



# UNIVERSITÀ DEGLI STUDI DI PALERMO

PhD in Biologia Cellulare e Scienze Tecnologiche del Farmaco  
Curriculum: “ Scienze Farmaceutiche”

Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche  
SSD: CHIM/08

## POLYCYCLIC PYRROLO-THIAZOLE SYSTEMS WITH BIOLOGICAL ACTIVITY

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CYCLE XXVI  
A.A. 2014-2015

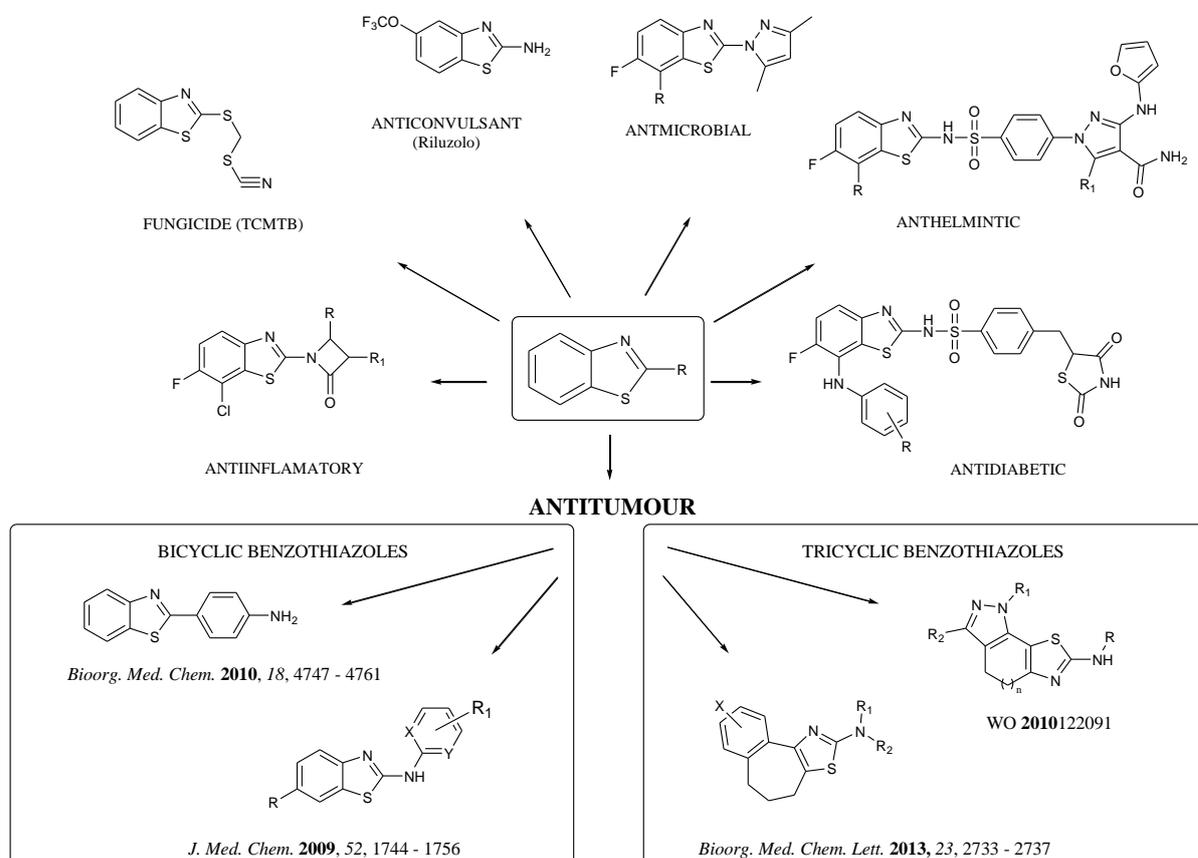
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# 1. INTRODUCTION

Benzothiazole (BTA) and its derivatives are one of the most important heterocyclic compounds, found in a variety of natural products and pharmaceutical agents. BTA shows a variety of pharmacological properties, and its analogues offer a high degree of structural diversity that has proven useful for the search of new therapeutic agents. BTA-based compounds are found to possess wide range of biological activities<sup>[1-3]</sup> such as anticancer,<sup>[4-6]</sup> antifungal, antibacterial,<sup>[7-9]</sup> antiinflammatory,<sup>[10]</sup> analgesic,<sup>[11]</sup> antiviral,<sup>[12]</sup> antioxidant, anticonvulsant, antitubercular,<sup>[13]</sup> antidiabetic,<sup>[14,15]</sup> anthelmintic,<sup>[16]</sup> antihistaminic, antimalarial, antiparkinson<sup>[17]</sup> activity. The broad spectrum of pharmacological activity in individual BTA derivative indicates that, this series of compounds is of an undoubted interest. The related research and developments in BTA-based medicinal chemistry have become a rapidly developing and increasingly active topic. Particularly, numerous BTA-based compounds as clinical drugs have been extensively used in practice to treat various diseases with high therapeutic potency (Chart 1).

**Chart 1**



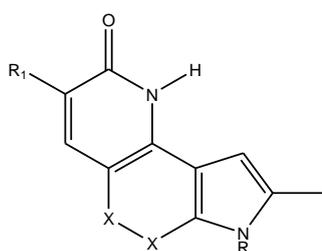
In the past two decades BTA have been largely studied mainly for their remarkable anticancer activity. In particular 2-(4-aminophenyl)benzothiazoles have showed notable anticancer activity against breast, colon and ovarian cell lines in *in vitro* anticancer screening. The anticancer activity of these molecules seems to be due to the formation of reactive intermediates that can bind covalently the DNA.<sup>[18]</sup> Furthermore, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203, NCS 703786) showed high sensitivity towards the tumor cells by acting through aryl hydrocarbon receptor (AhR) signal pathway.<sup>[19]</sup> BTA derivatives also showed some interesting efficacy as kinase, topoisomerase I/II and trans-retinoic acid metabolism inhibitors.<sup>[20]</sup> It must be emphasized that combination of 2-aminobenzothiazoles with other heterocyclic is a well known approach to design new drug like molecules, which allows achieving new pharmacological profile, action, toxicity lowering. For example, tricyclic compounds incorporating the thiazole nucleus, such as thiazolyl-dihydro-indazoles, are described as kinases inhibiting<sup>[21]</sup> and a new class of tricyclic benzothiazole has emerged as angiogenesis inhibitors<sup>[22]</sup>. Furthermore some tricyclic thiazolopyrazole derivatives of type **3** (Chart 4), have recently described as potent and selective modulators of metabotropic Glutamate Receptor 4 (mGluR4).<sup>[23]</sup> In addition, a role for glutamate signaling in cancer was investigated and a correlation between compounds that act on metabotropic glutamate receptors and their selective action on malignant tumors, including brain tumors, was found.<sup>[24]</sup>

## 2. AIM OF THE WORK

In our laboratory much efforts have been devoted, through the years, towards the synthesis of pyrrole fused heterocycles, mainly with antitumor properties (Chart 2).<sup>[25-33]</sup> Thus, in the light of the proven activity of tricyclic benzothiazole systems with different therapeutic targets, it was planned an investigation of new chemotypes containing the amino benzothiazole scaffold condensed with a pyrrole unit. In fact, pyrrole condensed systems are characterized by a wide structural variability depending on the position occupied by the nitrogen atom and allow the introduction of property-modulating functionality. Having in mind the promising activity of some lead compounds, susceptible of further modifications, we planned the synthesis of pyrrole benzothiazoles either as Central Nervous System (CNS) and as anticancer drug candidates. In particular, compounds of type **1** (Chart 3) would be the ideal key products for our purpose as they can be properly decorated, thus switching the proposed activities, being at the same time valuable *building blocks* for further investigations as antitumor agents, according to chosen lead structures.

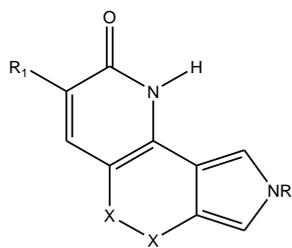
In this contest, one branch of my research project was the synthesis of compounds of type **2**, analogues of tricyclic thiazolopyrazole derivatives of type **3**, owned by Addex Therapeutics and recently emerged as potent Positive allosteric modulator (PAM) of metabotropic glutamate 4 (mGlu4) receptors, replacing the pyrazole ring with a pyrrole ring that would be potentially active on CNS. The second branch of the project was the synthesis of tetracyclic analogues **4** of quizartinib, currently in Phase 3 of clinical trials for the treatment of acute myeloid leukaemia (AML).

## Chart 2



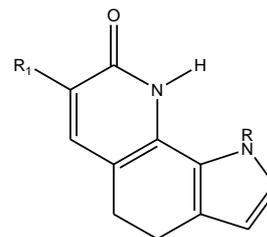
Pyrrolo[2,3-*h*]quinolinones

*Bioorg. Med. Chem.* **2006**, *14*, 8712



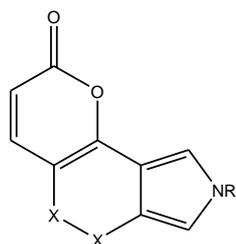
Pyrrolo[3,4-*h*]quinolinones

*Bioorg. Med. Chem.* **2011**, *19*, 2326



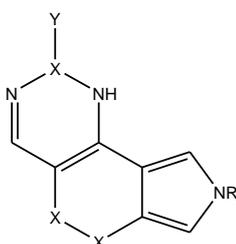
Pyrrolo[3,2-*h*]quinolinones

*Bioorg. Med. Chem.* **2010**, *18*, 4830;  
*PCT INT WO* **2011**013159



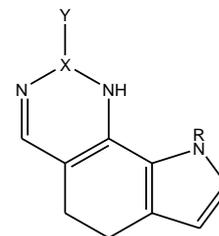
Pyrano[2,3-*e*]isoindolones

*Bioorg. Med. Chem. Lett.* **2009**, *19*, 1711



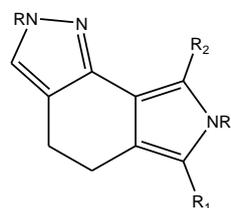
Pyrrolo[3,4-*h*]quinazolin-2-ones

*J. Med. Chem.* **2014**, *74*, 340



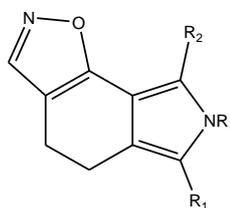
Pyrrolo[3,2-*h*]quinazolines

*Chem. Med. Chem.* **2011**, *6*, 1238

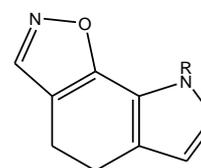


Pyrrolo[3,4-*g*]indazoles

*Tetrahedron* **2013**, *69*, 9839



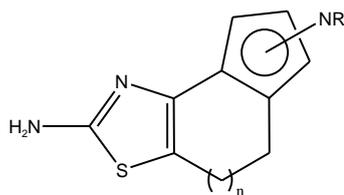
Isoxazolo[5,4-*e*]isoindoles



[1,2]oxazolo[4,5-*g*]indoles

*Chem. Med. Chem.* **2012**, *7*, 1901

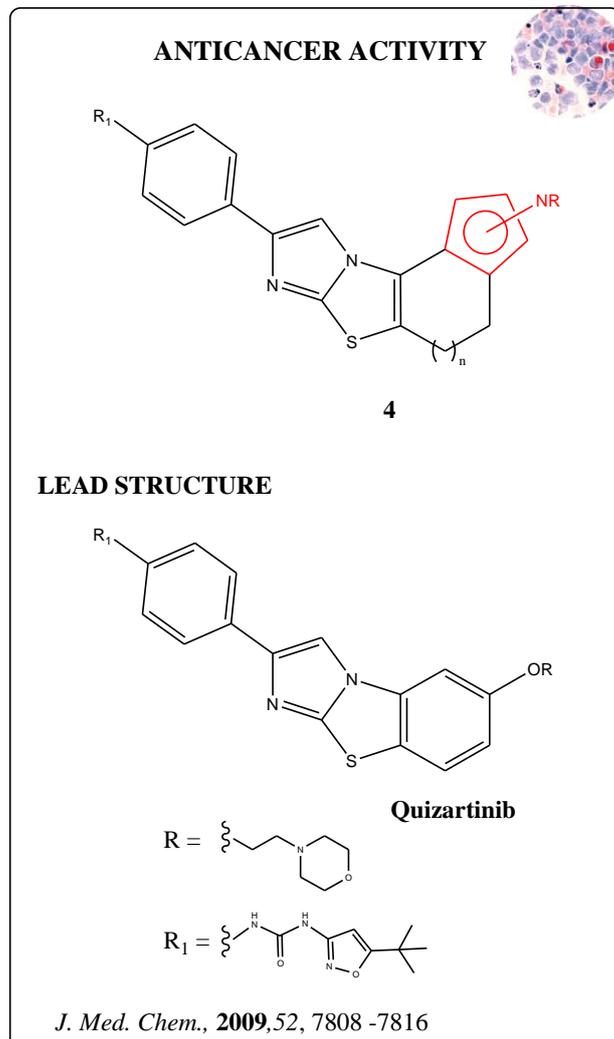
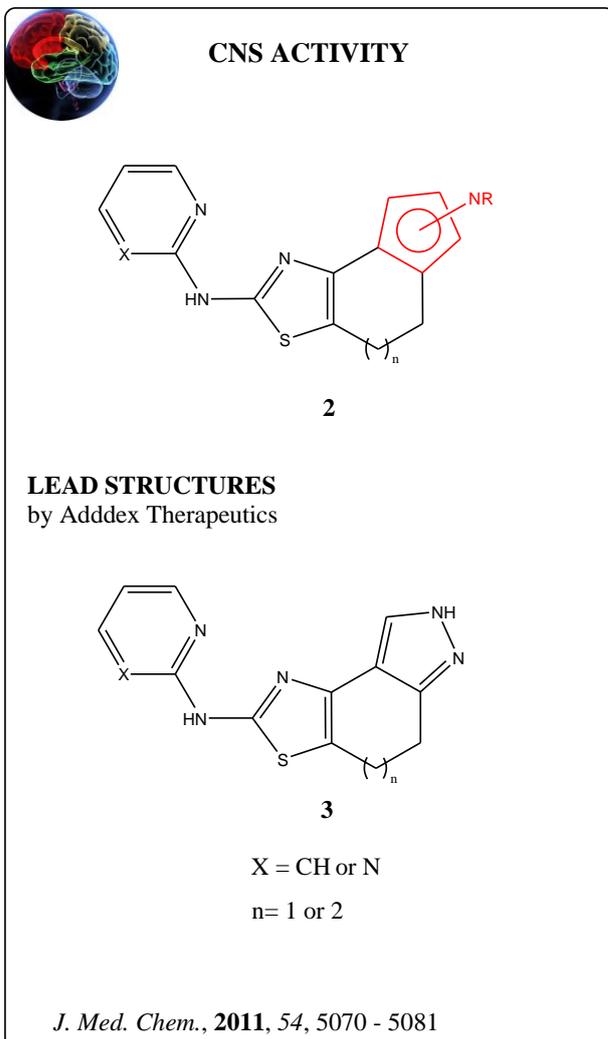
## Chart 3



**1**

$n = 1$  or  $2$

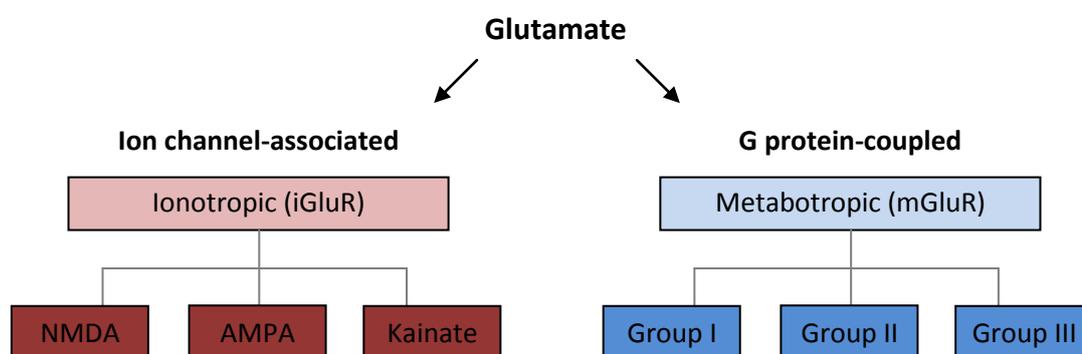
## Chart 4



### 3. PYRROLO BENZOTHAZOLES AS CNS DRUG CANDIDATES

Glutamate is the main excitatory neurotransmitter in the CNS.<sup>[34]</sup> It is important for neural communication, memory, learning implicated in a number of neurological conditions. Glutamate exerts its effects through the activation of several receptor subtypes. Glutamate receptors can be divided into two groups according to the mechanism, by which their activation gives rise to a postsynaptic current (Figure 1). Their classification has been possible due to the different affinity to specific agonists and antagonists. Ionotropic glutamate receptors (iGluRs) form the ion channel pore that activates when glutamate binds to the receptor and their activation evokes fast synaptic event. Metabotropic glutamate receptors (mGluRs) indirectly activate ion channels on the plasma membrane through a signalling cascade that involves G proteins and are associated with a more prolonged stimulus.

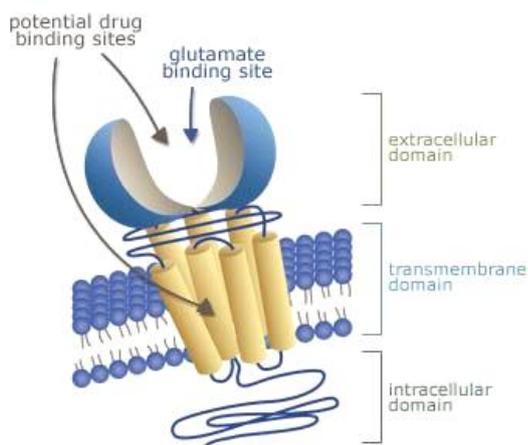
**Figure 1. Glutamate Receptors Classification**



Glutamatergic transmission is primarily mediated by ionotropic glutamate receptors, subdivided into three groups (AMPA, NMDA and Kainate receptors). Upon binding, the agonist will stimulate direct action of the central pore of the receptor, allowing ion flow ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) and causing excitatory postsynaptic current (EPSC). This current is depolarizing and, if enough glutamate receptors are activated, may trigger an action potential in the postsynaptic neuron. All of them produce excitatory postsynaptic current, but the speed and duration of the current is different for each type. However, glutamate shows a prominent modulatory role of the fast excitatory tone set by the ionotropic receptors by activation of metabotropic glutamate receptor family.<sup>[35]</sup> Metabotropic glutamate receptors (mGluRs) are

G-protein coupled receptors (GPCRs) that show the typical structure of seven transmembrane domains (Figure 2). The mGluRs possess a large bi-lobed extracellular N-terminal domain, to contain the glutamate binding site, linked via a cysteine-rich region to the transmembrane heptahelical domain responsible for G-protein activation.

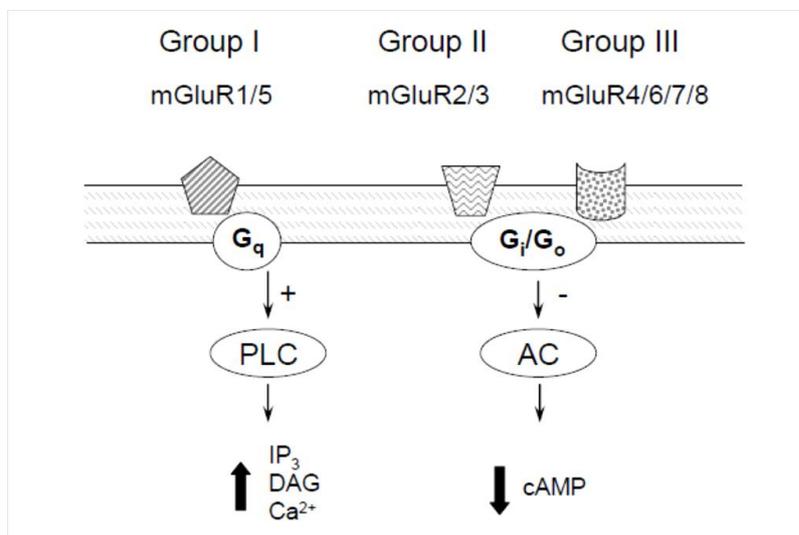
**Figure 2. Metabotropic Glutamate Receptor: typical structure**



Eight different types of mGluRs have been identified and classified into three groups according to their sequence homology, pharmacological properties and intracellular signal transduction pathways (Figure 3). Group I includes mGluR1 and mGluR5 and are positively coupled to phospholipase C through G-protein of the Gq/G<sub>11</sub> type; activation of Group I receptors leads to stimulation of phospholipase C, production of inositol triphosphate, release of Ca<sup>2+</sup> from intracellular stores and production of diacylglycerol, which in turn activates protein kinase C. Group II, consisting of mGluR2 and mGluR3 and group III which include mGluR4, mGluR6, mGluR7 and mGluR8 are mainly coupled to the inhibition of adenylyl cyclase activity through G-protein of Gi/G<sub>0</sub> type.<sup>[36-38]</sup> According to current knowledge on the molecular mechanism of action, the therapeutic effects of drugs targeting mGluRs involve, in the majority of cases, a reduction in the excitatory drive either through deactivation of Group I mGluRs or activation of Group II and III mGluRs. Pharmacological modulation of ionotropic glutamate receptor has been widely investigated as a potential therapeutic strategy for the treatment of several disorders associated with glutamatergic dysfunction. However, blockade of ionotropic glutamate receptor might be accompanied by severe side effects due to their vital role in many important physiological function. Targeting glutamatergic

neurotransmission through modulation of the mGluRs holds great promise with the advantage of potential fewer side effects.

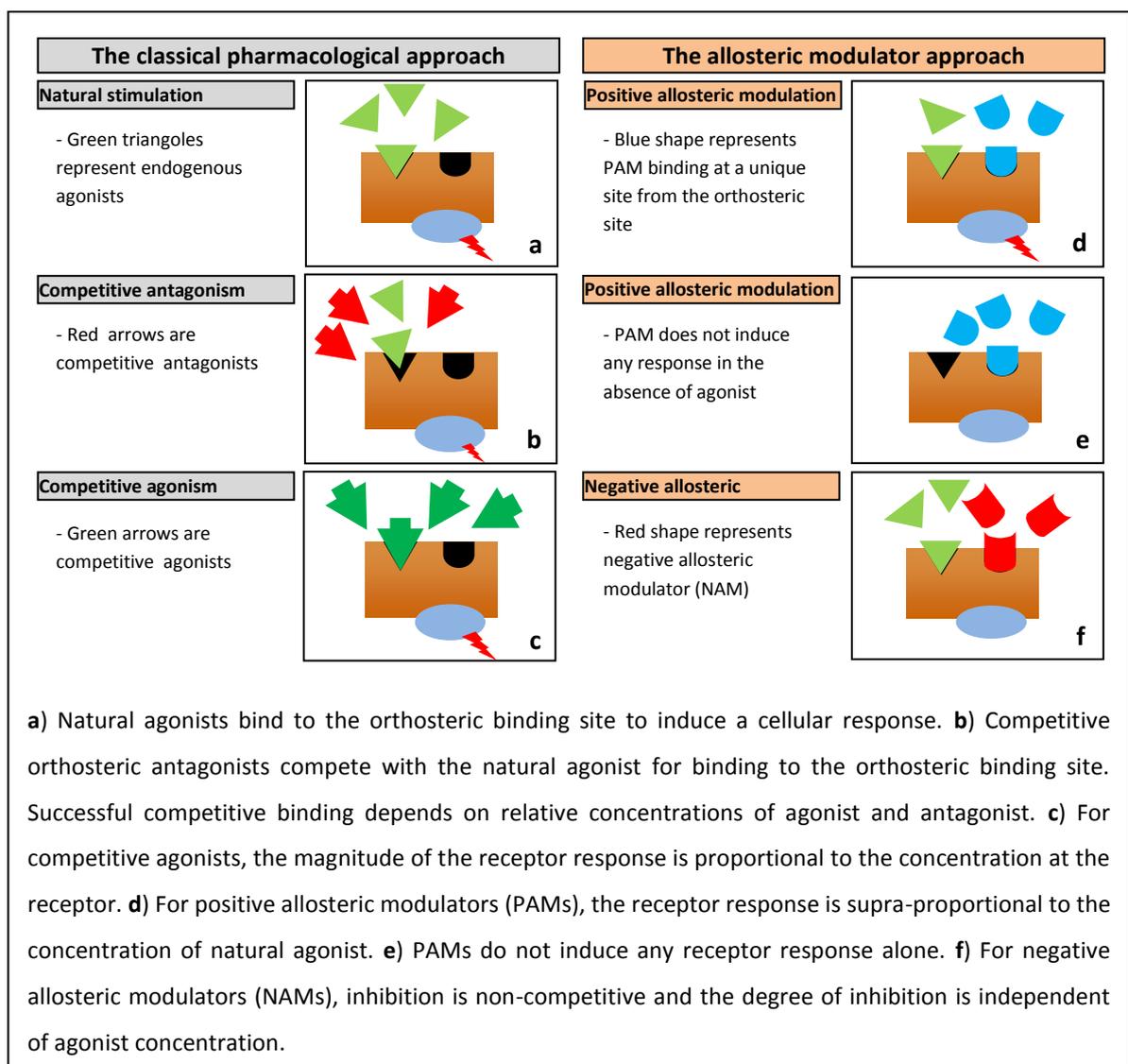
**Figure 3. Metabotropic Glutamate Receptor: classification**



Traditional approaches to drug discovery focus on mimicking the actions of the endogenous ligand for a target receptor. This could be done by the design and synthesis of small molecule agonists or antagonists that act in a competitive manner through interaction with the same binding site as the endogenous ligand, termed the “orthosteric” binding site. Historically, drug discovery programmes for mGluRs ligands have been dominated by efforts to develop molecules that act at the orthosteric site for glutamate. Unfortunately, targeting binding site where glutamate is itself docked, proved exceptionally difficult because the glutamate binding site is highly conserved in all glutamate receptors (ionotropic, metabotropic and transport systems) and it was nearly impossible to develop a selective molecule against any particular glutamate receptor. However, in recent years, there have been tremendous advances in the discovery of novel ligands for mGluRs that act at allosteric sites. The word “allosteric” literally translated from its Greek roots means “other site”. In contrast to orthosteric compounds, allosteric modulators interact with binding sites that are topographically distinct from the binding site of the endogenous ligand.<sup>[39]</sup> This implies that allosteric modulators are non-competitive and may only alter receptor responses in the presence of the endogenous ligand, preserving normal physiological signaling patterns. Usually allosteric modulators induce a conformational change within the protein structure which alters the activity of the receptor. A positive allosteric modulator (PAM) lead to an amplification of the orthosteric ligand's effect; a negative modulators (NAM) reduces the effects of the orthosteric ligand

(Figure 4).<sup>[40]</sup> Moreover, allosteric modulators are not limited to simply turning a receptor on or off, the way most drugs are. Instead, they act more like a dimmer switch, offering control over the intensity of activation or deactivation, while allowing the body to retain its natural control over initiating receptor activation. mGluRs are implicated in different aspects of CNS physiology, including motor control, motor coordination, sensory perception and pain transmission, learning and memory processes, and developmental plasticity.<sup>[41]</sup> Individual mGluR subtypes are considered as targets for drugs of potential use in a variety of disorders of CNS.<sup>[42,43]</sup> In addition, experimental evidence indicates that glutamate receptors may limit tumor growth mediating malignant tumor biology in pediatric brain tumors and therefore may be attractive targets for therapeutic intervention.<sup>[44]</sup>

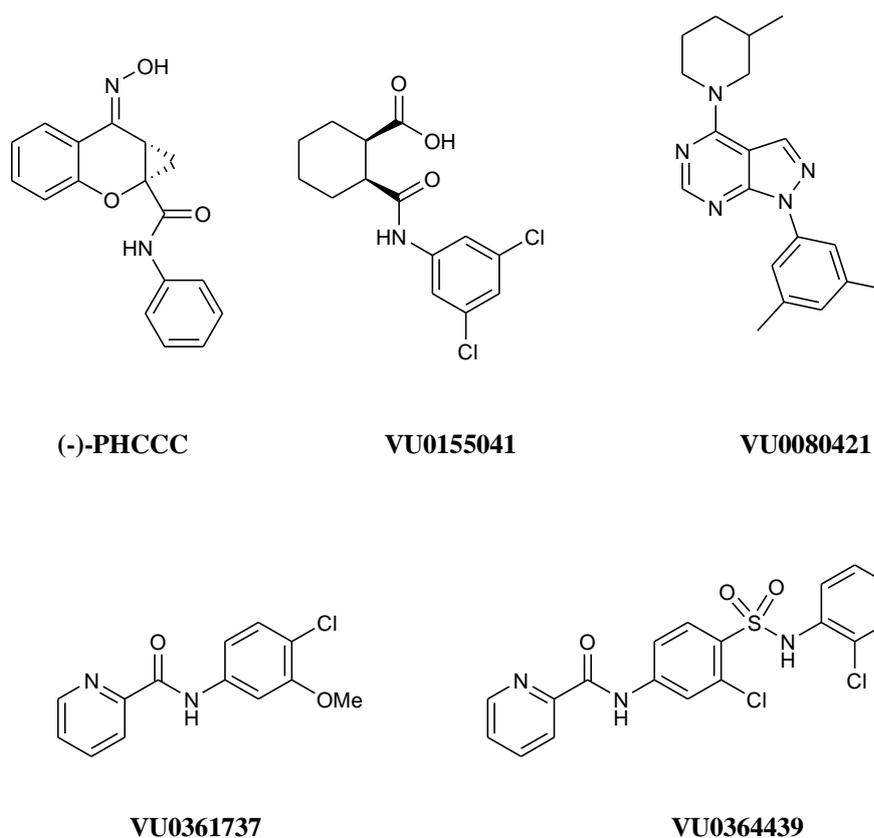
**Figure 4. Orthosteric vs. Allosteric approach**



Some glutamate receptor modulators were proven to have anticancer action *in vitro*, and/or *in vivo* in various cancers, including brain gliomas.<sup>[45]</sup> Recently, an increasing amount of evidence has been accumulating to support the hypothesis that activation of the mGlu4 receptor, either with an orthosteric agonist or a positive allosteric modulator PAM, may provide impactful pharmacological interventions in diseases such as Parkinson's<sup>[46]</sup> and anxiety.<sup>[47]</sup> Furthermore mGlu4 receptors are emerging as promising drug target in the treatment of epilepsy.<sup>[48]</sup> Although inhibition of adenylyl cyclase is related to classic functions of mGlu4 receptors, such as the modulation of glutamate release from the nerve terminals,<sup>[49]</sup> the role of mitogen activated protein kinase (MAPK) activation in mGlu4 receptor function is a topic for future investigation. Studies carried out in cultured cerebellar granule cells show that activation of mGlu4 receptor stimulate also the MAPK and phosphoinositide-3-kinase (PI-3-K) pathways.<sup>[50]</sup> In the CNS, mGlu4 receptors have predominantly presynaptic location where act as auto and hetero receptors. They are widely expressed in the striatopallidal synapses within the basal ganglia, a key area involved in motor control as well as in the pathophysiology of Parkinson's disease. Positive modulation of mGlu4 receptors could restore the imbalance among neuronal circuits at the level of the basal ganglia, typical of the Parkinson's disease, improving motor symptoms in both early and late stages, as well as offering neuroprotection in early stages.<sup>[51]</sup> Despite over the past several years, a number of mGlu4 PAMs have been reported (Chart 5),<sup>[52-58]</sup> allosteric modulators were not easy to find and when they were occasionally found they weren't particularly drug-like.

As part of common effort in identifying therapeutics for CNS diseases, the biopharmaceutical company Addex Therapeutics, focusing on the development of small molecules as allosteric modulators, identified a series of tricyclic thiazolopyrazole derivatives with potent and selective mGlu4 PAM affinity. In particular some of these compounds, bearing a six or seven membered central ring and a pyridine or pyrimidine moiety decorating the thiazole ring (Chart 3), were active at nanomolar concentrations ( $EC_{50} = 7 - 9 \text{ nM}$ ).<sup>[23]</sup>

## Chart 5



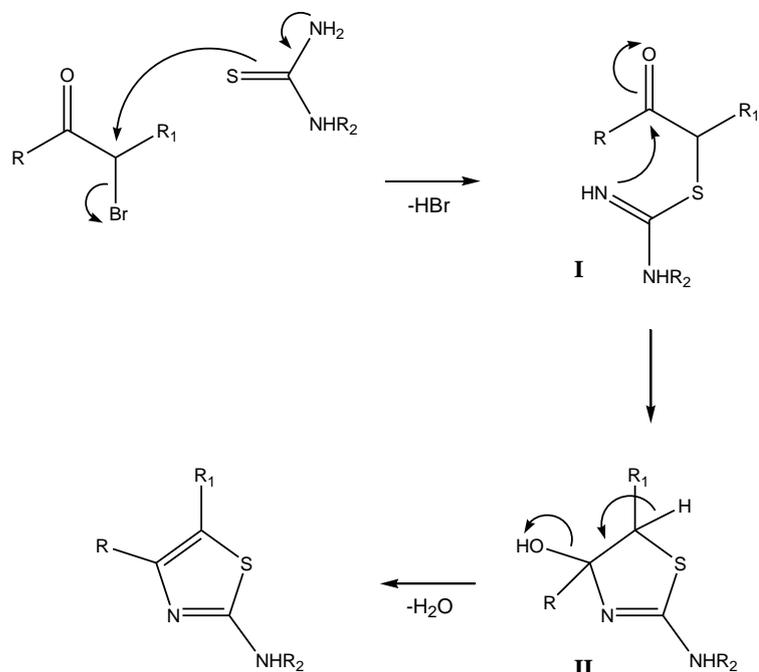
### 3.1 Synthetic approach to 5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amines and 4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amines

In the light of the experience gained from my research team on the synthesis of heterocyclic compounds containing the pyrrole ring and given the growing interest in allosteric modulation, especially on the CNS, the first aim of my research project (Chart 6) was the synthesis of analogues of the compounds owned from Addex Therapeutics (**3**) replacing the pyrazole ring with a pyrrole ring. To approach the synthesis of the target compounds **2**, incorporating an esocyclic ring, at first we focused on the synthesis of 5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amino **5** (Chart 7) then, with the aim to synthesize molecules more similar to the best lead compounds, we planned the synthesis of 4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amines **6** in which the six terms central ring was enlarged to seven. Moreover, in order to increase the number of derivatives and aid in the exploration of the structure-activity relationship (SAR), we planned the synthesis of two series of derivatives: one with an hydrogen atom in  $\alpha$  position to pyrrole



nucleophilic substitution at the ketone to form **II**, which undergoes rapid base-catalyzed elimination resulting in the formation of 2-aminothiozole.<sup>[59]</sup>

**Scheme 1**

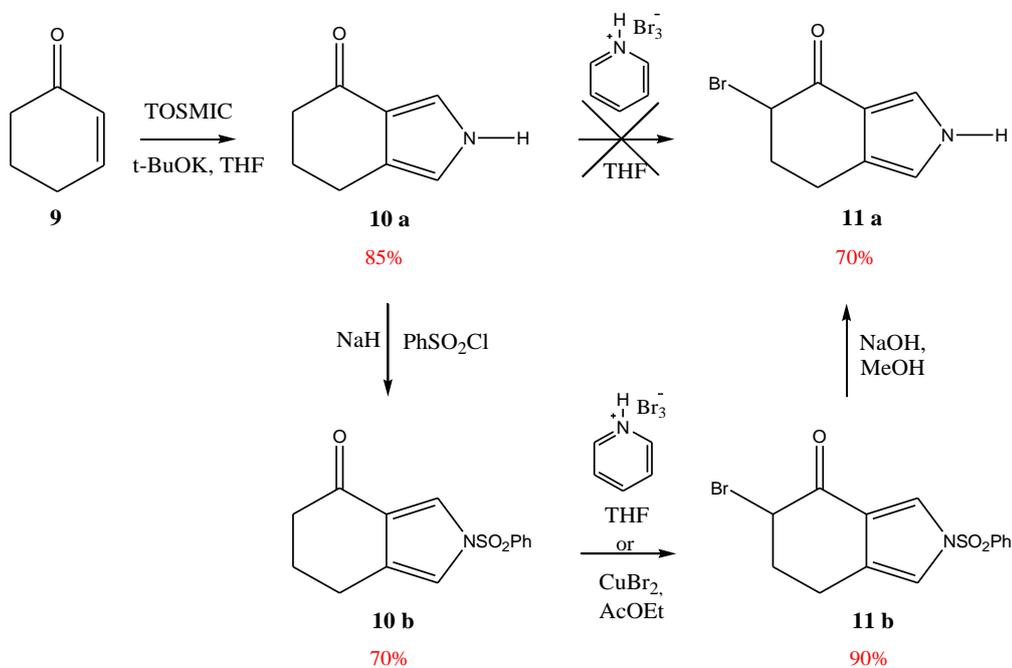


### 3.1.1 Synthesis of 5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amino

The synthesis of the 5-bromo-tetrahydroisoindole-4-one derivative, suitable precursor for the *new ring system* 5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amino **5**, was carried out according to the following synthetic schemes (Schemes 2 and 3). In particular, the reaction of 2-cyclohexen-1-one, a good Michael acceptor, with TOSMIC afforded the unsubstituted tetrahydroisoindole-4-one **10a** in only one step and good yield (85%)<sup>[60]</sup> (Scheme 2). Some preliminary attempts of bromination, using the most common brominating agents, such as bromine, N-bromosuccinimide or copper(II) bromide, revealed the difficulty of performing a  $\alpha$  selective bromination of **7a** because of the higher reactivity of the pyrrole positions. As suggested by the literature<sup>[61]</sup> the presence of an electron withdrawing group to the pyrrole nitrogen atom was crucial to direct the bromination towards the  $\alpha$  position of the carbonyl. Thus we decided to introduce a phenylsulphonyl group obtaining compound **10b** which was easily subjected to bromination to the  $\alpha$  position of the carbonyl. Bromination was carried out either with pyridine hydrobromide perbromide in THF at room temperature or with copper(II) bromide in ethyl acetate at reflux giving compound **11b** with good yield and avoiding other

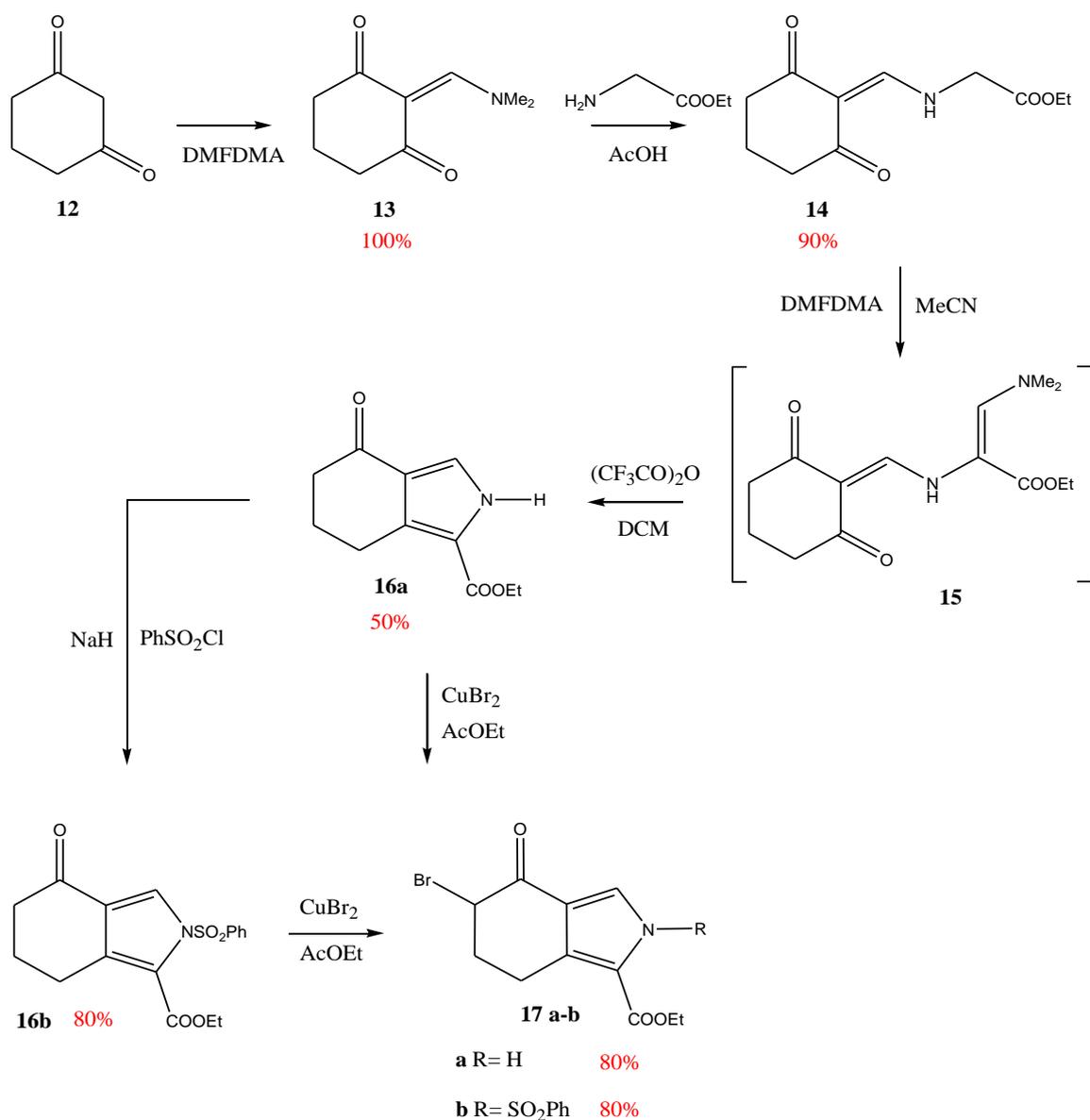
bromination by-products. Finally, deprotection with sodium hydroxide gave the  $\alpha$ -bromo-ketone derivative **11a** bearing the free NH.

**Scheme 2**



Tetrahydroisindole-4-ones belonging to the ethoxycarbonyl series were prepared following a multistep pathway (Scheme 3) starting from 1,3-cyclohexanedione **12** reacted with *N,N*-dimethylformamide dimethyl acetal (DMFDMA) in large excess to obtain the enamino derivative **13**. This latter, was refluxed in glacial acetic acid with glycine ethyl ester hydrochloride leading to compound **14** which was reacted with DMFDMA obtaining the intermediate **15** which was used in the next step without further purification. Finally, the reaction of the previous intermediate with trifluoroacetic anhydride (TFAA) led to compound **16a** according to a proposed mechanism of cyclization reported in literature.<sup>[62]</sup> *N*-phenylsulphonylation was carried out in the same conditions used for the analogues of the unsubstituted series obtaining the derivative **16b**. With regard to the  $\alpha$  bromination, it seems that the ethoxycarbonyl group make less reactive the pyrrole position also in the absence of a electron withdrawing group, driving bromination towards the  $\alpha$  position of the carbonyl. In fact, bromination of both NH and *N*-phenylsulphonyl ketone derivatives **17a** and **17b**, was successfully performed with good yields using copper(II) bromide in ethyl acetate at reflux. Having obtained the *building blocks* for the annelation of 2-amino-5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isindole of both planned series,  $\alpha$ -bromo-ketones of types **11** and **17** were subjected to the next *step* according to the retro-synthetic scheme anticipated in the Chart 7.

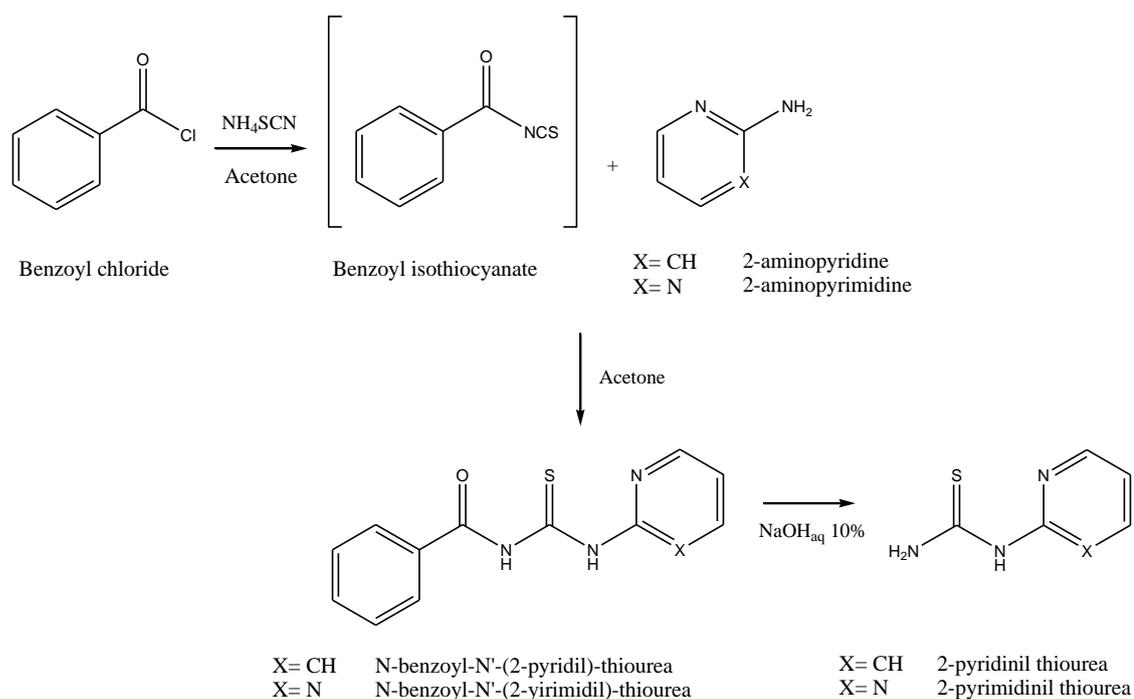
### Scheme 3



At first we decided to explore the reactivity of  $\alpha$ -bromo-ketone derivatives towards unsubstituted thiourea obtaining compounds bearing the free amino group in 2-position of the thiazole ring (Scheme 4). Despite the different “lead-like” decoration, these compounds would be themselves suitable final products as well as potentially prone to functionalization, similarly to lead compounds, through nucleophilic aromatic substitution  $\text{S}_{\text{N}}\text{Ar}$  or Buchwald coupling. The cyclization of the amino thiazole ring on  $\alpha$ -carbonyl systems is widely described in the literature under a variety of conditions. The method we have chosen<sup>[63]</sup> allowed to obtain derivatives **18a-d** in high yields (80-100%) and without any further purification (Table 1). Compounds **11a-b** and **17a-b** were reacted in dry DMF with thiourea

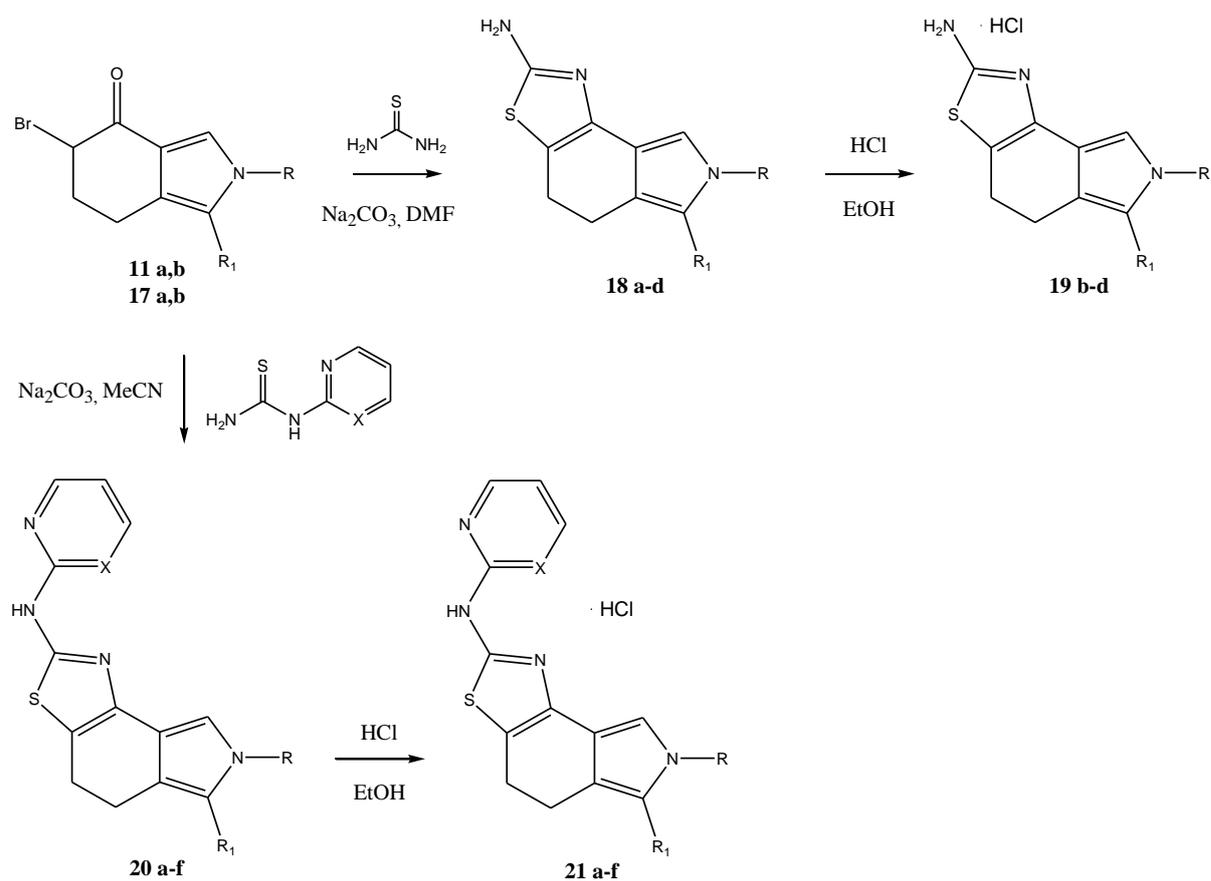
in the presence of an excess of  $\text{Na}_2\text{CO}_3$  at room temperature overnight. Then, pouring the reaction mixture into ice and brine the desired products precipitated, and were collected by filtration. Since some instability phenomenon was observed for the compound **18a** it was excluded from biological evaluation. Furthermore, in order to improve water solubility of synthesized compounds and facilitate biological experiments, we also transformed compounds **18b-d** into the corresponding salified forms **19b-d** by treatment with concentrated hydrochloric acid. With the purpose evaluating the effect on biological activity, the substitution pattern of the exocyclic nitrogen atom was extended mimicking compounds tested by Addex Therapeutics as mGlu4R PAM. Because among these the most active compounds bear 2-pyridyl or 2-pyrimidyl ring, we have chosen to decorate our tricyclic system in the same way. Rather than further functionalize the  $\text{NH}_2$  of compound type **18**, we found more convenient the reaction of bromo derivatives **11a-b** and **17a-b** with 2-pyridinyl and 2-pyrimidinyl thioureas. Synthesis of these heteroarylthioureas was performed with good yields from the corresponding commercially available amines, 2-aminopyridine and 2-aminopyrimidine, following a two-steps procedure reported in literature (Scheme 4).<sup>[64]</sup> It consists of the reaction of benzoyl isothiocyanate, generated in situ from benzoyl chloride and ammoniumthiocyanate, with an appropriate aniline and further debenzoylation of the resultant N-aryl-N'-benzoylthiourea by alkaline hydrolysis.

**Scheme 4**



In our first attempt of obtaining *N*-(pyridin-2-yl) and *N*-(pyrimidin-2-yl) 5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amines we used the same conditions adopted for the synthesis of derivatives of type **18** although, in most cases, a mild heating was necessary. Some of the desired products **20** were isolated with low yields, after a tedious workup procedure and a tricky chromatographic purification. An improvement of yields and a great simplification in the workup and purification was possible using MeCN at reflux instead of DMF. Also in this case, we took into account the transformation of compounds **20a-f** into the corresponding hydrochlorides **21a-f** to facilitate water solubility.

### Scheme 5



**Table 1**

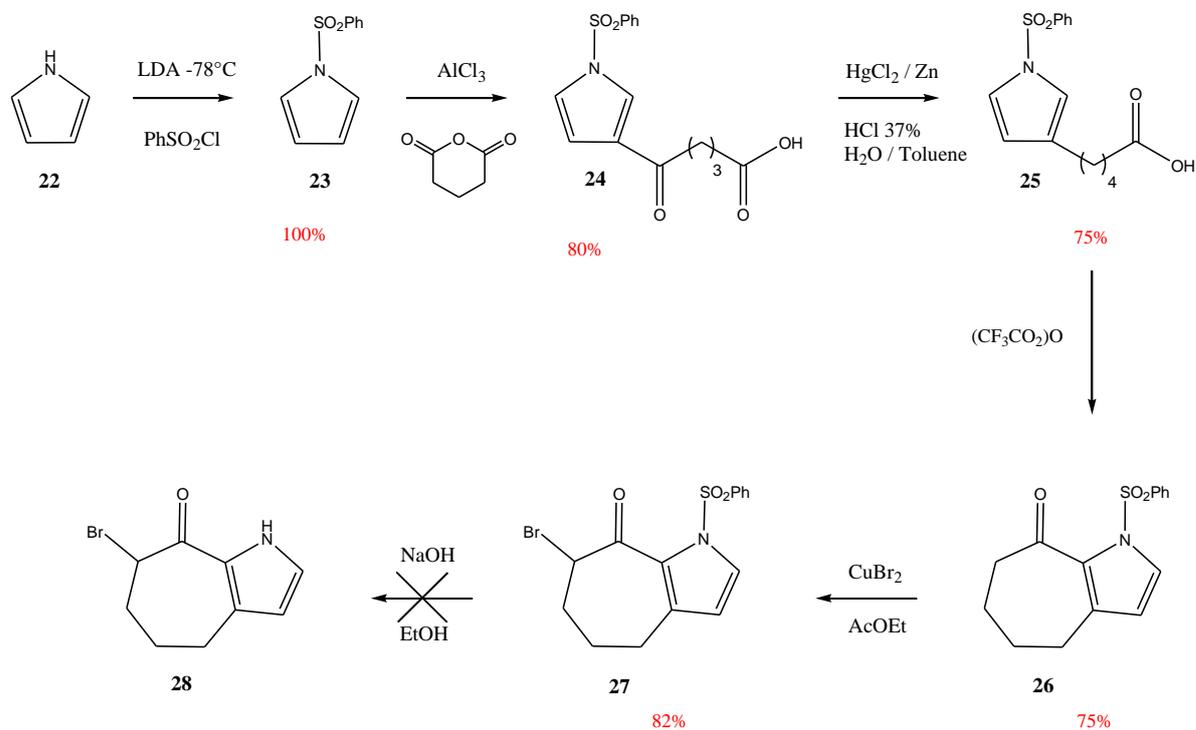
<b>Cmp</b>	<b>R</b>	<b>R<sub>1</sub></b>	<b>X</b>	<b>Yield</b>
<b>18a</b>	H	H	/	80 %
<b>18b</b>	SO <sub>2</sub> Ph	H	/	100 %
<b>18c</b>	H	COOEt	/	100 %
<b>18d</b>	SO <sub>2</sub> Ph	COOEt	/	95 %
<b>19b</b>	SO <sub>2</sub> Ph	H	/	80 %
<b>19c</b>	H	COOEt	/	85 %
<b>19d</b>	SO <sub>2</sub> Ph	COOEt	/	90 %
<b>20a</b>	H	H	CH	45 %
<b>20b</b>	H	H	N	30 %
<b>20c</b>	SO <sub>2</sub> Ph	H	CH	60 %
<b>20d</b>	SO <sub>2</sub> Ph	H	N	50 %
<b>20e</b>	H	COOEt	CH	50 %
<b>20f</b>	H	COOEt	N	45 %
<b>21a</b>	H	H	CH	66 %
<b>21b</b>	H	H	N	78 %
<b>21c</b>	SO <sub>2</sub> Ph	H	CH	100 %
<b>21d</b>	SO <sub>2</sub> Ph	H	N	80 %
<b>21e</b>	H	COOEt	CH	80 %
<b>21f</b>	H	COOEt	N	80 %

### 3.1.2 Synthesis of 4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amines

Considering that the most potent compounds as mGluR4 PAM synthesized by Addex Therapeutics present a seven atom carbocycle ( $n = 2$ , Chart 2), as previously anticipated, we decided to prepare two additional series of compounds with pyrrolo [3', 2' : 6,7] cyclohepta [1,2-*d*][1,3] thiazol-2-amine scaffold, without any substituent at the nitrogen pyrrole ring, in order to maintain a closer similarity to the most potent lead compounds.

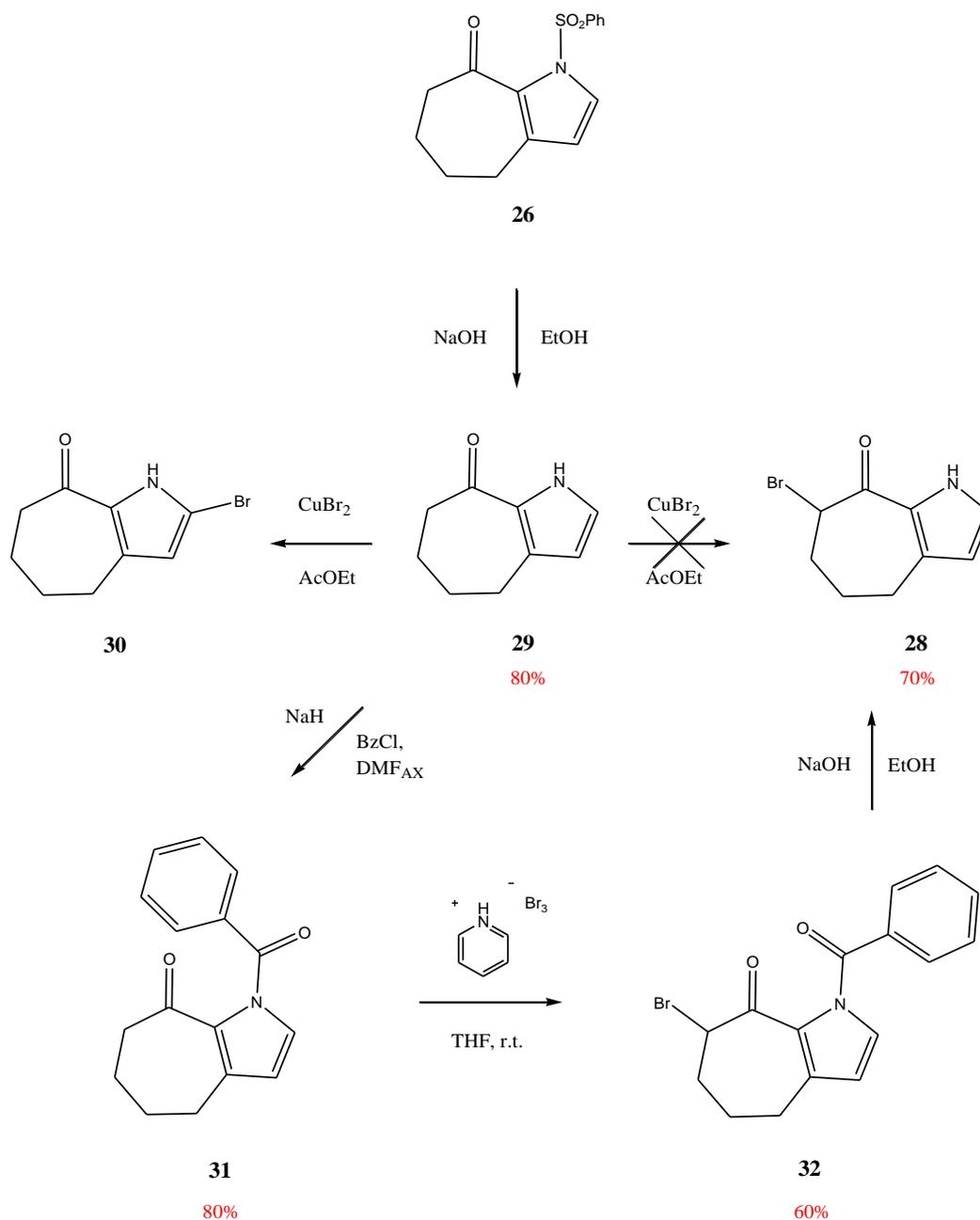
The synthesis of the *new ring system* pyrrolo [3', 2' : 6,7] cyclohepta [1,2-*d*] [1,3] thiazol-2-amine was possible starting from  $\alpha$ -bromo ketones of type **7** ( $n=2$ , Chart 6) which were also in this case valid intermediates to perform the annelation of the aminothiazole ring. The  $\alpha$ -bromo tetrahydrocycloheptapyrrol-8-ones were achieved according to the synthetic pathways illustrated in the Schemes 6,7 and 8. Tetrahydrocycloheptapyrrol-8-one **26** was obtained starting from 1-(phenylsulfonyl)-1*H*-pyrrole **23**, which although commercially available, was prepared in quantitative yield according to the methods used in the past in our group (Scheme 6). Pyrrole dissolved in anhydrous THF at  $-78^{\circ}\text{C}$ , was deprotonated with LDA, and subsequently reacted with benzenesulfonyl chloride to give **22** in quantitative yield. The second step was a Friedel Crafts acylation with glutaric anhydride and  $\text{AlCl}_3$ . Also in this case the reaction proceeds with excellent yields giving **24**. The reduction step was optimized using the Clemmensen reaction, which involves the use of an amalgam of zinc and mercuric chloride in concentrated HCl and toluene at reflux condition, allowing to obtain the desired product **25** in 75% yield. Different attempts were done to avoid the use of mercury salt and to have the access to a more eco-friendly reaction but any of these were successful. For example, the reduction with trifluoroacetic acid and triethylsilane led to a secondary product of pyrrole ring opening and the reduction with  $\text{NaBH}_4$  or with  $\text{H}_2/\text{Pd}$ , did not lead to the desired product. Finally the dehydration reaction with trifluoroacetic anhydride, led to the isolation of **26** (75 %), which in turn was deprotected with NaOH at reflux in ethanol to obtain the NH derivative. Based on what we had previously experienced in the  $\alpha$ -selective bromination of tetrahydroisindole-4-one **10a**, we decided to perform the bromination directly on the N-phenylsulfonyl derivative **26**, and unmask the free NH pyrrole later, on the bromo derivative **27**. As expected, the selective bromination succeeded using copper(II) bromide whereas surprisingly, deprotection of the phenylsulfonyl group was not possible under basic condition because of the degradation of the starting material.

## Scheme 6



So, although aware of the higher reactivity of the pyrrole positions in the absence of electron withdrawing substituents on the nitrogen atom, we tried the bromination directly on the ketone bearing the free NH **29**, which was easily obtained by reaction with NaOH in ethanol at reflux (Scheme 7). Nevertheless, when **29** was reacted with copper(II) bromide, the bromine atom was introduced preferentially at the position 2 of the pyrrole ring rather than the  $\alpha$  position of the carbonyl furnishing **30** as exclusive product. So we decided to insert a different protecting group to the pyrrole nitrogen in order to direct the bromination to the desired position, which could be more easily removed later. Thus, by reaction with NaH and benzoylchloride, a benzoyl group was introduced to furnish ketone **31**, which was then subjected to bromination. However, bromination gave **32** with better yield carried out with pyridine hydrobromide perbromide in THF at room temperature, than with copper(II) bromide in ethyl acetate at reflux (65% versus 18%). The deprotection step, also in this case performed with NaOH solution at room temperature, gave **28** with good yield.

## Scheme 7

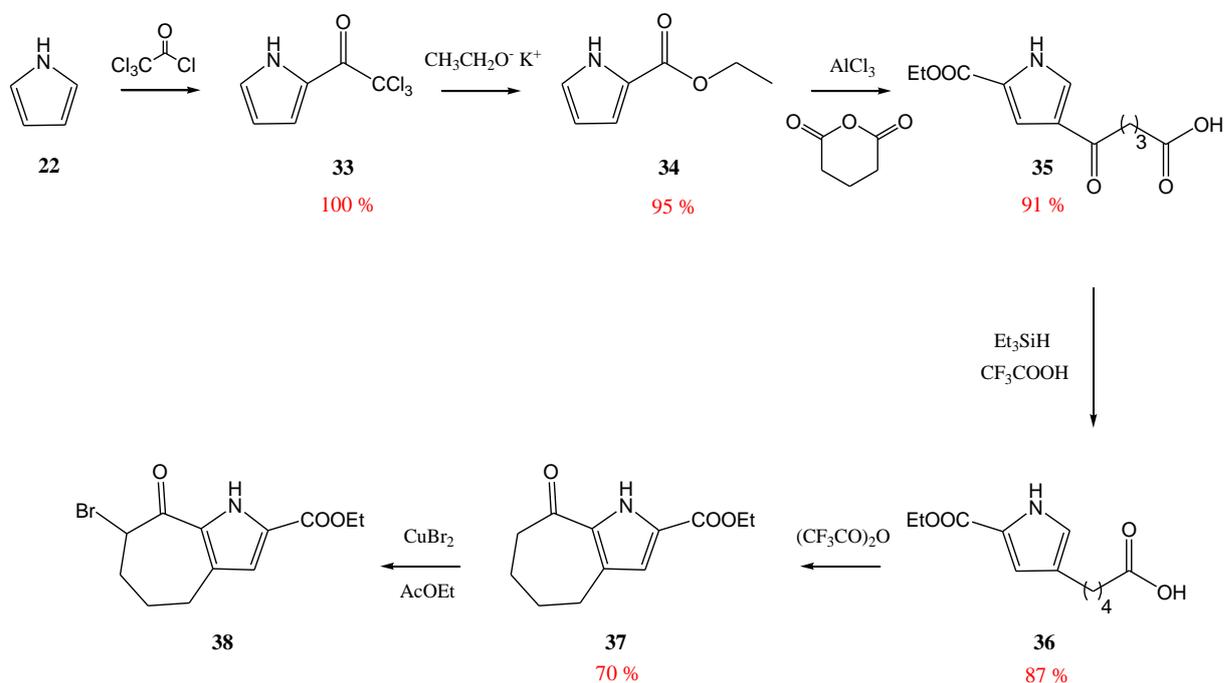


The analogue bromo ketone **38** belonging to the ethoxycarbonyl series was obtained according to the Scheme 8. Pyrrole was reacted with trichloroacetyl chloride obtaining the trichloroacetyl pyrrole **33** in quantitative yield, which in turn undergoes nucleophilic substitution with potassium ethylate giving the ethyl 1*H*-pyrrole-2-carboxylate **34**.<sup>[65]</sup>

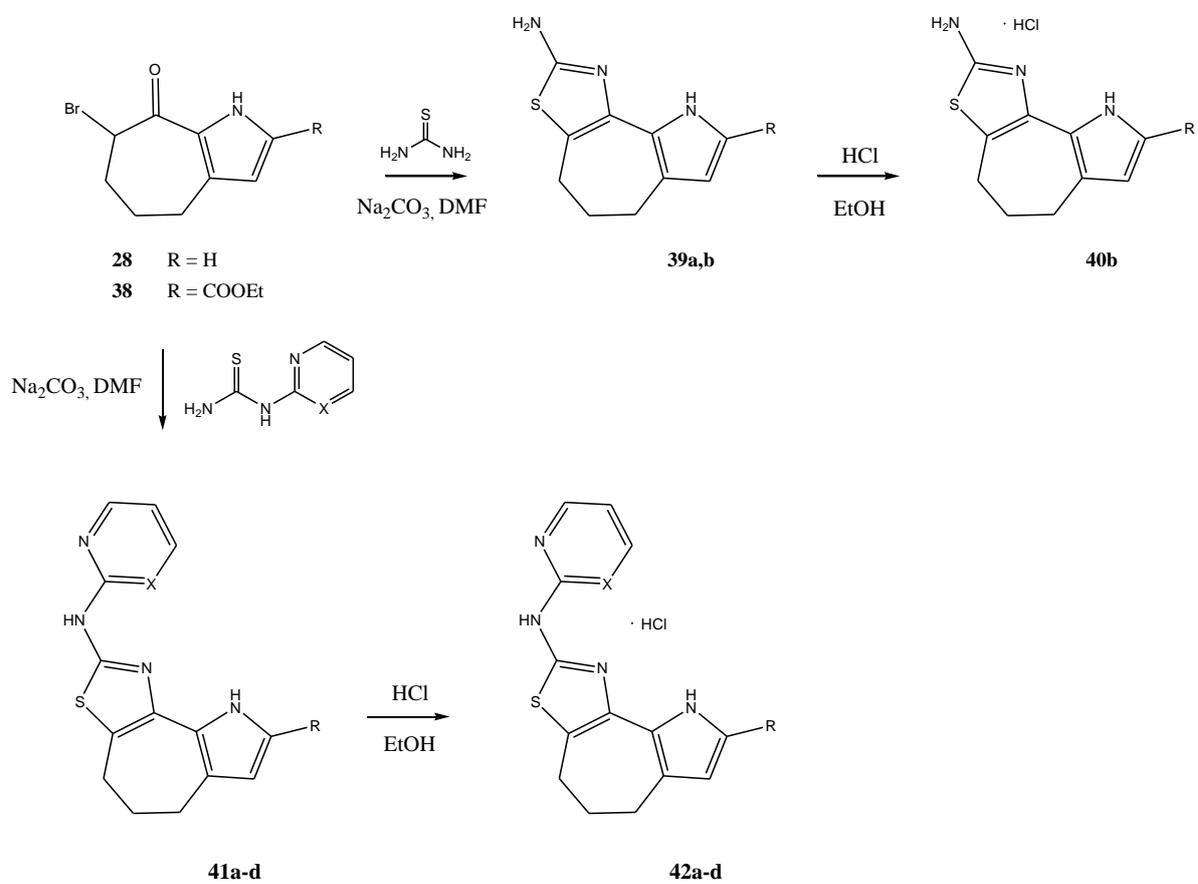
The three position of the pyrrole was acylated by a Friedel-Crafts acylation using AlCl<sub>3</sub> as Lewis acid, and glutaric anhydride as acylating agent. The so obtained derivate **35**, was subsequently subjected to reduction of the carbonyl group to methylene, through the use of triethylsilane in trifluoroacetic acid to give **36**, which in turn was cyclized by dehydration

using an excess of trifluoroacetic anhydride, with the subsequent ring closure to the seven members ring, giving ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate **37** in high yield. As expected, in the presence of the ethoxycarbonyl group that drives the bromination selectively toward the  $\alpha$  position of the carbonyl, bromination proceeded smoothly with copper(II) bromide obtaining compound **38** which was used directly in the next *step* without any purification. Cyclization to aminothiazoles **39** and **41** was carried out in the same condition used for the preparation of six-membered analogues of type **18**, reacting  $\alpha$ -bromo-ketones **28** and **38** with unsubstituted thiourea and heteroarylthioureas, according to the Scheme 9. Upon pouring the reaction mixture into ice and brine the resulted precipitate was purified by column chromatography. Pure compounds **39b** and **41a-d** also in this case were converted into the corresponding hydrochloride forms **40b** and **42a-d** to improve water solubility. Derivative **39a**, in analogy to **18a**, demonstrated quite unstable so it was excluded from the set of tested compounds.

### Scheme 8



## Scheme 9



**Table 2**

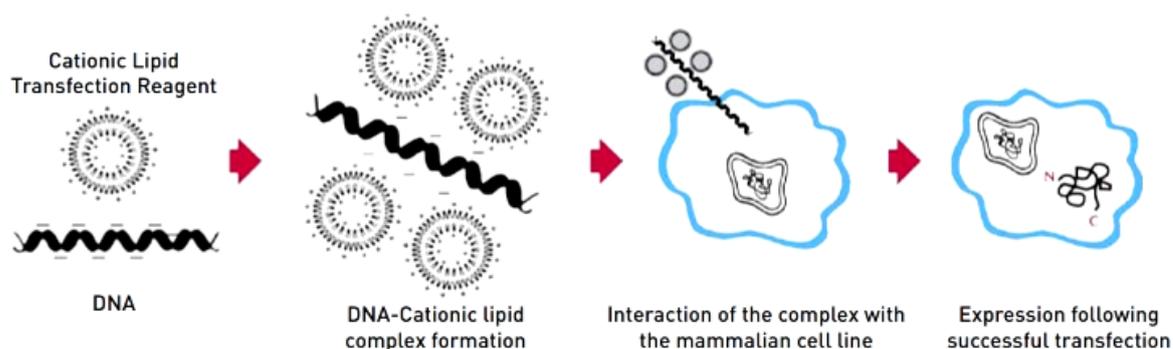
<b>Cmp.</b>	<b>R</b>	<b>X</b>	<b>Yield</b>
<b>39a</b>	H	/	64 %
<b>39b</b>	COOEt	/	52 %
<b>40b</b>	COOEt	/	66 %
<b>41a</b>	H	CH	45 %
<b>41b</b>	H	N	50 %
<b>41c</b>	COOEt	CH	60 %
<b>41d</b>	COOEt	N	60 %
<b>42a</b>	H	CH	79 %
<b>42b</b>	H	N	61 %
<b>42c</b>	COOEt	CH	70 %
<b>42d</b>	COOEt	N	70 %

### 3.2 Biological evaluation

The totality of 30 final compounds were sent to “Neuromed” (Pozzilli, IS, Italy), under the direction of Prof. F. Nicoletti, in collaboration of the department of Human and Physiology and Pharmacology, University of Rome “ La Sapienza” to assess the potential activity on the CNS, in particular toward the activation of mGluR4 since they were designed as analogues of potent and selective PAM of this mGlu receptor subtype. Tests are performed on human embryonic kidney (HEK) 293 cells which express transiently mGlu4 receptors. The expression of mGluR4 is selectively induced by transfection, the process by which specific nucleic acids are introduced into mammalian cells. HEK293 cells are transfected with cDNA encoding for mGlu4 receptor using a lipid transfection method. Specially designed cationic lipids, such as the Lipofectamine® Transfection Reagents, facilitate DNA and siRNA delivery into cells. The basic structure of cationic lipids consists of a positively charged head group and one or two hydrocarbon chains. The charged head group governs the interaction between the lipid and the phosphate backbone of the nucleic acid, and facilitates DNA

condensation (Figure 5). The positive surface charge of the liposomes mediates the interaction of the nucleic acid and the cell membrane, allowing for fusion of the liposome/nucleic acid transfection complex with the negatively charged cell membrane. The transfection complex is thought to enter the cell through endocytosis. Endocytosis is the process where a localized region of the cellular membrane uptakes the DNA/liposome complex by forming a membrane bound/intracellular vesicle. Once inside the cell, the complex must escape the endosomal pathway, diffuse through the cytoplasm and enter the nucleus for gene expression. Cationic lipids are thought to facilitate transfection during the early steps of the process by mediating DNA condensation and DNA/cellular interactions.

**Figure 5 Lipid-based cell transfection**



The expression of mGlu4 receptors is assessed by Western blotting. Since the activation of mGlu4 receptor is associated with the inhibition of adenylyl cyclase activity and the stimulation of the MAPK pathway, mGluR4 receptor-expressing HEK293 cells are used to examine these pathways. After transfection, cells are incubated with tested compounds and the measurement of Forskolin (FSK)-stimulated cAMP formation and of phosphorylated ERK1/2 are carried out according to the protocols. The selective agonist L-2-amino-4-phosphonobutyric acid (L-AP4) is used as reference drug.

The group of the University of Rome selected compounds presenting the most similar decoration of lead compounds of type **3** (Chart 4), bearing the pyridine and pyrimidine moieties, and the hydrochlorides were used. Thus, **42a-c**, as first set of compounds, followed by **21a** and **21e** were tested at increasing concentrations (1 nM - 10  $\mu$ M) on HEK293 transfected cells. Herein, I report the experimental protocol set for these preliminary studies (Table 3 and Table 4). At the moment studies of **21b-d** and **21f** are in progress, as well as the evaluation of cAMP level at Neuromed.

**Table 3**

<b>Samples</b>	<b>Drugs</b>	<b>Samples</b>	<b>Drugs</b>
1-4	Basal	1-4	Basal
5-9	FSK	5-9	FSK
10-12	FSK + L-AP4 200 $\mu$ M	10-12	FSK + L-AP4 200 $\mu$ M
3-15	FSK + <b>42a</b> 1 nM	3-15	FSK + <b>42b</b> 1 nM
16-18	FSK + <b>42a</b> 10 nM	16-18	FSK + <b>42b</b> 10 nM
19-21	FSK + <b>42a</b> 100 nM	19-21	FSK + <b>42b</b> 100 nM
22-24	FSK + <b>42a</b> 1 $\mu$ M	22-24	FSK + <b>42b</b> 1 $\mu$ M
25-27	FSK + <b>42a</b> 10 $\mu$ M	25-27	FSK + <b>42b</b> 10 $\mu$ M
29-30	FSK + <b>42c</b> 1 nM	29-30	FSK + <b>42d</b> 1 nM
31-33	FSK + <b>42c</b> 10 nM	31-33	FSK + <b>42d</b> 10 nM
34-36	FSK + <b>42c</b> 100 nM	34-36	FSK + <b>42d</b> 100 nM
37-39	FSK + <b>42c</b> 1 $\mu$ M	37-39	FSK + <b>42d</b> 1 $\mu$ M
40-42	FSK + <b>42c</b> 10 $\mu$ M	40-42	FSK + <b>42d</b> 10 $\mu$ M
43-45	<b>42a</b> 10 $\mu$ M	43-45	<b>42b</b> 10 $\mu$ M
46-48	<b>42c</b> 10 $\mu$ M	46-48	<b>42d</b> 10 $\mu$ M

[FSK]= 5 x 10<sup>-5</sup> M; Volume sample= 220 ml; Stimulus duration= 20 min

**Table 4**

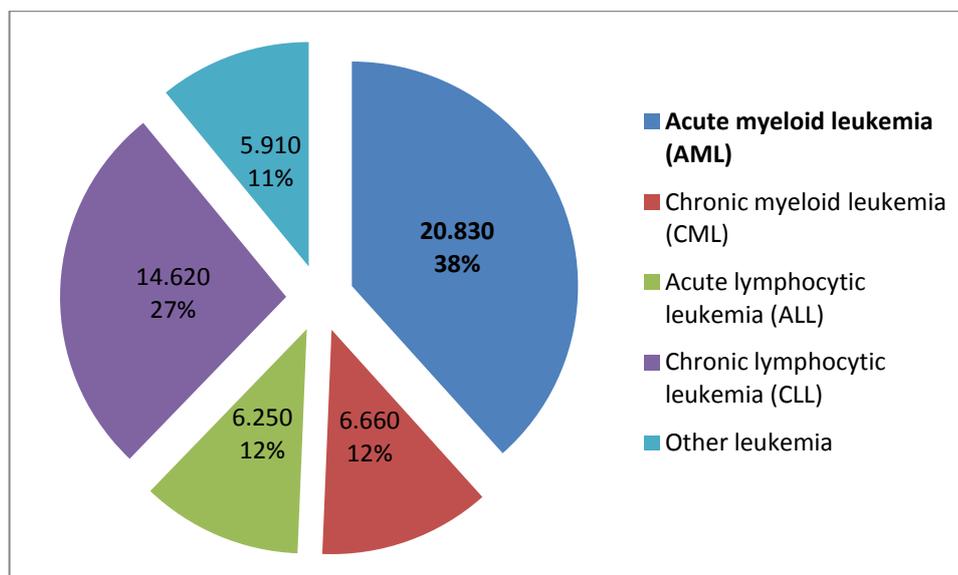
<b>Samples</b>	<b>Drugs</b>
1-4	Basal
5-9	FSK
10-12	FSK + L-AP4 200 $\mu$ M
3-15	FSK + <b>21a</b> 1 nM
16-18	FSK + <b>21a</b> 10 nM
19-21	FSK + <b>21a</b> 100 nM
22-24	FSK + <b>21a</b> 1 $\mu$ M
25-27	FSK + <b>21a</b> 10 $\mu$ M
29-30	FSK + <b>21e</b> 1 nM
31-33	FSK + <b>21e</b> 10 nM
34-36	FSK + <b>21e</b> 100 nM
37-39	FSK + <b>21e</b> 1 $\mu$ M
40-42	FSK + <b>21e</b> 10 $\mu$ M
43-45	<b>21a</b> 10 $\mu$ M
46-48	<b>21e</b> 10 $\mu$ M

[FSK]= 5 x 10<sup>-5</sup> M; Volume sample= 220 ml; Stimulus duration= 20 min

#### 4. PYRROLO BENZOTHAZOLES AS ANTICANCER DRUGS

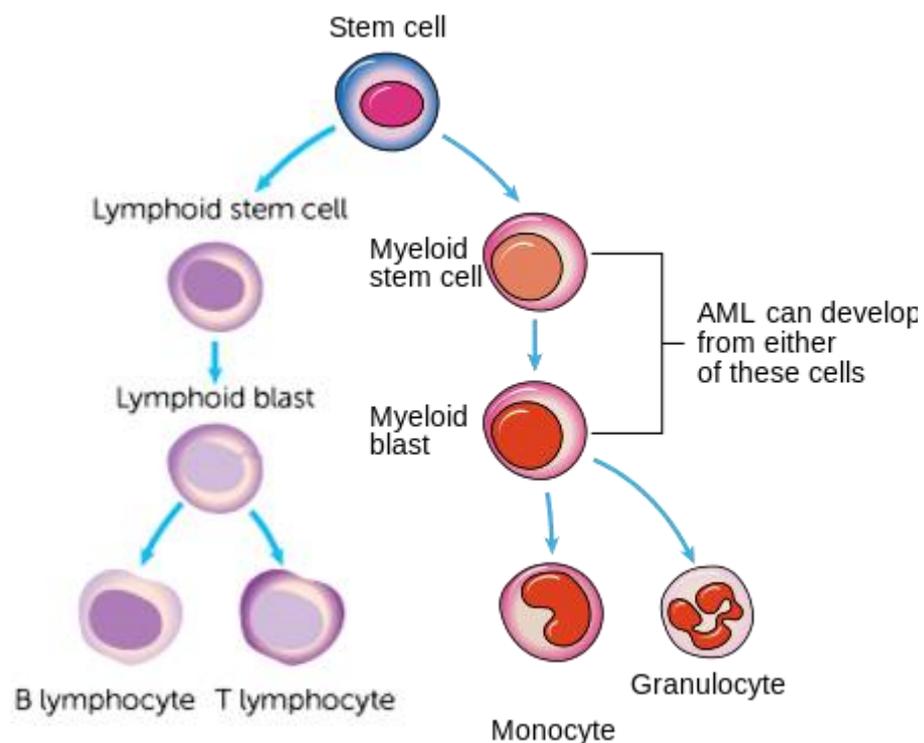
Acute myeloid leukemia (AML), also known as acute myelogenous leukemia, is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. Although AML is a relatively rare disease, accounting for roughly 1.3% of all new cancer cases in the United States, its incidence is expected to increase as the population ages.<sup>[66]</sup> According to the American Cancer Society in their annual report “Cancer Facts & Figures 2015”, AML is the most common type of leukemia diagnosed in both sexes. This year, an estimated 20.830 people of all ages (12.730 men and boys and 8.100 women and girls) in the United States will be diagnosed with AML. Figure 6 shows a breakdown of the number of cases specific to each group of leukemia and what percentage of expected leukemia cases the group makes up. The median age at diagnosis is 67 years and the prognosis for individual patients varies greatly but is generally poor, in fact the 5-year survival rate, which is the percentage of people who live at least 5 years after diagnosis, is about 25% based on 2008-2012 cases and deaths.

**Figure 6. Estimated new cases of leukemia in the United States in 2015.**



The malignant cell in AML is the myeloblast (Figure 7). In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells which will gradually mature into a mature white blood cell. In AML the myeloblast accumulates genetic changes which “freeze” the cell in its immature state and prevent differentiation. Such a mutation alone does not cause leukemia, however, when such a “differentiation arrest” is combined with other mutations which hyperactivate the signal transduction pathways promoting proliferation, the result is the uncontrolled growth of an immature clone of cells, called leukemic blasts, leading to the clinical entity of AML.<sup>[67]</sup>

**Figure 7. Illustration of blood cells maturing from stem cells.**

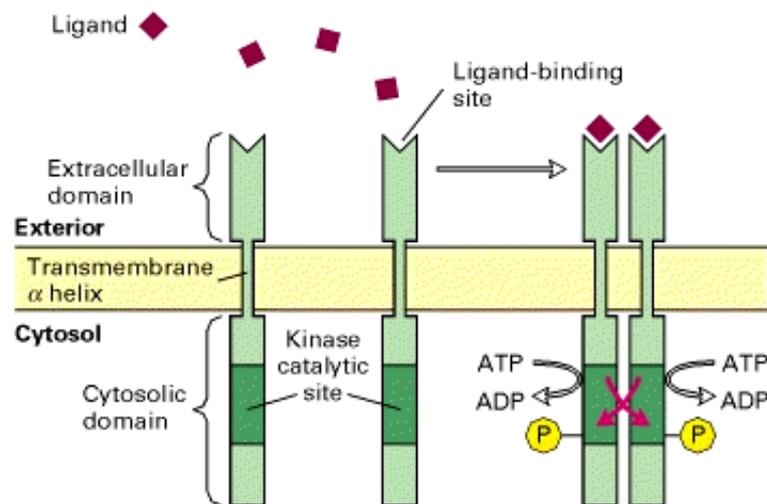


The most common mutations in AML occur in the FMS-like tyrosine kinase 3 (FLT3) gene which promotes cell growth and proliferation.<sup>[68]</sup> Mutations are present in up to 30% of AML patients, implicating FLT3 as a potential target for kinase inhibitor therapy.<sup>[69]</sup> FLT3 protein is a member of receptor-tyrosine kinase (RTK) family. RTKs are the high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones. Binding of a ligand to this type of receptor stimulates the receptor’s intrinsic protein-tyrosine kinase activity, which subsequently stimulates a signal-transduction cascade leading to changes in cellular physiology and/or patterns of gene expression. RTK signaling pathways have a wide spectrum of functions including regulation of cell proliferation and differentiation, promotion of cell survival and modulation of cellular metabolism. RTKs have been shown not only to be

key regulators of normal cellular processes but also to have a critical role in the development and progression of many types of cancer. In fact, therapeutic approaches based on compounds targeting selectively oncogenic RTKs, to which cells are dependent, are constantly developed.<sup>[70]</sup>

All RTKs consist of an extracellular domain containing a ligand-binding site, a single hydrophobic transmembrane  $\alpha$  helix, and a cytosolic domain that includes a region with protein-tyrosine kinase activity (Figure 8). The biochemical activity of a kinase involves transferring a phosphate group from a nucleoside triphosphate (usually ATP) and covalently attaching it to specific amino acids with a free hydroxyl group like a tyrosine. Binding of ligand causes most RTKs to dimerize; the protein kinase of each receptor monomer then phosphorylates a distinct set of tyrosine residues in the cytosolic domain of its dimer partner, a process called *autophosphorylation*. Autophosphorylation occurs in two stages. First, tyrosine residues in the phosphorylation lip near the catalytic site are phosphorylated. This leads to a conformational change that facilitates binding of ATP in some receptors (e.g., the insulin receptor) and binding of protein substrates in other receptors (e.g., FGF receptor). The receptor kinase activity then phosphorylates other sites in the cytosolic domain; the resulting phosphotyrosines serve as docking sites for other proteins involved in RTK-mediated signal transduction.<sup>[71]</sup>

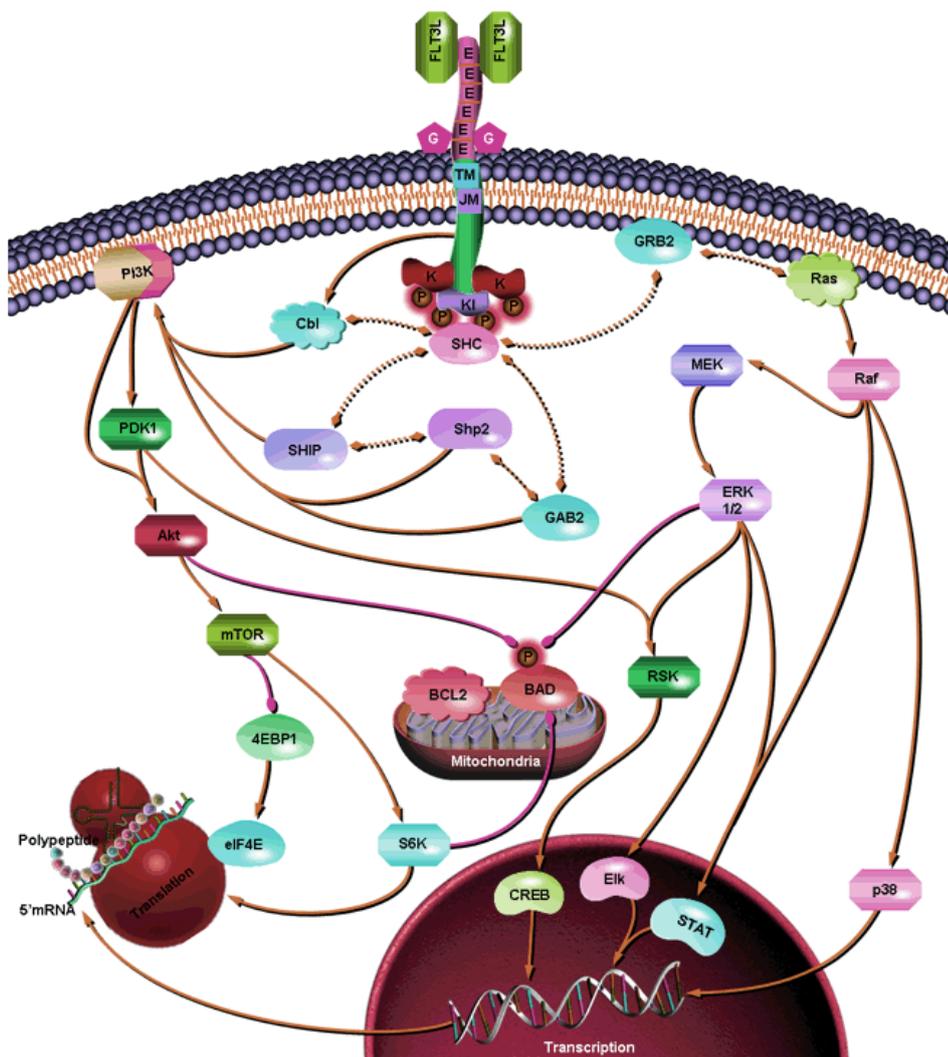
**Figure 8. Structure and activation of RTKs**



Approximately 20 different RTK classes have been identified and FLT3 belongs to the class III RTK sharing a high degree of structural homology with the other members of the same class as such as KIT, FMS and Platelet-Derived Growth Factor (PDGF) receptors. FLT3

contains five extracellular immunoglobulin-like domains (E), a transmembrane (TM) domain, a juxtamembrane (JM) domain and two tyrosine-kinase domains (K) that are linked through KI (tyrosine-kinase insert). Cytoplasmic FLT3 undergoes glycosylation (G), which promotes localization of the receptor to the membrane. The ligand for FLT3 (FLT3L) binds the receptor and induces receptor dimerization which in turn promotes phosphorylation of K, thereby activating the receptor and downstream effectors. (Figure 9)

**Figure 9. FLT3 signaling pathways**



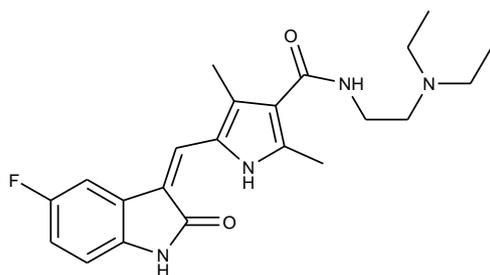
Although the FLT3 signaling cascade has not been definitively characterized, the binding of FLT3L to FLT3 triggers multiple downstream signaling pathways including the PI3K (Phosphatidylinositol 3-Kinase) and Ras/mitogen-activated protein kinase (MAPK) pathways, leading to increased cell proliferation and the inhibition of apoptosis. Activated FLT3 also associates with GRB2 (Growth Factor Receptor-Bound Protein-2) that activates Ras and

stimulates downstream effectors such as Raf, MEK (MAPK/ERK Kinases), p38, ERK1/2 (Extracellular-signal Regulated Kinase), and RSK (Ribosomal protein S6 Kinase). These downstream effectors activate CREB (cAMP Response Element Binding protein), Elk and STAT (Signal Transducer and Activators of Transcription), which lead to the transcription of FLT3 mRNA and translation to FLT3 protein.<sup>[72]</sup> Mutant FLT3 is often expressed at higher levels and is associated with ligand-independent autophosphorylation and activation of downstream signaling. The most common activating mutations of FLT3 are internal tandem duplication (ITD) and point mutations.<sup>[73]</sup> Both genetic aberrations were found in some acute myeloid leukemia cell lines and may lead to the constitutive activation of the receptor, providing the molecular basis for a persisting growth stimulus.<sup>[74]</sup>

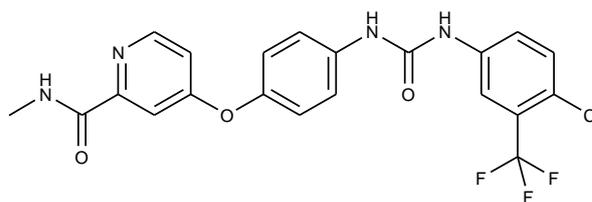
- Point mutations occur in approximately 7% of AML new cases. Most frequently mutations are observed at codon 835 where a tyrosine residue replaces aspartic acid, stabilizing the receptor in the ATP-bound configuration and promoting constitutive activation.<sup>[75]</sup>
- ITD mutations, impacting the JM portion of the receptor may be of variable length and location and occur in approximately 23% of patients with de novo AML. FLT3-ITD mutations are associated with a much higher relapse rate, a poor response to therapy and outcome, but patients harboring these mutations may be more responsive to FLT3-directed therapies.<sup>[76]</sup>

Therefore, FLT3 has been considered a valid therapeutic target in AML treatment and many pharmaceutical companies and research institutes have been involved in the discovery of FLT3 inhibitors. Early FLT3 inhibitors (Chart 8) including **Sunitinib** (SU-11248), **Sorafenib** (BAY-43-9006), **Midostaurin** (PKC412), and **Lestaurtinib** (CEP-701), demonstrated promising in preclinical models of FLT3 mutant AML<sup>[77-80]</sup>. Unfortunately many of these compounds are found to be not optimal for treatment of AML.<sup>[81]</sup> For example, first clinical trials evaluating safety and efficacy of sunitinib in AML, showed a transient antileukemic effect, but this was limited by short duration and significant toxicities.<sup>[82]</sup> Moreover, Sorafenib has demonstrated less toxicity and a reliable reduction blast count, particularly in patients harboring FLT3-ITD, although a clinical benefit has not been established.<sup>[83]</sup>

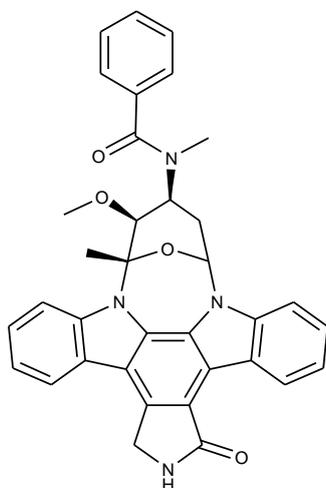
## Chart 8



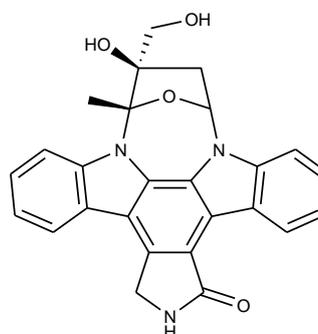
**Sunitinib (SU-11248)**



**Sorafenib (BAY-43-9006)**



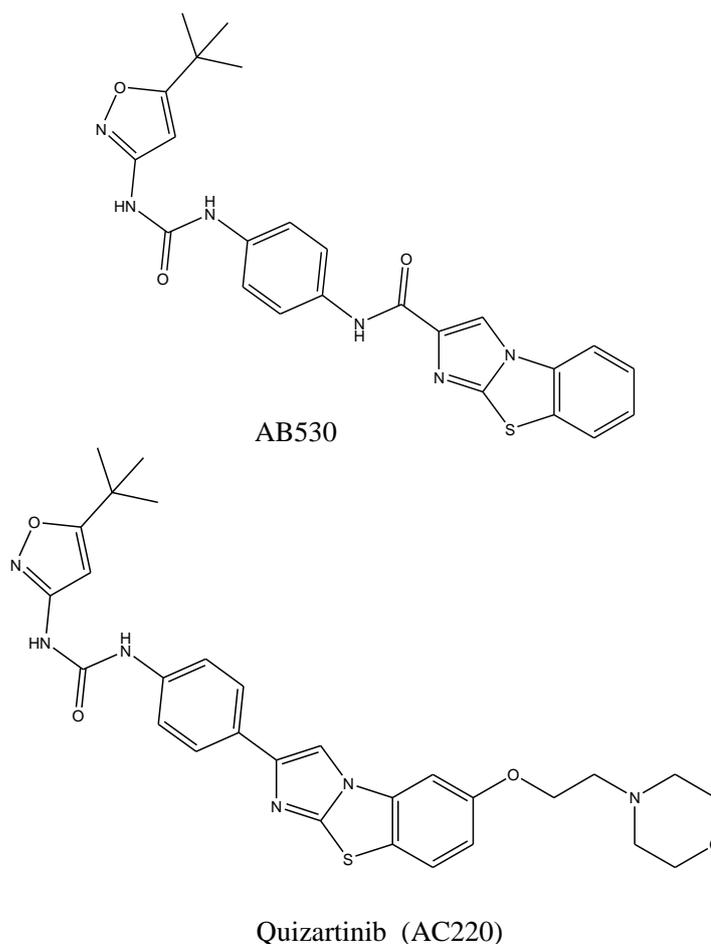
**Midostaurin (PKC412)**



**Lestaurtinib (CEP-701)**

This led to the development of the second generation FLT3 inhibitors represented by quizartinib (AC220) (Chart 9). Quizartinib was identified from a lead optimization study starting from compound AB530 an extremely potent and selective FLT3 inhibitor. However its aqueous solubility and oral pharmacokinetic properties were not promising for clinical development. Removal of the carboxyamido group and the addition of a water solubilising group led to quizartinib which not only retained potency and selectivity of AB530 but also showed improved solubility and pharmacokinetic profile. Clinical trials revealed an acceptable toxicity profile and enhanced clinical activity in FLT3-ITD, although reversible QT prolongation, which may be related to off targets effects on other RTKs, emerged as the most relevant side effect.<sup>[84]</sup>

## Chart 9

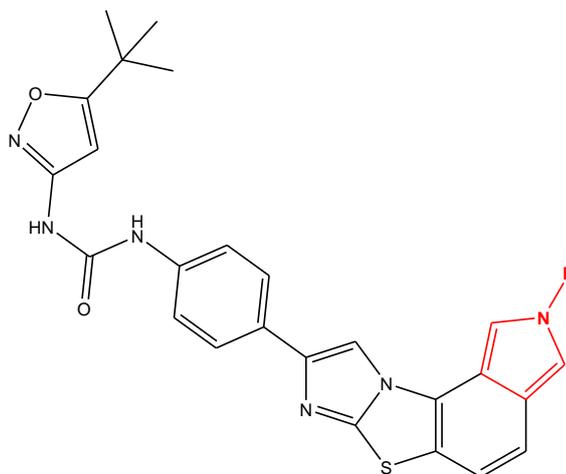


### 4.1 Synthesis of 1-(5-*tert*-butyl-1,2-oxazol-3-yl)-3-[4-substituted(4,5-dihydro-2*H*-imidazo [2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl)phenyl]ureas

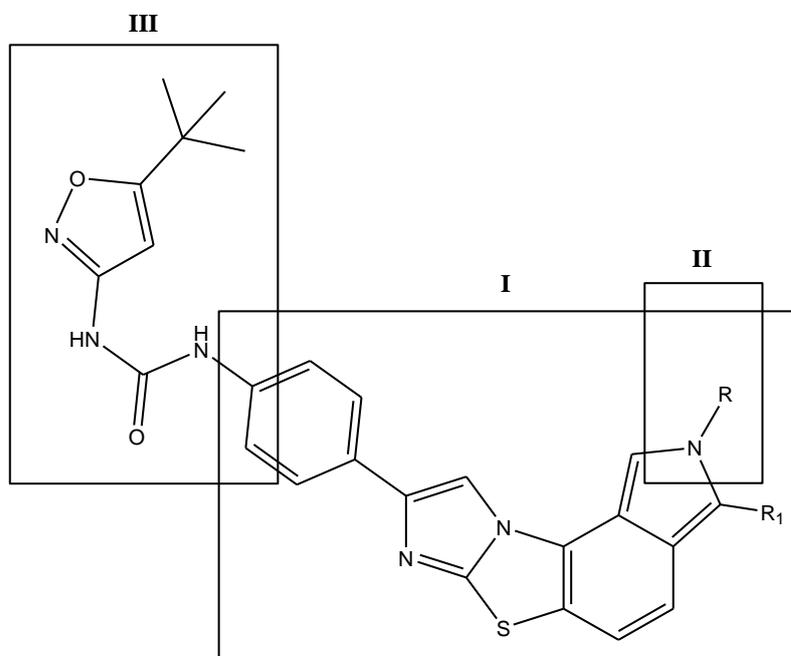
In the light of developing new therapeutic candidates against AML we have planned the synthesis of a novel series of FLT3 inhibitors as analogues of quizartinib. As previously mentioned, we thought to a new tetracyclic ring system in which a pyrrole unit is condensed to the imidazobenzothiazole scaffold of quizartinib. Moreover the pyrrole nitrogen atom allows the introduction of water solubilising groups for a closer mimicking AC220 decoration. At first we focused on isoindole derivatives type **43** (Chart 10) but we reserve to evaluate the effect of different condensation of pyrrole in the tetracyclic system.

Our synthetic strategy was first the synthesis of the new ring system with the tetracyclic core (**I**), then the introduction of water solubilising groups at the pyrrole nitrogen atom (**II**) and finally the introduction of the ureido moiety (**III**).

## Chart 10



43



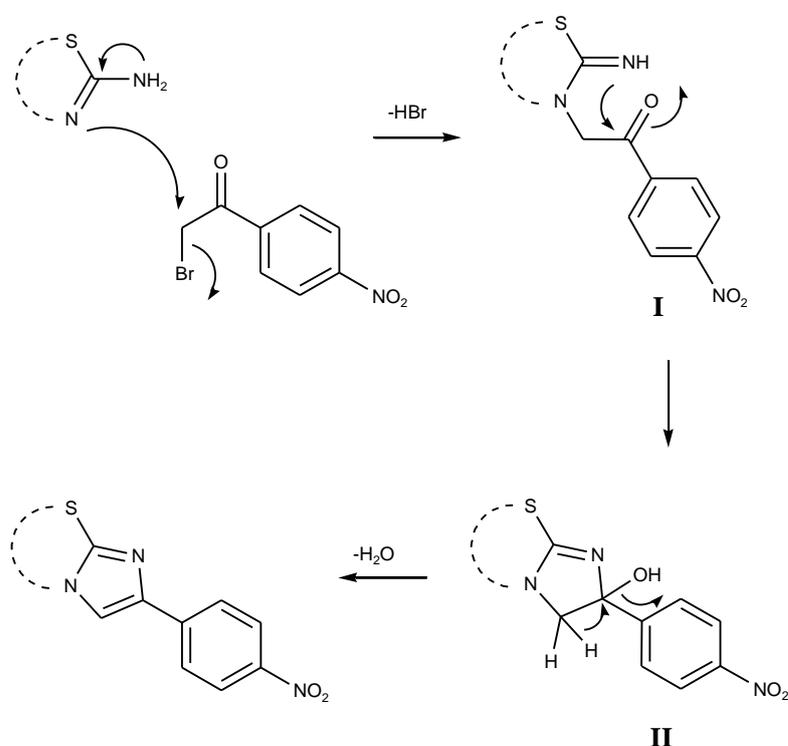
R = water solubilizing group

R<sub>1</sub> = H or COOEt

2-Amino-benzothiazole derivatives of type **18** (Scheme 5), previously prepared as final product in the first branch of the project, represented suitable *building blocks* to synthesize the tetracyclic core by reaction with bromo *p*-nitroacetophenone. Having already obtained 2-amino-benzothiazole of two different series, in order to increase the number of derivatives, we thought to use both of them as substrate in the cyclization to imidazole ring to create also a

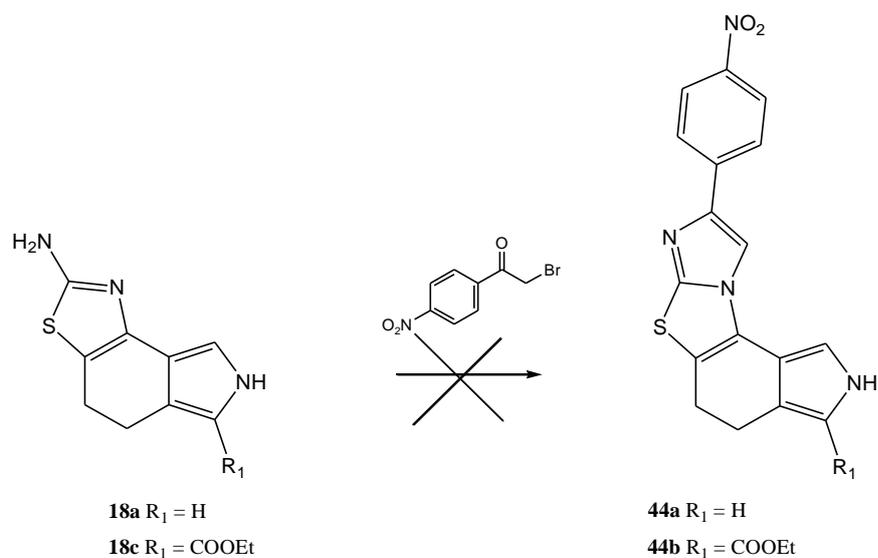
series of analogues of quizartinib bearing an ethoxycarbonyl functionality. Proposed mechanism for the synthesis of fused imidazo heterocycles is depicted in Scheme 10. The first step is the formation of an acyclic intermediate (**I**) by the nucleophilic attack of nitrogen of thiazole ring on the bromine-carrying carbon of bromo-*p*-nitroacetophenone resulting in the formation of the N - C bond with simultaneous transfer of the amino lone pair accompanied by the elimination of HBr. In the next step, an attack by the lone pair from imino nitrogen atom to the carbonyl yields a cyclic intermediate (**II**) which loses a water molecule leading to the formation of fused imidazoderivatives.

**Scheme 10**



At first we thought it would be better to use derivatives **18a** and **18c** (Table 1), bearing free pyrrole nitrogen, because if the cyclization to imidazole ring was successful we would have the tetracyclic substrates to be readily functionalized at the pyrrole nitrogen atom. Unfortunately, when these compounds were reacted with bromo *p*-nitroacetophenone heating up to reflux in ethanol<sup>[85]</sup> (Scheme 11) no condensation products were obtained, despite several attempts were made (Table 5) changing solvent, temperature,<sup>[86, 87]</sup> time and other reaction conditions, such as the use of microwave irradiation,<sup>[88]</sup> catalytic amount of bases or acids.<sup>[89, 90]</sup>

**Scheme 11**



**Table 5**

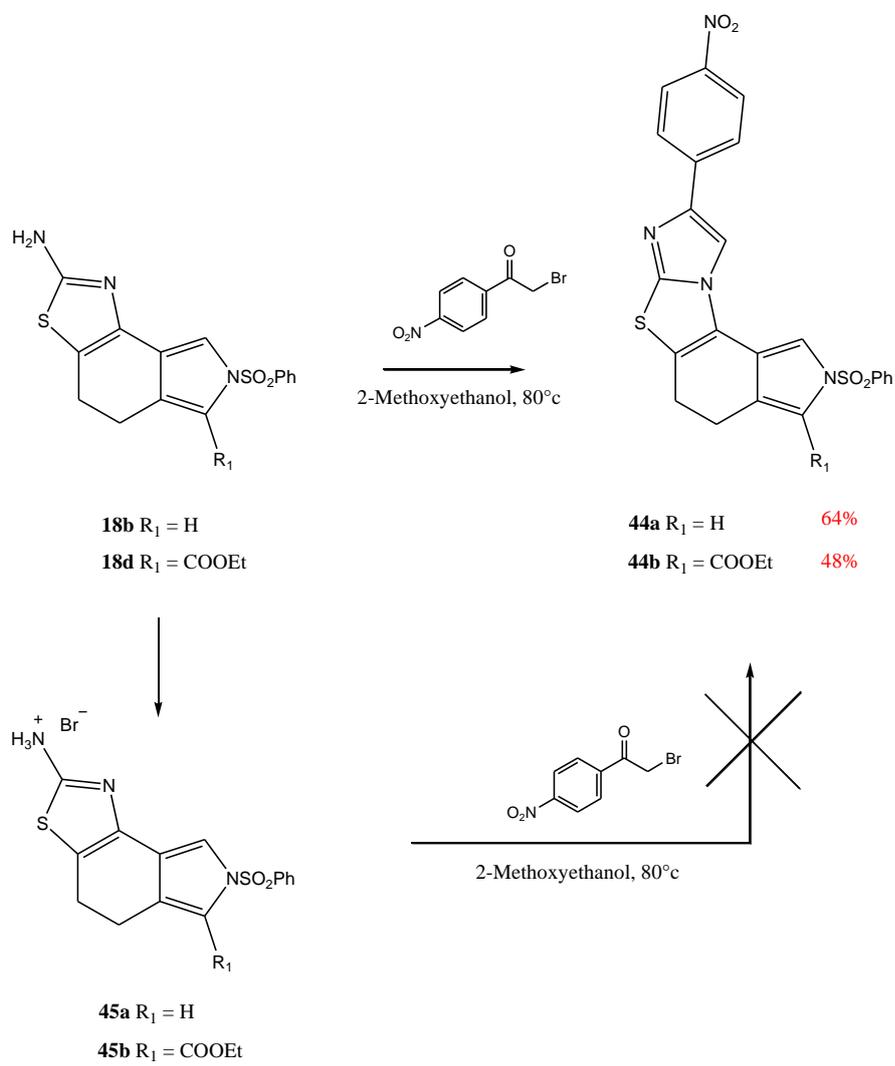
<b>Cmp.</b>	<b>Reactant (eq.)</b>	<b>Acid/Base (eq.)</b>	<b>Solvent</b>	<b>Temperature</b>	<b>Time</b>
<b>18a</b>	2	-	EtOH	reflux	16h
<b>18a</b>	1.1	AcOH (1.1)	EtOH	reflux	16h
<b>18a</b>	2	NaHCO <sub>3</sub> (1.1)	<i>i</i> -PrOH	reflux	16h
<b>18a</b>	2	-	Acetone	reflux	16h
<b>18a</b>	2	NaHCO <sub>3</sub> (1.1)	MeCN	60 °C	24h
<b>18a</b>	1.1	NEt <sub>3</sub> (1.5)	MeCN	60 °C	48h
<b>18a</b>	2	-	Toluene	r.t.	48h
<b>18c</b>	2	-	EtOH	reflux	48h
<b>18c</b>	1.1	-	EtOH	100 °C (MW)	4 x 30'
<b>18c</b>	1.1	AcOH (1.1)	EtOH	reflux	48h
<b>18c</b>	1.1	NEt <sub>3</sub> (1.5)	EtOH	reflux	16h
<b>18c</b>	2	-	Acetone	reflux	16h
<b>18c</b>	2	-	Toluene	reflux	24h
<b>18c</b>	2	-	2-Methoxyethanol	100 °C	16h

Thus, we considered that the presence of a bulky electron withdrawing group could have a positive effect in the desired cyclization. We investigated the imidazole ring closure on the corresponding phenylsulfonyl derivatives prepared as described in the SNC section (Scheme 5 and Table 1). As a result, heating compound **18b** and **18d** with bromo *p*-nitroacetophenone in different solvents (ethanol, acetone, 2-methoxyethanol, acetonitrile, dimethylformamide) we finally managed to isolate the tetracyclic derivatives **44a** and **44b** (Scheme 12). Despite the reaction has never reached completeness in any of the above solvents, neither increasing the temperature nor the stoichiometric ratio with the reagent, the best results were obtained using 2-methoxyethanol at 80 °C. In this condition the desired products were collected as yellow solids by filtration, without any further purification, with good yields. The starting material was recovered as salt, probably hydrobromide formed by mean of HBr developed during the reaction. However, we observed that the salified forms of **18b** and **18d** were not able to give the corresponding fused imidazoderivatives even in the presence of bases such as (Et)<sub>3</sub>N, Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub>.

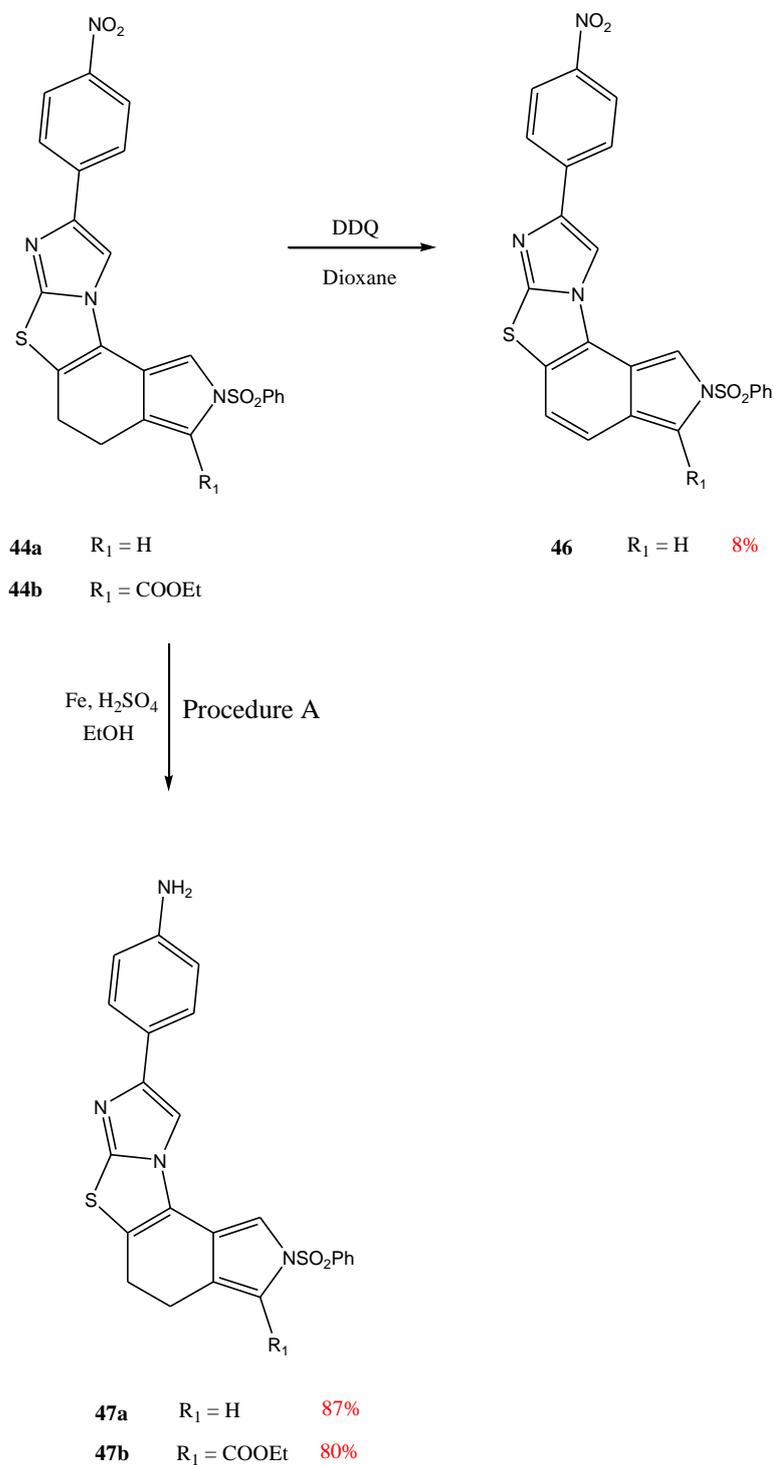
Considering that our compounds were supposed to be fully aromatic in analogy to the lead compound quizartinib we attempted the oxidation of derivative **44a** using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane at 80 °C (Scheme 13). Unfortunately compound **46** was isolated only in traces making difficult further steps. Another aromatization attempt with palladium on carbon in dioxane at reflux didn't lead to the desired product. However, some preliminary computational studies, based on quizartinib docked within FLT3 kinase domain,<sup>[91]</sup> showed that a completely aromatic system was not a necessary condition for proper binding as likely the imidazothiazole system is primarily involved in pi-interactions within a wide hydrophobic pocket. So, thinking that the lack of an unsaturation would not have affected the potential activity of our compounds, we decided to use the dihydro substrates **44a** and **44b** in the next steps.

Reduction of the nitro group was performed using iron and catalytic amount of sulphuric acid in ethanol obtaining the amino derivatives **47a** and **47b** (Scheme 13, Procedure A).<sup>[92]</sup>

## Scheme 12



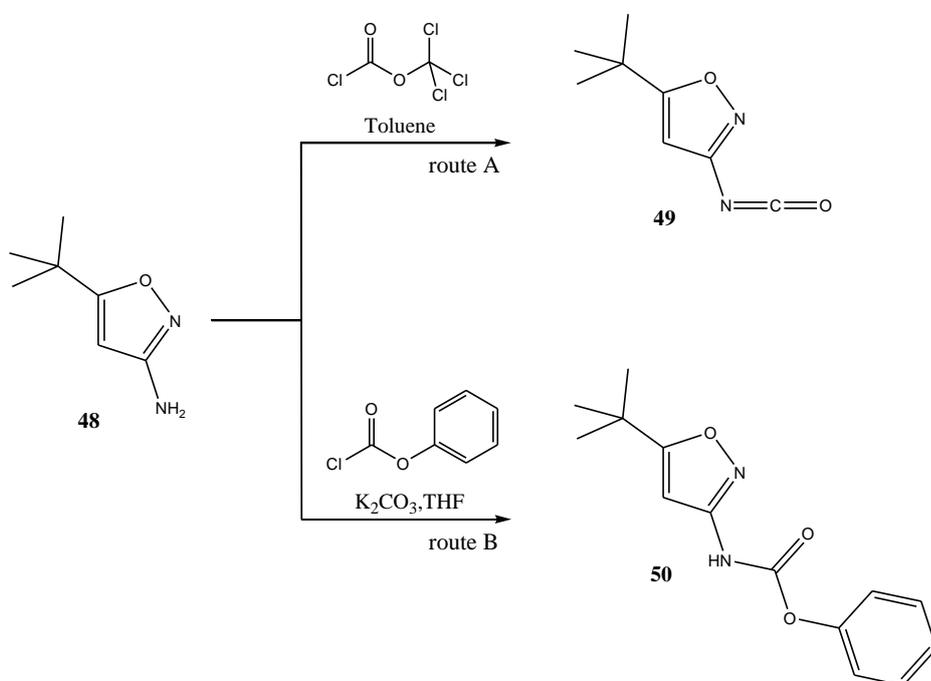
### Scheme 13



With regard to the introduction of the ureido functionality into the tetracyclic derivatives, an extensive literature searching, suggested two methods. One of these involved the use of the isocyanate derivative **49** synthesized from the commercially available 3-amino-5-*tert*-

butylisoxazole **48** by treatment with diphosgene (Scheme 14, route A).<sup>[93]</sup> The other method, involving the synthesis of the appropriate phenyl carbamate derivative **50** from the same amine by treatment with phenyl chloroformate, resulted more straightforward. Moreover carbamates are easier to handle and stock than isocyanates. Thus we chose to react amino derivatives **47a** and **47b** with phenyl 5-*tert*-butylisoxazol-3-ylcarbamate **50**, previously prepared according to a method described in literature (Scheme 14, route B).

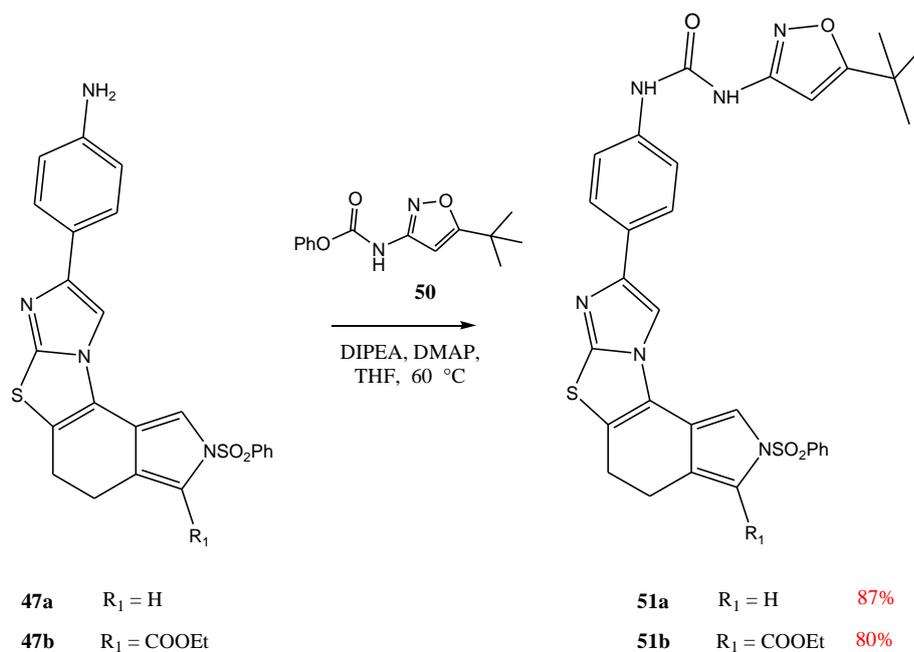
#### Scheme 14



The reaction, carried out in THF at 60 °C in the presence of DIPEA and DMAP, gave desired products **51a** and **51b** in excellent yields, without further chromatography purification (Scheme 15).<sup>[94]</sup> Encouraged by these results we focused on the introduction of water solubilising groups on the pyrrole nitrogen, after removal of the phenylsulfonyl group (Scheme 16). This latter reaction required a large excess of potassium hydroxide (substrate : KOH 1: 35) when conducted on compound **44a**. Unfortunately the same reaction on the analogue **44b**, belonging to the ethoxycarbonyl series, was not successful. Extensive attempts to cleave the phenylsulfonyl group using different bases under various conditions or

$\text{Bu}_4\text{NF}^{[95]}$  proved unsuccessful resulting either in recovery or in decomposition of the starting material.

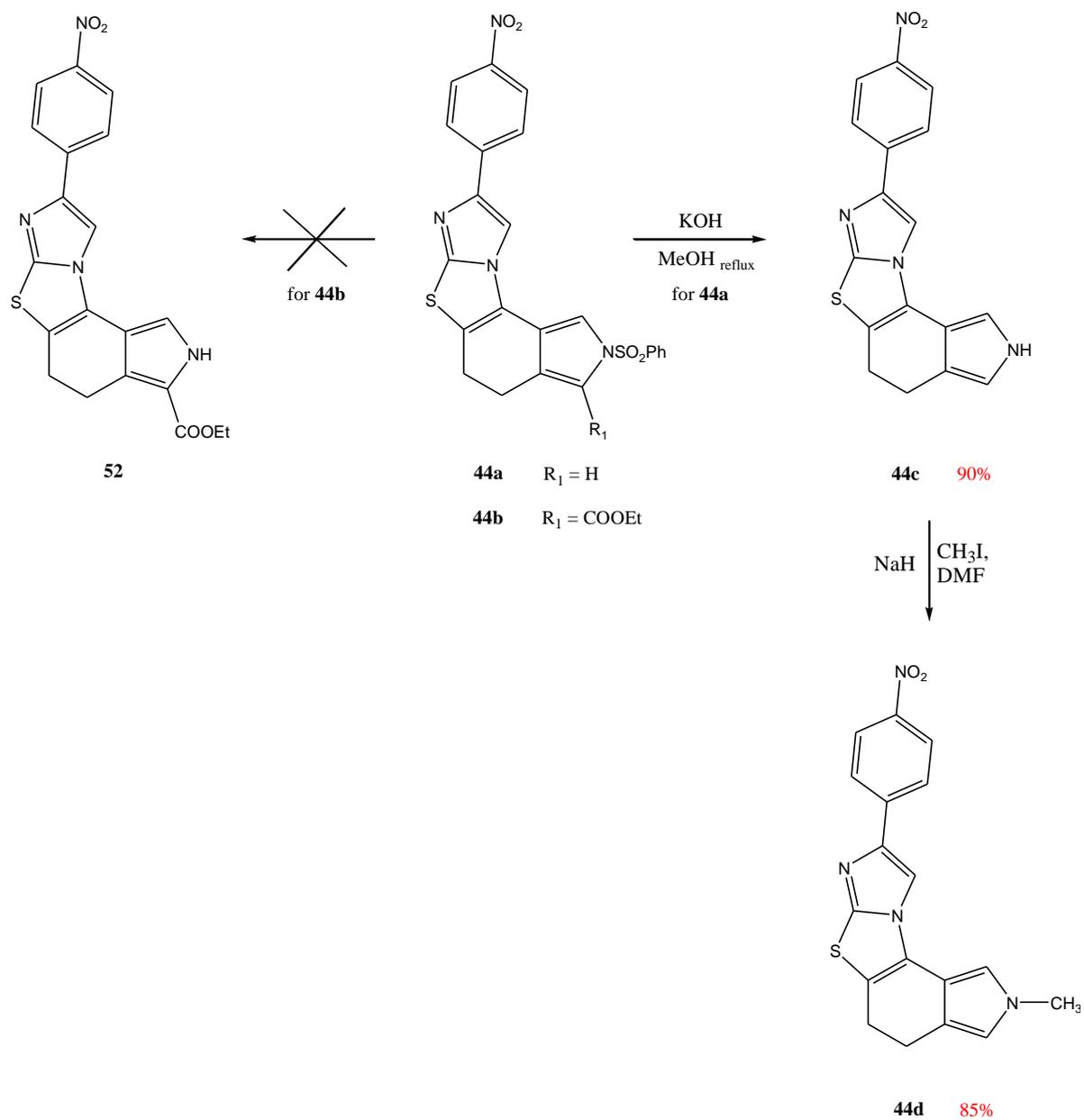
### Scheme 15



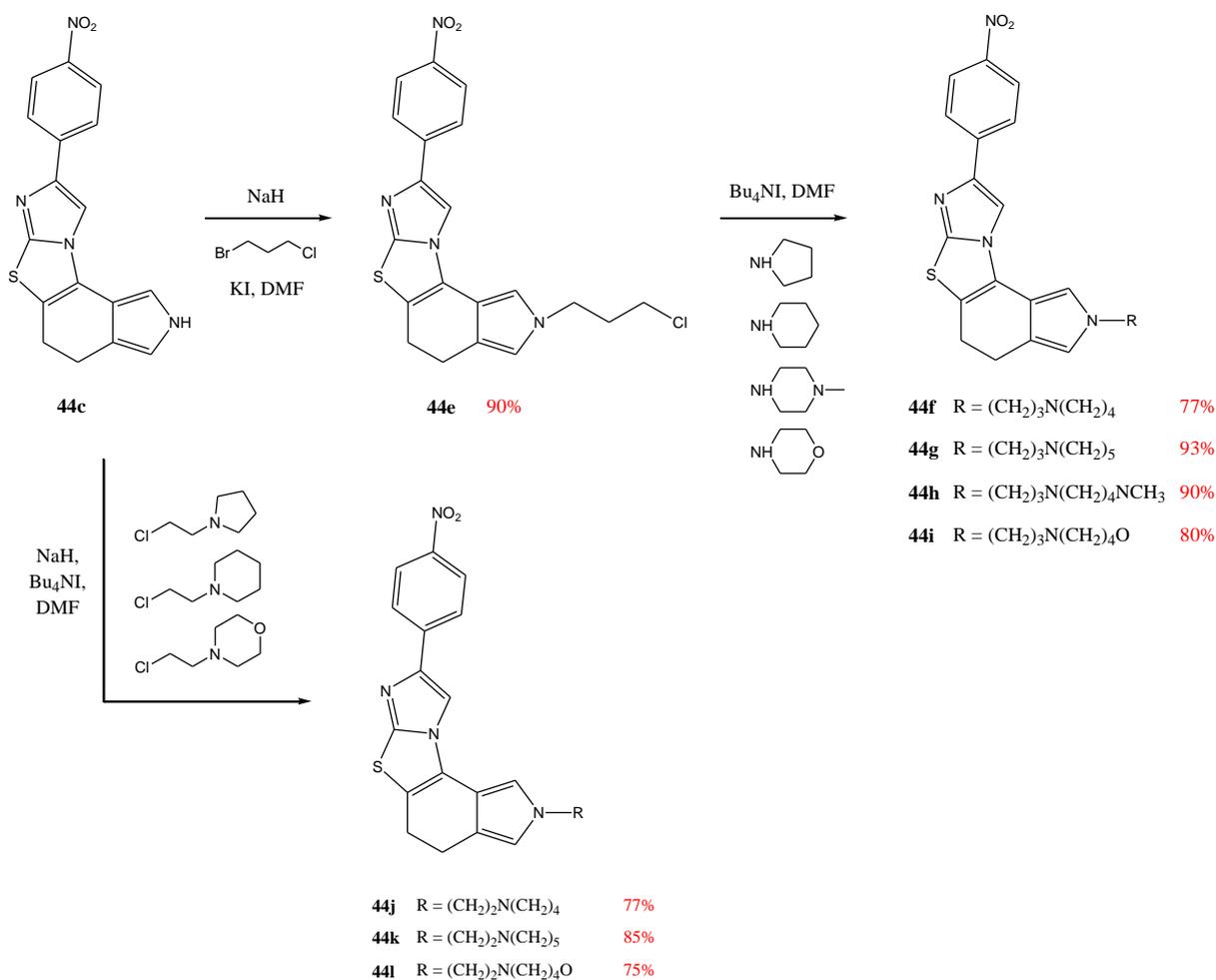
Thus, considering these synthetic difficulties, we decided to focus only on the unsubstituted series. For further functionalization of the pyrrole nitrogen atom we started with a simple methyl group and finishing with different water solubilising groups. The selection of the side chains was based on the best activity showed by lead compounds.

The N-methylation was easily performed by reaction of compound **44a** with NaH and then with methyl iodide (Scheme 16). To synthesize derivatives with three carbon atoms in the side chain we used a two steps method where compound **44c** was reacted first with 1-bromo-3-chloropropane and then with the appropriate nitrogen heterocyclic compound. (Scheme 17)

## Scheme 16

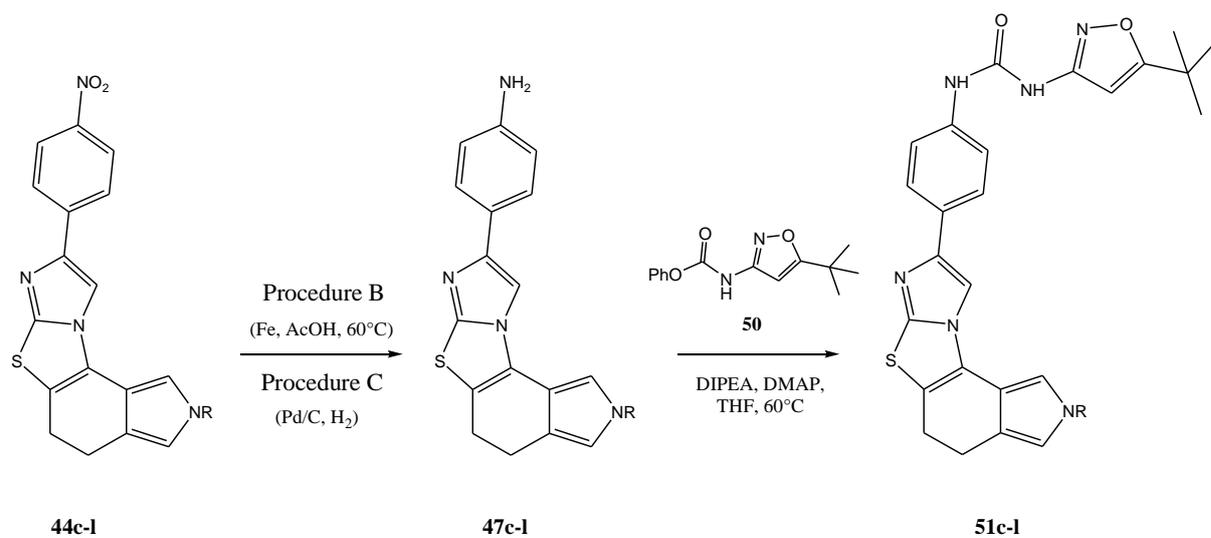


## Scheme 17



To obtain derivatives bearing two carbon atoms in the side chain, the anion of compound **44c**, generated in situ with NaH, was reacted directly with the proper chain as free-base obtained from commercially available salts by treatment with sodium hydroxide (Scheme 17). Nitro-derivatives **44c-l** were reduced using iron in acetic acid under ultrasonic irradiation (Procedure B)<sup>[96]</sup> or palladium on carbon and hydrogen (Procedure C) (Scheme 18) obtaining the corresponding amino-derivatives **47c-l**, used in some cases without any purification in the next step. Finally coupling reaction with the carbamate derivative **50**, in the same condition previously described, gave the ureido compounds properly decorated **51c-l**, in good yield (30 - 84 %) (Table 6).

### Scheme 18



**Table 6**

<b>Cmp.</b>	<b>Yield</b>	<b>Cmp.</b>	<b>Yield</b>	<b>R</b>
<b>47c</b>	53 %	<b>51c</b>	72 %	—H
<b>47d</b>	44 %	<b>51d</b>	84 %	—CH <sub>3</sub>
<b>47e</b>	/	<b>51e</b>	43 %	
<b>47f</b>	55 %	<b>51f</b>	42 %	
<b>47g</b>	38 %	<b>51g</b>	60 %	
<b>47h</b>	30 %	<b>51h</b>	53 %	
<b>47i</b>	60 %	<b>51i</b>	75 %	
<b>47j</b>	/	<b>51j</b>	60 %	
<b>47k</b>	/	<b>51k</b>	30 %	
<b>47l</b>	33 %	<b>51l</b>	45 %	

## 4.2 Biological evaluation

From a strictly chemical point of view, as the so obtained tetracyclic structure is a *New Ring System*, we thought it was worth testing every compound presenting the tetracyclic core. Thus, we submitted for the biological evaluation all the nitro derivatives **44a-l**, the amino-derivatives of type **47** (where isolated with an appropriate purity grade), and finally all the ureido derivatives **51a-l** more similar to quizartinib. A series of 30 compounds (Table 7) was submitted to “Oncotest” in Frieberg (Germany) for cell-based anti-proliferative screens. It was evaluated the inhibition of proliferation on twelve haematological cell lines, one derived from ALL (MOLT-4), nine from AML (HL-60, KG-1, KG-1a, MOLM-13, MV4-11, NOMO-1, OCI-AML2, PL-21, THP-1) and two from CML (K-562, KCL-22). **Dovitinib**, **Quizartinib** and compound **TCS 359**<sup>[97]</sup> (Chart 11), which target FLT3-ITD, were used as reference drugs and results were reported in terms of IC<sub>50</sub> and showed in a heat map (Table 8). The geometric mean of the IC<sub>50</sub> values was chosen to sort compounds because, unlike an arithmetic mean, it tends to dampen the effect of very high or low values.

Among the 30 compounds tested, five (**44d**, **51c**, **51d**, **51g**, **51h**) were identified with a very good potency, exhibiting a geometric mean IC<sub>50</sub> lower than 1 μM. Analysing the results from a structure -activity point of view it is possible to notice that among the ureido derivatives, compound **51c**, bearing the free NH on the pyrrole ring, was the most active compound of the entire series. Similarly to Quizartinib, it was particularly effective against the cell lines MOLM-13 and MV4-11, expressing the constitutively activated mutant FLT3-ITD, reaching the nanomolar range (0.011- 0.014 μM). However, it got responses also in the other cell lines showing IC<sub>50</sub> in the submicromolar and low micromolar range (0.265 - 1.582 μM). The same behaviour was observed for the N-Methyl derivative **51d**, which was almost as potent as the NH derivative retaining the selectivity towards MOLM-13 and MV4-11 (0.035 - 0.058 μM) and the activity on the other cell lines at submicromolar/micromolar concentrations (0.812 - 4.783 μM). The presence of water solubilising groups seemed to produce a decrease in the activity although compounds **51e-l** (0.101 - 17.15 μM) retained the selectivity towards MOLM-13 and MV4-11 (0.101 - 0.578 μM) showing IC<sub>50</sub> values in the submicromolar range and a good potency against the other cell lines. Whereas, almost inactive resulted both derivatives bearing the phenylsulfonyl group even if **51a** belonging to the unsubstituted series showed IC<sub>50</sub> in the low micromolar range selectively on MOLM-13 (5.468 μM) and MV4-11 (14.9 μM).

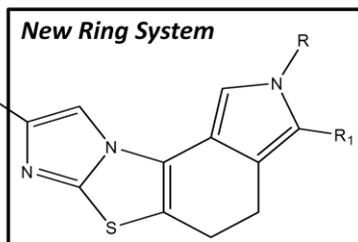
**Table 7**

**NITRO - DERIVATIVES**

$R_2 = -NO_2$

Cmp.	R	R <sub>1</sub>
44a		H
44b		COOEt
44c	-H	H
44d	-CH <sub>3</sub>	H
44e		H
44f		H
44g		H
44h		H
44i		H
44j		H
44k		H
44l		H

**New Ring System**

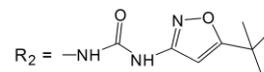


**AMINO - DERIVATIVES**

$R_2 = -NH_2$

Cmp.	R	R <sub>1</sub>
47a		H
47b		COOEt
47c	-H	H
47d	-CH <sub>3</sub>	H
47f		H
47i		H

**UREIDO - DERIVATIVES**



Cmp.	R	R <sub>1</sub>
51a		H
51b		COOEt
51c	-H	H
51d	-CH <sub>3</sub>	H
51e		H
51f		H
51g		H
51h		H
51i		H
51j		H
51k		H
51l		H

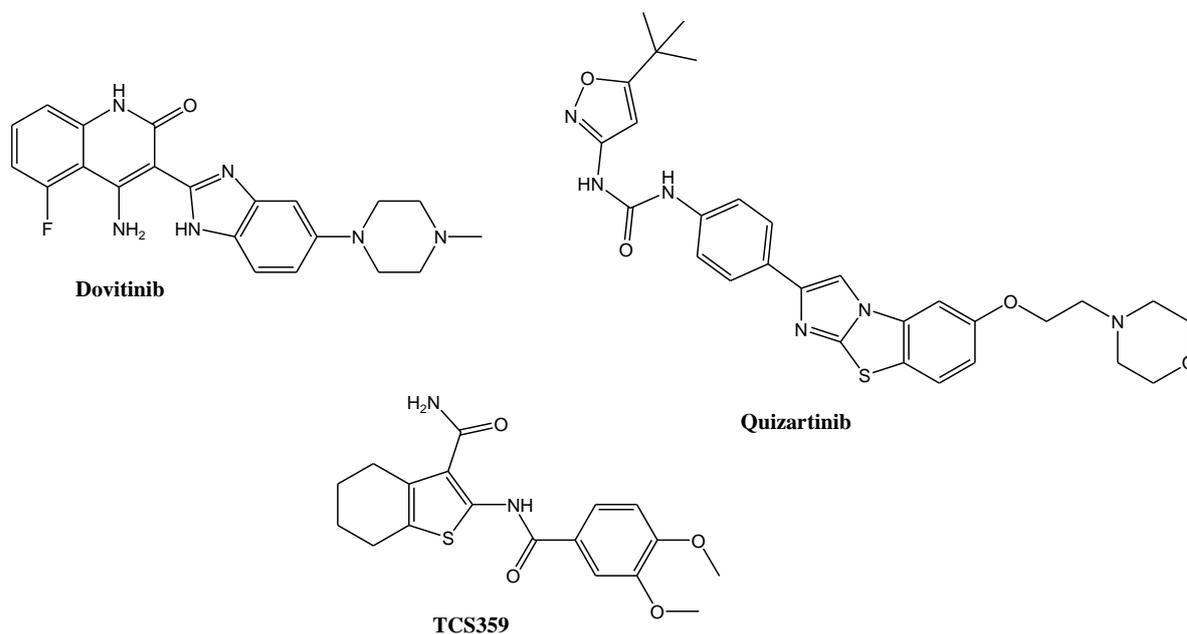
With regard to nitro derivatives, of special note are compounds **44f**, **44g**, **44h**, **44j** and **44k** which surprisingly, despite missing the selectivity, showed a geometric mean IC<sub>50</sub>, lower than Quizartinib. Among the nitro derivatives, **44e** bearing the chloropropyl chain and the NH derivative **44c** showed a certain selectivity against KG-1 (0.536 and 3.868 μM respectively) and KG-1a (0.876 and 2.985 μM respectively) cell lines presenting IC<sub>50</sub> values in the low micromolar range. Finally, with regards to the amino derivatives, they seemed to inhibit proliferation at micromolar concentrations, but only two of them, **47i** (2.281 and 4.39 μM respectively) and **47d** (2.36 and 4.558 μM), resulted active selectively against KG-1 and KG-1a cell lines reaching the low micromolar range.

Table 8

Cell Line / Compound	MOLT-4 (ALL)	HL-60 (AML)	KG-1 (AML)	KG-1a (AML)	MOLM-13 (AML)	MV4-11 (AML)	NOMO-1 (AML)	OCI-AML2 (AML)	PL-21 (AML)	THP-1 (AML)	K-562 (CML)	KCL-22 (CML)	Geometric mean abs. IC <sub>50</sub> values [ $\mu$ M]
Dovitinib	0.266	0.911	0.035	0.033	0.005	0.004	1.493	0.568	2.85	1.936	2.018	2.552	0.268
Quizartinib	50.28	100	33.53	100	0.003	0.003	100	0.45	100	100	100	100	9.687
TCS 359	100	100	100	100	0.31	0.322	100	100	100	100	100	100	38.306
51c	0.591	0.608	0.265	0.904	0.014	0.011	1.541	0.519	0.616	1.454	1.582	0.709	0.385
44d	1.842	1.318	0.415	0.587	0.358	0.378	0.409	0.375	0.94	1.985	1.373	1.36	0.769
51h	0.586	1.432	0.677	1.345	0.169	0.111	2.483	0.691	1.1	2.539	1.309	1.478	0.845
51d	1.008	1.761	0.812	4.783	0.058	0.035	2.499	0.799	0.86	2.271	4.151	2.229	0.945
51g	0.644	1.437	0.821	1.756	0.262	0.116	1.389	1.644	1.618	2.438	1.633	1.454	0.988
51k	0.943	1.672	1.533	2.585	0.42	0.29	1.749	1.096	1.882	2.798	3.332	3.046	1.438
51f	0.711	2.624	1.747	3.243	0.355	0.353	2.064	1.517	1.798	2.675	3.237	2.563	1.531
44k	0.498	4.306	2.229	1.362	1.502	1.152	3.448	1.253	1.362	1.361	6.416	2.459	1.831
51j	1.088	2.818	2.367	3.772	0.39	0.394	2.565	1.919	3.492	5.201	3.866	3.747	2.048
51l	1.983	4.128	1.969	7.007	0.105	0.157	6.857	2.311	2.145	4.519	13.2	4.438	2.249
44f	0.407	4.063	3.015	2.525	3.259	2.108	3.962	1.272	1.653	1.375	5.328	4.79	2.328
51e	1.877	4.619	1.258	13.58	0.578	0.469	3.769	1.074	1.37	8.288	100	8.258	3.275
44g	2.433	4.256	2.529	2.685	2.687	4.431	4.3	3.041	6.284	14.62	4.168	4.314	4.025
44j	1.927	4.173	2.507	1.282	5.292	2.249	5.135	2.108	5.062	6.945	19.33	9.95	4.099
51i	9.356	13.89	7.389	25.1	0.242	0.101	22.37	9.82	15.33	25.4	13.86	17.15	6.916
47i	11.57	14.45	2.281	2.36	9.759	7.775	14.07	17.58	15.34	20.93	12.52	22.79	10.370
47f	7.799	19.92	6.146	7.289	7.65	8.714	17.35	9.612	12.98	12.92	11.85	16.46	10.800
47d	14.12	27.46	4.39	4.558	5.654	2.959	29.94	25.98	18.32	19.64	11	18.71	11.824
47c	18.14	37.01	8.552	7.605	4.085	8.522	13.28	19.77	28.39	53.75	16.97	37.23	16.421
44e	0.743	100	0.536	0.876	15.36	48.66	90	100	100	100	50.63	100	21.857
47a	62.75	100	25.22	18.48	13.29	26.88	87.66	73.57	100	100	70.4	70.97	51.357
44c	100	62.22	3.868	2.985	58.36	100	100	100	100	100	100	100	52.305
51a	100	100	100	100	5.468	14.9	100	100	100	100	17.14	100	57.820
44d	100	100	58	75.82	100	67.55	100	100	100	100	100	100	90.379
44i	68.69	100	100	100	100	100	100	100	100	100	100	100	96.918
44a	100	100	91.56	78.83	100	100	100	100	100	100	100	100	97.319
51b	100	100	100	100	97.53	100	100	100	100	100	100	100	99.792
44l	100	100	100	100	100	100	100	100	100	100	100	100	100.000
44b	100	100	100	100	100	100	100	100	100	100	100	100	100.000
47b	100	100	100	100	100	100	100	100	100	100	100	100	100.000



## Chart 11



From the evaluation of these data, it is crucial to notice that the pronounced activity towards the cell lines MOLM-13 and MV4-11 indicates inhibition of FLT3 and the presence of ureido moiety plays a key role in driving the antiproliferative activity towards these cell lines. Moreover, the presence of water solubilising groups lead to a decrease in the activity even if these substituents could improve the pharmacokinetic properties. On the contrary the phenylsulfonyl and ethoxycarbonyl functionalities on the pyrrole ring seems not to be convenient in terms of activity even if evaluation of the real contribution of the ethoxycarbonyl group to lack of activity will require further investigation.

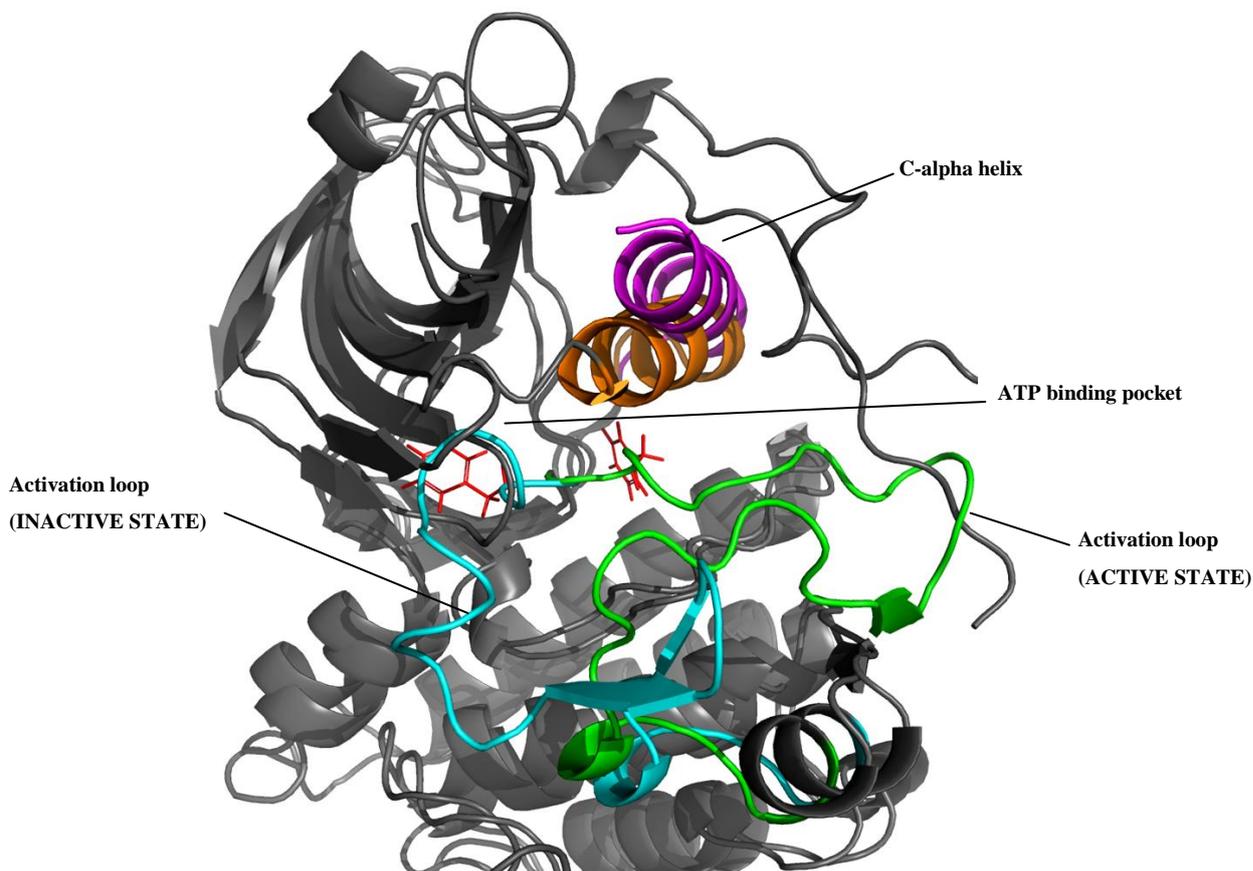
### 4.3 Molecular Modelling

In order to help to rationalize SAR and drive future synthesis, a molecular modelling approach was attempted. In parallel to chemistry and biology experimental efforts dedicated to this series of compounds, computer model of the corresponding protein - ligand complexes were generated. It appeared that kinase inhibitors could be clustered into two distinct families, type I and II, corresponding to the active state and the inactive state of the kinase, respectively. There are subtle differences in their binding modes that are based on the conformation of the conserved DFG (Aspartic acid - Phenylalanine - Glutamic acid) motif in

the activation loop. Particularly, the position of the Phenylalanine (Phe) residue of the DFG motif determines the conformation of the activation loop. When the phenyl group of the Phe residue is oriented outside of the ATP binding site, the DFG motif adopts the “in” conformation (DFG-in); alternatively, this motif adopts the “out” conformation if the phenyl group of the Phe residue is oriented inside of the ATP binding site (DFG-out). The switch between active (DFG-in) and inactive (DFG-out) states is coupled also with C-alpha helix movement altering significantly the cleft conformation. Inhibitors that bind to the DFG-in active conformation are termed type-I inhibitors, and those that bind to the DFG-out inactive conformation are referred to as type-II inhibitors. Type-II inhibitors, in addition to binding to the ATP site, also bind to an additional region termed the “back-pocket” region, that provides another handle for tuning kinase selectivity.<sup>[98]</sup>

In order to optimally exploit the bound conformation space, I carried out some docking studies, using the skills I acquired from previous experiences in molecular modelling. Considering that compounds we have designed possess structural properties typical of inactive-state-binding kinase inhibitors and are structural closely related to Quizartinib which is known as type-II tyrosine kinase inhibitor, I performed docking against the FLT3 inactive state. However, considering that there is not any crystal structure representative of the FLT3 active state, I also created a homology model of FLT3 active conformation for the purely illustrative purposes of our analysis. C-Kit kinase (pdb: 1PKG) was chosen as template because of the high homology and identity (58%) between the two proteins. Once the FLT3-modeled active structure was built, the structural differences between the DFG-in and DFG-out conformations were compared by superimposition of the FLT3-modeled active structure on the inactive structure (pdb: 1RJB) (Figure 10). The superimposition clearly showed that the activation loop (green) in the active structure flipped away from the ATP binding pocket, leading to the placement of the Phe away from this site, while in the DFG-out structure (inactive), the activation loop (cyan) flipped over to the active site, resulting in the placement of Phe in ATP binding pocket. The different arrangement of the activation loop, particularly the placement of the Phe residue, has important implications for the binding of different types of inhibitors to FLT3 kinase.

**Figure 10. Superimposition of inactive state and homology model active of FLT3. Green activation loop and orange C-alpha helix in the ACTIVE STATE; cyan activation loop and purple C-alpha helix in the INACTIVE STATE**

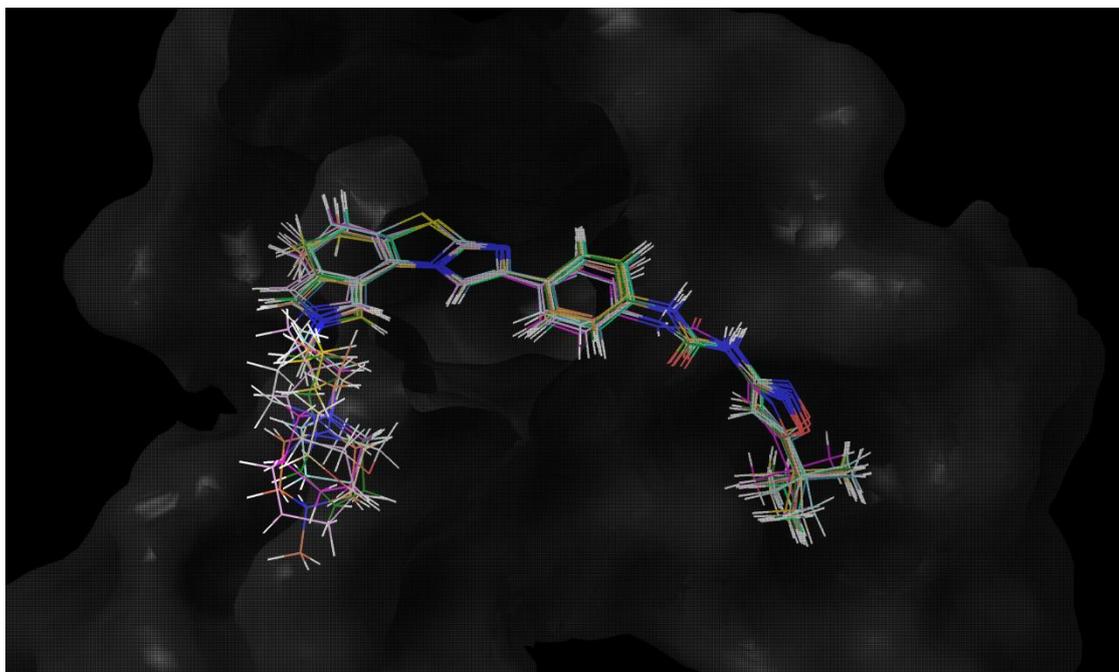


To date, three FLT3 structures for the inactive state are available in the Protein Data Bank (PDB). 1RJB (Resolution: 2.1 Å) was the first to be released which is an auto-inhibited DFG-out conformation without any inhibitors.<sup>[99]</sup> Recently two more crystal structure of FLT3 DFG-out conformation in complex with quizartinib was released on PDB (4RT7; Resolution: 3.1 Å and 4XUF; Resolution: 3.2 Å).

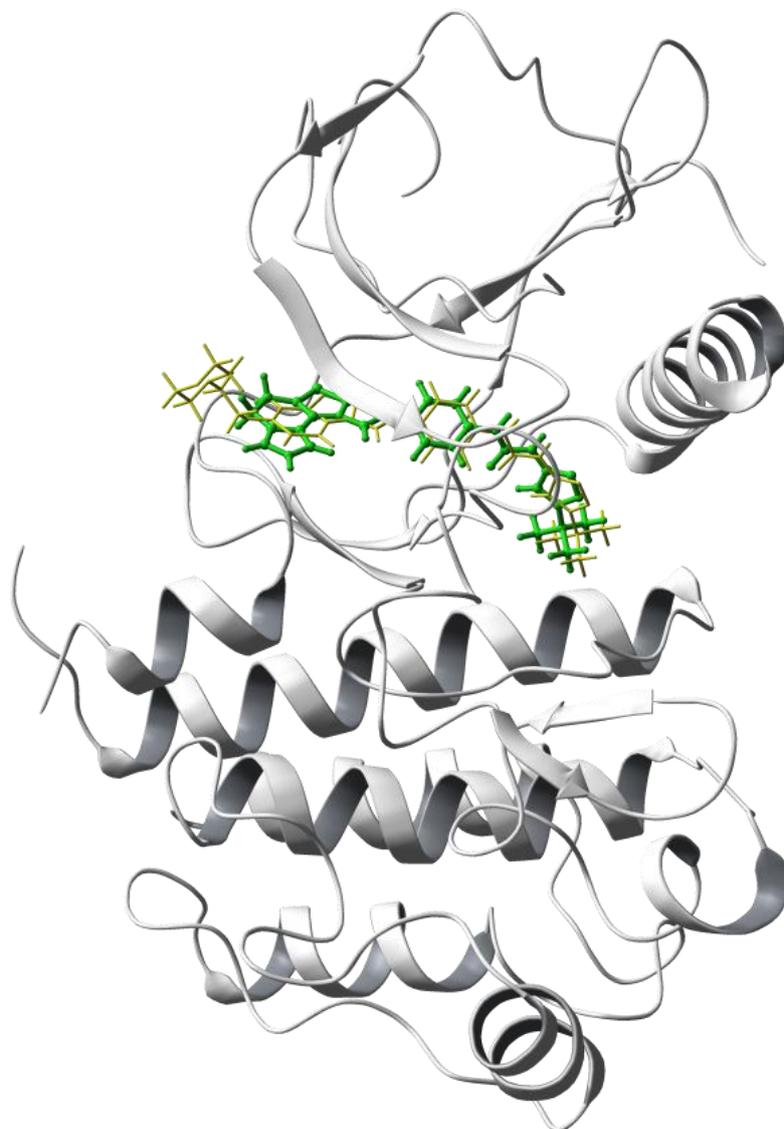
4RT7, the first co-crystal structure of FLT3 with a kinase inhibitor (Quizartinib)<sup>[100]</sup> was selected as representative of DFG-out inactive state, allowing to derive a more reliable molecular modeling for characterization of the interactions. Docking calculations were performed using the “Extra Precision” (XP) mode of Glide.<sup>[101]</sup> In a preliminary step, the ability of Glide to reproduce the bound poses of quizartinib in the FLT3 crystal structure was investigated. Docking gave a Root Mean Square Deviation (RMSD) of less than 1 Å indicating a good reliability for our docking model. Then, after energy minimization, ureido

compounds of type **51**, which resulted the most active and selective on MV4-11, were subjected to docking in order to determine a low energy binding mode. The docking calculations showed a common binding mode for all ureido derivatives. (Figure 11) Since water solubilizing groups seems to assume a position within a region freely accessible to the solvent, at the edge of the receptor, they could not strongly influence the binding mode. Thus for our analysis we reported a low-energy binding pose for compound **51c** as representative of a common binding mode shared from compounds we synthesized. As it is possible to see from the illustration of the active site with superimposition of Quizartinib (yellow molecule) and a low energy binding pose of **50** (green molecule) (Figure 12), the relative position of the molecules within the active site are very similar and occupy the same plane. In analogy to Quizartinib, compound **15c** seems to be oriented so that the tetracyclic scaffold occupy the adenine binding pocket. Even if a planar and aromatic system is preferred, it seems that a completely aromatic system was not a necessary condition for proper binding within this hydrophobic pocket. The *t*-butyl-isoxazole moiety lies in the allostreric back pocket of the kinase where it is surrounded by hydrophobic side chain.

**Figure 11. Superimposition of the docked poses of the ureido-compounds 51c-51l**

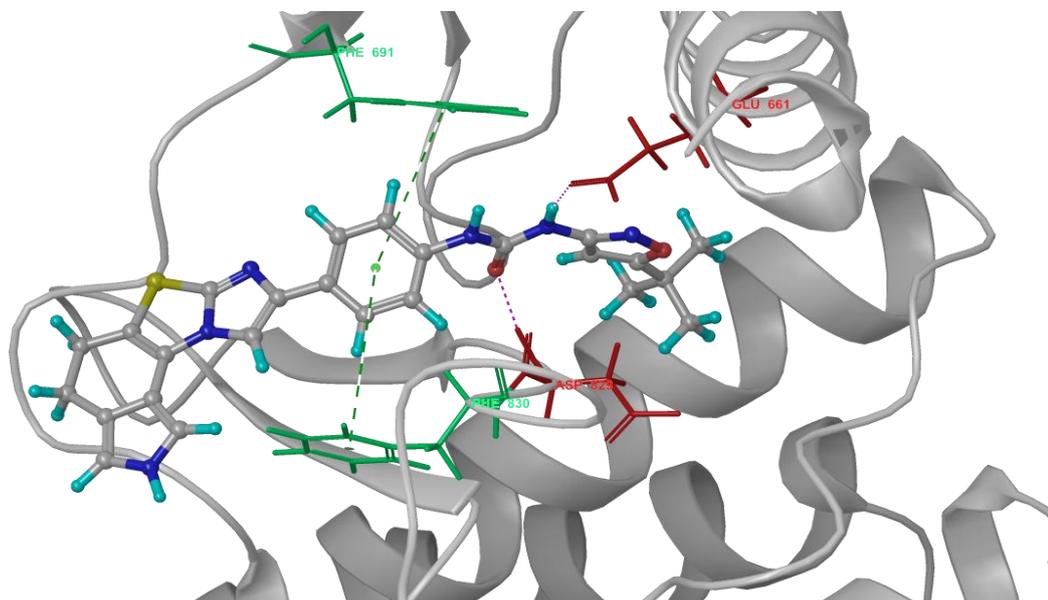


**Figure 12. Overview of FLT3 in complex with Quizartinib (yellow molecule) and predicted low energy binding pose of compound 51c (green molecule)**

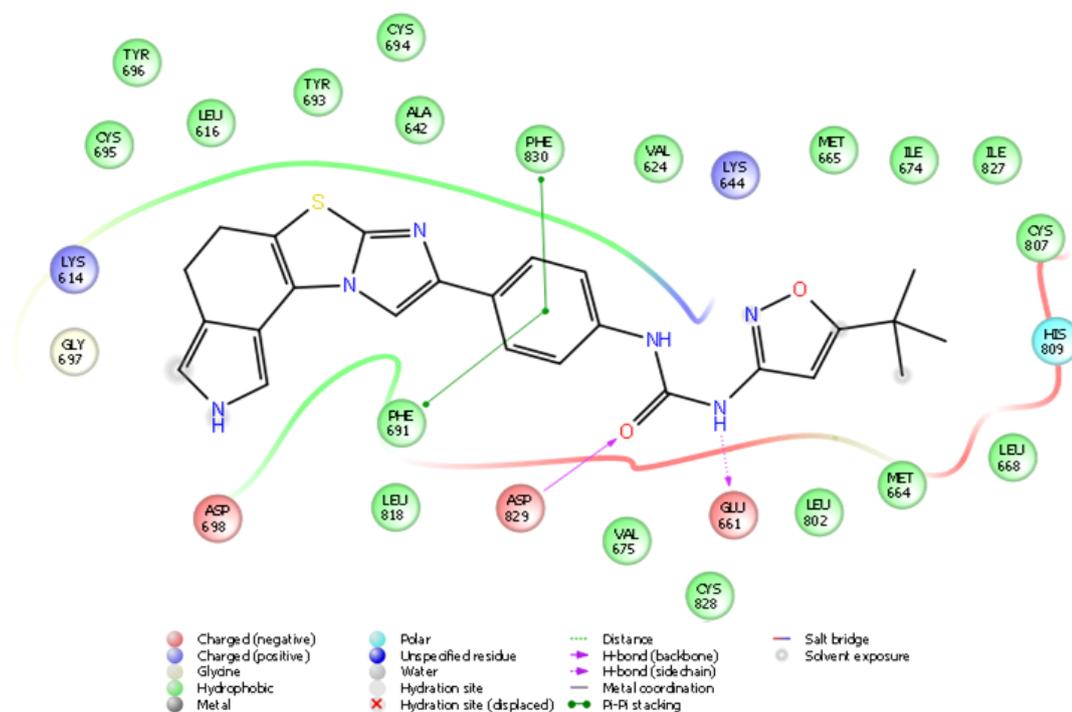


The ureido moiety is hydrogen bonded to E661 of the alphaC helix and to D829 of the DFG motif, a pattern shared by other urea- and amide -containing type II kinase inhibitors similarly to Imatinib and Sorafenib. In analogy to Quizartinib, the phenyl ring forms edge-to-face interactions with both gatekeeper F691 and F830 of the DFG motif (Figures 13 and 14).

**Figure 13. Predicted low-energy pose of compound 15c inside FLT3**



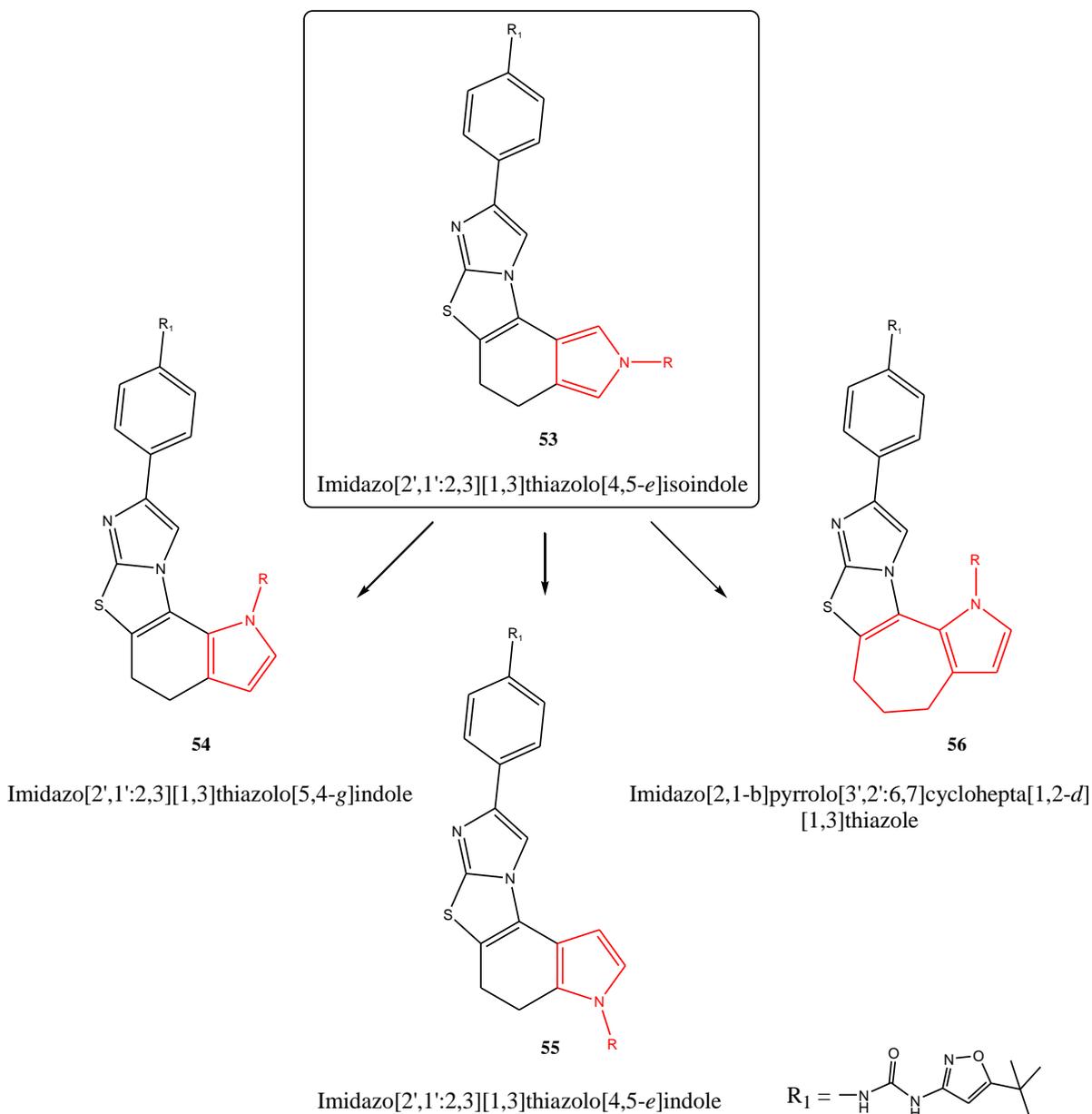
**Figure 14. Interaction diagram of the docked conformation of compound 51c with the key active site residues**



## 4.4 Perspectives

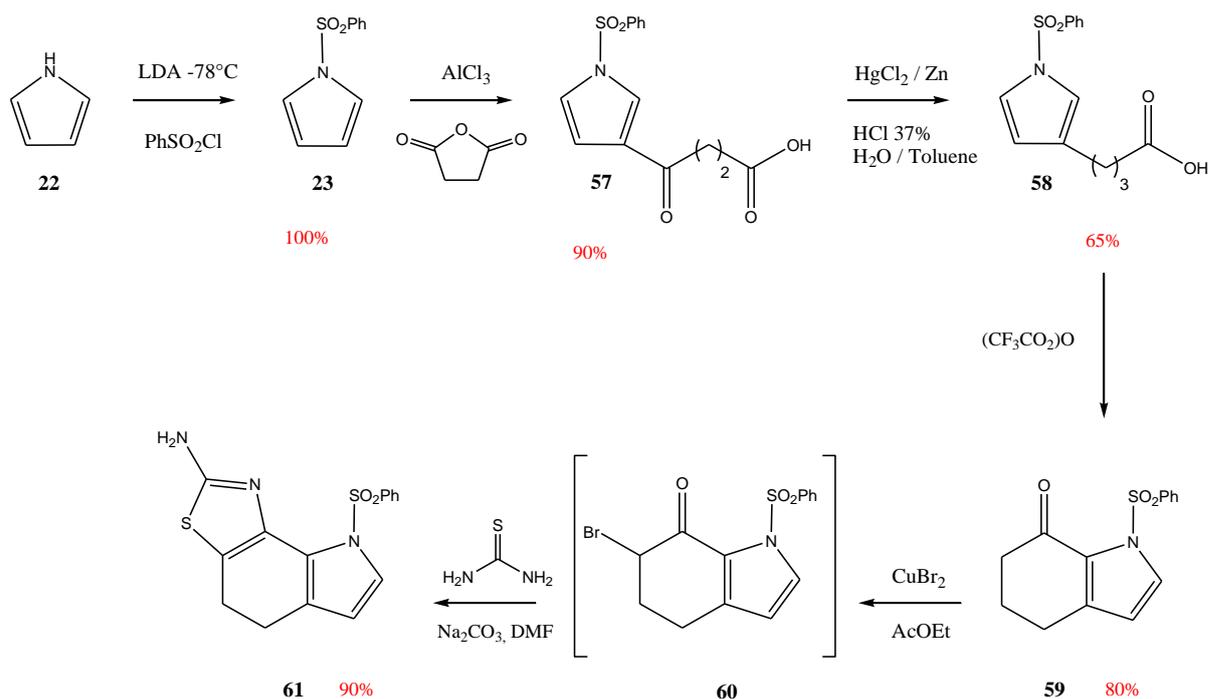
Encouraged from the pronounced antiproliferative activity showed by the ureido derivatives containing the imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole scaffold **53** (Chart 12), further structural investigations were planned such as a variation of the condensation of the pyrrole ring and an enlargement of the central ring generating two positional isomers **54** and **55** and the new ring system **56** starting from the previously investigated cyclohepta system **27** (Scheme 6).

Chart 12



Thus, we had to synthesize the corresponding 2-aminothiazole derivatives **61** and **66**, suitable substrates for the imidazole ring cyclization, according to the following synthetic routes (Scheme 19 and 20). To synthesize the 5,8-dihydro-4*H*-[1,3]thiazolo[5,4-*g*]indol-2-amine system **61** (Scheme 19) we tried to use similar methods and reaction conditions previously optimized in the first branch of the project. Thus pyrrole sulphonylation, followed by a Friedel Crafts acylation with succinic anhydride and AlCl<sub>3</sub> gave **57**. The reduction of the carbonyl group to methylene was performed using the Clemmensen reaction. Finally, the dehydration reaction with trifluoroacetic anhydride, led to compound **59**.<sup>[102]</sup> This latter was refluxed with copper(II) bromide in ethyl acetate then, when the bromointermediate was formed, the reaction mixture was filtered and the filtrate was evaporated and directly subjected to cyclization with thiourea in DMF obtaining **61** in excellent yield.

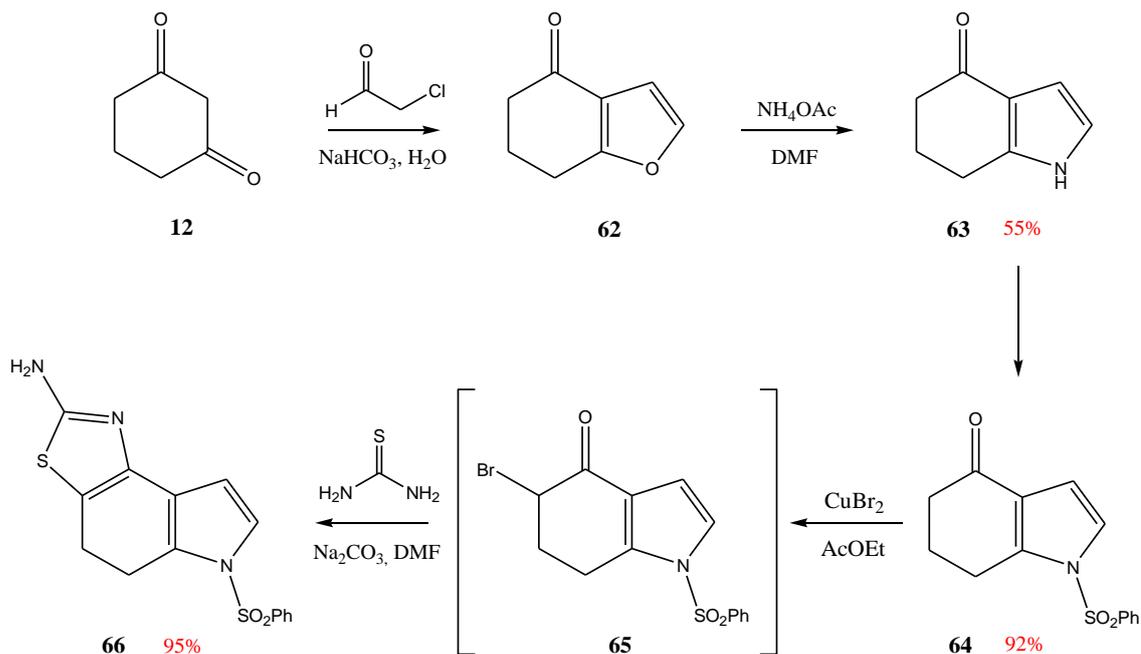
**Scheme 19**



Synthesis of the 5,6-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]indol-2-amine system **66** (Scheme 20) involved first the cyclization of chloroacetaldehyde to 1,3-cyclohexandione for the formation of 4-oxotetrahydrobenzofuran **62**.<sup>[103]</sup> Then the heating with ammonia, generated in situ from ammonium acetate, led to 4-oxo-tetrahydroindole through an ANRORC (Addition of the Nucleophile, Ring Opening and Ring Closure) type mechanism.<sup>[104, 105]</sup> The phenylsulphonyl

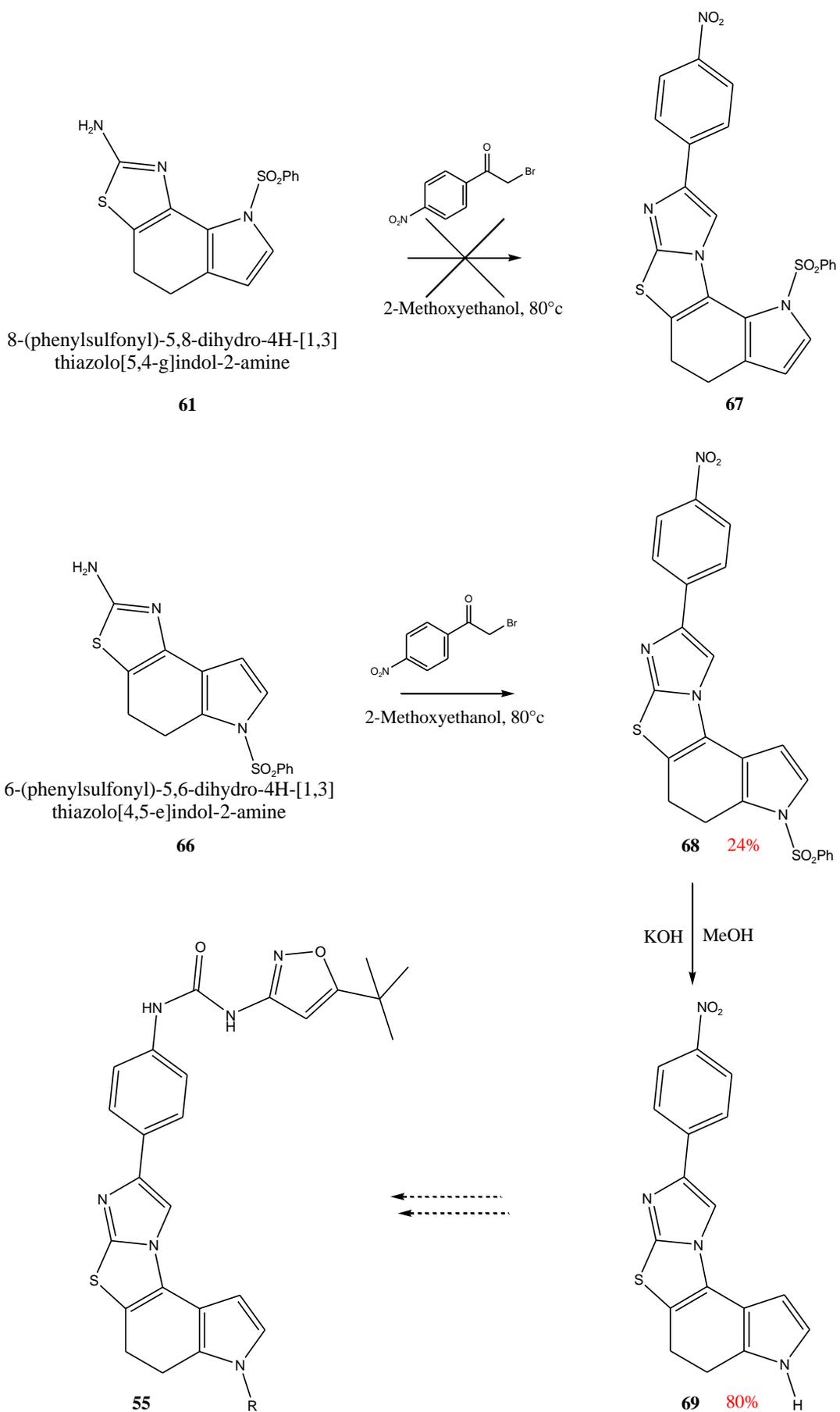
group was introduced on the nitrogen atom by reaction with NaH and benzenesulfonyl chloride obtaining **64** which was easily brominated with copper(II) bromide. The brominated intermediate was used in the next step without further purification and reacted with thiourea and sodium carbonate in DMF obtaining the 2-aminothiazole derivative **66** in high yield.

### Scheme 20



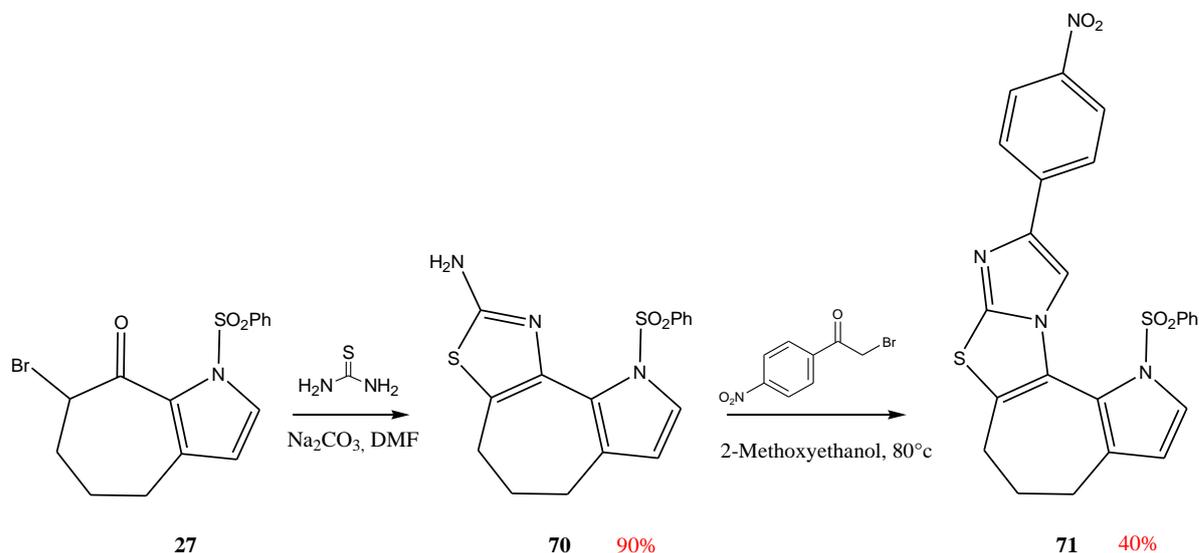
2-Aminothiazole derivative **61** was reacted with bromo-*p*-nitroacetophenone (Scheme 21) but contrary to our expectations, applying the same operating conditions previously used in the imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole series, we didn't manage to isolate the tetracyclic derivative **67**, recovering only starting material. On the contrary, the same conditions applied to **66** led to isolate the fused imidazoderivative **68** in low yield (24%). Despite that, we tried with success the removal of the phenylsulphonyl group using 2 equivalents of potassium hydroxide in methanol. The so obtained nitro derivative **69** represents an important intermediate for future reactions that will lead to the corresponding ureido derivatives NH and N-substituted. However, disappointed by the low reactivity exhibited by these derivatives, we decided to investigate on a different substrate. We thought to synthesize another series of analogues of quizartinib presenting the tetracyclic core of type **56** (Chart 12) in which a different condensation of the pyrrole unit is accompanied by the expansion to seven member of the central cycle.

**Scheme 21**



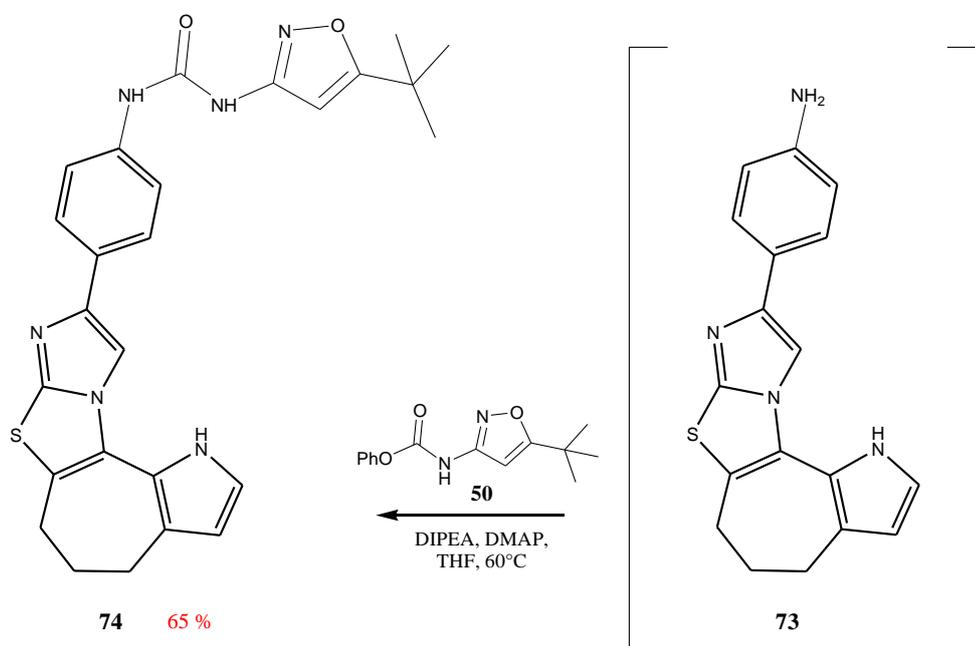
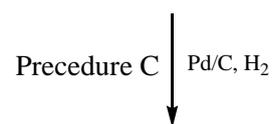
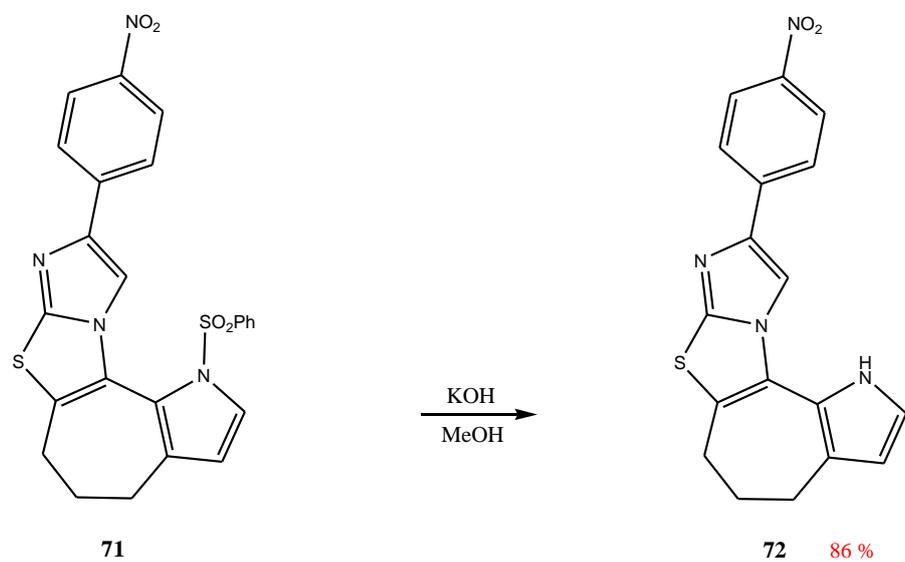
We started from the bromointermediate **27**, readily available for the cyclization to the aminothiazole derivative **70** which in turn was subjected to the imidazole closure with bromo-*p*-nitroacetophenone (Scheme 22).

### Scheme 22



In this case the fused imidazoderivative **71** was obtained in better yield so we decided to go through the synthetic route. At first we focused only on the NH and N-Methyl ureido derivatives, which resulted the most active in previous series of tested compounds. Thus, we performed the removal of the phenylsulphonyl group, using 1.5 equivalents of KOH in methanol (Scheme 23). Nitro derivative **72** was subjected to reduction using palladium on carbon and hydrogen (Procedure C), then the crude was directly used for the copulation with the carbamate derivative **50** obtaining **74** in good yield. The synthesis of the N-methyl derivative is still in progress. Further studies will be required to complete and better investigate the desired ring closure of the new pyrrole-thiazole systems. However these preliminary results represents the seeds for future work.

### Scheme 23



## 5. PROJECT IN COLLABORATION WITH THE UNIVERSITY OF CARDIFF

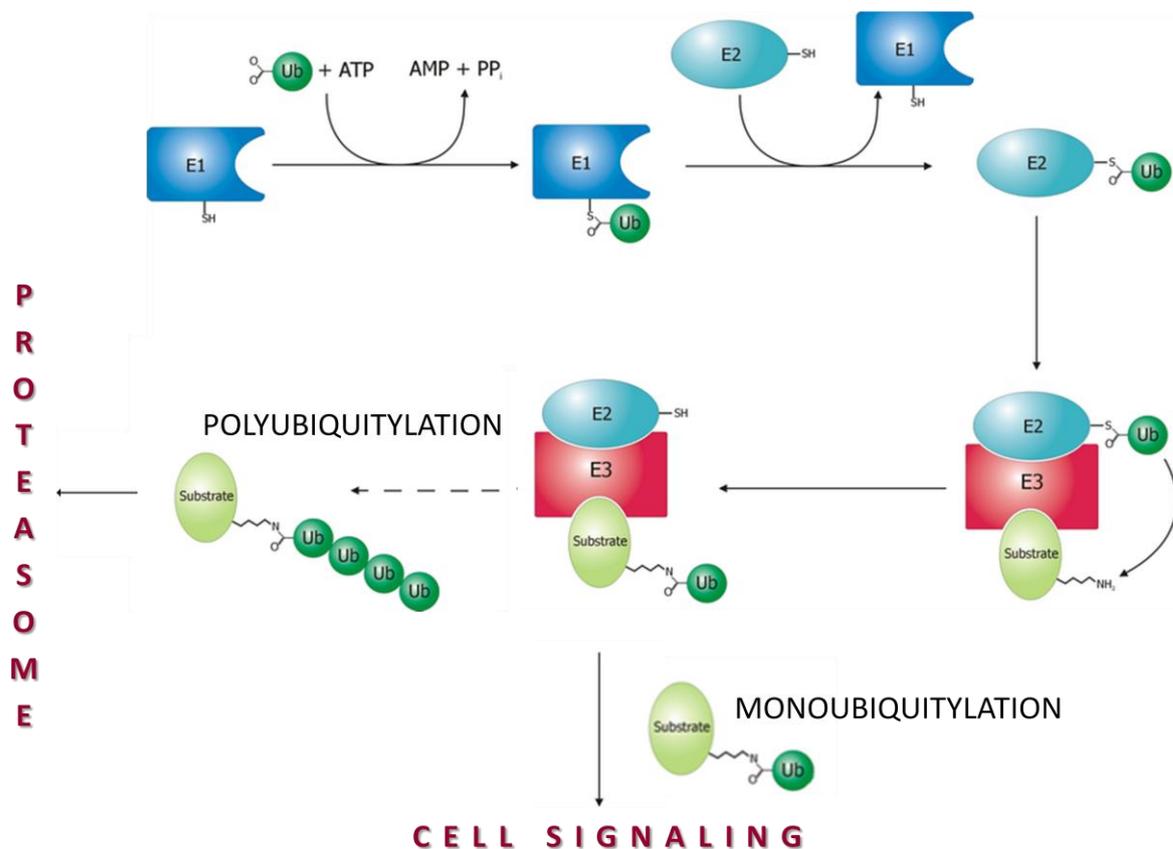
During my PhD studies, I spent 6 months (July - December 2014) at the School of Pharmacy and Pharmaceutical Sciences of Cardiff University under the supervision of Prof. Andrew D. Westwell. In this period I worked on a project about the synthesis of potential inhibitor of the breast cancer-associated protein 2 (BCA2) as antitumor agents. In addition I have found time to learn techniques in computational molecular modelling, and to work on an analytical chemistry project around new psychoactive substances (NPS), called Wedinos project.

### 5.1 Introduction

Ubiquitin-Proteasome System (UP-S) is the major degradation pathway for cellular proteins. The addition of a chain of multiple copies of ubiquitin (UB), a peptide of 76 aminoacids, targets specific proteins for destruction by the intracellular protease known as proteasome, a large complex that breaks down proteins to their constituent amino acids for reuse. The proteins targeted by this system are short-lived proteins, many of which are regulatory proteins, whose actions are controlled in part by rapid synthesis and degradation, much like an on/off switch. In addition, proteasome targets misfolded, damaged or mutant proteins with abnormal conformations that could be harmful to the cell. It is important for a cell to be able to select specific proteins for degradation so as to avoid degrading proteins vital to the functioning of the cell.<sup>[106]</sup> The majority (80%) of cellular proteins is destroyed by the UP-S after ubiquitin tagging<sup>[107]</sup>. The attachment of UB to a target protein (ubiquitination) requires the action of three enzymes, called E1 (UB-activating enzymes), E2 (UB-conjugating enzymes) and E3 (UB ligases), which work sequentially in a cascade (Figure 14). E3-ligase governs the final step of the ubiquitination process. The ubiquitin system is hierarchically structured and confers specificity for protein substrates through a multitude of E3 ubiquitin ligases. A few E1 ubiquitin-activating enzymes exist; however, at least 50 E2 ubiquitin-conjugating enzymes and about 500 E3 ubiquitin ligases are present in the human genome<sup>[108, 109]</sup>. In this contest, UB ligation provides the key step of substrate selection and UB transfer to the protein target by the E3 ligases is responsible for substrate specificity and regulation of the ubiquitination process. Additional UB molecules can be linked to the first one to form a poly-UB chain, which occurs through a particular type of E3-ligase.<sup>[110]</sup> While polyubiquitination

targets proteins for degradation by the proteasome, monoubiquitination alters subcellular localization and can change their activity. Important transcription factors are regulated by ubiquitination and their alteration is frequently involved in tumorigenesis. For that reason, a deregulated E3 system has been shown to be a feature in some form of cancer. An example of well-studied E3-ligase strongly correlated with tumorigenesis is Breast Cancer Associated protein 2 (BCA2) which has been shown to be over-expressed in 56 % of invasive breast cancers but not expressed in most of normal tissues. BCA2 is expressed in both the nucleus and cytoplasm of breast cancer cells, implying multiple functions and its down-regulation has been shown to reduce the growth and invasiveness of the breast cancer cell line MCF-7.<sup>[111]</sup> In hormone-responsive breast tumors, nuclear BCA2 expression appears to be under the control of estrogen, although its tumor-promoting function may not be completely dependent on estrogen signaling.<sup>[112]</sup>

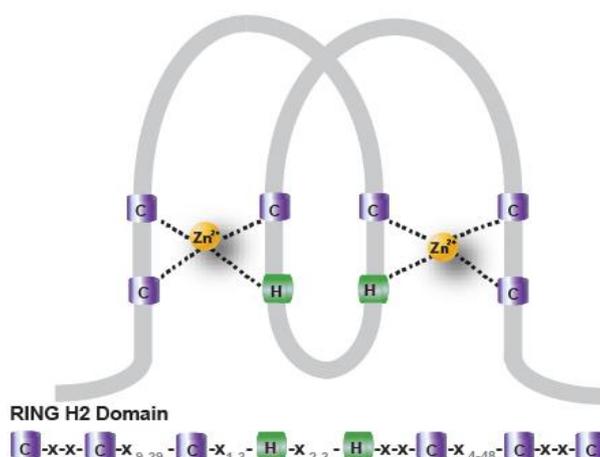
**Figure 14 Overview of the ubiquitin-mediated protein degradation pathway**



BCA2 can be classified as a Really Interesting New Gene (RING)-finger protein characterized by the presence of a double  $Zn^{2+}$ -binding motif arranged in a cross-brace structure essential for ubiquitin E3 ligase catalytic activity. Each zinc ion is coordinated by three cysteine residues and one histidine organized in a particular conformation called *cross-brace*. (Figure 15) The zinc ions are indispensable to maintain the protein's tertiary structure and their exclusion from the protein would disrupt the three-dimensional structure and hence the biological function. The crucial role played by zinc ions is demonstrated by the fact that mutations of residues involved in the coordination of zinc lead to the abolition of the BCA2.<sup>[113]</sup>

Given the crucial role of zinc ions in the catalytic domain of BCA2, a series of “*zinc-affinic*” compounds from National Cancer Institute database have been screened (2006) for their ability to inhibit BCA2. These studies led to the identification of Disulfiram<sup>[114, 115]</sup> (DSF) (Chart 12) a registered drug for the treatment of alcoholism by virtue of its aldehyde dehydrogenase (ALDH) inhibitory activity. In addition to the ability of the DSF and its analogues to complex zinc and copper ions it was reported an alternative mechanism for removal of zinc ions from the RING domain called “*zinc-ejection*”.

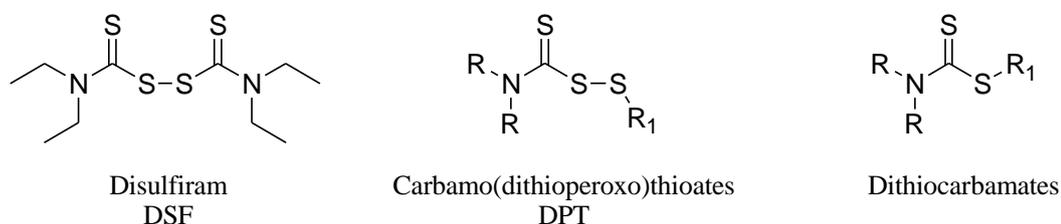
**Figure 15 Cross-brace structure of the RING domain as found in BCA2. C, cysteine (purple); H, histidine (green); zinc ion (yellow).**



According to this mechanism, some changes of the cysteine residues would cause the expulsion of  $Zn^{2+}$  from the active site. In the specific case of the DSF it seems that inhibition

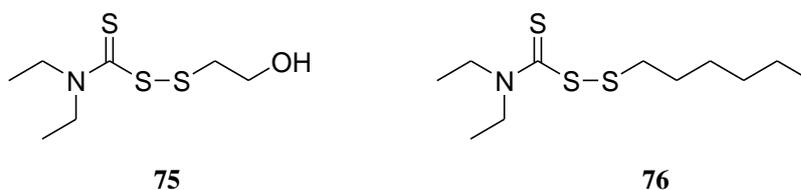
of BCA2 is not related to the ability to complex zinc ions but rather to a mechanism of type zinc-ejection. DSF is clinically well tolerated, with few side effects, but its structure considerably unstable, the whole pharmacokinetic profile and its inhibitory activity against dell'ALDH have limited its development as antitumor agent. In order to further elucidate the structure-activity relationship, other studies have led to the identification of two classes of potent and selective BCA2 inhibitor: DSF analogues and carbamo(dithioperoxo)thioates (DPT) (Chart 13).<sup>[116]</sup>

### Chart 13



Such a study conducted on a small number of these compounds, in comparison with the DSF and with other potential inhibitors of BCA2, showed excellent results in particular for the two derivatives **75** and **76** (Chart 14). Both resulted selectively active on breast cancer cell lines, expressing different levels of BCA2 (MCF-7, MDA-MB-231 / ER, T47D) with IC<sub>50</sub> values in the sub-micromolar range, while they resulted inactive on BCA2-negative cell lines (MDA-MB-231) and on normal cells of breast epithelium (MCF10A) (Table9). In addition, this two compounds, unlike the DSF, have the advantage of showing only low levels of inhibition of ALDH (Table 10).

### Chart 14



**Table 9**

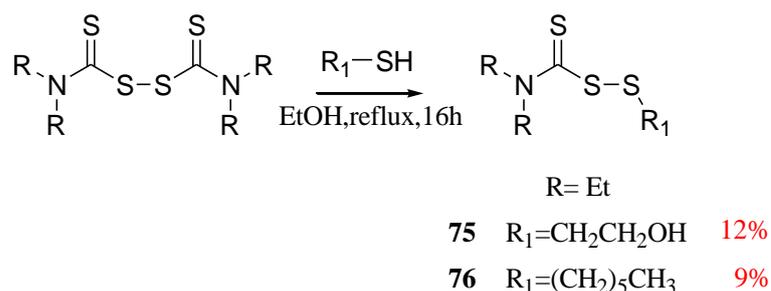
IC <sub>50</sub> (mean) ± SD (μM)					
<b>Cmp.</b>	<b>MCF-7</b>	<b>MDA-MB-231/ER</b>	<b>T47D</b>	<b>MDA-MB-231</b>	<b>MCF10A</b>
<b>DSF</b>	0.1 ± 0.01	0.32 ± 0.14	0.17 ± 0.03	> 10	10 ± 0.2
<b>75</b>	0.5 ± 0.24	2.75 ± 0.17	0.30 ± 0.03	> 10	> 10
<b>76</b>	0.43 ± 0.1	0.35 ± 0.38	0.23 ± 0.02	> 10	> 10

**Table 10**

<b>Compound (15 μM)</b>	<b>ALDH inhibition</b>
<b>BAAA</b> (positive control)	0 %
<b>DEAB</b> (negative control)	98.68 %
<b>DSF</b>	57.26 %
<b>75</b>	8.6 %
<b>76</b>	9.8 %

The same study suggested that the presence of the N(C = S)S-S motif seems to be necessary for the BCA2 inhibitory activity, as demonstrated by the fact that the dithiocarbamates (Chart 13), lacking the disulfide bridge, showed inactive on the above cell lines. DPTs have this important structural feature and they also allow to changes both R and R<sub>1</sub> group in order to further investigate the basis of the selective inhibition of BCA2 and ALDH enzyme. However, one hurdle in the development of disulfiram analogues and specially DPT compounds, lies in the synthesis process. Conventional method (Scheme 24) involved heating a mixture of disulfiram analogue with an appropriate thiol in ethanol under refluxing conditions for 16 hours. This procedure led to the required DPTs in low yield following extensive column chromatography alongside a number of mixed disulfide-based by-products.

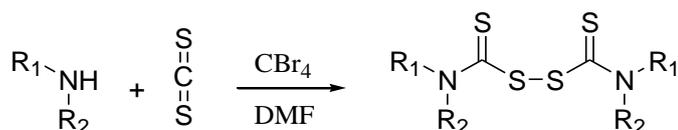
## Scheme 24



## 5.2 Synthesis of substituted carbamo(dithioperoxy)thioates as potential BCA2-inhibitory anticancer agents

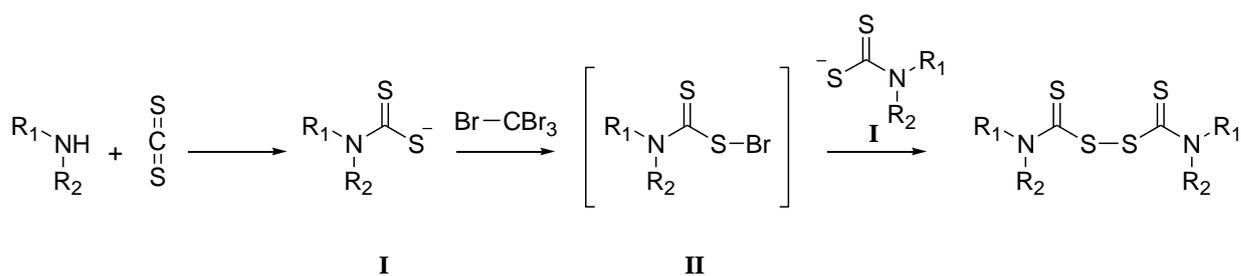
Given the biological relevance of the DPTs in the context of anticancer drug discovery, and the difficulties in obtaining products through the previously described method, a new synthetic protocol for reliable and high yielding access to this class of compound was required. For the development of a new synthetic approach to DPTs of crucial relevance was a study by *Liang et al.* in which an alternative synthesis for DSF analogues was reported. This method involves the use of CBr<sub>4</sub> to promote the formation of a disulfide bridge via reaction of amines with carbon disulfide according to the Scheme 25.<sup>[117]</sup>

## Scheme 25



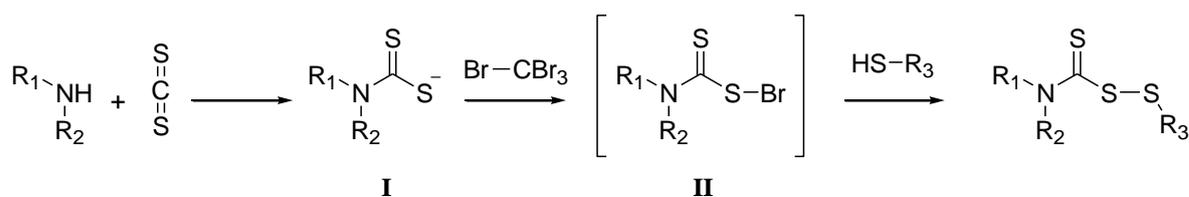
They also proposed a possible mechanism (Scheme 26) where an intermediate sulfenil bromide (**II**) is the direct responsible of the S-S coupling. The last step in this mechanism works like a bimolecular nucleophilic substitution (S<sub>N</sub>2), as bromine anion is a good leaving group.

### Scheme 26



On the basis of that possible mechanism we thought that a thiol, instead of a dithiocarbamate (I), could have displaced the bromine atom on the sulfenyl bromide leading to a DPT (Scheme 27).

### Scheme 27

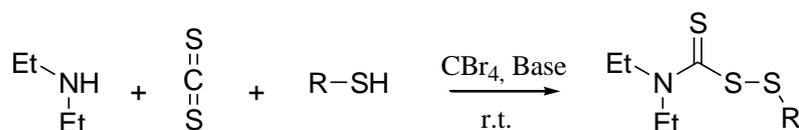


Two model reaction between diethylamine, and 2-mercaptoethanol or 1-hexanethiol respectively, were examined under various conditions (Table 11).

A first adaptation of the method developed by *Liang et al.* to synthesize DSF analogues was performed for the synthesis of DPTs. Diethylamine, the appropriate thiol, carbon disulfide and 1 equivalent of CBr<sub>4</sub> were mixed in DMF at 0 °C. The mixture was left to stir for 4 hours at room temperature and after extraction with ethyl acetate the crude was purified by column chromatography in order to isolate the desired products. However yields, in both cases, were a little higher than those obtained with the conventional method (Table 11, entry 1 and 2). In order to improve the method, further researches led us to think that a non-nucleophilic base (i.e. triethylamine) could have made the thiol more reactive towards the sulfenyl bromide. Another study conducted by the same authors of the above, regarding the use of CBr<sub>4</sub> to promote the formation of C-S bonds<sup>[118]</sup>, confirmed that DCM instead of DMF could be used without affecting the reactivity of the intermediate sulfenyl bromide, simplifying also the workup. Adopting these conditions, moderate yields were obtained (entry 5 and 6). To further improve the new synthetic protocol of DPTs, other attempts were made. The use of two equivalents of the alkyl thiol and triethylamine surprisingly didn't lead to the formation of the desired products instead yielded the corresponding dithiocarbamates as by-products (entry 7 and 8).

In the light of this unexpected result we took a step back to understand better the crucial role of CBr<sub>4</sub>. Considering that in absence of CBr<sub>4</sub> DPTs weren't obtained (entry 3 and 4), we confirmed its role in affecting the outcome of the reaction suggesting that an excess could have improved yields. In fact, adding two equivalents of CBr<sub>4</sub> to a mixture of diethylamine, alkyl thiol, carbon disulfide and triethylamine in DCM, higher yields were obtained (entry 9 and 10).

**Table 11**



Entry	(Et) <sub>2</sub> NH/CS <sub>2</sub> /RSH/CBr <sub>4</sub> (eq.)	R	Base (eq.)	Solv.	Time	Yield
1	1.0/1.0/1.0/1.0	CH <sub>2</sub> CH <sub>2</sub> OH	-	DMF	4h	15 %
2	1.0/1.0/1.0/1.0	<i>n</i> -Hex	-	DMF	4h	27 %
3	1.0/1.0/1.0/0	CH <sub>2</sub> CH <sub>2</sub> OH	NEt <sub>3</sub> (1.1)	DCM	2h	-
4	1.0/1.0/1.0/0	<i>n</i> -Hex	NEt <sub>3</sub> (1.1)	DCM	2h	-
5	1.0/1.0/1.0/1.0	CH <sub>2</sub> CH <sub>2</sub> OH	NEt <sub>3</sub> (1.1)	DCM	2h	38 %
6	1.0/1.0/1.0/1.0	<i>n</i> -Hex	NEt <sub>3</sub> (1.1)	DCM	2h	48 %
7	1.0/1.0/2.0/1.0	CH <sub>2</sub> CH <sub>2</sub> OH	NEt <sub>3</sub> (2.0)	DCM	2h	-
8	1.0/1.0/2.0/1.0	<i>n</i> -Hex	NEt <sub>3</sub> (2.0)	DCM	2h	-
9	1.0/1.0/1.0/2.0	CH <sub>2</sub> CH <sub>2</sub> OH	NEt <sub>3</sub> (1.1)	DCM	2h	58 %
10	1.0/1.0/1.0/2.0	<i>n</i> -Hex	NEt <sub>3</sub> (1.1)	DCM	2h	85 %

Finally, under the optimized conditions, a range of reaction between various secondary amines and alkyl thiols were investigated. We chose six secondary amines **77a-f** and four thiols **78a-d** (Scheme 28) achieving a series of 24 compounds (**79a-x**, Table 12), included compounds **75** and **76**, previously synthesized and reported in Table 12 as **79a** and **79c** respectively. Thus, with my new synthetic strategy, I obtained a new set of carbamo(dithioperoxo)thioates with yields over than 60 % in most cases, in mild conditions and in short reaction time.

Scheme 28

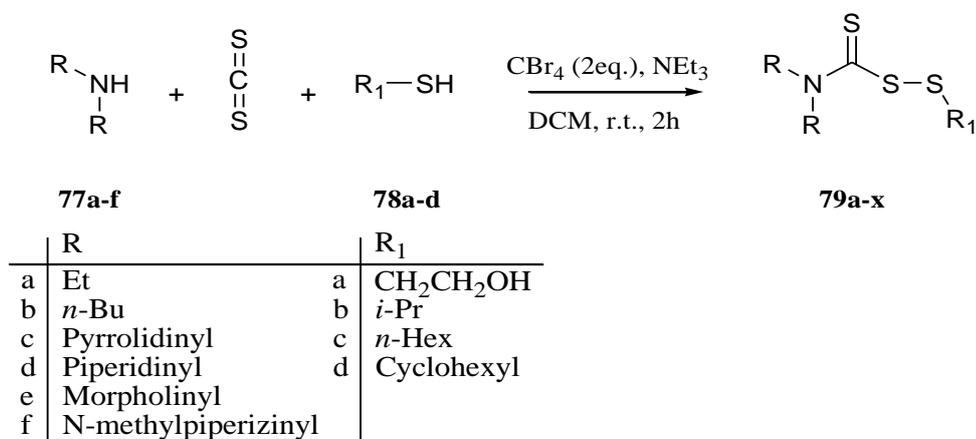


Table 12

Cmp.	R	R <sub>1</sub>	Yield	Cmp.	R	R <sub>1</sub>	Yield
79a	Et	CH <sub>2</sub> CH <sub>2</sub> OH	58 %	79m	-(CH <sub>2</sub> ) <sub>5</sub> -	CH <sub>2</sub> CH <sub>2</sub> OH	65 %
79b	Et	<i>i</i> -Pr	78 %	79n	-(CH <sub>2</sub> ) <sub>5</sub> -	<i>i</i> -Pr	68 %
79c	Et	<i>n</i> -Hex	85 %	79o	-(CH <sub>2</sub> ) <sub>5</sub> -	<i>n</i> -Hex	80 %
79d	Et	Cyclohexyl	68 %	79p	-(CH <sub>2</sub> ) <sub>5</sub> -	Cyclohexyl	90 %
79e	Bu	CH <sub>2</sub> CH <sub>2</sub> OH	64 %	79q	-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	CH <sub>2</sub> CH <sub>2</sub> OH	25 %
79f	Bu	<i>i</i> -Pr	68 %	79r	-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	<i>i</i> -Pr	75 %
79g	Bu	<i>n</i> -Hex	80 %	79s	-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	<i>n</i> -Hex	85 %
79h	Bu	Cyclohexyl	80 %	79t	-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	Cyclohexyl	90 %
79i	-(CH <sub>2</sub> ) <sub>4</sub> -	CH <sub>2</sub> CH <sub>2</sub> OH	60 %	79u	-(CH <sub>2</sub> ) <sub>2</sub> NMe(CH <sub>2</sub> ) <sub>2</sub> -	CH <sub>2</sub> CH <sub>2</sub> OH	30 %
79j	-(CH <sub>2</sub> ) <sub>4</sub> -	<i>i</i> -Pr	90 %	79v	-(CH <sub>2</sub> ) <sub>2</sub> NMe(CH <sub>2</sub> ) <sub>2</sub> -	<i>i</i> -Pr	76 %
79k	-(CH <sub>2</sub> ) <sub>4</sub> -	<i>n</i> -Hex	85 %	79w	-(CH <sub>2</sub> ) <sub>2</sub> NMe(CH <sub>2</sub> ) <sub>2</sub> -	<i>n</i> -Hex	70 %
79l	-(CH <sub>2</sub> ) <sub>4</sub> -	Cyclohexyl	95 %	79x	-(CH <sub>2</sub> ) <sub>2</sub> NMe(CH <sub>2</sub> ) <sub>2</sub> -	Cyclohexyl	75 %

### 5.3 Biological evaluation

On a small selection of DPTs, biologist group of the School of Pharmacy and Pharmaceutical Sciences (Cardiff University), carried out a preliminary *in vitro* evaluation across human breast cancer cell lines where BCA2 expression status was known. The mean IC<sub>50</sub> (μM) values for compounds **79i**, **79k**, **79o**, **79s** and **79w** in human breast cancer cell lines MCF-7 (BCA2-positive), MDA-MB-231 (BCA2-negative) and MCF-10A (non-cancerous control) were determined. The results presented in Table 9 indicate selective sub-micromolar IC<sub>50</sub> activity selectively within MCF-7 cells, consistent with previously synthesized analogues of this broad class. However, further studies on the anticancer properties of this new series are ongoing at Cardiff Univeristy.

**Table 9**

<b>Cmp.</b>	<b>MCF-7</b>	<b>MDA-MB-231</b>	<b>MCF10A</b>
<b>79i</b>	0.5	> 10	> 10
<b>79k</b>	0.9	> 10	> 10
<b>79o</b>	0.6	> 10	> 10
<b>79s</b>	1	10	> 10
<b>79w</b>	0.9	> 10	> 10

Considering that DPTs are not extensively explored either for the chemistry and for their anticancer activity, this work, which led to a new and efficient synthetic protocol for DPTs, has been published by us.<sup>[119]</sup>

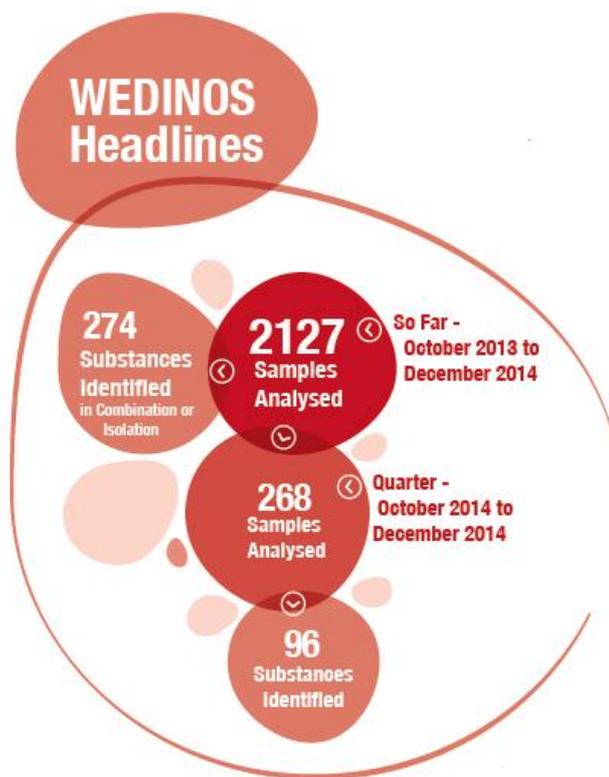
## 5.4 The Wedinos project

The Welsh word *wedinos* means “after dark” but also serves as an acronym for Welsh Emerging Drugs and Identification of Novel Substances. The Wedinos project is a free government-funded service which has been designed for the collection and testing of substances and, most importantly dissemination of pragmatic evidence based harm reduction information for users. Based at Llandough Hospital in Cardiff, in partnership with Cardiff and Vale University Health Board, the project has been established in response to an increase in presentations at emergency departments, and self reports/provider reports of unexpected/ill effects by users of NPSs, new combinations of “established” substances, new combinations of licit and illicit and new combinations performance/image enhancing substances. Since 2013, Wedinos allows the public and professionals (organisations such as substance misuse services, housing and hostels, youth clubs and young people’s services, education services, night clubs and bars, mental health community teams, local authorities, ambulance service and police) to send away samples of unknown substances to have them analysed. Following analysis of the samples, timely and accurate information regarding the chemical profile of the samples tested, based on the content and legal context, will be made available through a variety of means including the website, health alerts via press release and the quarterly bulletin “PHILTRE”.<sup>[120]</sup> Samples of unknown or unidentified substances are tested using innovative technologies including TOF mass spectrometry, high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), UV-Vis absorption spectroscopy and NMR spectroscopy.

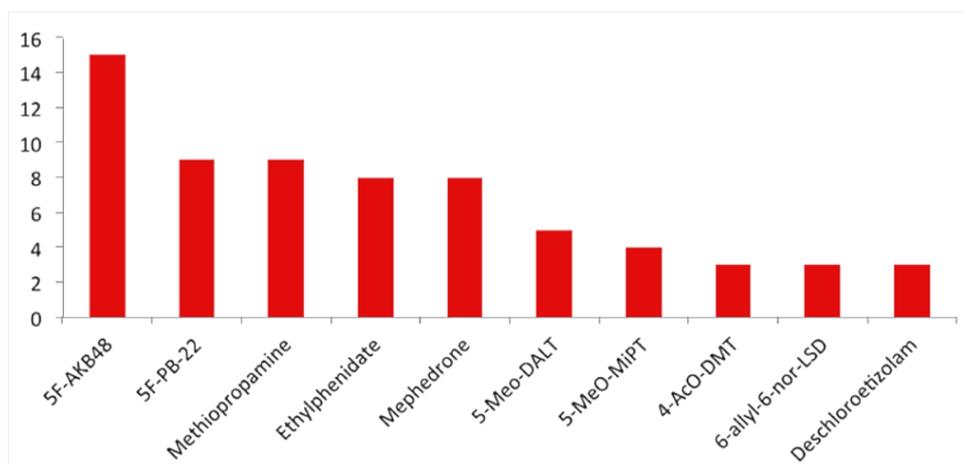
In the period I have worked as Wedinos analyst (October - December 2014), I contributed to analyze roughly 268 samples through a first rapid screening using TOF mass spectrometry with Direct Sample Analysis (DSA) system and gas chromatography-mass spectrometry (GC/MS) or NMR spectroscopy. NMR analysis ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{19}\text{F}$ ) revealed particularly useful to unequivocally determine the chemical structure in case of isomerism. This led to the identification of 96 substances either in combination or isolation (Figure 16).

Figure 17 shows that 5F-AKB48 and 5F-PB-22, (Chart 15) belonging to the group of Synthetic Cannabinoid Receptor Agonists (SCRA), are the most commonly identified psychoactive substances. Both of them are not currently controlled in UK and are frequently sold as “legal”, on-line and via “head-shops”, marked “not for human consumption”. The majority of SCRA compounds have been found in ready-to-smoke products\herbal products, but some have occurred as pure substances in powder form.

**Figure 16. Samples analysed and substances identified**



**Figure 17. Ten most commonly identified New Psychoactive Substances**

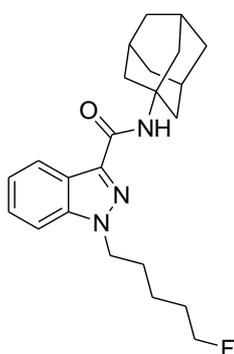


The term “synthetic cannabinoids” covers all synthetic substances that bind to one of the two known cannabinoid receptors (CB1 or CB2). Most of SCRA compounds have higher affinities for the CB1 receptor than Tetrahydrocannabinol (THC) and are full agonists of this site.<sup>[121]</sup> THC in comparison acts as a partial agonist.<sup>[122]</sup> According to The European

Monitoring Centre for Drugs and Drug Addiction (EMCDDA), despite these substances are not chemically similar to cannabis, they can be extremely potent and their use may result in different and potentially more serious health consequences.<sup>[123]</sup>

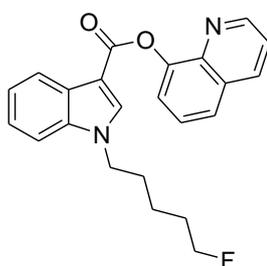
The Welsh government says the Wedinos project is vital to protect people and providing timely and accurate information this service can save lives. Next steps include working with other healthcare providers, including the pharmacy service and accident and emergency departments, to discuss how they might contribute.

### Chart 15



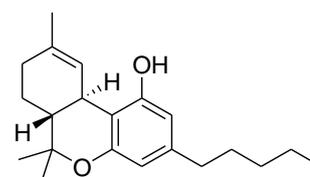
**5F-AKB48**

N-(Adamantan-1-yl)-1-(5-fluoropentyl)-*1H*-indazole-3-carboxamide



**5F-PB-22**

1-Pentyfluoro-1*H*-indole-3-carboxylic acid 8-quinolinyl ester



**THC**

Tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]piran-1-ol

## 6. EXPERIMENTALS

### 6.1 Central Nervous System drug candidates: Chemistry

All melting points were taken on a Buchi-Tottoli capillary apparatus and were uncorrected. IR spectra were determined with a Shimadzu IR Affinity-1 spectrophotometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured in  $\text{DMSO-}d_6$  or  $\text{CDCl}_3$  solutions, unless otherwise specified, at 200 and 50.3 MHz respectively, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM or with a SEPACORE BÜCHI chromatography apparatus or with BIOTAGE 40i chromatography apparatus. Elemental Analysis (C, H, N) were within  $\pm 0.4\%$  of the theoretical values.

#### 6.1.1 Synthesis of 2,5,6,7-tetrahydro-4H-isoindol-4-ones (10a and 10b)

**Preparation of 2,5,6,7-tetrahydro-4H-isoindol-4-one (10a).** To a solution of *p*-toluenesulfonylmethyl isocyanide (TOSMIC) (40 mmol) in dry THF (50 mL), 2-cyclohexen-1-one **9** (40 mmol) was added at room temperature, followed by the dropwise addition of a solution of *t*-BuOK (49 mmol) in the same solvent (50 mL). After 1 hour stirring at room temperature water was added and the solution was extracted with ethyl acetate. The organic extracts were dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane : ethyl acetate 90 : 10). Yield: 85%. The spectroscopic data are in agreement with the literature.<sup>[60]</sup>

**Preparation of 2-(phenylsulfonyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (10b).** To a solution of **10a** (7 mmol) in dry DMF (5 mL), NaH (7.7 mmol) was added at 0 °C and the reaction was stirred for 1 hour at room temperature. Benzenesulfonyl chloride (7.7 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 3 hours. Then the reaction was poured into ice and brine, and the resulted precipitate was collected by filtration. The crude product was purified by chromatography column (dichloromethane). Yield: 70%; pale brown solid; mp: 115.4 - 116.2 °C; IR: 1664 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.84 - 1.98 (2H, m,  $\text{CH}_2$ ), 2.34 - 2.47 (2H, m,  $\text{CH}_2$ ), 2.50 - 2.68 (2H, m,  $\text{CH}_2$ ), 7.27 (1H, d,  $J = 1.5$  Hz, H-1), 7.60 - 7.90 (4H, m, H-3', H-4', H-5' and H-3), 8.06 - 8.15 (2H, m, H-2' and H-

6');  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 20.70 (t), 23.60 (t), 38.97 (t), 116.42 (d), 120.79 (d), 125.15 (s), 127.26 (d x 2), 129.14 (s), 130.07 (d x 2), 135.14 (d), 137.25 (s), 194.42 (s). Anal Calcd. for  $\text{C}_{14}\text{H}_{13}\text{NO}_3\text{S}$ : C, 61.07; H, 4.76; N, 5.09. Found: C, 60.98; H, 4.62; N, 4.94.

### 6.1.2 Synthesis of ethyl 4-oxo-4,5,6,7-tetrahydro-2H-isoindoles-1-carboxylate (16a and 16b)

**Preparation of 2-[(dimethylamino)methylidene]cyclohexane-1,3-dione (13).** A solution of 1,3-cyclohexanedione **12** (70 mmol) in N,N-dimethylformamide dimethyl acetal (20 mL) was refluxed for 1 hour. The reaction was left to reach room temperature and solvent was evaporated under reduced pressure. Residue was triturated with diethyl ether and filtered off. Solid was directly used in the next step without any further purification. Yield: 96%; brown solid, mp: 101.7 - 102.4 °C; IR: 1660 (CO), 1585 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.90 - 2.03 (2H, m,  $\text{CH}_2$ ), 2.46 (4H, s,  $\text{CH}_2$  x 2), 3.18 (3H, s,  $\text{CH}_3$ ), 3.41 (3H, s,  $\text{CH}_3$ ), 8.05 (1H, s, CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 19.33 (t), 37.92 (q x 2), 44.42 (t), 48.32 (t), 109.11(d), 162.05 (s), 162.12 (s), 195.83 (s). Anal Calcd. for  $\text{C}_9\text{H}_{13}\text{NO}_2$ : C, 64.65; H, 7.84; N, 8.38. Found: C, 64.78; H, 7.52; N, 8.60.

**Preparation of ethyl [(2,6-dioxocyclohexylidene)methyl]amino}acetate (14).** To a solution of **13** (50 mmol) in glacial acetic acid (40 mL) glycine ethyl ester hydrochloride (55 mmol) was added and the mixture was refluxed for 2 hours. The reaction was left to reach room temperature and solvent was evaporated under reduced pressure. Residue was triturated with ethanol and filtered off. Solid was directly used in the next step without any further purification. Yield: 90%; yellow solid; mp: 58.8 - 59.4 °C; IR: 3434 (NH), 1747 (CO), 1713 (CO), 1667 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.21 (3H, t,  $J = 7.1$  Hz,  $\text{CH}_3$ ), 1.74 - 1.91 (2H, m,  $\text{CH}_2$ ), 2.27 - 2.48 (4H, m,  $\text{CH}_2$  x 2), 4.14 (2H, q,  $J = 7.1$  Hz,  $\text{CH}_2$ ), 4.37 (2H, d,  $J = 5.6$  Hz,  $\text{CH}_2$ ), 8.07 (1H, d,  $J = 14.2$  Hz, CH), 10.79 - 10.98 (1H, m, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 13.99 (q), 19.39 (t), 37.03 (t), 37.39 (t), 49.96 (t), 60.97 (t), 108.05 (s), 159.45 (d), 169.03 (s), 195.215 (s), 198.34 (s). Anal Calcd. for  $\text{C}_{11}\text{H}_{15}\text{NO}_4$ : C, 58.66; H, 6.71; N, 6.22. Found: C, 58.98; H, 6.56; N, 6.43.

**Preparation of ethyl 4-oxo-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (16a).** To a solution of **14** (10 mmol) in dry acetonitrile (15 mL), N,N-dimethylformamide dimethyl acetal (6.7 mL, 50 mmol) was added. The reaction was refluxed for 2 hours, then solvent was evaporated under reduced pressure. The oily residue of ethyl-3-(dimethylamino)-2-[(2,6-dioxocyclohexylidene)methyl]amino}prop-2-enoate **15** was directly used for the next step

without any further purification. The crude was solubilised in dry dichloromethane (5 mL), trifluoroacetic anhydride (11 mmol) was added dropwise at 0° C and the reaction mixture was stirred for 16 hours at room temperature. The solvent was evaporated under reduced pressure and the oily residue was neutralized with a solution of NaHCO<sub>3</sub> added dropwise at 0° C. The aqueous solution was extracted with ethyl acetate and the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated. Purification required chromatography (cyclohexane : ethyl acetate 50 : 50) and further crystallization from ethyl acetate. Yield: 50%; white solid; mp: 184.1 - 184.9 °C (EtOAc); IR: 3435 (NH), 1687 (CO), 1662 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.28 (3H, t, *J* = 7.0 Hz, CH<sub>3</sub>), 1.82 - 2.08 (2H, m, CH<sub>2</sub>), 2.30 - 2.45 (2H, m, CH<sub>2</sub>), 2.85 - 2.98 (2H, m, CH<sub>2</sub>), 4.26 (2H, q, *J* = 7.0 Hz, CH<sub>2</sub>), 7.44 (1H, s, H-3), 12.42 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 13.40 (q), 22.03 (t), 24.02 (t), 38.47 (t), 59.73 (t), 118.00 (s), 122.70 (s), 123.59 (d), 133.95 (s), 160.48 (s), 193.80 (s). Anal. Calcd. for C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub> C, 63.76; H, 6.32; N, 6.76. Found: C, 63.62; H, 6.35; N, 6.66.

**Preparation of ethyl 4-oxo-2-(phenylsulfonyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (16b).** To a solution of **16a** (20 mmol) in dry DMF (15 mL), NaH (22 mmol) was added at 0 °C and the reaction was stirred for 1 hour at room temperature. Benzenesulfonyl chloride (30 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 3 hours. Then the reaction was poured into ice and brine, and, the resulted precipitate was collected by filtration. The crude product was purified by chromatography (dichloromethane). Yield: 80 %; pale yellow solid; mp: 135.5 - 136.3 °C; IR : 1720 (CO), 1680 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.19 (3H, t, *J* = 7.0 Hz, CH<sub>3</sub>), 1.90 - 2.08 (2H, m, CH<sub>2</sub>), 2.40 - 2.55 (2H, m, CH<sub>2</sub>), 2.80 - 2.90 (2H, m, CH<sub>2</sub>), 4.18 (2H, q, *J* = 7.0 Hz, CH<sub>2</sub>), 7.60 - 7.85 (3H, m, H-3', H-4' and H-5'), 8.06 - 8.15 (2H, m, H-2' and H-6'), 8.27 (1H, s, H-3); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 13.95 (q), 22.23 (t), 22.93 (t), 38.51 (t), 60.83 (t), 120.35 (s), 122.58 (s), 128.00 (d), 128.09 (d x 2), 129.38 (d x 2), 134.80 (d), 137.40 (s), 138.02 (s), 158.60 (s), 193.74 (s). Anal. Calcd. for C<sub>17</sub>H<sub>17</sub>NO<sub>5</sub>S: C, 58.78; H, 4.93; N, 4.03. Found: C, 58.89; H, 4.99; N, 4.18.

### 6.1.3 Synthesis of 4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-ones (26 and 29)

**Preparation of 1-(phenylsulfonyl)-1*H*-pyrrole (23).** To a solution of pyrrole **22** (2.72 mL, 40 mmol) in dry THF (28 mL), under N<sub>2</sub> atmosphere at -78 °C, 24 mL of LDA (2M in THF/heptanes/ethylbenzene, 48 mmol) were added and the mixture was heated until room

temperature and stirred for 2 hours. The reaction mixture was then cooled again at  $-78\text{ }^{\circ}\text{C}$ , and a solution of benzenesulfonyl chloride (5.6 mL, 48 mmol) in dry THF (25 mL) was added. After 2 hours and half, the reaction was heated to room temperature, and 5 mL of water were added. The reaction mixture was distilled *in vacuo* and the residue was washed with a solution of  $\text{NH}_4\text{Cl}$  (20 g) in HCl 0.1 M (140 mL). The solution was extracted with dichloromethane (3 x 100 mL) and the organic phase was dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane). Quantitative yield. The spectroscopic data are in agreement with the structure of the commercial product.

**Preparation of 5-oxo-5-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]pentanoic acid (24).** A suspension of  $\text{AlCl}_3$  (8 g, 60 mmol) and glutaric anhydride (2.3 g, 20 mmol) in dry dichloromethane, (40 mL) was stirred at room temperature for 1 hour. A solution of **23** (2 g, 10 mmol) in dry dichloromethane (20 mL), was added and the reaction mixture was stirred for 1 hour and 30 minutes. Ice and water were added (80 mL), and the two phases were separated. The aqueous fraction was extracted with dichloromethane (3 x 40 mL). The organic phases were collected, and dried over  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure to give the crude product that was purified by chromatography (dichloromethane : ethyl acetate 60 : 40). Yield: 80%, light brown solid; mp:  $111.6 - 111.8\text{ }^{\circ}\text{C}$ ; IR: 3550 (OH), 1706 (CO), 1675 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.72 - 1.83 (2H, m,  $\text{CH}_2$ ), 2.26 (2H, t,  $J = 7.3\text{ Hz}$ ,  $\text{CH}_2$ ), 2.85 (2H, t,  $J = 7.3\text{ Hz}$ ,  $\text{CH}_2$ ), 6.67 (1H, dd,  $J = 3.3, 1.8\text{ Hz}$ , H-4), 7.45 (1H, dd,  $J = 3.3, 1.8\text{ Hz}$ , H-5), 7.64 - 7.86 (3H, m, H-3', H-4' and H-5'), 8.06 - 8.13 (2H, m, H-2' and H-6'), 8.21 (1H, s, H-2), 12.07 (1H, s, OH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 19.2 (t), 32.8 (t), 38.0 (t), 112.0 (d), 122.2 (d), 125.4 (d), 127.2 (d x 2), 128.6 (s), 130.1 (d x 2), 135.1 (d), 137.3 (s), 174.1 (s), 194.7 (s). Anal. Calcd. for  $\text{C}_{15}\text{H}_{15}\text{NO}_5\text{S}$ : C, 56.06; H, 4.70; N, 4.36. Found: C, 56.23; H, 4.79; N, 4.45.

**Preparation of 5-[1-(Phenylsulfonyl)-1H-pyrrol-3-yl]pentanoic acid (25).** An amalgam of zinc (8.5 g, 130 mmol) and mercury (II) chloride (2.96 g, 10.9 mmol) in water (12 mL) and HCl 12 M (0.7 mL) was prepared; after 30 minutes the aqueous phase was eliminated and **24** (3.5 g, 10.9 mmol), water (5 mL), toluene (50 mL) and HCl 12 M (12.5 mL) were added. The reaction mixture was heated to reflux for 4 hours, and the two phases were separated. The aqueous phase was extracted with dichloromethane (3 x 30 mL) and the organic fractions were collected, dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane : ethyl acetate 90 : 10). Yield: 75%; white

solid; mp: 84.0 - 84.2 °C; IR: 3548 (OH), 1701 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.46 (4H, t,  $J = 7.2$  Hz,  $\text{CH}_2 \times 2$ ), 2.19 (2H, t,  $J = 7.2$  Hz,  $\text{CH}_2$ ), 2.34 (2H, t,  $J = 7.2$  Hz,  $\text{CH}_2$ ), 6.24 (1H, dd,  $J = 2.9, 1.5$  Hz, H-4), 7.10 (1H, s, H-2), 7.24 (1H, dd,  $J = 2.9, 1.5$  Hz, H-5), 7.57 - 7.79 (3H, m, H-3', H-4' and H-5'), 7.89 - 7.95 (2H, m, H-2' and H-6'), 11.99 (1H, s, OH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 24.0 (t), 25.7 (t), 28.8 (t), 33.3 (t), 115.1 (d), 117.5 (d), 121.2 (d), 126.5 (d  $\times 2$ ), 129.3 (s), 129.7 (d  $\times 2$ ), 134.3 (d), 138.3 (s), 174.4 (s). Anal. Calcd. for  $\text{C}_{15}\text{H}_{17}\text{NO}_4\text{S}$ : C, 58.61; H, 5.57; N, 4.56. Found: C, 58.64; H, 5.71; N, 4.48.

**Preparation of 1-(phenylsulfonyl)-4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one (26).**

Trifluoroacetic anhydride (5.4 mL, 39 mmol) was added to a solution of **25** (2 g, 6.5 mmol) in dry dichloromethane (20 mL), and the reaction mixture was stirred for three hours at room temperature. The solvent was evaporated and a saturated solution of  $\text{NaHCO}_3$  (40 mL) was added to residue. The solid formed was filtered and purified by chromatography (dichloromethane). Yield: 75%; white solid; mp: 99.3 - 99.6 °C; IR: 1659 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.63 - 1.78 (4H, m,  $\text{CH}_2 \times 2$ ), 2.53 (2H, t,  $J = 5.9$  Hz,  $\text{CH}_2$ ), 2.77 (2H, t,  $J = 5.9$  Hz,  $\text{CH}_2$ ), 6.39 (1H, d,  $J = 3.2$  Hz, H-3), 7.59 - 7.76 (3H, m, H-3', H-4' and H-5'), 7.81 (1H, d,  $J = 3.2$  Hz, H-2), 7.96 (2H, dd,  $J = 8.2, 1.6$  Hz, H-2' and H-6');  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 21.0 (t), 24.4 (t), 25.8 (t), 40.9 (t), 113.3 (d), 127.5 (d  $\times 2$ ), 129.0 (d  $\times 2$ ), 129.1 (d), 131.0 (s), 133.8 (d), 139.0 (s), 139.6 (s), 190.2 (s). Anal. Calcd. for  $\text{C}_{15}\text{H}_{15}\text{NO}_3\text{S}$ : C, 62.26; H, 5.23; N, 4.84. Found: C, 62.34; H, 5.19; N, 4.91.

**Preparation of 4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one (29).**

A solution of NaOH (1.66 g, 41.52 mmol) in ethanol (15 mL) was added to a suspension of **26** (3 g, 10.4 mmol) in ethanol (15 mL), and the resulting mixture was heated to reflux for 3 hours. The reaction mixture was concentrated under reduce pressure and the residue poured into ice and water (20 mL) and acidified with HCl 6 M. The solution was extracted with dichloromethane (3  $\times$  30 mL) and the organic phases was collected, dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography (dichloromethane) Yield: 80%; white solid; mp: 55.1 - 55.3 °C; IR: 3438 (NH), 1617 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.69 - 1.90 (4H, m,  $\text{CH}_2 \times 2$ ), 2.56 (2H, t,  $J = 6.2$  Hz,  $\text{CH}_2$ ), 2.79 (2H, t,  $J = 6.2$  Hz,  $\text{CH}_2$ ), 6.01 (1H, d,  $J = 2.8$  Hz, H-3), 6.95 (1H, d,  $J = 2.8$  Hz, H-2), 11.38 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 22.32 (t), 26.05 (t), 27.14 (t), 41.41 (t), 110.61 (d), 124.51 (d), 129.35 (s), 132.15 (s), 190.51 (s). Anal. Calcd. for  $\text{C}_9\text{H}_{11}\text{NO}$ : C, 72.46; H, 7.43; N, 9.39. Found: C, 72.49; H, 7.56; N, 9.52.

#### 6.1.4 Synthesis of ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (37)

**Preparation of 2,2,2-trichloro-1-(1*H*-pyrrol-2-yl)ethanone (33).** To 8 mL of diethyl ether, 5.5 mL of trichloroacetyl chloride (49.2 mmol) were added, and the solution was stirred at room temperature for 3 hours. A solution of pyrrole **22** (3 g, 44.7 mmol) in diethyl ether (25 mL) was added and the mixture was stirred for 1 hour. Very slowly the reaction mixture was neutralized with a solution of K<sub>2</sub>CO<sub>3</sub> (3.9 g in 11.7 mL of H<sub>2</sub>O). The two phases were separated and the organic fraction was filtered on celite. The filtrate was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Quantitative yield. The spectroscopic data are in agreement with the literature.<sup>[65]</sup>

**Preparation of ethyl 1*H*-pyrrole-2-carboxylate (34).** To a solution of potassium ethylate (4 g, 49.2 mmol) in ethanol (120 mL), a solution of **33** (9.5 g, 44.7 mmol) in dichloromethane (75 mL) was added. After 30 minutes at room temperature, the reaction mixture was evaporated. The residue was dissolved with HCl 2 N (45 mL) and diethyl ether (105 mL). The two phases were separated and the aqueous fraction was extracted with diethyl ether (2 x 80 mL). The organic phase was washed with a saturated solution of Na<sub>2</sub>CO<sub>3</sub> (100 mL). The organic fraction was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to give the crude product that was purified by chromatography (dichloromethane). Yield 95%. The spectroscopic data are in agreement with the literature.<sup>[65]</sup>

**Preparation of 5-[5-(ethoxycarbonyl)-1*H*-pyrrol-3-yl]-5-oxopentanoic acid (35).** A suspension of AlCl<sub>3</sub> (12 g, 90 mmol) and glutaric anhydride (3.40 g, 30 mmol) in dry dichloromethane, (60 mL) was stirred at room temperature for 1 hour. A solution of **34** (2.1 g, 15 mmol) in dry dichloromethane (30 mL) was added and the reaction mixture was stirred for 1 hour and 30 minutes. Ice and water were added (80 mL), and the two phases were separated. The aqueous fraction was extracted with ethyl acetate (3 x 60 mL). The organic phases were collected, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure to give the crude product that was purified by chromatography (dichloromethane : ethyl acetate 50 : 50). Yield: 91%; light brown solid; mp: 81.4 - 82.3 °C; IR: 3337 (OH broad), 1702 (CO), 1696 (CO), 1653 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.29 (3H, t, *J* = 6.7 Hz, CH<sub>3</sub>), 1.70 - 1.86 (4H, m, CH<sub>2</sub> x 2), 2.27 (2H, t, *J* = 6.2 Hz, CH<sub>2</sub>), 2.80 (2H, t, *J* = 6.2 Hz, CH<sub>2</sub>), 4.26 (2H, q, *J* = 6.7 Hz, CH<sub>2</sub>), 7.13 (1H, s, H-4), 7.72 (1H, s, H-2), 12.07 (1H, s, OH), 12.51 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.23 (q), 19.59 (t), 32.87 (t), 37.89 (t), 60.12 (t), 114.02 (d),

123.64 (s), 125.77 (s), 127.76 (d), 160.11 (s), 174.22 (s), 194.65 (s). Anal. Calcd. for C<sub>12</sub>H<sub>15</sub>NO<sub>5</sub>: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.86; H, 5.85; N, 5.69.

**Preparation of 5-[5-(ethoxycarbonyl)-1*H*-pyrrol-3-yl]pentanoic acid (36).** To a solution of **35** (1.5 g, 6 mmol) in trifluoroacetic acid (13 mL), triethylsilane (3.3 mL, 21 mmol) was added and the reaction mixture was stirred at room temperature for 16 hours. The solvent was evaporated and brine (40 mL) was added to the residue. The solid formed was filtered and purified by chromatography (dichloromethane : ethyl acetate 50 : 50). Yield: 80%; brown solid; mp: 84.6 - 85.4 °C; IR: 3355 (OH broad), 1700 (CO), 1653 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.26 (3H, t, *J* = 7.1 Hz, CH<sub>3</sub>), 1.41 - 1.62 (4H, m, CH<sub>2</sub> x 2), 2.21 (2H, t, *J* = 6.4 Hz, CH<sub>2</sub>), 2.39 (2H, t, *J* = 6.4 Hz, CH<sub>2</sub>), 4.20 (2H, q, *J* = 7.1 Hz, CH<sub>2</sub>), 6.60 (1H, s, H-4), 6.79 (1H, s, H-2), 11.55 (1H, s, OH), 11.99 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.36 (q), 24.12 (t), 25.69 (t), 29.87 (t), 33.48 (t), 59.31 (t), 114.52 (d), 121.53 (s), 121.68 (d), 124.51 (s), 160.43 (s), 174.54 (s). Anal. Calcd. for C<sub>12</sub>H<sub>17</sub>NO<sub>4</sub>: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.33; H, 7.25; N, 5.72.

**Preparation of ethyl 8-oxo-1,4,5,6,7,8-hexahydrocyclohepta[*b*]pyrrole-2-carboxylate (37).** Trifluoroacetic anhydride (3.5 mL, 25.2 mmol) was added to a solution of **36** (1 g, 4.2 mmol), in dry dichloromethane (10 mL), and the reaction mixture was stirred for 3 hours at room temperature. The solvent was evaporated and a saturated solution of NaHCO<sub>3</sub> (40 mL) was added to the residue. The solid formed, was filtered and purified by chromatography (dichloromethane). Yield: 70%; white solid; mp: 76.7 - 76.9 °C; IR: 3415 (NH), 1709 (CO), 1633 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.28 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 1.60 - 1.97 (4H, m, CH<sub>2</sub> x 2), 2.63 (2H, t, *J* = 6.6 Hz, CH<sub>2</sub>), 2.79 (2H, t, *J* = 6.6 Hz, CH<sub>2</sub>), 4.24 (2H, q, *J* = 6.9 Hz, CH<sub>2</sub>), 6.67 (1H, s, H-3), 11.91 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.1 (q), 21.5 (t), 25.4 (t), 26.0 (t), 41.2 (t), 60.2 (t), 115.7 (d), 125.9 (s), 131.9 (s), 132.7 (s), 160.0 (s), 192.1 (s). Anal. Calcd. for C<sub>12</sub>H<sub>15</sub>NO<sub>3</sub>: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.31; H, 6.78; N, 6.42.

**6.1.5 Synthesis of 5-bromo-2,5,6,7-tetrahydro-4*H*-isoindol-4-ones (11a).** To a solution of **11b** (5 mmol) in methanol (100 mL), sodium hydroxide solution 5 M (1.1 mL, 5.5 mmol) was added and the reaction mixture was stirred at room temperature for 1 hour. The solution was concentrated, cooled at 0° C and acidified using HCl 6 M. The aqueous phase was extracted with dichloromethane (3 x 20 mL) and the organic phase was washed with a solution of 5%

aq. NaHCO<sub>3</sub>. Then the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The residue was triturated with diethyl ether or further purified by chromatography (dichloromethane : ethyl acetate 95 : 5). Yield: 70%; grey solid; mp: 133.8 - 134.6 °C; IR: 3438 (NH), 1661 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR: 2.25-2.50 (2H, m, CH<sub>2</sub>), 2.65 - 2.79 (2H, m, CH<sub>2</sub>), 4.67 - 4.78 (1H, m, CH), 6.68 (1H, s, H-1), 7.44 (1H, s, H-3), 11.66 (1H, bs, NH); <sup>13</sup>C NMR : 18.13 (t), 33.70 (t), 53.51 (d), 114.67 (d), 117.92 (s), 121.74 (d), 123.57 (s), 186.43 (s). Anal. Calcd. for C<sub>8</sub>H<sub>8</sub>BrNO: C, 44.89; H, 3.77; N, 6.54. Found: C, 44.71; H, 4.02; N, 6.69.

**6.1.6 General synthesis for 5-bromo-tetrahydroisoindol-4-ones (11b, 17a and 17b).** To a suspension of CuBr<sub>2</sub> (5.4 mmol) in dry ethyl acetate (30 mL) the appropriate ketone **10b**, **16a** and **16b** (3 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the CuBr formed and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography.

**5-Bromo-2-(phenylsulfonyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (11b).** This compound was obtained from reaction of **10b**. Purification was performed by chromatography (dichloromethane). Yield: 90%; white solid; mp: 134.6 - 135.3 °C; IR: 1683 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.15 - 2.50 (2H, m, CH<sub>2</sub>), 2.65 - 2.78 (2H, m, CH<sub>2</sub>), 4.85 - 4.85 (1H, m, CH), 7.34 (1H, s, H-1), 7.65 - 7.90 (3H, m, H-3', H-4' and H-5'), 8.04 (1H, s, H-3), 8.08 - 8.19 (2H, m, H-2' and H-6'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 17.93 (t), 32.27 (t), 52.26 (d), 116.83 (d), 122.12 (s), 123.09 (d), 127.14 (s), 127.44 (d x 2), 130.15 (d x 2), 135.35 (d), 136.99 (s) 186.70 (s). Anal. Calcd. for C<sub>14</sub>H<sub>12</sub>BrNO<sub>3</sub>S: C, 47.47; H, 3.41; N, 3.95. Found: C, 47.56; H, 3.57; N, 4.03

**Ethyl 5-bromo-4-oxo-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (17a).** This compound was obtained from reaction of **16a**. Purification was performed by flash column chromatography (dichloromethane : ethyl acetate 95: 5). Yield: 80%; white solid; mp: 189.6 - 190.0 °C; IR: 3248 (NH), 1728 (CO), 1686 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.31 (3H, t, *J* = 7.0 Hz, CH<sub>3</sub>), 2.25 - 2.60 (2H, m, CH<sub>2</sub>), 2.80 - 3.20 (2H, m, CH<sub>2</sub>), 4.28 (2H, q, *J* = 7.0 Hz, CH<sub>2</sub>), 4.75 - 4.85 (1H, m, CH), 7.63 (1H, s, H-3), 12.69 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.28 (q), 19.34 (t), 32.71 (t), 52.45 (d), 59.94 (t), 118.39 (s), 119.41 (s), 125.78 (d), 132.05 (s), 160.27 (s), 186.30 (s). Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>BrNO<sub>3</sub>: C, 46.18; H, 4.23; N, 4.90. Found: C, 45.98; H, 4.38; N, 5.22.

**Ethyl 5-bromo-4-oxo-2-(phenylsulfonyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylate (17b).** This compound was obtained from reaction of **16b**. Purification was performed by chromatography (dichloromethane). Yield: 80%; white solid; mp: 122.8 - 123.6 °C; IR: 1723 (CO), 1687 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.21 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.25 - 2.65 (2H, m,  $\text{CH}_2$ ), 2.75 - 3.15 (2H, m,  $\text{CH}_2$ ), 4.17 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 4.85 - 4.98 (1H, m, CH), 7.60 - 7.85 (3H, m, H-3', H-4' and H-5'), 8.10 - 8.22 (2H, m, H-2' and H-6'), 8.45 (1H, s, H-3);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 13.94 (q), 19.72 (t), 31.50 (t), 51.53 (d), 61.05 (t), 119.58 (s), 120.53 (s), 128.32 (d x 2), 129.42 (d x 2), 130.17 (d), 135.01 (d), 135.93 (s), 137.06 (s), 158.40 (s), 186.13 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{16}\text{BrNO}_5\text{S}$ : C, 47.90; H, 3.78; N, 3.29. Found: C, 47.99; H, 3.57; N, 3.53.

### 6.1.7 Synthesis of 7-bromo-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-ones (27, 32 and 28)

**7-Bromo-1-(phenylsulfonyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (27).** To a suspension of  $\text{CuBr}_2$  (5.4 mmol) in dry ethyl acetate (30 mL), **26** (3 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the  $\text{CuBr}$  formed and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography (dichloromethane). Yield: 82 %; colourless oil; IR: 1622 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.78 - 2.35 (4H, m,  $\text{CH}_2$  x 2), 2.63 - 3.00 (2H, m,  $\text{CH}_2$ ), 4.64 - 4.75 (1H, m, CH), 6.16 (1H, d,  $J = 2.8$  Hz, H-3), 7.48 - 7.63 (3H, m, H-3', H-4' and H-5'), 7.70 (1H, d,  $J = 2.8$  Hz, H-2), 7.94 - 8.04 (2H, m, H-2' and H-6');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 22.80 (t), 28.03 (t), 32.24 (t), 55.22 (d), 113.08 (d), 128.04 (d x 2), 128.78 (d x 2), 129.00 (s), 129.67 (d), 133.63 (d), 137.46 (s), 139.39 (s), 184.83 (s). Anal. Calcd. for  $\text{C}_{15}\text{H}_{14}\text{BrNO}_3\text{S}$ : C, 48.92; H, 3.83; N, 3.80. Found: 49.03; H, 3.90; N, 4.00.

**1-(Benzoyl)-4,5,6,7-tetrahydrocyclohepta[*b*]pyrrol-8(1*H*)-one (31).** To a solution of **29** (6 mmol) in dry DMF (12 mL),  $\text{NaH}$  (6.6 mmol) was added at 0 °C and the reaction was stirred for 1 hour and 30 minutes at room temperature. Benzoyl chloride (9 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 3 hours. Then the reaction was poured into ice and brine (40 mL), and the aqueous solution was extracted with dichloromethane (3 x 40 mL). The organic phase was dried over  $\text{Na}_2\text{SO}_4$  and the solvent

evaporated under reduced pressure. The crude product was purified by chromatography (dichloromethane). Yield: 82 %; colourless oil; IR: 1699 (CO) 1645 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.83 - 2.01 (4H, m,  $\text{CH}_2 \times 2$ ), 2.59 (2H, t,  $J = 6.1$  Hz,  $\text{CH}_2$ ), 2.81 - 2.90 (2H, m,  $\text{CH}_2$ ), 6.13 (1H, d,  $J = 2.4$  Hz, H-3), 7.17 (1H, d,  $J = 2.4$  Hz, H-2), 7.40 - 7.73 (5H, m, Ar);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 22.41 (t), 26.04 (t), 27.58 (t), 42.12 (t), 112.42 (d), 128.18 (d), 128.54 (d  $\times 2$ ), 129.57 (d  $\times 2$ ), 130.11 (s), 133.18 (d), 133.77 (s), 137.21 (s), 169.04 (s), 192.12 (s). Anal. Calcd. for  $\text{C}_{16}\text{H}_{15}\text{NO}_2$ : C, 75.87; H, 5.97; N, 5.53. Found: 76.03; H, 6.12; N, 5.44.

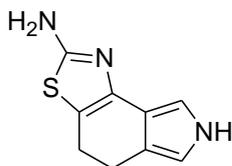
**1-(Benzoyl)-7-bromo-4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one (32).** To a solution of **31** (5 mmol) in dry THF (10 mL), pyridine hydrobromide perbromide (5 mmol) dissolved in dry THF (5 mL), was added and the reaction mixture was stirred at room temperature for 6 hours. The solid formed was filtered and eliminated, and the organic phases was concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 mL), washed with a solution at 5% of  $\text{NaHCO}_3$  (10 mL), dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography column (dichloromethane). Yield: 60 %; yellow oil; IR: 1711 (CO) 1636 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.93 - 2.02 (2H, m,  $\text{CH}_2$ ), 2.33 - 2.42 (2H, m,  $\text{CH}_2$ ), 2.82 - 3.09 (2H, m,  $\text{CH}_2$ ), 4.63 - 4.70 (1H, m, CH), 6.12 (1H, d,  $J = 2.6$  Hz, H-3), 7.24 (1H, d,  $J = 2.6$  Hz, H-2), 7.41 - 7.76 (5H, m, Ar);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 22.81 (t), 28.53 (t), 32.32 (t), 55.27 (d), 112.46 (d), 128.61 (d), 129.77 (d  $\times 2$ ), 129.92 (d  $\times 2$ ), 130.14 (s), 133.02 (s), 133.38 (d), 136.29 (s), 168.58 (s), 184.62 (s). Anal. Calcd. for  $\text{C}_{16}\text{H}_{14}\text{BrNO}_2$ : C, 57.85; H, 4.25; N, 4.22. Found: 57.95; H, 4.32; N, 4.01.

**7-Bromo-4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one (28).** To a solution of **32** (5 mmol) in ethanol (15 mL), sodium hydroxide (10 mmol) was added and the reaction mixture was stirred at room temperature for 4 hours. The solution was concentrated, cooled at  $0^\circ\text{C}$  and acidified using HCl 6 M. The aqueous phase was extracted with dichloromethane (3  $\times$  50 mL) and the organic phase was collect and dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The residue was purified by chromatography column (dichloromethane). Yield: 72 %; pale brown solid, mp: 99.9 - 100.5 ; IR: 3425 (NH), 1622 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.94 - 2.03 (2H, m,  $\text{CH}_2$ ), 2.33 - 2.39 (2H, m,  $\text{CH}_2$ ), 2.85 - 3.08 (2H, m,  $\text{CH}_2$ ), 4.83 - 4.90 (1H, m, CH), 6.10 (1H, d,  $J = 2.4$  Hz, H-3), 7.03 (1H, d,  $J = 2.4$  Hz, H-2), 9.45 (1H, bs, NH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 23.3 (t), 28.5 (t), 32.8 (t), 55.0 (d), 112.2 (d), 126.3 (d), 127.0

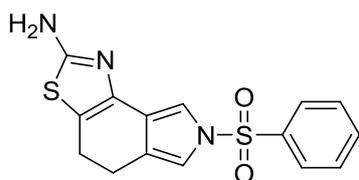
(s), 133.0 (s), 185.2 (s). Anal. Calcd. for  $C_9H_{10}BrNO$ : C, 47.39; H, 4.42; N, 6.14. Found: 47.40; H, 4.27; N, 6.38.

### 6.1.8 General synthesis for 2-amino-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindoles (18a-d)

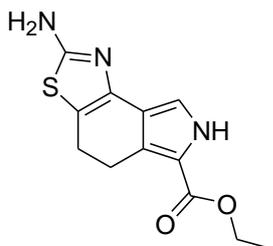
To a solution of the appropriate bromo derivative **11a,b** and **17a,b** (0.5 mmol) in dry DMF (5 mL),  $Na_2CO_3$  (1 mmol) and thiourea (1 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and the precipitate was filtered. No further purification was needed.



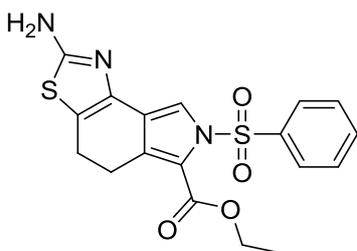
**5,7-Dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (18a).** This compound was obtained from reaction of **11a**. Yield: 80%; grey solid;  $R_f=0.45$  ( $CH_2Cl_2/EtOAc$  70:30); mp: 63.6 - 63.9 °C; IR: 3455 - 3386 ( $NH_2$ ), 3287 (NH)  $cm^{-1}$ ;  $^1H$  NMR ( $DMSO-d_6$ ) (ppm): 2.68 (4H, s,  $CH_2 \times 2$ ), 6.51 (1H, s, H-6), 6.63 (1H, s, H-8) 10.36 (1H, s, NH);  $^{13}C$  NMR ( $DMSO-d_6$ ) (ppm): 20.86 (t), 22.58 (t), 110.47 (d), 110.82 (s), 113.69 (d), 116.93 (s), 117.57 (s), 145.33 (s), 166.16 (s). Anal. Calcd. for  $C_9H_9N_3S$ : C, 56.52; H, 4.74; N, 21.97. Found: C, 56.28; H, 4.59; N, 22.06.



**7-(Phenylsulfonyl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (18b).** This compound was obtained from reaction of **11b**. Yield: 100%; light grey solid;  $R_f=0.31$  ( $CH_2Cl_2/EtOAc$  95:5); mp: 209.7 - 210.1 °C; IR: 3407 - 3276 ( $NH_2$ )  $cm^{-1}$ ;  $^1H$  NMR ( $DMSO-d_6$ ) (ppm): 2.65 - 2.75 (4H, m,  $CH_2 \times 2$ ), 6.91 (2H, s,  $NH_2$ ), 7.03 (1H, d,  $J = 2.0$  Hz, H-6), 7.11 (1H, d,  $J = 2.0$  Hz, H-8), 7.55 - 7.80 (3H, m, H-3', H-4' and H-5'), 7.95 (2H, d,  $J = 7.0$  Hz, H-2' and H-6');  $^{13}C$  NMR ( $DMSO-d_6$ ) (ppm): 20.06 (t), 21.56 (t), 111.79 (d), 116.91 (d), 117.16 (s), 123.18 (s), 124.61 (s), 126.61 (d  $\times 2$ ), 129.78 (d  $\times 2$ ), 134.30 (d), 138.11 (s), 139.33 (s), 166.86 (s). Anal. Calcd. for  $C_{15}H_{13}N_3O_2S_2$ : C, 54.36; H, 3.95; N, 12.68. Found: C, 54.22; H, 3.75; N, 12.78.



**Ethyl 2-amino-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate (18c).** This compound was obtained from reaction of **17a**. Yield: 100%; light grey solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  60:40); mp: 201.4 - 202.1 °C; IR : 3442 - 3388 ( $\text{NH}_2$ ), 3327 (NH), 1687 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.29 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.70 - 2.85 (2H, m,  $\text{CH}_2$ ), 2.90 - 3.08 (2H, m,  $\text{CH}_2$ ), 4.23 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 6.84 (3H, s, H-8 and  $\text{NH}_2$ ), 11.45 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 14.38 (q), 21.21 (t), 21.73 (t), 59.36 (t), 111.86 (s), 115.82 (d), 117.77 (s), 119.53 (s), 125.27 (s), 114.16 (s), 160.77 (s), 166.63 (s). Anal. Calcd. for  $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ : C, 54.74; H, 4.98; N, 15.96. Found: C, 55.02; H, 4.85; N, 15.66.

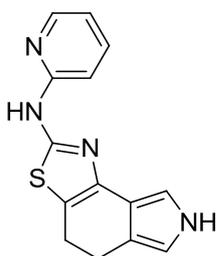


**Ethyl 2-amino-7-(phenylsulfonyl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate (18d).** This compound was obtained from reaction of **17b**. Yield: 95%; light grey solid;  $R_f=0.32$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp: 212.7 - 213.3 °C; IR: 3450 - 3353 ( $\text{NH}_2$ ), 1698 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.18 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.70 - 3.10 (4H, m,  $\text{CH}_2 \times 2$ ), 4.16 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 7.04 (2H, s,  $\text{NH}_2$ ), 7.52 (1H, s, H-8), 7.60 - 7.80 (3H, m, H-3', H-4' and H-5'), 7.95 - 8.10 (2H, m, H-2' and H-6');  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 13.90 (q), 21.03 (t), 21.16 (t), 60.72 (t), 117.18 (s), 118.88 (d), 120.22 (s), 120.92 (s), 127.19 (d  $\times 2$ ), 129.30 (d  $\times 2$ ), 133.16 (s), 134.15 (d), 138.21 (s), 138.51 (s), 158.99 (s), 167.26 (s). Anal. Calcd. for  $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_4\text{S}_2$ : C, 53.58; H, 4.25; N, 10.41. Found: C, 53.82; H, 4.57; N, 10.29.

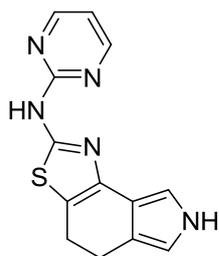
**6.1.9 General synthesis for pyridin-2-yl-thiourea and pyrimidin-2-yl-thiourea.** Benzoyl chloride (10 mmol) was added dropwise to a freshly prepared solution of  $\text{NH}_4\text{SCN}$  (11 mmol) in dry acetone (10 mL) and the mixture was heated under reflux for 15 minutes. Heating was stopped and a solution of the commercially available amine (2-aminopyridine or 2-

aminopyrimidine, 10 mmol) in acetone (10 mL) was added. Following the addition, the mixture was heated under reflux for 30 minutes, then poured onto cracked ice with vigorous stirring. The N-benzoyl thiourea intermediate precipitate was collected by filtration and washed with more water. This crude material was added in one portion to a preheated (80 - 85 °C) stirring solution of 10 % aq. NaOH (10 mL) for 5-10 minutes, then the mixture was poured onto excess ice containing excess aq. HCl 6M (5 mL). After the acidification, the pH was adjusted to 8 - 8.5 with NaHCO<sub>3</sub>. The desired products was collected, washed with water and dried. Yields and spectroscopic data are in agreement with the literature.<sup>[23]</sup>

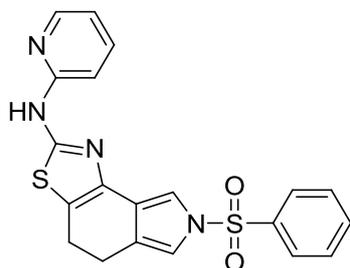
**6.1.10 General synthesis for N-(pyridin-2-yl) and N-(pyrimidin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amines (20a-f).** To a solution of the appropriate bromoderivative **11a,b** or **17a** (1 mmol) in dry acetonitrile (10 mL), pyridin-2-yl-thiourea or pyrimidin-2-yl-thiourea (1.1 mmol) and Na<sub>2</sub>CO<sub>3</sub> (1.1 mmol) were added and the mixture was refluxed for 16 hours. Solvent was evaporated and crude residue was purified by chromatography. In most cases further purification by crystallization was needed.



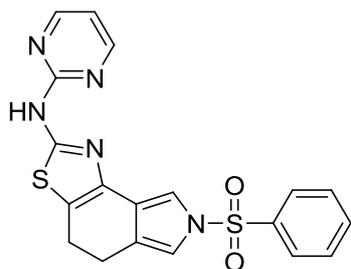
**N-(Pyridin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (20a).** This compound was obtained from reaction of **11a** with pyridin-2-ylthiourea. Purification required flash column chromatography (dichloromethane : ethyl acetate 85 : 15) and further crystallization from ethanol. Yield: 45%; brown solid; R<sub>f</sub>=0.32 (EtOAc); mp: 118.3 - 119.1 °C (EtOH); IR: 3469 (NH), 3405 (NH) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.65 - 2.82 (4H, m, CH<sub>2</sub> x 2), 6.57 (1H, s, H-6), 6.75 (1H, s, H-8), 6.87 (1H, t, *J* = 6.0 Hz, H-5''), 6.99 (1H, d, *J* = 8.3 Hz, H-3''), 7.66 (1H, t, *J* = 7.4 Hz, H-4''), 8.26 (1H, d, *J* = 3.1 Hz, H-6''), 10.47 (1H, s, NH), 11.23 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 20.80 (t), 22.34 (t), 110.40 (d), 110.51 (d), 113.96 (d), 115.26 (s), 115.41 (d), 117.25 (s), 117.30 (s), 137.60 (d), 141.56 (s), 146.49 (d), 151.89 (s), 157.06 (s). Anal. Calcd. for C<sub>14</sub>H<sub>12</sub>N<sub>4</sub>S: C, 62.66; H, 4.51; N, 20.88. Found: C, 62.72; H, 4.59; N, 20.68.



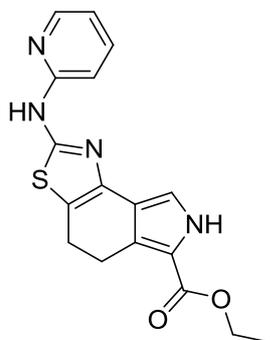
**N-(Pyrimidin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (20b).** This compound was obtained from reaction of **11a** with pyrimidin-2-yl-thiourea. Purification was performed by flash column chromatography (cyclohexane : ethyl acetate 40 : 60). Yield: 30%; light brown solid;  $R_f=0.18$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp  $>400$  °C; IR: 3466 (NH), 3312 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.60 - 2.90 (4H, m,  $\text{CH}_2 \times 2$ ), 6.58 (1H, s, H-6), 6.77 (1H, s, H-8), 6.98 (1H, t,  $J = 4.7$  Hz, H-5''), 8.58 (2H, d,  $J = 4.7$  Hz, H-4'' and H-6''), 10.50 (1H, s, NH), 11.62 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.62 (t), 22.23 (t), 110.73 (d), 113.67 (d), 114.06 (d), 116.79 (s), 116.87 (s), 117.36 (s), 141.94 (s), 156.75 (s), 156.84 (s), 157.94 (d x 2). Anal. Calcd. for  $\text{C}_{13}\text{H}_{11}\text{N}_5\text{S}$ : C, 57.97; H, 4.12; N, 26.00. Found: C, 58.15; H, 4.29; N, 25.88.



**7-(Phenylsulfonyl)-N-(pyridin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (20c).** This compound was obtained from reaction of **11b** with pyridin-2-yl-thiourea. Purification required flash column chromatography (dichloromethane : ethyl acetate 95 : 5) and further crystallization from methanol. Yield: 60%; pale yellow solid;  $R_f=0.25$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  90:10); mp: 225.0 - 226.4 °C (MeOH); IR: 3222 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 2.80 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 6.72 (1H, d,  $J = 8.3$  Hz, H-3''), 6.85 (1H, t,  $J = 5.2$  Hz, H-5''), 6.96 (1H, s, H-6), 7.21 (1H, s, H-8), 7.30 - 7.60 (4H, m, H-3', H-4', H-5' and H-4''), 7.80 - 7.90 (2H, m, H-2' and H-6'), 8.33 (1H, d,  $J = 4.1$  Hz, H-6''), 9.79 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 20.71 (t), 22.71 (t), 110.54 (d), 112.73 (d), 116.32 (d), 116.56 (d), 121.89 (s), 122.57 (s), 124.84 (s), 126.81 (d x 2), 129.28 (d x 2), 133.58 (d), 137.73 (d), 138.45 (s), 138.98 (s), 146.77 (d), 151.25 (s), 159.35 (s). Anal. Calcd. for  $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2\text{S}_2$ : C, 58.80; H, 3.95; N, 13.72. Found: C, 58.98; H, 4.08; N, 13.96.

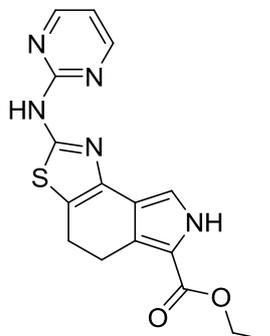


**7-(Phenylsulfonyl)-N-(pyrimidin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindol-2-amine (20d).** This compound was obtained from reaction of **11b** with pyrimidin-2-ylthiourea. Purification required flash column chromatography (dichloromethane : ethyl acetate 90 : 10) and further crystallization from ethanol. Yield: 50%; light brown solid;  $R_f=0.15$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 249.5 - 250.7 °C (EtOH); IR: 3228 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.65 - 2.90 (4H, m,  $\text{CH}_2 \times 2$ ), 7.02 (H, t,  $J = 4.6$  Hz, H-5''), 7.17 (1H, s, H-6), 7.20 (1H, s, H-8), 7.55 - 7.85 (3H, m, H-3', H-4' and H-5'), 7.95 - 8.08 (2H, d,  $J = 7.1$  Hz, H-2' and H-6'), 8.60 (2H, d,  $J = 4.6$  Hz, H-4'' and H-6''), 11.71 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 19.92 (t), 21.29 (t), 111.91 (d), 113.95 (d), 117.13 (d), 122.57 (s), 122.74 (s), 124.82 (s), 126.67 (d  $\times 2$ ), 129.87 (d  $\times 2$ ), 134.45 (d), 137.97 (s), 138.66 (s), 156.76 (s), 158.00 (d  $\times 2$ ), 159.21 (s). Anal. Calcd. for  $\text{C}_{19}\text{H}_{15}\text{N}_5\text{O}_2\text{S}_2$ : C, 55.73; H, 3.69; N, 17.10. Found: C, 55.89; H, 3.76; N, 16.96.

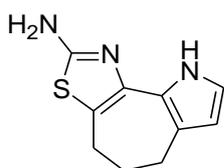


**Ethyl 2-(pyridin-2-ylamino)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindole-6-carboxylate (20e).** This compound was obtained from reaction of **17a** with pyridin-2-ylthiourea. Purification required flash column chromatography (dichloromethane : ethyl acetate 50 : 50) and further crystallization from ethanol. Yield: 50%; light brown solid;  $R_f=0.46$  (EtOAc); mp: 278.4 - 279.1 °C (EtOH); IR: 3286 (NH), 3252 (NH), 1683 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.31 (3H, t,  $J = 6.9$  Hz,  $\text{CH}_3$ ), 2.85 - 3.15 (4H, m,  $\text{CH}_2 \times 2$ ), 4.26 (2H, q,  $J = 6.9$  Hz,  $\text{CH}_2$ ), 6.82 - 7.10 (3H, m, H-8, H-5'' and H-3''), 7.67 (1H, t,  $J = 7.0$  Hz, H-4''), 8.24 (1H, d,  $J = 3.5$  Hz, H-6''), 11.28 (1H, s, NH), 11.56 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 14.36 (q), 21.09 (t), 21.45 (t), 59.49 (t), 110.53 (d), 115.59 (d), 115.73 (d), 116.33 (s), 117.79 (s),

119.14 (s), 125.62 (s), 137.73 (d), 140.06 (s), 146.46 (d), 151.64 (s), 157.43 (s), 160.76 (s).  
Anal. Calcd. for C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>S: C, 59.98; H, 4.74; N, 16.46. Found: C, 60.08; H, 4.89; N, 16.37.

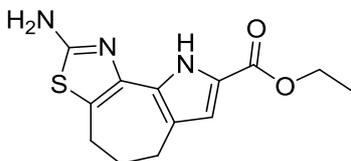


**Ethyl 2-(pyrimidin-2-ylamino)-5,7-dihydro-4H-[1,3]thiazolo[4,5-e]isoindole-6-carboxylate (20f).** This compound was obtained from reaction of **17a** with pyrimidin-2-ylthiourea. Purification was performed by flash column chromatography (ethyl acetate). Yield: 45%; light brown solid; R<sub>f</sub>=0.50 (EtOAc); mp: 313.4 - 314.6 °C; 3291 (NH), 3239 (NH), 1679 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.31 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 2.75 - 3.20 (4H, m, CH<sub>2</sub> x 2), 4.25 (2H, q, *J* = 6.9 Hz, CH<sub>2</sub>), 6.80 - 7.10 (2H, m, H-8 and H-5''), 8.60 (2H, d, *J* = 4.6 Hz, H-4'' and H-6''), 11.45 - 11.90 (2H, m, NH x 2); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.34 (q), 20.99 (t), 21.40 (t), 59.53 (t), 113.74 (d), 115.86 (d), 117.74 (s), 118.93 (s), 125.63 (s), 140.55 (s), 156.83 (s), 157.20 (s), 157.96 (d x 2), 158.13 (s), 160.75 (s). Anal. Calcd. for C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>2</sub>S: C, 56.29; H, 4.43; N, 20.51. Found: C, 56.44; H, 4.59; N, 20.39.



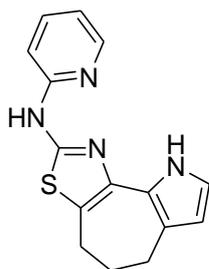
**6.1.11 Synthesis of 4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (39a).** To a solution of **28** (0.5 mmol) in dry DMF (5 mL), K<sub>2</sub>CO<sub>3</sub> (1 mmol) and thiourea (1 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine (30 mL) and the precipitate was filtered off. The solid was purified by crystallization from ethanol. Yield: 64 %; brown solid; R<sub>f</sub>=0.54 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 70:30); mp: 66.4 - 67.2 °C (EtOH); IR: 3455 - 3416 (NH<sub>2</sub>), 3287 (NH) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) (ppm): 2.02 - 2.09 (2H, m, CH<sub>2</sub>), 2.87 - 2.94 (4H, m, CH<sub>2</sub> x 2), 4.97 (2H, bs, NH<sub>2</sub>), 5.99 (1H, d, *J* = 2.9 Hz, H-7), 6.58 (1H, d, *J* = 2.9 Hz, H-8), 9.27 (1H, bs, NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 24.31 (t), 27.57 (t), 28.52 (t), 110.28 (d), 116.18 (d), 117.02 (s), 120.53

(s), 124.79 (s), 139.16 (s), 144.61 (s). Anal. Calcd. for C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>S: C, 58.51; H, 5.40; N, 20.47. Found: C, 58.65; H, 5.53; N, 20.30.

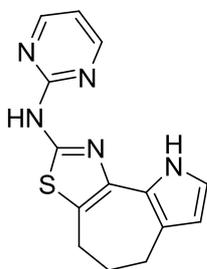


**6.1.12 Synthesis of ethyl 2-amino-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (39b).** To a suspension of CuBr<sub>2</sub> (1.8 mmol) in dry ethyl acetate (10 mL) ketone **37** (1 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the CuBr formed. The solvent was evaporated and the residue dissolved in dry DMF (8 mL). K<sub>2</sub>CO<sub>3</sub> (2 mmol) and thiourea (2 mmol) were added and the new mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and aqueous solution was extracted with dichloromethane (3 x 30 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure. The crude product was then purified by chromatography (dichloromethane : ethyl acetate 80 : 20). Yield: 52%; white solid; R<sub>f</sub>=0.42 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 70:30); mp: 190.3 - 191.1 °C; IR: 3370 (NH), 3202 - 3156 (NH<sub>2</sub>), 1692 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.27 (3H, t, *J* = 7.1 Hz, CH<sub>3</sub>), 1.85 - 1.99 (2H, m, CH<sub>2</sub>), 2.75 - 2.85 (4H, m, CH<sub>2</sub> x 2), 4.22 (2H, q, *J* = 7.1 Hz, CH<sub>2</sub>), 6.63 (1H, s, H-7), 6.88 (2H, s, NH<sub>2</sub>), 9.64 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.33 (q), 23.91 (t), 27.04 (t), 27.64 (t), 59.55 (t), 116.41 (d), 118.95 (s), 119.33 (s), 121.62 (s), 129.87 (s), 137.86 (s), 159.91 (s), 165.52 (s). Anal. Calcd. for C<sub>13</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>S: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.16; H, 5.57; N, 15.30.

**6.1.13 General synthesis for *N*-(pyridin-2-yl) and *N*-(pyrimidin-2-yl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (41a and 41b).** To a solution of **28** (1 mmol) in dry DMF (5 mL), K<sub>2</sub>CO<sub>3</sub> (1 mmol) and 1-(pyridin-2-yl)thiourea or 1-(pyrimidin-2-yl)thiourea (1 mmol) were added and the mixture was stirred 16 hours. The reaction was poured into ice and brine and the solid formed was filtered and purified by flash column chromatography.

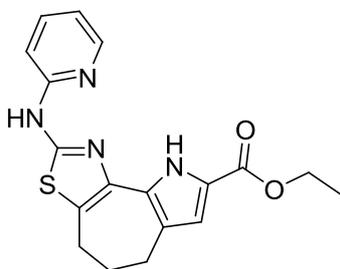


***N*-(Pyridin-2-yl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (41a).** This compound was obtained from reaction of **28** with pyridin-2-yl-thiourea. Purification was performed by flash column chromatography (dichloromethane : ethyl acetate 95: 5). Yield: 45%; white solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 192.5 - 193.0 °C ; IR: 3455 (NH), 3403 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.85 - 2.10 (2H, m,  $\text{CH}_2$ ), 2.75 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 5.85 - 5.92 (1H, m, H-7), 6.55 - 6.65 (1H, m, H-8), 6.90 (1H, t,  $J = 6.1$  Hz, H-5''), 7.21 (1H, d,  $J = 8.2$  Hz, H-3''), 7.68 (1H, t,  $J = 7.0$  Hz, H-4''), 8.26 (1H, d,  $J = 3.4$  Hz, H-6''), 10.38 (1H, s, NH), 10.87 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 24.13 (t), 26.59 (t), 28.27 (t), 109.77 (d), 110.79 (d), 115.72 (d), 116.69 (d), 117.48 (s), 119.78 (s), 124.30 (s), 137.71 (d), 138.18 (s), 146.39 (d), 151.87 (s), 155.95 (s); Anal. Calcd. for  $\text{C}_{15}\text{H}_{14}\text{N}_4\text{S}$ : C, 63.80; H, 5.00; N, 19.84. Found: C, 64.02; H, 5.23; N, 19.76.

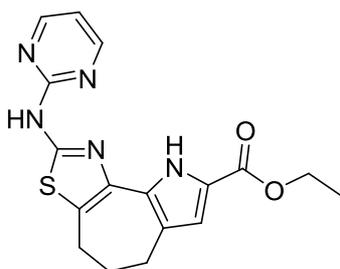


***N*-(Pyrimidin-2-yl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (41b).** This compound was obtained from reaction of **28** with pyrimidin-2-yl-thiourea. Purification was performed by flash column chromatography (dichloromethane : ethyl acetate 90: 10). Yield: 50%; white solid;  $R_f=0.21$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 215.5 - 216.0 °C; IR: 3475 (NH), 3405 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.85 - 2.10 (2H, m,  $\text{CH}_2$ ), 2.75 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 5.85 - 5.92 (1H, m, H-7), 6.55 - 6.65 (1H, m, H-8), 7.02 (1H, t,  $J = 4.6$  Hz, H-5''), 8.62 (2H, d,  $J = 4.6$  Hz, H-4'' and H-5''), 10.07 (1H, s, NH), 11.36 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 24.07 (t), 26.53 (t), 28.24 (t), 109.77 (d), 113.73 (d), 116.91 (d), 118.86 (s), 119.91 (s), 124.08 (s), 138.65 (s), 155.61 (s), 157.01 (s), 157.95 (d x 2). Anal. Calcd. for  $\text{C}_{14}\text{H}_{13}\text{N}_5\text{S}$ : C, 59.34; H, 4.62; N, 24.72. Found: C, 59.08; H, 4.39; N, 24.89.

**6.1.14 General synthesis for ethyl 2-(pyridin-2-ylamino) and 2-(pyrimidin-2-ylamino)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (41c and 41d).** To a suspension of CuBr<sub>2</sub> (1.8 mmol) in dry ethyl acetate (10 mL) **37** (1 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under reduced pressure remove the CuBr formed. The solvent was evaporated and the residue dissolved in dry DMF (8 mL). K<sub>2</sub>CO<sub>3</sub> (1 mmol) and pyridin-2-yl-thiourea or pyrimidin-2-yl-thiourea (1 mmol) were added and the new mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and the solid formed was filtered and purified by chromatography (dichloromethane : ethyl acetate 95 : 5). Further purification was performed by crystallization from ethanol.



**Ethyl 2-(pyridin-2-ylamino)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (41c).** This compound was obtained from reaction of **37** with pyridin-2-yl-thiourea. Yield: 60%; orange solid; R<sub>f</sub>=0.37 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: 165.5 - 167.0 °C (EtOH); IR: 3473 (NH), 3308 (NH), 1684 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.29 (3H, t, *J* = 7.1 Hz, CH<sub>3</sub>), 1.85 - 2.05 (2H, m, CH<sub>2</sub>), 2.75 - 3.15 (4H, m, CH<sub>2</sub> x 2), 4.24 (2H, q, *J* = 7.1 Hz, CH<sub>2</sub>), 6.68 (1H, s, H-7), 6.93 (1H, t, *J* = 6.4 Hz, H-5''), 7.02 (1H, d, *J* = 8.3 Hz, H-3''), 7.71 (1H, t, *J* = 6.9 Hz, H-4''), 8.28 (1H, d, *J* = 3.9 Hz, H-6''), 9.71 (1H, s, NH), 11.23 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.37 (q), 23.81 (t), 26.59 (t), 27.66 (t), 55.98 (t), 110.67 (d), 116.00 (d), 116.57 (d), 119.33 (s), 122.11 (s), 123.21 (s), 129.75 (s), 136.03 (s), 137.94 (d), 146.40 (d), 151.43 (s), 156.74 (s), 160.02 (s). Anal. Calcd. for C<sub>18</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub>S: C, 61.00; H, 5.12; N, 15.81. Found: C, 61.18; H, 5.24; N, 15.73.



**Ethyl 2-(pyrimidin-2-ylamino)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate (41d).** This compound was obtained from reaction of **37** with pyrimidin-2-yl-thiourea. Yield: 60%; light brown solid;  $R_f=0.35$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  90:10); mp: 257.1 - 257.9 °C (EtOH); IR: 3470 (NH), 3229 (NH), 1694 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.29 (3H, t,  $J = 6.9$  Hz,  $\text{CH}_3$ ), 1.90 - 2.10 (2H, m,  $\text{CH}_2$ ), 2.75 - 3.10 (4H, m,  $\text{CH}_2 \times 2$ ), 4.25 (2H, q,  $J = 6.9$  Hz,  $\text{CH}_2$ ), 6.68 (1H, s, H-7), 7.04 (1H, t,  $J = 4.5$  Hz, H-5''), 8.63 (2H, d,  $J = 4.5$  Hz, H-4'' and H-6''), 9.77 (1H, s, NH), 11.60 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 14.38 (q), 23.72 (t), 26.53 (t), 27.64 (t), 59.69 (t), 114.02 (d), 116.58 (d), 119.47 (s), 122.18 (s), 124.36 (s), 129.63 (s), 136.59 (s), 156.69 (s), 156.76 (s), 158.05 (d x 2), 160.10 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$ : C, 57.45; H, 4.82; N, 19.70. Found: C, 57.56; H, 4.94; N, 19.98.

**6.1.15 General procedure for preparation of hydrochloride salts (19b-d, 21a-f, 40b and 42a-d).** The free base (0.3 mmol) was dissolved or suspended in ethanol (5-8 mL). A solution of conc. HCl (1.1 eq.) was added dropwise. The precipitate was collected by filtration to give the corresponding hydrochloride form in some cases after the addition of diethyl ether.

**7-(Phenylsulfonyl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindol-2-amine hydrochloride (19b).** This compound was obtained from reaction of **18b**. Yield: 80%; light grey solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: >400 °C; IR: 2300 - 2900 ( $\text{NH}_3^+$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 2.48 - 2.59 (4H, m,  $\text{CH}_2 \times 2$ ), 7.27 (1H, d,  $J = 1.9$  Hz, H-6), 7.56 (1H, d,  $J = 1.9$  Hz, H-8), 7.62 - 7.84 (3H, m, H-3', H-4' and H-5'), 7.96 (2H, d,  $J = 7.1$  Hz, H-2' e H-6'), 9.26 (2H, bs,  $\text{NH}_2$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 19.36 (t), 21.42 (t), 113.98 (d), 115.52 (s), 116.97 (s), 117.34 (d), 123.41 (s), 126.65 (d x 2), 128.83 (s), 130.02 (d x 2), 134.71 (d), 137.75 (s), 168.51 (s). Anal. Calcd. for  $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_2\text{S}_2$ : C, 48.97; H, 3.84; N, 11.42. Found: C, 49.06; H, 3.94; N, 11.91.

**Ethyl 2-amino-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate hydrochloride (19c).** This compound was obtained from reaction of **18c**. Yield: 85%; grey solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  60:40); mp: > 400 °C; IR: 3269 (NH), 2500 - 2900 ( $\text{NH}_3^+$ ), 1683 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.30 (3H, t,  $J = 6.8$  Hz,  $\text{CH}_3$ ), 2.76 - 2.87 (2H, m,  $\text{CH}_2 \times 2$ ), 2.90 - 3.18 (2H, m,  $\text{CH}_2 \times 2$ ), 4.24 (2H, q,  $J = 6.8$  Hz,  $\text{CH}_2$ ), 7.34 (1H, s, H-8), 9.22 (2H, bs,  $\text{NH}_2$ ), 11.79 (1H, s, NH).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 14.32 (q), 20.41 (t), 21.39 (t), 59.73 (t), 110.00 (s), 112.92 (s), 117.68 (d), 118.36 (s), 124.71 (s), 129.77 (s), 160.43 (s), 168.61 (s). Anal. Calcd. for  $\text{C}_{12}\text{H}_{14}\text{ClN}_3\text{O}_2\text{S}$ : C, 48.08; H, 4.71; N, 14.02. Found: C, 48.01; H, 4.78; N, 13.96.

**Ethyl 2-amino-7-(phenylsulfonyl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate hydrochloride (19d).** This compound was obtained from reaction of **18d**. Yield: 90%, light grey solid;  $R_f=0.30$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp: 149.1 - 150.8 °C; IR: 2400 - 3000 ( $\text{NH}_3^+$ ), 1725 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.18 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.78 - 2.97 (2H, m,  $\text{CH}_2$ ), 3.00 - 3.10 (2H, m,  $\text{CH}_2$ ), 4.16 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 7.60 - 7.84 (3H, m, H-3', H-4' and H-5'), 7.91 - 8.00 (2H, m, H-2' and H-6'), 8.08 (1H, s, H-8), 9.25 (2H, bs,  $\text{NH}_2$ );  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 13.90 (q), 20.61 (t), 20.87 (t), 60.88 (t), 114.82 (s), 115.73 (s), 120.84 (d), 121.00 (s), 127.24 (d x 2), 128.61 (s), 129.44 (d x 2), 132.09 (s), 134.37 (d), 138.29 (s), 158.59 (s), 168.65 (s). Anal. Calcd. for  $\text{C}_{18}\text{H}_{18}\text{ClN}_3\text{O}_4\text{S}_2$ : C, 49.14; H, 4.12; N, 9.55. Found: C, 48.87; H, 4.36; N, 9.69.

***N*-(Pyridin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindol-2-amine hydrochloride (21a).** This compound was obtained from reaction of **20a**. Yield: 66%; grey solid;  $R_f=0.27$  ( $\text{EtOAc}$ ); mp: 243.5 - 244.0 °C; IR: 3290 (NH), 2600 - 3000 ( $\text{NH}_2^+$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.70 - 2.92 (4H, m,  $\text{CH}_2 \times 2$ ), 6.63 (1H, s, H-6), 7.03 - 7.21 (2H, m, H-8 and H-5''), 7.33 (1H, d,  $J = 8.4$  Hz, H-3''), 7.91 (1H, t,  $J = 7.0$  Hz, H-4''), 8.40 (1H, d,  $J = 4.2$  Hz, H-6''), 10.73 (1H, s, NH), 12.65 (1H, bs, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.33 (t), 22.25 (t), 112.18 (d), 112.41 (d), 113.88 (s), 114.39 (d), 115.21 (s), 117.09 (s), 117.25 (d), 137.27 (s), 139.66 (d), 144.51 (d), 150.08 (s), 158.25 (s); Anal. Calcd. for  $\text{C}_{14}\text{H}_{13}\text{ClN}_4\text{S}$ : C, 55.17; H, 4.30; N, 18.38. Found: C, 55.29; H, 4.21; N, 18.29.

***N*-(Pyrimidin-2-yl)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindol-2-amine hydrochloride (21b).** This compound was obtained from reaction of **20b**. Yield: 78%; light grey solid;  $R_f=0.24$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  60:40); mp: >400 °C; IR: 3233 (NH), 2300 - 2900 ( $\text{NH}_2^+$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$

NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.60 - 2.90 (4H, m, CH<sub>2</sub> x 2), 6.62 (1H, s, H-6), 6.97 (1H, s, H-8), 7.11 (1H, t, *J* = 4.8 Hz, H-5''), 8.74 (2H, d, *J* = 4.8 Hz, H-4'' and H-6''), 10.73 (1H, s, NH), 13.10 (1H, vbs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 20.31 (t), 22.36 (t), 111.81 (d), 112.71 (d), 113.51 (s), 114.45 (d), 115.81 (s), 117.10 (s), 136.85 (s), 155.71 (s), 157.78 (d x 2), 160.66 (s). Anal. Calcd. for C<sub>13</sub>H<sub>12</sub>ClN<sub>5</sub>S: C, 51.06; H, 3.96; N, 22.90. Found: C, 50.87; H, 3.89; N, 22.87.

**7-(Phenylsulfonyl)-*N*-(pyridin-2-yl)-5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amine hydrochloride (21c).** This compound was obtained from reaction of **20c**. Yield: 100%; white solid; *R*<sub>f</sub>=0.25 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: > 400 °C; IR: 2500 - 3000 (NH<sub>2</sub><sup>+</sup>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO) (ppm): 2.65 - 3.00 (4H, m, CH<sub>2</sub> x 2), 7.07 (1H, t, *J* = 6.3 Hz, H-5''), 7.08 - 7.30 (2H, m, H-3'' and H-6), 7.42 (1H, s, H-8), 7.60 - 7.90 (4H, m, H-3', H-4', H-5' and H-4''), 7.92 - 8.02 (2H, m, H-2' and H-6'), 8.36 (1H, d, *J* = 4.3 Hz, H-6''), 12.22 (1H, bs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 19.87 (t), 21.38 (t), 112.09 (d), 112.80 (d), 116.48 (d), 117.16 (d), 121.70 (s), 121.88 (s), 124.56 (s), 126.60 (d x 2), 129.90 (d x 2), 134.46 (d), 137.68 (s), 138.05 (s), 139.93 (d), 143.72 (d), 150.38 (s), 158.34 (s). Anal. Calcd. for C<sub>20</sub>H<sub>17</sub>ClN<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: C, 53.99; H, 3.85; N, 12.59. Found: C, 54.25; H, 4.10; N, 12.68.

**7-(Phenylsulfonyl)-*N*-(pyrimidin-2-yl)-5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindol-2-amine hydrochloride (21d).** This compound was obtained from reaction of **20d**. Yield: 80%; light brown solid; *R*<sub>f</sub>=0.23 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: > 400 °C; IR: 2300 - 3000 (NH<sub>2</sub><sup>+</sup>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.70 - 2.98 (4H, m, CH<sub>2</sub> x 2), 7.02 (H, t, *J* = 4.6 Hz, H-5''), 7.22 (2H, s, H-6 and H-8), 7.58 - 7.81 (3H, m, H-3', H-4' and H-5'), 8.00 (2H, d, *J* = 7.1 Hz, H-2' and H-6'), 8.62 (2H, d, *J* = 4.6 Hz, H-4'' and H-6''), 13.51 (1H, vbs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 19.87 (t), 21.35 (t), 112.18 (d), 113.63 (d), 117.18 (d), 121.92 (s), 122.34 (s), 124.66 (s), 126.68 (d x 2), 129.88 (d x 2), 134.48 (d), 137.90 (s), 137.98 (s), 156.59 (s), 158.89 (d x 2), 158.70 (s). Anal. Calcd. for C<sub>19</sub>H<sub>16</sub>ClN<sub>5</sub>O<sub>2</sub>S<sub>2</sub>: C, 51.17; H, 3.62; N, 15.70. Found: C, 51.19; H, 3.73; N, 15.82.

**Ethyl 2-(pyridin-2-ylamino)-5,7-dihydro-4*H*-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate hydrochloride (21e).** This compound was obtained from reaction of **20e**. Yield: 80%; yellow solid; *R*<sub>f</sub>=0.41 (EtOAc); mp: 219.1 - 220.4 °C; IR: 3239 (NH), 2500 - 2900 (NH<sub>2</sub><sup>+</sup>), 1690 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.31 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 2.85 - 3.20 (4H, m, CH<sub>2</sub> x 2), 4.26 (2H, q, *J* = 6.9 Hz, CH<sub>2</sub>), 7.01 - 7.24 (3H, m, H-8, H-5'' and H-3''), 7.89 (1H,

t,  $J = 6.7$  Hz, H-4''), 8.24 (1H, d,  $J = 3.3$  Hz, H-6''), 11.68 (1H, s, NH), 12.26 (1H, bs, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 14.38 (q), 20.88 (t), 21.46 (t), 59.54 (t), 112.24 (d), 116.60 (d), 116.97 (d), 117.49 (s), 117.59 (s), 118.03 (s), 125.41 (s), 138.48 (d), 139.90 (s), 146.85 (d), 150.30 (s), 158.15 (s), 160.70 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$ : C, 54.18; H, 4.55; N, 14.87. Found: C, 54.35; H, 4.76; N, 15.02.

**Ethyl 2-(pyrimidin-2-ylamino)-5,7-dihydro-4H-[1,3]thiazolo[4,5-*e*]isoindole-6-carboxylate hydrochloride (21f).** This compound was obtained from reaction of **20f**. Yield: 80%, yellow solid;  $R_f=0.47$  (EtOAc); mp: 319.5 - 320.3 °C; IR: 3238 (NH), 2500 - 2800 ( $\text{NH}_2^+$ ), 1690 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.31 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.79 - 3.00 (2H, m,  $\text{CH}_2$ ), 3.00 - 3.18 (2H, m,  $\text{CH}_2$ ), 4.25 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 6.98 - 7.16 (2H, m, H-8 and H-5''), 8.70 (2H, d,  $J = 5.0$  Hz, H-4'' and H-6''), 11.70 (1H, s, NH), 13.39 (1H, vbs, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 14.36 (q), 20.80 (t), 21.48 (t), 59.56 (t), 112.95 (d), 116.58 (d), 117.13 (s), 118.14 (s), 125.44 (s), 137.52 (s), 156.09 (s), 157.97 (s), 159.95 (s), 160.65 (s), 161.01 (d x 2). Anal. Calcd. for  $\text{C}_{16}\text{H}_{16}\text{ClN}_5\text{O}_2\text{S}$ : C, 50.86; H, 4.27; N, 18.53. Found: C, 51.01; H, 4.40 N, 18.72.

**Ethyl 2-amino-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate hydrochloride (40b).** This compound was obtained from reaction of **39b**. Yield: 66%; white solid;  $R_f=0.40$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp: 244.7 - 245.5 °C; IR: 3300 (NH), 2500 - 3000 ( $\text{NH}_3^+$ ), 1691 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.27 (3H, t,  $J = 7.1$  Hz,  $\text{CH}_3$ ), 1.85 - 1.99 (2H, m,  $\text{CH}_2$ ), 2.75 - 2.95 (4H, m,  $\text{CH}_2$  x 2), 4.25 (2H, q,  $J = 7.1$  Hz,  $\text{CH}_2$ ), 6.68 (1H, s, H-7), 9.13 (2H, bs,  $\text{NH}_2$ ), 11.53 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 14.33 (q), 22.94 (t), 27.27 (t), 27.45 (t), 59.92 (t), 116.50 (d), 117.26 (s), 118.13 (s), 121.27 (s), 123.94 (s), 124.84 (s), 160.03 (s), 167.38 (s). Anal. Calcd. for  $\text{C}_{13}\text{H}_{16}\text{ClN}_3\text{O}_2\text{S}$ : C, 49.76; H, 5.14; N, 13.39. Found: C, 49.85; H, 5.29 N, 13.58.

***N*-(Pyridin-2-yl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine hydrochloride (42a).** This compound was obtained from reaction of **41a**. Yield: 79%; green solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 268.7 - 269.3 °C; IR: 3403 (NH), 2300 - 3000 ( $\text{NH}_2^+$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.80 - 2.00 (2H, m,  $\text{CH}_2$ ), 2.80 - 3.00 (4H, m,  $\text{CH}_2$  x 2), 5.92 - 5.96 (1H, m, H-7), 6.72 - 6.77 (1H, m, H-8), 7.24 (1H, t,  $J = 6.2$  Hz, H-5''), 7.45 (1H, d,  $J = 8.5$  Hz, H-3''), 8.10 (1H, t,  $J = 7.2$  Hz, H-4''), 8.53 (1H, d,  $J = 5.0$  Hz, H-6''), 11.19 (1H, s, NH), 13.87 (1H, bs, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 23.70 (t), 26.66 (t),

28.16 (t), 110.15 (d), 113.72 (d), 116.49 (d), 117.17 (d), 118.72 (s), 120.83 (s), 123.06 (s), 139.07 (d), 140.29 (d), 148.54 (s), 148.97 (s), 156.55 (s). Anal. Calcd. for C<sub>15</sub>H<sub>15</sub>ClN<sub>4</sub>S: C, 56.51; H, 4.74; N, 17.57. Found: C, 56.25; H, 4.89; N, 17.65.

***N*-(Pyrimidin-2-yl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine hydrochloride (42b).** This compound was obtained from reaction of **41b**. Yield: 61%; green solid; R<sub>f</sub>=0.26 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: > 400 °C; IR: 3458 (NH), 2600 - 3000 (NH<sub>2</sub><sup>+</sup>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.85 - 2.10 (2H, m, CH<sub>2</sub>), 2.75 - 3.00 (4H, m, CH<sub>2</sub> x 2), 5.91 (1H, s, H-7), 6.66 (1H, s, H-8), 7.07 (1H, t, *J* = 4.8 Hz, H-5''), 8.62 (2H, d, *J* = 4.8 Hz, H-4'' and H-5''), 10.43 (1H, s, NH), 13.47 (1H, vbs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 23.86 (t), 26.78 (s), 28.15 (t), 107.09 (d), 110.02 (d), 113.25 (d), 117.35 (s), 118.61 (s), 125.16 (s), 130.80 (s), 148.47 (s), 156.34 (s), 158.01 (d x 2). Anal. Calcd. for C<sub>14</sub>H<sub>14</sub>ClN<sub>5</sub>S: C, 52.58; H, 4.41; N, 21.90. Found: C, 52.79; H, 4.55; N, 22.02.

**Ethyl 2-(pyridin-2-ylamino)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate hydrochloride (42c).** This compound was obtained from reaction of **41c**. Yield: 70%; yellow solid; R<sub>f</sub>=0.35 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: 239.4 - 240.2 °C; IR: 3357 (NH), 2300 - 3000 (NH<sub>2</sub><sup>+</sup>), 1693 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.29 (3H, t, *J* = 7.0 Hz, CH<sub>3</sub>), 1.85 - 2.05 (2H, m, CH<sub>2</sub>), 2.75 - 2.93 (2H, m, CH<sub>2</sub>), 3.00 - 3.15 (2H, m, CH<sub>2</sub>), 4.24 (2H, q, *J* = 7.0 Hz, CH<sub>2</sub>), 6.68 (1H, s, H-7), 7.22 (1H, t, *J* = 6.3 Hz, H-5''), 7.39 (1H, d, *J* = 8.3 Hz, H-3''), 8.04 (1H, t, *J* = 6.9 Hz, H-4''), 8.40 (1H, d, *J* = 3.9 Hz, H-6''), 11.06 (1H, bs, NH), 13.10 (1H, bs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.38 (q), 23.57 (t), 26.82 (t), 27.68 (t), 59.62 (t), 113.26 (d), 116.70 (d), 116.94 (d), 120.19 (s), 122.80 (s), 122.89 (s), 124.49 (s), 128.90 (s), 136.95 (d), 141.02 (d), 149.03 (s), 156.90 (s), 160.24 (s). Anal. Calcd. for C<sub>18</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>2</sub>S: C, 55.31; H, 4.90; N, 14.33. Found: C, 55.57; H, 5.06; N, 14.47.

**Ethyl 2-(pyrimidin-2-ylamino)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole-8-carboxylate hydrochloride (42d).** This compound was obtained from reaction of **41d**. Yield: 70%; yellow solid; R<sub>f</sub>=0.33 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: 258.0 - 258.6 °C; IR: 3457 (NH), 2400 - 3000 (NH<sub>2</sub><sup>+</sup>), 1691 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.29 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 1.90 - 2.10 (2H, m, CH<sub>2</sub>), 2.75 - 3.10 (4H, m, CH<sub>2</sub> x 2), 4.25 (2H, q, *J* = 6.9 Hz, CH<sub>2</sub>), 6.68 (1H, s, H-7), 7.04 (1H, t, *J* = 4.5 Hz, H-5'') 8.63 (2H, d, *J* = 4.5 Hz, H-4'' and H-6''), 9.72 (1H, s, NH), 11.60 (1H, bs, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 14.39 (q), 23.70 (t), 26.55 (t), 27.64 (t), 59.69 (t), 114.02 (d), 116.59 (d), 119.49 (s), 122.20 (s), 124.35 (s),

129.61 (s), 136.54 (s), 156.69 (s), 156.74 (s), 158.06 (d x 2), 160.10 (s). Anal. Calcd. for C<sub>17</sub>H<sub>18</sub>ClN<sub>5</sub>O<sub>2</sub>S: C, 52.10; H, 4.63; N, 17.87. Found: C, 52.04; H, 4.71; N, 17.92.

## 6.2 Potential antileukemic agents: Chemistry

**6.2.1 Synthesis of 1-(phenylsulfonyl)-1,4,5,6-tetrahydro-7H-indol-7-one (59).** The synthesis of the titled compound was performed according to literature procedures.<sup>[102]</sup>

### 6.2.2 Synthesis of 1,5,6,7-tetrahydro-4H-indol-4-ones (63 and 64)

**Preparation of 1,5,6,7-tetrahydro-4H-indol-4-one (63).** To a solution of NaHCO<sub>3</sub> (4.5 g, 62 mmol) in H<sub>2</sub>O (60 mL), a solution of chloroacetaldehyde 45% in H<sub>2</sub>O (8.33 mL, 58 mmol) and tetrabutylammonium iodide (1.5 g, 4 mmol) were added. Then a solution of 1,3-cyclohexanedione (5 g, 44 mmol) in H<sub>2</sub>O (50 mL) was added dropwise at 0 °C. The resulting mixture was stirred at room temperature for 70 hours. Following the addition of AcOEt (50 mL), to the mixture was added H<sub>2</sub>SO<sub>4</sub> 12 M in order to adjust the pH of the aqueous layer to 1. After 1 hour, the mixture was extracted with AcOEt. The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. To the oily residue containing 6,7-dihydro-1-benzofuran-4(5H)-one **62**, was solubilized in dry DMF (20 mL) and NH<sub>4</sub>OAc (10.2 g, 132 mmol) was added. The mixture was heated to 110 °C for 6 hours. The reaction was poured into water and the mixture was extracted with AcOEt. The extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography (petroleum ether : ethyl acetate 40 : 60). Yield: 55 %; The spectroscopic data are in agreement with the structure of the commercial product or reported in literature.<sup>[103, 104]</sup>

**Preparation of 1-(phenylsulfonyl)-1,5,6,7-tetrahydro-4H-indol-4-one (64).** To a solution of **63** (4 mmol) in dry DMF (15 mL), NaH (4.4 mmol) was added at 0 °C and the reaction was stirred for 1 hour at room temperature. Benzenesulfonyl chloride (6 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 4 hours. Then the reaction was poured into ice and brine and the resulted precipitate was collected by filtration and further purified by trituration with diethyl ether. Yield: 92%; white solid; mp: 125.3 - 126.0 °C; IR: 1668 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.92 - 2.08 (2H, m, CH<sub>2</sub>), 2.30 - 2.40 (2H, m,

CH<sub>2</sub>), 2.93 - 3.05 (2H, m, CH<sub>2</sub>), 6.56 (1H, d, *J* = 3.5 Hz, H-8), 7.46 (1H, d, *J* = 3.5 Hz, H-7), 8.00 - 7.59 (3H, m, H-3', H-4' and H-5'), 8.04 (2H, d, *J* = 7.4 Hz, H-2' and H-6'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 22.34 (t), 22.89 (t), 36.77 (t), 108.40 (d), 122.81 (d), 124.09 (s), 127.15 (d x 2), 130.14 (d x 2), 135.20 (d), 137.42 (s), 143.77 (s), 193.58 (s). Anal. Calcd. for C<sub>14</sub>H<sub>13</sub>NO<sub>3</sub>S: C, 61.07; H, 4.76; N, 5.09. Found: C, 61.18; H, 4.82; N, 4.94.

### 6.2.3 Synthesis of 8-(phenylsulfonyl)-5,8-dihydro-4H-[1,3]thiazolo[5,4-g]indol-2-amine

**(61).** To a suspension of CuBr<sub>2</sub> (1.8 mmol) in dry ethyl acetate (10 mL) ketone **59** (1 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the CuBr formed. The solvent was evaporated under reduced pressure and the residue dissolved in dry DMF (8 mL). Na<sub>2</sub>CO<sub>3</sub> (2 mmol) and thiourea (2 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and the solid formed was filtered and purified by crystallization from ethanol. Yield: 90%; pale yellow solid; mp: 79.2 - 80.1 °C (EtOH); IR: 3415 - 3275 (NH<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.65 - 2.75 (4H, m, CH<sub>2</sub> x 2), 6.26 (1H, d, *J* = 3.2 Hz, H-7), 6.84 (2H, s, NH<sub>2</sub>), 7.31 (1H, d, *J* = 3.2 Hz, H-8), 7.53 - 7.75 (3H, m, H-3', H-4' and H-5'), 8.24 - 8.31 (2H, m, H-2' and H-6'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 21.52 (t), 22.47 (t), 110.87 (d), 114.78 (s), 120.99 (d), 123.55 (s), 125.39 (s), 128.78 (d x 2), 129.08 (d x 2), 139.97 (d), 137.55 (s), 138.19 (s), 164.89 (s); Anal. Calcd. for C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>: C, 54.36; H, 3.95; N, 12.68. Found: C, 54.12; H, 4.08; N, 12.79.

### 6.2.4 Synthesis of 6-(phenylsulfonyl)-5,6-dihydro-4H-[1,3]thiazolo[4,5-e]indol-2-amine

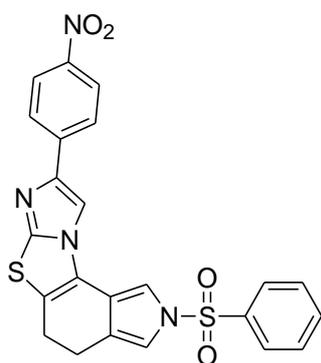
**(66).** To a suspension of CuBr<sub>2</sub> (1.8 mmol) in dry ethyl acetate (10 mL) ketone **64** (1 mmol) was added and the reaction mixture was heated to reflux for 2 hours. After cooling the reaction mixture was filtered under vacuum to remove the CuBr formed. The solvent was evaporated under reduced pressure and the residue dissolved in dry DMF (8 mL). Na<sub>2</sub>CO<sub>3</sub> (2 mmol) and thiourea (2 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and the solid formed was filtered and purified by crystallization from ethanol. Yield: 90%; pale yellow solid; mp: 216.9 - 217.3 °C (EtOH); IR: 3410 - 3275 (NH<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.70 - 2.92 (2H, m, CH<sub>2</sub>), 2.95 - 3.12 (2H, m, CH<sub>2</sub>), 6.36 (1H, d, *J* = 3.2 Hz, H-8), 6.91 (2H, s, NH<sub>2</sub>), 7.30 (1H, d, *J* = 3.2 Hz, H-7), 7.55 - 7.80 (3H, m, H-3', H-4' and H-5'), 7.87 - 7.98 (2H, m, H-2' and H-6'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 21.55 (t), 21.71 (t), 108.25 (d), 110.97 (s), 121.71 (s), 121.77 (d), 126.56 (d x 2), 127.45 (s), 130.02 (d x 2), 134.60 (d), 137.92 (s), 141.25 (s), 167.29 (s).

Anal. Calcd. for C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>: C, 54.36; H, 3.95; N, 12.68. Found: C, 54.58; H, 3.87; N, 12.82.

**6.2.5 Synthesis of 9-(phenylsulfonyl)-4,5,6,9-tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-2-amine (70).** To a solution of bromo derivative **28** (0.5 mmol) in dry DMF (5 mL), Na<sub>2</sub>CO<sub>3</sub> (1 mmol) and thiourea (1 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction was poured into ice and brine and the precipitate was filtered. No further purification was needed. Yield: 90 %; yellow solid; mp: 173.3 - 174.1 °C; IR: 3252 - 3143 (NH<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) (ppm): 2.01 - 2.11 (2H, m, CH<sub>2</sub>), 2.41 (2H, t, *J* = 6.7 Hz, CH<sub>2</sub>), 2.45 (2H, t, *J* = 6.7 Hz, CH<sub>2</sub>), 4.68 (2H, s, NH<sub>2</sub>), 6.17 (1H, d, *J* = 3.0 Hz, H-7), 7.35 (1H, d, *J* = 3.0 Hz, H-8), 7.40 - 7.55 (3H, m, H-3', H-4' and H-5'), 7.86 (2H, d, *J* = 7.0 Hz, H-2' and H-6'); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 24.0 (t), 25.0 (t), 31.8 (t), 113.5 (d), 123.2 (d), 125.3 (s), 126.7 (s), 127.2 (d x 2), 128.4 (d x 2), 130.5 (s), 133.0 (d), 138.3 (s), 140.1 (s), 162.7 (s); Anal Calcd. for C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>: C, 55.63; H, 4.38; N, 12.16. Found: C, 55.91; H, 4.42; N, 12.43.

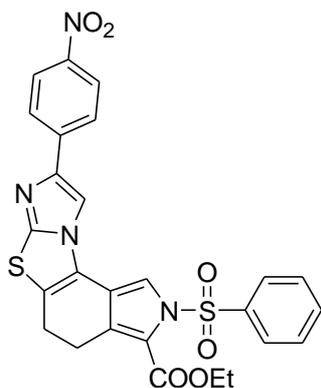
### 6.2.6 General synthesis for the nitro-derivatives **44a**, **44b**, **68** and **71**

A mixture of the appropriate 2-aminothiazole (4.5 mmol) and 2-bromo-4'-nitroacetophenone (4.95 mmol) was heated to 70 °C in dry 2-methoxyethanol for 13 hours. The mixture was cooled to 0° C and the solid was collected by filtration and washed with 2-methoxyethanol to afford the product without any further purification.

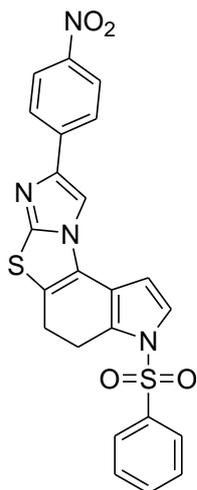


**8-(4-Nitrophenyl)-2-(phenylsulfonyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole (44a).** This compound was obtained from reaction of **18b**. Yield: 64%; yellow solid; R<sub>f</sub>=0.68 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 95:5); mp: 261.1 - 261.5 °C; IR: 1505 - 1333 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.80 - 3.07 (4H, m, CH<sub>2</sub> x 2), 7.36 (1H, s, H-3), 7.55 - 7.90 (3H, m, H-3', H-4' and H-5'), 8.05 (2H, d, *J* = 7.2 Hz, H-2' and H-6'), 8.03 (1H, s, H-1), 8.18 (2H, d,

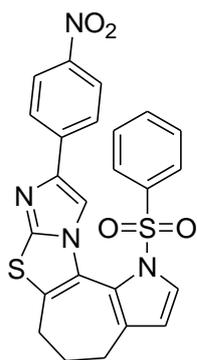
$J = 8.5$  Hz, H-2'' and H-6''), 8.30 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 9.07 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 19.58 (t), 23.17 (t), 112.05 (d), 113.58 (d), 115.99 (s), 117.79 (d), 121.36 (s), 123.00 (s), 124.13 (d x 2), 125.19 (d x 2), 126.65 (d x 2), 128.90 (s), 130.00 (d x 2), 134.68 (d), 137.92 (s), 140.82 (s), 143.87 (s), 145.84 (s), 148.08 (s). Anal. Calcd. for  $\text{C}_{23}\text{H}_{16}\text{N}_4\text{O}_4\text{S}_2$ : C, 57.97; H, 3.38; N, 11.76. Found: C, 58.10; H, 3.65; N, 11.66.



**Ethyl 8-(4-nitrophenyl)-2-(phenylsulfonyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindole-3-carboxylate (44b).** This compound was obtained from reaction of **18d**. Yield: 48; pale yellow solid;  $R_f=0.50$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  98:2); mp: 249.7 - 250.2 °C; IR: 1726 (CO), 1505 - 1333 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.22 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.97 - 3.19 (4H, m,  $\text{CH}_2$  x 2), 4.22 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 7.59 - 7.84 (3H, m, H-3', H-4' and H-5'), 8.05 (2H, d,  $J = 6.8$  Hz, H-2' and H-6'), 8.20 (2H, d,  $J = 8.6$  Hz, H-2'' and H-6''), 8.30 (2H, d,  $J = 8.6$  Hz, H-3'' and H-5''), 8.51 (1H, s, H-1), 9.16 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 13.93 (q), 20.63 (t), 22.55 (t), 61.06 (t), 113.83 (s), 120.60 (d), 121.67 (s), 123.34 (s), 124.18 (d x 2), 125.28 (d x 2), 127.66 (d x 2), 129.48 (d x 2), 132.07 (d), 134.42 (d), 138.36 (s), 140.89 (s), 143.90 (s), 145.87 (s), 147.62 (s), 148.25 (s), 157.05 (s), 158.84 (s). Anal. Calcd. for  $\text{C}_{26}\text{H}_{20}\text{N}_4\text{O}_6\text{S}_2$ : C, 56.92; H, 3.67; N, 10.21. Found: C, 56.70; H, 3.75; N, 10.56.

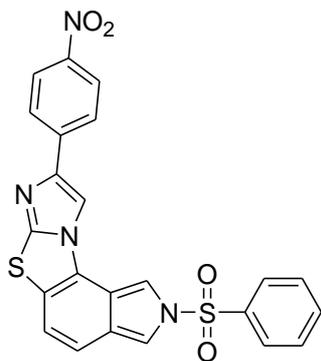


**8-(4-Nitrophenyl)-3-(phenylsulfonyl)-4,5-dihydro-3H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]indole (68).** This compound was obtained from reaction of **66**. Yield: 24%; yellow solid;  $R_f=0.65$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 241.3 - 242.0 °C; IR: 1519 - 1342 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.98 - 3.33 (4H, m,  $\text{CH}_2 \times 2$ ), 7.15 (1H, d,  $J = 3.3$  Hz, H-1), 7.60 (1H, d,  $J = 3.3$  Hz, H-2), 7.58 - 7.90 (3H, m, H-3', H-4' and H-5'), 8.05 (2H, d,  $J = 7.3$  Hz, H-2' and H-6'), 8.12 (2H, d,  $J = 8.8$  Hz, H-2'' and H-6''), 8.25 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 9.07 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 21.27 (t), 22.92 (t), 107.03 (d), 111.28 (d), 114.86 (s), 116.91 (s), 122.51 (d), 122.92 (s), 124.06 (d x 2), 125.11 (d x 2), 126.85 (d x 2), 128.59 (s), 130.18 (d x 2), 134.99 (d), 137.56 (s), 140.77 (s), 143.68 (s), 145.71 (s), 148.76 (s). Anal. Calcd. for  $\text{C}_{23}\text{H}_{16}\text{N}_4\text{O}_4\text{S}_2$ : C, 57.97; H, 3.38; N, 11.76. Found: C, 57.99; H, 3.41; N, 11.79.

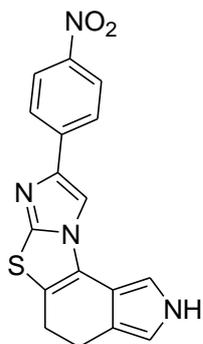


**9-(4-Nitrophenyl)-1-(phenylsulfonyl)-1,4,5,6-tetrahydroimidazo[2,1-b]pyrrolo[3',2':6,7]cyclohepta[1,2-d][1,3]thiazole (71).** This compound was obtained from reaction of **70**. Yield: 40 %; yellow solid;  $R_f=0.65$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 230.2 - 230.9 °C; IR: 1519 - 1341 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.65 - 2.33 (4H, m,  $\text{CH}_2 \times 2$ ), 2.52 - 2.74 (2H, m,  $\text{CH}_2$ ), 6.58 (1H, d,  $J = 3.2$  Hz, H-3), 7.52 - 7.63 (4H, m, H-3', H-4', H-5' and H-2), 7.68 - 7.80 (2H, m, H-2' and H-6'), 8.18 (2H, d,  $J = 8.3$  Hz, H-2'' and H-6''), 8.30 (2H, d,  $J = 8.3$

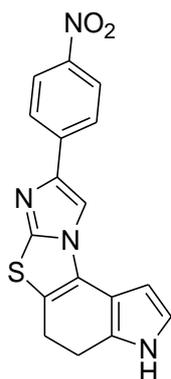
Hz, H-3'' and H-5''), 8.61 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 22.51 (t), 23.74 (t), 34.48 (t) 113.79 (d), 118.01 (d), 120.37 (s), 120.72 (s), 124.15 (d x 2), 125.34 (d x 2), 126.37 (d x 2), 126.53 (d), 129.62 (d x 2), 130.21 (s), 134.79 (d), 136.01 (s), 136.18 (s), 140.51 (s), 141.87 (s), 145.81 (s), 147.30 (s). Anal. Calcd. for  $\text{C}_{24}\text{H}_{18}\text{N}_4\text{O}_4\text{S}_2$ : C, 58.76; H, 3.70; N, 11.42. Found: C, 58.67; H, 3.65; N, 11.39.



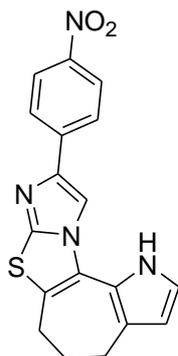
**6.2.7 Synthesis of 8-(4-nitrophenyl)-2-(phenylsulfonyl)-2H-imidazo [2',1':2,3] [1,3] thiazolo[4,5-*e*]isoindole (46).** To a suspension of **44a** (0.2 mmol) in dry 1,4-dioxane (30 mL), DDQ (0.4 mmol) was added and the reaction was refluxed for 3 hours. Then the solvent was removed under reduced pressure and the residue was purified by chromatography (dichloromethane : ethyl acetate 98 : 2). Yield: 8%, brown solid;  $R_f=0.71$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 289.3 - 290.1 °C; IR: 1503 - 1335 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 7.53 (1H, d,  $J = 7.6$  Hz, H-5), 7.74 (1H, d,  $J = 7.6$  Hz, H-4), 8.01 - 8.26 (10H, m, Ar), 8.80 (1H, s, H-1), 9.37 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 109.12 (d), 112.67 (d), 114.13 (d), 114.32 (s), 118.93 (d), 119.34 (d), 121.63 (s), 122.64 (s), 124.12 (d x 2), 124.89 (s), 125.17 (d x 2), 127.15 (d x 2), 130.22 (d x 2), 135.40 (d), 137.25 (s), 140.51 (s), 144.40 (s), 145.81 (s), 148.30 (s). Anal. Calcd. for  $\text{C}_{23}\text{H}_{14}\text{N}_4\text{O}_4\text{S}_2$ : C, 58.22; H, 2.97; N, 11.81. Found: C, 58.13; H, 3.16; N, 11.79.



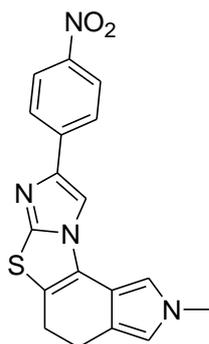
**6.2.8 Synthesis of 8-(4-nitrophenyl)-4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3] thiazolo[4,5-*e*]isoindole (44c).** To a suspension of **44a** (1.5 mmol) in methanol (120 mL), potassium hydroxide (52.5 mmol) was added portion wise and the mixture was refluxed for 16 hours. After cooling the solvent was removed under reduced pressure and the residue was suspended in water and the solid was filtered off. Purification was performed with flash column chromatography (dichloromethane). Yield: 90%; orange solid;  $R_f=0.54$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 277.1 - 278.0 °C; IR: 3270 (NH), 1503 - 1335 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.80 - 3.05 (4H, m,  $\text{CH}_2 \times 2$ ), 6.75 (1H, s, H-3), 7.52 (1H, s, H-1), 8.17 (2H, d,  $J = 8.8$  Hz, H-2'' and H-6''), 8.29 (2H, d,  $J = 8.8$  Hz, H-3'' and H-5''), 8.88 (1H, s, H-imidaz), 11.02 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.29 (t), 24.01 (t), 110.38 (s), 111.55 (d), 112.05 (d), 115.01 (d), 115.88 (s), 116.46 (s), 124.13 (d x 2), 124.23 (s), 125.08 (d x 2), 141.05 (s), 143.54 (s), 145.65 (s), 148.38 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ : C, 60.70; H, 3.60; N, 16.66. Found: C, 61.05; H, 3.55; N, 16.85.



**6.2.9 Synthesis of 8-(4-nitrophenyl)-4,5-dihydro-3*H*-imidazo[2',1':2,3][1,3] thiazolo[4,5-*e*]indole (69).** To a suspension of **68** (0.2 mmol) in methanol (15 mL), potassium hydroxide (0.4 mmol) was added and the mixture was refluxed for 3 hours. After cooling the solvent was removed under reduced pressure and the residue was suspended in water and the solid was filtered off. Purification was performed by crystallization from ethanol. Yield: 80%; orange solid;  $R_f=0.43$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 293.5 - 294.3 °C (EtOH); IR: 3367 (NH), 1507 - 1339 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.93 - 3.08 (4H, m,  $\text{CH}_2 \times 2$ ), 6.75 (1H, s, H-1), 6.81 (1H, s, H-2), 8.19 (2H, d,  $J = 9.0$  Hz, H-2'' and H-6''), 8.26 (2H, d,  $J = 9.9$  Hz, H-3'' and H-5''), 8.87 (1H, s, H-imidaz), 11.27 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 21.36 (t), 23.45 (t), 101.82 (d), 108.33 (s), 111.11 (d), 111.64 (s), 117.04 (d), 124.06 (d x 2), 125.14 (d x 2), 125.61 (s), 128.24 (s), 141.05 (s), 143.53 (s), 145.63 (s), 148.90 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ : C, 60.70; H, 3.60; N, 16.66. Found: C, 60.95; H, 3.76; N, 16.85.

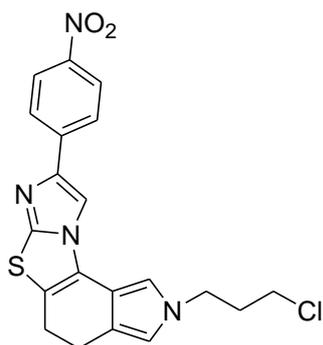


**6.2.10 Synthesis of 9-(4-nitrophenyl)-1,4,5,6-tetrahydroimidazo[2,1-*b*]pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazole (72).** To a suspension of **71** (1.0 mmol) in methanol (70 mL), potassium hydroxide (1.5 mmol) was added and the mixture was refluxed for 3 hours. After cooling the solvent was removed under reduced pressure and the residue was suspended in water and the solid was filtered off. Purification was performed by crystallization from ethanol. Yield: 86%; orange solid;  $R_f=0.61$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 268.6 - 269.2 °C (EtOH); IR: 3438 (NH), 1507 - 1339 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.70 - 2.15 (2H, m,  $\text{CH}_2$ ), 2.80 - 3.12 (4H, m,  $\text{CH}_2 \times 2$ ), 6.13 (1H, s, H-2), 6.96 (1H, s, H-3), 8.11 (2H, d,  $J = 8.7$  Hz, H-2'' and H-6''), 8.31 (2H, d,  $J = 8.7$  Hz, H-3'' and H-5''), 8.90 (1H, s, H-imidaz), 11.01 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 25.31 (t), 27.28 (t), 28.74 (t), 110.67 (d), 112.39 (d), 116.78 (s), 119.65 (d), 121.10 (s), 121.57 (s), 124.19 (d x 2), 124.76 (s), 125.04 (d x 2), 140.79 (s), 143.04 (s), 145.77 (s), 147.89 (s). Anal. Calcd. for  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_2\text{S}$ : C, 61.70; H, 4.03; N, 15.99. Found: C, 61.75; H, 4.23; N, 16.17.



**6.2.11 Synthesis of 2-methyl-8-(4-nitrophenyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole (44d).** To a solution of **44c** (0.5 mmol) in dry DMF (5 mL), NaH (0.55 mmol) was added at 0 °C and the reaction was stirred for 2 hours at room temperature. Iodomethane (0.75 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 3 hours. Then the reaction was poured into ice and brine and the solid formed was filtered and purified by flash column chromatography (dichloromethane). Yield: 85%;

orange solid;  $R_f=0.63$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  95:5); mp: 271.8 - 272.7 °C; IR: 1509 - 1339 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.75 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 3.69 (3H, s,  $\text{CH}_3$ ), 6.70 (1H, s, H-3), 7.46 (1H, s, H-1), 8.16 (2H, d,  $J = 8.4$  Hz, H-2'' and H-6''), 8.29 (2H, d,  $J = 8.4$  Hz, H-3'' and H-5''), 8.81 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.18 (t), 23.89 (t), 35.92 (q), 110.54 (s), 111.34 (d), 115.63 (d), 115.93 (s), 116.88 (s), 119.26 (d), 123.86 (s), 124.25 (d x 2), 125.21 (d x 2), 141.06 (s), 143.68 (s), 145.74 (s), 148.44 (s). Anal. Calcd. for  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_2\text{S}$ : C, 61.70; H, 4.03; N, 15.99. Found: C, 61.68; H, 4.12; N, 16.14.

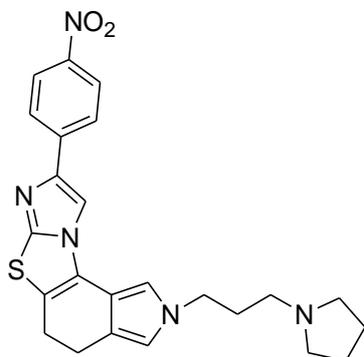


**6.2.12 Synthesis of 2-(3-chloropropyl)-8-(4-nitrophenyl)-4,5-dihydro-2H-imidazo [2',1':2,3][1,3] thiazolo[4,5-e]isoindole (44e).** To a solution of **44c** (1.5 mmol) in dry DMF (15 mL), NaH (1.65 mmol) was added portion wise at 0 °C and the reaction was stirred for 2 hours at room temperature. Potassium iodide (0.5 mmol) and 1-bromo-3-chloropropane (4.5 mmol) were added and the reaction mixture was heated at 70° C in an ultrasound bath for 2 hours. Reaction was poured into ice and brine and the solid formed was filtered and purified by flash column chromatography (dichloromethane). Yield: 100%; yellow solid;  $R_f=0.48$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  98:2); mp: 227.2 - 227.5 °C; IR: 1509 - 1339 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.10 - 2.40 (2H, m,  $\text{CH}_2$ ), 2.75 - 3.08 (4H, m,  $\text{CH}_2 \times 2$ ), 3.60 - 3.68 (2H, t,  $J = 6.2$  Hz,  $\text{CH}_2$ ), 4.07 (2H, t,  $J = 6.6$  Hz,  $\text{CH}_2$ ), 6.78 (1H, s, H-3), 7.54 (1H, s, H-1), 8.15 (2H, d,  $J = 8.8$  Hz, H-2'' and H-6''), 8.29 (2H, d,  $J = 8.8$  Hz, H-3'' and H-5''), 8.82 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.18 (t), 23.84 (t), 33.51 (t), 42.34 (t), 46.20 (t), 110.69 (s), 111.36 (d), 114.63 (d), 116.22 (s), 116.97 (s), 118.33 (d), 123.71 (s), 124.15 (d x 2), 125.06 (d x 2), 140.99 (s), 143.58 (s), 145.69 (s), 148.40 (s). Anal. Calcd. for  $\text{C}_{20}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$ : C, 58.18; H, 4.15; N, 13.57. Found: C, 58.35; H, 4.23; N, 13.74.

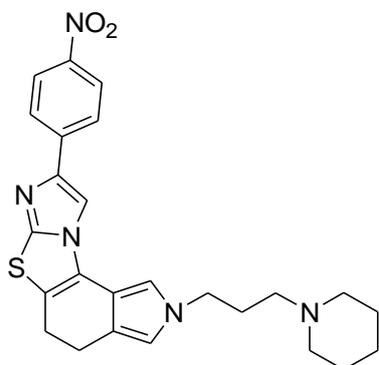
### 6.2.13 General synthesis for the nitro derivatives 44f-i

To a solution of **44e** (1 mmol) in dry DMF (8 mL), tetrabutylammonium iodide (1.5 mmol) and the appropriate heterocyclic amine (4 mmol) were added. The reaction mixture was

heated at 70° C up to completeness. Then the reaction was poured into ice and brine, and the solid formed was filtered. The crude product was purified by chromatography.

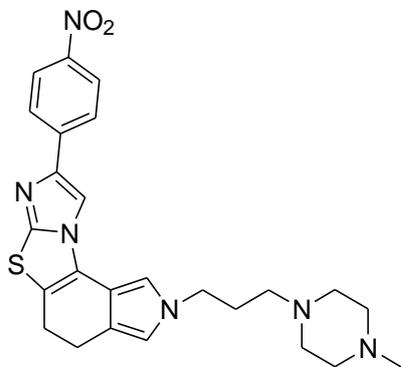


**8-(4-Nitrophenyl)-2-[3-(pyrrolidin-1-yl)propyl]-4,5-dihydro-2H-imidazo [2',1':2,3] [1,3] thiazolo[4,5-*e*]isoindole (44f).** This compound was obtained from reaction of **44e** with pyrrolidine in 3 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 95 : 5). Yield: 77%; orange solid;  $R_f=0.42$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 171.1 - 171.8 °C; IR: 1511 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.83 - 1.94 (4H, m,  $\text{CH}_2 \times 2$ ), 2.02 - 2.20 (2H, m,  $\text{CH}_2$ ), 2.50 - 2.75 (6H, m,  $\text{CH}_2 \times 3$ ), 2.3 (4H, s,  $\text{CH}_2 \times 2$ ), 4.03 (2H, t,  $J = 6.8$  Hz,  $\text{CH}_2$ ), 6.55 (1H, s, H-3), 6.98 (1H, s, H-1), 7.94 (1H, s, H-imidaz), 8.02 (2H, d,  $J = 9.0$  Hz, H-2'' and H-6''), 8.24 (2H, d,  $J = 9.0$  Hz, H-3'' and H-5'');  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.78 (t), 23.49 (t x 2), 24.67 (t), 30.03 (t), 47.57 (t), 52.90 (t), 54.13 (t x 2), 109.04 (d), 111.76 (s), 113.09 (d), 117.57 (s), 117.58 (s), 118.05 (d), 123.93 (s), 124.18 (d x 2), 125.22 (d x 2), 140.99 (s), 144.42 (s), 146.37 (s), 149.86 (s). Anal. Calcd. for  $\text{C}_{24}\text{H}_{25}\text{N}_5\text{O}_2\text{S}$ : C, 64.41; H, 5.63; N, 15.65. Found: C, 64.45; H, 5.78; N, 15.92.



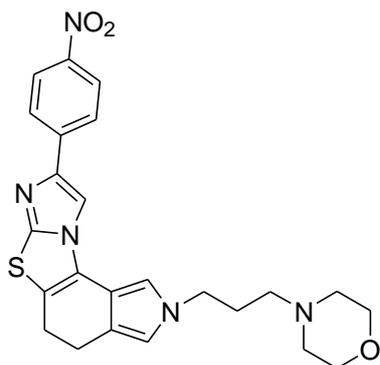
**8-(4-Nitrophenyl)-2-[3-(piperidin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3] thiazolo[4,5-*e*]isoindole (44g).** This compound was obtained from reaction of **44e** with piperidine in 16 hours. Purification was possible with flash column chromatography

(dichloromethane : methanol 97 : 3). Yield: 93%; yellow solid;  $R_f=0.58$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 290.2 - 290.8 °C; IR: 1510 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.25 - 1.63 (6H, m,  $\text{CH}_2 \times 3$ ), 1.83 - 2.02 (2H, m,  $\text{CH}_2$ ), 2.20 - 2.50 (6H, m,  $\text{CH}_2 \times 3$ ), 2.72 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 3.93 (2H, t,  $J = 6.9$  Hz,  $\text{CH}_2$ ), 6.74 (1H, s, H-3), 7.50 (1H, s, H-1), 8.13 (2H, d,  $J = 9.0$  Hz, H-2'' and H-6''), 8.27 (2H, d,  $J = 9.0$  Hz, H-3'' and H-5''), 8.78 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.19 (t x 2), 23.86 (t x 2), 25.33 (t), 28.01 (t), 47.16 (t), 53.90 (t x 2), 55.28 (t), 110.41 (s), 111.33 (d), 114.53 (d), 115.85 (s), 116.57 (s), 118.31 (d), 123.82 (s), 124.10 (d x 2), 125.00 (d x 2), 140.98 (s), 143.52 (s), 145.61 (s), 148.37 (s). Anal. Calcd. for  $\text{C}_{25}\text{H}_{27}\text{N}_5\text{O}_2\text{S}$ : C, 65.05; H, 5.90; N, 15.17. Found: C, 65.31; H, 5.82; N, 15.02.



**2-[3-(4-Methylpiperazin-1-yl)propyl]-8-(4-nitrophenyl)-4,5-dihydro-2H-imidazo**

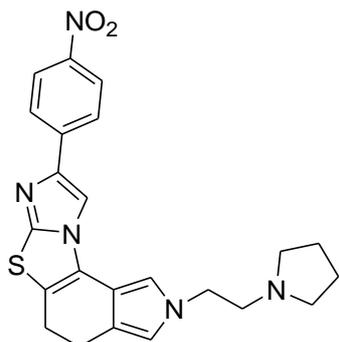
**[2',1':2,3][1,3]thiazolo[4,5-e]isoindole (44h).** This compound was obtained from reaction of **44e** with 1-methylpiperazine in 18 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 96 : 4). Yield: 90%; orange solid;  $R_f=0.48$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 194.0 - 194.8 °C; IR: 1512 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.90 - 2.09 (2H, m,  $\text{CH}_2$ ), 2.27 - 2.70 (13H, m,  $\text{CH}_2 \times 5$  and  $\text{CH}_3$ ), 2.92 (4H, m,  $\text{CH}_2 \times 2$ ), 3.98 (2H, t,  $J = 6.8$  Hz,  $\text{CH}_2$ ), 6.54 (1H, s, H-3), 6.89 (1H, s, H-1), 7.85 (1H, s, H-imidaz), 7.98 (2H, d,  $J = 7.1$  Hz, H-2'' and H-6''), 8.22 (2H, d,  $J = 7.1$  Hz, H-3'' and H-5'');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 20.75 (t), 24.65 (t), 28.52 (t), 45.97 (q), 47.55 (t), 52.97 (t x 2), 54.76 (t), 55.09 (t x 2), 108.81 (d), 111.59 (s), 113.08 (d), 117.54 (s), 117.89 (s), 118.05 (d), 123.90 (s), 124.14 (d x 2), 125.12 (d x 2), 140.92 (s), 144.34 (s), 146.32 (s), 149.88 (s). Anal. Calcd. for  $\text{C}_{25}\text{H}_{28}\text{N}_6\text{O}_2\text{S}$ : C, 63.00; H, 5.92; N, 17.63. Found: C, 63.31; H, 6.13; N, 17.75.



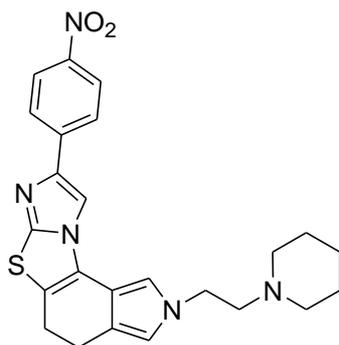
**2-[3-(Morpholin-4-yl)propyl]-8-(4-nitrophenyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindole (44i).** This compound was obtained from reaction of **44e** with morpholine in 8 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 98 : 2). Yield: 80%; orange solid;  $R_f=0.42$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 203.2 - 203.8 °C; IR: 1511 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.92 - 2.09 (2H, m,  $\text{CH}_2$ ), 2.23 - 2.50 (6H, m,  $\text{CH}_2 \times 3$ ), 2.86 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 3.68 - 3.82 (4H, m,  $\text{CH}_2 \times 2$ ), 3.95 - 4.10 (2H, t,  $J = 6.8$  Hz,  $\text{CH}_2$ ), 6.55 (1H, s, H-3), 6.88 (1H, s, H-1), 7.82 (1H, s, H-imidaz), 7.97 (2H, d,  $J = 8.9$  Hz, H-2'' and H-6''), 8.22 (2H, d,  $J = 8.9$  Hz, H-3'' and H-5'');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) (ppm): 20.74 (t), 24.64 (t), 28.25 (t), 47.51 (t), 53.66 (t x 2), 55.29 (t), 66.98 (t x 2), 108.74 (d), 111.64 (s), 112.96 (d), 117.61 (s), 117.92 (s), 118.08 (d), 123.87 (s), 124.13 (d x 2), 125.10 (d x 2), 140.89 (s), 144.34 (s), 146.31 (s), 149.87 (s). Anal. Calcd. for  $\text{C}_{24}\text{H}_{25}\text{N}_5\text{O}_3\text{S}$ : C, 62.18; H, 5.44; N, 15.11. Found: C, 61.97; H, 5.41; N, 15.18.

#### 6.2.14 General synthesis for the nitro derivatives 44j-l

To a solution of **44c** (1.2 mmol) in dry DMF (8 mL), NaH (1.32 mmol) was added portion wise at 0 °C and the reaction was stirred for 2 hour at room temperature. Tetrabutylammonium iodide (1.32 mmol) and the proper alkyl halide as free base (4.8 mmol) in DMF (2 mL) were added and the mixture was stirred at room temperature or heated up to 50 °C overnight. (Free base forms of alkyl halides were extemporaneously prepared from commercially available salts by treatment with sodium hydroxide aqueous solution and extraction with DCM). Reaction was poured into ice and brine and the solid formed was filtered and purified by chromatography.

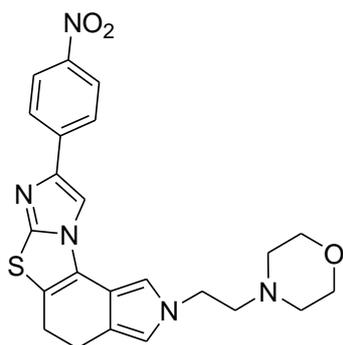


**8-(4-Nitrophenyl)-2-[2-(pyrrolidin-1-yl)ethyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole (44j).** This compound was obtained from reaction of **44c** with 1-(2-chloroethyl)pyrrolidine in 20 hours at room temperature. Purification was possible with flash column chromatography (dichloromethane : methanol 98 : 2) and further crystallization from ethanol. Yield: 77%; orange solid;  $R_f=0.38$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 171.1 - 171.8 °C (EtOH); IR: 1512 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.63 - 1.72 (4H, m,  $\text{CH}_2 \times 2$ ), 2.45 - 2.53 (4H, m,  $\text{CH}_2 \times 2$ ), 2.77 - 3.00 (6H, m,  $\text{CH}_2 \times 3$ ), 4.03 (2H, t,  $J = 6.9$  Hz,  $\text{CH}_2$ ), 6.78 (1H, s, H-3), 7.53 (1H, s, H-1), 8.16 (2H, d,  $J = 9.0$  Hz, H-2'' and H-6''), 8.30 (2H, d,  $J = 9.0$  Hz, H-3'' and H-5''), 8.82 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.20 (t), 23.12 (t  $\times 2$ ), 23.88 (t), 48.39 (t), 53.59 (t  $\times 2$ ), 56.52 (t), 110.39 (d), 111.34 (s), 114.80 (d), 115.92 (s), 116.57 (s), 118.51 (d), 123.84 (s), 124.16 (d  $\times 2$ ), 125.09 (d  $\times 2$ ), 141.01 (s), 143.58 (s), 145.73 (s), 148.43 (s). Anal. Calcd. for  $\text{C}_{23}\text{H}_{23}\text{N}_5\text{O}_2\text{S}$ : C, 63.72; H, 5.35; N, 16.15. Found: C, 64.01; H, 5.28; N, 16.33.



**8-(4-Nitrophenyl)-2-[2-(piperidin-1-yl)ethyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole (44k).** This compound was obtained from reaction of **44c** with 1-(2-chloroethyl)piperidine in 20 hours at room temperature. Purification was possible with flash column chromatography (dichloromethane : methanol 99 : 1) and further crystallization from ethanol. Yield: 85%; yellow solid;  $R_f=0.18$  (EtOAc); mp: 231.4 - 232.0 °C (EtOH); IR: 1505 - 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 1.32 - 1.62 (6H, m,  $\text{CH}_2 \times 3$ ), 2.37 - 2.50 (4H, m,

CH<sub>2</sub> x 2), 2.68 (2H, t, *J* = 6.8 Hz, CH<sub>2</sub>), 2.85 (4H, s, CH<sub>2</sub> x 2), 4.00 (2H, t, *J* = 6.8 Hz, CH<sub>2</sub>), 6.52 (1H, s, H-3), 6.89 (1H, s, H-1), 7.79 (1H, s, H-imidaz), 7.91 (2H, d, *J* = 8.6 Hz, H-2'' and H-6''), 8.16 (2H, d, *J* = 8.6 Hz, H-3'' and H-5''); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 20.76 (t), 24.09 (t), 24.66 (t), 25.88 (t x 2), 47.64 (t), 54.87 (t x 2), 59.96 (t), 108.83 (d), 111.74 (s), 113.29 (d), 117.63 (s), 118.01 (s), 118.26 (d), 123.89 (s), 124.16 (d x 2), 125.18 (d x 2), 140.94 (s), 144.38 (s), 146.37 (s), 149.89 (s). Anal. Calcd. for C<sub>24</sub>H<sub>25</sub>N<sub>5</sub>O<sub>2</sub>S: C, 64.41; H, 5.63; N, 15.65. Found: C, 64.83; H, 5.81; N, 15.90.



**2-[2-(Morpholin-4-yl)ethyl]-8-(4-nitrophenyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole (44l).** This compound was obtained from reaction of **44c** with 4-(2-chloroethyl)morpholine in 20 hours at 50 °C . Purification was possible with flash column chromatography (dichloromethane : methanol 98 : 2). Yield: 75%; orange solid; *R*<sub>f</sub>=0.18 (EtOAc); mp: 258.0 - 258.4 °C; IR: 1511 - 1340 (NO<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.38 - 2.50 (4H, m, CH<sub>2</sub> x 2), 2.70 (2H, t, *J* = 6.7 Hz, CH<sub>2</sub>), 2.76 - 3.00 (4H, m, CH<sub>2</sub> x 2), 3.53 - 3.63 (4H, m, CH<sub>2</sub> x 2), 4.05 (2H, t, *J* = 6.7 Hz, CH<sub>2</sub>), 6.79 (1H, s, H-3), 7.53 (1H, s, H-1), 8.15 (2H, d, *J* = 9.0 Hz, H-2'' and H-6''), 8.30 (2H, d, *J* = 9.0 Hz, H-3'' and H-5''), 8.80 (1H, s, H-imidaz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 20.19 (t), 23.87 (t), 46.41 (t), 53.27 (t x 2), 58.94 (t), 66.14 (t x 2), 110.43 (s), 111.30 (d), 114.81 (d), 115.95 (s), 116.58 (s), 118.58 (d), 123.81 (s), 124.16 (d x 2), 125.06 (d x 2), 140.99 (s), 143.57 (s), 145.69 (s), 148.41 (s). Anal. Calcd. for C<sub>23</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>S: C, 61.45; H, 5.16; N, 15.58. Found: C, 61.37; H, 5.41; N, 15.77.

### 6.2.15 General synthesis for the amino derivatives 47a-l

#### Procedure A

To a suspension of **44a** or **44b** (0.45 mmol) in ethanol/water (5:1, 15 mL), iron powder (9 mmol) and H<sub>2</sub>SO<sub>4</sub> (12 N, 0.5 mL) were added and the mixture was heated at 60°C up to completeness. The solution was filtered through celite and washed with hot ethanol. The solvent was removed under reduced pressure and the crude material was extracted by saturated solution of NaHCO<sub>3</sub> and ethyl acetate. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>,

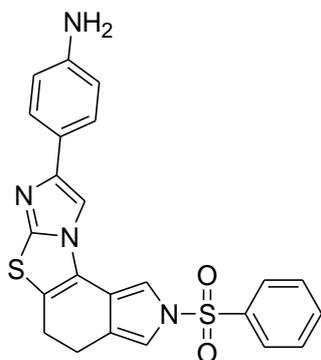
filtered and evaporated under reduced pressure. The crude product was then purified by chromatography.

### Procedure B

To a solution of the appropriate nitro derivative (0.5 mmol) in glacial acetic acid (5 ml) iron powder (5 mmol) was added. Then the mixture was heated up to 60°C in an ultrasound bath up to completeness. The mixture was filtered through celite and the filtrate was concentrated. The residue was neutralized with NaHCO<sub>3</sub> (or Na<sub>2</sub>CO<sub>3</sub>) and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the crude product which was purified by chromatography.

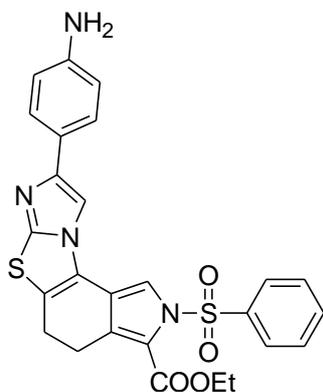
### Procedure C

To a solution/suspension of the appropriate nitro derivative (0.5 mmol) in ethanol (20 mL), Pd/C (10% w/w) was added. An H<sub>2</sub> atmosphere was created and the reaction was left to stir for 24 hours at room temperature. Upon complete the reaction mixture was filtered under vacuum. The filtrate was concentrated under reduced pressure, and the residue was purified by chromatography (**47f**) or directly used in the next step without any purification (**47e**, **47j-k** and **73**).

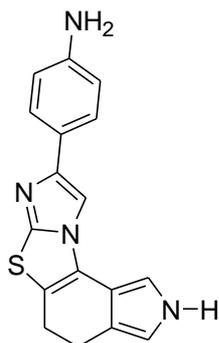


**4-[2-(Phenylsulfonyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl]aniline (**47a**)**. This compound was obtained from reaction of **44a** according to the procedure A in 20 hours. Purification was possible with flash column chromatography (cyclohexane : ethyl acetate 60 : 40). Yield: 50%; pink solid; R<sub>f</sub>=0.33 (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 70:30); mp: 223.6 - 224.1 °C; IR: 3461 - 3472 (NH<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 2.80 - 3.00 (4H, m, CH<sub>2</sub> x 2), 5.19 (2H, s, NH<sub>2</sub>), 6.61 (2H, d, *J* = 7.9 Hz, H-3'' and H-5''), 7.33 (1H, s, H-3), 7.58 - 7.85 (5H, m, H-3', H-4', H-5', H-2'', H-6''), 8.02 (2H, d, *J* = 7.0 Hz, H-2' and H-6'), 8.13 (1H, s, H-1), 8.53 (1H, s, H-imidaz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 19.69 (t),

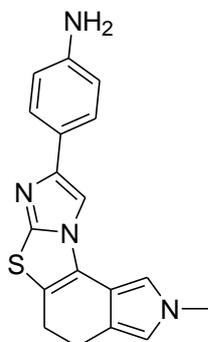
23.06 (t), 106.75 (d), 108.62 (d), 113.41 (s), 113.69 (d x 2), 116.40 (s), 117.66 (d), 120.72 (s), 121.41 (s), 122.11 (s), 123.30 (s), 125.71 (d x 2), 126.70 (d x 2), 129.95 (d x 2), 134.60 (s), 137.94 (d), 147.29 (s), 147.94 (s). Anal. Calcd. for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: C, 61.86; H, 4.06; N, 12.55. Found: C, 61.97; H, 4.30; N, 12.41.



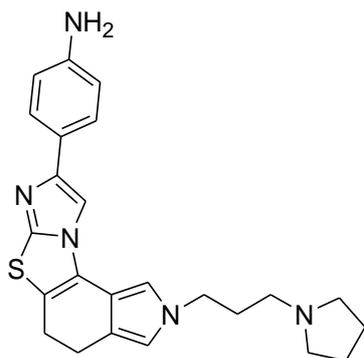
**Ethyl 8-(4-aminophenyl)-2-(phenylsulfonyl)-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole-3-carboxylate (47b).** This compound was obtained from reaction of **44b** according to the procedure A heating the mixture to 60°C in an ultrasound bath for 6 hours. Purification was possible with flash column chromatography (dichloromethane : ethyl acetate 90 : 10). Yield: 80%; pale yellow solid;  $R_f=0.25$  (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 90:10); mp: 259.6 - 260.2 °C; IR: 3486 - 3398 (NH<sub>2</sub>), 1715 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.22 (3H, t,  $J = 7.0$  Hz, CH<sub>3</sub>) 2.93 - 3.19 (4H, m, CH<sub>2</sub> x 2), 4.22 (2H, q,  $J = 7.0$  Hz, CH<sub>2</sub>), 5.18 (2H, s, NH<sub>2</sub>), 6.61 (2H, d,  $J = 8.4$  Hz, H-3'' and H-5''), 7.58 - 7.85 (5H, m, H-3', H-4', H-5', H-2'' and H-6''), 8.11 (2H, d,  $J_n = 6.7$  Hz, H-2' and H-6'), 8.47 (1H, s, H-1), 8.62 (1H, s, H-imidaz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 13.94 (q), 20.72 (t), 22.48 (t), 61.05 (t), 106.92 (d), 113.70 (d x 2), 114.11 (s), 119.71 (d), 120.56 (s), 121.02 (s), 121.51 (s), 122.08 (s), 125.77 (d x 2), 127.43 (d x 2), 129.42 (d x 2), 131.29 (s), 134.42 (d) 138.37 (s), 146.30 (s), 147.36 (s), 148.00 (s), 158.92 (s). Anal. Calcd. for C<sub>26</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub>: C, 60.21; H, 4.28; N, 10.80. Found: C, 60.57; H, 4.30; N, 11.01.



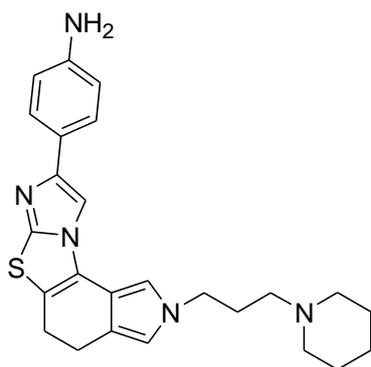
**4-(4,5-Dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl)aniline (47c).** This compound was obtained from reaction of **44c** according to the procedure B in 3 hours. Purification was possible with flash column chromatography (dichloromethane : ethyl acetate 80 : 20). Yield: 53%; pink solid;  $R_f=0.15$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  90:10); mp: 264.5 - 265.4 °C; IR: 3462 - 3368 ( $\text{NH}_2$ ), 3209 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.80 - 2.95 (4H, m,  $\text{CH}_2 \times 2$ ), 5.14 (2H, s,  $\text{NH}_2$ ), 6.59 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 6.72 (1H, s, H-3), 7.46 (1H, s, H-1), 7.60 (2H, d,  $J = 8.5$  Hz, H-2'' and H-6''), 7.82 (1H, s, H-imidaz), 10.96 (1H, bs, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.44 (t), 23.97 (t), 106.13 (d), 110.81 (s), 111.86 (d), 113.66 (s), 113.76 (d x 2), 114.85 (d), 116.52 (s), 122.42 (s), 124.31 (s), 125.66 (d x 2), 146.53 (s), 146.93 (s), 147.78 (s). Anal. Calcd. for  $\text{C}_{17}\text{H}_{14}\text{N}_4\text{S}$ : C, 66.64; H, 4.61; N, 18.29. Found: C, 66.58; H, 4.92; N, 18.55.



**4-(2-Methyl-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl)aniline (47d).** This compound was obtained from reaction of **44d** according to the procedure B in 1 hour. Purification was possible with flash column chromatography (dichloromethane : ethyl acetate 85 : 15). Yield: 44%; pale orange solid;  $R_f=0.23$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  80:20); mp: 109.8 - 110.7 °C; IR: 3451 - 3370 ( $\text{NH}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 2.73 - 2.98 (4H, m,  $\text{CH}_2 \times 2$ ), 3.66 (1H, s,  $\text{CH}_3$ ), 5.20 (2H, bs,  $\text{NH}_2$ ), 6.60 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 6.67 (1H, s, H-3), 7.45 (1H, s, H-1), 7.58 (2H, d,  $J = 8.5$  Hz, H-2'' and H-6''), 8.22 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.33 (t), 23.82 (t), 35.85 (q), 105.96 (d), 110.93 (s), 113.61 (s), 113.84 (d x 2), 115.58 (d), 116.95 (s), 119.05 (d), 122.42 (s), 123.87 (s), 125.61 (d x 2), 146.54 (s), 146.93 (s), 147.68 (s). Anal. Calcd. for  $\text{C}_{18}\text{H}_{16}\text{N}_4\text{S}$ : C, 67.47; H, 5.03; N, 17.49. Found: C, 67.67; H, 5.31; N, 17.71.

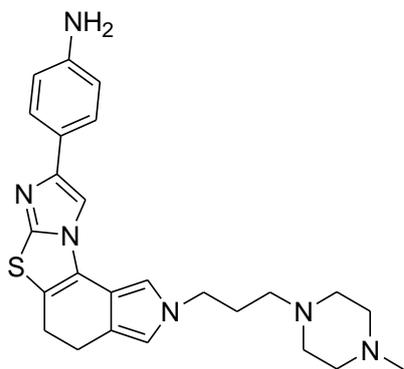


**4-{2-[3-(pyrrolidin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl}aniline (47f).** This compound was obtained from reaction of **44f** according to the procedure C. Purification was possible with flash column chromatography (dichloromethane : methanol 95 : 5) and further crystallization from diethyl ether. Yield: 55%; pale brown solid;  $R_f=0.17$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90:10); mp: 164.7 - 165.5 °C ( $\text{Et}_2\text{O}$ ); IR: 3415 - 3387 ( $\text{NH}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.73- 1.89 (4H, m,  $\text{CH}_2 \times 2$ ), 1.97 - 2.20 (2H, m,  $\text{CH}_2$ ), 2.65 - 3.00 (10H, m,  $\text{CH}_2 \times 5$ ), 3.99 (2H, t,  $J = 6.6$  Hz,  $\text{CH}_2$ ), 5.16 (2H, bs,  $\text{NH}_2$ ), 6.60 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 6.76 (1H, s, H-3), 7.53 (1H, s, H-1), 7.58 (2H, d,  $J = 8.5$  Hz, H-2'' and H-6''), 8.23 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.34 (t), 22.86 (t x 2), 22.87 (t), 23.81 (t), 46.64 (t), 51.94 (t), 53.24 (t x 2), 105.95 (d), 110.97 (s), 113.76 (d x 2), 113.78 (s), 114.49 (d), 116.78 (s), 118.14 (d), 122.31 (s), 123.86 (s), 125.62 (d x 2), 146.56 (s), 146.98 (s), 147.85 (s). Anal. Calcd. for  $\text{C}_{24}\text{H}_{27}\text{N}_5\text{S}$ : C, 69.03; H, 6.52; N, 16.77. Found: C, 69.28; H, 6.43; N, 16.65.

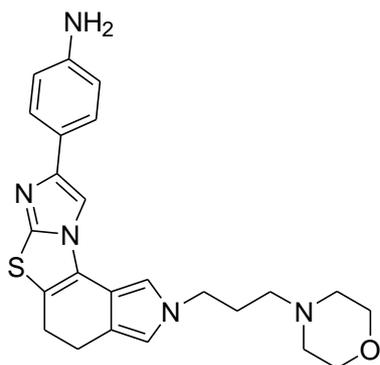


**4-{2-[3-(Piperidin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl}aniline (47g).** This compound was obtained from reaction of **44g** according to the procedure B in 2 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 95 : 5). Yield: 38%; pale orange solid;  $R_f=0.38$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90:10); mp: 201.8 - 202.3 °C; IR: 3446 - 3380 ( $\text{NH}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.25 -

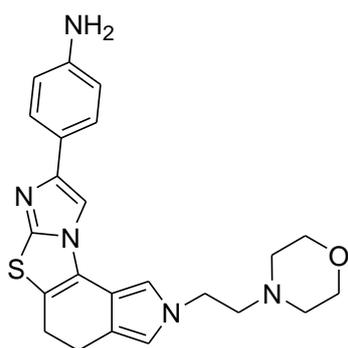
1.53 (6H, m, CH<sub>2</sub> x 3), 1.83 - 2.02 (2H, m, CH<sub>2</sub>), 2.10 - 2.50 (6H, m, CH<sub>2</sub> x 3), 2.62 - 2.98 (4H, m, CH<sub>2</sub> x 2), 3.80 - 4.00 (2H, m, CH<sub>2</sub>), 5.13 (2H, bs, NH<sub>2</sub>), 6.59 (2H, d, *J* = 8.1 Hz, H-3'' and H-5''), 6.71 (1H, s, H-3), 7.48 (1H, s, H-1), 7.57 (2H, d, *J* = 8.1 Hz, H-2'' and H-6''), 8.22 (1H, s, H-imidaz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 20.35 (t), 23.82 (t), 24.05 (t), 25.50 (t x 2), 28.11 (t), 47.13 (t), 54.00 (t x 2), 55.40 (t), 105.96 (d), 110.80 (s), 113.58 (s), 113.73 (d x 2), 114.48 (d), 116.55 (s), 118.19 (d), 122.31 (s), 123.93 (s), 125.60 (d x 2), 146.53 (s), 146.95 (s), 147.84 (s). Anal. Calcd. for C<sub>25</sub>H<sub>29</sub>N<sub>5</sub>S: C, 69.57; H, 6.77; N, 16.23. Found: C, 69.84; H, 6.93; N, 16.51.



**4-{2-[3-(4-Methylpiperazin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl}aniline (47h).** This compound was obtained from reaction of **44h** according to the procedure B in 3 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 95 : 5). Yield: 30%; light brown solid; *R*<sub>f</sub>=0.32 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5); mp: 219.3 - 220.4 °C; IR: 3443 - 3374 (NH<sub>2</sub>) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) (ppm): 1.88 - 2.00 (2H, m, CH<sub>2</sub>), 2.30 - 2.40 (5H, m, CH<sub>2</sub> and CH<sub>3</sub>), 2.42 - 2.68 (8H, m, CH<sub>2</sub> x 4), 2.90 (4H, s, CH<sub>2</sub> x 2), 3.95 (2H, t, *J* = 6.8 Hz, CH<sub>2</sub>), 4.60 (2H, bs, NH<sub>2</sub>), 6.50 (1H, s, H-3), 6.73 (2H, d, *J* = 8.6 Hz, H-3'' and H-5''), 6.86 (1H, s, H-1), 7.61 (1H, s, H-imidaz), 7.67 (2H, d, *J* = 8.6 Hz, H-2'' and H-6''); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 20.87 (t), 24.57 (t), 28.40 (t), 45.48 (q), 47.43 (t), 52.45 (t x 2), 54.63 (t x 3), 105.24 (d), 112.05 (s), 113.06 (d), 115.27 (d x 2), 115.91 (s), 117.73 (d), 117.99 (s), 123.88 (s), 125.22 (s), 126.24 (d x 2), 145.61 (s), 147.09 (s), 148.47 (s). Anal. Calcd. for C<sub>25</sub>H<sub>30</sub>N<sub>6</sub>S: C, 67.23; H, 6.77; N, 18.82. Found: C, 67.51; H, 6.83; N, 18.95.

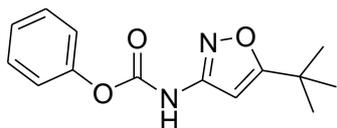


**4-{2-[3-(Morpholin-4-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}aniline (47i).** This compound was obtained from reaction of **44i** according to the procedure B in 5 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 98 : 2) and further crystallization from ethyl acetate. Yield: 60%; pale yellow solid;  $R_f=0.25$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 207.1 - 207.5 °C (AcOEt); IR: 3410 - 3324 ( $\text{NH}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.83 - 2.02 (2H, m,  $\text{CH}_2$ ), 2.20 - 2.43 (6H, m,  $\text{CH}_2 \times 3$ ), 2.73 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 3.52 - 3.58 (4H, m,  $\text{CH}_2 \times 2$ ), 3.83 - 3.98 (2H, t,  $J = 6.8$  Hz,  $\text{CH}_2$ ), 5.15 (2H, s,  $\text{NH}_2$ ), 6.60 (2H, d,  $J = 8.4$  Hz, H-3'' and H-5''), 6.72 (1H, s, H-3), 7.49 (1H, s, H-1), 7.58 (2H, d,  $J = 8.5$  Hz, H-2'' and H-6''), 8.23 (1H, s, H-imidaz);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.35 (t), 23.82 (t), 27.74 (t), 47.03 (t), 53.29 (t  $\times$  2), 55.13 (t), 66.16 (t  $\times$  2), 105.96 (d), 110.82 (s), 113.61 (s), 113.74 (d  $\times$  2), 114.48 (d), 116.58 (s), 118.20 (d), 122.32 (s), 123.92 (s), 125.60 (d  $\times$  2), 146.54 (s), 146.95 (s), 147.84 (s). Anal. Calcd. for  $\text{C}_{24}\text{H}_{27}\text{N}_5\text{OS}$ : C, 66.48; H, 6.28; N, 16.15. Found: C, 66.75; H, 6.43; N, 16.27.



**4-{2-[2-(Morpholin-4-yl)ethyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}aniline (47l).** This compound was obtained from reaction of **44l** according to the procedure B in 3 hours. Purification was possible with flash column chromatography (dichloromethane : methanol 98 : 2). Yield: 33%; light brown solid;  $R_f=0.32$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 225.4 - 226.2 °C; IR: 3443 - 3375 ( $\text{NH}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (ppm): 2.43 - 2.55 (4H, m,  $\text{CH}_2 \times 2$ ), 2.73 (2H, t,  $J = 6.7$  Hz,  $\text{CH}_2$ ), 2.89 (4H, s,  $\text{CH}_2 \times 2$ ), 3.47 (2H, s,  $\text{NH}_2$ ),

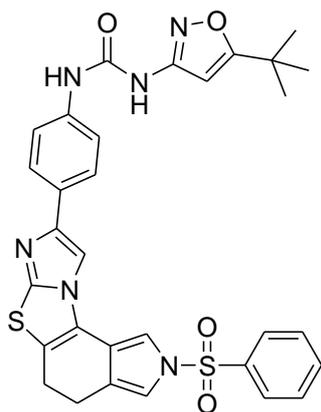
3.65 - 3.75 (4H, m, CH<sub>2</sub> x 2), 4.01 (2H, t, *J* = 6.7 Hz, CH<sub>2</sub>), 6.56 (1H, s, H-3), 6.73 (2H, d, *J* = 8.5 Hz, H-3'' and H-5''), 6.88 (1H, s, H-1), 7.59 (1H, s, H-imidaz), 7.66 (2H, d, *J* = 8.5 Hz, H-2'' and H-6''); <sup>13</sup>C NMR (CDCl<sub>3</sub>) (ppm): 20.87 (t), 24.59 (t), 47.37 (t), 53.83 (t x 2), 59.62 (t), 66.96 (t x 2), 105.28 (d), 112.28 (s), 113.13 (d), 115.33 (d x 2), 116.11 (s), 118.03 (d), 118.16 (s), 123.88 (s), 125.20 (s), 126.29 (d x 2), 145.68 (s), 147.15 (s), 148.50 (s). Anal. Calcd. for C<sub>23</sub>H<sub>25</sub>N<sub>5</sub>OS: C, 65.84; H, 6.01; N, 16.69. Found: C, 65.79; H, 6.21; N, 16.56.



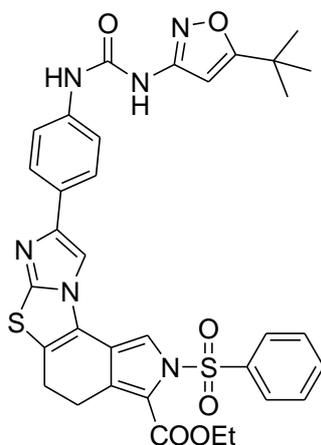
**6.2.16 Preparation of phenyl 5-*tert*-butylisoxazol-3-ylcarbamate (50).** To a suspension of 5-*tert*-butylisoxazol-3-amine (3.5 mmol) and anhydrous potassium carbonate (7 mmol) in dry THF (10 mL), a solution of phenyl chloroformate (5.25 mmol) in THF (5 mL) was added dropwise at 0 °C under N<sub>2</sub> atmosphere. The reaction was left to stir for 3 hours at room temperature. The suspension was filtered and the filter was rinsed with THF. The filtrate was vacuum distilled. To the residue water and ethanol (4 : 1) were added and the mixture was stirred at 10° C for 2 hours. The resulting solid was collected by filtration and washed with a mixture of water and ethanol (4 : 1). No further purification was needed. Yield: 90%. The spectroscopic data are in agreement with the literature.<sup>[94]</sup>

#### 6.2.17 General synthesis for the ureido derivatives 51a-l and 74

To a stirred solution of pure or crude amino derivatives **47** or **73** (0.3 mmol) in dry THF (5 mL) were added phenyl 5-*tert*-butylisoxazol-3-ylcarbamate (0.33 mmol), DIPEA (0.45 mmol) and DMAP (0.15 mmol). The solution was stirred at 60 °C for 1-3 hours. The mixture was concentrated under reduced pressure and the crude residue was purified by chromatography or crystallization from ethanol or THF.

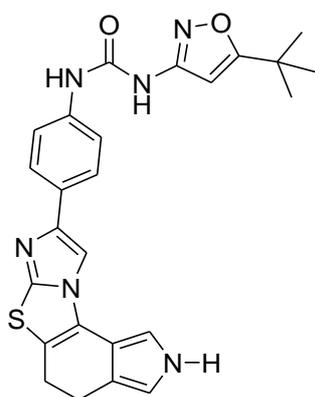


**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-{4-[2-(phenylsulfonyl)-4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl]phenyl}urea (51a).** This compound was obtained from reaction of **44a** in 2 hours and crude material was purified by crystallization from THF. Yield: 87%; pink solid;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  80:20); mp: 258.9 - 259.8 °C (THF); IR: 3315 (NH), 3287 (NH), 1690 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.31 (9H, s,  $\text{CH}_3 \times 3$ ), 2.80 - 3.03 (4H, m,  $\text{CH}_2 \times 2$ ), 6.54 (1H, s, CH-isoxaz), 7.35 (1H, s, H-3), 7.52 (2H, d,  $J = 8.6$ , H-2'' and H-6''), 7.58 - 7.83 (3H, m, H-3', H-4' and H-5'), 7.89 (2H, d,  $J = 8.6$  Hz, H-3'' and 5''), 8.02 - 8.11 (2H, m, H-2' and H-6'), 8.16 (1H, s, H-1), 8.75 (1H, s, CH-imidaz), 8.90 (1H, s, NH), 9.56 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 23.04 (t), 25.03 (t), 28.25 (q  $\times 3$ ), 32.42 (s), 92.39 (d), 108.47 (d), 113.31 (d), 116.21 (s), 117.64 (d), 118.54 (d  $\times 2$ ), 121.30 (s), 121.83 (s), 123.31 (s), 125.27 (d  $\times 2$ ), 126.70 (d  $\times 2$ ), 128.57 (s), 129.97 (d  $\times 2$ ), 134.70 (d), 137.64 (s), 137.76 (s), 145.81 (s), 147.01 (s), 151.15 (s), 158.20 (s), 180.42 (s). Anal. Calcd. for  $\text{C}_{31}\text{H}_{28}\text{N}_6\text{O}_4\text{S}_2$ : C, 60.77; H, 4.61; N, 13.72. Found: C, 60.81; H, 4.52; N, 13.68.

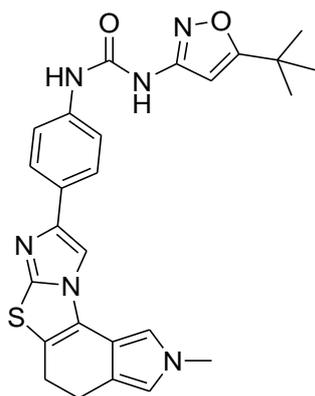


**Ethyl 8-(4-[(5-*tert*-butyl-1,2-oxazol-3-yl)carbamoyl]amino)phenyl)-2-(phenylsulfonyl)-4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindole-3-carboxylate (51b).** This compound was obtained from reaction of **47b** in 1 hour and crude product was purified by

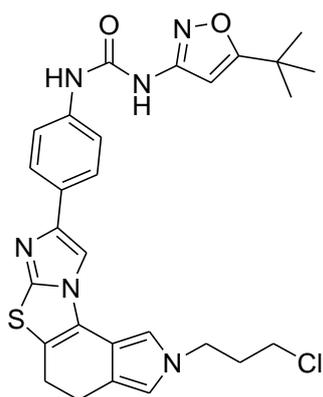
crystallization from ethanol. Yield: 80%; pale yellow solid;  $R_f=0.25$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp: 264.1 - 264.9 °C (EtOH); IR: 3315 (NH), 3278 (NH), 1719 (CO), 1689 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.28 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 1.37 (9H, s,  $\text{CH}_3 \times 3$ ), 3.02 - 3.25 (4H, m,  $\text{CH}_2 \times 2$ ), 4.29 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_2$ ), 6.60 (1H, s, CH-isoxaz), 7.58 (2H, d,  $J = 8.7$  Hz, H-2'' and H-6''), 7.70 - 7.92 (3H, m, H-3', H-4' and H-5'), 7.97 (2H, d,  $J = 8.7$  Hz, H-3'' and 5''), 8.02 - 8.11 (2H, m, H-2' and H-6'), 8.56 (1H, s, H-1), 8.91 (1H, s, CH-imidaz), 8.96 (1H, s, NH), 9.61 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 13.94 (q), 20.75 (t), 22.59 (t), 28.25 (q x 3), 32.45 (s), 61.14 (t), 92.50 (d), 106.86 (s), 108.75 (d), 113.99 (s), 118.46 (d x 2), 120.59 (d), 121.87 (s), 125.31 (d x 2), 127.40 (d x 2), 128.69 (s), 129.44 (d x 2), 131.31 (s), 134.43 (d), 137.88 (s), 138.39 (s), 146.00 (s), 146.92 (s), 149.70 (s), 151.32 (s), 158.36 (s), 158.89 (s), 180.17 (s). Anal. Calcd. for  $\text{C}_{34}\text{H}_{32}\text{N}_6\text{O}_6\text{S}_2$ : C, 59.63; H, 4.71; N, 12.27. Found: C, 59.89; H, 4.79; N, 12.52.



**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-[4-(4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl)phenyl]urea (51c).** This compound was obtained from reaction of **47c** in 1 hour and the crude product was purified by crystallization from ethanol. Yield: 72%; pink solid;  $R_f=0.17$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  80:20); mp: > 400 °C (EtOH); IR: 3315 (NH), 3285 (NH), 3142 (NH), 1654 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.31 (9H, s,  $\text{CH}_3 \times 3$ ), 2.80 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 6.54 (1H, s, CH-isoxaz), 6.74 (1H, s, H-3), 7.47 - 7.53 (3H, m, H-1, H-2'' and H-6''), 7.87 (2H, d,  $J = 8.5$  Hz, H-3'' and 5''), 8.75 (1H, s, CH-imidaz), 8.90 (1H, s, NH), 9.56 (1H, s, NH), 10.99 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.39 (t), 23.98 (t), 28.32 (q x 3), 32.45 (s), 92.42 (d), 107.97 (d), 110.67 (s), 111.95 (d), 114.40 (s), 114.92 (d), 116.50 (s), 118.48 (d x 2), 124.30 (s), 125.17 (d x 2), 128.94 (s), 137.65 (s), 145.58 (s), 147.09 (s), 151.24 (s), 158.36 (s), 180.14 (s). Anal. Calcd. for  $\text{C}_{25}\text{H}_{24}\text{N}_6\text{O}_2\text{S}$ : C, 63.54; H, 5.12; N, 17.78. Found: C, 63.85; H, 5.42; N, 18.03.

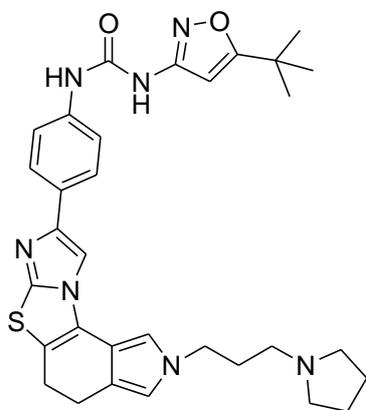


**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-[4-(2-methyl-4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl)phenyl]urea (51d).** This compound was obtained from reaction of **47d** in 1 hour and the crude product was purified by crystallization from THF. Yield: 84%; pale yellow solid;  $R_f=0.34$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  80:20); mp: 243.4 - 243.9 °C (THF); IR: 3335 (NH), 3278 (NH), 1711 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.31 (9H, s,  $\text{CH}_3 \times 3$ ), 2.77 - 2.98 (4H, m,  $\text{CH}_2 \times 2$ ), 3.68 (3H, s,  $\text{CH}_3$ ), 6.54 (1H, s, CH-isoxaz), 6.68 (1H, s, H-3), 7.46 - 7.53 (3H, m, H-1, H-2'' and H-6''), 7.85 (2H, d,  $J = 8.5$  Hz, H-3'' and 5''), 8.45 (1H, s, CH-imidaz), 8.89 (1H, s, NH), 9.57 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 20.28 (t), 23.85 (t), 28.32 (q  $\times 3$ ), 32.44 (s), 35.88 (q), 92.43 (d), 107.74 (d), 110.83 (s), 114.35 (s), 115.61 (d), 116.95 (s), 118.52 (d  $\times 2$ ), 119.12 (d), 123.88 (s), 125.13 (d  $\times 2$ ), 128.90 (s), 137.71 (s), 145.65 (s), 147.12 (s), 151.26 (s), 158.37 (s), 180.13 (s). Anal. Calcd. for  $\text{C}_{26}\text{H}_{26}\text{N}_6\text{O}_2\text{S}$ : C, 64.18; H, 5.39; N, 17.27. Found: C, 64.45; H, 5.24; N, 17.53.

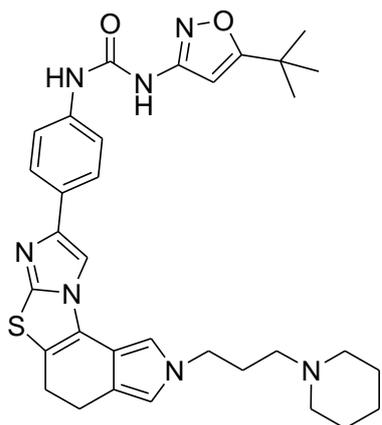


**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-[4-[2-(3-chloropropyl)-4,5-dihydro-2*H*-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl]phenyl]urea (51e).** This compound was obtained from reaction of **47e** (crude) in 1 hour and the crude product was purified by flash column chromatography (petroleum ether : ethyl acetate 70 : 30). Yield: 43%; pale yellow solid;  $R_f=0.48$  ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  70:30); mp: 253.3 - 253.9 °C; IR: 3325 (NH), 3284 (NH), 1720 (CO)

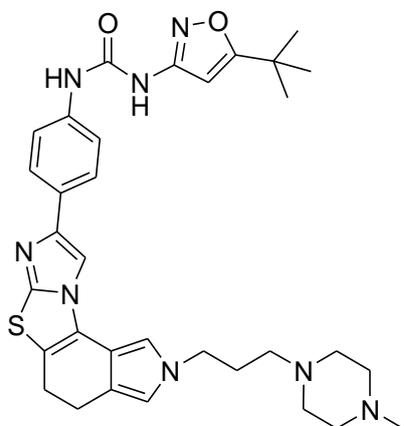
cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.31 (9H, s, CH<sub>3</sub> x 3), 2.20 - 2.40 (2H, m, CH<sub>2</sub>), 2.82 - 3.02 (4H, m, CH<sub>2</sub> x 2), 3.69 (2H, t, *J* = 6.2 Hz, CH<sub>2</sub>), 4.06 - 2.20 (2H, m, CH<sub>2</sub>), 6.59 (1H, s, CH-isoxaz), 6.82 (1H, s, H-3), 7.52 - 7.61 (3H, m, H-1, H-2'' and H-6''), 7.91 (2H, d, *J* = 8.3 Hz, H-3'' and 5''), 8.52 (1H, s, CH-imidaz), 8.93 (1H, s, NH), 9.60 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 20.28 (t), 23.85 (t), 28.32 (q x 3), 32.45 (s), 33.52 (t), 42.36 (t), 46.17 (t), 92.43 (d), 107.80 (d), 111.00 (s), 114.72 (d), 116.95 (s), 118.26 (s), 118.49 (d x 2), 118.51 (d), 123.79 (s), 125.14 (d x 2), 128.90 (s), 137.71 (s), 145.63 (s), 147.12 (s), 151.24 (s), 158.35 (s), 180.15 (s). Anal. Calcd. for C<sub>28</sub>H<sub>29</sub>ClN<sub>6</sub>O<sub>2</sub>S: C, 61.25; H, 5.32; N, 15.31. Found: C, 61.53; H, 5.54; N, 15.25.



**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-(4-{2-[3-(pyrrolidin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}phenyl)urea (51f).** This compound was obtained from reaction of **47f** in 2 hours and the crude product was purified by flash column chromatography (dichloromethane : methanol 95 : 5) and further crystallization from ethanol. Yield: 42%; white solid; *R*<sub>f</sub>=0.52 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90:10); mp: 230.1 - 230.2 °C (EtOH); IR: 3341 (NH), 3303 (NH), 1718 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.31 (9H, s, CH<sub>3</sub> x 3), 1.64 - 1.78 (4H, m, CH<sub>2</sub> x 2), 1.90 - 2.10 (2H, m, CH<sub>2</sub>), 2.40 - 2.60 (6H, m, CH<sub>2</sub> x 3), 2.80 - 3.00 (4H, m, CH<sub>2</sub> x 2), 3.96 (2H, t, *J* = 6.6 Hz, CH<sub>2</sub>), 6.53 (1H, s, CH-isoxaz), 6.74 (1H, s, H-3), 7.47 - 7.55 (3H, m, H-1, H-2'' and H-6''), 7.85 (2H, d, *J* = 8.6 Hz, H-3'' and 5''), 8.46 (1H, s, CH-imidaz), 8.96 (1H, s, NH), 9.57 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 20.32 (t), 23.06 (t x 2), 23.85 (t), 28.33 (q x 3), 29.99 (t), 32.44 (s), 47.15 (t), 52.52 (t), 53.54 (t x 2), 92.44 (d), 107.77 (s), 110.71 (d), 114.39 (s), 114.53 (d), 116.65 (s), 118.21 (d), 118.47 (d x 2), 123.93 (s), 125.14 (d x 2), 128.89 (s), 137.75 (s), 145.64 (s), 147.13 (s), 151.28 (s), 158.36 (s), 180.13 (s). Anal. Calcd. for C<sub>32</sub>H<sub>37</sub>N<sub>7</sub>O<sub>2</sub>S: C, 65.84; H, 6.39; N, 16.80. Found: C, 65.99; H, 6.66; N, 17.07.

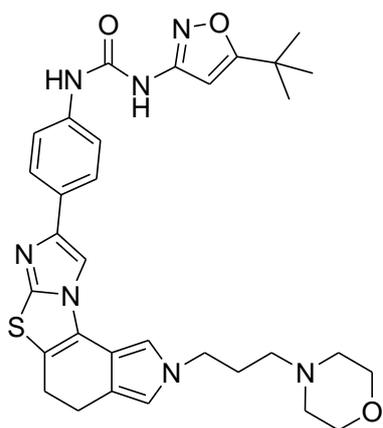


**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-(4-{2-[3-(piperidin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}phenyl)urea (51g).** This compound was obtained from reaction of **47g** in 2 hours and the crude product was purified by flash column chromatography (dichloromethane : methanol 93 : 7). Yield: 60%; pale yellow solid;  $R_f=0.55$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90:10); mp: 255.5 - 255.7 °C; IR: 3342 (NH), 3280 (NH), 1701 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.20 - 1.68 (15H, m,  $\text{CH}_3 \times 3$  and  $\text{CH}_2 \times 3$ ), 1.90 - 2.13 (2H, m,  $\text{CH}_2$ ), 2.32 - 2.50 (6H, m,  $\text{CH}_2 \times 3$ ), 2.75 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 3.86 - 4.03 (2H, m,  $\text{CH}_2$ ), 6.54 (1H, s, CH-isoxaz), 6.74 (1H, s, H-3), 7.48 - 7.54 (3H, m, H-2'', H-6'' and H-1), 7.85 (2H, d,  $J = 8.4$  Hz, H-3'' and H-5''), 8.46 (1H, s, CH-imidaz), 9.10 (1H, s, NH), 9.64 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.31 (t), 23.42 (t), 23.85 (t), 24.83 (t x 2), 27.45 (t), 28.33 (q x 3), 32.44 (s), 46.95 (t), 53.57 (t x 2), 54.96 (t), 92.43 (d), 107.73 (d), 110.78 (s), 114.44 (d), 114.50 (s), 116.67 (s), 118.23 (d), 118.42 (d x 2), 123.91 (s), 125.14 (d x 2), 128.84 (s), 137.80 (s), 145.66 (s), 147.13 (s), 151.31 (s), 158.34 (s), 180.10 (s). Anal. Calcd. for  $\text{C}_{33}\text{H}_{39}\text{N}_7\text{O}_2\text{S}$ : C, 66.30; H, 6.58; N, 16.40. Found: C, 66.12; H, 6.53; N, 16.73.



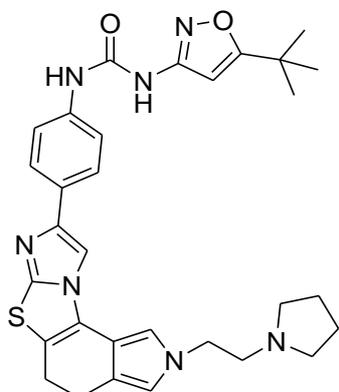
**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-(4-{2-[3-(4-methylpiperazin-1-yl)propyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}phenyl)urea (51h).** This compound

was obtained from reaction of **47h** in 2 hours and the crude product was purified by crystallization from ethanol. Yield: 53%; pale yellow solid;  $R_f=0.40$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 227.3 - 228.0 °C (EtOH); IR: 3334 (NH), 3279 (NH), 1719 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.30 (9H, s,  $\text{CH}_3$  x 3), 1.80 - 2.50 (15H, m,  $\text{CH}_2$  x 6 and  $\text{CH}_3$ ), 2.75 - 3.00 (4H, m,  $\text{CH}_2$  x 2), 3.86 - 3.98 (2H, m,  $\text{CH}_2$ ), 6.54 (1H, s, CH-isoxaz), 6.74 (1H, s, H-3), 7.49 - 7.54 (3H, m, H-2'', H-6'' and H-1), 7.85 (2H, d,  $J = 7.9$  Hz, H-3'' and H-5''), 8.46 (1H, s, CH-imidaz), 8.88 (1H, s, NH), 9.56 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 20.31 (t), 23.86 (t), 28.12 (t), 28.32 (q x 3), 32.44 (s), 45.71 (q), 47.11 (t), 52.65 (t x 2), 54.71 (t x 2), 54.72 (t), 92.42 (d), 107.77 (d), 110.69 (s), 114.35 (s), 114.52 (d), 116.56 (s), 118.21 (d), 118.48 (d x 2), 123.93 (s), 125.14 (d x 2), 128.91 (s), 137.71 (s), 145.63 (s), 147.13 (s), 151.24 (s), 158.36 (s), 180.13 (s). Anal. Calcd. for  $\text{C}_{33}\text{H}_{40}\text{N}_8\text{O}_2\text{S}$ : C, 64.68; H, 6.58; N, 18.29. Found: C, 64.89; H, 6.73; N, 18.53.

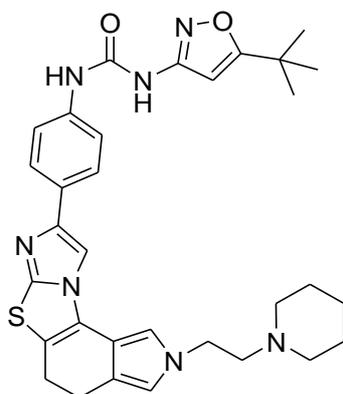


**1-(5-tert-Butyl-1,2-oxazol-3-yl)-3-(4-{2-[3-(morpholin-4-yl)propyl]-4,5-dihydro-2H-imidazo [2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl}phenyl)urea (51i)**. This compound was obtained from reaction of **47i** in 2 hours and the crude product was purified by crystallization from ethanol. Yield: 75%; pale yellow solid;  $R_f=0.44$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 238.1 - 238.5 °C (EtOH); IR: 3340 (NH), 3280 (NH), 1720 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ) (ppm): 1.34 (9H, s,  $\text{CH}_3$  x 3), 1.87 - 2.00 (2H, m,  $\text{CH}_2$ ), 2.20 - 2.40 (6H, m,  $\text{CH}_2$  x 3), 2.80 - 3.00 (4H, m,  $\text{CH}_2$  x 2), 3.52 - 3.68 (4H, m,  $\text{CH}_2$  x 2), 3.86 - 4.03 (2H, m,  $\text{CH}_2$ ), 6.54 (1H, s, CH-isoxaz), 6.73 (1H, s, H-3), 7.47 - 7.54 (3H, m, H-2'', H-6'' and H-1), 7.85 (2H, d,  $J = 7.9$  Hz, H-3'' and H-5''), 8.46 (1H, s, CH-imidaz), 8.88 (1H, s, NH), 9.55 (1H, s, NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) (ppm): 20.31 (t), 23.84 (t), 27.76 (t), 28.32 (q x 3), 32.45 (s), 47.06 (t), 53.29 (t x 2), 55.13 (t), 66.16 (t x 2), 92.42 (d), 107.76 (d), 110.70 (s), 114.38 (s), 114.52 (d), 116.58 (s), 118.26 (d), 118.48 (d x 2), 123.92 (s), 125.13 (d x 2), 128.89 (s), 137.71 (s), 145.61 (s),

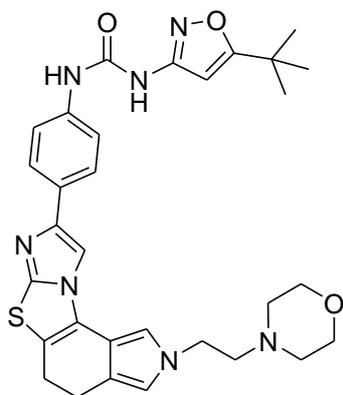
147.12 (s), 151.24 (s), 158.35 (s), 180.14 (s). Anal. Calcd. for  $C_{32}H_{37}N_7O_3S$ : C, 64.08; H, 6.22; N, 16.35. Found: C, 64.09; H, 6.44; N, 16.53.



**1-(5-tert-butyl-1,2-oxazol-3-yl)-3-(4-{2-[2-(pyrrolidin-1-yl)ethyl]-4,5-dihydro-2H-imidazo[2',1':2,3][1,3]thiazolo[4,5-e]isoindol-8-yl}phenyl)urea (51j).** This compound was obtained from reaction of **47j** (crude) in 3 hours and the crude product was purified by flash column chromatography (dichloromethane : methanol 95 : 5). Yield: 60%; pale yellow solid;  $R_f=0.55$  ( $CH_2Cl_2/MeOH$  90:10); mp: 250.4 - 251.0 °C; IR: 3347 (NH), 3296 (NH), 1726 (CO)  $cm^{-1}$ ;  $^1H$  NMR ( $DMSO-d_6$ ) (ppm): 1.37 (9H, s,  $CH_3 \times 3$ ), 1.71 - 1.78 (4H, m,  $CH_2 \times 2$ ), 2.50 - 2.60 (2H, m,  $CH_2 \times 2$ ), 2.80 - 3.05 (6H, m,  $CH_2 \times 3$ ), 4.08 (2H, t,  $J = 6.7$  Hz,  $CH_2$ ), 6.59 (1H, s, CH-isoxaz), 6.83 (1H, s, H-3), 7.52 - 7.61 (3H, m, H-1, H-2'' and H-6''), 7.92 (2H, d,  $J = 8.5$  Hz, H-3'' and 5''), 8.51 (1H, s, CH-imidaz), 8.96 (1H, s, NH), 9.62 (1H, s, NH);  $^{13}C$  NMR ( $DMSO-d_6$ ) (ppm): 20.31 (t), 23.12 (t x 2), 23.84 (t), 28.32 (q x 3), 32.45 (s), 48.35 (t), 53.58 (t x 2), 56.54 (t), 92.42 (d), 107.78 (d), 110.64 (s), 114.38 (s), 114.77 (d), 116.55 (s), 118.49 (d x 2), 118.50 (d), 123.92 (s), 125.14 (d x 2), 128.89 (s), 137.72 (s), 145.62 (s), 147.13 (s), 151.26 (s), 158.36 (s), 180.14 (s). Anal. Calcd. for  $C_{31}H_{35}N_7O_2S$ : C, 65.35; H, 6.19; N, 17.21. Found: C, 65.29; H, 6.21; N, 17.53.

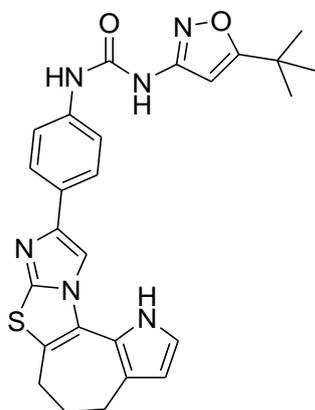


**1-(5-*tert*-butyl-1,2-oxazol-3-yl)-3-(4-{2-[2-(piperidin-1-yl)ethyl]-4,5-dihydro-2*H*-imidazo [2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}phenyl)urea (51k)**. This compound was obtained from reaction of **47k** (crude) in 1 hours and the crude product was purified by flash column chromatography (dichloromethane : methanol 98 : 2) and further crystallization from diethyl ether. Yield: 30%; pale yellow solid;  $R_f=0.43$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 230.6 - 231.1 °C ( $\text{Et}_2\text{O}$ ); IR: 3326 (NH), 3284 (NH), 1712 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.31 (9H, s,  $\text{CH}_3 \times 3$ ), 1.30 - 1.58 (6H, m,  $\text{CH}_2 \times 3$ ), 2.38 - 2.50 (4H, m,  $\text{CH}_2 \times 2$ ), 2.60 - 2.75 (2H, m,  $\text{CH}_2$ ), 2.80 - 3.00 (4H, m,  $\text{CH}_2 \times 2$ ), 4.02 (2H, t,  $J = 6.3$  Hz,  $\text{CH}_2$ ), 6.53 (1H, s, CH-isoxaz), 6.76 (1H, s, H-3), 7.48 - 7.54 (3H, m, H-2'', H-6'' and H-1), 7.85 (2H, d,  $J = 8.6$  Hz, H-3'' and H-5''), 8.44 (1H, s, CH-imidaz), 8.88 (1H, s, NH), 9.55 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.31 (t), 23.86 (t), 25.50 (t x 2), 28.32 (q x 3), 32.44 (s), 46.71 (t), 54.06 (t x 2), 54.16 (t), 59.33 (t), 92.43 (d), 107.70 (d), 110.67 (s), 114.39 (s), 114.77 (d), 116.57 (s), 118.45 (d), 118.50 (d x 2), 123.91 (s), 125.14 (d x 2), 128.90 (s), 137.72 (s), 145.64 (s), 147.15 (s), 151.25 (s), 158.36 (s), 180.15 (s). Anal. Calcd. for  $\text{C}_{32}\text{H}_{37}\text{N}_7\text{O}_2\text{S}$ : C, 65.84; H, 6.39; N, 16.80. Found: C, 66.12; H, 6.28; N, 16.98.



**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-(4-{2-[2-(morpholin-4-yl)ethyl]-4,5-dihydro-2*H*-imidazo [2',1':2,3][1,3]thiazolo[4,5-*e*]isoindol-8-yl}phenyl)urea (51l)**. This compound was obtained from reaction of **47l** in 2 hours and the crude product was purified by crystallization from ethanol. Yield: 45%; white solid;  $R_f=0.48$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5); mp: 247.9 - 248.1 °C ( $\text{EtOH}$ ); IR: 3326 (NH), 3286 (NH), 1712 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 1.31 (9H, s,  $\text{CH}_3 \times 3$ ), 2.42 - 2.50 (4H, m,  $\text{CH}_2 \times 2$ ), 2.70 (2H, t,  $J = 6.4$  Hz,  $\text{CH}_2$ ), 2.75 - 2.97 (4H, m,  $\text{CH}_2 \times 2$ ), 3.52 - 3.62 (4H, m,  $\text{CH}_2 \times 2$ ), 4.04 (2H, t,  $J = 6.4$  Hz,  $\text{CH}_2$ ), 6.53 (1H, s, CH-isoxaz), 6.78 (1H, s, H-3), 7.47 - 7.54 (3H, m, H-2'', H-6'' and H-1), 7.85 (2H, d,  $J = 8.5$  Hz, H-3'' and H-5''), 8.44 (1H, s, CH-imidaz), 8.88 (1H, s, NH), 9.55 (1H, s, NH);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ) (ppm): 20.30 (t), 23.84 (t), 28.32 (q x 3), 32.45 (s), 46.33 (t), 53.27 (t x 2), 58.93

(t), 66.15 (t x 2), 92.43 (d), 107.71 (d), 110.70 (s), 114.44 (s), 114.80 (d), 116.59 (s), 118.50 (d x 3), 123.90 (s), 125.14 (d x 2), 128.89 (s), 137.72 (s), 145.63 (s), 147.15 (s), 151.25 (s), 158.36 (s), 180.15 (s). Anal. Calcd. for C<sub>31</sub>H<sub>35</sub>N<sub>7</sub>O<sub>3</sub>S: C, 63.57; H, 6.02; N, 16.74. Found: C, 63.42; H, 6.30; N, 16.80.



**1-(5-*tert*-Butyl-1,2-oxazol-3-yl)-3-[4-(1,4,5,6-tetrahydroimidazo[2,1-*b*]pyrrolo[3',2':6,7]cyclohepta[1,2-*d*][1,3]thiazol-9-yl)phenyl]urea (74).** This compound was obtained from reaction of **73** (crude) in 3 hours and the crude product was purified by crystallization from ethanol. Yield: 65%; pale yellow solid;  $R_f=0.63$  (AcOEt); mp: 194.7 - 195.3 °C (EtOH); IR : 3273 (NH), 3251 (NH), 3144 (NH), 1690 (CO) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) (ppm): 1.36 (9H, s, CH<sub>3</sub> x 3), 2.01 - 2.12 (2H, m, CH<sub>2</sub>), 2.92 - 3.10 (4H, m, CH<sub>2</sub> x 2), 6.17 (1H, s, H-3), 6.59 (1H, s, CH-isoxaz), 6.99 (1H, s, H-2), 7.58 (2H, d,  $J = 8.7$  Hz, H-2'' and H-6''), 7.87 (2H, d,  $J = 8.7$  Hz, H-3'' and 5''), 8.63 (1H, s, CH-imidaz), 8.94 (1H, s, NH), 9.62 (1H, s, NH), 11.04 (1H, s, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) (ppm): 25.37 (t), 27.34 (t), 28.32 (q x 3), 28.71 (t), 32.44 (s), 35.88 (q), 92.40 (d), 108.79 (d), 110.61 (d), 117.06 (s), 118.56 (d x 2), 119.49 (d), 119.78 (s), 121.60 (s), 124.49 (s), 125.13 (d x 2), 128.69 (s), 137.80 (s), 145.16 (s), 146.66 (s), 151.25 (s), 158.35 (s), 180.15 (s). Anal. Calcd. for C<sub>26</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>S: C, 64.18; H, 5.39; N, 17.27. Found: C, 64.15; H, 5.42; N, 17.33.

### 6.3 Breast cancer drug candidates: Chemistry

All reactions were carried out in oven-dried glassware, with magnetic stirring. All reagents, solvents, and starting materials were purchased from commercial suppliers and used without purification. Flash chromatography was carried out on silica gel (230-400 mesh), using mixtures of hexane and dichloromethane as eluents. NMR spectra were recorded on a Bruker 500 MHz spectrometer and were referenced to the residual solvent (CDCl<sub>3</sub> at 7.26 ppm).

Coupling constants are reported in Hertz (Hz). Mass spectra were recorded using a Bruker microTOF instrument and high-resolution mass spectra (HRMS, ESI) were recorded as a service at the EPSRC National Mass Spectrometry facility (Swansea, Wales, U.K.). Microanalyses (% CHNS, in duplicate) were recorded as a service by Medac Ltd (Chobham, Surrey, U.K.).

### 6.3.1 General synthesis of substituted carbamo(dithioperoxo)thioates (79a-x)

Following mixing of equimolar quantities of secondary amine (**77a-f**) and alkyl thiol (**78a-d**) in anhydrous dichloromethane, the reaction mixture was cooled in an ice bath. Carbon disulfide (1 equiv.) was then added dropwise, followed by slow addition of triethylamine (1.1 equiv.). After a further five minutes, a solution of CBr<sub>4</sub> (2 equiv.) in dichloromethane was added, followed by stirring at room temperature for two hours. The solution was washed with water (x3) and purified by column chromatography (dichloromethane and hexane as eluent) to yield the product carbamo(dithioperoxo)thioates (**3a-x**).

**2-Hydroxyethyl diethylcarbamo(dithioperoxo)thioate (79a).**<sup>[116]</sup> Yellow oil, 58% yield; R<sub>f</sub>=0.30(CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.28 - 1.43 (m, 6H, CH<sub>3</sub> x 2), 2.91 - 3.01 (m, 2H, SCH<sub>2</sub>), 3.64 (t, *J* = 7.3, 1H, OH), 3.71 - 3.78 (m, 2H, CH<sub>2</sub>OH), 3.86 (q, *J* = 7.1, 2H, NCH<sub>2</sub>), 4.09 (q, *J* = 7.1, 2H, NCH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 11.39 (CH<sub>3</sub>), 13.06 (CH<sub>3</sub>), 43.44 (SCH<sub>2</sub>), 47.25 (NCH<sub>2</sub>), 52.31 (NCH<sub>2</sub>), 58.18 (CH<sub>2</sub>OH), 196.99 (C=S). MS (ESI) *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>7</sub>H<sub>16</sub>NOS<sub>3</sub> 226.0; Found 226.0 [M + H]<sup>+</sup>, 248.0[M + Na]<sup>+</sup>.

**Isopropyl diethylcarbamo(dithioperoxo)thioate (79b).**<sup>[116]</sup> Yellow oil, 78% yield; R<sub>f</sub>=0.45 (hexane/CH<sub>2</sub>Cl<sub>2</sub> 1:1); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.16 - 1.36 (m, 12H, CH<sub>3</sub> x 4), 3.17 - 3.26 (m, 1H, SCH), 3.76 - 3.83 (m, 2H, NCH<sub>2</sub>), 3.83 - 3.92 (m, 2H, NCH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 11.47 (CH<sub>3</sub>), 13.16 (CH<sub>3</sub>), 22.12 (CH<sub>3</sub> x 2), 41.06 (SCH), 46.97 (NCH<sub>2</sub>), 51.64 (NCH<sub>2</sub>), 196.57 (C=S). MS (ESI) *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>7</sub>H<sub>16</sub>NOS<sub>3</sub> 224.1; Found 224.1 [M + H]<sup>+</sup>, 248.0 [M + Na]<sup>+</sup>.

**Hexyl diethylcarbamo(dithioperoxo)thioate (79c).**<sup>[116]</sup> Yellow oil, 85% yield; R<sub>f</sub>=0.32 (hexane/CH<sub>2</sub>Cl<sub>2</sub> 70:30); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.91 (t, *J* = 7.0, 3H, CH<sub>3</sub>), 1.25 - 1.53 (m, 12H, CH<sub>3</sub> x 2, CH<sub>2</sub> x 3), 1.62 - 1.81 (m, 2H, CH<sub>2</sub>), 2.82 - 2.98 (m, 2H, SCH<sub>2</sub>), 3.80 - 3.90 (m, 2H, NCH<sub>2</sub>), 4.08 - 4.15 (m, 2H, NCH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 11.15 (CH<sub>3</sub>), 12.93 (CH<sub>3</sub>), 13.80 (CH<sub>3</sub>), 21.97 (CH<sub>2</sub>), 27.47 (CH<sub>2</sub>), 27.83 (CH<sub>2</sub>), 30.82 (CH<sub>2</sub>), 38.00 (SCH<sub>2</sub>), 46.78 (NCH<sub>2</sub>), 51.08 (NCH<sub>2</sub>), 193.96 (C=S). MS (ESI) *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>7</sub>H<sub>16</sub>NOS<sub>3</sub> 266.1; Found 266.1 [M + H]<sup>+</sup>, 288.1 [M + Na]<sup>+</sup>.

**Cyclohexyl diethylcarbamo(dithioperoxo)thioate (79d).**<sup>[116]</sup> Yellow oil, 68% yield;  $R_f=0.58$  (hexane/ $\text{CH}_2\text{Cl}_2$  1:1);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 - 1.46 (m, 11H,  $\text{CH}_3 \times 2$ ,  $\text{CH}_2 \times 2$ , CH), 1.58 - 1.67 (m, 1H, CH), 1.74 - 1.89 (2H, m,  $\text{CH}_2$ ), 2.09 (d,  $J = 10.0$ , 2H,  $\text{CH}_2$ ), 3.00 - 3.10 (m, 1H, SCH), 3.80 - 3.95 (m, 2H,  $\text{NCH}_2$ ), 4.00 - 4.18 (m, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  11.50 ( $\text{CH}_3$ ), 13.16 ( $\text{CH}_3$ ), 25.71 ( $\text{CH}_2$ ), 25.83 ( $\text{CH}_2$ ), 32.35 ( $\text{CH}_2 \times 2$ ), 46.93 ( $\text{NCH}_2$ ), 49.13 (SCH), 51.64 ( $\text{NCH}_2$ ), 196.93 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{22}\text{NS}_3$  264.1; Found 264.1  $[\text{M} + \text{H}]^+$ , 286.1  $[\text{M} + \text{Na}]^+$ .

**2-Hydroxyethyl dibutylcarbamo(dithioperoxo)thioate (79e).** Yellow oil, 64 % yield;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.85 - 0.95 (m, 6H,  $\text{CH}_3 \times 2$ ), 1.23 - 1.41 (m, 4H,  $\text{CH}_2 \times 2$ ), 1.62 - 1.73 (4H, m,  $\text{CH}_2 \times 2$ ), 2.85 (m, 2H, SCH<sub>2</sub>), 3.50 (t,  $J = 7.3$ , 1H, OH), 3.59 - 3.76 (m, 4H,  $\text{CH}_2\text{OH}$ ,  $\text{NCH}_2$ ), 3.85 - 3.99 (m, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  13.67 ( $\text{CH}_3$ ), 13.77 ( $\text{CH}_3$ ), 20.06 ( $\text{CH}_2 \times 2$ ), 28.18 ( $\text{CH}_2$ ), 29.95 ( $\text{CH}_2$ ), 43.46 (SCH<sub>2</sub>), 52.95 ( $\text{CH}_2$ ), 57.72 ( $\text{CH}_2$ ), 58.24 ( $\text{CH}_2\text{OH}$ ), 197.32 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{24}\text{NOS}_3$  282.1; Found 282.1  $[\text{M} + \text{H}]^+$ , 304.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{24}\text{NOS}_3$  282.1015, Found 282.1015.

**Isopropyl dibutylcarbamo(dithioperoxo)thioate (79f).** Yellow oil, 68% yield;  $R_f=0.32$  (hexane/ $\text{CH}_2\text{Cl}_2$  70:30);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.86 - 1.04 (m, 6H,  $\text{CH}_3 \times 2$ ), 1.24 - 1.51 (m, 10H,  $\text{CH}_3 \times 2$ ,  $\text{CH}_2 \times 2$ ), 1.68 - 1.75 (m, 4H,  $\text{CH}_2 \times 2$ ), 3.20 - 3.32 (m, 1H, SCH), 3.85 - 3.66 (m, 2H,  $\text{NCH}_2$ ), 3.87 - 4.09 (m, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  13.71 ( $\text{CH}_3$ ), 13.81 ( $\text{CH}_3$ ), 20.08 ( $\text{CH}_2$ ), 22.11 ( $\text{CH}_3 \times 2$ ), 28.26 ( $\text{CH}_2$ ), 30.05 ( $\text{CH}_2$ ), 41.05 (SCH), 52.69 ( $\text{NCH}_2$ ), 57.08 ( $\text{NCH}_2$ ), 196.88 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{26}\text{NS}_3$  280.1; Found 280.1  $[\text{M} + \text{H}]^+$ , 302.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{26}\text{NS}_3$  280.1222, Found 280.1227.n

**Hexyl dibutylcarbamo(dithioperoxo)thioate (79g).** Orange oil, 80% yield;  $R_f=0.35$  (hexane/ $\text{CH}_2\text{Cl}_2$  70:30);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (t,  $J = 7.0$ , 3H,  $\text{CH}_3$ ), 0.95 - 1.05 (m, 6H,  $\text{CH}_3 \times 2$ ), 1.23 - 1.49 (m, 10H,  $\text{CH}_2 \times 5$ ), 1.60 - 1.84 (m, 6H,  $\text{CH}_2 \times 3$ ), 2.88 (t,  $J = 7.4$ , 2H, SCH<sub>2</sub>), 3.61 - 3.85 (m, 2H,  $\text{NCH}_2$ ), 3.90 - 4.19 (m, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  13.71 ( $\text{CH}_3$ ), 13.81 ( $\text{CH}_3$ ), 20.08 ( $\text{CH}_2$ ), 22.11 ( $\text{CH}_3 \times 2$ ), 28.26 ( $\text{CH}_2$ ), 30.05 ( $\text{CH}_2$ ), 41.05 (SCH), 52.69 ( $\text{NCH}_2$ ), 57.08 ( $\text{NCH}_2$ ), 196.88 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{15}\text{H}_{32}\text{NS}_3$  322.2; Found 322.2  $[\text{M} + \text{H}]^+$ , 344.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{15}\text{H}_{32}\text{NS}_3$  322.1691, Found 322.1687.

**Cyclohexyl dibutylcarbamo(dithioperoxo)thioate (79h).** Yellow oil, 80% yield;  $R_f=0.30$  (hexane/ $\text{CH}_2\text{Cl}_2$  70:30);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.93 - 1.08 (m, 6H,  $\text{CH}_3 \times 2$ ), 1.22 - 1.53 (m, 9H,  $\text{CH}_2 \times 4$ , CH), 1.58 - 1.67 (m, 1H, CH), 1.72 - 1.91 (m, 6H,  $\text{CH}_2 \times 3$ ), 2.05 - 2.16 (m, 2H,  $\text{CH}_2$ ), 3.00 - 3.12 (m, 1H, SCH), 3.65 - 3.88 (m, 2H,  $\text{NCH}_2$ ), 3.89 - 4.16 (m, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  13.71 ( $\text{CH}_3$ ), 13.81 ( $\text{CH}_3$ ), 20.12 ( $\text{CH}_2 \times 2$ ), 25.72 ( $\text{CH}_2$ ), 25.84 ( $\text{CH}_2 \times 2$ ), 28.29 ( $\text{CH}_2$ ), 30.04 ( $\text{CH}_2$ ), 32.36 ( $\text{CH}_2 \times 2$ ), 49.18 (SCH), 52.70 ( $\text{NCH}_2$ ), 57.14 ( $\text{NCH}_2$ ), 197.27 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{15}\text{H}_{30}\text{NS}_3$  320.2; Found 320.2  $[\text{M} + \text{H}]^+$ , 342.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{15}\text{H}_{30}\text{NS}_3$  322.1535, Found 322.1538.

**2-Hydroxyethyl pyrrolidine-1-carbo(dithioperoxo)thioate (79i).** Yellow oil, 60% yield;  $R_f=0.25$  ( $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.95 (quintet,  $J = 6.7$ , 2H,  $\text{CH}_2$ ), 2.08 (quintet,  $J = 6.8$ , 2H,  $\text{CH}_2$ ), 2.85 - 2.97 (m, 2H, SCH<sub>2</sub>), 3.61 - 3.79 (m, 5H, OH,  $\text{CH}_2\text{OH}$ ,  $\text{NCH}_2$ ), 3.91 (t,  $J = 7.0$ , 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  24.17 ( $\text{CH}_2$ ), 26.53 ( $\text{CH}_2$ ), 43.47 (SCH<sub>2</sub>), 50.81 ( $\text{NCH}_2$ ), 57.22 ( $\text{NCH}_2$ ), 58.20 ( $\text{CH}_2\text{OH}$ ), 193.87 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_7\text{H}_{14}\text{NOS}_3$  224.0; Found 224.0  $[\text{M} + \text{H}]^+$ , 246.0  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_7\text{H}_{14}\text{NOS}_3$  224.0232, Found 224.0234.

**Isopropyl pyrrolidine-1-carbo(dithioperoxo)thioate (79j).** Pale yellow solid, 90% yield;  $R_f=0.38$  (hexane/ $\text{CH}_2\text{Cl}_2$  60:40); m.p. 59-60°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.26 (d,  $J = 6.8$ , 6H,  $\text{CH}_3 \times 2$ ), 1.93 (quintet,  $J = 7.0$ , 2H,  $\text{CH}_2$ ), 2.05 (quintet,  $J = 6.8$ , 2H,  $\text{CH}_2$ ), 3.17 - 3.26 (m, 1H, SCH), 3.71 (t,  $J = 6.8$ , 2H,  $\text{NCH}_2$ ), 3.90 (t,  $J = 7.0$ , 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  22.16 ( $\text{CH}_3 \times 2$ ), 24.19 ( $\text{CH}_2$ ), 26.52 ( $\text{CH}_2$ ), 41.18 (SCH), 50.58 ( $\text{NCH}_2$ ), 56.68 ( $\text{NCH}_2$ ), 193.42 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_8\text{H}_{16}\text{NS}_3$  222.0; Found 222.0  $[\text{M} + \text{H}]^+$ , 244.0  $[\text{M} + \text{Na}]^+$ . Anal. calcd. for  $\text{C}_8\text{H}_{15}\text{NS}_3$  C, 43.40; H, 6.83; N, 6.32; S, 43.45. Found C, 43.39; H, 6.90; N, 6.31; S, 43.59.

**Hexyl pyrrolidine-1-carbo(dithioperoxo)thioate (79k).** Yellow oil, 85% yield;  $R_f=0.32$  (hexane/ $\text{CH}_2\text{Cl}_2$  70:30);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.90 (t,  $J = 7.0$ , 3H,  $\text{CH}_3$ ), 1.25 - 1.50 (m, 6H,  $\text{CH}_2 \times 3$ ), 1.65 - 1.74 (m, 2H,  $\text{CH}_2$ ), 2.02 (quintet,  $J = 6.8$ , 2H,  $\text{CH}_2$ ), 2.14 (quintet,  $J = 6.8$ , 2H,  $\text{CH}_2$ ), 2.80 - 3.00 (m, 2H, SCH<sub>2</sub>), 3.77 (t,  $J = 6.9$ , 2H,  $\text{NCH}_2$ ), 3.99 (t,  $J = 7.0$ , 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.01 ( $\text{CH}_3$ ), 22.51 ( $\text{CH}_2$ ), 24.19 ( $\text{CH}_2$ ), 26.30 ( $\text{CH}_2$ ), 26.51 ( $\text{CH}_2$ ), 28.24 ( $\text{CH}_2$ ), 28.60 ( $\text{CH}_2$ ), 31.39 ( $\text{CH}_2$ ), 38.72 (SCH<sub>2</sub>), 50.53 ( $\text{NCH}_2$ ), 56.62 ( $\text{NCH}_2$ ), 192.99 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{22}\text{NS}_3$  264.1; Found 264.1  $[\text{M} + \text{H}]^+$ , 286.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{22}\text{NS}_3$  264.0909, Found 264.0913.

**Cyclohexyl pyrrolidine-1-carbo(dithioperoxo)thioate (79l).**<sup>[116]</sup> Pale yellow solid, 95% yield;  $R_f=0.55$  (hexane/ $\text{CH}_2\text{Cl}_2$  1:1); m.p. 44-45°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.13 - 1.38 (m, 5H,  $\text{CH}_2 \times 2$ , CH), 1.49 - 1.60 (m, 1H, CH), 1.65 - 1.77 (m, 2H,  $\text{CH}_2$ ), 1.90 - 1.96 (m, 2H,  $\text{CH}_2$ ), 2.03 (m, 4H,  $\text{CH}_2 \times 2$ ), 2.96 (m, 1H, SCH), 3.71 (t,  $J = 6.9$ , 2H,  $\text{NCH}_2$ ), 3.90 (t,  $J = 7.0$ , 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  24.19 ( $\text{CH}_2$ ), 25.68 ( $\text{CH}_2$ ), 25.86 ( $\text{CH}_2 \times 2$ ), 26.53 ( $\text{CH}_2$ ), 32.39 ( $\text{CH}_2 \times 2$ ), 49.32 (SCH), 50.55 ( $\text{NCH}_2$ ), 56.71 ( $\text{NCH}_2$ ), 193.67 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{20}\text{NS}_3$  262.1; Found 262.1  $[\text{M} + \text{H}]^+$ , 284.1  $[\text{M} + \text{Na}]^+$ . Anal. calcd. for  $\text{C}_{11}\text{H}_{19}\text{NS}_3$  C, 50.53; H, 7.32; N, 5.35; S, 36.80. Found C, 50.44; H, 7.80; N, 5.40; S, 37.11.

**2-Hydroxyethyl piperidine-1-carbo(dithioperoxo)thioate (79m).** Pale brown solid, 65% yield;  $R_f=0.28$  ( $\text{CH}_2\text{Cl}_2/\text{AcOEt}$  95:5); m.p. 39-40°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.76 (s, 6H,  $\text{CH}_2 \times 3$ ), 2.90 - 3.01 (m, 2H,  $\text{SCH}_2$ ), 3.50 (t,  $J = 7.2$ , 1H, OH), 3.69 - 3.83 (m, 2H,  $\text{CH}_2\text{OH}$ ), 4.02 (bs, 2H  $\text{NCH}_2$ ), 4.36 (bs, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  24.09 ( $\text{CH}_2$ ), 25.48 ( $\text{CH}_2$ ), 26.32 ( $\text{CH}_2$ ), 43.44 ( $\text{SCH}_2$ ), 52.09 ( $\text{NCH}_2$ ), 55.99 ( $\text{NCH}_2$ ), 58.32 ( $\text{CH}_2\text{OH}$ ), 197.00 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_8\text{H}_{16}\text{NOS}_3$  238.0; Found 238.0  $[\text{M} + \text{H}]^+$ , 260.0  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_8\text{H}_{16}\text{NOS}_3$  238.0389, Found 238.0391.

**Isopropyl piperidine-1-carbo(dithioperoxo)thioate (79n).** Pale yellow solid, 87% yield;  $R_f=0.45$  (hexane/ $\text{CH}_2\text{Cl}_2$  1:1); m.p. 40-41°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.26 (d,  $J = 6.8$ , 6H,  $\text{CH}_3 \times 2$ ), 1.66 (s, 6H,  $\text{CH}_2 \times 2$ ), 3.17 - 3.26 (m, 1H, SCH), 3.96 (bs, 2H,  $\text{NCH}_2$ ), 4.23 (bs, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  22.14 ( $\text{CH}_3 \times 2$ ), 24.20 ( $\text{CH}_2$ ), 25.50 ( $\text{CH}_2$ ), 26.32 ( $\text{CH}_2$ ), 41.27 (SCH), 51.94 ( $\text{NCH}_2$ ), 55.37 ( $\text{NCH}_2$ ), 196.83 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_9\text{H}_{18}\text{NS}_3$  236.1; Found 236.1  $[\text{M} + \text{H}]^+$ , 258.1  $[\text{M} + \text{Na}]^+$ . Anal. Calcd. for  $\text{C}_9\text{H}_{17}\text{NS}_3$  C, 45.92; H, 7.28; N, 5.95; S, 40.85. Found C, 45.86; H, 7.14; N, 5.92; S, 40.67.

**Hexyl piperidine-1-carbo(dithioperoxo)thioate (79o).** Yellow oil, 80% yield;  $R_f=0.60$  (hexane/ $\text{CH}_2\text{Cl}_2$  1:1);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82 (t,  $J = 6.8$ , 3H,  $\text{CH}_3$ ), 1.16 - 1.29 (m, 4H,  $\text{CH}_2 \times 2$ ), 1.33 (m, 2H,  $\text{CH}_2$ ), 1.53 - 1.75 (m, 8H,  $\text{CH}_2 \times 4$ ), 2.79 (t,  $J = 7.4$ , 2H,  $\text{SCH}_2$ ), 3.91 (bs, 2H,  $\text{NCH}_2$ ), 4.23 (bs, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.01 ( $\text{CH}_3$ ), 22.51 ( $\text{CH}_2$ ), 24.18 ( $\text{CH}_2$ ), 25.44 ( $\text{CH}_2$ ), 26.28 ( $\text{CH}_2$ ), 28.25 ( $\text{CH}_2$ ), 28.56 ( $\text{CH}_2$ ), 31.39 ( $\text{CH}_2$ ), 38.84 ( $\text{SCH}_2$ ), 51.83 ( $\text{NCH}_2$ ), 55.17 ( $\text{NCH}_2$ ), 196.41 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{24}\text{NS}_3$  278.1; Found 278.1  $[\text{M} + \text{H}]^+$ , 300.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{24}\text{NS}_3$  278.1065, Found 278.1069.

**Cyclohexyl piperidine-1-carbo(dithioperoxo)thioate (79p).**<sup>[116]</sup> Pale yellow solid, 90% yield;  $R_f=0.55$  (hexane/ $\text{CH}_2\text{Cl}_2$  1:1); m.p. 60-61 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 - 1.50 (m, 5H,  $\text{CH}_2 \times 2$ , CH), 1.58 - 1.65 (m, 1H, CH), 1.68 - 1.89 (m, 8H,  $\text{CH}_2 \times 4$ ), 2.03 - 2.13 (m, 2H,  $\text{CH}_2$ ), 3.05 (m, 1H, SCH), 4.05 (bs, 2H,  $\text{NCH}_2$ ), 4.35 (bs, 2H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  24.20 ( $\text{CH}_2$ ), 25.42 ( $\text{CH}_2$ ), 25.70 ( $\text{CH}_2$ ), 25.86 ( $\text{CH}_2 \times 2$ ), 26.21 ( $\text{CH}_2$ ), 32.36 ( $\text{CH}_2 \times 2$ ), 49.37 (SCH), 51.87 ( $\text{NCH}_2$ ), 55.30 ( $\text{NCH}_2$ ), 197.12 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{22}\text{NS}_3$  276.1; Found 276.1  $[\text{M} + \text{H}]^+$ , 298.1  $[\text{M} + \text{Na}]^+$ . Anal. Calcd. for  $\text{C}_{12}\text{H}_{21}\text{NS}_3$  C, 52.32; H, 7.68; N, 5.08; S, 34.92. Found C, 52.19; H, 8.13; N, 5.10; S, 35.23.

**2-Hydroxyethyl morpholine-1-carbo(dithioperoxo)thioate (79q).** Brown solid, 25% yield;  $R_f=0.45$  (AcOEt); m.p. 58-59 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  2.92 - 3.05 (m, 2H,  $\text{SCH}_2$ ), 3.32 (t,  $J = 7.1$ , 1H, OH), 3.73 - 3.93 (m, 6H,  $\text{CH}_2\text{OH}$ ,  $\text{OCH}_2 \times 2$ ), 3.97 - 4.65 (m, 4H,  $\text{NCH}_2 \times 2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  43.28 ( $\text{SCH}_2$ ), 58.54 ( $\text{CH}_2\text{OH}$ ), 66.27 ( $\text{OCH}_2 \times 2$ ), 198.83 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_7\text{H}_{14}\text{NO}_2\text{S}_3$  240.0; Found 240.0  $[\text{M} + \text{H}]^+$ , 262.0  $[\text{M} + \text{Na}]^+$ . Anal. Calcd. for  $\text{C}_7\text{H}_{13}\text{NO}_2\text{S}_3$  C, 35.15; H, 5.47; N, 5.85; S, 40.18. Found C, 35.14; H, 5.48; N, 5.76; S, 39.82.

**Isopropyl morpholine-1-carbo(dithioperoxo)thioate (79r).** Pale yellow solid, 75% yield;  $R_f=0.38$  ( $\text{CH}_2\text{Cl}_2$ ); m.p. 47-48 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.26 (d,  $J = 6.8$ , 6H,  $\text{CH}_3$ ), 3.17 - 3.27 (m, 1H,  $\text{SCH}_2$ ), 3.66 - 3.78 (m, 4H,  $\text{OCH}_2$ ), 4.16 (bs, 4H,  $\text{NCH}_2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  22.17 ( $\text{CH}_3 \times 2$ ), 41.39 (SCH), 66.34 ( $\text{OCH}_2 \times 2$ ), 198.69 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_8\text{H}_{16}\text{NOS}_3$  238.0; Found 238.0  $[\text{M} + \text{H}]^+$ , 260.0  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_8\text{H}_{16}\text{NOS}_3$  238.0389, Found 238.0391.

**Hexyl morpholine-1-carbo(dithioperoxo)thioate (79s).** Yellow oil, 85% yield;  $R_f=0.40$  ( $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (t,  $J = 6.9$ , 3H,  $\text{CH}_3$ ), 1.21 - 1.52 (m, 6H,  $\text{CH}_2 \times 3$ ), 1.63 - 1.75 (m, 2H,  $\text{CH}_2$ ), 2.84 - 3.00 (m, 2H,  $\text{SCH}_2$ ), 3.68 - 3.92 (m, 4H,  $\text{OCH}_2 \times 2$ ), 4.22 (bs, 4H,  $\text{NCH}_2 \times 2$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.00 ( $\text{CH}_3$ ), 22.50 ( $\text{CH}_2$ ), 28.23 ( $\text{CH}_2$ ), 28.59 ( $\text{CH}_2$ ), 31.37 ( $\text{CH}_2$ ), 38.79 ( $\text{CH}_2$ ), 66.31 ( $\text{OCH}_2 \times 2$ ), 198.24 (C=S). MS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{22}\text{NOS}_3$  280.1; Found 280.1  $[\text{M} + \text{H}]^+$ , 302.1  $[\text{M} + \text{Na}]^+$ . HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{11}\text{H}_{22}\text{NOS}_3$  280.0858, Found 280.0862.

**Cyclohexyl morpholine-1-carbo(dithioperoxo)thioate (79t).** Pale yellow solid, 90% yield;  $R_f=0.42$  ( $\text{CH}_2\text{Cl}_2$ ); m.p. 46-47 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.21 - 1.50 (m, 5H,  $\text{CH}_2 \times 2$ , CH), 1.59 - 1.70 (m, 1H, CH), 1.72 - 1.88 (m, 2H,  $\text{CH}_2$ ), 2.04 - 2.12 (m, 2H,  $\text{CH}_2$ ), 3.03 - 3.09 (m, 1H, SCH), 3.93 - 3.71 (m, 4H,  $\text{NCH}_2 \times 2$ ), 4.00 - 4.55 (m, 4H,  $\text{OCH}_2 \times 2$ ).  $^{13}\text{C}$  NMR (126

MHz, CDCl<sub>3</sub>)  $\delta$  25.64 (CH<sub>2</sub>), 25.85 (CH<sub>2</sub>), 32.39 (CH<sub>2</sub> x 2), 49.50 (SCH), 66.33 (OCH<sub>2</sub> x 2), 198.96 (C=S). MS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>11</sub>H<sub>20</sub>NOS<sub>3</sub> 278.1; Found 278.1 [M + H]<sup>+</sup>, 300.1 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>11</sub>H<sub>19</sub>NOS<sub>3</sub> C, 47.62; H, 6.90; N, 5.05; S, 34.67. Found C, 47.82; H, 7.10; N, 5.02; S, 34.85.

**2-Hydroxyethyl 4-methylpiperazine-1-carbo(dithioperoxo)thioate (79u).** Yellow oil, 30% yield; R<sub>f</sub>=0.20 (AcOEt); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  2.36 (s, 3H, NCH<sub>3</sub>), 2.55 (s, 4H, NCH<sub>2</sub> x 2), 2.89 - 3.05 (m, 2H, SCH<sub>2</sub>), 3.41 (bs, 1H, OH), 3.83 - 3.69 (m, 2H, CH<sub>2</sub>OH), 4.08 (bs, 2H, NCH<sub>2</sub>), 4.42 (bs, 2H, NCH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  43.33 (SCH<sub>2</sub>), 45.56 (NCH<sub>3</sub>), 54.43 (NCH<sub>2</sub> x 2), 58.45 (CH<sub>2</sub>OH), 198.25 (C=S). MS (ESI)  $m/z$ : [M + H]<sup>+</sup> calcd for C<sub>8</sub>H<sub>17</sub>N<sub>2</sub>OS<sub>3</sub> 253.0; Found 253.0 [M + H]<sup>+</sup>, 275.0 [M + Na]<sup>+</sup>. HRMS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>8</sub>H<sub>17</sub>N<sub>2</sub>OS<sub>3</sub> 253.0498, Found 253.0501.

**Isopropyl 4-methylpiperazine-1-carbo(dithioperoxo)thioate (79v).** Yellow oil, 76% yield; R<sub>f</sub>=0.33 (AcOEt); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.34 (d,  $J$  = 6.8, 6H, CH<sub>3</sub> x 2), 2.35 (s, 3H, NCH<sub>3</sub>), 2.60 - 2.49 (m, 4H, NCH<sub>2</sub> x 2), 3.25 - 3.37 (m, 1H, SCH<sub>2</sub>), 4.10 (s, 2H, broad, NCH<sub>2</sub>), 4.40 (s, 2H, broad, NCH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  22.15 (CH<sub>3</sub> x 2), 41.33 (SCH), 45.61 (NCH<sub>3</sub>), 54.49 (NCH<sub>2</sub> x 2), 198.07 (C=S). MS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>9</sub>H<sub>19</sub>N<sub>2</sub>S<sub>3</sub> 251.1; Found 251.1 [M + H]<sup>+</sup>, 273.1 [M + Na]<sup>+</sup>. HRMS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>9</sub>H<sub>19</sub>N<sub>2</sub>S<sub>3</sub> 251.0705, Found 253.0701.

**Hexyl 4-methylpiperazine-1-carbo(dithioperoxo)thioate (79w).** Yellow oil, 70% yield; R<sub>f</sub>=0.42 (AcOEt); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.91 (t,  $J$  = 6.9, 3H, CH<sub>3</sub>), 1.23 - 1.52 (m, 6H, CH<sub>2</sub> x 3), 1.64 - 1.70 (m, 2H, CH<sub>2</sub>), 2.35 (s, 3H, NCH<sub>3</sub>), 2.44 - 2.69 (m, 4H, NCH<sub>2</sub> x 2), 2.82 - 3.02 (m, 2H, SCH<sub>2</sub>), 3.95 - 4.50 (bs, 4H, NCH<sub>2</sub> x 2). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  14.01 (CH<sub>3</sub>), 22.50 (CH<sub>2</sub>), 28.23 (CH<sub>2</sub>), 28.60 (CH<sub>2</sub>), 31.37 (CH<sub>2</sub>), 38.79 (CH<sub>2</sub>), 45.39 (NCH<sub>3</sub>), 54.40 (NCH<sub>2</sub> x 2), 197.67 (C=S). MS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>12</sub>H<sub>25</sub>N<sub>2</sub>S<sub>3</sub> 293.1; Found 293.1 [M + H]<sup>+</sup>, 315.1 [M + Na]<sup>+</sup>. HRMS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for C<sub>12</sub>H<sub>25</sub>N<sub>2</sub>S<sub>3</sub> 293.1174, Found 293.1171.

**Cyclohexyl 4-methylpiperazine-1-carbo(dithioperoxo)thioate (79x).** Yellow oil, 75% yield; R<sub>f</sub>=0.45 (AcOEt); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.21 - 1.48 (m, 5H, CH<sub>2</sub> x 2, CH), 1.58 - 1.63 (m, 1H, CH), 1.73 - 1.86 (m, 2H, CH<sub>2</sub>), 2.00 - 2.14 (m, 2H, CH<sub>2</sub>), 2.35 (s, 3H, NCH<sub>3</sub>), 2.49 - 2.61 (m, 4H, NCH<sub>2</sub> x 2), 3.05 (m, 1H, SCH<sub>2</sub>), 4.00 - 4.50 (bs, 4H, NCH<sub>2</sub> x 2). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  25.67 (CH<sub>2</sub>), 25.85 (CH<sub>2</sub> x 2), 32.38 (CH<sub>2</sub> x 2), 45.62 (NCH<sub>3</sub>), 49.43 (SCH), 54.49 (NCH<sub>2</sub> x 2), 198.37 (C=S). MS (ESI)  $m/z$ : [M + H]<sup>+</sup> Calcd for

$C_{12}H_{23}N_2S_3$  291.1; Found 291.1  $[M + H]^+$ , 313.1  $[M + Na]^+$ . HRMS (ESI)  $m/z$ :  $[M + H]^+$   
Calcd for  $C_{12}H_{23}N_2S_3$  291.1018, Found 291.1014.

## 6.4 Central Nervous System drug candidates: Biology

Polyclonal anti-mGlu4 receptor and monoclonal anti-GRK2/3 (clone C5/1) were purchased from Upstate Biotechnology (Lake Placid, NY); polyclonal anti-ERK1/2 were from Santa Cruz Biotechnology (Santa Cruz, CA); monoclonal anti-phospho-ERK1/2 was from Cell Signaling Technology (Beverly, MA); L-2-Amino-4-phosphonobutanoate (L-AP4) was purchased from Tocris Cookson (Bristol, UK); all other drugs were purchased from Sigma-Aldrich (Milan, Italy); mGlu4 receptor and GFP-mGlu4 receptor cDNA were kindly provided by J. P. Pin (Montpellier, France).

**Cell Culture and Transfection.** Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin, 100  $\mu$ g/ml streptomycin). Cells were transfected in 10-mm Falcon dishes using 8  $\mu$ l of LipofectAMINE2000 (Invitrogen, Carlsbad, CA) in OptiMEM medium, and 10  $\mu$ g of cDNA for 4 h. The cells used for determination of cAMP were cotransfected with 2.5  $\mu$ g/dish of adenylyl cyclase type V cDNA. One day later, cells were split into six-well dishes previously coated with poly(L-lysine) (0.01%), and the experiments were performed 72 h after transfection.

**Immunoblotting.** At the end of the final incubation, cells were rapidly rinsed in ice-cold PBS and solubilized in Triton X-lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10  $\mu$ g/ml leupeptin, 10  $\mu$ g/ml aprotinin, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM  $\beta$ -glycerophosphate). Protein cell lysates (80  $\mu$ g) were separated by SDS-PAGE electrophoresis, blotted onto nitrocellulose, and probed using specific antibodies. The antibodies were used at the following dilution: anti-phospho-ERK1/2, 1:1000; anti-ERK1/2, 1:2000; anti-mGlu4 receptor. The immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) using horseradish peroxidase-conjugated secondary antibodies.

**cAMP Assay.** Cultures were incubated in Hanks' balanced salt solution buffer, pH 7.4, containing 0.5 mM 3-isobutyl-1-methylxanthine. L-AP4 (or vehicle) was added 10 min before forskolin (FSK) stimulation (10 mM). After 20 min, the reaction was stopped by substituting the buffer with ice-cold ethanol. Extraction and measurement of cAMP was carried out as described previously.<sup>[124]</sup>

## **6.5 Potential antileukemic agents: Biology**

Cell growth inhibition studies were carried out according to protocols on 12 hematological cell lines: 1 derived from ALL (MOLT-4), 9 from AML (HL-60, KG-1, KG-1a, MOLM-13, MV4-11, NOMO-1, OCI-AML2, PL-21, THP-1) and 2 from CML (K-562, KCL-22). Dovitinib, Quizartinib (AC220) and TCS 359, which are described to selectively target FLT3, were used as reference drugs. Proliferation, viability and cytotoxicity assays were conducted using Promega's CellTiter-Blue Viability Assay. Compounds were tested at 10 concentrations in duplicates in half-log increments up to 100  $\mu$ M. If an  $IC_{50}$  value could not be determined within the examined dose range (because a compound was either too active or lacked activity), the lowest or highest concentration studied was used for calculation of the geometric mean  $IC_{50}$  value. Results were presented as  $IC_{50}$  heatmap presentation.

## **6.6 Breast cancer drug candidates: Biology**

Cell growth inhibition studies were carried out according to published protocols.<sup>[125]</sup> To account for different growth rates, cells were seeded in black 96 well plates at densities which provided 70% confluency over 72 hours. After 24 hours, cells were treated with the indicated concentration of analogues (dissolved to 10 mM in DMSO) or diluent control for 72 hours. Viability studies were then conducted using the CellTiter Blue assay (Promega, Southampton, UK) according to manufacturer's protocol. Experiments were run on three separate occasions, and mean  $IC_{50}$  values are reported.

## 6.7 Potential antileukemic agents: Molecular Modelling

Ligands 2D structure were built using ChemDraw 7.0, then imported in Maestro v. 10.1 and energy minimized with OPLS\_2005 force field generating possible state of ionization using Epik v. 3.1 (pH  $7.35 \pm 0.05$ ). The FLT3 receptor structure was prepared from the original PDB file (pdb: 4RT7; Resolution: 3.1 Å) by using Maestro. Docking calculations were performed using the “Extra Precision” (XP) mode of Glide 6.6 program. The number of docking runs per ligand was set to 30. The reliability of the docking protocol was tested by retrieving the binding mode of quizartinib as cocrystallized in the 4RT7, giving a minor deviation from the crystallographic resolved position (RMSD= 0.899 Å). Homology models of FLT3 active state was built using the c-kit crystal structure (pdb: 1PKG; Resolution: 2.1 Å) as template (identity percentage = 58% ). The structure alignment was manually adjusted to remove gaps in helices. Highly conserved residues were anchored, and the models were generated in Prime v. 3.9. Some representations were prepared via PyMOL v. 1.6.

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