at 0 °C for 1 hour and then at room temperature for 16 hours. DIEA (17.4 mL, 100.0 mmol) was added, and the mixture was stirred at 50 °C for 5 h. The mixture was cooled to room temperature and diluted with CH₂Cl₂; the CH₂Cl₂ solution was washed with water and dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated to dryness, and the crude product was purified using silica gel column chromatography (hexane:ethyl acetate 100/0 to 60/40) to give **152** as a white solid (1.894 g, 59%).

Data of 152:

Mp: 148-149 °C

Elemental analysis calcd (%) for C₁₉H₁₇BrCl₂N₂O₃: C 48.33, H 3.63, N 5.93; found: C 47.91, H 3.48, N 6.06.

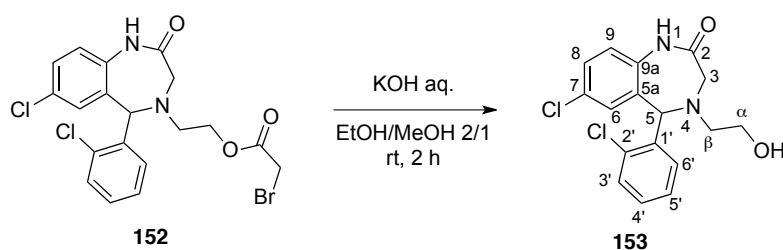
IR (neat) ν 2948 (N-H), 1733 (C-1''=O), 1664 (C-2=O), 1025 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.84-3.00 (m, 1H, one H of CH₂- β), 3.00-3.15 (m, 1H, one H of CH₂- β), 3.47 (d, J = 15.5 Hz, 1H, one H of CH₂-3), 3.58 (d, J = 15.5 Hz, 1H, one H of CH₂-3), 3.85 (s, 2H, CH₂-2), 4.14-4.29 (m, 1H, one H of CH₂- α), 4.33-4.49 (m, 1H, one H of CH₂- α), 5.31 (s, 1H, CH-5), 7.05 (d, J = 8.5 Hz, 1H, CH-9), 7.25 (dd, J = 8.3, 2.2 Hz, 1H, CH-8), 7.45-7.29 (m, 4H, CH-6, CH-4', CH-5' and CH-6'), 7.60 (dd, J = 7.3, 1.5 Hz, 1H, CH-3'), 9.12 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 172.39 (C-2=O), 167.45 (C-1''=O), 137.26 (C-1'*), 137.02 (C-5a*), 134.90 (C-9a), 132.78 (C-6'*), 131.14 (CHAR), 130.93 (C-7*),

130.68 (CHAr), 130.50 (CHAr), 129.93 (CHAr), 129.34 (CHAr), 127.52 (CHAr), 122.56 (CHAr), 65.45 (CH-5), 64.17 (CH₂-α), 53.23 (CH₂-β), 52.30 (CH₂-3), 26.18 (CH₂-2'') ppm.

9.6.1.4 Synthesis of 7-chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1H-benzo[e][1,4]diazepin-2(3H)-one (**153**)



To a solution of **152** (1.794 g, 3.8 mmol) in 15 mL of EtOH/MeOH (2:1) at room temperature was added a 2N KOH aqueous solution (0.853 g, 15.2 mmol in 7.6 mL of water) and the mixture was stirred at room temperature for 2h. At the end of the reaction (as monitored by TLC) 2N HCl aqueous solution was added to neutralize the reaction and then the crude was extracted with CH₂Cl₂ (3x30 mL); the organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to give pure **153** as a white solid (1.334 g, 100%).

Data of 153:

Mp: 157-158 °C

Elemental analysis calcd (%) for C₁₇H₁₆Cl₂N₂O₂: C 58.13, H 4.59, N 7.98; found: C 58.05, H 4.60, N 8.05.

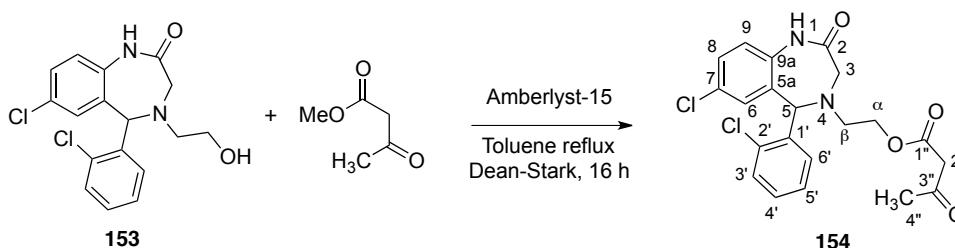
IR (neat) ν 3390 (O-H), 2927 (N-H), 1672 (C=O), 1038 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 2.47-2.53 (m, 1H, OH), 2.80-3.02 (m, 2H, CH₂-β), 3.53 (s, 2H, CH₂-3), 3.62-3.83 (m, 2H, CH₂-α), 5.40 (s, 1H, CH-5), 6.82 (d, *J* = 2.2 Hz, 1H, CH-6), 7.00 (d, *J* = 8.5 Hz, 1H, CH-8), 7.18-7.36 (m, 4H, CH-9, CH-4', CH-5' and CH-6'), 7.45 (dd, *J* = 5.9, 3.4 Hz, 1H, CH-3'), 8.61 (s, 1H, CONH) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 173.87 (CONH), 137.37 (C-1'*), 136.21 (C-5a*), 134.67 (C-9a), 131.69 (C-2'*), 131.40 (CHAR), 131.01 (CHAR), 130.93 (CHAR), 130.33 (C-7*), 130.02 (CHAR), 129.43 (CHAR), 127.66 (CHAR), 122.33 (CHAR), 66.09 (CH-5), 59.13 (CH_2 - α), 55.40 (CH_2 - β), 52.82 (CH_2 -3) ppm.

9.6.2 Synthesis of the hybrid 153-nimodipine *via* a multicomponent process

9.6.2.1 Synthesis of 2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4] diazepin-4-yl)ethyl 3-oxobutanoate (**154**)



A mixture of methyl acetoacetate (0.332 g, 2.85 mmol), **153** (1.053 g, 3 mmol) and Amberlyst-15 (10% by weight of β -ketoester, 0.033g) in toluene (10 mL) was heated to 110 °C in a flask provided with a distillation condenser (Dean-Stark) to remove MeOH. After completion of the reaction (as monitored by TLC), the catalyst was filtered off and the filtrate concentrated and purified using silica gel column chromatography (dichlorometane:ethyl acetate from 100:0 to 75:25) to give **154** as a light brown solid (0.640 g, 49%).

Data of 154:

Mp 68-69 °C

Elemental analysis calcd (%) for $\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_4$: C 57.94, H 4.63, N 6.44; found: C 57.82, H 4.78, N 6.33.

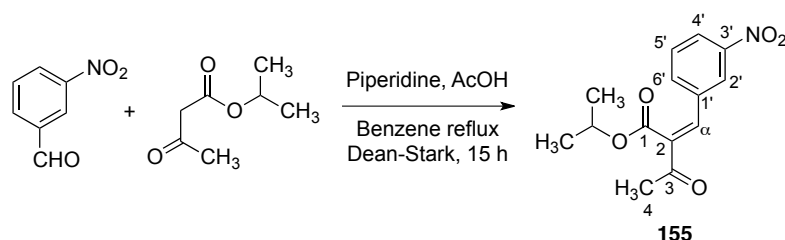
IR (neat) ν 2918 (N-H), 1712 (C-3''=O), 1665 (C-2=O), 1034 (C-Cl) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 2.27 (s, 3H, CH_3 -4''), 2.80-2.96 (m, 1H, one H of

CH₂-β), 2.97-3.11 (m, 1H, one H of CH₂-β), 3.36-3.67 (m, 4H, CH₂-3 and CH₂-2''), 4.12-4.28 (m, 1H, one H of CH₂-α), 4.28-4.42 (m, 1H, one H of CH₂-α), 5.29 (s, 1H, CH-5), 6.61 (d, *J* = 2.3 Hz, 1H, CH-6), 6.98 (d, *J* = 8.5 Hz, 1H, CH-8), 7.18-7.47 (m, 4H, CH-9, CH-4', CH-5' and CH-6'), 7.54 (dd, *J* = 7.2, 2.2 Hz, 1H, CH-3'), 8.38 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 200.88 (C-3''=O), 172.62 (C-2=O), 167.38 (C-1''=O), 137.35 (C-1'*), 136.93 (C-5a*), 134.86 (C-9a), 132.80 (C-2'*), 131.10 (CHAR), 130.86 (C-7*), 130.67 (CHAR), 130.50 (CHAR), 129.89 (CHAR), 129.29 (CHAR), 127.49 (CHAR), 122.55 (CHAR), 65.42 (CH-5), 63.33 (CH₂-α), 53.24 (CH₂-β), 52.34 (CH₂-3), 50.35 (CH₂-2''), 30.66 (CH₃-4'') ppm.

9.6.2.2 Synthesis of isopropyl 2-(3-nitrobenzylidene)-3-oxobutanoate (**155**)



A solution of 3-nitrobenzaldehyde (1.511 g, 10 mmol), isopropyl acetoacetate (1.442 g, 10 mmol), 0.5 mL of glacial AcOH, 0.1 mL of piperidine and benzene (5 mL) was refluxed for 15 hours, using a Dean-Stark trap to remove water as it was generated. The dark yellow solution was cooled to room temperature and solidification occurred. Filtration followed by washing with a mixture of petroleum ether and ethyl ether afforded **155** as a yellow solid (2.240 g, 81%).

Data of 155:

Mp: 94-95 °C

IR (neat) ν 1715 (C-3=O), 1662 (C-1=O), 1530 (NO₂), 1349 (NO₂), 1096 (C-Cl) cm⁻¹.

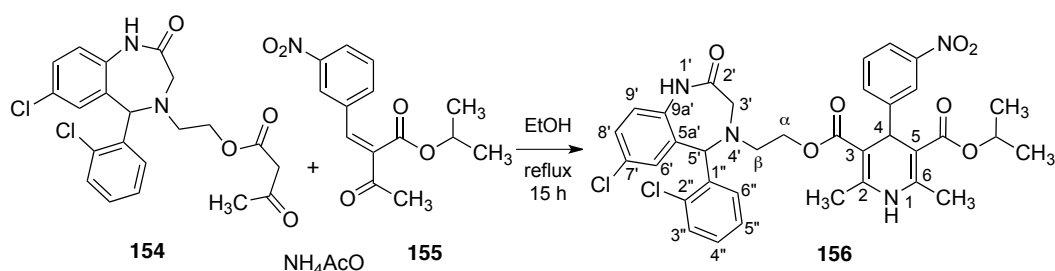
¹H NMR (250 MHz, CDCl₃) δ 1.30 (d, *J* = 6.3 Hz, 6H, OCH(CH₃)₂), 2.44 (s, 3H, CH₃-4), 5.28 (hept, *J* = 6.3 Hz, 1H, OCH(CH₃)₂), 7.54-7.64 (m, 2H, CH-5' and

CH-6'), 7.77 (d, $J = 7.8$ Hz, 1H, CH-4'), 8.26 (ddd, $J = 8.2, 2.1, 0.9$ Hz, 1H, CH-2'), 8.35 (t, $J = 1.7$ Hz, 1H, CH- α) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 194.33 (C-3=O), 166.88 (C-1=O), 148.82 (C-3'), 137.95 (CH- α), 137.50 (C-1'), 135.65 (CHAr), 135.25 (C-2), 130.28 (CHAr), 125.21 (CHAr), 124.13 (CHAr), 70.70 ($\text{OCH}(\text{CH}_3)_2$), 27.34 (CH_3 -4), 21.93 ($\text{OCH}(\text{CH}_3)_2$) ppm.

These data are consistent with those described in the literature.²⁹⁴

9.6.2.3 Synthesis of 3-(2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-4-yl)ethyl) 5-isopropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**156**)



A solution of **154** (192 mg, 0.44 mmol) and ammonium acetate (68 mg, 0.88 mmol) in ethanol (2 mL) was stirred for 30 min at room temperature; **155** (122 mg, 0.44 mmol) was then added and the mixture was heated under reflux and stirred for 15 hours. After this time, the mixture was diluted with CH_2Cl_2 (20 mL), washed with water followed by brine, and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure left a crude that was purified using silica gel column chromatography (dichloromethane:ethyl acetate from 100:0 to 90:10) to give **156** as a light yellow solid (218 mg, 72%).

Data of **156**:

Mp: 149-150°C

²⁹⁴ Kobayashy, T.; Inoue, T.; Nishino, S.; Fujihara, Y.; Oizumi, K.; Kimura, T. *Chem. Pharm. Bull.* **1995**, *43*, 797-817

Elemental analysis calcd (%) for $C_{35}H_{34}Cl_2N_4O_7$: C 60.61, H 4.94, N 8.08; found: C 60.21, H 4.94, N 8.10.

IR (neat) ν 1675 (C=O), 1526 (NO₂), 1348 (NO₂), 1101 (C-Cl) cm^{-1} .

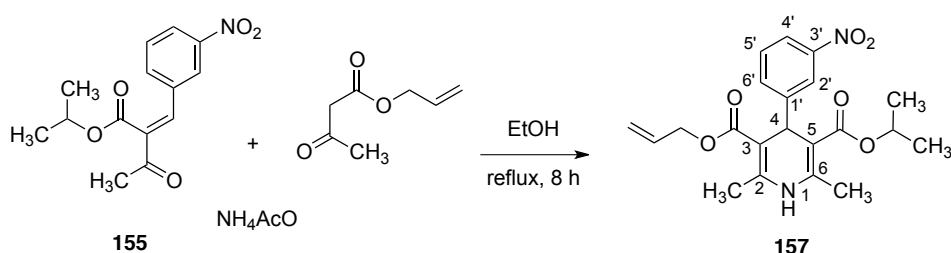
¹H NMR (250 MHz, CDCl₃) 2 isomers δ 1.06 (d, $J = 6.2$ Hz, 3H, OCH(CH₃)₂ one isomer), 1.07 (d, $J = 6.2$ Hz, 3H, OCH(CH₃)₂ one isomer), 1.22 (d, $J = 6.0$ Hz, 3H, OCH(CH₃)₂ one isomer), 1.24 (d, $J = 5.8$ Hz, 3H, OCH(CH₃)₂ one isomer), 2.32 (s, 6H, C-6CH₃ two isomers), 2.37 (s, 3H, C-2CH₃ one isomer) 2.38 (s, 3H, C-2CH₃ one isomer), 2.77-3.04 (m, 4H, CH₂- β , two isomers), 3.35-3.56 (m, 4H, CH₂-3' two isomers), 4.06-4.14 (m, 2H, CH₂- α one isomer), 4.23-4.39 (m, 2H, CH₂- α one isomer), 4.89-5.00 (m, 2H, OCH(CH₃)₂ two isomers), 5.06 (s, 1H, CH-4 one isomer), 5.12 (s, 1H, CH-4 one isomer), 5.16 (s, 1H, CH-5' one isomer), 5.28 (s, 1H, CH-5' one isomer), 5.73 (bs, 2H, NH-1 two isomers), 6.43 (d, $J = 2.0$ Hz, 1H, NH-1' one isomer), 6.62 (d, $J = 2.1$ Hz, 1H, NH-1' one isomer), 6.94 (d, $J = 5.5$ Hz, 1H, ArH one isomer), 6.98 (d, $J = 5.5$ Hz, 1H, ArH one isomer), 7.16-7.39 (m, 11H, ArH two isomers), 7.52-7.55 (m, 1H, ArH one isomer), 7.64 (d, $J = 7.5$ Hz, 2H, ArH two isomers), 7.92-7.97 (m, 2H, ArH two isomers), 8.23-8.04 (m, 4H, ArH two isomers) ppm.

¹³C NMR (63 MHz, CDCl₃) 2 isomers δ 172.35 (C-2'=O one isomer), 171.83 (C-2'=O one isomer), 167.26 (C-5C=O* one isomer), 167.22 (C-5C=O* one isomer), 166.96 (C-3C=O* one isomer), 166.91 (C-3C=O* one isomer), 150.28 (C-6* one isomer), 150.22 (C-6* one isomer), 148.53 (C-2* one isomer), 148.47 (C-2* one isomer), 145.64 (CNO₂* one isomer), 145.45 (CNO₂* one isomer), 144.84 (CAr* one isomer), 144.78 (CAr* one isomer), 137.46 (C-1''* one isomer), 137.30 (C-1''* one isomer), 137.02 (C-5a* one isomer), 136.82 (C-5a* one isomer), 135.01 (CHAR one isomers), 134.95 (CHAR one isomer), 134.82 (C-9a* one isomer), 134.81 (C-9a* one isomer), 133.01 (C-2'''* one isomer), 132.75 (C-2'''* one isomer), 131.05 (CHAR one isomer), 130.96 (C-7 one isomer), 130.77 (CHAR + C-7 one isomer), 130.64 (CHAR one isomer), 130.55 (CHAR one isomer), 130.50 (CHAR one isomer), 130.33 (CHAR one isomer), 129.75 (CHAR one isomer), 129.67 (CHAR one isomer), 129.22 (CHAR one isomer), 128.99 (CHAR one isomer), 128.94 (CHAR one isomer), 127.50 (CHAR one isomer), 127.30 (CHAR one isomer), 123.59 (CHAR one isomer), 123.53 (CHAR one isomer), 122.46 (CHAR two isomers), 121.76 (CHAR two isomers), 104.24 (C-5 two isomers), 103.47 (C-3 one isomer), 103.29 (C-3 one isomer), 67.84

(OCH(CH₃)₂ two isomers), 65.35 (CH-5' one isomer), 65.07 (CH-5' one isomer), 61.74 (CH₂-α one isomer), 61.15 (CH₂-α one isomer), 53.30 (CH₂-β one isomer), 52.68 (CH₂-β one isomer), 52.48 (CH₂-3' two isomers), 40.41 (CH-4 one isomer), 40.33 (CH-4 one isomer), 22.52 (C-6CH₃ two isomers OCH(CH₃)₂ one isomer), 22.19 (C-2CH₃ one isomer), 20.37 (OCH(CH₃)₂ one isomer), 20.21 (OCH(CH₃)₂ one isomer), 20.04 (OCH(CH₃)₂ two isomer) ppm.

9.6.3 Linear synthesis of the hybrid 153-nimodipine

9.6.3.1 Synthesis of 3-allyl 5-isopropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**157**)



A solution of allyl acetoacetate (0.710 g, 5.0 mmol) and ammonium acetate (0.578 g, 7.5 mmol) in ethanol (5 mL) was stirred for 30 min at room temperature; **155** (1.385 g, 5.0 mmol) was then added and the mixture was heated under reflux and stirred for 8 hours. After completion of the reaction (checked by TLC), the mixture was diluted with CH₂Cl₂ (20 mL), washed with water followed by brine, and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure left a crude that was purified using silica gel column chromatography (*n*-hexane:ethyl acetate from 100:0 to 70:30) to give **157** as a yellow solid (1.420 g, 71%).

Data of 157:

Mp: 89-90 °C

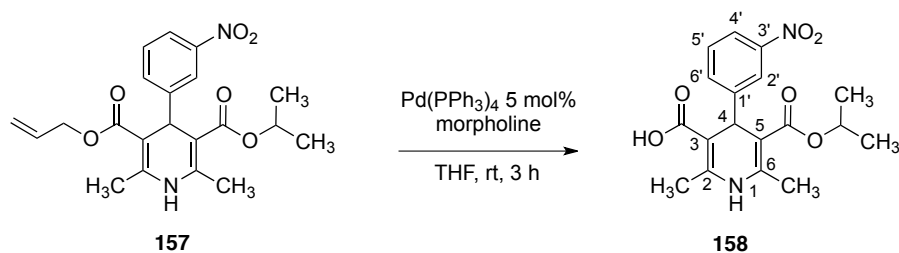
Elemental analysis calcd (%) for C₂₁H₂₄N₂O₆: C 62.99, H 6.04, N 7.00; found: C 63.18, H 6.10, N 6.81.

IR (neat) ν 1692 (C=O), 1651 (C=O), 1623 (C=CH₂), 1522 (NO₂), 1346 (NO₂) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 1.09 (d, J = 6.2 Hz, 3H, OCH(CH₃)₂), 1.26 (d, J = 6.2 Hz, 3H, OCH(CH₃)₂), 2.36 (s, 3H, C-2CH₃*), 2.37 (s, 3H, C-6CH₃*), 4.45-4.68 (m, 2H, OCH₂CH=CH₂), 4.88-5.01 (m, 1H, OCH(CH₃)₂), 5.03-5.24 (m, 3H, OCH₂CH=CH₂, CH-4, NH), 5.67-5.97 (m, 2H, OCH₂CH=CH₂), 7.36 (t, J = 7.8 Hz, 1H, CH-5'), 7.64 (dt, J = 7.8, 1.0 Hz, 1H, CH-6'), 8.00 (ddd, J = 7.8, 2.0, 1.0 Hz, 1H, CH-4'), 8.12 (t, J = 2.0 Hz, 1H, CH-2') ppm.

¹³C NMR (63 MHz, CDCl₃) two isomers δ 167.27, 167.18 (C-5C=O*), 167.14, 167.04, (C-3C=O*), 150.58, 150.35 (C-2*), 148.48, 148.36 (C-6*), 145.88, 145.83 (C-1'), 145.10, 145.06 (C-3'), 135.12, 135.03 (CHAr), 132.85, 132.81 (OCH₂CH=CH₂), 129.00, 128.92 (CHAr), 123.73, 123.59 (CHAr), 121.72, 121.63 (CHAr), 118.34, 118.18 (OCH₂CH=CH₂), 104.06, 103.88 (C-3*), 103.38, 103.21 (C-5*), 67.79, 67.74 (OCH(CH₃)₂), 65.20, 65.13 (OCH₂CH=CH₂), 40.55, 40.36 (CH-4), 22.50 (C-2CH₃*), 22.20 (C-6CH₃*), 20.02, 19.96, 19.90, 19.84 (OCH(CH₃)₂) ppm.

9.6.3.2 Synthesis of 5-(isopropoxycarbonyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (**158**)



A solution of **157** (1.128 g, 2.82 mmol) and morpholine (2.46 mL, 28.2 mmol) in THF (15 mL) was degassed under Ar for 10 min. Pd(PPh₃)₄ (0.163 g, 0.14 mmol) was added and the solution was stirred at room temperature for 3 hours. The reaction was diluted with AcOEt, washed with 1M HCl and brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude was purified using silica gel column chromatography (dichloromethane:ethyl acetate

from 100:0 to 85:15) to give **158** as a light yellow solid (0.630 g, 62%).

Data of 158:

Mp: 179-180 °C

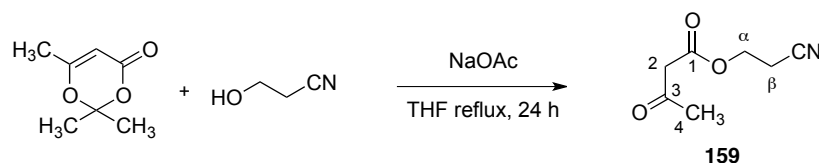
IR (neat) ν 3358 (N-H), 1650 (C=O), 1531 (NO₂), 1347 (NO₂) cm⁻¹.

¹H NMR (250 MHz, DMSO-*d*₆) δ 1.03 (d, *J* = 6.2 Hz, 3H, OCH(CH₃)₂), 1.19 (d, *J* = 6.2 Hz, 3H, OCH(CH₃)₂), 2.26 (s, 3H, C-2CH₃*), 2.27 (s, 3H, C-6CH₃*), 4.82 (hept, *J* = 6.2 Hz, 1H, OCH(CH₃)₂), 4.94 (s, 1H, CH-4), 7.50-7.64 (m, 2H, CH-5' and CH-6'), 7.93– 8.05 (m, 2H, CH-2' and CH-4'), 8.89 (s, 1H, NH), 11.81 (bs, 1H, COOH) ppm.

¹³C NMR (63 MHz, DMSO-*d*₆) δ 168.79 (C-3C=O), 166.42 (C-5C=O), 150.75 (C-2*), 147.72 (C-6*), 146.75 (C-1'*), 146.16 (C-3'*), 134.63 (CHAr), 129.93 (CHAr), 122.26 (CHAr), 121.35 (CHAr), 101.96 (C-3*), 101.41 (C-5*), 66.59 (OCH(CH₃)₂), 39.70 (CH-4), 22.23 (C-2CH₃*), 21.89 (C-6CH₃*), 18.58 (OCH(CH₃)₂) ppm.

These data are consistent with those described in the literature.²⁹⁵

9.6.3.3 Synthesis of 2-cyanoethyl 3-oxobutanoate (**159**)



A mixture of 3-hydroxypropanenitrile (1.065 g, 15 mmol), 2,2,6-trimethyl-4H-1,3-dioxin-4-one (2.772 g, 19.5 mmol) and anhydrous NaOAc (1.230 g, 15 mmol) in THF (10 mL) is heated under reflux for 24 hours. After completion of the reaction, the mixture is cooled, diluted with Et₂O and the solution is washed with water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude was purified using silica gel column chromatography (petroleum ether:ethyl acetate from 95:5 to 50:50) to give **159** as a colorless liquid

²⁹⁵ Ogawa, T.; Hatayama, K.; Maeda, H.; Kita, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1579-1589.

(1.191 g, 51%).

Data of 159:

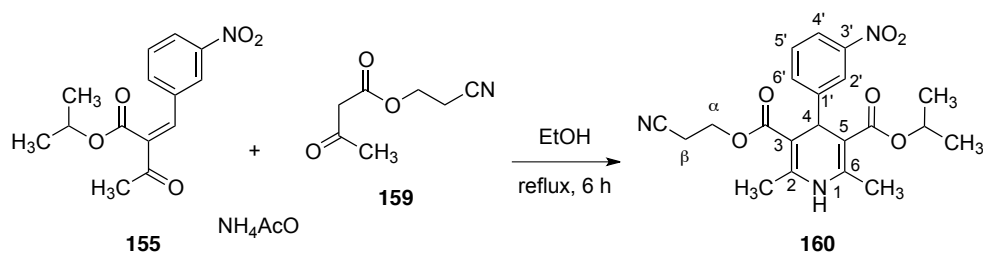
IR (neat) ν 2249 (CN), 1664 (C=O) cm^{-1} .

^1H NMR (250 MHz, CDCl_3) δ 1.95 (d, $J = 0.4$ Hz, 3H enol, CH_3 -4), 2.25 (s, 3H keto, CH_3 -4), 2.72 (t, $J = 6.3$ Hz, 2H keto+enol, CH_2 - β), 3.51 (s, 2H keto, CH_2 -2), 4.32 (t, $J = 6.3$ Hz, 2H keto+enol, CH_2 - α), 5.02 (d, $J = 0.6$ Hz, 1H enol, C=CH), 11.76 (s, 1H enol, C(OH)) ppm.

^{13}C NMR (63 MHz, CDCl_3) δ 200.41 (keto, C-3=O), 177.38 (enol, C(OH)), 172.11 (enol, C-1=O), 166.95 (keto, C-1=O), 117.13 (keto+enol, CN), 89.46 (enol, C=CH), 59.80 (keto, CH_2 - α), 58.58 (enol, CH_2 - α), 49.96 (keto, CH_2 -3), 30.68 (keto+enol, CH_3 -4) 18.48 (enol, CH_2 - β), 18.31 (keto, CH_2 - β) ppm.

These data are consistent with those described in the literature.²⁹⁶

9.6.3.4 Synthesis of 3-(2-cyanoethyl) 5-isopropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (160)



A solution of 2-cyanoethyl 3-oxobutanoate (1.085 g, 7.0 mmol) and ammonium acetate (0.809 g, 10.5 mmol) in ethanol (7.5 mL) was stirred for 30 min at room temperature; **155** (1.939 g, 7.0 mmol) was then added and the mixture was heated under reflux and stirred for 6 hours. After completion of the reaction (checked by TLC), the mixture was diluted with CH_2Cl_2 (20 mL), washed with water followed by brine, dried over anhydrous magnesium sulfate and the solvent was finally

²⁹⁶ Dollé, F.; Hinnen, F.; Valette, H.; Fuseau, C.; Duval, R.; Pégliion, J.-L.; Crouzel, C. *Bioorg. Med. Chem.* **1997**, *5*, 749-764.

evaporated under reduced pressure. The crude was purified using silica gel column chromatography (*n*-hexane:ethyl acetate from 100:0 to 85:15) to give **160** as a yellow solid (1.450 g, 50%).

Data of 160:

Mp: 129-130 °C;

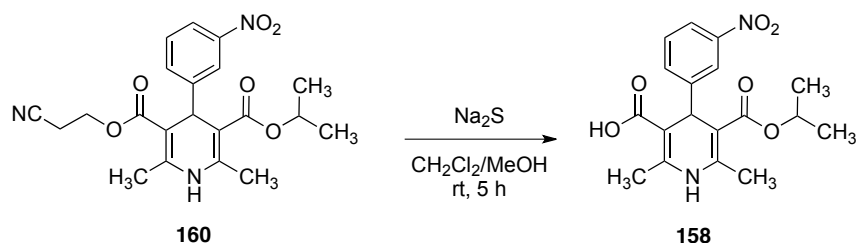
IR (neat) ν 3366 (N-H), 2279 (CN), 1691 (C=O), 1523 (NO₂), 1347 (NO₂) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 1.11 (d, *J* = 6.2 Hz, 3H, OCH(CH₃)₂), 1.26 (d, *J* = 6.2 Hz, 3H, OCH(CH₃)₂), 2.37 (s, 3H, C-2CH₃*), 2.39 (s, 3H, C-6CH₃*), 2.65 (t, *J* = 6.1 Hz, 2H, CH₂- β), 4.25 (td, *J* = 6.2, 2.2 Hz, 2H, CH₂- α), 4.95 (hept, *J* = 6.3 Hz, 1H, OCH(CH₃)₂), 5.08 (s, 1H, CH-4), 5.82 (s, 1H, NH), 7.40 (t, *J* = 8.0 Hz, 1H, CH-5'), 7.67 (dd, *J* = 8.0, 1.1 Hz, 1H, CH-6'), 8.01 (ddd, *J* = 8.0, 2.2, 1.1 Hz, 1H, CH-4'), 8.12 (d, *J* = 2.2 Hz, 1H, CH-2')

¹³C NMR (63 MHz, CDCl₃) δ 166.79 (C-3C=O*), 166.73 (C-5C=O*), 150.07 (C-2*), 148.61 (C-6*), 147.09 (C-1'*), 144.64 (C-3'*), 135.00 (CHAr), 129.18 (CHAr), 123.49 (CHAr), 121.90 (CHAr), 117.49 (CN), 104.59 (C-3*), 102.27 (C-5*), 67.95 (OCH(CH₃)₂), 58.76 (CH₂- α), 40.27 (CH-4), 22.50 (C-2CH₃*), 22.18 (C-6CH₃*), 20.38 and 19.78 (OCH(CH₃)₂), 18.55 (CH₂- β) ppm.

These data are consistent with those described in the literature.²⁹⁷

9.6.3.5 Synthesis of 5-(isopropoxycarbonyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (**158**)



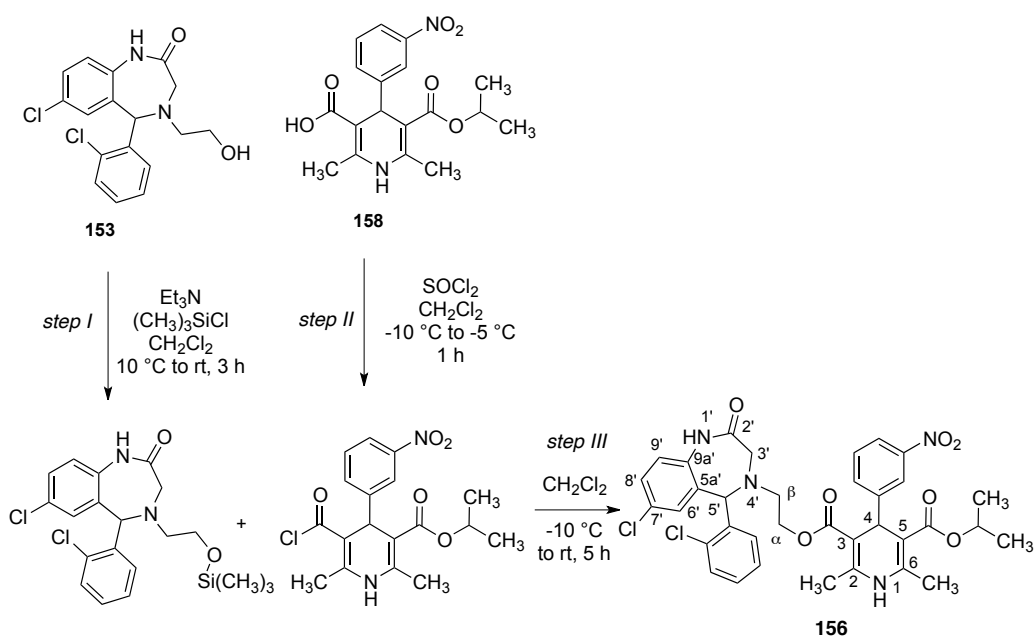
Sodium sulfide hydrate (0.195 g, 2.5 mmol) was added to a CH₂Cl₂/MeOH (8/4

²⁹⁷ Ogawa, T.; Nakazato, A.; Tsuchida, K.; Hatayama, K. *Chem. Pharm. Bull.* **1993**, *41*, 108-116.

mL) solution of **160** (1.033 g, 2.5 mmol) and the reaction was stirred at room temperature for 5 hours. The reaction mixture was diluted with water and extracted with CH_2Cl_2 . The aqueous layer was acidified with 1N HCl and the formed precipitate was filtered off and recrystallized from EtOH to give **158** as a light yellow solid (0.638 g, 71%).

Data of **158** are identical to those described in Section 9.6.3.2

9.6.3.6 Synthesis of 3-(2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethyl) 5-isopropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**156**)



Step I:

Compound **153** (173 mg, 0.49 mmol) was dissolved in anhydrous CH_2Cl_2 (1 mL) at room temperature; triethylamine (0.10 mL, 0.73 mmol) was added and the contents were cooled to 10 °C under Ar atmosphere. TMSiCl (74 mg, 0.68 mmol) was slowly added to the reaction mass, while maintaining the temperature between 10-15 °C. The mass temperature is raised to room temperature and stirred

for 3 hours at this temperature to give the silyl derivative.

Step II:

Compound **158** (169 mg, 0.47 mmol) was suspended in anhydrous CH₂Cl₂ (3 mL); anhydrous DMF (0.5 mL) was added and then the mixture was cooled to -10 °C under Ar atmosphere. Thionyl chloride (0.05 mL, 0.70 mL) was slowly added to the reaction mass at -10 °C and maintained at the same temperature for 1 hour to obtain the acetyl chloride derivative.

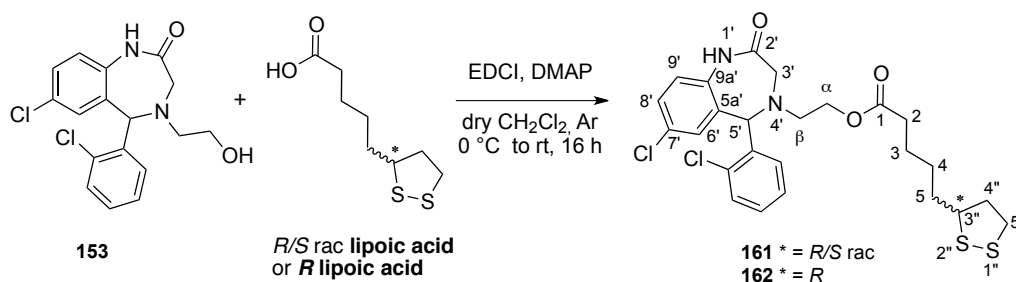
Step III:

The reaction mass obtained in the step I was slowly added to the solution obtained in step II. The mass temperature was slowly raised to room temperature and stirring continued for 5 hours at the same temperature. Then the reaction mixture was cooled to 5 °C, water was added and stirring continued for 15 min. The resulting organic layer was washed with 20% w/w NaCl solution, dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude was purified using silica gel column chromatography (*n*-hexane:ethyl acetate from 90:10 to 40:60) to give **156** as a light yellow solid (176 mg, 54%).

Data of **156** were identical to those described above in Section **9.6.2.3**

9.6.4 Synthesis of the hybrid 153-Lipoic acid

9.6.4.1 Synthesis of 2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-4-yl)ethyl 5-(1,2-dithiolan-3-yl)pentanoate (**161** and **162**)



Under argon flow, **153** (70 mg, 0.2 mmol) was dissolved in anhydrous dichloromethane (3 mL), and α -lipoic acid (racemic mixture or (*R*)-(+)-enantiomer) (50 mg, 0.24 mmol) and DMAP (73 mg, 0.6 mmol) were then added at room temperature. After cooling at 0 °C, a solution of EDCI (46 mg, 0.24 mmol) in anhydrous dichloromethane (2 mL) was added dropwise to the solution; the reaction was allowed to warm slowly to room temperature and kept at this condition for 16 hours. At the end of reaction, as monitored by TLC, the reaction mixture was diluted with dichloromethane, washed with water followed by brine solution, dried over Na₂SO₄ and evaporated under reduced pressure. The crude was purified using silica gel column chromatography (*n*-hexane:ethyl acetate from 100:0 to 60:40) to give **161** or **162** as pale yellow viscous oils (87 mg, 74%).

Data of **161**:

Elemental analysis calcd (%) for C₂₅H₂₈Cl₂N₂O₃S₂: C 55.65, H 5.23, N 5.19, S 11.89; found: C 55.47, H 5.08, N 5.16, S 11.75.

IR (neat) ν 2923 (N-H), 1729 (C-1=O), 1664 (C-2'=O), 1070 (C-Cl) cm⁻¹.

¹H NMR (250 MHz, CDCl₃) δ 1.38-1.54 (m, 2H, CH₂-4), 1.56-1.77 (m, 4H, CH₂-3 and CH₂-5), 1.83-1.96 (m, 1H, one H of CH₂-4''), 2.32 (t, *J* = 7.2 Hz, 2H, CH₂-3)

2), 2.38-2.51 (m, 1H, one H of CH₂-4''), 2.78-2.93 (m, 1H, CH-3''), 2.97-3.26 (m, 3H, one H of CH₂-β and CH₂-5''), 3.39-3.64 (m, 3H, CH₂-3' and one H of CH₂-β), 4.07-4.22 (m, 1H, one H of CH₂-α), 4.22-4.35 (m, 1H, one H of CH₂-α), 5.30 (s, 1H, CH-5'), 6.61 (d, *J* = 2.3 Hz, 1H, ArH), 7.02 (d, *J* = 8.5 Hz, 1H, ArH), 7.24 (dd, *J* = 8.1, 2.0 Hz, 1H, ArH), 7.29-7.47 (m, 3H, ArH), 7.50-7.62 (m, 1H, ArH), 8.83 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 173.70 (CONH), 172.62 (C-1=O), 137.44 (CAr*), 137.03 (C-5a'*), 134.89 (C-9a'*), 132.89 (CCl*), 131.13 (CHAr), 130.81 (C-7'*), 130.66 (CHAr), 130.47 (CHAr), 129.85 (CHAr), 129.26 (CHAr), 127.42 (CHAr), 122.52 (CHAr), 65.40 (CH-5'), 62.38 (CH₂-α), 56.74 (CH-3''), 53.33 (CH₂-β), 52.51 (CH₂-3'), 40.65 (CH₂-4''), 38.91 (CH₂-5''), 35.02 (CH₂-5), 34.42 (CH₂-2), 29.22 (CH₂-3), 24.99 (CH₂-4) ppm.

Data of 162:

Elemental analysis calcd (%) for C₂₅H₂₈Cl₂N₂O₃S₂: C 55.65, H 5.23, N 5.19, S 11.89; found: C 55.55, H 5.24, N 5.11, S 11.92.

IR (neat) ν 2920 (N-H), 1728 (C-1=O), 1664 (C-2'=O), 1070 (C-Cl) cm⁻¹.

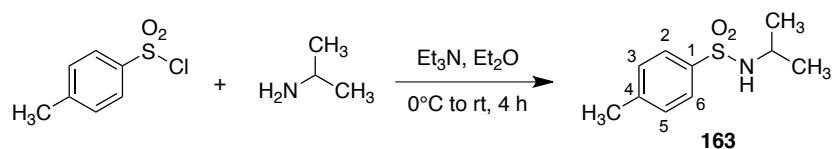
¹H NMR (250 MHz, CDCl₃) δ 1.38-1.53 (m, 2H, CH₂-4), 1.57-1.78 (m, 4H, CH₂-3 and CH₂-5), 1.80-1.98 (m, 1H, one H of CH₂-4''), 2.32 (t, *J* = 7.2 Hz, 2H, CH₂-2), 2.38-2.51 (m, 1H, one H of CH₂-4''), 2.77-2.96 (m, 1H, CH-3''), 2.96-3.28 (m, 3H, one H of CH₂-β, CH₂-5''), 3.40-3.66 (m, 3H, CH₂-3', one H of CH₂-β), 4.07-4.22 (m, 1H, one H of CH₂-α), 4.22-4.39 (m, 1H, one H of CH₂-α), 5.30 (s, 1H, CH-5'), 6.61 (d, *J* = 2.3 Hz, 1H, ArH), 7.01 (d, *J* = 8.5 Hz, 1H, ArH), 7.24 (dd, *J* = 8.0, 1.9 Hz, 1H, ArH), 7.29-7.46 (m, 3H, ArH), 7.53-7.63 (m, 1H, ArH), 8.66 (s, 1H, CONH) ppm.

¹³C NMR (63 MHz, CDCl₃) δ 173.69 (CONH), 172.20 (C-1=O), 137.41 (CAr*), 136.95 (C-5a'*), 134.89 (C-9a'*), 132.96 (CCl*), 131.10 (CHAr), 130.89 (C-7'*), 130.66 (CHAr), 130.50 (CHAr), 129.86 (CHAr), 129.26 (CHAr), 127.43 (CHAr), 122.46 (CHAr), 65.37 (CH-5'), 62.38 (CH₂-α), 56.75 (CH-3''), 53.27 (CH₂-β), 52.51 (CH₂-3'), 40.64 (CH₂-4''), 38.91 (CH₂-5''), 35.02 (CH₂-5), 34.41 (CH₂-2), 29.22 (CH₂-3), 24.98 (CH₂-4) ppm.

9.7 SULFONAMIDE AS DIRECTING GROUP FOR THE C-H BOND FUNCTIONALIZATION

9.7.1 Synthesis of the starting benzenesulfonamides.

9.7.1.1 Synthesis of *N*-isopropyl-4-methylbenzenesulfonamide (**163**)



p-Toluenesulfonyl chloride (1.906 g, 10 mmol) was dissolved in Et₂O (20 mL) and treated with isopropylamine (0.90 mL, 11 mmol) and Et₃N (1.53 mL, 11 mmol) at 0 °C, and the mixture was stirred at room temperature for 4 h. After removal of the solvent *in vacuo*, the residue was purified by silica gel packed flash column chromatography (eluent: EtOAc:hexane from 25:75 to 50:50) to afford **163** as a colorless solid (1.795 g, 84%).

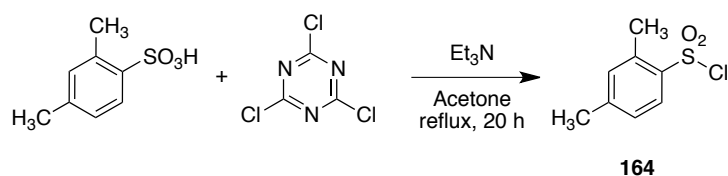
Data of **163**:

¹H NMR (300 MHz, CDCl₃) δ 1.05 (d, *J* = 6.5 Hz, 6H, CH(CH₃)₂), 2.40 (s, 3H, ArCH₃), 3.33-3.51 (m, 1H, CH(CH₃)₂), 4.57 (d, *J* = 7.4 Hz, 1H, SO₂NH), 7.27 (d, *J* = 7.9 Hz, 2H, CH-3 and CH-5), 7.72-7.78 (m, 2H, CH-2 and CH-6) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 143.07 (C-1), 138.06 (C-4), 129.55 (2xCHAR), 126.94 (2xCHAR), 46.02 (CH(CH₃)₂), 23.74 (CH(CH₃)₂), 21.51 (C-4CH₃) ppm.

These data are consistent with those described in the literature.²⁹⁸

²⁹⁸ Hamura, S.; Oda, T.; Shimizu, Y.; Matsubara, K.; Nagashima, H. *J. Chem. Soc., Dalton Trans.* **2002**, 1521-1527.

9.7.1.2 Synthesis of 2,4-dimethylbenzenesulfonyl chloride (**164**)

To a solution of 2,4-dimethylbenzenesulfonic acid (1.862 g, 10 mmol) and triethylamine (1.012 g, 10 mmol) in 20 mL of acetone was added cyanuric chloride (1.844 g, 10 mmol) and the mixture was heated under reflux for 20 hours. After cooling to room temperature the solution was filtered through a celite pad; solvent was removed *in vacuo* and the crude mixture was purified by silica gel packed flash column chromatography (eluent: EtOAc:hexane from 0:100 to 3:97) to afford **164** as a colorless liquid (1.705 g, 83%).

Data of 164:

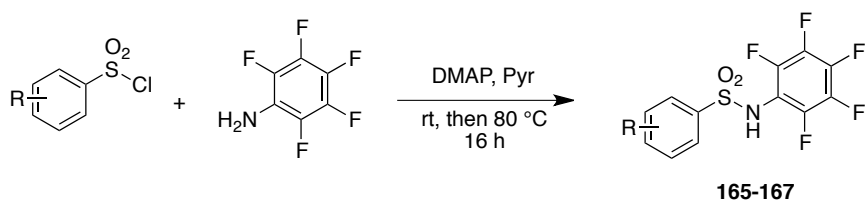
MS (EI) m/z (relative intensity) 204 (30) [M^+], 169 (52), 151 (13), 105 (100), 103 (25), 79 (33), 51 (13).

HR-MS (EI) m/z calcd for $C_8H_9ClO_2S$ 204.0012, found 204.0012.

IR (neat) ν 1364 (SO_2) cm^{-1} .

1H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, C-4 CH_3), 2.74 (s, 3H, C-2 CH_3), 7.16-7.24 (m, 2H, CH-3 and CH-5), 7.93 (d, $J = 8.1$ Hz, 1H, CH-6).

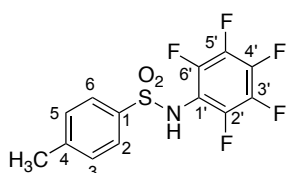
^{13}C NMR (126 MHz, $CDCl_3$) δ 146.5 (C-4), 140.2 (C-1), 137.8 (C-2), 134.0 (CH-3), 128.9 (CH-6*), 127.3 (CH-5*), 21.4 (C-2 CH_3), 20.1 (C-4 CH_3).

9.7.1.3 Synthesis of benzenesulfoanilides 165-167. General procedure

Compound	R
165	4-CH ₃
166	2,4-diCH ₃
167	3-CH ₃

To a solution of benzenesulfonyl chloride (10.0 mmol, 1 equiv) in pyridine (30 mL) was added DMAP (5.0 mmol, 0.5 equiv). After the mixture was stirred for 15 min at room temperature, 2,3,4,5,6-pentafluoroaniline (11.0 mmol, 1.1 equiv) was added, and then the mixture was stirred overnight at 80 °C. After removing the solvent (pyridine) *in vacuo*, the residue was dissolved in dichloromethane (100 mL) and washed with H₂O (3x30 mL) and brine (20 mL) successively. The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by a silica gel packed flash chromatography column (eluent: EtOAc/hexane) to afford benzenesulfoanilides **165-167**.

Data of compounds **165-167** and the experimental conditions employed for their synthesis are indicated below.

4-Methyl-*N*-(pentafluorophenyl)benzenesulfonamide (165)

Prepared from 4-methylbenzenesulfonyl chloride (2.86 g, 15.0 mmol), 2,3,4,5,6-pentafluoroaniline (3.02 g, 16.5 mmol) and DMAP (0.92 g, 7.5 mmol) in pyridine (50 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 80:20, v/v).

Yield: 1.92 g (38%). White solid.

Data of 165:

Mp: 151-152 °C

MS (EI) *m/z* (relative intensity) 337 (23) [M⁺], 182 (7), 155 (89), 91 (100), 65 (29).

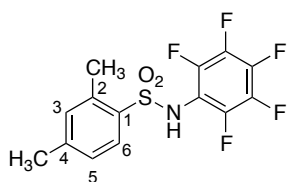
HR-MS (ESI) calcd for C₁₃H₈F₅NO₂S+Na⁺ [M+Na]⁺ 360.0088, found 360.0088.

IR (neat) ν 3267 (N-H), 1344 (SO₂) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 2.46 (s, 3H, C-4CH₃), 6.37 (s, 1H, NH), 7.32 (d, *J* = 8.2 Hz, 2H CH-3 and CH-5), 7.73 (d, *J* = 8.0 Hz, 2H, CH-2 and CH-6) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 144.8 (C-1), 144.1 (dm, *J* = 250 Hz, C-4'), 140.7 (dm, *J* = 262 Hz, C-3' and C-5'), 137.6 (dm, *J* = 250 Hz, C-2' and C-4'), 135.8 (C-4), 129.8 (2xCHAR), 127.3 (2xCHAR), 111.3 (m, C-1'), 21.7 (C-4CH₃) ppm.

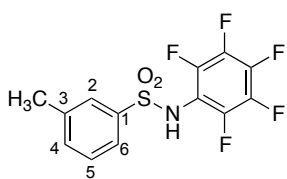
¹⁹F NMR (282 MHz, CDCl₃) δ -145.0 (m, 2F), -154.05 (t, *J* = 21.6 Hz, 1F), -161.5 (m, 2F) ppm.

2,4-Dimethyl-*N*-(pentafluorophenyl)benzenesulfonamide (166)

Prepared from 2,4-dimethylbenzenesulfonyl chloride (1.64 g, 8.0 mmol), 2,3,4,5,6-pentafluoroaniline (1.61 g, 8.8 mmol) and DMAP (0.49 g, 4.0 mmol) in pyridine (25 mL).

Purification: *n*-hexane: ethyl acetate (from 100:0 to 90:10, v/v).

Yield: 1.090 g (39%). White solid.

Data of 166:**Mp:** 156-157 °C**MS (EI)** *m/z* (relative intensity) 351 (12) [M^+], 182 (6), 169 (50), 155 (10), 105 (100), 77 (22).**HR-MS (EI)** *m/z* calcd for $C_{14}H_{10}F_5NO_2S$ 351.0352, found 351.0354.**IR** (neat) ν 3249 (N-H), 1326 (SO₂) cm^{-1} .**¹H NMR** (300 MHz, CDCl₃) δ 2.38 (s, 3H, C-4CH₃), 2.67 (s, 3H, C-2CH₃), 6.34 (s, 1H, NH), 7.07 (d, *J* = 8.2 Hz, 1H, CH-5), 7.15 (s, 1H, CH-3), 7.72 (d, *J* = 8.1 Hz, 1H, CH-6) ppm.**¹³C NMR** (126 MHz, CDCl₃) δ 144.7 (C-1), 143.9 (dm, *J* = 250 Hz, C-4'), 140.5 (dm, *J* = 262 Hz, C-3' and C-5'), 137.5 (dm, *J* = 250 Hz, C-2' and C-6'), 137.4 (C-2), 136.5 (C-4), 133.9 (CHAr), 129.7 (CHAr), 126.7 (CHAr), 111.3 (m, C-1'), 21.4 (C-2CH₃), 20.4 (C-4CH₃) ppm.**¹⁹F NMR** (282 MHz, CDCl₃) δ -145.1 (m, 2F), -154.48 (t, *J* = 21.6 Hz, 1F), -161.4 (m, 2F) ppm.**3-Methyl-*N*-(pentafluorophenyl)benzenesulfonamide (167)**

Prepared from 3-methylbenzenesulfonyl chloride (2.86 g, 15.0 mmol), 2,3,4,5,6-pentafluoroaniline (3.02 g, 16.5 mmol) and DMAP (0.92 g, 7.5 mmol) in pyridine (50 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 80:20, v/v).

Yield: 2.010 g (40%). White solid.

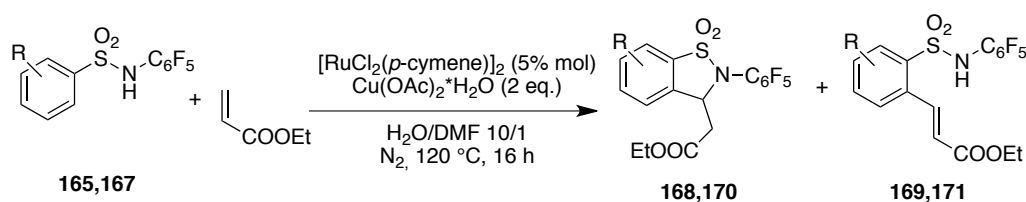
Data of 167:**Mp:** 156-157 °C**MS (EI)** *m/z* (relative intensity) 337 (18) [M^+], 182 (5), 155 (51), 91 (100), 65 (24).**HR-MS (EI)** *m/z* calcd for $C_{13}H_8F_5NO_2S$ 337.0196, found 337.0202.**IR** (neat) ν 3239 (N-H), 1350 (SO₂) cm^{-1} .**¹H NMR** (300 MHz, CDCl₃) δ 2.41 (s, 3H, C-3CH₃), 6.44 (s, 1H, NH), 7.35-7.46 (m, 2H, CH-4 and CH-5), 7.61 (d, *J* = 7.4 Hz, 1H, CH-6), 7.66 (s, 1H, CH-2)

ppm.

¹³C NMR (126 MHz, CDCl₃) δ 144.1 (dm, *J* = 250 Hz, C-4'), 140.6 (dm, *J* = 262 Hz, C-3' and C-5'), 139.5 (C-1), 138.6 (C-3), 137.6 (dm, *J* = 250 Hz, C-2' and C-6'), 134.5 (CHAr), 129.0 (CHAr), 127.4 (CHAr), 124.3 (CHAr), 111.2 (m, C-1'), 21.4 (CH₃) ppm.

¹⁹F NMR (282 MHz, CDCl₃) δ -145.4 (m, 2F), -153.96 (t, *J* = 21.6 Hz, 1F), -161.7 (m, 2F) ppm.

9.7.2 Ruthenium-catalyzed oxidative alkenylation of benzenesulfonamides. General procedure

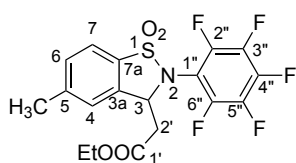


Compound	R
168	4-CH ₃
169	4-CH ₃
170	3-CH ₃
171	3-CH ₃

A mixture of *N*-(pentafluorophenyl)benzenesulfonamide (0.25 mmol, 1.0 equiv.), ethyl acrylate (0.75 mmol, 3.0 equiv.), [RuCl₂(*p*-cymene)]₂ (5 mol%), and Cu(OAc)₂·H₂O (0.50 mmol, 2.0 equiv.) in H₂O (2.0 mL) and DMF (0.2 mL) was stirred at 120 °C under N₂ for 16 hours. After cooling to ambient temperature, the reaction was quenched with a mixture of sat. aq. NH₄Cl/NH₃ (1:1, 15 mL) and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic phase was washed with brine (50 mL) and dried over anhydrous Na₂SO₄. After filtration and evaporation of the solvents *in vacuo*, the crude product was purified by column chromatography on silica gel to give pure compounds **167-169**.

Data of compounds **167-169** and the experimental conditions employed for their synthesis are indicated below.

Ethyl 2-(5-methyl-1,1-dioxido-2-(pentafluorophenyl)-2,3-dihydrobenzo[*d*]isothiazol-3-yl)acetate (168)



Prepared from 4-methyl-*N*-(pentafluorophenyl) benzenesulfonamide (83.4 mg, 0.25 mmol) and ethyl acrylate (75.0 mg, 0.75 mmol), [RuCl₂(*p*-cymene)]₂ (7.7 mg, 5 mol %), and Cu(OAc)₂·H₂O (99.8 mg, 0.50 mmol) in H₂O (2.0 mL) and DMF (0.2 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 70:30, v/v).

Yield: 13 mg (12%). Colorless solid.

Data of 168:

Mp: 109-111 °C

MS (EI) *m/z* (relative intensity) 436 (5) [M+H]⁺, 371 (31), 348 (100), 342 (30) 297 (32), 284 (70), 234 (27), 166 (14).

HR-MS (ESI) calcd for C₁₈H₁₅F₅NO₄S [M+H]⁺ 436.0636, found 436.0634.

IR (neat) ν 1734 (C=O), 1305 (SO₂) cm⁻¹.

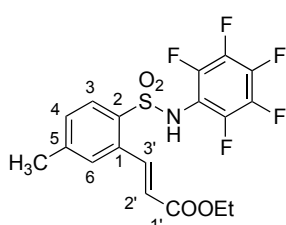
¹H NMR (300 MHz, CDCl₃) δ 1.21 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.50 (s, 3H, C-5CH₃), 2.88-2.97 (m, 2H, CH₂-2'), 4.08 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 5.30-5.37 (m, 1H, CH-3), 7.21-7.27 (m, 1H, CH-4), 7.42 (d, *J* = 8.0 Hz, 1H, CH-6), 7.74 (d, *J* = 8.0 Hz, 1H, CH-7) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 169.8 (C-1'=O), 144.8 (C-3a), 142.1 (dm, *J* = 262 Hz, C-3' and C-5'), 137.8 (dm, *J* = 250 Hz, C-2' and C-6'), 136.6 (C-5), 131.3 (C-7a), 130.8 (CHAr), 124.2 (CHAr), 121.4 (CHAr), 111.3 (m, C-1'), 61.4 (OCH₂CH₃), 60.3 (CH-3), 41.2 (CH₂-2'), 22.0 (C-5CH₃), 14.0 (OCH₂CH₃) ppm. C-4'' is missing.

¹⁹F NMR (282 MHz, CDCl₃) δ -141.72 (bs, 1F), -142.82 (bs, 1F), -151.1 (tt, *J* = 21.5, 2.5 Hz, 1F), -(161.0-161.8) (m, 2F) ppm.

Ethyl 3-(5-methyl-2-(*N*-(pentafluorophenyl)sulfamoyl)phenyl)acrylate (169)

Prepared from 4-methyl-*N*-(pentafluorophenyl) benzenesulfonamide (83.4 mg, 0.25 mmol) and ethyl acrylate (75.0 mg, 0.75 mmol), [RuCl₂(*p*-cymene)]₂ (7.7 mg, 5 mol %), and Cu(OAc)₂·H₂O (99.8 mg, 0.50 mmol) in H₂O (2.0 mL) and



DMF (0.2 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 70:30, v/v).

Yield: 50 mg (46%). Colorless solid.

Data of 169:

Mp: 149-151 °C

MS (EI) *m/z* (relative intensity) 435 (3) [M^+], 390 (13), 362 (10), 253 (55), 225 (20), 181 (100), 155 (13), 115 (44).

HR-MS (EI) *m/z* calcd for $C_{18}H_{14}F_5NO_4S$ 435.0564, found 435.0562.

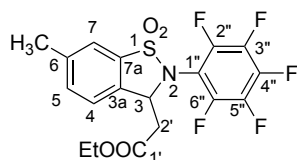
IR (neat) ν 3155 (N-H), 1692 (C=O), 1345 (SO₂) cm^{-1} .

¹H NMR (300 MHz, CDCl₃) δ 1.33 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.46 (s, 3H, C-5CH₃), 4.25 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 6.35 (d, *J* = 15.8 Hz, 1H, CH-2'), 6.98 (s, 1H, NH), 7.28 (dd, *J* = 8.1, 1.0 Hz, 1H, CH-4), 7.47 (d, *J* = 1.0 Hz, 1H, CH-6), 7.85 (d, *J* = 8.1 Hz, 1H, CH-3), 8.41 (d, *J* = 15.8 Hz, 1H, CH-3') ppm.

¹³C NMR (126 MHz, CDCl₃) δ 166.1 (C-1'=O), 144.8 (C-5), 144.3 (dm, *J* = 250 Hz, C-4'), 140.7 (dm, *J* = 262 Hz, C-3' and C-5'), 140.4 (CH-3'), 137.6 (dm, *J* = 250 Hz, C-2' and C-6'), 134.6 (C-2*), 134.1 (C-1*), 130.2 (CH), 129.8 (CH), 129.4 (CH), 123.1 (CH-2'), 111.0 (m, C-1'), 61.1 (OCH₂CH₃), 21.6 (C-5CH₃), 14.2 (OCH₂CH₃) ppm.

¹⁹F NMR (282 MHz, CDCl₃) δ -144.7 (m, 2F), -154.1 (t, *J* = 21.5 Hz, 1F), -161.7 (m, 2F) ppm.

Ethyl 2-(6-methyl-1,1-dioxido-2-(perfluorophenyl)-2,3-dihydrobenzo[*d*]isothiazol-3-yl)acetate (170)

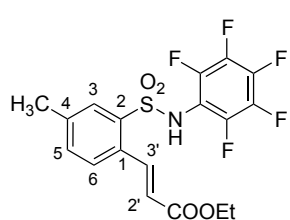


Prepared from 3-methyl-*N*-(pentafluorophenyl) benzenesulfonamide (83.4 mg, 0.25 mmol) and ethyl acrylate (75.0 mg, 0.75 mmol), [RuCl₂(*p*-cymene)]₂ (7.7 mg, 5 mol %), and Cu(OAc)₂·H₂O (99.8 mg, 0.50 mmol)

in H₂O (2.0 mL) and DMF (0.2 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 70:30, v/v).

Yield: 23 mg (21%). Colorless solid.

Data of 170:**Mp:** 116-117 °C**MS (EI)** *m/z* (relative intensity) 436 (5) [M+H]⁺, 371 (25), 348 (100), 297 (30), 284 (80), 234 (35), 166 (10).**HR-MS (ESI)** calcd for C₁₈H₁₈F₅N₂O₄S [M+NH₄]⁺ 453.0902, found 453.0910.**IR** (neat) ν 1730 (C=O), 1316 (SO₂) cm⁻¹.**¹H NMR** (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.49 (s, 3H, C-6CH₃), 2.91-2.95 (m, 2H, CH₂-2'), 4.08 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 5.30-5.38 (m, 1H, CH-3), 7.34 (d, *J* = 8.1 Hz, 1H, CH-5), 7.51 (d, *J* = 8.0 Hz, 1H, CH-4), 7.66 (s, 1H, CH-7) ppm.**¹³C NMR** (126 MHz, CDCl₃) δ 169.8 (C-1'=O), 142.1 (dm, *J* = 262 Hz, C-3' and C-5'), 140.7 (C-3a), 137.7 (dm, *J* = 250 Hz, C-2' and C-6'), 134.7 (CHAr), 134.0 (C-6*), 133.6 (C-7a*), 123.7 (CHAr), 121.6 (CHAr), 111.2 (m, C-1''), 61.4 (OCH₂CH₃), 60.3 (CH-3), 41.2 (CH₂-2'), 21.4 (C-6CH₃), 14.0 (OCH₂CH₃) ppm. C-4' is missing.**¹⁹F NMR** (282 MHz, CDCl₃) δ -141.75 (bs, 1F), -142.9 (bs, 1F), -151.0 (tt, *J* = 21.5, 2.6 Hz, 1F), -(160.8-161.6) (m, 2F) ppm.**Ethyl 3-(4-methyl-2-(*N*-(pentafluorophenyl)sulfamoyl)phenyl)acrylate (171)**

Prepared from 3-methyl-*N*-(pentafluorophenyl) benzenesulfonamide (83.4 mg, 0.25 mmol) and ethyl acrylate (75.0 mg, 0.75 mmol), [RuCl₂(*p*-cymene)]₂ (7.7 mg, 5 mol %), and Cu(OAc)₂·H₂O (99.8 mg, 0.50 mmol) in H₂O (2.0 mL) and DMF (0.2 mL).

Purification: *n*-hexane: ethyl acetate (from 95:5 to 70:30, v/v).

Yield: 55 mg (51%). Colorless solid.

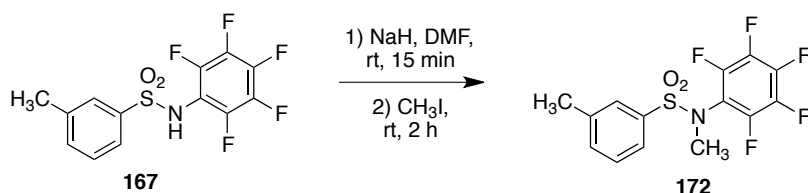
Data of 171:**Mp:** 140-142 °C**MS (EI)** *m/z* (relative intensity) 435 (2) [M⁺], 390 (13), 362 (10), 253 (64), 225 (26), 181 (100), 161 (15), 115 (50).**HR-MS (EI)** *m/z* calcd for C₁₈H₁₄F₅NO₄S 435.0564, found 435.0556.**IR** (neat) ν 3138 (N-H), 1692 (C=O), 1349 (SO₂) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 1.33 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 2.42 (s, 3H, C-4CH₃), 4.24 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 6.34 (d, *J* = 15.8 Hz, 1H, CH-2'), 7.17 (s, 1H, NH), 7.44 (dd, *J* = 7.9, 1.0 Hz, 1H, CH-5), 7.59 (d, *J* = 7.9 Hz, 1H, CH-6), 7.80 (d, *J* = 1.0 Hz, 1H, CH-3), 8.40 (d, *J* = 15.8 Hz, 1H, CH-3') ppm.

¹³C NMR (126 MHz, CDCl₃) δ 166.3 (C-1'=O), 144.3 (dm, *J* = 250 Hz, C-4'), 140.8 (dm, *J* = 262 Hz, C-3' and C-5'), 140.5 (C-4), 140.1 (CH-3'), 137.6 (dm, *J* = 250 Hz, C-2' and C-6'), 137.3 (C-2), 134.4 (CHAR), 131.1 (C-1), 130.0 (CHAR), 128.6 (CHAR), 122.3 (CH-2'), 111.0 (m, C-1'), 61.1 (OCH₂CH₃), 21.3 (C-5CH₃), 14.2 (OCH₂CH₃) ppm.

¹⁹F NMR (282 MHz, CDCl₃) δ -144.7 (m, 2F), -154.0 (t, *J* = 21.6 Hz, 1F), -161.5 (m, 2F) ppm.

9.7.3 Synthesis of *N*,3-dimethyl-*N*-(pentafluorophenyl)benzenesulfonamide (**172**)



In a 25-mL two-neck round bottom flask under nitrogen, a solution of 3-methyl-*N*-(pentafluorophenyl)benzenesulfonamide **167** (270 mg, 0.8 mmol) in 1 mL of anhydrous DMF was added to a solution of NaH (35 mg, 1.44 mmol) in 1 mL of anhydrous DMF. The mixture was stirred at room temperature for 15 min and then methyl iodide (136 mg, 0.96 mmol) was added dropwise, and the mixture was stirred at the same temperature for additional 2 hour. Reaction mixture was quenched by addition of H₂O (15 mL) and the organic layer was extracted with EtOAc (3x20 mL), washed with brine (20 mL) and dried over anhydrous Na₂SO₄. After filtration and evaporation of the solvents *in vacuo*, the crude product was purified by column chromatography on silica gel (*n*-hexane:ethyl acetate from 100:0 to 90:10) to yield **172** (248 mg, 88%) as a white solid.

Data of 172:

Mp: 129-130 °C

MS (EI) *m/z* (relative intensity) 351 (21) [M⁺], 249 (3), 196 (12), 155 (38), 91 (100), 65 (20), 58 (16), 43 (61).

HR-MS (EI) *m/z* calcd for C₁₄H₁₀F₅NO₂S 351.0352, found 351.0346.

IR (neat) ν 1349 (SO₂) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ , 2.45 (s, 3H, C-3CH₃), 3.18 (s, 3H, NCH₃), 7.39-7.49 (m, 2H, CH-2 and CH-4) 7.58-7.65 (m, 2H, CH-5 and CH-6) ppm.

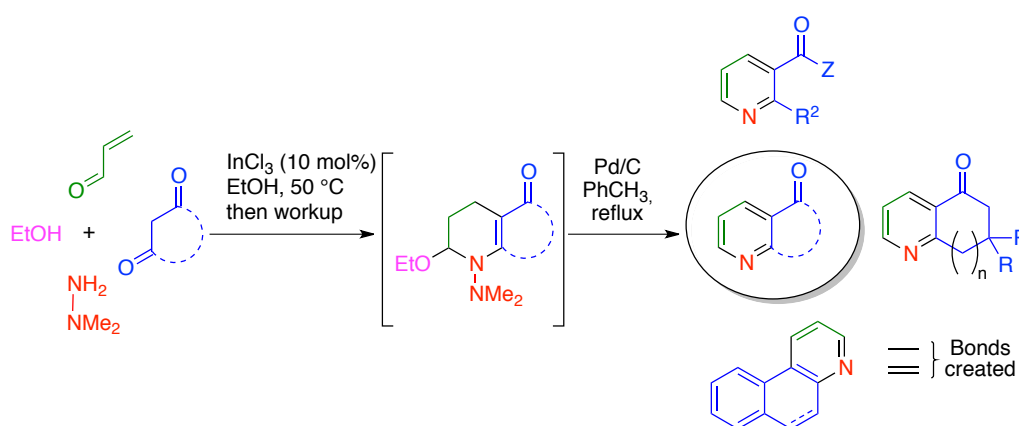
¹³C NMR (126 MHz, CDCl₃) δ 145.9 (dm, *J* = 250 Hz, C-4'), 141.4 (dm, *J* = 250 Hz, C-3' and C-5'), 139.4 (C-1), 137.7 (dm, *J* = 250 Hz, C-2' and C-6'), 137.5 (C-3), 134.2 (CHAR), 129.0 (CHAR), 127.8 (CHAR), 124.7 (CHAR), 116.1 (m, C-1'), 37.6 (NCH₃), 21.4 (C-3CH₃) ppm.

¹⁹F NMR (282 MHz, CDCl₃) δ -142.8 (m, 2F), -152.8 (tt, *J* = 21.6, 2.1 Hz, 1F), -

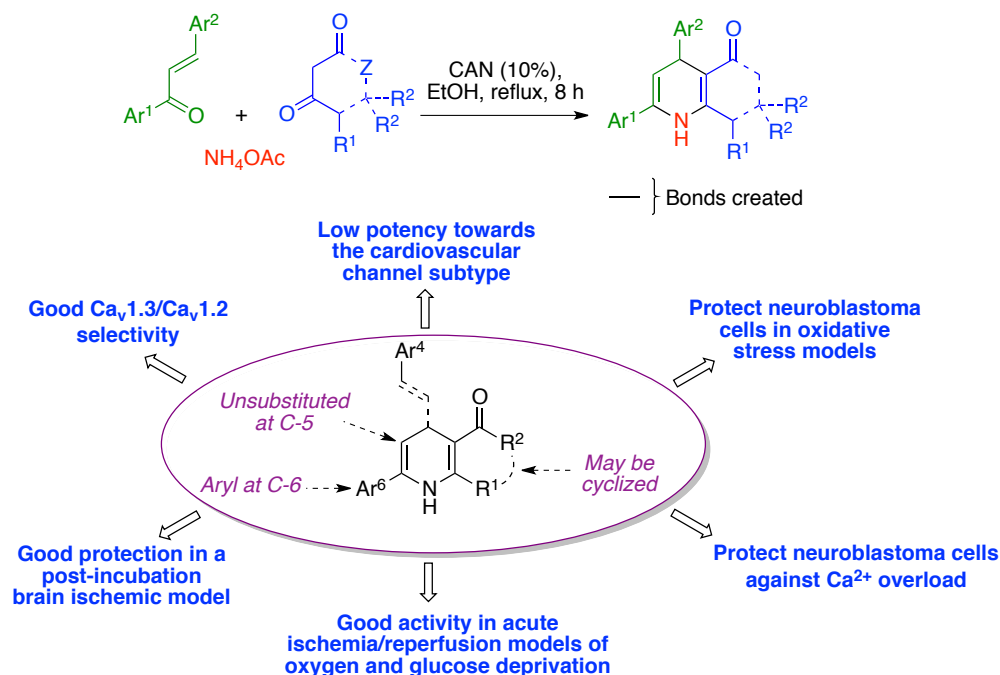
161.6 (m, 2F) ppm.

10. Conclusions

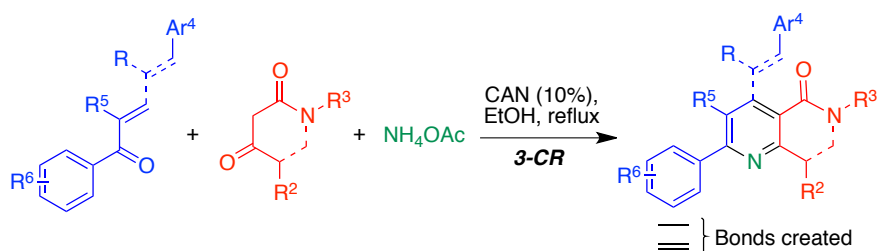
1. The indium trichloride-catalysed four-component reaction between β -dicarbonyl compounds, dimethylhydrazine, acroleins and ethanol affords 6-ethoxy-1,4,5,6-tetrahydropyridine derivatives. This transformation was telescoped with a double elimination reaction, without the need for any intermediate purification stage, to yield a process that affords 2,3-disubstituted pyridines and which could also be adapted to the preparation of several types of fused pyridines.



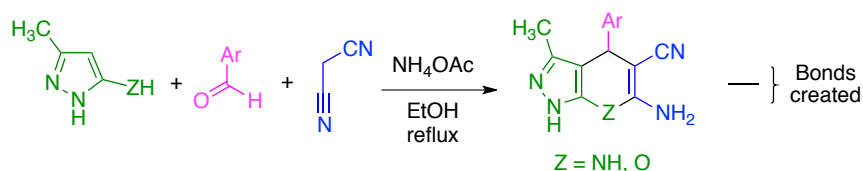
2. The three-component reaction between β -dicarbonyl compounds, chalcones and ammonium acetate in refluxing ethanol, in the presence of a catalytic amount of cerium(IV) ammonium nitrate, affords 4,6-diaryl-1,4-dihydropyridines. These compounds showed improved selectivity towards $\text{Ca}_v1.3$ neuronal calcium channels with regard to previously known dihydropyridines and behave as neuroprotective agents against ischemic injury.



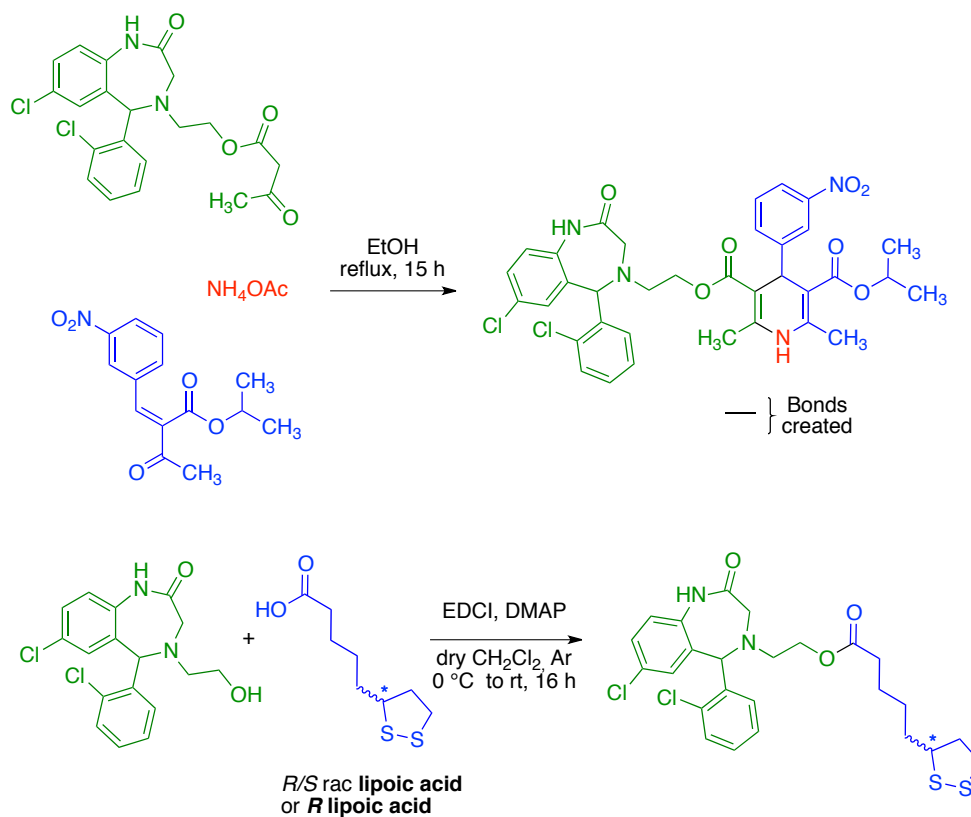
3. The three-component reaction summarized in Conclusion 2 was applied to the synthesis of nicotinamides from β -ketoamides, chalcones and ammonium acetate under similar conditions (CAN as catalyst in refluxing ethanol). In this case, the reaction directly afforded aromatic compounds via a 3CR-air oxidation sequence.



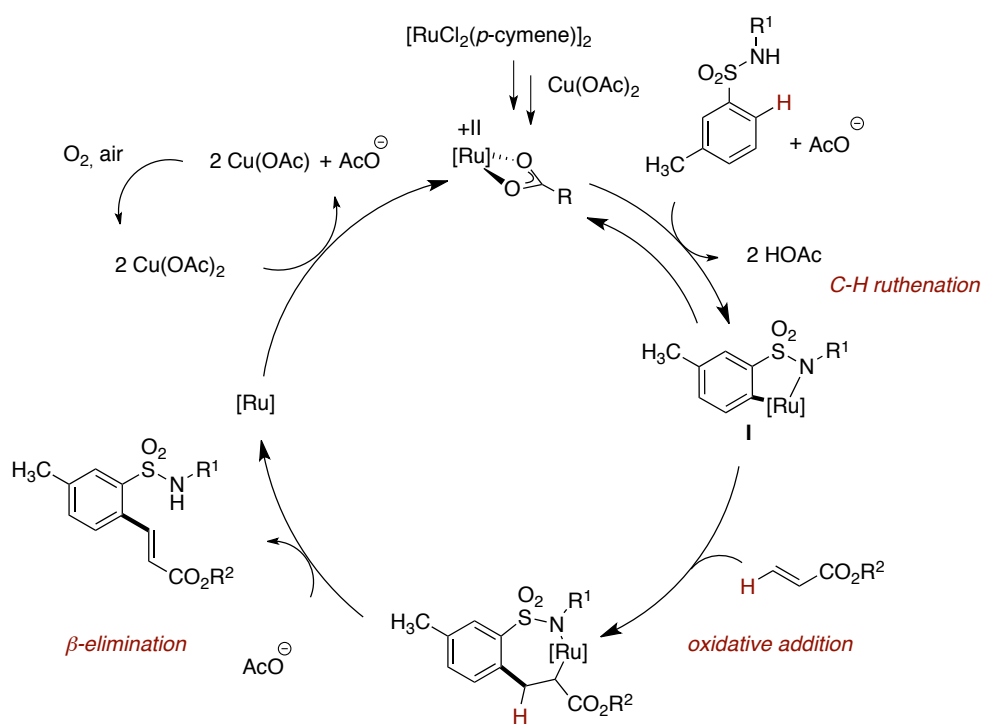
4. The synthesis of fused dihydropyridines and their oxygen heteroanalogues was achieved *via* Hantzsch-like three-component reactions between malononitrile, aromatic aldehydes and the suitable (lactam or aminine) C5-functionalized pyrazoles. These compounds are currently being studied as anti-Alzheimer agents *via* inhibition of GSK3 β .



5. Hybrid structures related to nimodipine and CGP-37157 were designed as potential anti-Alzheimer compounds aimed at stabilizing neuronal and mitochondrial calcium cycling by a multitarget approach. These compounds were prepared via a modified Hantzsch dihydropyridine synthesis. Hybrids of a CGP-37157 aza analogue and lipoic acid, a well-known antioxidant able to cross the blood-brain barrier, were also synthesized. These compounds are currently being studied as anti-Alzheimer agents.



6. During a stay at the group of Professor Lutz Ackermann at Goettingen University as part of the requirements for the European Mention to the Ph. D. title, a Ru(II)-catalyzed oxidative C-H alkenylation of arylsulfonamides was developed.



Appendix. Representative spectra

