Synthesis of new heteropolycyclic compounds with potential antitumor activity

Preparació de nous compostos fenòlics i derivats amb potencial activitat antitumoral

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SYNTHESIS OF NEW HETEROPOLYCYCLIC COMPOUNDS
WITH POTENTIAL ANTITUMOR ACTIVITY.
(PREPARACIÓ DE NOUS COMPOSTOS FENÓLICS I
DERIVATS AMB POTENCIAL ACTIVITAT ANTITUMORAL).

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UNIVERSITAT DE BARCELONA

SYNTHESIS OF NEW HETEROPOLYCYCLIC COMPOUNDS WITH POTENTIAL ANTITUMOR
ACTIVITY

Doctoral thesis submitted by Richard Soucek, Master's degree in Chemistry to obtain the PhD degree by the university of Barcelona.

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Barcelona, 28 of june 2013.

Thesis Supervisor: PhD student:

Dra. M. Dolors Pujol Dilmé   **Richard Soucek**

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Table of content

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Preparació de nous compostos fenólics i derivats amb potencial activitat antitumoral

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Abbreviations

ν : frequence

δ: chemical shift

(E): *engegen* (Trans conformation)

μg: microgram

(Z): Z-isomer (CIS conformation)

^{13}C NMR: carbon Nuclear Magnetic Resonnance

^{1}H NMR: proton Nuclear Magnetic Resonance

 Ac: acetyl

AIBN: α,α'-azoisobutironitrile

APJ: Apelin Receptor

Ar: aromatic

ATP: adenosine triphosphate

BINAP: 2,2'-*bis*(diphenylphosphino)-1,1'-binaphthyl

Bn: benzyl

Bu: butyl

BuLi: butyl lithium

cAMP: cycline adenosine monophosphate

cat: catalyst

CGRP: Calcitonin Gene-Related Peptide

CDK: Cyclin-Dependent Kinases

COSY: correlation spectroscopy

CSA: (+)-Camphor Sulfonic Acid

d: doublet

DBU: 1,8-diazabiciclo[5.4.0]undec-7-ene

DCC: diciclohexylcarbodiimide

DCM: dichloromethane

dd: doublet doublet

DEPT: Distortionless Enhancement by Polarization Transfer

DMF: dimethylformamide

DMSO: dimethylsulfoxyde

DNA: deoxyribonucleic acid

dt: doublet triplet

EGF: epidermial Growth Factor

EI: electronic Impact

eq: equivalent

ESI: electrospray

Et: ethyl

EtOAc: ethyl acetate

FGF: Fibroblast Growth Factor

g: gram

GDP: guanosine diphosphate

GEFs: guanine nucleotide exchange factors

Glucagon-like peptide 1 (GLP-1)

GPCRs: G protein-coupled receptors

GTP: guanosine triphosphate

h: hour

HIV: Human Immunodeficiency Virus

HOBt: hydroxybenzotriazole

HSQC: Heteronuclear Single Quantum Coherence

Hz: hertz

IC_{50}: half maximal inhibitory concentration

Inhib: inhibition

IR: infrared

J: coupling constant

K-Ras (or Ras): kirsten rat sarcoma

LDA: Lithium diisopropylamide

m/z: mass-to-charge ratio

m: multiplet

mCPBA: metachloroperbenzoic acid

Me: methyl

MeOH: methanol

mg: milligram

mGluR: metabotropic Glutamate Receptors

MHz: mega hertz

min: minute

mL: millilitre

mmol: millimole

mp.: melting point

MS: mass spectroscopy

N: normal aqueous solution

NBS: *N*-bromosuccinimide

NCS: *N*-chlorosuccinimide

ng: nanogram

NMP: *N*-metilpirrolidona

NMR: Nuclear Magnetic Resonnance

o/n: overnight

Pd/C: palladium on charcoal

PDGF: Platelet Derived Growth Factor

ppm: parts per million

PTSA: *p*-toluensulfònic acid

q: quadruplet

qt: quintuplet

Ras (or K-Ras): rat sarcoma

RBF: round bottom flask

R_f: retention factor

RMN-^{1}H: Proton Nuclear Magnetic Resonnance

RNA: ribonucleic acid

rt: room temperature

s: singlet

SAR: Structure Activity Relationship

sext: sextuplet

SM: starting material

Stim: stimulation

TLC: Thin Layer Chromatography

t: triplet

t-BuOK: potassium tertiary butoxyde

Tf: triflate

TFA: trifluoroacetic acid

TFAA: trifluoroacetic acid anydride

THF: tetrahydrofuran

TPI: Tubulin Polymerization Inhibition.

UV: Ultraviolet

VEGF: Vascular Endothelial Growth Factor

1. Introduction

1. Introduction

1.1. Cancer

1.1.1. Definition of cancer

Cancer represents a broad group of various diseases, all involving unregulated cell growth. Cells divide and grow uncontrollably, forming malignant tumors and invading nearby parts of the body through metastasis.[1] There are more than 100 different types of cancer. Most of them are named for the organ or type of cell in which they start. For example, cancers that begin in the colon are called colon cancer, cancers that begin in basal cells of the skin are called basal cell carcinoma. The main categories of cancer include:

- Carcinoma - cancer that begins in the skin or in tissues that line or cover internal organs.
- Sarcoma - cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other cconnective or supportive tissue.
- Leukemia - cancer that starts in blood-forming tissues such as the bone marrow and ccauses large numbers of abnormal blood cells to be produced and enter the blood.
- Lymphoma and myeloma - cancers that begin in the cells of the immune system.
- Central nervous system cancers - cancers that begin in the tissues of the brain and sspinal cord.[2]

1.1.2. Endogenous and exogenous factors: limits to cancer prevention

Most cancer cases comes from exogenous factors (90-95%). Few comes from endogenous factors (5-10%).[3] The exogenous factors include:

- Diet, physical unactivity and obesity (30-35% of cancer cases).[4, 5, 6, 7]

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[1] http://www.cancerresearchuk.org/cancer-help/about-cancer/.
[2] http://www.cancer.gov/cancertopics/types/alphalist.
[3] P. Anand, A. B. Kunnumakkara. *Pharm. Res*. **2008**, *25*, 2097-2116.
[4] L. H. Kushi, T. Byers, C. Doyle. *Cancer. J. Clin.* **2006**, *56*, 254-281.
[5] H. Brenner, D. Rothenbacher, V. Arndt. *Epidemiology of stomach cancer*. Edited by Mukesh Verna, Humana press. **2009**, *472*, 467-477.
[6] I. Lee, Y. Oguma, D. Schottenfeld, J. F. Fraumeni. *Cancer Epidemiology and Prevention*. 3rd ed. New York Oxford University Press **2006**. Chap. 1.
[7] F. Bianchini, R. Kaaks, H. Vainio. *IARC Handbooks of Cancer Prevention* **2002**, *6*, 5-8.

- Chemical exposure (25-30% of cancer cases).[7] This includes tobacco smoking[8,9, 10,] (one in three of all cancer death is the develloped world),[11] alcohol[12] (3.5% of cancer deaths)[13] and exposure to carcinogenic substances in one's workplace or occupation (200,000 death per year).[14]

- Infections[15] (18% of cancer cases)[7] from viruses (herpes, hepatitis or human papillomaviruses), bacterias[16,17] or parasites.[18]

- Radiations[1, 19] (10% of cancer cases).[20]

- Aging. One of the principal cancer risk factors is aging. Two third of cancer happens after 65 years old, mostly due to the accumulation of nonlethal DNA mutation that is passed on to subsequent cell divisions.

The endogenous factors are due to an inherited genetic defect that can trigger cancer. The most common types of cancer that can be genetically transmitted include breast, colorectal, gynecologic and endocrine cancers.[21] For example, mutations in the genes *BRCA1* and *BRCA2* cause 75% more risk of breast cancer and ovarian cancer.[22] Hormones are also an important endogenous factor in human cancer. The available epidemiological evidence suggests a hormonal role in the pathogenesis of testis, thyroid and breast cancer.

[8] H. K. Biesalski, B. Bueno de Mesquita, A. Chesson. *Cancer J. Clin.* **1998**, *3*, 167-176.

[9] S. Dubey, C. A. Powell. *Am. J. Respir. Crit. Care Med*. **2007**, *9*, 941-946.

[10] H. Kuper, P. Boffetta, H. Adami. *J. Int. Med.* **2002**, *3*, 206-224.

[11] H. Kuper, P. Boffetta, H. Adami. *J. Int. Med.* **2002**, *6*, 455-466.

[12] A. Benedetti, M. Parent, J. Siemiatycki. *Canc. Det. Prev.* **2009**, *32*, 352-362.

[13] P. Boffetta, M. Hashibe, C. La Vecchia, W. Zatonski, J. Rehm. *Int. J. Cancer* **2006***, 4*, 884-887.

[14] *WHO calls for prevention of cancer through healthy workplaces*. World Health Organization **2007**.

[15] H. Zur Hausen. *Science* **1991**, *5035*, 1167-1173.

[16] S. Peter, C. Beglinger. *Digestion* **2007**, *1*, 25-35.

[17] C. Wang, Y. Yuan, R. Hunt. *Gastroenterol.* **2007**, *8*, 1789-1798.

[18] R. C. Bast, D. W. Kufe, R. E. Pollock. *Cancer Med.* **2000**.

[19] R. C. Bast, D. W. Kufe, R. Weichselbaum, J. F. Holland, E. Frei. *Holland-Frei Cancer. Med*. Edited by BC Decker. 5th edition. **2003**. Chap 14.

[20] http://www.ehrs.upenn.edu/programs/radiation/nonionizing_faq.html.

[21] http://www.mdanderson.org/patient-and-cancer-information/cancer-information/cancer-topics/prevention-and-screening/hereditary-cancer-syndromes/index.html.

[22] D. H. Roukos. *Expert. Rev. Anticanc. Therap.* **2009**, *4*, 389-392.

1.1.3. Caracteristics of tumor cells

The cancer pathology is triggered by qualitative or quantitative gene modification. Three types of genes are associated to cancer pathologies:

- *Oncogenes*: They are positive regulators of cell proliferation. When excessively expressed, they trigger tissue proliferation, forming a tumor (Figure 1). There are about 100 different known oncogenes.
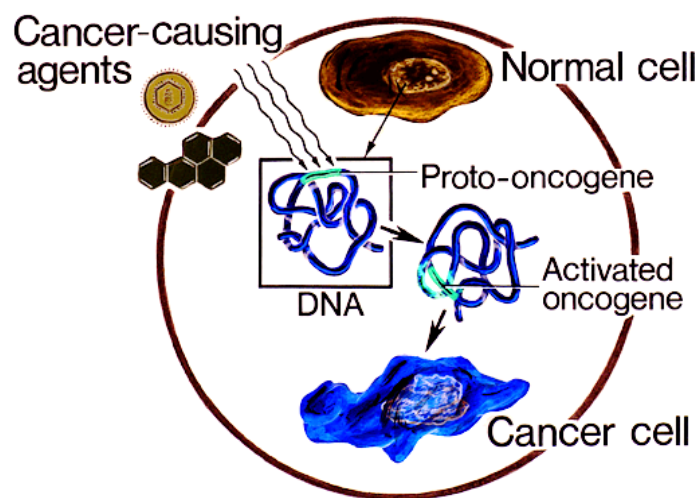
Figure 1. Oncogene formation[23]

- *Tumor suppressor genes*: They are negative cell regulators that inhibit cell proliferation. If those genes are inactivated, cell proliferation occurs. Inactivation of the tumor suppressor gene p53 greatly increases the risk of several types of cancers. p53 gene involvement in tumors is more frequent than any other known tumor suppressor or dominant proto-oncogene.[19]
- *DNA repair genes*: They detect and repair DNA lesions. Inactivation of these genes results in more DNA abnormality and a higher risk of develloping cancer.

Cancer is a multigenic pathology. Each cancer is triggered by the alteration of about 10-20 genes. These alterations occur in a succesive way, each one favouring the apparition of the next one. What happens is a chronology of alteration. At some point a healthy cell gets a non detected genetic damage that favours its uncontrolled replication. This geneticaly modified cell

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[23]http://www.sekmchd.org/cms/Services/ChronicDiseaseControl/Cancer/PancreaticCancerFAQs/tabid/1340/Default.aspx. 27/06/2013

has a selective advantage and a higher risk to contract other gene modifications. Thus, there is an accumulation of growth favouring gene mutations that lead to a highly cancerigenic cell (Figure 2).
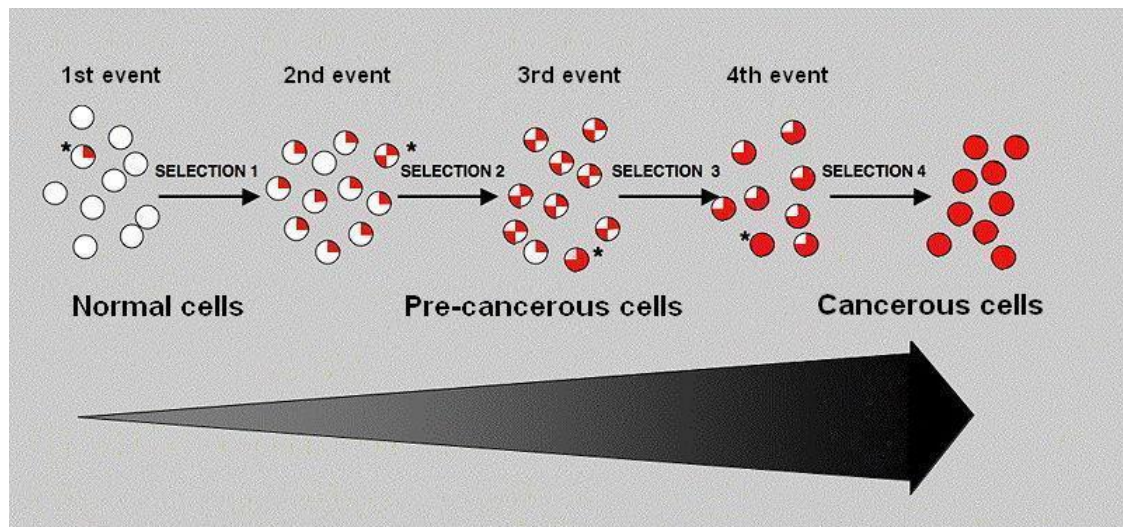
Figure 2. Chronology of alteration of normal cells

Finally, The tissue looses its life and death balance system. Due to inactivation of the tumor suppressor and DNA repair genes, the cancerigenic cells don't die of apoptosis, don't repare their DNA and begin to proliferate. Then, depending on the environment, the tumor can evolve or stop growing. If the environment is not favorable, the tumor doesn't receive anymore nutrients or blood vessels to grow. If the environment is cooperative, the tumor receives what it needs to grow. It stimulates blood vessel formations by angiogenesis, links itself to the bloodstream and become invasive by metastasis (Figure 3 and 4). Depending on the type of cancer, some specific genes are altered and others not.[24,25]

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[24] A. G. Knudson. *Nat. Rev.* **2001**, *2*, 157-162.
[25] C. M. Croce. *New. Eng. J. Med.* **2008**, *5*, 502-511.
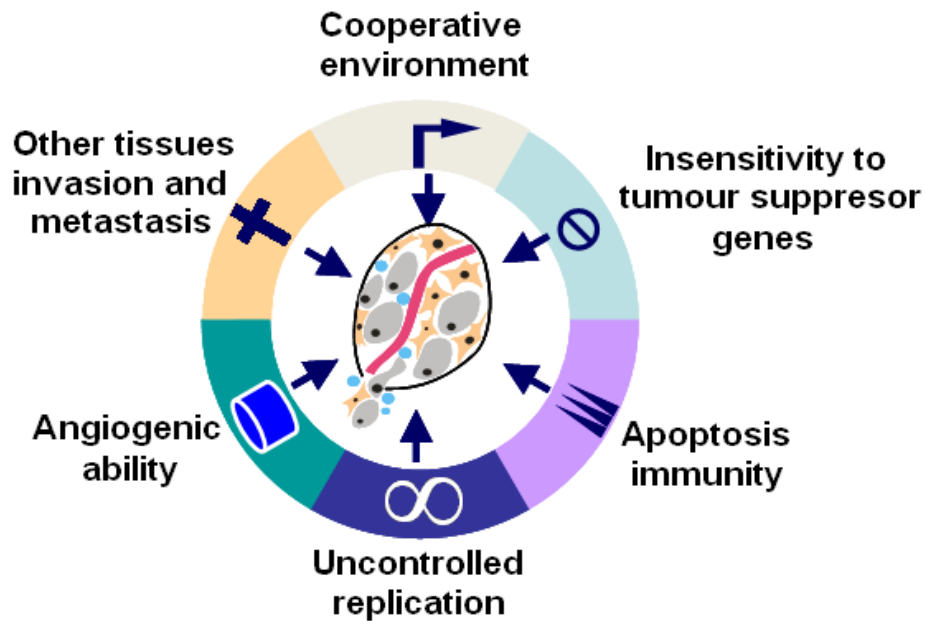
Figure 3. Cancer cells caracteristics
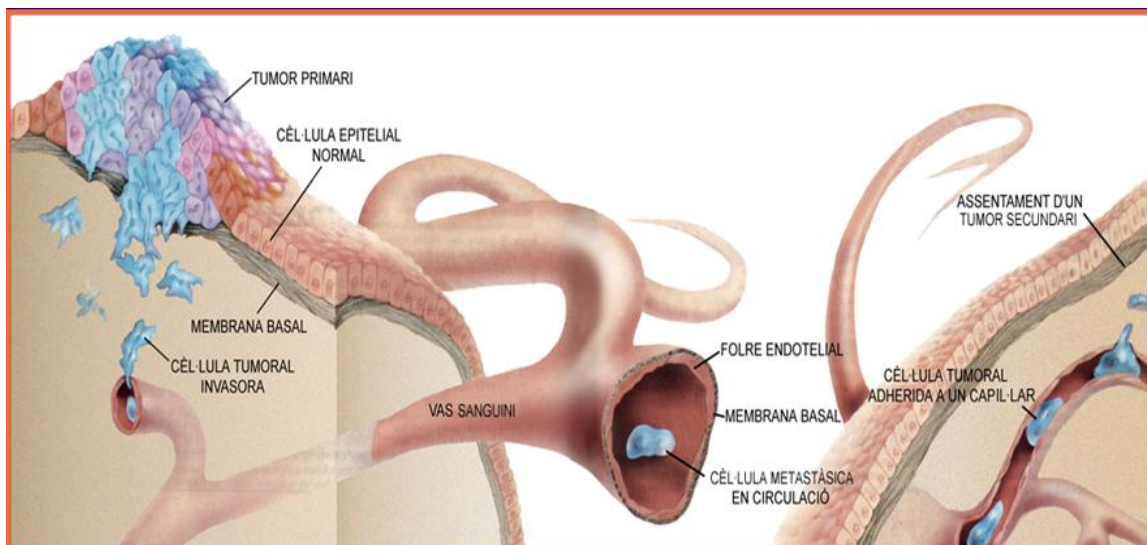
Figure 4. Metastasis of a malignous tumor

1.2. Impact and prevalence

In 2008, 12.7 million cancers were diagnosed and 7.6 million people died of cancer, which is 13% of the annual death proportion.[26] It is the second death cause in the developed world and the third death cause in the developing world. The most commun cancers are lung cancer,

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[26] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, D. Forman. *A Cancer. J. Clinic.* **2011**, 61-65.

stomach cancer, liver cancer, colorectal cancer and breast cancer. The deadliest cancers are lung cancer, colorectal cancer, breast cancer, prostate cancer and pancreatic cancer (Figure 5).[27]
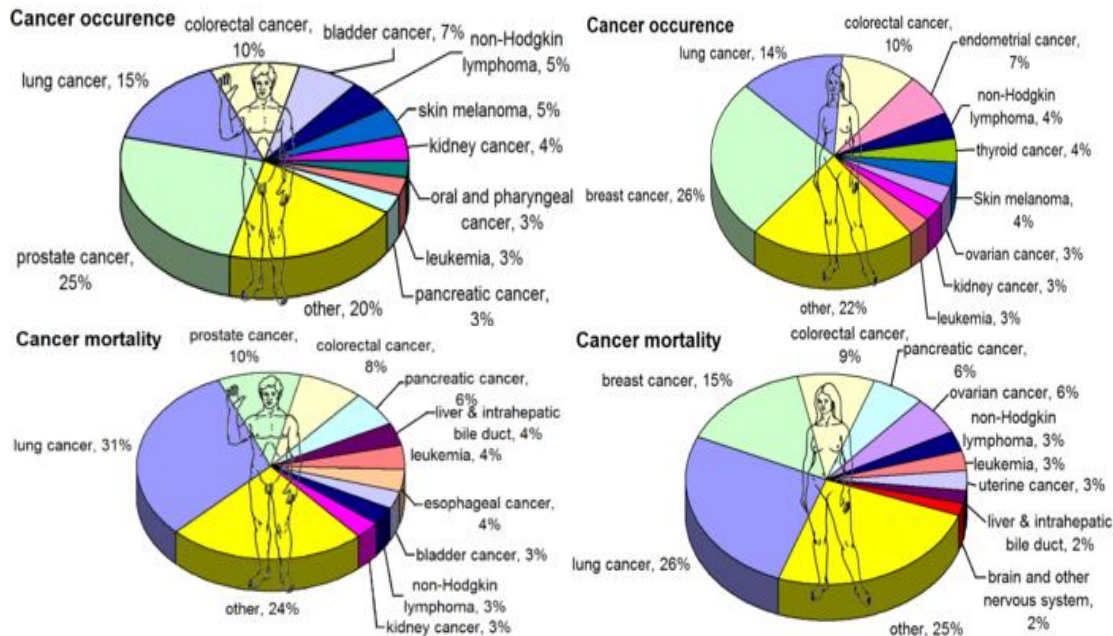
Figure 5. Cancer occurrence and mortality in US male and female, by occurrence[3]

The most commun childhood cancers are leukemia (34%), brain tumor (23%) and lymphomas (12%). Rate of childhood cancer has increased by 1.1% per year between 1978 and 1997 in Europe.[28]

1.3. Cancer treatment

1.3.1. Cancer staging

Cancer staging is the determination of the extent the cancer has spread. It is necessary to stage cancer to determine which type of treatment will receive the patient. They are 5 cancer levels from stage 0, which is a precursor form of cancer, to stage IV, which is metastasis formation (Figure 6).

[27] A. Jemal, R. Siegel, E. Ward. *Cancer. J. Clin.* **2008**, *2*, 71-96.
[28] P. Kaatsch. *Cancer Treat. Rev.* **2010**, *4*, 277-285.
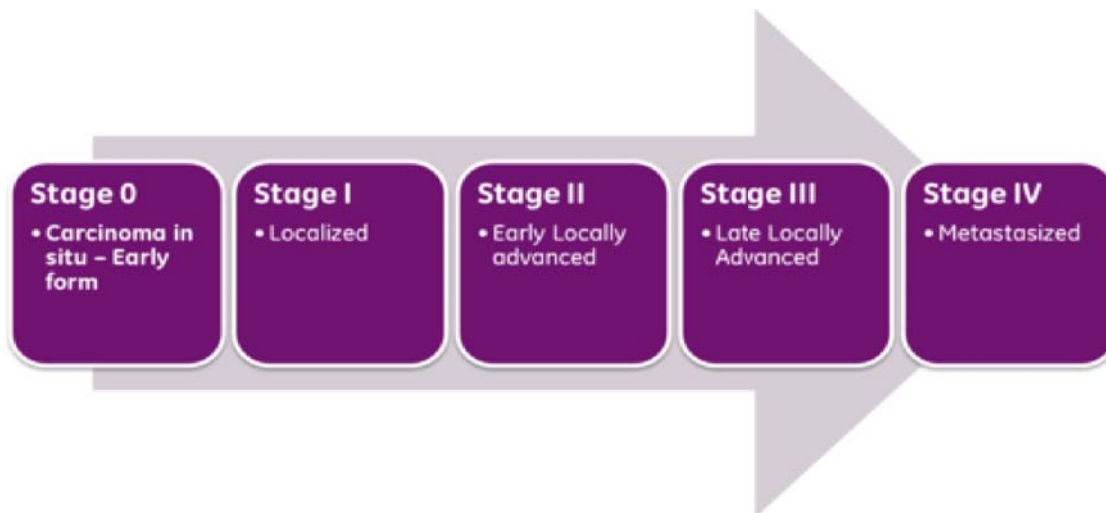
Figure 6. The 5 different cancer stages

1.3.2. Surgery

Surgery is an efficient treatment for solid localized tumor from stage 0 to II. The surgeon tries to remove the entire tumor along with, in certain cases, the lymph nodes in the area.[29]

1.3.3. Radiations

Radiation therapy involves the use of a ionizing radiation to burn tumors. It is either used alone to treat cancer, or in addition to a chirurgical operation to improve its results.[30]

1.3.4. Chemotherapy

1.3.4.1. Antecedents

Chemotherapy is used alone or in combination to surgery. Antitumor agents are administred to the patient to chemically kill cancer cells. It is useful in a lot of different cancer types such as cancer of breast, colon, pancreas, testicles, ovaries and lung. However, the effectiveness of chemotherapy is often limited by its high toxicity due to the low selectivity of the antitumour agents to other tissues in the body. Actual chemotherapeutic agents are not selective of cancer cells. They also attack healthy cells, especially cells in the bone marrow, digestive tract, and hair follicles. This lack of selectivity in chemotherapy causes side-effects such as hair loss, nausea,

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[29] W. Kufe, M. D. Raphael, E. Pollock, R. Weichselbaum, M. D. R. Bast, M. D. Gansler, J. Holland, E. Frei. Holland-Frei Cancer Med, 6th edition. **2003**, Chap.40.

[30] W. Kufe, M. D. Raphael, E. Pollock, R. Weichselbaum, M. D. R. Bast, M. D. Gansler, J. Holland, E. Frei. Holland-Frei Cancer Med, 6th edition. **2003**, Chap.41.

myelosuppression (decreased production of blood cells), suppression of the immune system and mucositis (inflammation of the lining of the digestive tract). In many cases, the drug effects can contribute to the ultimate cause of death. Fortunately, because cancer cells spend more time dividing than other cells, inhibiting cell division harm tumor cells much more than other cells. Another problem comes from possible tumor resistance to some chemotherapeutic agents. In fact, cancer cells having lots of different mutations, they are more likely to bear some mutated cells that develloped a resistance against some drugs. That is why new chemotherapy treatments consist in administrating a combination of different antitumor agents to lower the tumor resistance probability to the drug.[30]

1.3.4.2. New antitumor target

1.3.4.2.1. Introduction

Cancer represents a broad group of various diseases, all involving unregulated cell growth. Cells divide and grow uncontrollably, forming malignant tumors and invading nearby parts of the body through metastasis. Cancer chemotherapy began in 1940 with the use of nitrogen mustards and folic acid antagonists (antifolates). Many agents used in clinical therapy for the treatment of cancer were essentially poisons.[31] Targeted therapy developed later on, but many of the principles and limitations of chemotherapy encountered by the early researchers still apply. The classic example of targeted therapy is *imatinib*, a small molecule which inhibits kinase enzymes (Figure 1).[32] *Combretastatin* A-4 is a good exemple of a natural compound that possesses several mechanisms of action that can be used in cancer treatment. In general, antitumor agents cause severe side-effects that limit the dose which can be administered and hence limit their beneficial effects. Researching and studying new anticancer agents is crucial to reduce side-effects and improve cancer chemotherapy.
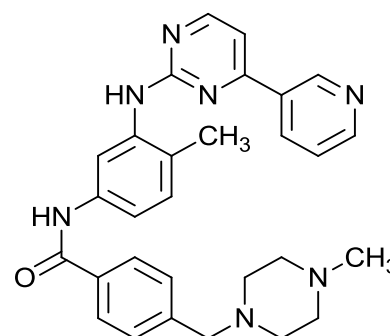
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[31] http://www.cancerresearchuk.org/cancer-help/about-cancer/ 28/04/2013.
[32] H. J. Droogendijk, H. J. Klin-Nelemons, A. P. Orange. *Cancer* **2000**, *24*, 935-942.

1.3.4.2.2. DNA Intercalants

DNA intercalant compounds do not form a covalent bond but insert and bound to DNA through electrostatic interactions. Intercalation occurs when ligands of an appropriate size and chemical nature fit themselves between pairs of DNA bases. These ligands are mostly polycyclic, aromatic, and planar.[33] Examples include *ellipticine* and *proflavine* (Figure 2).

Ellipticine

Proflavine
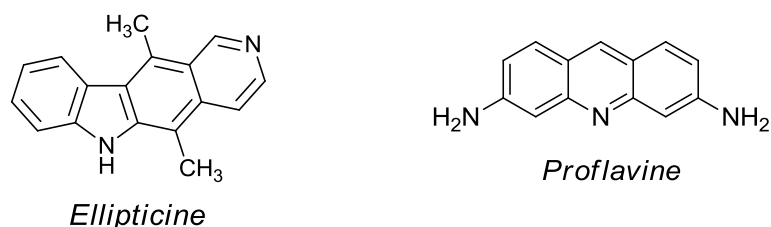
Figure 2. DNA intercalants compounds

Most studies showed that *ellipticine* mode of action is based on DNA intercalation and topoisomerase II inhibition. It has been found that *ellipticine* metabolises through the action of cytochrome P450 into 9-hydroxyellipticine derivatives, which are responsible of its antitumour activity.[34]

1.3.4.2.3. Topoisomerase II inhibitors

Topoisomerase II, a DNA gyrase, is an enzyme that regulates DNA supercoiling during the cell cycle. It is a homodimeric protein, associated with the mitotic chromosome portion. The crystal structure of a large fragment of topoisomerase II reveals a heart-shaped dimeric protein with a large central hole. It provides a molecular model of the enzyme as an ATP-modulated clamp with two sets of jaws at opposite ends, connected by multiple points (Figure 3).

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[33] A. D. Richards, A. Rodgers. *Chem. Soc. Rev*. **2007**, *36*, 471-483.
[34] M. Stiborova, J. Sejbal, L. Borek-Dohalska, D. Aimova, J. Poljakova, K. Forsterova, M. Rupertova, J. Wiesner, J. Hudecek, M. Wiessler, E. Frei. *Canc. Res.* **2004**, *64*, 8374-8380.
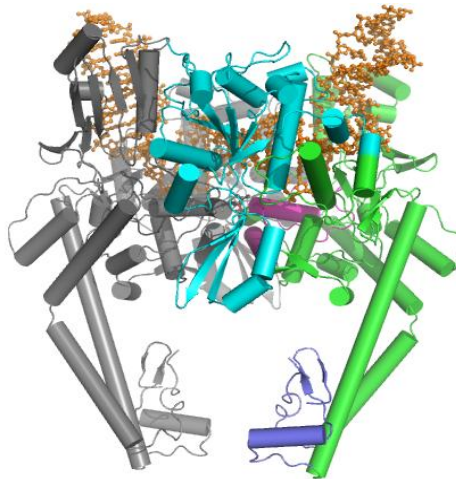
Figure 3. Structure of yeast topoisomerase II bound to DNA[35]

Topoisomerase II regulates DNA topology by first binding to DNA in a reversible way and then performing a concerted strand-breaking and religation process.[36] Thereby, it changes the linking number of DNA, relaxing supercoiling.[37] Topoisomerase II can also repair DNA helices that are not superimposed correctly. Indeed, by cutting the DNA helix, weak interactions between nucleotides can be broken more easily. Therefore, the nucleotides can be re-superimposed at the right location. This mechanism allows mutations during cell anaphase to be prevented (Figure 4).
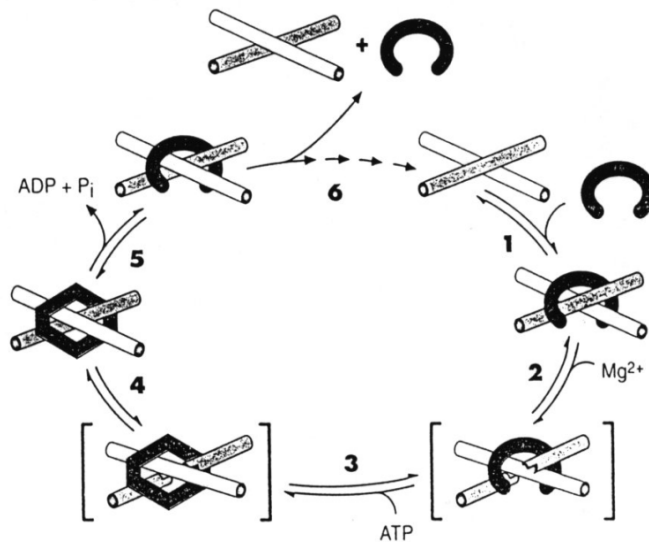
Figure 4. General Figure of DNA cleavage with Topoisomerase II

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[35] http://helicase.pbworks.com/w/page/17605643/Justin-Neese (28-04-2013).
[36] J. M. Berger, S. J. Gamlin, S. C. Harrison, J. C. Wong. *Nature* **1996**, *379*, 225-232.
[37] J. M. Berger. *Curr. Opin. Struct. Biol*. **1998**, *8*, 26-32.

Topoisomerase II inhibitors have the capacity, via the formation of a ternary drug-enzyme-DNA complex, to inhibit the religation step, resulting in increased formation of lethal DNA double breaks. They stabilize an intermediate in the complex formed by DNA and topoisomerase II and thereby prevent them from separating. This prevents topoisomerase II from repairing DNA correctly and causes cell death. *Etoposide* and *teniposide* are examples of topoisomerase II inhibitors actually used in lung cancer clinical chemotherapy (Figure 5).

Etoposide   *Teniposide*
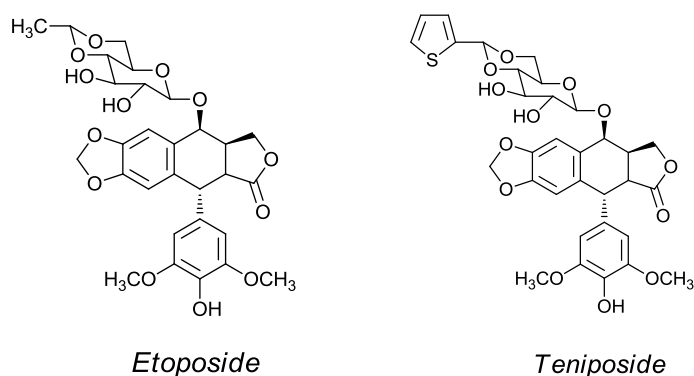
Figure 5. *Etoposide* and *teniposide* topoisomerase II inhibitors[38]

1.3.4.2.4. Apoptosis promoters

Apoptosis is the process of programmed cell death that occurs in multicellular organisms. Apoptosis promoters are pro-apoptotic compounds involved in imitating apoptosis.[39] *Combretastatin* A4 is one of the most potent apoptosis promoter which binds to tubulin and targets existing tumor blood vessels (Figure 6).
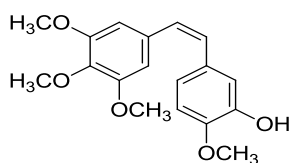
Figure 6. *Combretastatin* A4

Combretastatin A4 also inhibits cell tubulin polymerization at what is called the colchicine site. Microtubules are essential to cytoskeleton production, intercellular movement, cell movement, and mitotic spindle formation used in chromosome segregation and cellular division.

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[38] K. R. Hande. *Eur. J. Cancer* **1998**, *34*, 1514-1521.
[39] J. Lei, H. Jiege. *J. Mol. Onc.* **2012**, *20*, 643-645.

Endothelial cells treated with *combretastatin* A4 rapidly result in necrosis of the tumor core.[40] The BCL-2-associated death promoter is encoded by the BCL-2 gene.[41]

1.3.4.2.5. Inhibitors of Cyclin Dependant Kinases (CDKs)

CDKs are enzymes that, once activated by cyclins, catalyse the phosphorylation of proteins in the cell cycle. These enzymes are responsible of the cell cycle checkpoints. There are four checkpoints and one restriction point, each serving as a biological hurdle to prevent any DNA damage incurred at a specific phase of the cell cycle from being replicated and passed onto new generations of cells. They are controlled by a serie of highly specific CDKs, which control progression first through the cell cycle phase and then on through the next checkpoint (Figure 7).[38]
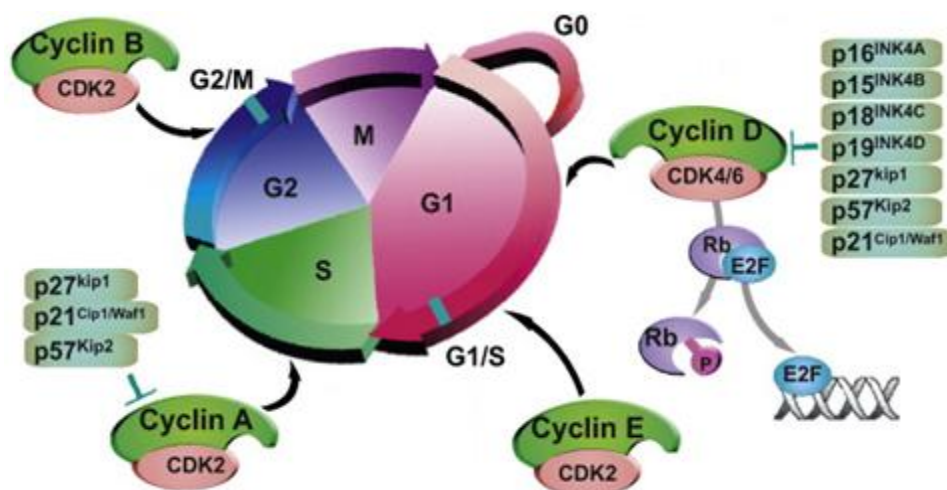
Figure 7. Checkpoints and restriction points of CDKs in the cell cycle

The cell cycle is composed of 5 different phases:

1) The G_0 phase: It is a resting phase where the new cell leave the cycle. The G_1 restriction point allows re-entry of G_0 cells into cell cycle.

2) The G_1 phase: The cell dimension increases. The G_1 checkpoint ensures that the G_1 phase has been accurately completed. If not, the G_1 checkpoint delays division or put the cell back to the G_0 phase.

[40] W. Yan, Z. Liyuan, L. Chang, L. Liangping, Y. Shaobi, W. Mingxing. *J. Sun Yat-sen Univ*. **2012**, *33*, 8-15. DOI: 158:234040.

[41] A. M. Petros, A. Medek. *Proc. Natl. Acad. Sci.* **2001**, *98*, 3012-3017.

3) The S phase: The S phase is when DNA replication takes place. The double stranded DNA molecule opens and each DNA strands serves as template for the production of the new complementary strand.

4) The G_2 phase: The cell continues to grow and gets ready for replication. The G_2 checkpoint ensures that the cell is ready for mitosis. If not, the cell cycle is stopped.

5) The M phase: In this phase, the cell divides by mitosis. Mitosis is composed of 5 different phases:

a) Prophase: DNA coils into homologous chromosomes which all have two identical copies of each chromosome.

b) Metaphase: Each homologuous chromosome align in the middle of the cell. The M checkpoint ensures that chomosomes are correctly aligned. If not, cell replication is stopped.

c) Anaphase: During anaphase, chromosomes move to the opposite poles of the cell.

d) Telophase: In telophase, the last stage of mitosis, the chromosomes have reached the poles and begin to uncoil and become less condensed. Two new nuclear enveloppes begin to form around each of the two separated sets of unreplicated chromosomes.

e) Cytokinesis: The cytoplasm is divided into two daughter cells.

CDK inhibitors interrupt CDK phosphorylation catalysis and stop cell replication. Among the numerous existing CDKs, CDK-1, CDK-2, CDK-4, CDK-6 and CDK-9 play a very important role on cell division. Recent studies show that CDK-4 and CDK-6 are overexpressed in tumours, which means that their inhibition would dramatically decrease tumour growth. Therefore, it is commun that biological assays look for CDK-4 and CDK-6 inhibition as a possible antitumour mechanism. CDK-4 and CDK-6 act only in late G_1 phase. Examples of CDK inhibitors include *flavopyridol* and *roscovitine* (Figure 8).[42]

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[42] N. Liu, H. Jiang, A. Ben-Shlomo, K. Wawrowsky, X. Fan, S. Lin, S. Melmed. *Natl. Acad. Sci. USA*. **2011**, *108*, 8414-8419.
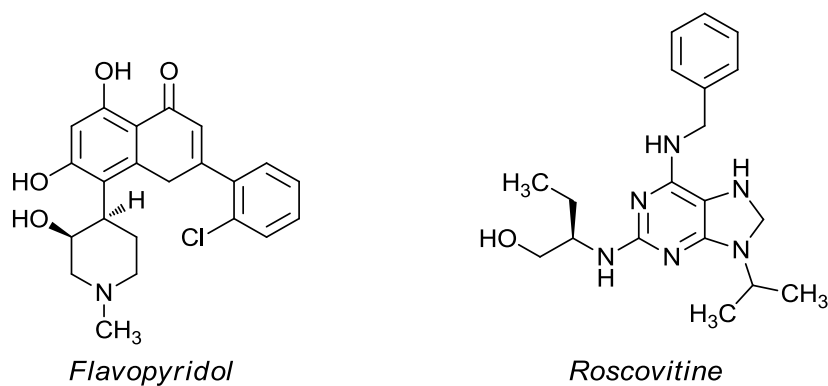
Figure 8. *Flavopyridol* and *roscovitine* CDK-9 inhibitors

1.3.4.2.6. Pharnesyl-transferase inhibitors

Pharnesyl-transferase inhibitors inhibit the pharnesyl-transferase enzyme responsible of the K-Ras protein binding to the cell membrane. The K-Ras protein (or Ras protein) is an oncoprotein that, once activated, is a positive regulator of cell proliferation. The K-ras protein is activated with the action of the GEFs (GDP/GTP exchange factors) that phosphorylate a particular guanosine diphosphate into a guanosine triphosphate. Conversely, the K-ras protein inactivation is triggered by the GAP (GTPase activating protein). In healthy cells, these activation/inactivation proceses act as cell proliferation regulators. However, in 30% of human tumors, an inactivation of the GAP is observed, maintaining the K-Ras protein in its activated state. In order to be active the K-Ras protein has to previously bind to the cell membrane with the action of the pharnesyl-transferase. Inhibiting the pharnesyl-transferase enzyme results in the inactivation of the K-Ras protein which stops the uncontrolled cell proliferation (Figure 9).[43, 44]

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[43] C. W. Reuter, M. A. Morgan, L. Bergmann. *Blood* **1996**, *5*, 1655-1669.
[44] G. Anuj, R. Agrawal, R. Rakesh. *Farnesyltransferase Inhibitor in Cancer Treatment, Current Cancer Treatment*. Edited by Öner Özdemir. **2011**, 150-160.
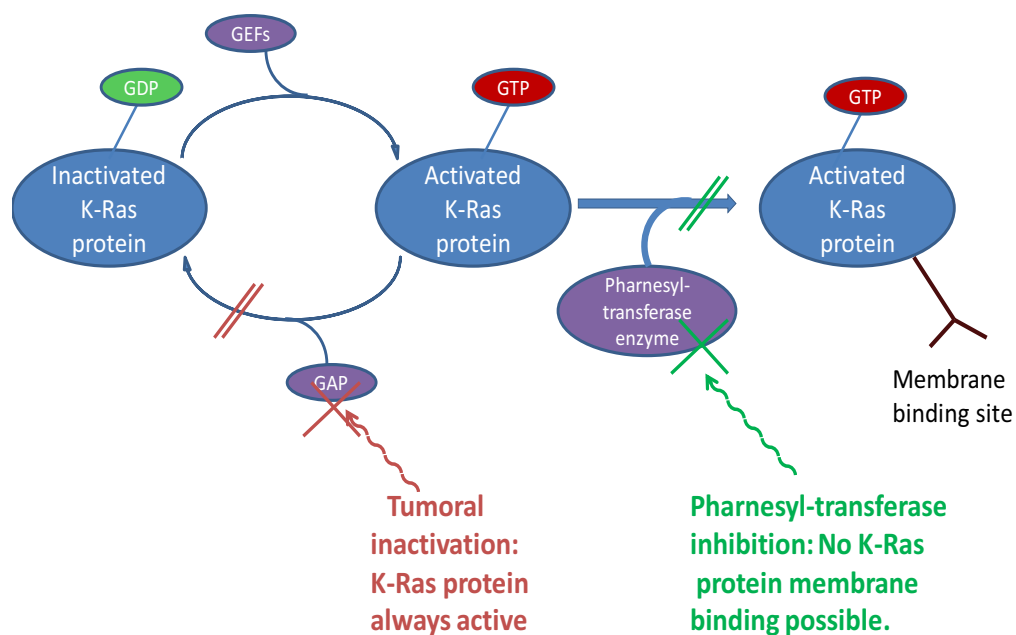
Figure 9. Mechanism of action of pharnesyl-transferase inhibitors

Examples include *tipifamib* (R115777), a farnesyl-transferase inhibitor that entered phase III clinical trials (Figure 10).[45]
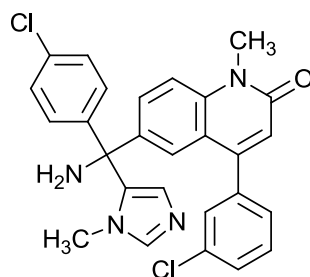
Figure 10. *Tipifamib* structure

1.3.4.2.7. Anti-angiogenic agents

Pathological events such as solid tumor growth, metastasis and other nonmalignant diseases such as arthritis, psoriasis or molecular degeneration have been found to be related to the angiogenesis process. Anti-angiogenic agents target the processes that lead to new blood vessels formation in tumours. When solid cancer tumors are small, they are supplied with

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[45] J. A. Sparano, S. Moulder, A. Kazi. *Clin. Cancer. Res.* **2009**, *15*, 2942-2948.

nutrients by diffusion from nearby blood vessels. In order to grow larger, they need their own blood vessels, which they create by angiogenesis promoters (Figure 11).[46]
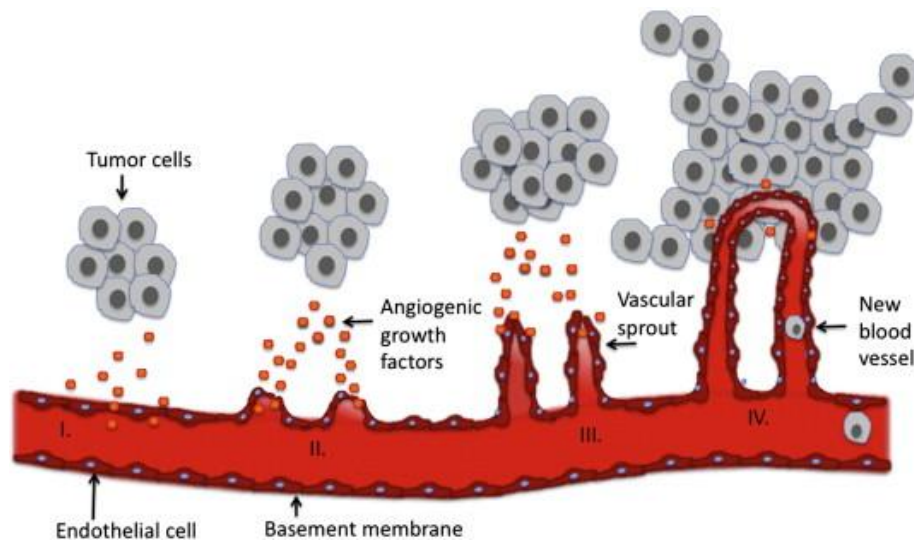
Figure 11. Mechanism of angiogenesis

Examples of angiogenesis promoters include Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF).[46] VEGF's normal function is to create new blood vessels after injury, during embryonic development or in muscles after doing exercise, and to bypass blocked vessels. The most important member is VEGF-A. Other members are Placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D. Solid cancers cannot grow beyond a limited size without an adequate blood supply. Cancers that can express VEGF are able to grow and metastasize. Drugs that interrupt that process show promise in treating cancer.[47] Anti-VEGF therapies are important in the treatment of certain cancers. Examples of monoclonal antibodies are *bevacizumab* (*Avastin*®) and antibody derivatives such as *ranibizumab* (*Lucentis*®), that both inhibit VEGF-A (Figure 12).[48]

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[46] R. Airley. *Cancer Chemotherapy, Basic Science to the Clinic*. Edited by Wiley-Blackwell. **2009**, 199-209.
[47] E. C. Hayden. *Nature* **2009**, *458,* 686-687.
[48] H. S. Kim. *Cytogenet. Cell Genet*. **1998**, *83*, 1-2.
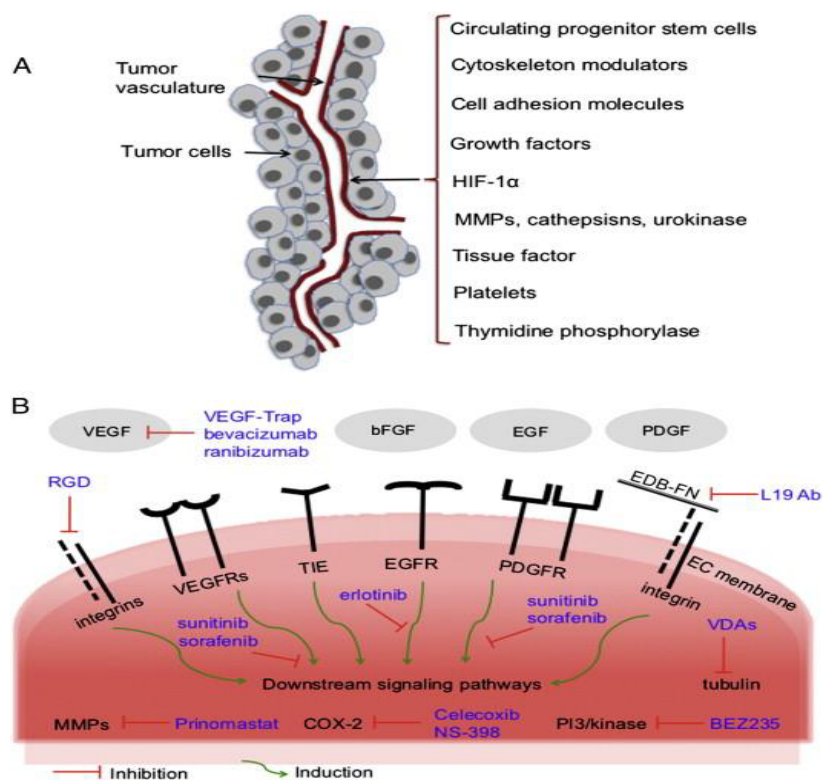
Figure 12. Angiogenesis growth factors and anti-VEGF agents

Other examples of anti-angiogenic agents include *sorafenib*, *sunitinib* and *thalidomide* (Figure 13).[49,50]

Sorafenib

Sunitinib
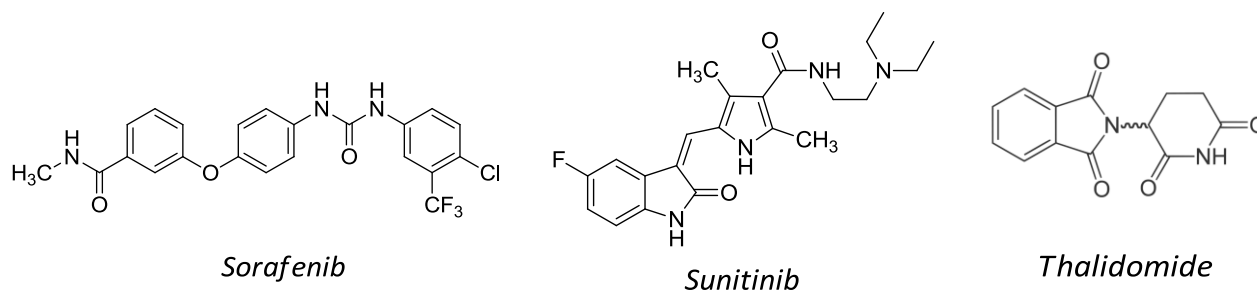
Thalidomide

Figure 13. *Sorafenib*, *sunitinib* and *thalidomide* anti-angiogen

[49] S. M. Wilhelm, L. Adnane, P. Newell, A. Villanueva, J. M. Llovet, M. Lynch. *Mol. Canc. Ther.* **2008**, *10*, 3129-3140.
[50] R. J. Amato, M. S. Loughnan, E. Flynn, J. Folhman. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 4082-4085.

2.1. Synthesis of new topoisomerase II inhibitors

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology. In agreement with precedents of our research group our aim consists in the synthesis of new topoisomerase II inhibitors possessing a dioxino-isoquinoline nucleus in its general structure (Figure 14). These compounds must not be planar to possess non intercalating DNA properties, and show
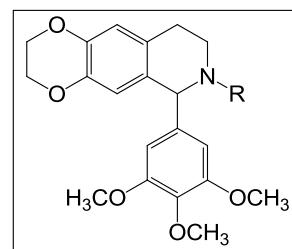
Figure 14. General dioxinoisoquinoline structure

great affinity for the topoisomerase II. These compounds are of particular interest since they have been reported to selectively target pulmonary cells and to have strong inhibitory properties on topoisomerase II with an IC_{50} of only 0.2 µm for the tetrahydroisoquinoline **1** in human NCI-H460 cell line (Figure 15). [51,52]

1	**2**	**3**
$IC_{50} = 0.2$ µM 72 h	$IC_{50} > 10$ µM 72 h	$IC_{50} = 21{,}1$ µM 72 h
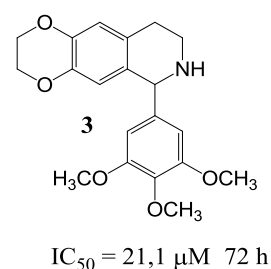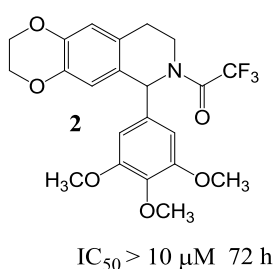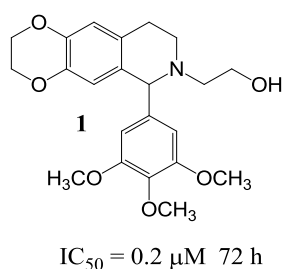
Figure 15. IC_{50} of compounds **1-3**

These values show that a polar group on the side chain bounded to the isoquinoline nitrogen atom improves the IC_{50} significantly. Therefore, a new investigation is needed to change the side chain on the nitrogen atom (Scheme 1 and 2).

1. R = CH$_2$-CH$_2$-OH, 32%
4. R = CH$_2$-CN, 78%
5. R = CH$_2$-CH$_2$-NH$_2$, 52%
6. R = CH$_2$-CH$_2$-N(CH$_3$)$_2$, 8%
7. R = CH$_2$-CH$_2$-CH(OEt)$_2$, 15%
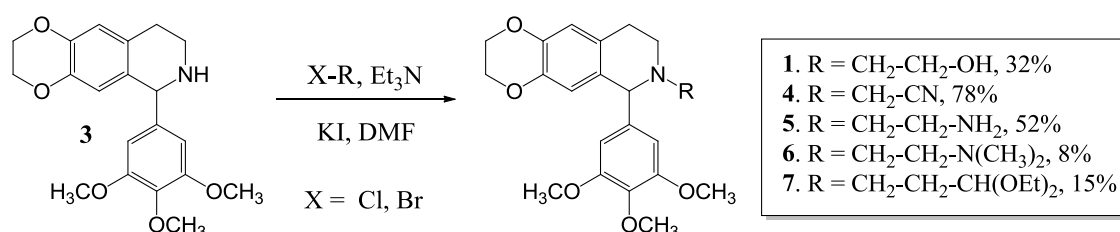
Scheme 1. Alkylation of the dioxinoisoquinoline **3**

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[51] *deaoptcancer.ptt.* presentation from M. D. Pujol group **2007**.
[52] A. S. Capilla, M. Romero, M. D. Pujol, D. H. Caignard and P. Renard. *Tetrahedron* **2001**, *57*, 8297-8303.
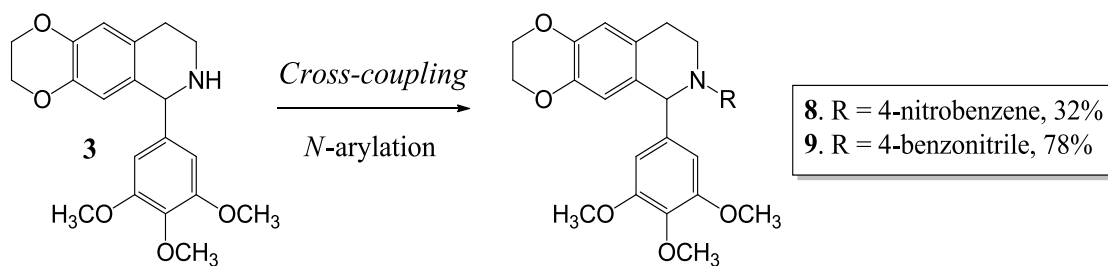
Scheme 2. *N*-arylation of the dioxinoisoquinoline **3** in *cross coupling* condition

The chain that fits best in the complex active site of the DNA topoisomerase complex will have the best IC_{50} value. To obtain novel compounds and to carry out a structure activity relationship study (SAR), we were interested in developing a ready access to **1** and analogous compounds. We were also interested in changing the 1,4-benzodioxan nucleus by a dimethoxyphenyl group in order to determine the influence of this modification in its biological activity (Figure 16).
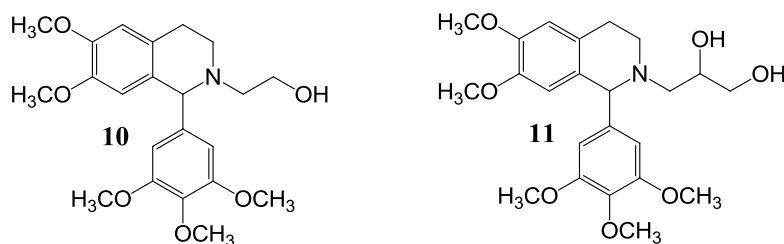
Figure 16. The dimethoxy-isoquinoline analogues **10** and **11**

The difference of antitumor activity between **1** and **10** should assess the importance of the 1,4-benzodioxan subunit compared to the dimethoxyphenyl nucleus.

2.2. Synthesis of *combretastatin* A-4 derivatives

Combretastatin A-4 is a natural product known for its strong anticancerigenic activity attributable to different mechanisms of action. *Combretastatin* A-4 was isolated from the tree *Combretum caffrum*, in South Africa (Figure 17).[53] This polymethoxylated stilbene strongly inhibits
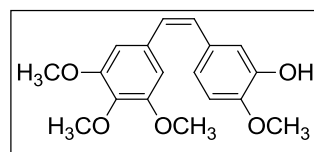
Figure 17. *Combretastatin* A-4

tubulin polymerization and also shows anti-angiogenic effects. It has been shown to be a cytotoxic agent against a wide variety of tumor cells, including multidrug-resistant tumors.[54] The work presented here describes new *combretastatin* A-4 analogues which contain a disubstituted cyclopropane, an oxirane or a 5-6 atom cycle instead of its central double bond (Figure 18).

12. X = CH$_2$
13. X = O

14. R = Bn
15. R = H

16. R = Bn
17. R = H
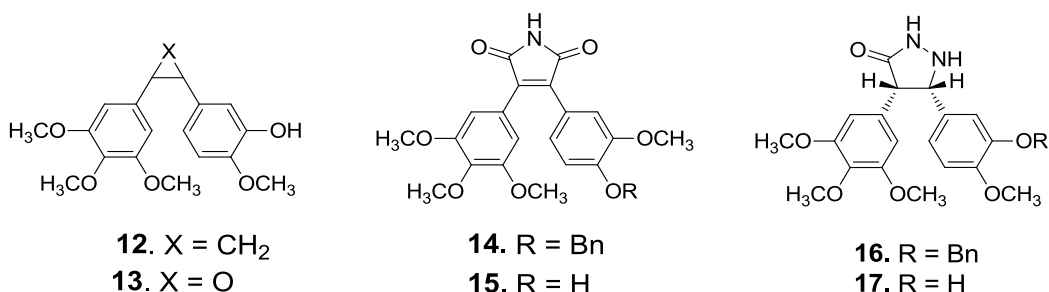
Figure 18. New *combretastatin* A-4 analogues

Replacing the central double bond with a cycle causes a change in the molecule bond angles. This implies that there are changes in the interactions between the compound and the site of action that can lead to an improved activity or a different mechanism of action. In all cases in order to mimic the double bound, the *(Z)* configuration is required. Indeed, it has been reported that the *(Z)-combretastatin* A-4 has nanomolar activity on tubulin polymerization inhibition (TPI), whereas the *(E)* isomer have lower cytotoxic activity.[55]

Subsequently, we took an interest in the preparation of *combretastatin* A-4 analogues which contain a sulfonyl group instead of the trimethoxy moiety (Figure 19).

[53] K. Ohsumi, T. Hatanaka, K. Fujita, R. Nakagawa, Y. Fukuda, Y. Nihei, Y. Suga, Y. Morinaga, Y. Akiyama, T. Tsuji. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3153-3158.
[54] J. Lei, H. Jiege. *J. Mod. Oncol*. **2012**, *20*, 643-645.
[55] D. Simoni, R. Romagnoli, R. Baruchello, R. Rondanin, M. Rizzi, M. G. Pavani, D. Alloatti, G. Giannini, M. Marcellini, T. Riccioni, M. Castorina, M. B. Guglielmi, F. Bucci, P. Carminati, C. Pisano. *J. Med. Chem.* **2006**, *49*, 3143-3152.

18. X = CH$_2$.
19. X = O.
20. X = CONHNH
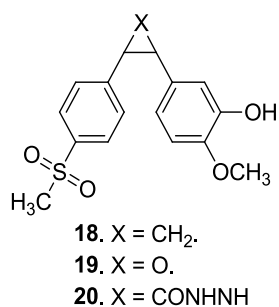
Figure 19. New sulfonyl *combretastatin* A-4 analogues

Determination of the activity of these sulfonyl combretastatin derivatives and comparing them with the trimethoxy *combretastatin* compounds should allow us to assess the importance of the sulfoxyde and trimethoxy group in the antitumor activity of **18**, **19** and **20**. Moreover, the SAR study of those compounds should give us information on whether electron withdrawing groups in the aromatic ring give a better or lower activity than electron donor groups.

2.3. Synthesis of 1,4-benzodioxan derivatives

Benzodioxan derivatives are new cytotoxic agents derived from [1,4]-benzodioxan previously described by Dr S. Capilla and Dr M. T. Vázquez.[56,57] These compounds were reported to have a micromolar antitumor activity against several cell lines, and are of great interest (Scheme 3).

Sergi Capilla Mies's doctoral thesis   Teresa Vazquez's doctoral thesis   Current work
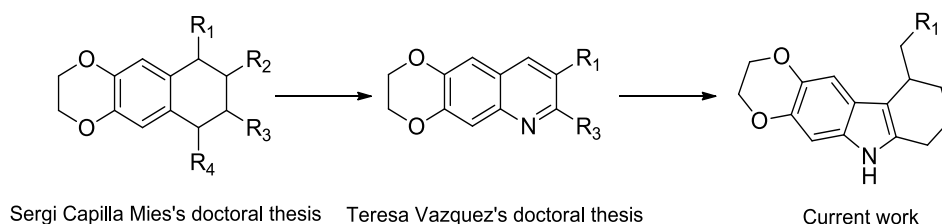
Scheme 3. Previous work in 1,4-benzodioxan derivatives synthesis

[56] Sergi Capilla Mies. *Estudi sintètic i estructural de nous sistemes policíclics derivats de la podofil.lotoxina que contenen el nucli de la 2,3-dihidro-1,4-benzodioxina. Agents amb potencial activitat antitumoral.* Tesi doctoral, **1998**, Facultad de Farmacia. Universidad de Barcelona.

[57] María Teresa Vázquez Fernández, *Estudio de estrategias sintéticas para la preparación de nuevos compuestos antiinflamatorios y antitumorales que contienen el nucleo de 2,3-dihidro-2,4-benzodioxino como subestructura*. Tesis doctoral, **1997**, Facultad de Farmacia. Universidad de Barcelona.

Tetracyclic fused heterocyclic systems represent a series of compounds of considerable medicinal importance.[58] The research work of Dr S. Capilla and Dr M. T. Vázquez showed that benzodioxan derivatives with a mobile side chain that bears an aromatic carbamate substituent were particularly active (Figure 20).
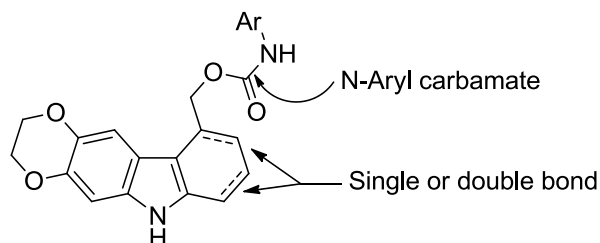
Figure 20. General structure of the desired dioxancarbazole derivatives

The work presented in this section reports the synthesis of fused dioxancarbazole compounds. A practical and efficient synthesis of dioxancarbazoles **21** and **22** was achieved (Figure 21).
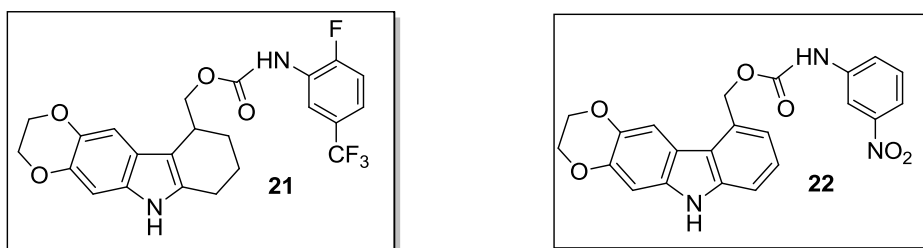
Figure 21. Dioxancarbazoles **21** and **22**

The carbamate aromatic substituents were inspired of known agents like *sorafenib* (*Nexavar*® Bayer) and potent antitumor agents synthesized in the doctoral thesis of Dr N. Mur Blanch.[59]

[58] Y. Tachibana, H. Kikuzaki, N. H. Lafis, N. Naketami. *J. Agric. Food Chem*. **2001**, *49*, 5589-5594.

[59] Núria Mur Blanch. *Disseny I síntesis de nous compostos de naturalesa heterocíclica amb potencial activitat anticancerígena. Síntesi de nous inhibidors de CDKS.* Tesis doctoral, **2011**, Facultad de Farmacia. Universidad de Barcelona.

2.4. Synthesis of *resveratrol* derivatives

Pharmaceutical studies suggested that the polyphenol *resveratrol* (3,4',5-trihydroxy-*trans*-stilbene) is one of the main wine grape components that protect from vascular and neurodegenerative diseases and cancers due to antioxidant properties (Figure 22).[60,61] The ability of *resveratrol* to
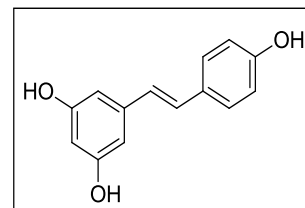
Figure 22. *Resveratrol*

prevent the occurrence of carcinomas was related to the inhibition of tumor cell cycle and induction of tumor cell death.[62,63] We focused on the synthesis of new structural *resveratrol* analogues replacing the central double bond by an unsaturated ring. This modification should allow us to assess the importance of this double bond in the antitumor activity of those products (Scheme 4).
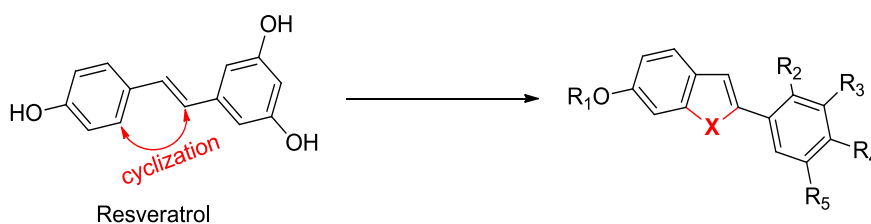
23. X = O, R_1 = R_2 = H, R_3 = R_4 = R_5 = OH
24. X = O, R_1 = R_2 = H, R_3 = R_4 = R_5 = OCH_3
25. X = NH, R_1 = Bn, R_2 = R_5 = OCH_3, R_3 = R_4 = H
26. X = NH, R_1 = R_3 = R_4 = H, R_2 = R_5 = OCH_3

Scheme 4. General structure of the *resveratrol* analogue

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[60] H. C. Hansen, F. S. Chiacchia, R. Patel, N. C. W. Wong, V. Khlebnikov, R. Jankowska, K. Patel, M. M. Reddy. *J. Org. Chem*. **2010**, *45*, 2018-2023.

[61] J. Lu, C. T. Ho, G. Ghai, K. Y. Chen. *Carcinogenesis* **2001**, *22,* 321-328.

[62] G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J. M, Schmidt, F. Hogan. *J. Med. Chem*. **1995**, *38*, 1666-1672.

[63] G. R. Pettit, A. Thornhill, N. Melody, J. C. Knight. *J. Nat. Prod*. **2009**, *72*, 380-388.

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3. Results and discussion

3.1. Introduction

Heterocyclic nuclei rings containing at least one atom of an element other than carbon are present as a subunit in an array of drug categories such as antitumor agents, antimicrobials, analgesics, antidepressants, etc. Heterocycles form by far the largest of classical divisions of organic chemistry and are of high importance biologically and industrially.[64] The majority of drugs with biologically active compounds are heterocyclic. For more than a century heterocycles have constituted one of the largest area of research in organic chemistry. Heterocyclic chemistry also contributed to the understanding of life processes, because the side groups of the most typical and essential constituents of living cells, DNA and RNA, are based on aromatic heterocycles. Between the heterocyclic compounds, those containing nitrogen, sulfur and oxygen have maintained the interest of researchers.[65] Our society is dependent of synthetic heterocycles such as drugs, plastics, pesticides or compounds related to nutrition.

3.2. Synthesis of new topoisomerase II inhibitors

3.2.1. Synthesis of dioxinoisoquinoline derivatives

3.2.1.1. Retrosynthetic analysis

Previous syntheses of the dioxinoisoquinoline **1** realized by our research group used 2,3-dihydro-[1,4]-benzodioxan **27** as starting material.[52] Isoquinoline **1** can be prepared by alkylation of the dioxinoisoquinoline **3**, which would be obtained by cyclization of the phenethylamine **28** with 3,4,5-trimethoxybenzaldehyde (**29**). The phenethylamine **28** could be synthesized from 2,3-dihydro-1,4-benzodioxin **27** under three different conditions (Scheme 5).

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[64] A. Katritzky, C. Ramsden, J. Joule, V. Zhdankin. *Handbook of heterocyclic Chemistry*. 3rd edition. Edited by Elsevier. **2010**.
[65] M. G. Valverde, T. Torroba. *Molecules* **2005**, *10*, 318-320.
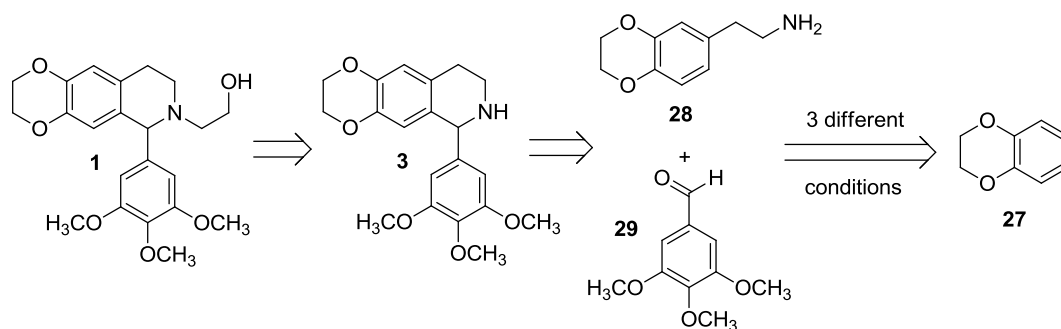
Scheme 5. Retrosynthetic analysis of 1 starting from 2,3-dihydro-[1,4]-benzodioxin 27

In this work, a large quantity of 3 was synthesized, and different functionalized chains were fixed at the *N*-atom. In this way, new methods to improve previous yields and reduce the number of steps of the synthesis of 3 and 28 were developed.

3.2.1.2. Preparation of the arylethylamine 28

In our search for a good methodology to the preparation of the arylethylamine 28, four methods were investigated, one of which was already developed by our research group (1st method).[52] This synthetic route offers three attractive alternatives such as the formylation of the 2,3-dihydro-[1,4]-benzodioxin followed by condensation with nitromethane under *Henry* reaction conditions or a *Friedel-Crafts* acylation of the dioxygenated heterocycle and its reduction (Scheme 6).
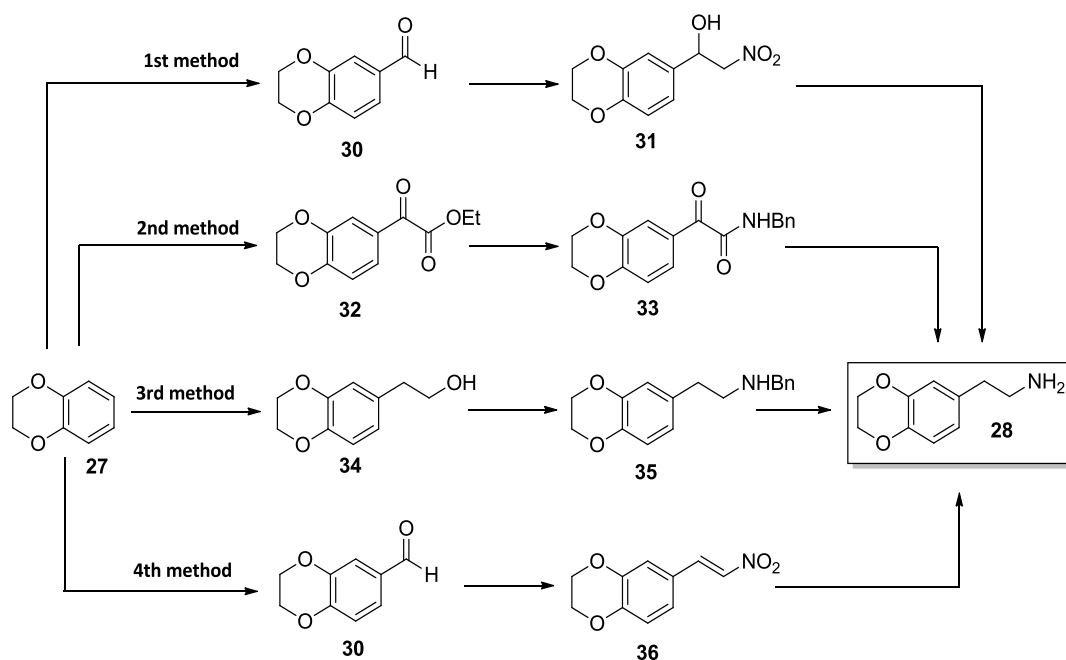
Scheme 6. Synthesis of the amine 28

1st Method

Following the procedure designed by our research group, 2,3-dihydro-[1,4]-benzodioxin (**27**) was brominated with NBS to give 6-bromo-2,3-dihydro-[1,4]-benzodioxin (**37**), which reacted with DMF after metal-halogen interchange using BuLi to afford 2,3-dihydro-[1,4]-benzodioxin-6-carbaldehyde (**30**) in appreciable yield. Treatment of nitromethane with LDA and addition to the aldehyde **30** under *Henry* reaction conditions gave the nitroalcohol **31** in low yield, which was reduced by catalytic hydrogenation with H_2 and 10% palladium on carbon to afford the arylethylamine **28** in appreciable yield. The amine **28** was identical in all respects with that previously reported (Scheme 7).[19]
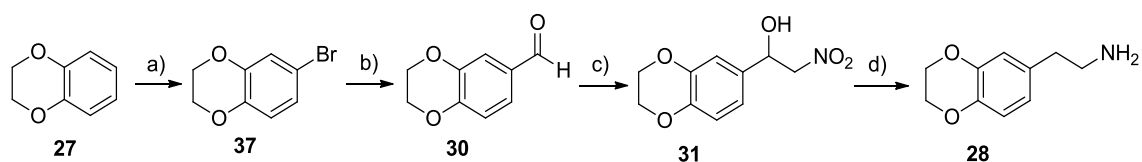
Scheme 7. Reagents and conditions: a) NBS (1 eq), MeOH (30 mL), rt, 16 h, 97%. b) 1) BuLi (1.5 eq), THF, rt, 1 h. 2) DMF, rt, 16 h, 60%. c) CH_3NO_2 (1.5 eq), LDA (1.5 eq), THF (10 mL), rt, 6 h, 20%. d) H_2, Pd/C (10% m/m), MeOH (50 mL), HCl (30 µL), rt, 32 h, 55%.

The overall yield of this synthetic route was 7%, which is comparable with the 10% overall yield described in the literature by our research group.[19]

2nd Method

The second method implies a *Friedel-Crafts* acylation reaction of 2,3-dihydro-[1,4]-benzodioxin (**27**) with ethyl oxalyl chloride to afford **32** in quantitative yield. Treatment of **32** with an excess of benzylamine in a nucleophilic substitution led to reaction in both the ester and the ketone electrophilic group to form **38** instead of **33** in high yield (Scheme 8).
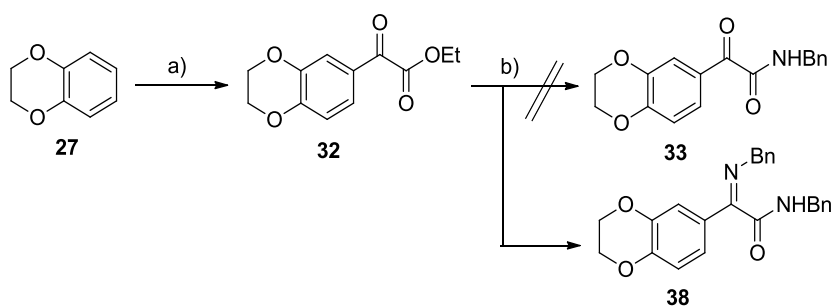
Scheme 8. Reagents and conditions: a) Ethyl oxalyl chloride (1.1 eq), TiCl$_4$ (1 mL), DCM (20 mL), rt, 3 h, quant. b) BnNH$_2$ (2 eq), Et$_3$N (2 eq), DMF (20 mL), 90 °C, 32 h, **38**, 95 %.

Other reaction conditions using less than 2 eq of benzylamine led to reaction on the ketone group first and then reaction on the ester group. This reactivity can be explained by the presence of the aromatic group in alpha of the ketone that favours the ketone amination. Attempts to hydrolyze the imine with HCl 5N led to the hydrolysis of the amide into the carboxylic acid. A possible solution would be to previously protect the ketone, but it would increase significantly the number of steps of this synthesis in comparison with the other reported routes.

3th Method

The third method consists in a *Friedel-Crafts* acylation reaction of 2,3-dihydro-[1,4]-benzodioxin (**27**) with ethyl oxalyl chloride, followed by a double reduction of the ester **32** with LiAlH$_4$ to form **34** in good yield. The hydroxyl group of **34** was converted to a good leaving group by treatment with SOCl$_2$, and a nucleophilic substitution with benzylamine afforded the secondary amine **35** in appreciable yield. Finally, a catalytic debenzylation of **35** in the presence of Pd/C under hydrogen atmosphere in acidic conditions led to the primary amine **28** in moderate yield (Scheme 9).
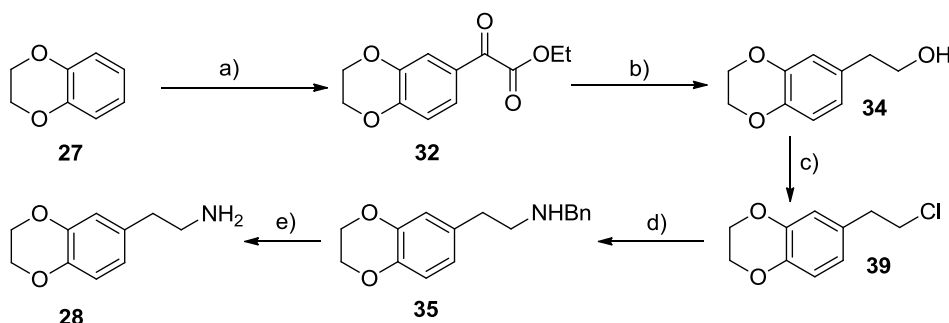
Scheme 9. Reagents and conditions: a) Ethyl oxalyl chloride (1.1 eq), TiCl$_4$ (1 mL), DCM (20 mL), rt, 3 h, 98%. b) LiAlH$_4$ (4 eq), THF, 4 h, 76%. c) SOCl$_2$ (1.66 mL), toluene (5 mL), 110 °C, 77%. d) BnNH$_2$ (1.5 eq), Et$_3$N (1.5 eq), DMF (20 mL), 90 °C, 32 h, 66%. e) H$_2$, Pd/C (20% m/m), MeOH (3 mL), HCl (30 µl), EtOAc (20 mL), rt, 32 h, 53%.

The overall yield of this 5-step alternative route was 20%, which was higher than the first method.

4st Method

In this fourth method, the aldehyde **30** reacted with nitromethane and ammonium acetate according to the literature procedure to afford **36** in excellent yield.[66] Reduction of the nitroethene **36** under hydrogen pressure in the presence of Pd/C led mostly to the vinyl amine and only traces of the amine **28**. A high pressure hydrogenation of **36** (6-8 atm) with Pd/C slightly improved the reaction to afford **28** in low yield. Finally, the reduction of **36** with LiAlH$_4$ under classical conditions afforded the amine **28** in acceptable yield (Scheme 10).
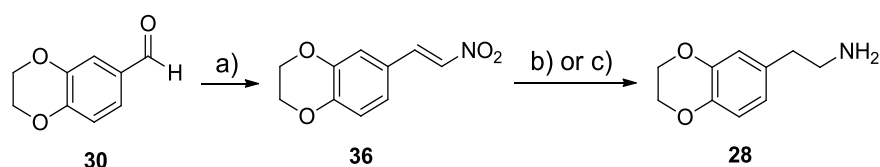
Scheme 10. Reagents and conditions: a) CH$_3$NO$_2$ (10 mL), CH$_3$COONH$_4$ (3.5 eq), 100 °C, 2 h, 90%. b) Pd/C (10% m/m), H$_2$ (7 atm), MeOH/EtOAc 1:3, 16 h, 24%. c) LiAlH$_4$ (4 eq), THF, rt, 16 h, 58%.

In order to optimize the preparation of 2,3-dihydro-[1,4]-benzodioxin-6-carbaldehyde (**30**), the commercially available 3,4-dihydroxybenzaldehyde (**40**) was alkylated with 1,2-dibromoethane and K$_2$CO$_3$ in acetone yielding **30** in 42% yield. When the solvent was substituted by DMF the desired aldehyde **30** was obtained in 81% yield (Scheme 11).[67] DMF was chosen to afford a higher reaction temperature.
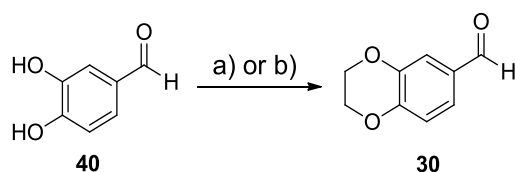
Scheme 11. Reagents and conditions: a) 1,2-dibromoethane (1.2 eq), K$_2$CO$_3$ (5 eq), acetone, 56 °C, 16 h, 42%. b) 1,2-dibromoethane (1.2 eq), K$_2$CO$_3$ (5 eq), DMF, 120 °C, 16 h, 81%.

On the basis of the results obtained, the best reaction conditions to yield the amine **28** were depicted on scheme 12. The 3-steps are: a) alkylation, b) condensation of the

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[66] R. Faust, P. J. Garratt, M. A. T. Pérez, V. J. D. Piccio. C, Madsen, A. Stenstrom, B. Frolund, K. Davidson, K. Teh, D. Sugden. *Bioorg. Med. Chem.* **2007**, *15*, 4543-4551.
[67] Taylor, W. I. *Helv. Chim. Acta*. **1950**, *33*, 164-168.

arylaldehyde **29** with nitromethane and c) reduction of the nitroolefin **36** to the arylethylamine **28**.
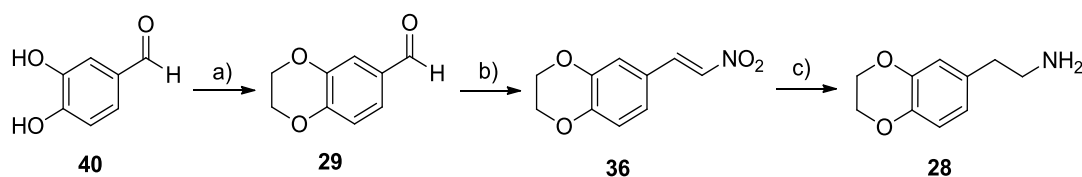
Scheme 12. Best reaction conditions to yield the arylethylamine **28.** Reagents and conditions: a) 1,2-dibromoethane (1.2 eq), K_2CO_3 (5 eq), DMF, 120 °C, 16 h, 81%. b) CH_3NO_2 (10 mL), CH_3COONH_4 (3.5 eq), 100 °C, 2 h, 90%. c) $LiAlH_4$ (4 eq), THF, rt, 16 h, 58%.

The overall yield of this 3-steps reaction was 42%. This method is more practical and efficient than the one previously described in the literature by our research group.[19] The overall yield of the synthesis of **28** was improved by 32%. The arylethylamine **28** was characterized by IR, ^1H NMR and ^{13}C NMR.

3.2.1.3. Synthesis of the isoquinoline 3 from the arylethylamine 28

Recognizing the medical value of nitrogen-containing heterocyclic compounds, scientists continue to devise new compounds and novel methods for their preparation.[68] The dioxinoisoquinoline **3** is a tricyclic heterocycle which can be used as scaffold in medicinal chemistry. Common syntheses of substitued isoquinolines involve traditional routes such as the *Bischler-Napieralski* reaction, an intramolecular electrophilic aromatic substitution reaction that allows the cyclisation of arylethylamides. This reaction involves a dichlorophorphoryl imine-ester intermediate (amide + $POCl_3$) (Scheme 13).[69]

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[68] J. S. Carey, D. Laflan, C. Thomson, M. T. Williams. *Org. Biomol. Chem.* **2006**, *4*, 2337-2347.
[69] J. J. Lee, Name reactions: A collection of Detailled mechanisms and Synthetic Applications. 4th edition. Edited by Springer. **2007**, 48-49.
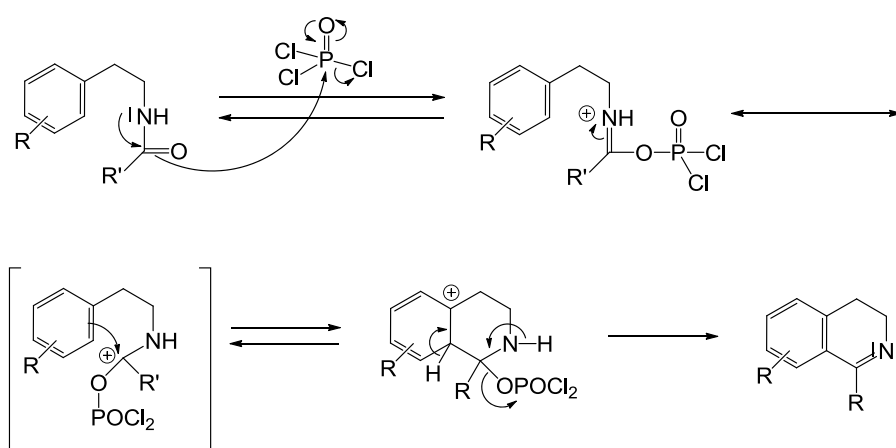
Scheme 13. *Bischler-Napieralski* mechanism.

An alternative mechanism considers the formation of the nitrilium ion after elimination and before cyclization. Different reaction conditions favor one or another mechanism (Scheme 14).
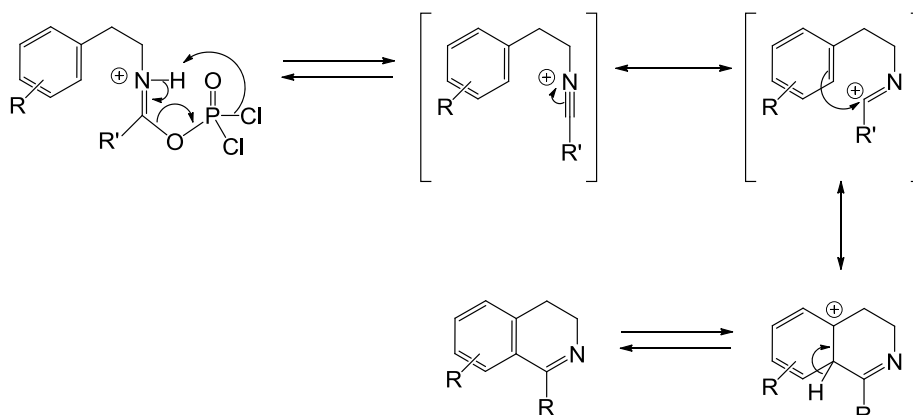
Scheme 14. Alternative *Bischler-Napieralski* mechanism

Previous work described by Dr. Sergi Capilla Mies and Dr. Manel Romero Balaguer showed that classical *Bischler-Napieralski* reactions were ineffective for cyclization of 1,4-benzodioxanethylamine structures.[70] Following the previous work methodology of our research group, a number of attempts were carried out for the preparation of the tetrahydroisoquinolines **3** and its optimization.[19] Amination of 3,4,5-trimetoxibenzaldehyde (**29**) with the arylethylamine **28** using PTSA or CSA in toluene

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[70] Manel Romero i Balaguer, *Preparació de nous agents antitumorals. Síntesi I avaluació citotoxica de sistemes polícíclics que contenen el nucli d'1,4-benzodioxina.* Tesis doctoral, **2001**, Facultad de Farmacia. Universidad de Barcelona.

did not yield the corresponding isoquinoline due to solubility problems. However, amination of **29** with **28** in EtOH at p*H* 6 in presence of molecular sieves 4 Å, followed by an intramolecular cyclization reaction of the resulting imine with TFA and TFAA gave the intermediate **2** in 14% yield, which was hydrolysed with NaOH 2N to afford the dioxine-isoquinoline **3** in 98% yield (Table 1). Replacing HCl by an acid resin did not improve the reaction.

Table 1. Formation of the dioxine-isoquinoline **3**
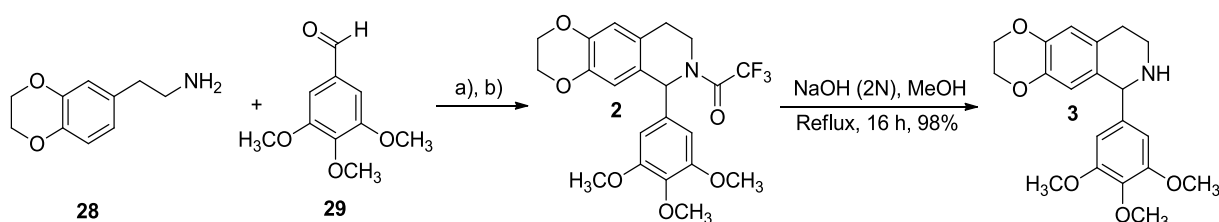
Entry	a)	b)	Results
1.	toluene, CSA, Dean Stark, 16 h, reflux	TFA, TFAA, rt, 72 h	Low solubility
2.	EtOH, HCl (p*H* 6), molecular sieves 4Å, 16 h, reflux	TFA, TFAA, reflux, 16 h	**2** (14%)
3.	EtOH, amberlyst® 15 ion-exchange resin, 72 h, reflux	TFA, TFAA, reflux, 48 h	No reaction

Another method was tried to improve the yield of **3**. Amination of the 3,4,5-trimetoxibenzaldehyde **29** with the arylethylamine **28** in a Dean Stark apparatus, followed by addition of H_3PO_4 (85% of an aqueous solution) afforded the isoquinoline **3** in 32% yield (Scheme 15).
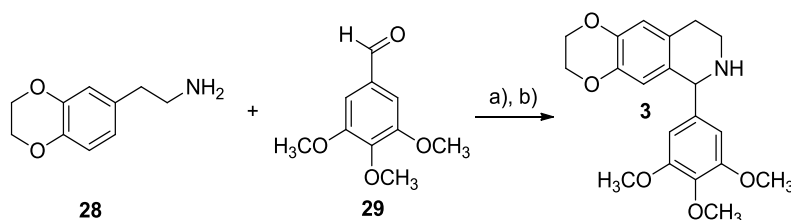
Scheme 15. Synthesis of the dioxinisoquinoline **3** using H_3PO_4. Reagents and conditions: a) 3,4,5-trimetoxybenzaldehyde (1.1 eq), benzene, Dean-Stark, 110°C, 3 h. b) H_3PO_4 (2 mL of a 85% aqueous solution), Dean-Stark, 110 °C, 4 h, 32% yield.

A possible mechanism for this intramolecular ciclyzation process implies protonation of the aldehyde, addition of the amine to the carbonyl group, dehydration and cyclization as indicated in Scheme 16.
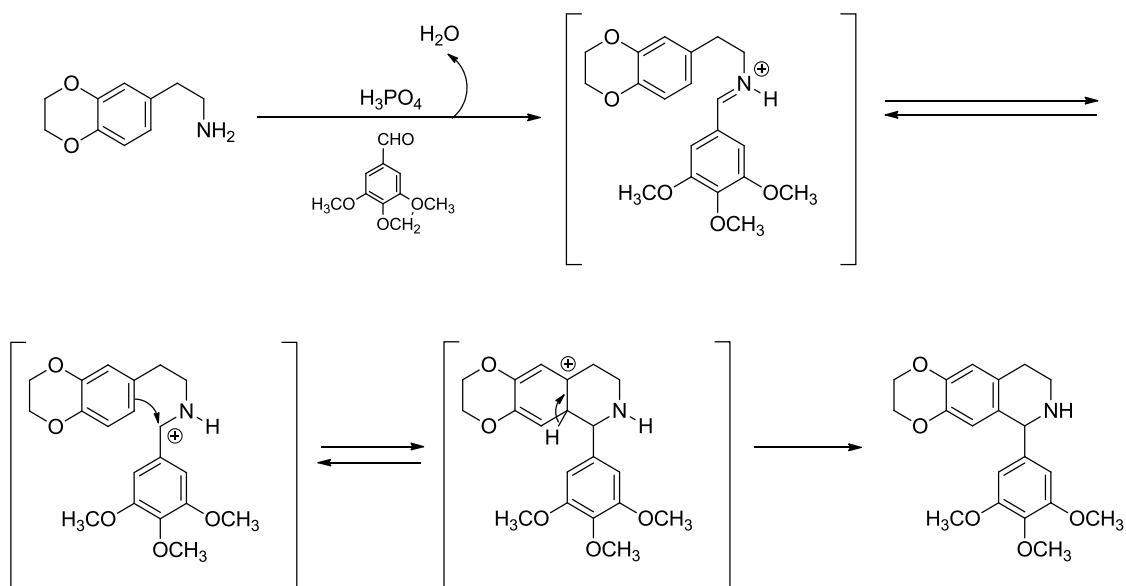
Scheme 16. H_3PO_4 mediated cyclization mechanism

The yield was improved by 18% compared to the previous method.

3.2.1.4. Alkylation of benzodioxanisoquinoline 3

In the present study and as a continuation of the synthesis of dioxanisoquinoline analogues, the isoquinoline **3** was involved in an alkylation process using classical conditions. In a general procedure, **3** is treated with the corresponding alkyle halide, Et_3N or K_2CO_3, KI and DMF obtaining the desired compound in low to satisfactory yields (Scheme 17).
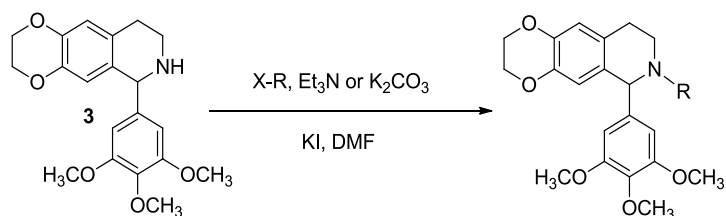
Scheme 17. General alkylation procedure of the tetrahydroisoquinoline **3**.

The results of a number of alkylation attempts of **3** carried out at both 80 °C and room temperature revealed that the alkylation reaction provided better yields when performed at room temperature despite of an increased reaction time. Indeed, alkylation of **3** at 80 °C were faster but favoured the oxidation of the tetrahydroisoquinoline **3** to the isoquinoline by-product **43** (Scheme 18).
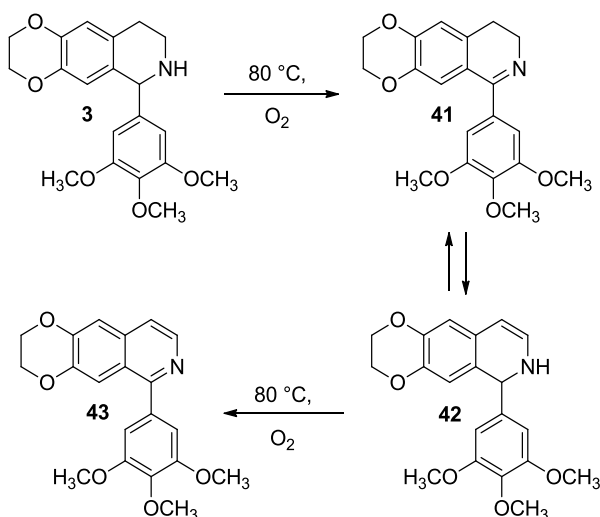
Scheme 18. Oxidation of **3** at 80°C forming the by-products **41**, **42** and **43**

3.2.1.5. Synthesis of compound 1 and 6

The tetrahydroisoquinoline **3** was alkylated with 2-chloroethanol under classical conditions in the presence of Et$_3$N and KI providing the alcohol **1** in 32% yield after purification (Scheme 19).
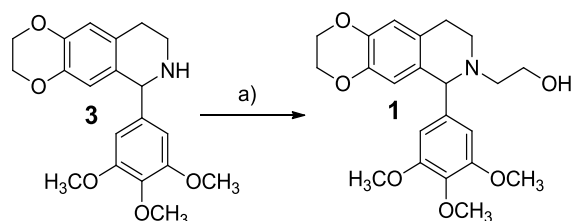
Scheme 19. Reagents and conditions: a) 2-chloroethanol (40 eq over 10 days), Et$_3$N (8 eq), KI (0,1 eq), rt, DMF, 7 days, 32%.

The tetrahydroisoquinoline **3** was involved in an *N*-alkylation reaction under the same reaction conditions as indicated above with 2-dimethylaminoethyl chloride, Et$_3$N and KI to afford **6** in 8% yield and recuperation of the starting material (Scheme 20). The low yield obtained was attributed to the low solubility of the 2-dimethylaminoethyl chloride in DMF.
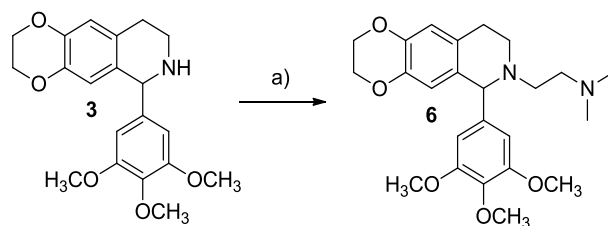
Scheme 20. Synthesis of amine **6**. Reagents and conditions: a) 2-dimethylaminoethyl chloride (15 eq over 5 days), Et₃N (8 eq), KI (0,1 eq), rt, 7 days, **6** (8%), **3** (83%).

3.2.1.6. Synthesis of the tetrahydroisoquinolines 4 and 5

An alkylation reaction of **3** using chloroacetonitrile and the conditions described above led to **4** in a satisfactory yield of 78% due to the high reactivity of the CH$_2$ group of the chloroacetonitrile in comparison with the low reactivity of other alkyl halides. The nitrile group of **4** was reduced with LiAlH$_4$ to yield **5** in acceptable yield (Scheme 21).
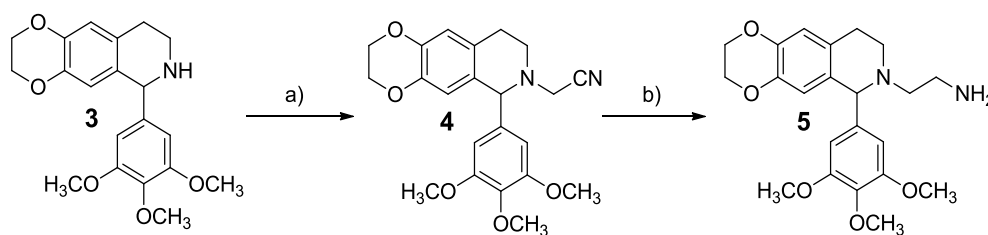
Scheme 21. Reagents and conditions: a) chloroacetonitrile (3 eq), KI (0.3 eq), K$_2$CO$_3$ (5 eq), DMF, rt, 10 days, 78%. b) LiAlH$_4$ (3 eq), THF, 3 h, 52%.

3.2.1.7. Synthesis of 7 and 44

The acetal **7** was synthesized by *N*-alkylation of **3** with the corresponding alkyl halide in low yield. Hydrolysis of the acetal **7** with HCl 2N was not successful due to decomposition of **7** into the tetrahydroisoquinoline **3** and its oxidized derivatives **41**, **42** and **43**. A second hydrolysis attempt at rt with HCl 2N and isopropanol did not improve the reaction. A third hydrolysis attempt with an acidic resin instead of HCl afforded starting material only (Scheme 22).
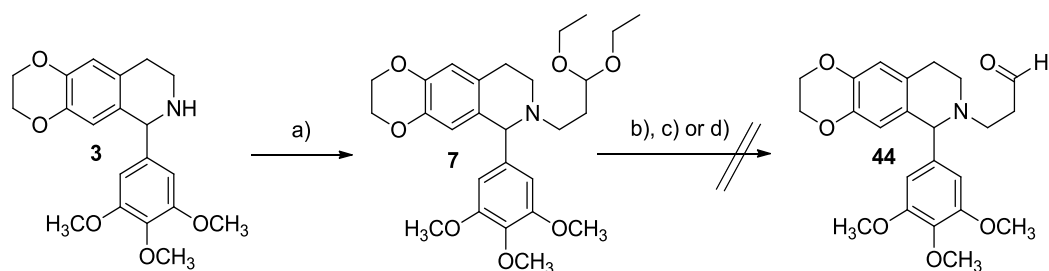
Scheme 22. Synthesis attempt of aldehyde **44**. Reagents and conditions: a) 3-Chloropropionaldehydediethylacetal (7.5 eq), Et$_3$N (4 eq), KI (0,1 eq), DMF, 5 days, 15%. b) HCl (8 mL of a 2N aqueous solution), EtOH, rt, 16 h, decomposition of **7** to **3**. C) HCl (8 mL of a 2N aqueous solution), isopropanol, rt, 16 h, no reaction. d) acidic resin (1 eq), EtOH, 16 h, no reaction.

3.2.1.8. Synthesis attempt of 46 and 47

Firstly, the acylation of the tetrahydroisoquinoline **3** with ethyl chloroformate and Et$_3$N led to the carbamate **45** in satisfactory yield. Nucleophilic substitution of the ester **45** with piperazine, HOBt and Et$_3$N in DCM at rt afforded starting material only. Replacing DCM by DMF and heating at 120 °C for 16 h did not improve the reaction. According to these results, an acylation reaction of **3** with 1-methylpiperazine instead of piperazine was attempted in the same conditions as before but starting material only was recovered. Replacing THF by DMF and heating to 100 °C for 16 h did not improve the reaction. An attempt to prepare the urea **47** from the amine **3** and ethyl-4-methyl-1-piperazinecarboxylate was carried out in presence of DMP at reflux of THF for 16 h, but only starting material was afforded (Scheme 23).
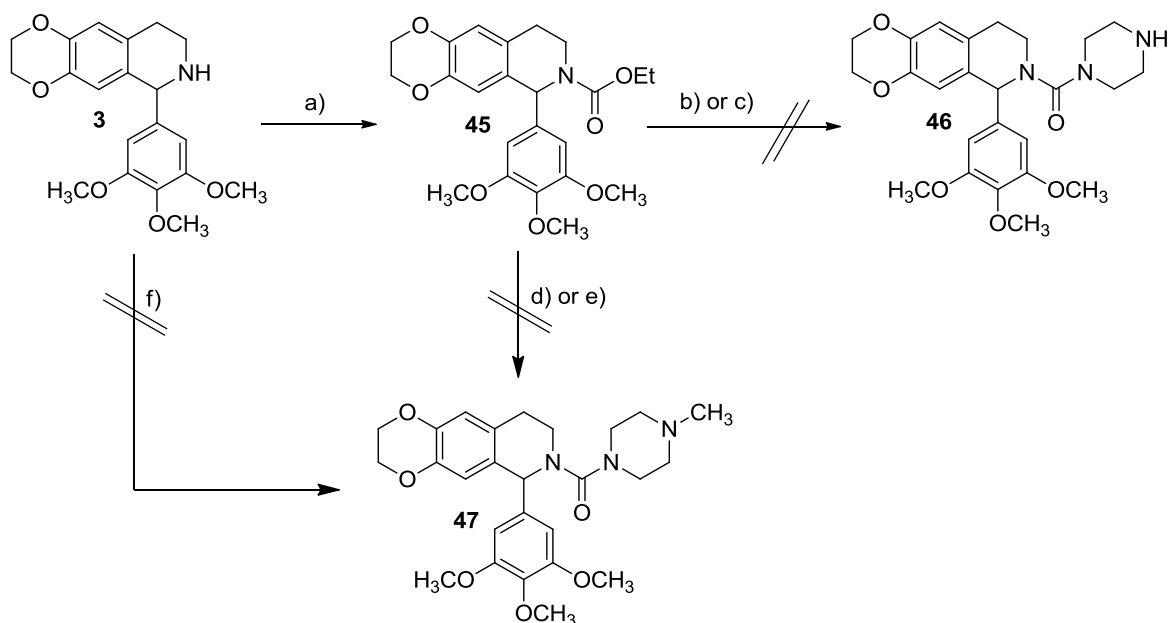
Scheme 23. Synthesis attempts of ureas **46** and **47**. Reagents and conditions: a) ethyl chloroformate (1.5 eq), Et$_3$N (1.5 eq), DCM, rt, 3 h, 53 %. b) Piperazine (3 eq), Et$_3$N (1.5 eq), HOBt (1.5 eq), DCM, reflux, 16 h, no reaction. c) Piperazine (3 eq), Et$_3$N (1.5 eq), HOBt (1.5 eq), DMF, reflux, 16 h, no reaction. d) 1-Methylpiperazine (3 eq), Et$_3$N (1.5 eq), HOBt (1.5 eq), THF, reflux, 16 h, no reaction. e) 1-Methylpiperazine (3 eq), Et3N (1.5 eq), HOBt (1.5 eq), DMF, reflux, 16 h, SM only. f) 4-Ethyl 4-methylpiperazine-1-carboxylate (1.5 eq), DMP (0.4 eq), THF, rt, 3 days, no reaction.

On the basis of these results, we came to the conclusion that the carbamate **45** is not reactive enough to undergo nucleophilic substitutions with secondary amines under the tested conditions.

3.2.1.9. Synthesis of the tetrahydroisoquinolines 8 and 9

The preparation of the tetrahydroisoquinolines **8** and **9** was accomplished by *N*-arylation of the tetrahydroisoquinoline **3**, following a general procedure previously described in the literature.[71,72] The tetrahydroisoquinoline **3** reacted with 1-bromo-4-nitrobenzene in the presence of Pd[(*o*-tolylP)$_3$P]$_2$Cl$_2$, (±)-BINAP and 2 equivalents of Cs$_2$O$_3$ in toluene yielding **8** in 33% yield. In the same way, the *N*-arylation of **3** with 4-bromobenzonitrile afforded **9** in 26% yield (Scheme 24). The low yields should be attributed to the hindrance provided by the trimethoxyphenyl substituent at the C-1

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[71] Y. Harrak, M. Romero, P. Constans, M. D. Pujol. *Lett. Org. Chem.* **2006**, *3*, 29-35.

[72] M. Romero, Y. Harrak, J. Basset, L. Ginet, P. Constans, M. D. Pujol. *Tetrahedron* **2006**, *60*, 9010-9016.

position as well as the high temperature required for this *N*-arylation that favoured the formation of the aromatized isoquinoline **43**.
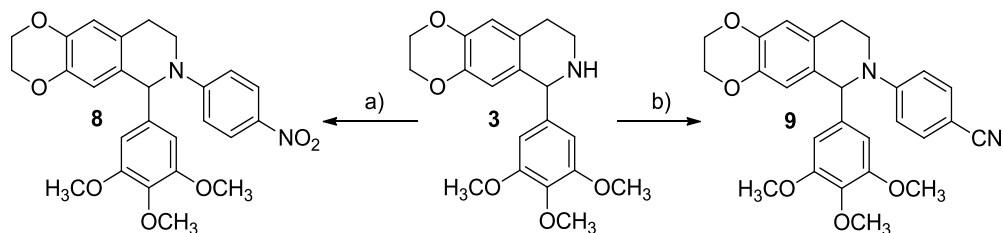
Scheme 24. Reagents and conditions: a) 1-bromo-4-nitrobenzene (1.2 eq), Pd[(*o*-tolyl)$_3$P]$_2$Cl$_2$ (cat), Cs$_2$CO$_3$ (2 eq), (±)-BINAP (cat), toluene, 110 °C, 72 h, 33% yield. b) 4-bromobenzonitrile (1.2 eq), Pd[(*o*-tolyl)$_3$P]$_2$Cl$_2$ (cat), Cs$_2$CO$_3$ (2 eq), (±) BINAP (cat), toluene, 110 °C, 24 h, 26% yield.

Compounds **1, 4, 5, 6, 7, 8** and **9** were submitted to the Lilly Laboratories (Indianapolis, USA) for biological testing to estimate their antitumor activities and to study their mechanisms of action.

3.2.1.10. Synthesis attempt of 48

The *N*-arylation of the tetrahydroisoquinoline **3** with 4-bromothioanisole, Pd[(*o*-tolyl)$_3$P]$_2$Cl$_2$, *(±)* BINAP and Cs$_2$O$_3$ only led to the aromatized isoquinoline **43** due to the lack of reactivity of 4-bromothioanisole compared to 1-bromo-4-nitrobenzene or 1-bromo-4-cyanobenzene (Scheme 25). Indeed, *N*-arylation reactions are more efficient when the aromatic halogen bears an electron withdrawing group in the *para* position and lower or no reactivity was found with an electron donor group.
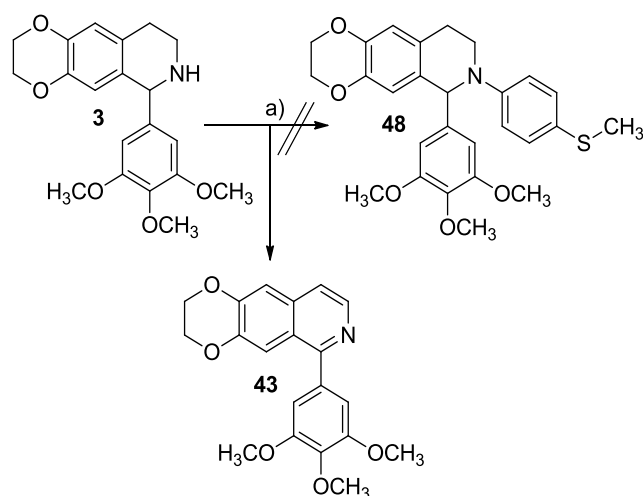
Scheme 25. Synthesis attempt of **48**. Reagents and conditions: a) 1-Bromo-4-cyanobenzene (1.2 eq), Pd[(*o*-tolyl)$_3$P]$_2$Cl$_2$ (cat), Cs$_2$CO$_3$ (2 eq), (±) BINAP (cat), toluene, 110 °C, 72 h, aromatization of **3** to **43**.

3.2.2. Synthesis of dimethoxy-isoquinoline analogues

3.2.2.1. Retrosynthetic analysis of the dimethoxy-isoquinoline 10 and 11

In order to determine if the substitution of the 1-4-dioxane nucleus by a dimethoxy group results in an increase or decrease in biological activity, two dimethoxy-isoquinoline analogues were synthesized from the commercially available 2-(3,4-dimethoxyphenyl)ethylamine **50** following the same experimental protocol used for the preparation of **3**. The dimethoxy-isoquinoline **10** and **11** can be made from the dimethoxy-isoquinoline intermediate **49**, which would be obtained by cyclization of the phenethylamine **50** with 3,4,5-trimethoxybenzaldehyde (**29**) (Scheme 26).
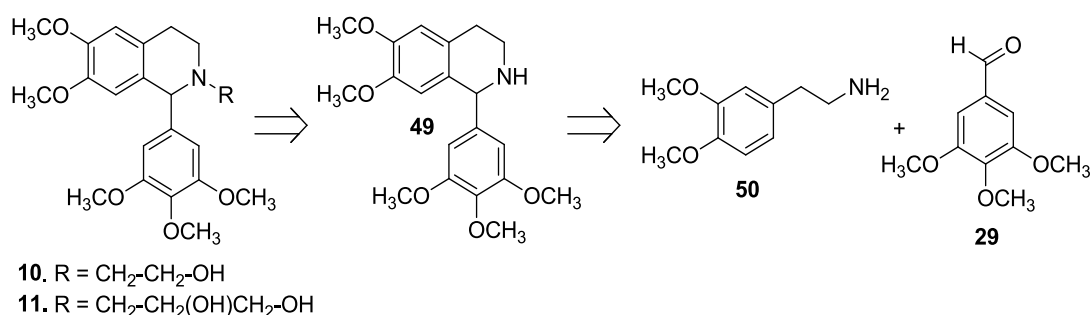
Scheme 26. Retrosynthetic analysis of the dimethoxy-isoquinolines **10** and **11**

3.2.2.2. Synthesis of the isoquinoline 49 from the arylethylamine 50

3,4,5-Trimethoxybenzaldehyde (**29**) and 2-(3,4-dimethoxyphenyl)ethylamine (**50**) were coupled in an amination reaction, followed by intramolecular cyclization of the corresponding imine with TFA and TFAA to afford the amide **51** in moderate yield. The tetrahydroisoquinoline **49** was prepared from hydrolysis of **51** with NaOH 2N in good yield (Scheme 27).
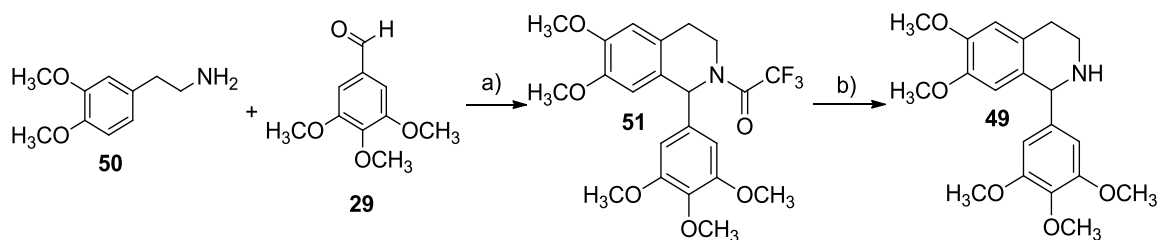
Scheme 27. Reagents and conditions: a) toluene, PTSA, Dean Stark, 16 h, reflux. b) TFA, TFAA, rt, 16 h, 33%. c) NaOH 2N/MeOH 7:3, reflux, 16 h, 97%.

3.1.2.3. Alkylation of the dimethoxy-isoquinoline 49

In continuation of our study of the synthesis of tetrahydroisoquinolines possessing potential cytotoxic activity, the dimethoxy-isoquinoline **49** was alkylated giving two new compounds. First, treatment of **49** with 2-bromoethanol in classical conditions led to the alcohol **10** in low yield (Scheme 28). The reaction was performed at room temperature because of the same stability problems than the amine **3**.
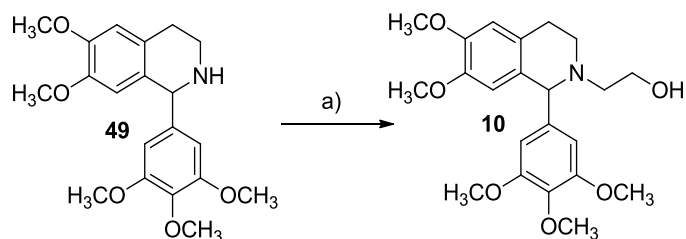
Scheme 28. Reagents and conditions: a) 2-chloroethanol (25 eq over 7 days), Et₃N (8 eq), KI (0,1 eq), rt, DMF, 10 days, 16%.

Similarly, the alkylation of **49** with *(±)*-epichlorohydrin in the presence of Et₃N and KI led to the intermediate epoxide **52** in moderate yield, which was hydrolyzed with NaOH 2N to afford the diol **11** in moderate yield (Scheme 29).
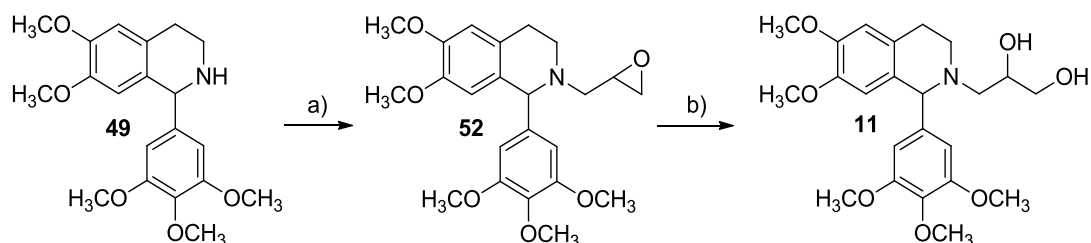
Scheme 29. Reagents and conditions: a) *(±)*-epichlorohydrin (9 eq over 2 days), Et₃N (8 eq), KI (0,1 eq), rt, DMF, 2 days, 45%. b) NaOH 2N/1,4-dioxane 5:2, rt, 2 days, 48%.

The alcohol **10** and the diol **11** were submitted to the Lilly Laboratories (Indianapolis, USA) for biological testing to evaluate their antitumor activities.

3.3. Synthesis of *combretastatin* A-4 derivatives

3.3.1. Synthesis of the carboxylic acid 53, ester 54 and olefin 55a

3.3.1.1. Retrosynthetic analysis

The cyclized *combretastatin* A-4 analogues can be prepared from the carboxylic acid **53**, which would come from 3,4,5-trimethoxyphenylacetic acid **56** by condensation with 3-benzyloxy-4-methoxybenzaldehyde **57** (Scheme 30). We expected that this molecular modification reveals some detailed and structural information to predict other series of analogues.

12. X = CH$_2$. **13.** X = O.
17. X = CONHNH

53

56

57
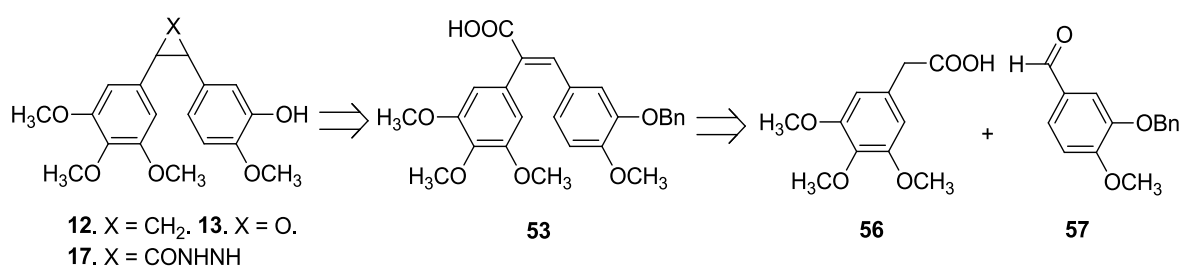
Scheme 30. Retrosynthetic analysis of *combretastatin* A-4 analogues from the monocyclic compounds **56** and **57**.

3.3.1.2. Synthesis of the carboxylic acid 53 and the ester 54

The multistep synthesis of the new *combretastatin* A-4 analogues required the preparation of the carboxylic acid **53** and the ester **54** as starting material. *O*-Benzylation of 3-hydroxy-4-methoxybenzaldehyde **58** with benzyl bromide provided **57** in quantitative yield. The corresponding aldehyde **57** was then coupled with 3,4,5-trimetoxyphenylacetic acid (**56**) in a modified *Perkin* reaction according to the literature procedure[73,74] to form the *trans* carboxylic acid **53** in moderate yield. Only the *trans* isomer **53** was isolated, while no sign of the *cis* isomer was detected according to the NMR data. The carboxylic acid **53** was treated with CH$_3$I and K$_2$CO$_3$ affording the alkyne ester **49** in quantitative yield (Scheme 31).

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[73] K. C. Sanko, L. Illes, K. Felfoeldi, J. Kiss, P. Sipos, I. Palinko. *J. Mol. Struct.* **2011**, *993*, 259-263.

[74] A. Khalaf, I. M. Awad, I. M. El-Emary, T. I. Abd El-Aal. *J. Ind. Chem. Soc.* **2010**, *87*, 595-600.
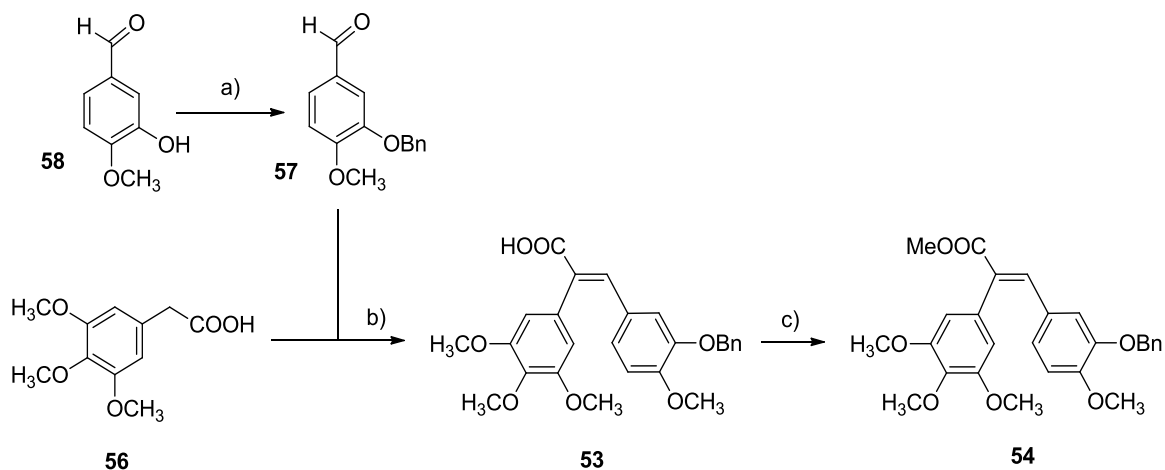
Scheme 31. Synthesis of carboxylic acid **53** and alkyne ester **54**. Reagents and conditions: a) BnBr (1.5 eq), K$_2$CO$_3$ (1.5 eq), DMF, 80 °C, 16 h, 94%. b) Ac$_2$O, Et$_3$N, 6 h, 140 °C, 27%. c) CH$_3$I (2.5 eq), K$_2$CO$_3$ (2 eq), DMF, rt, 16 h, 95%.

A mechanism of the modified *Perkin* reaction can be the below indicated (Scheme 32).
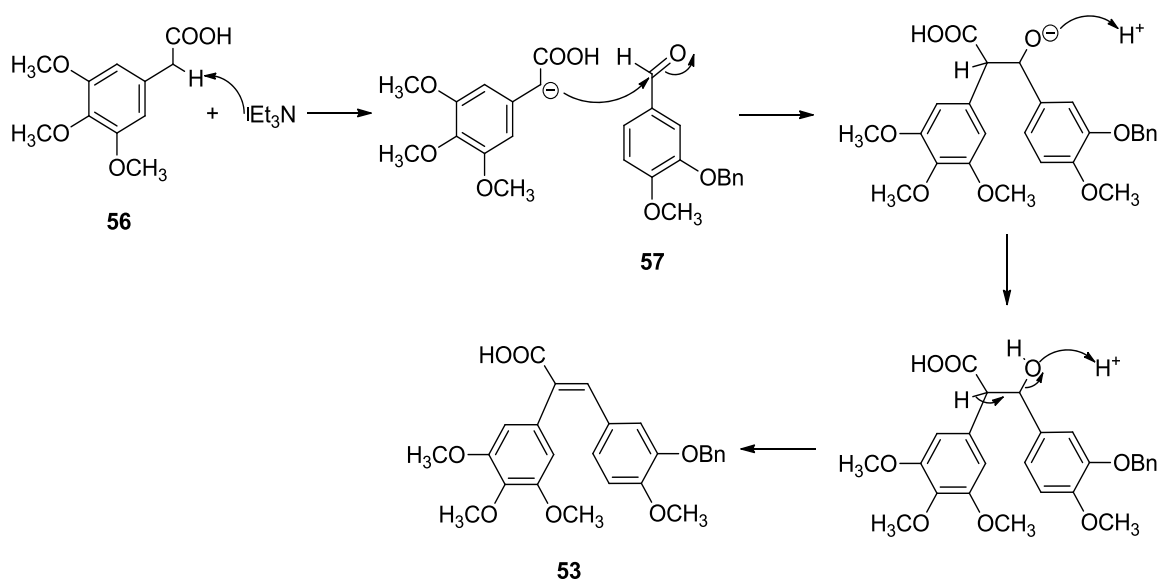
Scheme 32. Mechanism of the modified *Perkin* reaction

3.3.1.3. Synthesis of the olefin 55a

The carboxylic acid **53** was decarboxylated using quinoline and copper in a microwave mediated reaction to form a mixture of the *cis* olefin **55a** in 75% yield and the *trans* olefin **55b** in 20% yield. Alternatively, this reaction can be performed in standard heating conditions to form **55a** in 60% yield and **55b** in 19% yield (Scheme 34). **55b** was converted to **55a** in a photochemical isomerization reaction using benzil in 60% yield

according to the literature procedure (Scheme 33).[75] This conversion was confirmed by the corresponding NMR data (^{1}H and ^{13}C spectra).
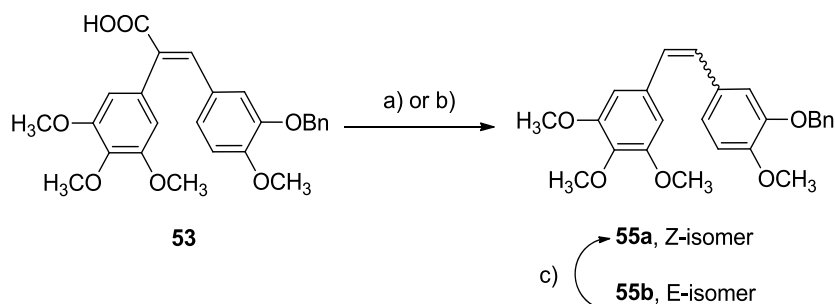
Scheme 33. Synthesis of olefin **55a**. Reagents and conditions: a) Quinoline, Cu (cat), microwave oven, 230 °C, 100 psi, 10 min, **55a** 75%, **55b** 21%. b) Quinoline, Cu (cat), 230 °C, 6 h, **55a** 60%, **55b** 22%. c) Benzil (5 eq), benzene, 254 nm UV, 5 h, 60%.

3.3.2. Synthesis attempts of the carbocylic acid 21, 22 and 23

3.3.2.1. Retrosynthetic analysis

Similarly, we took an interest in the preparation of *combretastatin* A-4 analogues which contain a sulfonyl group instead of the trimethoxy moiety (Scheme 34).
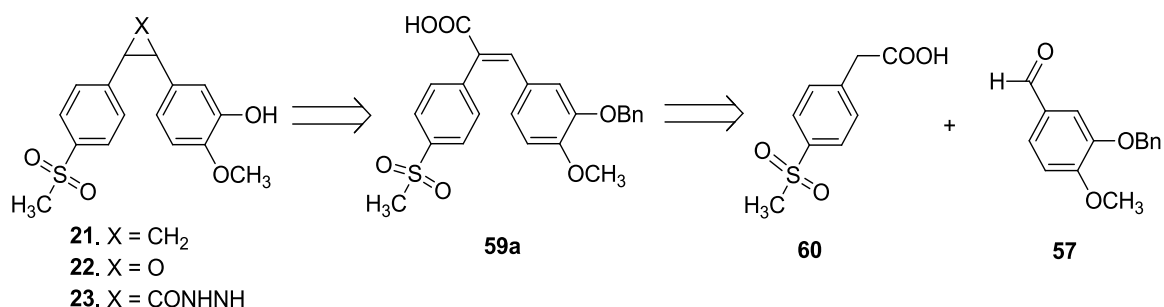
Scheme 34. Retrosynthetic analysis of sulfonyl *combretastatin* A-4 analogues from carboxylic acid **60** and benzaldehyde **57**.

3.3.2.2. Synthesis of 59a from the carboxylic acid 60

A number of attempts were made to synthesize the carboxylic acid **59a** from 4-(methylsulfonyl)phenylacetic acid (**60**). Firstly, a Perkin reaction with **60**, 3-benzyl-4-methylbenzaldehyde **57** and Et$_3$N in Ac$_2$O led to *(E)* and *(Z)* carboxylic acid **59a** and **59b** in very low yield. Starting material **60** and **57** were recovered. A *Perkin* reaction with DBU instead of Et$_3$N did not improve this condensation (Scheme 35).

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[75] G. R. Pettit, C. R. Anderson, D. L. Herald, M. K. Jung, D. J. Lee. *J. Med. Chem.* **2003**, *46*, 525-531.
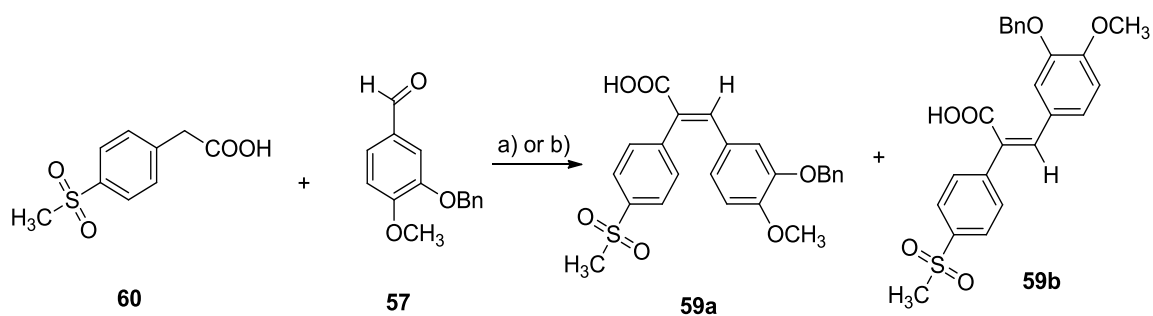
Scheme 35. Synthesis of **59**. Reagents and conditions: a) Et₃N (2 eq), Ac₂O, reflux, 8 h, **59a** and **59b** (5%). b) DBU (2 eq), Ac₂O, reflux, 8 h, **59a** and **59b** (3%).

The very low yield can be explained by the lack of reactivity of **60** due to the sulfone group which stabilizes the carbanion intermediate formed by deprotonation of the hydrogen in alpha of the carboxylic acid group.

3.3.2.3. Alternative synthesis attempts of the carboxylic acid 59a

On the basis of the results exposed in the previous section, an alternative route was tried to afford the carboxylic acid **59a**. A *Perkin* reaction of 4-(methylthio)phenylacetic acid (**62**) with 3-benzyl-4-methylbenzaldehyde (**57**) followed by oxidation of the corresponding methylthiobenzene **61** would afford the methylsulfone **59a** (Scheme 36).
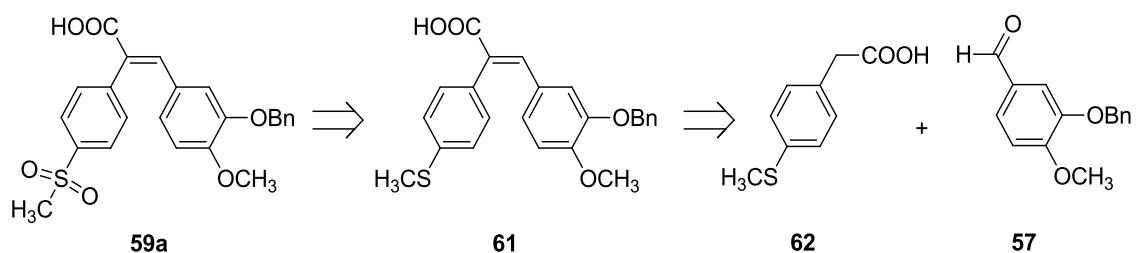
Scheme 36. Retrosynthetic analysis of the carboxylic acid 59a A-4 from the carboxylic acid **62** and the aldehyde **57**.

The 4-(methylthio)phenylacetic acid (**62**) was prepared in a 3-step synthesis starting from (methylthio)benzene (**63**). Acylation of **63** with mono-ethyl oxalyl chloride followed by hydrolysis of the corresponding ester (**64**) with NaOH led to 2-(4-(methylthiophenyl)-2-oxoacetic acid **65** in good yield. 2-(4-(Methylthiophenyl)acetic acid (**62**) was synthesized from **65** by a *Wolff-Kishner* reduction in satisfactory yield (Scheme 36).
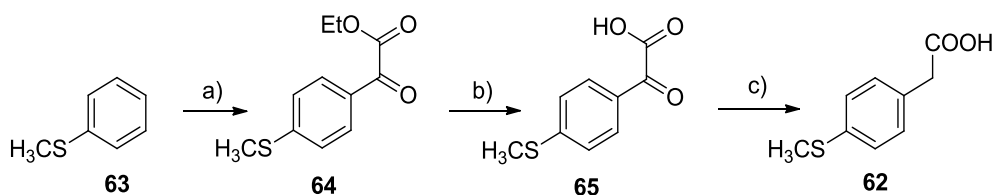
Scheme 36. Preparation of 2-(4-(methylthiophenyl)acetic acid (**61**) from (methylthio)benzene (**62**). Reagents and conditions: a) ethyl chlorooxoacetate (1.1 eq), TiCl$_4$ (3.5 mL), DCM, rt, 16 h, 50%. b) NaOH / EtOH (7:3), reflux, 16 h, 78%. c) KOH (2.5 eq), hydrazine hydrate (2.3 eq), diethylene glycol, 200 °C, 4 h, 79%.

Finally, a Perkin reaction with 4-(methylsulfonyl)phenylacetic acid (**62**) and 3-benzyl-4-methylbenzaldehyde (**57**) in the presence of Et$_3$N in Ac$_2$O led to recuperation of the starting materials only (Scheme 37).
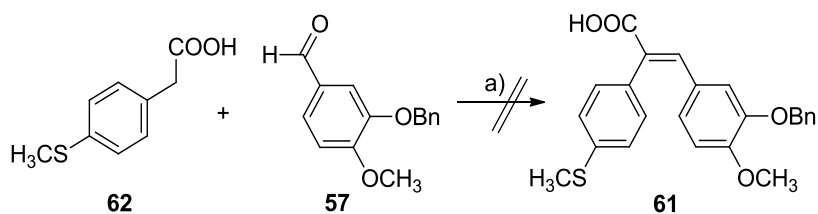
Scheme 37. Synthesis attempt of **65**. Reagents and conditions: a) Et$_3$N (2 eq), Ac$_2$O, reflux, 8 h, starting materials only.

These results show that the *Perkin* reaction is difficult to perform with methylsulfones and methylthio carboxylic acids.

3.3.3. Cyclopropanation attempts of the olefin 55a

3.3.3.1. Classical synthesis attempts of the cyclopropane 66

Several attempts were made to synthesize the cyclopropane **66** from the olefin **55a** using reaction conditions previously described in the literature[76,77,78] (Table 2). A Zn(Cu) catalyzed cyclopropanation with CH$_2$I$_2$ in DCM heated to reflux for 1 week recovered the starting material. A cyclopropanation reaction using trimethylsulfonium iodide and NaH in DMSO at 150 °C for 16 h recovered only the starting material. The microwave assisted cyclopropanation of **55a** according to the procedure described in

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[76] H. E. Simmons, D. S. Ronald. *J. Chem. Soc*. **1959**, *81*, 4256-4264.

[77] G. Chen, S. J. Cho, X. Huang, N. H. Jensen, A. Svennebring, M. F. Sassano, B. L. Roth, A. P. Kozikowski. *ACS Med. Chem. Lett*. **2011**, 929-932.

[78] A. G. Whittaker, D. M. P. Mingos. *J. Chem. Soc*. **2002**, 3967-3970.

the literature did not improve the reaction. Heating at 150 °C for one week showed partial consumption of the starting material and formation of a product that was identified by mass spectrum and ^{1}H NMR data as the C-2 iodinated compound derivative (MS (EI) m/z = 532). Replacing the base NaH by t-BuOK or MeONa in order to allow better deprotonation of the trimethylsulfonium iodide did not improve the reaction. The trimethylsulfonium iodide used in this reaction was previously prepared from dimethylsulfoxide and CH_3I as reported in the literature.[79] Finally, a cyclopropanation reaction of **55a** with Pd(OAc)$_2$, N-methyl-N-nitrosourea and NaOH was attempted according to the procedure described in the literature[80] to form diazomethane in-$situ$. This reaction led to (E)-isomerization of **55a** in a 26% yield. Starting material was also recovered.

Table 2. Reaction conditions of the cyclopropanation of olefin **15**
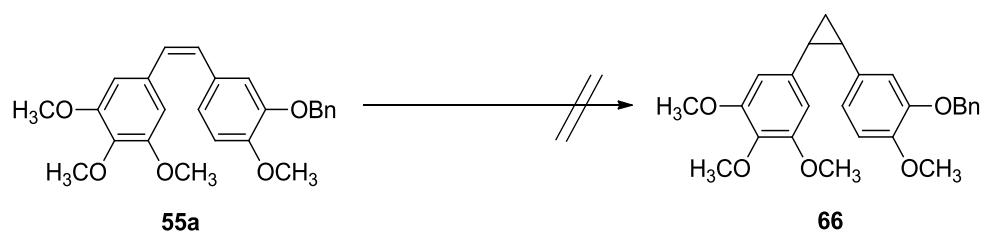
Entry	Reagents	Reaction conditions	Observations
1.	CH_2I_2, Zn(Cu), CH_2Cl_2	Reflux, 1 week	Starting material
2.	Trimethylsulfonium iodide, NaH, DMSO	150 °C, 16 h	Starting material
3.	Trimethylsulfonium iodide, NaH, DMSO	Microwaves, 150 °C, 10 min, 60 psi	Starting material
4.	Trimethylsulfonium iodide, t-BuOK or MeONa, DMSO	80 °C, 72 h	Starting material
5.	Trimethylsulfonium iodide, NaH, DMSO	150 °C, 1 week	Starting material and the C-2 iodinated by-product
6.	N-methyl-N-nitrosourea, Pd(OAc)$_2$, KOH, ether	4 h at 0 °C and 16 h at rt	Trans-isomerization and starting material only.

[79] C. Leslie, D. J. Salmon, W. Donald. $J.$ $Chem.$ $Soc.$ **1971**, 2, 304-312.
[80] F. D. Ozdermirhan, M. Celik, S. Ath, C. Tanyeli. $Tetrahedron$ **2006**, 17, 287-291.

3.3.3.2. Alternative cyclopropanation attempts

On the basis of these results, alternative cyclopropanations were attempted according to the literature procedure.[81] The use of ethyl diazoacetate catalyzed by copper did not afford the desired cyclopropane derivative **67**. Ethyl diazoacetate was prepared from the commercially available glycine ethyl ester hydrochloride, $NaNO_2$ and H_2SO_4 as reported in the literature (Scheme 38).[82]
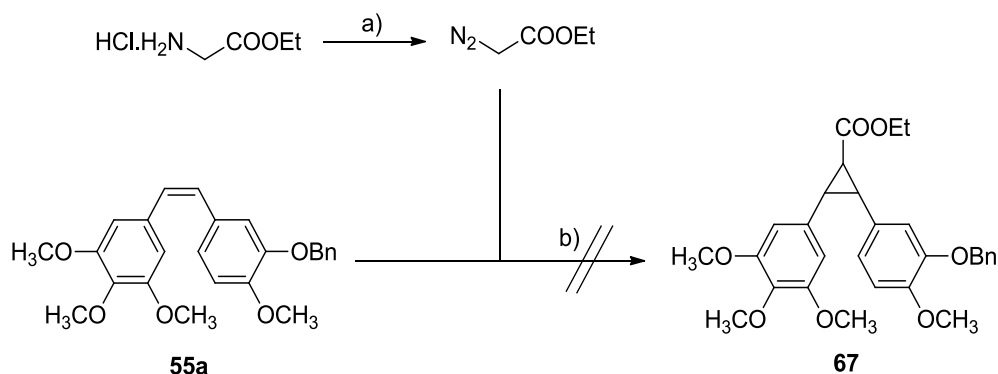
Scheme 38. Copper-catalyzed olefin cyclopropanation of **55a**. Reagents and conditions: a) $NaNO_2$ (1.1 eq), H_2SO_4 (5 mL of an aqueous solution), H_2O (2 mL), DCM (10 mL), rt, 15 min, 82%. b) Ethyl diazoacetate (24 eq), $Fe_2(CO)_9$ (1 eq), CuI (cat), DCM, rt, 16 h, inaltered starting material was recovered.

Secondly, the cyclopropanation reaction using dichlorocarbene prepared *in situ*, chloroform and KOH recovered starting material only.[83] Using *t*-BuOK instead of KOH did not improve the reaction (Scheme 39).
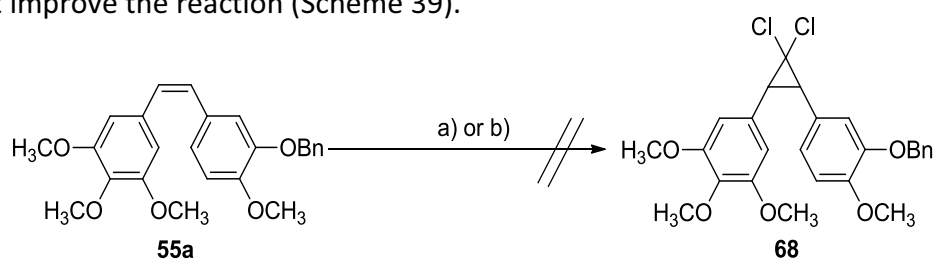
Scheme 39. Cyclopropanation reaction of **55a** with dichlorocarbene. Reagents and conditions: a) KOH (20 eq), $CHCl_3$ (10 mL), reflux, 3 days, starting material only. b) *t*-BuOK (20 eq), $CHCl_3$ (10 mL), reflux, 3 days, inaltered starting material was recovered.

These results showed that the central double bond is not reactive enough in the conditions applied to undergo cyclopropanation reactions. In the future, this reaction

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[81] M. M. Diaz-Requejo, A. Caballero, T. R. Belderrain, M. C. Nicasio, S. Trofimenko, P. J. Pérez. *J. Am. Chem. Soc*. **2002**, *124*, 978-983.

[82] K. Shanmugan, T. Balakrishan. *Ind. J. Org. Chem*. **2007**, *7*, 1069-1074.

[83] J. D. Clark, A. S. Shah, J. C. Peterson. *Thermochim. Acta* **2002**. 177-186.

will be attempted under microwave assistance at high pressure and temperature in an attempt to force the reaction conditions.

3.3.4. Epoxidation of the olefin 55a

Several epoxidation attempts of olefin **55a** were made under different classical conditions, as reported in the literature.[84,85] Firstly, the epoxidation reaction with *m*CPBA afforded a product that was identified by ^{1}H-NMR as the diol **70** obtained by hydrolysis of the epoxide **69**. Indeed, due to the bonded aromatic substituents, the epoxide **69** is unstable and reacts very easily with nucleophilic groups thereby increasing its stability. Addition of a base (K_2CO_3 or *t*-BuOK) did not improve this reaction. (Table 3).
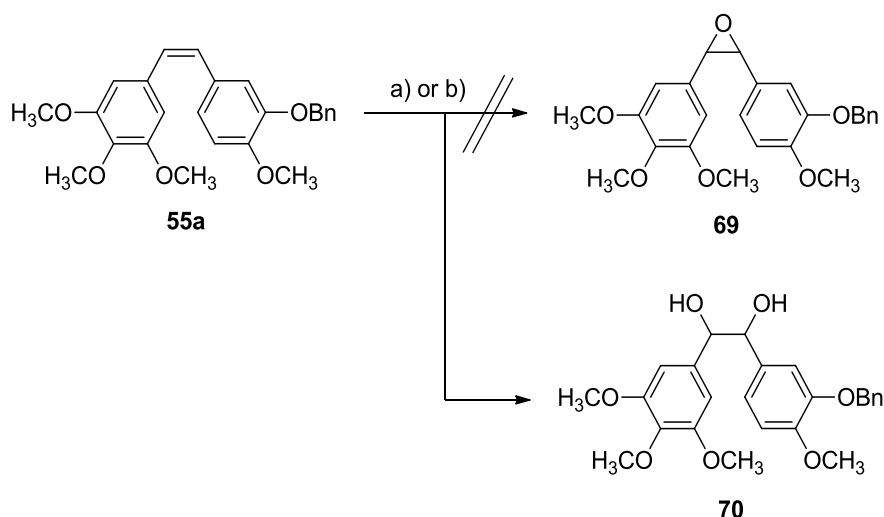
Scheme 40. Epoxidation attempts of olefin **55a**. Reagents and conditions: a) *m*CPBA (1.2 eq), CH_2Cl_2, rt, 48 h, **70**, 76%. b) *m*CPBA (1.2 eq), K_2CO_3 (1.2 eq) or *t*-BuOK (1.2 eq), rt, 48 h, **70**, 79%.

The results showed that the epoxide **69** is not stable enough and easily hydrolyzed in more stabilized compounds in the conditions applied.

Different reaction conditions employing NBS, $AcOH/H_2O$ 7:3, 1,4-dioxane and Na_2CO_3 led to the recuperation of the starting material and formation of a by-product that was identified by ^{1}H NMR and MS as the C-2 bromine derivative **71** (MS (EI) *m/z* = 486) (Scheme 41).

[84] C. Dallanoce, P. Magrone, P. Bazza, G. Grazioso, L. Rizzi, L. Riganti, C. Gotti, F. Clementi, K. Frydenvang, M. Amici. *Chem. Biodiversity* **2009**, *6*, 244-259.
[85] J. L. Garcia Ruano, C. Fajardo, A. Fraile, M. Rosario Martin. *J. Org. Chem*. **2005**, *70*, 4300-4306.
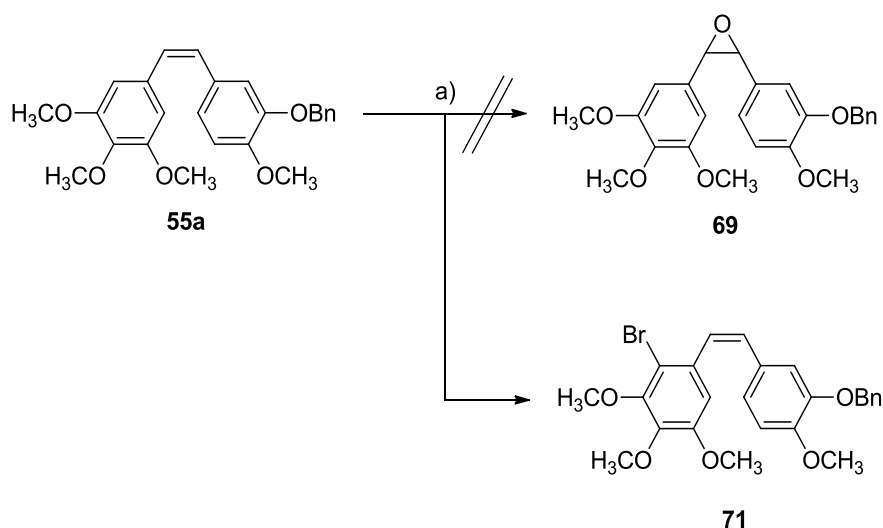
Scheme 41. Epoxidation attempts of olefin **55a**. Reagents and conditions: 1) NBS (1.2 eq, AcOH (1 eq), Dioxane/H$_2$O 7:3, 16 h. 2) Na$_2$CO$_3$ (2 eq), rt, *96* h, **55a** (65%), **71** (31%).

The results show that the C-2 position of **55a** is more reactive than the central olefin, making it difficult to selectively brominate the double bond in this position.

3.3.5. Preparation of pyrazolone derivatives

Pyrazolone derivatives are an important class of heterocyclic compounds present in many drug and synthetic products. The work presented in this section describes the preparation of pyrazolone derivatives from the previously synthesized carboxylic acid **53** and ester **54**. Firstly, a cyclization reaction with the ester **54** and phenylhydrazine as the nucleophile and solvent at 200 °C for 24 h afforded a new product that was identified by ^{1}H NMR as the non cyclized phenylhydrazide derivative. The intramolecular cyclization has not been detected. Secondly, a cyclization reaction was attempted with the ester **54,** hydroxylamine, HOB*t* and Na$_2$SO$_4$ in refluxing DCM for 1 week, which yielded only starting material. The addition of a base (K$_2$CO$_3$) did not improve this reaction. Finally, the cyclization of the carboxylic acid **53** with hydrazine hydrate, CSA and HOB*t* in MeOH at 66 °C for 72 hours yielded the corresponding five-membered-ring pyrazolone derivative **16** in 28% yield. Cyclization of **54** with an excess of hydrazine hydrate in MeOH at reflux temperature for 72 hours afforded **16** in 52% yield. (Table 4). [86,87]

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[86] M. F. Braña, A. Gradillas, A. G. Ovalles, B. Lopez, N. Acero, F. Llinares, D. M. Mingarro. *Bioorg. Med. Chem.* **2011**, *14*, 9-16.

Table 3. Reaction conditions of pyrazolone derivatives

53. R = H
54. R = Me

16. X = NH, Y = H
72. X = NH, Y = C$_6$H$_5$
73. X = O, Y = H
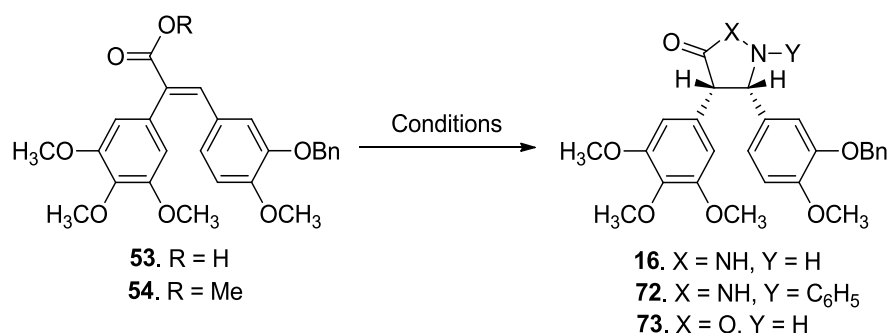
Entry	R	Reagents	Reaction conditions	Product X	Product Y	Observations
1.	Me	PhNHNH$_2$	200 °C, 24 h	NH	Ph	Non cyclized hydrazide
2.	Me	NH$_2$OH, HOBt, Na$_2$SO$_4$	DCM, reflux, 1 week	O	H	Starting material
3.	Me	NH$_2$OH, HOBt, K$_2$CO$_3$, Na$_2$SO$_4$	DCM, reflux, 1 week	O	H	Starting material
4.	H	NH$_2$NH$_2$.H$_2$O, CSA, HOBt	MeOH, reflux, 72 h	NH	H	**16** (28%)
5.	Me	NH$_2$NH$_2$.H$_2$O, Na$_2$SO$_4$	MeOH, reflux, 72 h	NH	H	**16** (52%)

^{1}H NMR (2.92 ppm (dd, 1H, *J* = 13 Hz, *J* = 6 Hz, CO-C<u>H</u>)) confirmed the (*Z*) configuration of **16** (Table 3). The (*E*) configuration isomer of **16** was not detected in any of the two studied cyclization reaction. The low yields obtained in entry 4 and 5 are explained by the formation of the vinyl pyrazolone by-product **74** in 14% and 16% yield respectively (Scheme 40).

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[87] B. Burja, M. Kocevar, S. Polanc. *Tetrahedron* **2009**, *65*, 8690-8696.
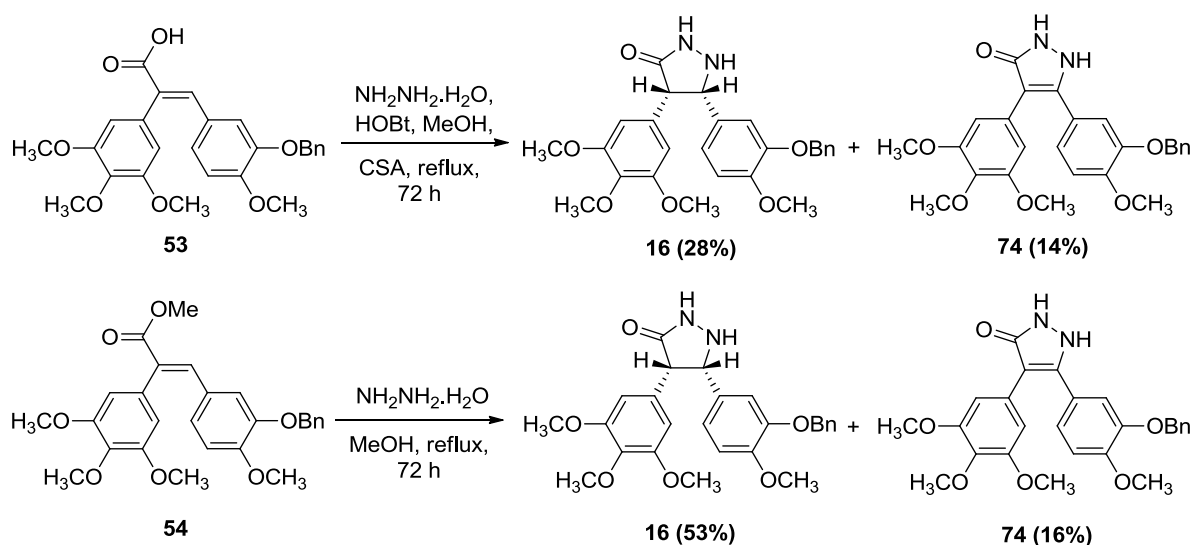
Scheme 40. Formation of the by-product **74**.

The pyrazolone **16** was *O*-debenzylated in a hydrogenolysis with addition of hydrogen gas catalyzed by palladium on charcoal to afford the corresponding deprotected phenol **17** in a moderate yield. Similary, the double reduction of the by-product **74** by hydrogenolysis catalyzed by palladium on charcoal afforded **17** in moderate yield (Scheme 41).
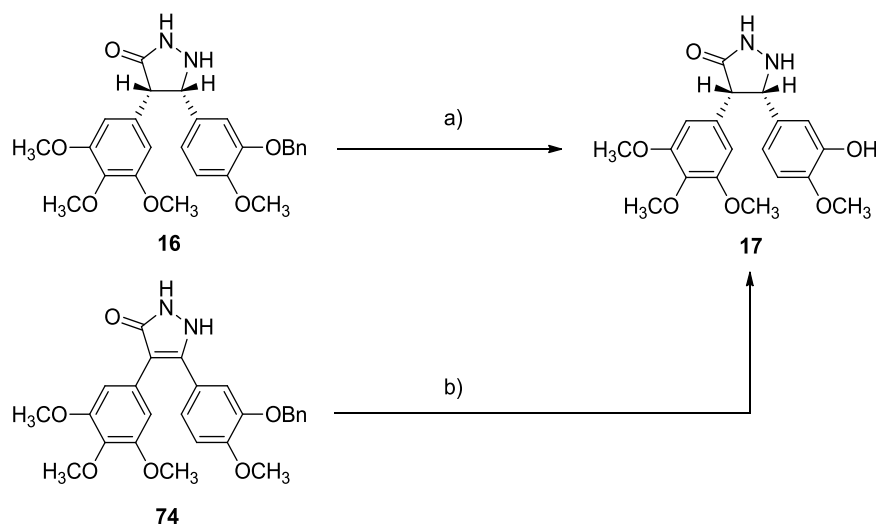
Scheme 41. Hydrogenation reaction of **16** and **74**. Reagents and conditions: a) Pd/C (10% m/m), HCl (40 µL of a 5N aqueous solution), H_2, EtOAc, rt, 16 h, 33%. b) Pd/C (10% m/m), HCl (40 µL of a 5N aqueous solution), H_2, EtOAc, rt, 16 h, 90%.

Compounds **16** and **17** were submitted to Lilly Laboratories (Indianapolis, USA) for biological testing.

3.3.6. Synthesis of 6 ring cyclized *combretastatin* analogues

3.3.6.1. Synthesis attempts to 1,2-oxazine derivatives

Several conditions were tested for the synthesis of 1,2-oxazine derivatives according to the literature procedure.[88] Firstly, a cyclization reaction of **54** with *N*-hydroxypropanimidoyl chloride and Et_3N in CH_2Cl_2 at rt for 5 h afforded starting material only. Another cyclization attempt with *N*-hydroxypropanimidoyl chloride, HOBt and DMAP in MeOH at 66 °C for 16 h yielded the C-2' chlorinated compound and no olefin cyclization was observed (Scheme 42). The *N*-hydroxypropanimidoyl chloride was previously prepared from commercially available propanal.[89]
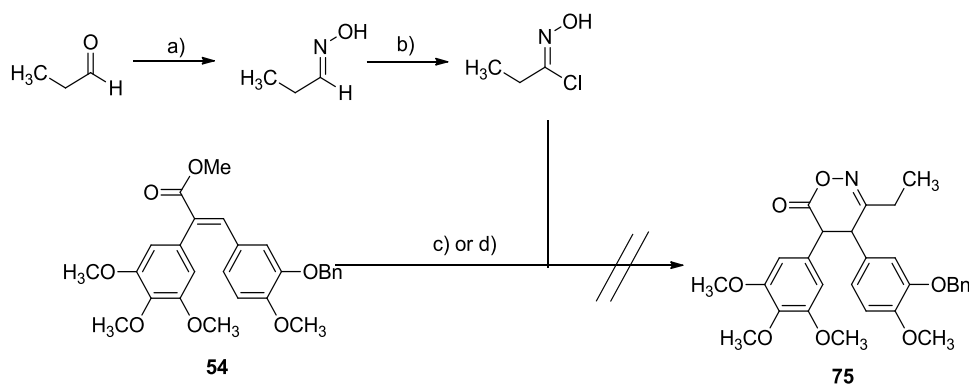
Scheme 42. Reagents and conditions: a) Hydroxylamine chloride (1 eq), NaOH (5 mL of a saturated aqueous solution), EtOH (15 mL), reflux, 2 h, 87%. b) NCS (1 eq), MeOH (20 mL), rt, 16 h, >100% (crude). c) *N*-hydroxypropanimidoyl chloride (20 eq), CH_2Cl_2 (30 mL , Et_3N (1.5 eq), 5 h, SM only. d) *N*-hydroxypropanimidoyl chloride (20 eq), HOBt (2 eq), MeOH (20 mL), 78 °C, 16 h, C-2'-chlorinated compound.

3.3.6.2. Synthesis of *combretastatin* naphtalene derivatives

A number of attempts to the synthesis of combretastatin naphtalene derivatives were carried out. Firstly, a *Diels Alder* reaction between **55a** and 1,3-diphenylisobenzofuran in toluene at 90 °C for 5 h yielded starting material **55a** and the diketone proceding from the opening of the diene (Scheme 43).

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[88] A. A. B. Robertson, N. P. Botting. *Tetrahedron* **1999**, 13269-13284.
[89] M. Gucma, W. M. Golebiewski, B. Morytz, H. Charville, A. Whitting. *Lett. Org. Chem.* **2010**, *7*, 502-507.
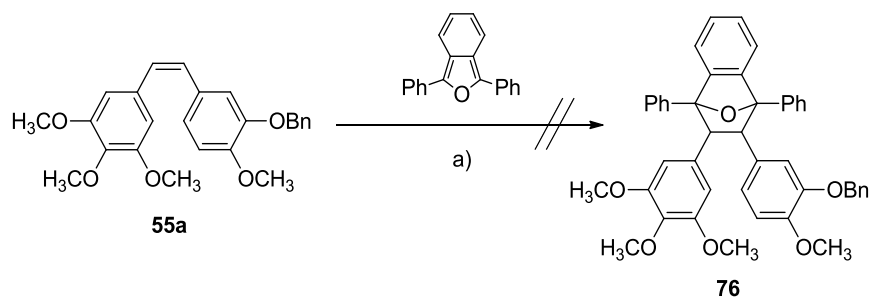
Scheme 43. *Diels Alder* cyclization reaction from **55a**. Reagents and conditions: 1,3-diphenylisobenzofuran (1 eq), toluene (10 mL), 90 °C, 5 h, SM and diketone only.

On the basis of these results, a second attempt with **55a** as starting material and Yb(OTf)$_3$ as catalyst in toluene at 90 °C during 5 h afforded starting material and the diketone only. Heating up to 100 °C for 72 h did not improve the reaction (Scheme 44).
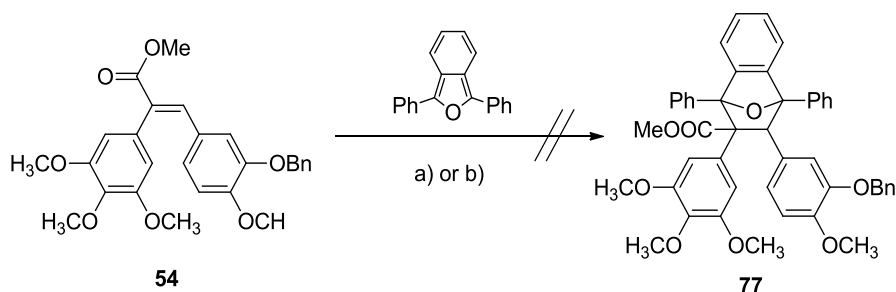
Scheme 44. Diels Alder cyclization reaction of ester **54**. Reagents and conditions: a) 1,3-diphenylisobenzofuran (1 eq), Yb(OTf)$_3$ (cat), toluene (10 mL), 90 °C, 5 h, SM and diketone only. b) 1,3-diphenylisobenzofuran (1 eq), Yb(OTf)$_3$ (cat), toluene (10 mL), 100 °C, 72 h, SM and diketone only.

3.3.7. Other combretastatin analogue synthesis

The benzoic condensation is a reaction between two aromatic aldehydes. This reaction is catalyzed by a nucleophile such as the cyanide anion (CN⁻) or other derivatives. The desired product is an aromatic acyloin. A possible mechanism of this reaction was proposed by A. J. Lapworth (Scheme 45).[90]

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[90] A. Lapworth. *J. Chem. Soc.* **1904**, *85*, 1206-14.
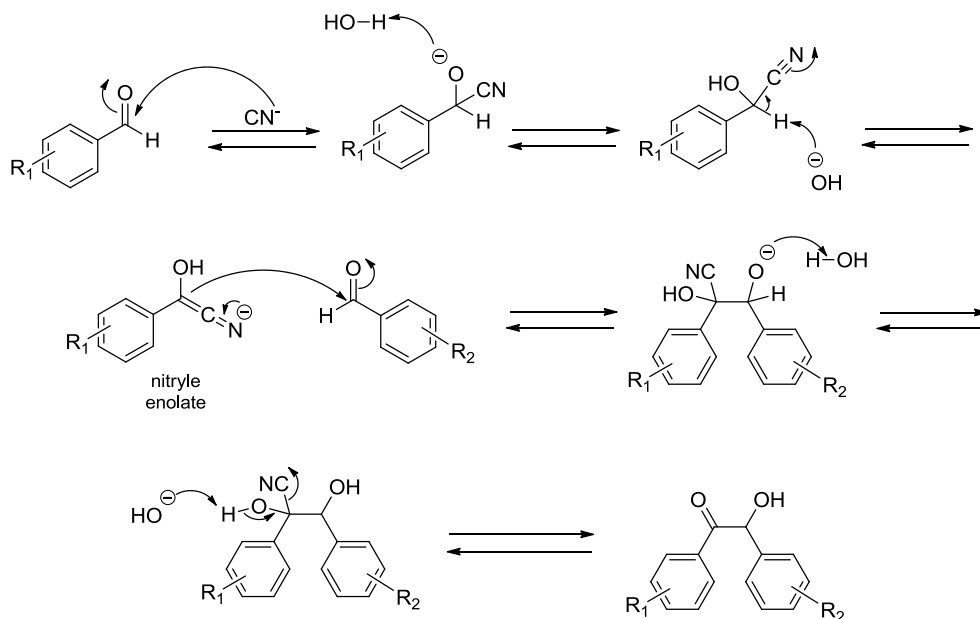
Scheme 45. Mechanism of the benzoic condensation.

A benzoic condensation attempt of 3,4,5-trimethoxybenzaldehyde (**29**) with 3-benzyl-4-methylbenzaldehyde (**57**) was carried out according to the literature procedure.[91] Treatment of the aldehydes **29** and **57** with NaCN and water in EtOH afforded 3 new products. The ^{1}H NMR spectrum of all the isolated products showed presence of the 2 starting materials and of 2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetonitrile, 2-(3-(benzyloxy)-4-methoxyphenyl)-2-hydroxyacetonitrile, and (*E*)-1,2-*bis*(3-benzyloxy)-4-methoxyphenyl)ethane (Scheme 46).
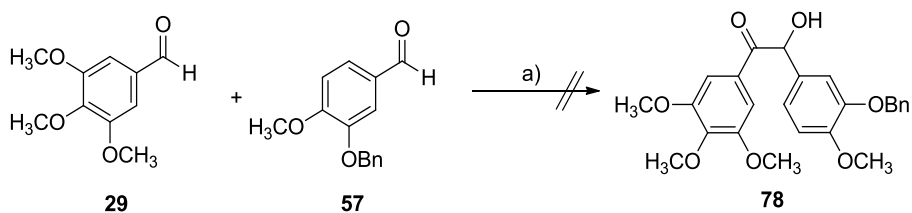
Scheme 46. Reagents and conditions: a) NaCN (1 eq), EtOH (20 mL), H_2O (10 mL), 78 °C, 16 h, complex mixture.

The singlet at 6 ppm of the CH in alpha of the alcohol of the desired product was not observed in any of the final products, which means that the desired product was not formed.

[91] J. H. Biel, E. P. Sprengeler, H. A. Leiser, J. Horner, A. Drukker, H. L. Friedman, *J. Am. Chem. Soc*, **1955,** *77*, 2250-2256.

3.3.8. Preparation of azoledione *combretastatin* derivatives

The azolediones, unsaturated imides, are interesting building blocks in organic synthesis and a class of compounds with several biological properties. In this section the preparation of new disubstituted azoledione derivatives or disubstitued maleimides related to *combretastatin* A-4 is reported. A very practical and efficient synthesis of the azoledione **15** was achieved from 2-oxo-2-(3,4,5-trimethoxyphenyl)acetic acid (**80**) and 4-benzyloxy-3-methoxyphenylacetic acid (**81**) (Scheme 47).
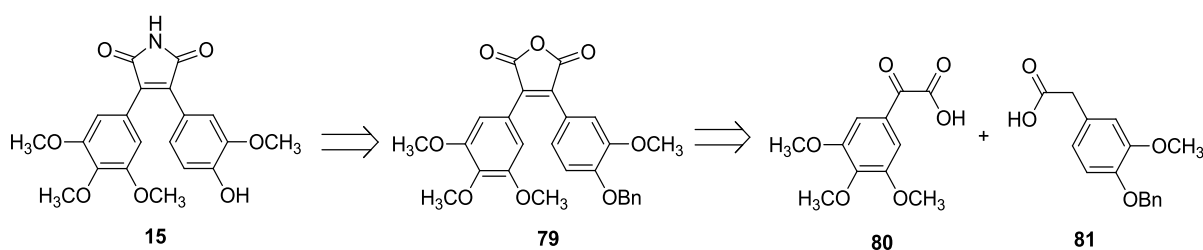
Scheme 47. Retrosynthetic analysis of the azoledione *combretastatin* analogue **15**.

This approach has been demonstrated to be efficient for the preparation of 2,3-disubstituted maleimides with hindered susbstituents.[92] This process implies formation of an anhydride which undergoes an intramolecular *Perkin*-type condensation to hydroxy imide followed by dehydratation to maleimide. Condensation of the ketoacid **80** with the arylacetic acid **81** in acetic anhydride at 130 °C for 3 h led to the isolation of the anhydride **79**. Due to stability problems of the anhydride group, the product was used crude without further purification. The treatment of the anhydride **79** with NH_3 (25% aqueous solution), NH_4Cl and NH_4OAc in DMF gave the disubstituted imide **14**, which was then involved in a hydrogenolysis reaction catalyzed by palladium on charcoal yielding **15** in satisfactory yield (Scheme 48).

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[92] Ruben Francisco Castillo. *Síntesis de maleimidas disubstituidas con potencial actividad antiangiogénica.* Tesis doctoral en curso*, **2013**, Facultad de Farmacia. Universidad de Barcelona.
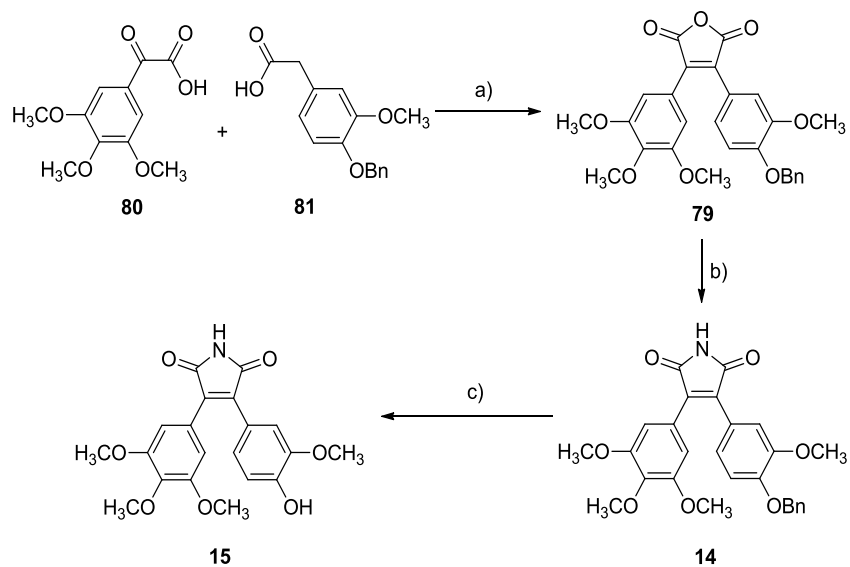
Scheme 48. Reagents and conditions: a) Ac$_2$O, 2 h, 150 °C (crude). b) NH$_3$ (25% aqueous solution) (20 eq), NH$_4$Cl (1 eq), NH$_4$OAc (1 eq), 125 °C, 16 h, 42% over two steps. c) Pd/C (10% m/m), HCl (40 µL of a 5N aqueous solution), EtOAc, H$_2$, 16 h, 92%.

Compounds **14** (*O*-protected) and **15** (*O*-deprotected) were submitted to the Lilly Laboratories (Indianapolis, USA) for biological testing to evaluate their biological properties and study the mechanism through which they produce antitumor activity.

3.4. Synthesis of dioxancarbazole derivatives

3.4.1. Retrosynthetic analysis

In our search for a good methodology orientated to the preparation of new dioxancarbazole derivatives, a retrosynthetic analytical approach starting from simple compounds is depicted in Scheme 32. The dioxancarbazole **21** and **22** were prepared from the corresponding alcohols **82** and **83**, which can be obtained by reduction of the esters **84** and **85** respectively. The intermediate esters **84** and **85** can be prepared in a single step from the aniline **86** and the bromoketoester **87** under the modified *Bischler* conditions (Scheme 49).[93] The *Bischler* indole synthesis is based on the monoalkylation of the corresponding aniline with the bromoketoester, followed by an intramolecular electrophilic cyclization.
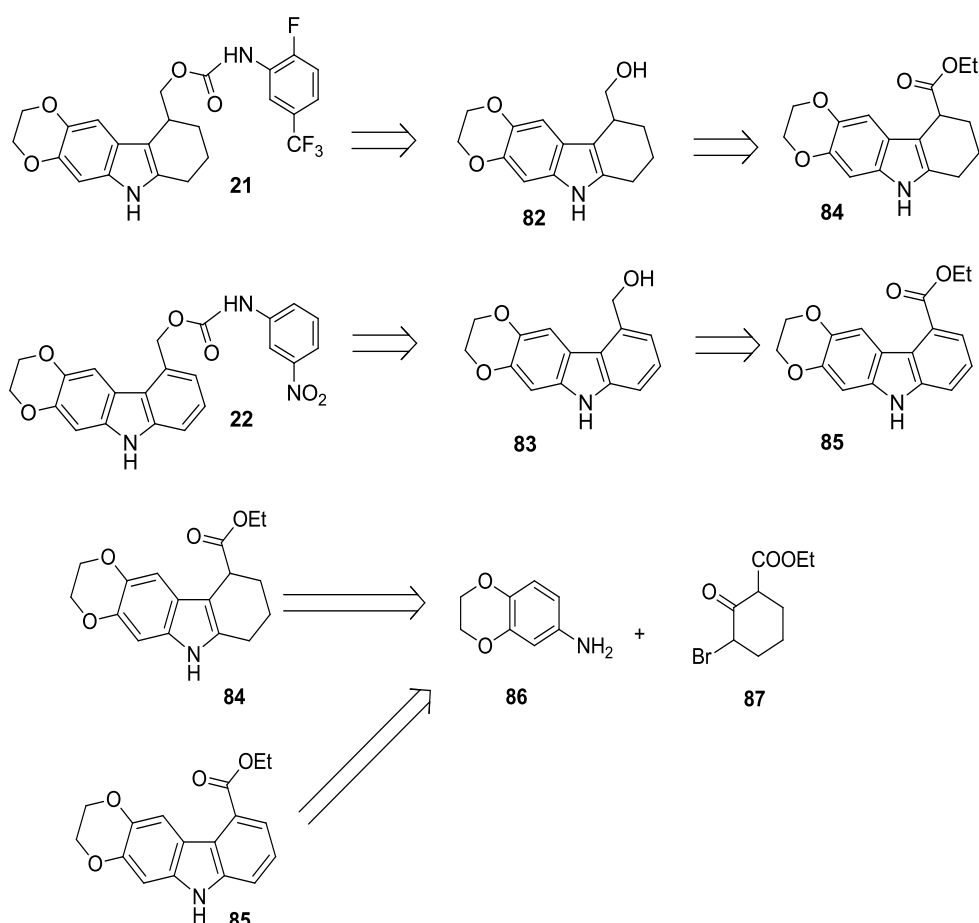
Scheme 49. Retrosynthetic analysis of **21** and **22**.

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[93] G. Fodor, S. Nagubendi. *Tetrahedron* **1980**, *36*, 1279-1300.

3.4.2. Synthesis of the dioxancarbazoles 21 and 22

This route began with bromination of the ketoester **88** with Br$_2$ according to the literature procedure to afford the bromo ketoester **87** in quantitative yield. **87** was identical in all aspects with that previously described.[94] The bromo ketoester **87** was condensed with 1,4-benzodioxan-6-amine in a modified *Bischler* reaction condition optimized recently by our research group (Scheme 50).[95]
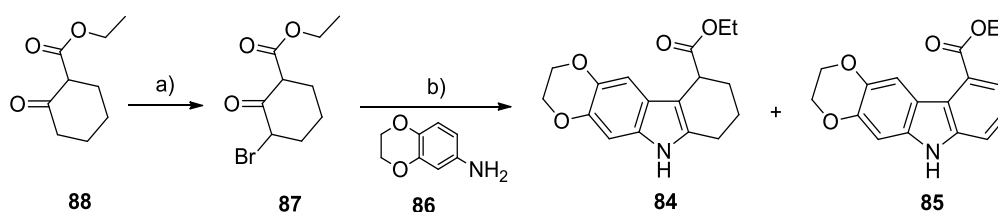
Scheme 50. Reagents and conditions: a) Br$_2$, CHCl$_3$, rt, 72 h, quant. b) 1,4-benzodioxan-6-amine (3 eq), *N*,*N*-dimethylaniline, 150 °C, 1 h, **84** (22%), **85**(12%).

The aliphatic ester **84** was obtained as the major product in 22% yield. The aromatic ester **85** was obtained in 12% yield. The ester **85** was formed because of the instability of **84** that easily aromatizes at high temperatures. The low yield obtained in this reaction is explained by the non-linear cyclization of the imine intermediate that leads to the orthogonal 1,4-dioxancarbazole by-product **89** (Scheme 51).
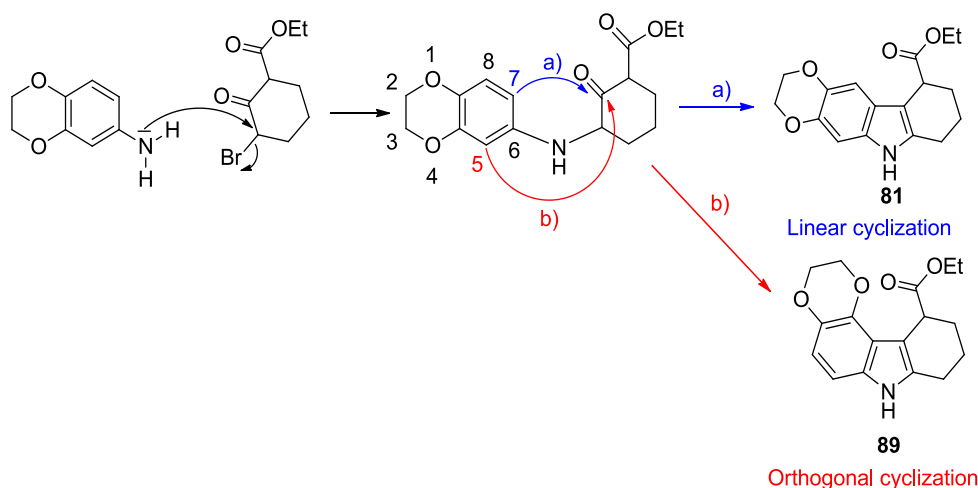
Scheme 51. Linear and orthogonal cyclization of the aniline intermediate under modified *Bischler* reaction conditions.

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[94] E. Campaigne, R. D. Lake. *J. Org. Chem*. **1959**, *24*, 478-487.
[95] M. Romero, M. D. Pujol. **2013**. Unpublished results.

The low yield of this reaction can also be explained by the hydrolysis of the esters formed to the corresponding carboxylic acid. (Scheme 52).
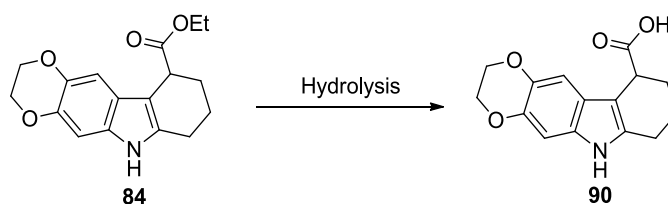
Scheme 52. Hydrolysis of ester **84** under the modified *bishler* reaction conditions

The separation of all the products formed by silica gel flash column chromatography led to the results and yields depicted on Scheme 53:
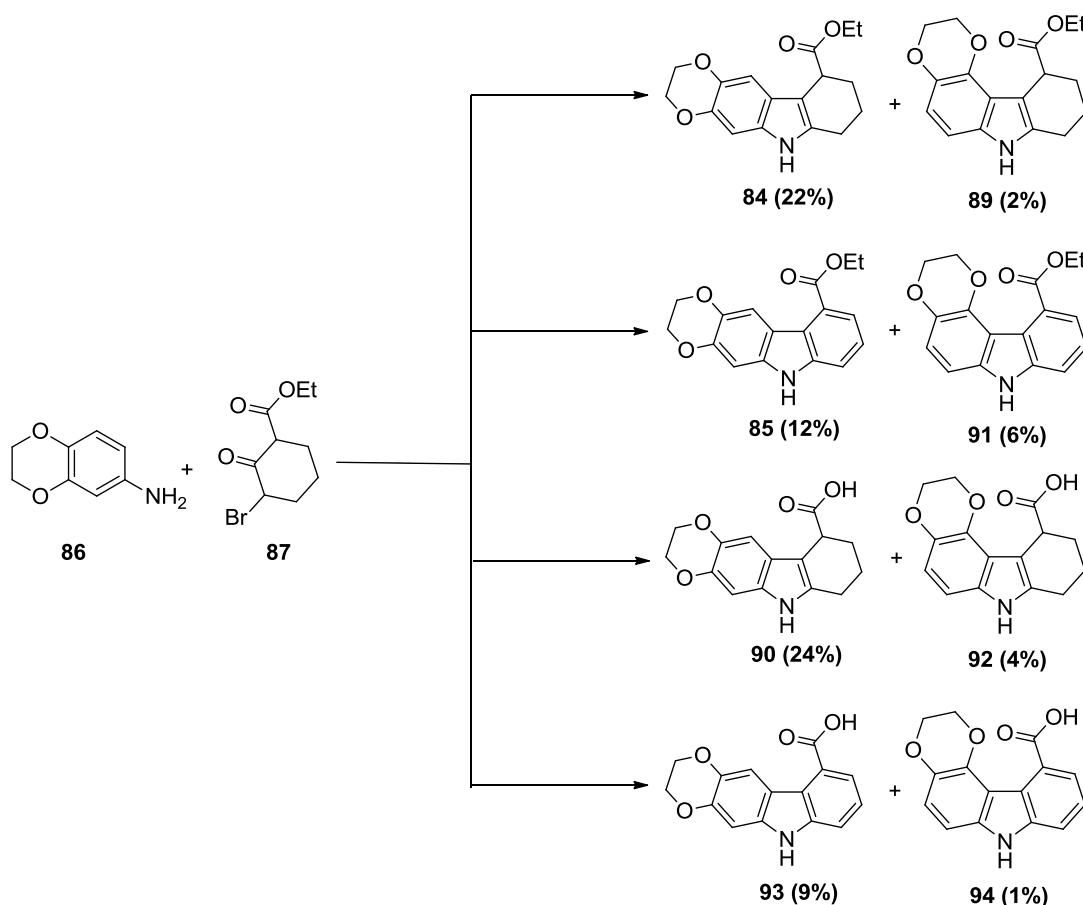
Scheme 53. Yields of all the obtained products of the modified Bishler reaction.

The esters **84** and **85** were reduced by LiAlH$_4$ to afford the corresponding alcohols **82** and **83** in good yields (Scheme 54).
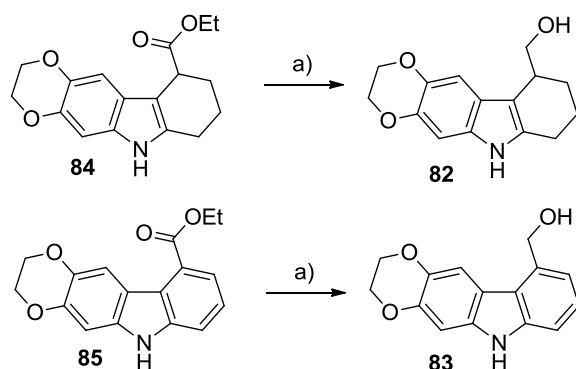
Scheme 54. Reagents and conditions: a) LiAlH₄ (4 eq), THF, rt, 4 h, **82** (83%), **83** (73%).

The alcohol **82** and 2-fluoro-5-(trifluoromethyl)phenyl isocyanate (**95**) were condensed in the presence of Et₃N to give the dioxancarbazole **22** in good yield. Similarly, the alcohol **83** was coupled with 3-nitrophenyl isocyanate (**96**) in the presence of Et₃N to provide **23** in low yield (Scheme 55).
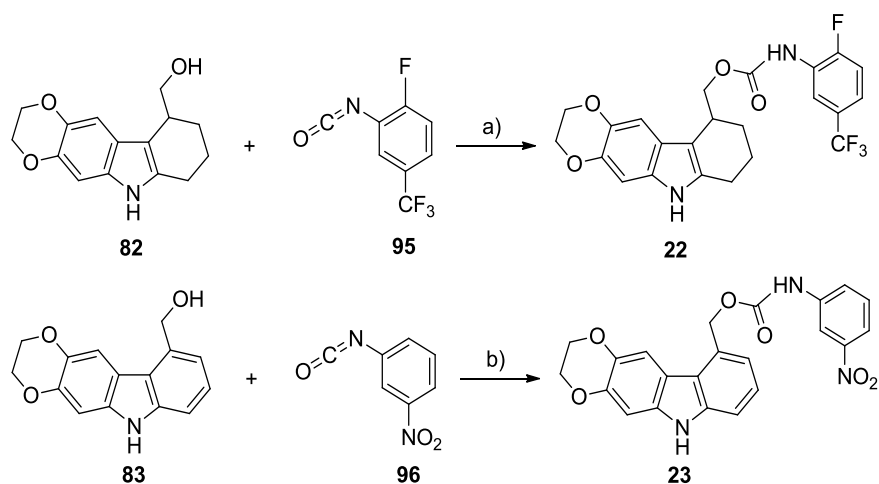
Scheme 55. Synthesis of carbamate **22** and **23**. Reagents and conditions: a) 2-fluoro-5-(trifluoromethyl)phenylisocyanate (1 eq), CH₂Cl₂, rt, 16 h, 78%. b) 3-nitrophenyl isocyanate (1 eq), CH₂Cl₂, rt, 16 h, 14%.

3.4.3. Synthesis attempts of side chain functionalization

In continuation of our current studies on the synthesis of functionalized dioxancarbazoles, we attempted to synthesize **97** from the alcohol **79** (Scheme 31). **79** was treated with Tf₂O and Et₃N giving the triflate **98** which was used without further purification because of its low stability. Treatment of the triflate **98** with 3-amine-2-chloropyridine (**99**) and K₂CO₃ led to decomposition of **98** and no sign of the desired product **97** (Scheme 56).
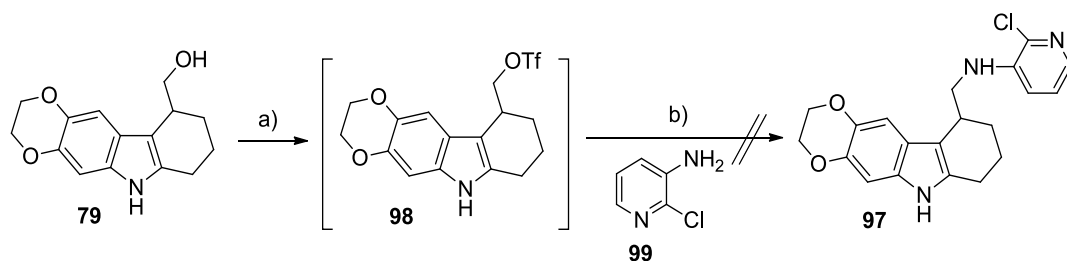
Scheme 56. Synthesis attempt of dioxancarbazole **97**. Reagents and conditions: a) Tf$_2$O, Et$_3$N, CH$_2$Cl$_2$, 3 h at -78 °C and 3 h at rt, >100% (crude) b) 3-amine-2-chloropyridine (1.5 eq), K$_2$CO$_3$ (2 eq), DMF, 80 °C, 16 h, decomposition of **98**.

The ^{1}H NMR (7.82 ppm, 2 H, C=C$\underline{H}_2$) and MS data (M = 365.1) revealed that the elimination product **100** was formed (Scheme 57).
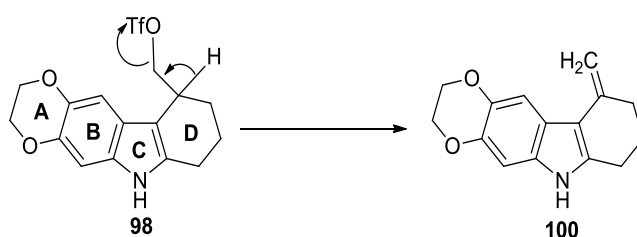
Scheme 57. Product of elimination of the triflate group.

On the basis of these results, an alternative synthesis of **97** and **101** was attempted forming the bromo- or chloro- derivative intermediate **102** and **103**. Treatment of alcohol **75** with PPh$_3$ and NBS led to decomposition of starting material. Similarly, treatment of alcohol **76** with SOCl$_2$ led to starting material decomposition and no sign of the desired chloro derivative **103** (Scheme 58).
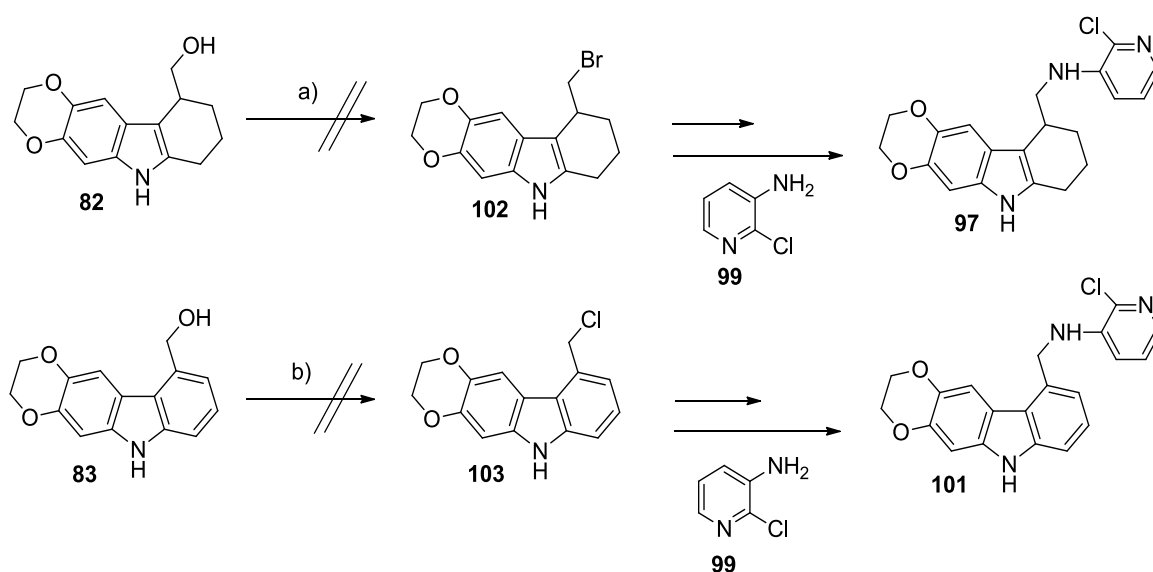
Scheme 58. Synthesis attempts of **97** and **101**. Reagents and conditions: a) NBS (2 eq), Ph$_3$P (2 eq), DMF, 40 °C, 6 h, SM decomposition. b) SOCl$_2$ (2 mL) used as reactive and solvent, starting material decomposition.

The ^{1}H NMR data of these reactions showed dissapearance of aliphatic protons and appearance of new aromatic signals, revealing formation of the fully aromatized dioxancarbazole **104** (2.44 ppm (s, 3H, CH_3-Ar), 7.95 (d, J = 7.5 Hz, 1H, CH-Ar), 7.43 (d, J = 7.5 Hz, 1H, CH-Ar), 7.28 (t, J = 7.5 Hz, 1H, CH-Ar). This can be explained by the fact that leaving groups in beta position of aromatic systems are very labile and are easily eliminated en more stable conjugated derivative that, in this case, ends up aromatizing the D ring (Scheme 59). The 3-amino-2-chloropyridine is here acting as a base and attacking a beta hydrogen of the halogenated chain (E_2 mechanism).
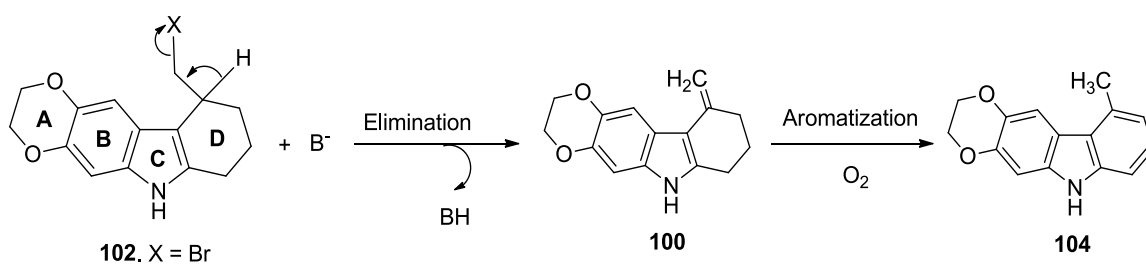
Scheme 59. Aromatization of bromo- or chloro- benzodioxancarbazole derivatives.

3.5. Preparation of *resveratrol* analogues

3.5.1. Preparation of benzofuran *resveratrol* analogues

3.5.1.1. Retrosynthetic analysis

At a first stage, two retrosynthetic analysis of a cyclic *resveratrol* analogue were outlined. The furan **23** can be obtained from the lactone **105**, which can be prepared by condensation of the carboxylic acid **56** with the aldehyde **106** under *Perkin*-type reaction conditions. The *Perkin* reaction is an organic reaction used to convert an aromatic aldehyde and a carboxylic acid (anhydride) to an α,β-unsaturated carboxylic acid or a coumarin.[96] Alternatively, the furan **23** could be synthesized from the *trans* stilbene **107**, which can be obtained by condensation of the aromatic aldehydes **108** and **29** (Scheme 60).
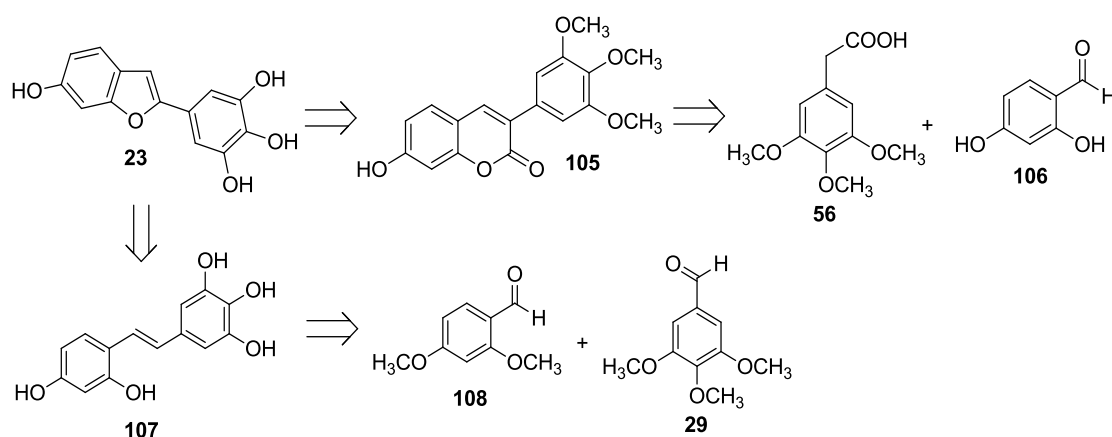
Scheme 60. Two retrosynthetic analysis of the furan *resveratrol* analogue **23**

3.5.1.2. Synthesis of the benzofuran 23

A multistep approach for the preparation of *resveratrol* analogues was proposed. Firstly, a condensation of 3,4,5-trimethoxyphenylacetic acid (**56**) with 2,4-dihydroxybenzaldehyde (**106**) under *Perkin*-type conditions with Ac_2O and Et_3N led to **105** in moderate yield. The corresponding lactone **105** was hydrolyzed and involved in an intramolecular cyclization reaction with HCl 2N to afford the benzofuran **24** in

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[96] W. H. Perkin. *J. Chem. Soc.* **1868**, *21*, 181-184.

moderate yield. The methoxyl groups of **24** were cleaved by treatment with BBr$_3$ at 0 °C affording **23** in satisfactory yield (Scheme 61).
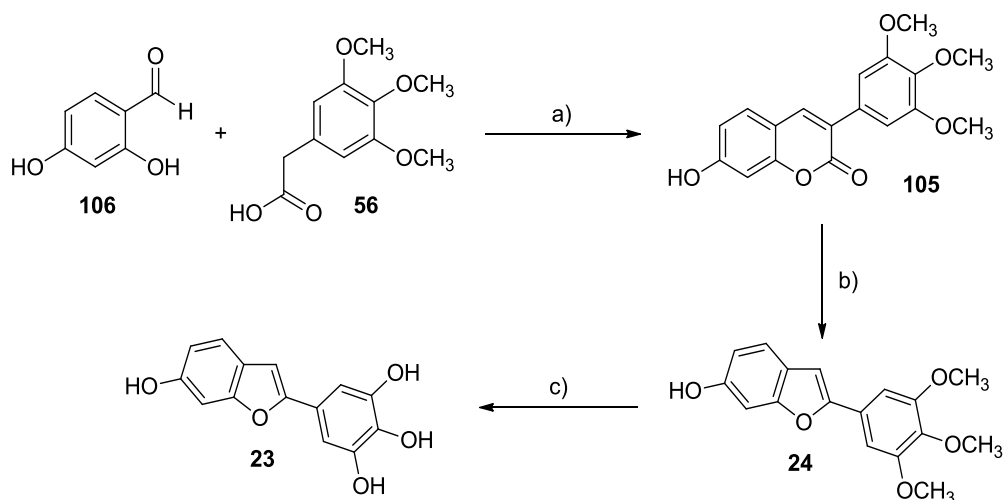
Scheme 61. Preparation of furan *resveratrol* analogue **23**. Reagents and conditions: a) Et$_3$N (5 eq), Ac$_2$O, reflux, 3 h, 47%. b) EtOH / HCl (2N) 8:2, reflux, 16 h, 29%. c) BBr$_3$ (20 eq), DCM, rt, 3 h, 51%.

A possible mechanism for the acid-mediated cyclization reaction is depicted in Scheme 62.
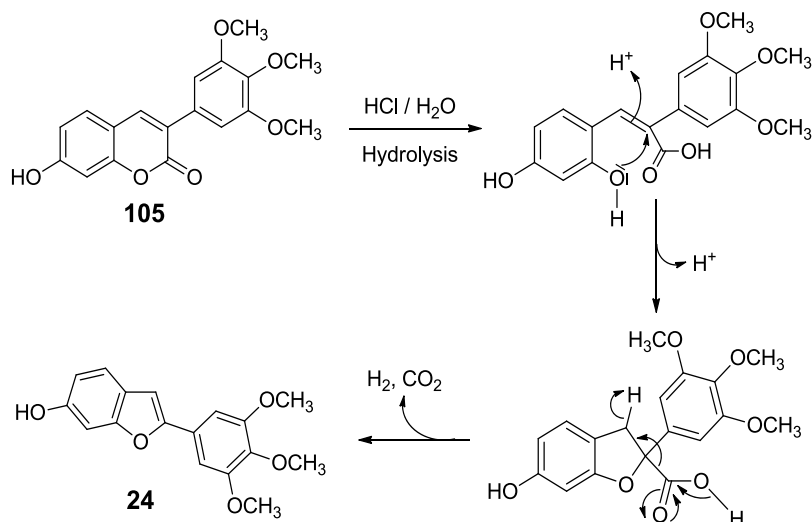
Scheme 62. Proposed mechanism of the acid-mediated cyclization reaction to form the furan **24**

Secondly, a *McMurry* reaction between 3,4,5-trimethoxyaldehyde (**29**) and 2,4-dimethoxybenzaldehyde (**108**) led to the *trans* stilbene **109** in moderate yield.[97] The *Mc Murry* reaction is known as an organic reaction in which two aryl ketones or arylaldehydes are condensed to give an alkene using TiCl$_4$ and Zn° as a reducing agent.

[97] J. E. Mc. Murry. *J. Am. Chem. Soc*. **1974**, *96*, 4708-4709.

This reaction consists in a reductive condensation of carbonyl compounds. A possible mechanism for the *Mc Murry* reaction is depicted in Scheme 63.
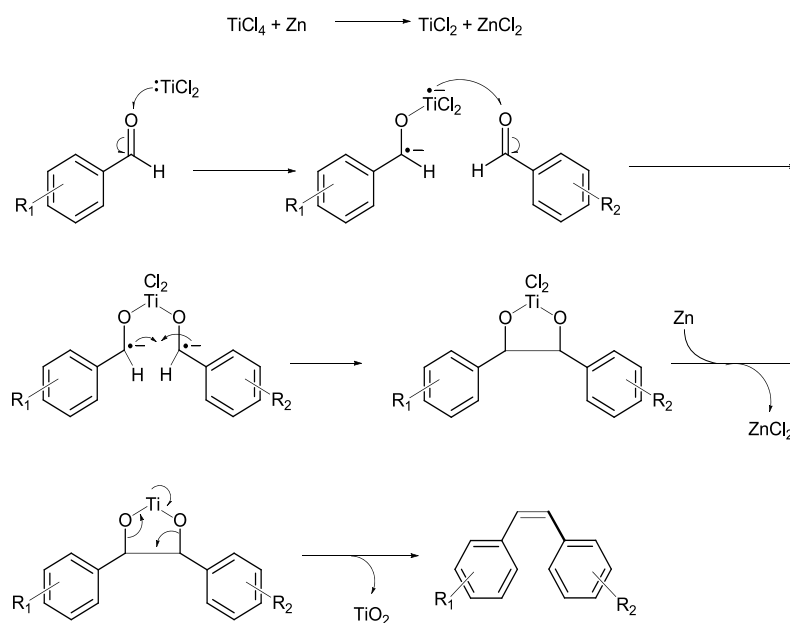
Scheme 63. Proposed mechanism of the *Mc Murry* reaction

The 5 methoxyl groups of **109** were then cleaved with BBr$_3$ to afford the pentaphenol stilbene **107** in quantitative yield (Scheme 64).
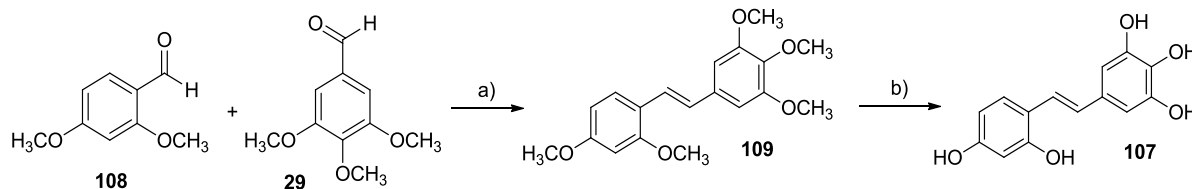
Scheme 64. Reagents and conditions: a) Zn (5 eq), TiCl$_4$ (2.5 eq), THF (30 mL), reflux, 72 h, 38%. b) BBr$_3$ (10 eq), 0 °C, 30 min, 98%.

Finally, an intramolecular cyclization reaction with PTSA in toluene led to decomposition of the starting material into 3,4,5-trihydroxybenzaldehyde (**110**) and 2,4-dihydroxybenzaldehyde (**106**). Another synthesis attempt of **23** from **107** using I$_2$ in an iodination reaction and performing the cyclization reaction with Et$_3$N in EtOH also led to the decomposition of starting material into **106** and **110** (Scheme 65).
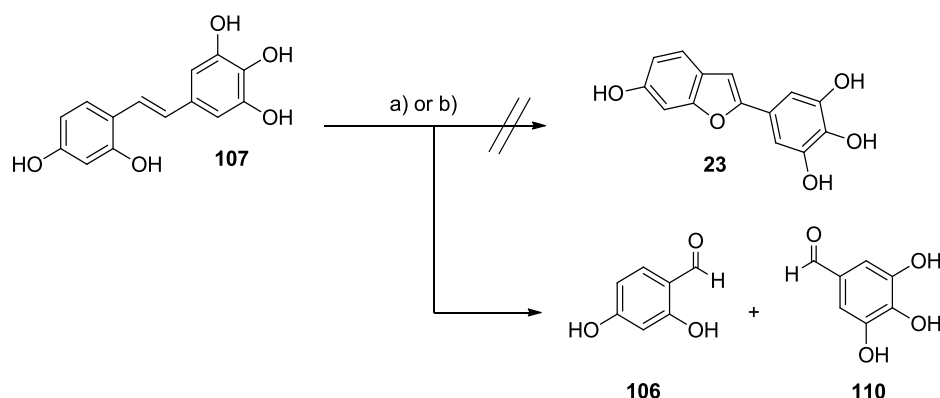
Scheme 65. Reagents and conditions: a) PTSA (0.2 eq), toluene, reflux, 72 h, decomposition into **106** and **110**. b) 1) I$_2$ (1.1 eq), DCM, acetone, rt, 16 h. 2) Et$_3$N (1.5 eq), EtOH, reflux, 16 h, decomposition into **106** and **110**.

3.5.2. Preparation of indole *resveratrol* analogues

3.5.2.1. Synthesis attempt of trimethoxyphenylindole *resveratrol* analogue 111

In continuation of our current studies on the synthesis of indole *resveratrol* analogue, the synthesis of the índole derivative **111** was attempted from 2-bromo-1-(3,4,5-dimethoxyphenyl)ethanone (**112**) and 3-benzyloxyaniline (**113**) (Scheme 66).
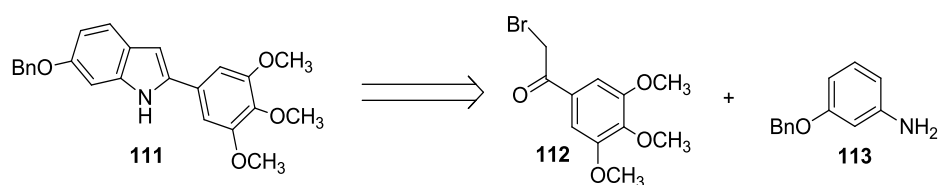
Scheme 66. Retrosynthetic analysis of the disubstitued indole analogue **111**

Firstly, bromination of the 3,4,5-trimethoxyacetophenone (**114**) with NBS in AcOH at 80°C led to both bromination on the alpha position of the ketone and on the aromatic ring. The same reaction at rt led to bromination on the aromatic ring only (Scheme 67). These results show that the C-2 and C-6 position of the aromatic ring are more reactive than the alpha position of the ketone.
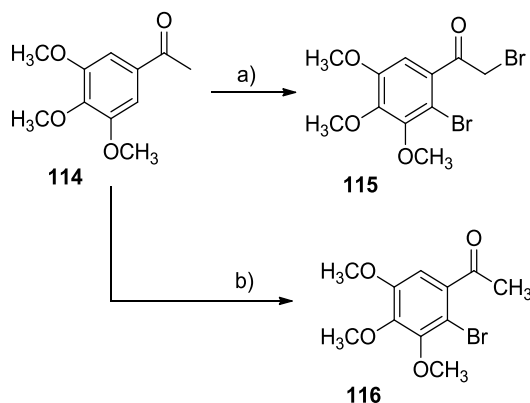
Scheme 67. Synthesis attempts of **112**. Reagents and conditions: a) NBS (1.2 eq), AcOH (1.2 eq), CCl$_4$, 80 °C, 22%. b) NBS (1.2 eq), AcOH (1.2 eq), CCl$_4$, rt, **115** (56%) and **116** (44%).

On the basis of these results, another synthetic route to obtain **112** was attempted from 1,2,3-trimethoxybenzene (**117**). Acylation of **117** with bromoacetyl bromide and TiCl$_4$ did not form **112** and only the 2-bromo-1-(2,3,4-trimethoxyphenyl)ethanone (**118**) was observed (Scheme 68).
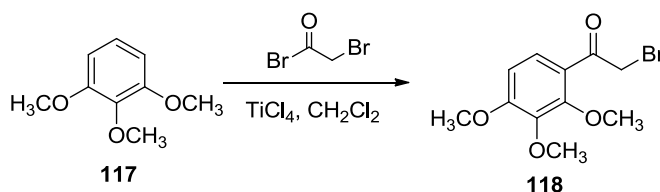
Scheme 68. Alternative synthesis attempt of **112**. Reagents and conditions: Bromoacetyl bromide (1.5 eq), TiCl$_4$ (excess, 1 mL), *N,N*-dimethylaniline, 165 °C, 1 h, SM (11%) and **118** (87%).

These results show that, despite of the sterical hindrance of the ortho position, this site is more electronegative and acylation exclusively occurs in this position.

3.5.2.2. Synthesis attempt of dimethoxyphenyl indole derivative.

3.5.2.2.1. Synthesis of 3,4-dimethoxyphenyl indole 119

A number of attempts were made to synthesize the indole *resveratrol* analogue **119** from 3-benzyloxyaniline and 2-chloro-1-(3,4-dimethoxyphenyl)ethanone (**120**) or 2-bromo-1-(3,4-dimethoxyphenyl)ethanone (**121**). Firstly, chloration of 3,4-dimethoxyacetophenone (**122**) with NCS and AcOH in CCl$_4$ afforded 2-chloro-1-(3,4-dimethoxyphenyl)ethanone (**120**) in quantitative yield. Treatment of **120** with 3-benzyloxyaniline in *N,N*-dimethylaniline yielded products of decomposition of the 3-

benzyloxyaniline and starting material only. An alternative synthesis was attempted to obtain **119**. The 2-bromo-1-(3,4-dimethoxyphenyl)ethanone (**121**) was prepared by bromination of **122** with NBS in AcOH in moderate yield. Cyclization of **121** with 3-benzyloxyaniline in *N,N*-dimethylaniline afforded products of decomposition of the 3-benzyloxyaniline and starting material only (Scheme 69).
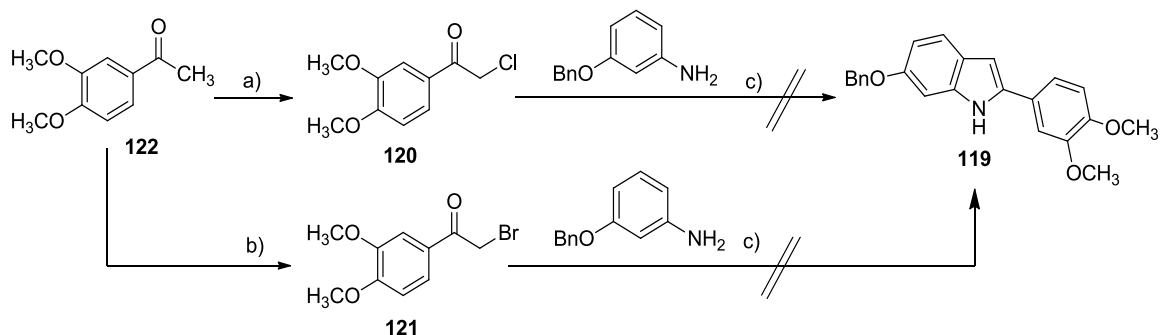
Scheme 69. Synthesis attempt of **119**. Reagents and conditions: a) NCS (1.5 eq), AcOH (1 eq), CCl$_4$, reflux, 92 h, 98%. b) NBS (3 eq), AcOH (3 eq), reflux, 72 h, 40%. c) *N,N*-dimethylaniline, 165 °C, 1 h, decomposition of the 3-benzyloxyaniline and SM.

3.5.2.2.2. Synthesis of 2,5-dimethoxyphenyl indole (26)

The preparation of indole **26** was carried out as depicted in Scheme 67. 3-Benzyloxyaniline (**113**) and 2-bromo-1-(2,5-dimethoxyphenyl)ethanone (**123**) were involved in a modified *Bischler* reaction with *N,N*-dimethylaniline yielding **25** in moderate yield.[98] Deprotection of the benzyl group in a catalytic hydrogenation by H$_2$, Pd/C in EtOAc and MeOH led to **26** in satisfactory yield (Scheme 70).
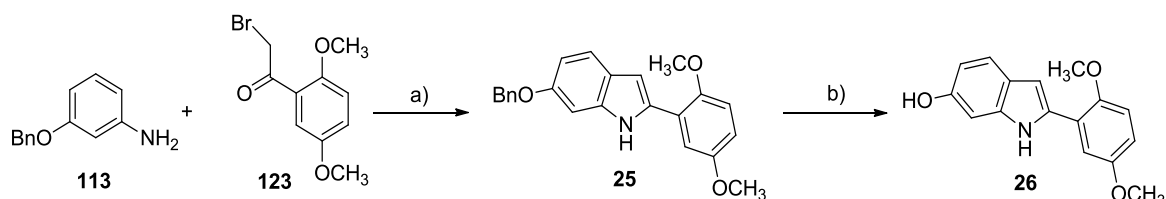
Scheme 70. Preparation of **23** and **24**. Reagents and conditions: a) *N,N*-dimethylaniline, 165 °C, 1 h, 53%. b) H$_2$, Pd/C, EtOAc, MeOH, rt, 16 h, 55%.

The substituted indoles **25** and **26** were submitted to the laboratory Lilly (Indianapolis, USA) for biological testing to evaluate their biological activities and to study the mechanism of action.

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[98] A. F. Crowther, F. G. Mann, D. Purdie. *J. Chem. Soc.* **1943**, *4*, 28-31

3.6. NMR data of the synthesized tetrahydroisoquinoline compounds

3.6.1. Benzodioxan-tetrahydroisoquinoline compounds.

Table 4. ^{1}H NMR Characterization of the dioxanisoquinoline compounds

6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolines

Comp.	1	2	3	4	5	6	7	8	9	45
R	CH$_2$CH$_2$OH	COCF$_3$	H	CH$_2$CN	CH$_2$CH$_2$NH$_2$	CH$_2$CH$_2$N(Me)$_2$	CH$_2$CH$_2$CH(OEt)$_2$	Ph-CN	Ph-NO$_2$	COOEt
CH$_2$-8	2.36-2.43, 2.52-2.61	2.75-2.82	2.83-3.03, 3.18-3.25	2.65-2.75, 2.85-3.00	2.40-2.56	2.80-2.95	2.20-2.35, 2.40-2.80	2.84-2.91	2.83-2.98	2.61-2.70
CH$_2$-9	2.95-3.05, 3.21-3.28	2.94-3.05, 3.18-3.26	2.54-2.65, 2.83-3.03	3.01-3.35	2.96-3.06, 3.15-3.22	3.20-3.29	2.40-2.80, 2.90-3.10	3.46-3.54	3.52-3.61	2.79-2.95, 3.17-3.27
OCH$_3$	3.81, 3.84	3.77 3.83	3.75, 3.77	3.80, 3.82	3.81, 3.84	3.78 , 3.82	3.81, 3.83	3.74, 3.80	3.74, 3.80	3.77, 3.82
CH$_2$-2, CH$_2$-3	4.15-4.25	4.20-4.25	4.13-4.16	4.09-4-26	4.14-4.20	4.20-4.30	4.12-4-22	4.21-4.31	4.21-4.31	4.20-4.26
H-6	4.40	6.69	4.81	4.45	4.29	4.28	4.26	5.66	5.74	4.19
H-5	6.26	6.57	6.22	6.18	6.25	6.57	6.24	6.70	6.72	6.68
H-2', H-6'	6.45	6.45	6.45	6.52	6.51	6.45	6.51	6.41	6.77	6.57
H-10	6.63	6.70	6.56	6.58	6.61	6.68	6.59	6.86	6.89	6.79

Table 5. ^{13}C NMR Characterization of the dioxanisoquinoline compounds

6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolines
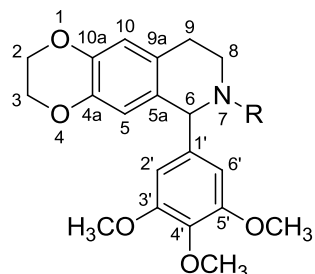
Compound	1	2	3	4	5	6	7	8	9	45
R	CH$_2$CH$_2$OH	COCF$_3$	H	CH$_2$CN	CH$_2$CH$_2$NH$_2$	CH$_2$CH$_2$N(Me)$_2$	CH$_2$CH$_2$CH(OEt)$_2$	Ph-CN	Ph-NO$_2$	COOEt
C-9	28.6	28.4	28.9	29.0	28.9	46.1	48.6	27.6	27.6	28.1
C-8	47.8	39.5	43.1	44.3	48.4	56.5	50.5	44.7	45.2	38.5
CH$_3$	56.5, 58.5	60.8, 64.4	56.0, 60.7	56.5, 61.1	56.4, 57.4	58.0, 61.1	56.4, 57.2	56.6, 62.2	56.6, 61.1	56.4, 61.0
C-2, C-3	64.7	64.5, 64.6	64.2, 64.3	64.6, 64.7	64.6, 64.7	57.8, 58.5	63.1, 64.7	64.2-65.1	64.7	64.6, 64.7
C-6	69.4	56.2	62.4	67.6	69.9	57.73	69.4	61.1	62.3	57.5
C-2', C-6'	106.7	106.2	105.6	106.3	106.7	106.1	106.7	104.2	104.2	106.0
C-5	116.4	116.5	115.9	116.4	116.4	116.9	116.3	116.4	116.5	116.9
C-10	117.1	116.8	116.6	116.9	117.0	117.1	116.5	116.8	116.9	116.9
C-5a	127.7	136.5	128.1	126.8	127.9	128.4	127.9	128.4	128.5	128.3
C-9a	131.3	137.8	131.3	130.7	131.8	137.7	132.9	130.3	130.1	137.6
C-1'	139.6	126.0	140.1	137.7	140.2	138.8	137.3	137.6	137.3	138.9
C-4a	141.9	142.3	141.4	142.0	141.8	142.2	141.7	142.4	142.5	142.2
C-10a	142.4	142.9	141.9	142.5	142.2	142.9	142.1	143.2	143.3	142.9
C-4'	153.5	152.9	152.8	153.4	153.4	153.2	153.9	152.6	153.9	153.2
C-3', C-5'	153.5	153.0	152.9	153.8	153.4	153.2	153.3	153.6	153.7	153.2

Table 6. ^{1}H NMR Characterization of the dimethoxyisoquinoline compounds

6,7-dimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinolines
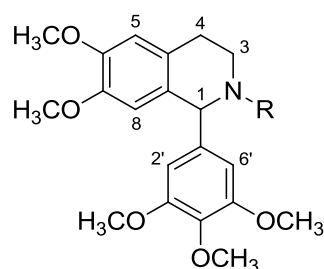
Compound	10	11	49	50
R	CH_2CH_2OH	$CH_2CH_2(OH)CH_2OH$	CF_3	H
CH_2-4	2.40-2.52, 2.52-2.65	2.60-2.90	2.70-2.85, 2.90-3.10	2.65-2.78, 2.90-3.10
CH_2-3	2.70-2.85, 2.86-3.00	2.92-3.20	3.35-3.50, 3.85-3.95	2.90-3.10, 3.20-3.30
OCH_3	3.61, 3.76, 3.81, 3.82	3.82, 3.83, 3.84, 3.85	3.68, 3.71, 3.77, 3.83	3.68, 3.81, 3.85, 3.88
H-1	4.45	4.20	6.62	4.97
H-5	6.22	6.59	6.46	6.31
H-2', H-6'	6.41	6.81	6.39	6.49
H-8	6.58	6.86	6.63	6.63

Table 7. ^{13}C NMR Characterization of the dimethoxyisoquinoline compounds

6,7-dimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinolines
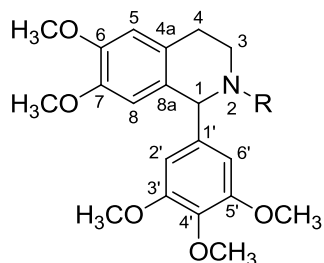
Compound	10	11	49	50
R	CH$_2$CH$_2$(OH)CH$_2$OH	CF$_3$	CH$_2$CH$_2$OH	H
C-4	30.0	28.9	28.1	29.0
C-3	47.9	39.6	46.9	39.7
OCH$_3$	56.1, 56.4, 56.5, 61.1	56.1, 56.3, 56.4, 56.8	56.1, 56.2, 56.4, 56.5	56.3, 56.5, 56.8, 60.9
C-1	75.8	61.0	68.6	61.11
C-2', C-6'	104.2	106.5	106.7	106.6
C-5	110.5	111.3	111.1	111.3
C-8	111.6	111.2	111.8	111.4
C-4a	126.9	125.1	126.8	125.2
C-8a	128.7	125.9	129.4	126.1
C-1'	140.7	138.2	139.6	136.8
C-7	147.8	148.8	147.4	148.2
C-6	148.7	148.1	147.9	148.9
C-4'	153.0	153.5	153.4	153.5
C-3', C-5'	153.3	153.5	153.4	153.5

4. Biological data

Thirteen compounds (**1**, **4**, **5**, **8**, **9**, **16**, **17**, **21**, **22**, **23**, **24**, **25** and **26**) were sent to the Lilly laboratories (Indianapolis, USA) to determine their activities on K-Ras protein inhibition, anti-angiogenesis and anti-osteoporosis. Five other compounds (**6, 7, 10, 11, 14, 15**) are to be tested soon. Topoisomerase II inhibition, CDK inhibition and toxicity of the above-mentionned compounds will also be tested. In order to determine if these compounds would have other interesting therapeutic properties, complementary assays on diabetes, neurologic disorders and other diseases were also carried out.

4.1. Antitumour activity assays

4.1.1. K-Ras, anti-angiogenesis and anti-osteoporosis biological results

4.1.1.1. Introduction

4.1.1.1.1. K-Ras/Wnt Synthetic Lethal

K-Ras is a protein that is encoded in humans by the K-Ras gene. K-Ras mutations are involved in the development of many cancers. Most colorectal cancers develop from benign lesions that are initiated by mutations in the protein adenomatous polyposis coli (APC). Progression to colorectal cancers requires a second event such as an activating KRas mutation, which is triggered by undefined interactions between the Wnt signaling pathways and the K-Ras protein. The Wnt signaling pathways are a group of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell. The goal is to identify small molecules that are selectively lethal to tumor cells that depend on this Wnt-KRas synergy.[99] In 2009, the FDA updated two drugs: *panitumab* and *cetuximab* (monoclonal antibodies) indicated for treatment of colorectal cancer and related to the K-Ras.[100]

The K-Ras synthetic lethal phenotypic module measures survival of colorectal cancers cells carrying mutations that activate both Wnt and K-Ras signaling relative to those

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[99] D. A. Chan, A. J. Giaccia. *Nat. Rev. Drug. Discov*. **2011**, *10*, 351-364.
[100] Cetuximab (Erbitux) and Ponitumab (Vectibix). U.S. *Food and Drug Administration* **2010-01-11**

with other driver mutations. Confirmed actives are tested in a battery of phenotypic assays that are regulated through Wnt and/or K-Ras-signaling pathways. This strategy provides an opportunity to discover targeted agents with improve cancer vs. normal cell selectivity. The overall goal is to develop targeted therapies directed towards colorectal cancers patients with mutant K-Ras tumors. The K-Ras activity of the thirteen tested compounds were carried out on 4 different colon cancer cell lines (HCT KrasSL, RKO KrasSL, Colo 320 KrasSL and SNU-C1 KrasSL) in three different concentrations (0.2 µM, 2 µM and 20 µM).[101] The activities of these compounds were compared to the Lilly lead compound **3b** which has a significant K-Ras activity.

4.1.1.1.2. Anti-angiogenesis assays

Angiogenesis and vasculogenesis consist in blood vessel sprouting and tube formation. Vascular disrupting agents cause rapid collapse and shut down of established tumour blood vessels leading to regional tumour ischaemia and necrosis.[102] The anti-angiogenesis agents can bind the extracellular domain of VEGF receptor-2[103] or the ligands VEGF-A, VEGF-C and VEGF-D.[104] Several anti-angiogenesis compounds are investigated in clinical trials for cancer treatment. The efficiency and safety of these agents have not been yet established.

Bevacizumab was the first angiogenesis inhibitor approved by the FDA. Other drugs with antiangiogenic activity were approved afterwards: *sorafenib* (Nexavar®), *sunitinib* (Stutent®) and *pazopenib* (Votrient®).[105] Although K-Ras promotes angiogenesis, the function of mutant K-Ras activity in tumor angiogenesis process remains poorly understood. The last results suggest that angiogenesis is initiated by secretion of chemokinas and VEGF downstream of activated oncogenic K-Ras, and the vascular maturation is also dependent on MEK ½ antibodies and C-Jun signaling proteins.[106]

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[101] https://openinnovation.lilly.com/dd/about-open-innovation/resources-links.html.

[102] D. Hanahan, R. A. Weinberg. *Cell* **2011**, *144*, 646-674.

[103] D. Lu. *J. Brol. Chem*. **2003**, *278*, 43496-43507.

[104] H. Youssoufian. *Clin. Canc. Res.* **2007**, *13*, 55445-55485.

[105] K. M. Cook, W. D. Figg. *Cancer. J. Clin.* **2010,** *60*, 222-243.

[106] Y. Matsuo, P. M. Campbell, R. A. Breckken, B. Sung. M. M. Ouellette, J. B. Flemming, B. B. Aggarwal, C. J. Der, S. Guha. *Mol. Canc. Res.* **2009**, *7*, 799-808.

4.1.1.1.3. Anti-osteoporosis promoters

Osteoporosis is a disease that weakens bones over time and increases the risks of fractures. The mechanism of bone loss is not well understood, but in practical effect, the disorder arises from an imbalance in the formation of new healthy bones and the decrease and resorption of bone tissue. Osteoporosis triggers a decrease of bone protein matrix and mineral content.
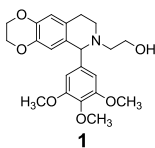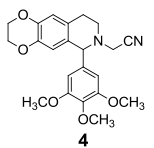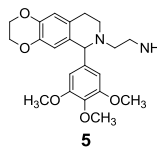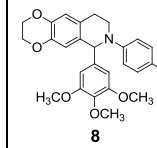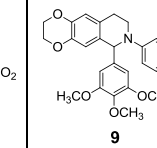
The Bone Formation phenotypic assay module tests compounds for their ability to differentiate murine C2C12 cells, a cell line with multi-lineage potential, to an osteoblast-like phenotype through beta-catenin-dependent stimulation of alkaline phosphatase activity. Secondary assays confirm the osteogenic activity of the compounds in both rodent and human multi-potential cell populations. Compounds of interest increase osteoblast formation in rodent and human cellular assays through a non-glycogen synthase kinase (GSK) mechanism.[107]

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[107] Y. Chen, B. A. Alman. *J. Cell. Biochem*. **2009**, *106*, 353-362.

4.1.1.2. Antitumour activity of the tetrahydroisoquinoline analogues

4.1.1.2.1. K-Ras inhibition (Table 8)

Table 8. K-Ras inhibition of the tetrahydroisoquinoline analogues

		Products / Assays	Lilly's lead compound **3b**	**1**	**4**	**5**	**8**	**9**
K-Ras Wnt Synthetic Lethal	HCT KrasSL	% Inhib 0.2 µM	31.2	18	12.1	0	0	35.9
		% Inhib 2 µM	1.2	12.1	17	16.2	19.3	16
		% Inhib 20 µM	52.4	46.5	49.1	56.9	55.9	45.2
	RKO KrasSL	% Inhib 0.2 µM	0	0	0	0	17.9	95.8
		% Inhib 2 µM	12.1	6.3	16.3	10.1	32.9	7.7
		% Inhib 20 µM	41	56.7	79.2	92.7	70.5	66.5
	Colo 320 KrasSL	% Inhib 0.2 µM	0	12.2	0	0	0	0
		% Inhib 2 µM	12.4	0	8.2	20.1	4	22.5
		% Inhib 20 µM	85.4	78.2	74.7	85.3	92.5	89.9
	SNU-C1 KrasSL	% Inhib 0.2 µM	21	12.3	10	12.2	17.7	6.6
		% Inhib 2 µM	14.2	11.5	6	2.4	35	32.5
		% Inhib 20 µM	18.8	19.7	59.8	57.1	42.7	43.5
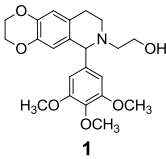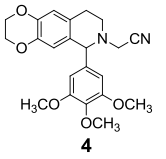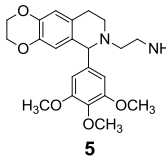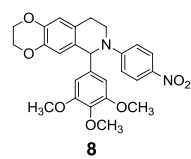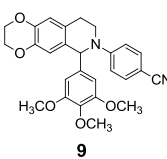
Footnote: SNU-C1, Colo 320, RKO and HCT are colon cancer cell lines.

The results show that all of the studied compounds have a higher overall K-Ras inhibition than the lead compound **3b**, which means that those products have a high K-Ras inhibition activity. Surprisingly, the alcohol **1** displayed a lower inhibition than the other isoquinoline analogues. The amine isoquinoline **5** presents the highest K-Ras activity of all the tested tetrahydroisoquinoline analogues, which reveals that a terminal ionic interaction results in an increase of K-Ras inhibition. The *N*-arylisoquinoline **8** and **9** have the highest K-Ras inhibition on Colo 320 Kras SL cell line, which suggests that a low electron density aromatic side chain structure results in a higher K-ras activity for this type of cancer cells. The isoquinoline **9** shows a surprisingly high K-Ras activity at 0.2 µM concentration (RKO KrasSL 95.8% inhib., HCT

KrasSL 35.9% inhib.) which suggests that its activity is not dose-dependant for these types of cancer lines.

4.1.1.2.2. Anti-angiogenesis and anti-osteoporosis activity (Table 9)

Table 9. Anti-angiogenesis and anti-osteoporosis activity of the tetrahydroisoquinoline analogues

Assays			**1**	**4**	**5**	**8**	**9**
Anti-angiogenesis	Angio Tube Area	% Inhib 2 µM	0	15	8.5	6	16.6
		% Inhib 10 µM	6.7	76.9	58.6	37.5	55.3
		IC$_{50}$	N.A	2.886 µM	6.539 µM	>10 µM	>10 µM
	Angio Nuc Area	IC$_{50}$	N.A	>10 µM	>10 µM	>10 µM	>10 µM
	Osteo bCat	% Stim 2 µM	0	4.6	0	0.3	27.2
		% Stim 10 µM	2,9	51,8	25,4	4,3	14,2
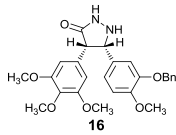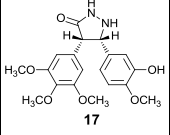
Footnote: Angiogenesis nuclear area, b-catenin osteoporosis and angiogenesis tube area (tracheal tube area)

The results show that the isoquinolines **4**, **5**, **8** and **9** posess a high anti-angiogenesis activity while compound **1** is little active. The nitrile isoquinoline **4** has the highest anti-angiogenesis and anti-osteoporosis activity, which suggests that a dipolar lipophilic terminal group such as a nitrile group results in an increase of anti-angiogenesis and anti-osteoporosis activity.

4.1.1.3. Antitumour activity of the *combretastatin* analogues

4.1.1.3.1. K-Ras inhibition (Table 10)

Table 10. K-Ras inhibition of the *combretastatin* analogues **16** and **17**
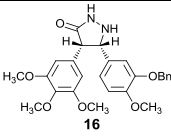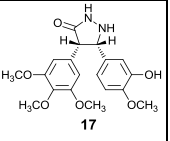
Assays		Products	Lilly's lead compound **3b**	**16**	**17**
K-Ras Wnt Synthetic Lethal	HCT KrasSL	% Inhib 0.2 µM	31.2	0	0
		% Inhib 2 µM	1.2	11.3	57.9
		% Inhib 20 µM	52.4	9.5	100
	RKO KrasSL	% Inhib 0.2 µM	0	1.5	33.7
		% Inhib 2 µM	12.1	2.6	62.4
		% Inhib 20 µM	41	44.3	97.3
	Colo 320 KrasSL	% Inhib 0.2 µM	0	0	21
		% Inhib 2 µM	12.4	18.4	54.2
		% Inhib 20 µM	85.4	29.5	73.1
	SNU-C1 KrasSL	% Inhib 0.2 µM	21	10.2	12.8
		% Inhib 2 µM	14.2	6.5	58.3
		% Inhib 20 µM	18.8	0	50.7

Footnote: SNU-C1, Colo 320, RKO and HCT are colon cancer cell lines.

According to the results, the *O*-benzylated pyrazolone **16** presents a moderate K-Ras activity on the RKO KrasSL and the Colo 320 KrasSL cell lines and low to no K-Ras activity for the two other tested cell lines. However, the *O*-debenzylated pyrazolone **17** shows the highest overall K-Ras activity of all tested compounds and the highest activity on the HCT KrasSL cell line. It also has a high K-ras activity at low concentrations compared to the other tested compounds. The structure activity relationship between analogues **16** and **17** reveals that the arylalcohol at the C-3' position plays an important role in its K-Ras activity. In other words, small electronegative groups at the C-3' position dramatically increases the compound K-Ras activity.

4.1.1.3.2. Anti-angiogenesis activity (Table 11)

Table 11. Anti-angiogenesis activity of the *combretastatin* analogues

Products / Assays			16	17
Anti-angiogenesis	Angio Tube Area	% Inhib 2 µM	9.8	100
		% Inhib 10 µM	38.7	100
		IC_{50}	>10 µM	0.507 µM
	Angio Nuc Area	IC_{50}	>10 µM	>10 µM
Wnt Pathway	Osteo bCat	% Stim 2 µM	0	78.6
		% Stim 10µM	4.4	65.2

Footnote: Angiogenesis nuclear area, b-catenin osteoporosis and angiogenesis tube area
(tracheal tube area)

The results show that the pyrazolone **17** presents a very high anti-angiogenesis effect with a 100% inhibition at a 2 µM concentration. It is the highest anti-angiogenesis and anti-osteoporosis activity of all the tested products with an IC_{50} of 507 nM. It also has the highest anti-osteoporosis activity of all the tested products. The *O*-benzylated pyrazolone **16** presents a lower anti-angiogenesis and anti-osteoporosis activity, which reveals that the arylalcohol at the C-3' position also plays an important role in its angiogenesis and anti-osteoporosis activity.

4.1.1.3.3. CDKs and cell cycle inhibition

Compound **17** being the most active of all tested compounds, complementary assays on CDKs and cell cycle inhibition were carried on as shown on table **12** and table **13.** The IC_{50} of analogue **17** was determined for each phase of the cell cycle, and to be more precise, for each G_2 sub-phase (Table 12).

Table **12**. Cell cycle inhibition of compound **17**

Assays / Product	Cell cycle (G$_2$/M)					
	G$_2$M 4N	G$_2$M 2N	G$_2$M MI	G$_2$M Cell Number	G$_2$M S Phase	G$_2$M G$_1$ Arrest
	IC$_{50}$	IC$_{50}$	IC$_{50}$	IC$_{50}$	IC$_{50}$	IC$_{50}$
17	0.192 µM	0.307 µM	0.292 µM	0.178 µM	0 µM	>10 µM

The results show that the pirazolone **17** possesses a high activity on the G$_2$M cell cycle phase and its sub-phase. Therefore, compound **17** has a different mechanism of action to *combretastatin* A-4, which has an antimitotic mechanism of action that essentially occurs much more in the M-phase (69.75%) and not much in the G$_2$/M-phase (30.25 %) (Scheme 71).[108]
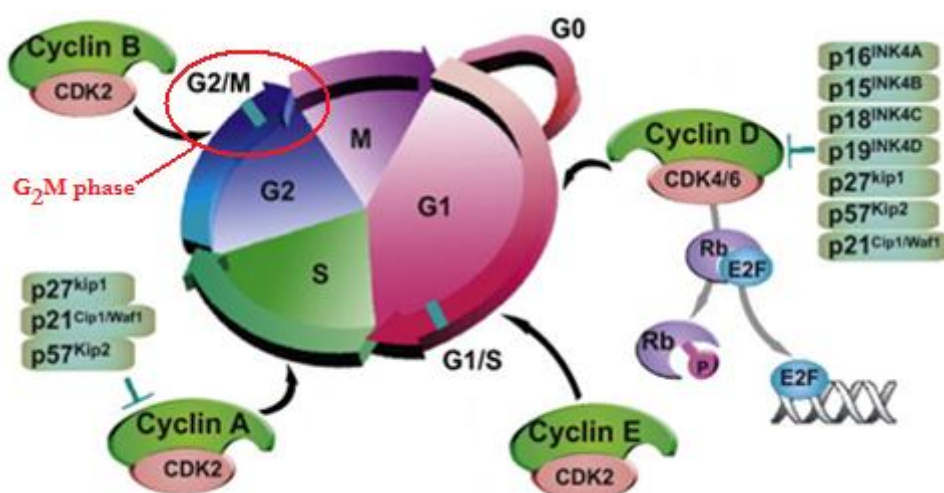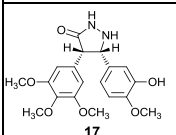
Scheme 71. Localisaion of the G$_2$M phase.[109]
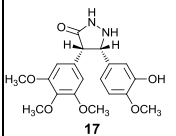
The antitumour activity of the pirazolone **17** was tested on various types of CDKs (Table 13)
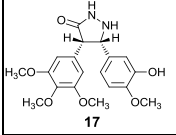
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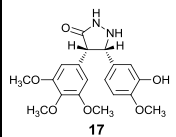
[108] H. L. Lin, S. H. Chiou, C. W. Wu, W. B. Lin, L. H. Shen, Y. P. Yang, M. L. Tsai, Y. H. Uen, J. P. Liou, C. W. Chi. *J.P.E.T.* **2007**, *323*, 365-373.
[109] Arturo Vinuesa Hernando. *Diseño y Síntesis de Nuevas Purinas 6,9-disustituidas con potencial actividad antitumoral por inhibición de CDKs*. Master experimental, **2012**, Facultad de Farmacia. Universidad de Barcelona.
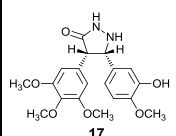
Table **13**. CDK inhibition of compound **17**
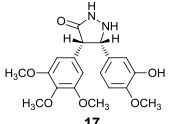
Product / Assays	Profiling kinase											
	hABL1 inhib			hALK4 inhib			HAMPKa			hAURA		
	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% nhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	0	0	0	0	4.65	0	0	0	0	0	1.97	7.33
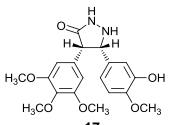
Product / Assays	Profiling kinase											
	hCDC2/CDK1 inhib			hCHK2 inhib			hCK1e inhib			hEGFR inhib		
	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	0	0	0	8.06	14.43	20.08	7.11	16.88	18.94	0	8.91	4.74

Product / Assays	Profiling kinase											
	hEPHB4 inhib			hERK2 inhib			hFGFR1 inhib			hFLT1 inhib		
	% Inhib 0.2 µM	% nhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	26.26	25.73	16.83	0	4.54	0	0	0	12.16	0	0	22.36

Product / Assays	Profiling kinase											
	hFLT3 inhib			hGSK3b inhib			hJAK2 inhib			hJNK1 inhib		
	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	0	7.65	4.23	0	11.66	19.10	0	0	0.15	0	0	0

Product / Assays	Profiling kinase											
	hKDR inhib			hKIT inhib			hMet inhib			hP38a inhib		
	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	0	0.19	0	0	13.11	18.21	0	1.20	0	0	0	1.65

Assays	Profiling kinase											
	Hp70S6K1 inhib			hPDGFRa inhib			hPKCb2 inhib			hPLK1 inhib		
Product	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	%I nhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	13.43	11.05	9.52	0	8.62	25.93	0	0	0	18.22	12.94	29.50

Assays	Profiling kinase					
	hRock2 inhib			hTIE2 inhib		
Product	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM	% Inhib 0.2 µM	% Inhib 2 µM	% Inhib 20 µM
17	0	2.30	0	0	0	0
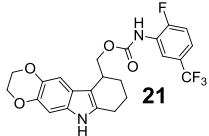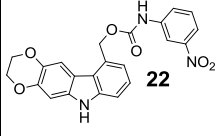
The results show that **17** have little to no activity on the studied CDKs (<30% of inhibit. at 20 µM). Therefore, compound **17** is not an inhibitor of these CDKs.

Biological trials from Dr Nuria Mur Blanch's thesis revealed that *combretastatin* A-4 has a nano molar antitumour activity on numerous cancer cell lines (B-16 mouse melanoma IC_{50} = 3 nM, K562 human leukemia IC_{50} = 3 nM, LoVo colon cancer IC_{50} = 0,015 nM, HT-29 human colon cancer IC_{50} = 0.006 nM, colo 205 colon cancer IC_{50} = 0.02 nM, DLD-1 colorectal adenocarcinoma IC_{50} = 0.015 nM and HCT-15 colon cancer IC_{50} = 0.003 nM)[59]. Therefore, the *combretastatin* A-4 analogue **17** seems to present a lower activity than *combretastatin* A-4. However, the K-Ras biological assays of compound **17** being quite different from the more general biological trial of *combretastatin* A-4, these two assays are difficult to compare with one another.

4.1.1.4. Antitumour activity of the dioxancarbazole analogues

4.1.1.4.1. K-Ras inhibition (Table 14)

Table 14. K-Ras inhibition of the dioxancarbazole analogues **21** and **22**
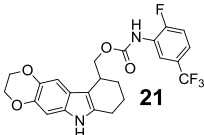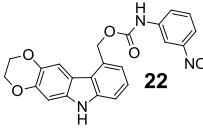
Assays / Products			Lilly's lead compound **3b**	**21**	**22**
K-Ras Wnt Synthetic Lethal	HCT KrasSL	% Inhib 0.2 µM	31.2	33.5	32.5
		% Inhib 2 µM	1.2	8.6	16.3
		% Inhib 20 µM	52.4	52.1	98.7
	RKO KrasSL	% Inhib 0.2 µM	0	5.2	6.5
		% Inhib 2 µM	12.1	5.6	20.6
		% Inhib 20 µM	41	53.6	100
	Colo 320 KrasSL	% Inhib 0.2 µM	0	0	0
		% Inhib 2 µM	12.4	0	0
		% Inhib 20 µM	85.4	37.2	71.1
	SNU-C1 KrasSL	% Inhib 0.2 µM	21	7.1	7.7
		% Inhib 2 µM	14.2	2.5	8.2
		% Inhib 20 µM	18.8	63.2	55.6

Footnote: SNU-C1, Colo 320, RKO and HCT are colon cancer cell lines.

According to the results, the dioxancarbazoles **21** and **22** have a high K-Ras activity. Compound **22** possesses the highest activity on RKO KrasSL cell line of all the tested compounds and a higher overall K-Ras activity than the dioxancarbazole **21**. Both compounds **21** and **22** present a high K-Ras activity on HCT KrasSL cell line at low concentration, which suggests that their activity is not dose-dependant for this type of cell line.

4.1.1.4.2. Anti-angiogenesis activity (Table 15)

Table 15. Anti-angiogenesis activity of the *combretastatin* analogues

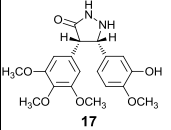| Products / Assays			21	22
Anti-angiogenesis	Angio Tube Area	% Inhib 2 µM	26.3	6.1
		% Inhib 10 µM	13.9	54.3
		IC_{50}	N.A	9.636
	Angio Nuc Area	IC_{50}	N.A	>10 µM
Wnt	Osteo bCat	% Stim 2 µM	2.5	0
		% Stim 10 µM	3.1	2.5
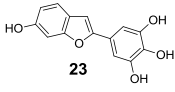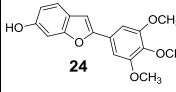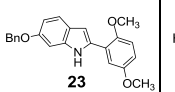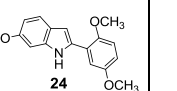
Footnote: Angiogenesis nuclear area, b-catenin osteoporosis and angiogenesis tube area (tracheal tube area)

The results reveal that the dioxancarbazole **22** presents a high anti-angiogenesis activity with an IC_{50} of 9.6 µM, while analogue **21** has no significant activity. Neither of compound **21** and **22** have a significant anti-osteoporosis activity on the carried out tests.

4.1.1.5. Antitumour activity of the *resveratrol* analogues

4.1.1.5.1. K-Ras inhibition (Table 16)

Table 16. K-Ras inhibition of the *resveratrol* analogues
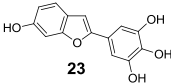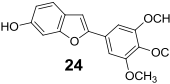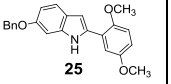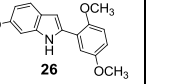
	Products / Assays		Lilly's lead compound **3b**	**23**	**24**	**23**	**24**
K-Ras Wnt Synthetic Lethal	HCT KrasSL	% Inhib 0.2 µM	31.2	15.6	0	0	0
		% Inhib 2 µM	1.2	0	0.5	6.9	0
		% Inhib 20 µM	52.4	0	0	28.9	6.3
	RKO KrasSL	% Inhib 0.2 µM	0	2.7	0	47.3	9.4
		% Inhib 2 µM	12.1	33	6.3	5.6	0
		% Inhib 20 µM	41	45.9	47.4	49.7	0
	Colo 320 KrasSL	% Inhib 0.2 µM	0	0	0	0	0
		% Inhib 2 µM	12.4	0	4.4	0	0
		% Inhib 20 µM	85.4	0	0	0	55.3
	SNU-C1 KrasSL	% Inhib 0.2 µM	21	5.8	8.7	7.4	0
		% Inhib 2 µM	14.2	3.1	8.7	13.9	0
		% Inhib 20 µM	18.8	11.2	14.9	34.5	30.3

According to the results, the four tested *resveratrol* analogues have a low to moderate K-ras activity depending on the cancer cell line. **23** and **24** have a moderate K-ras activity on the RKO KrasSL cell line and low or no activity on the other cancer cell lines. The trihydroxy group of **23** does not bring a better K-Ras activity compared to the trimethoxy group of **24**, which suggests that neither the polarity nor the geometry of this moiety are decisive for its K-Ras activity. **25** has a moderate K-ras activity on HCT KrasSL, RKO KrasSL and SNU-C1 KrasSL cell lines. **26** has a moderate K-ras activity on Colo 320 Kras SL and SNU-C1 KrasSL. The difference of activity of **25** and **26** suggests that the benzyl group is important for the selectivity of the activity of these compounds between the different colon cell lines. **25** has nearly the same K-Ras

activity on RKO KrasSL at high and low concentration, which suggests that its activity is not dose-dependent.

4.1.1.5.2. Antiangiogenesis activity (Table 17)

Table 17. Antiangiogenesis activity of the combretastatin analogues

	Products Assays		23	24	25	26
Anti-angiogenesis	Angio Tube Area	% Inhib 2 µM	0	0	0	25.2
		% Inhib 10 µM	13.8	15.7	0	43.7
		IC_{50}	N.A	N.A	N.A	>10 µM
	Angio Nuc Area	IC_{50}	N.A	N.A	N.A	>10 µM
Wnt Pathway	Osteo bCat	% Stim 2 µM	0	2	14.1	0.7
		% Stim 10 µM	5.5	10.2	5.4	8.1

Footnote: Angiogenesis nuclear area, b-catenin osteoporosis and angiogenesis tube area (tracheal tube area)

Results of the four tested combretastatin analogues showed that only **26** has a significant anti-angiogenesis activity. Non of them have a significant osteoporose activity.
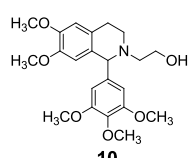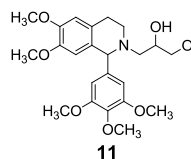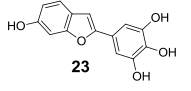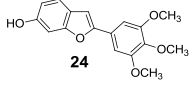
The K-Ras, antiangiogenesis and antiosteoporosis activity of these resveratrol analogues were compared to the resveratrol antitumour activities activities described in the litterature. However, as the cell lines activities found were different and described in dosis/duration instead of percentage of inhibition, it was not possible to make a clear comparation of these two studies.[110]

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[110] A. Bishayee. *Cancer Prev. Res*. **2009**, *2*, 409-418.

4.1.2. Complementary antitumor activity assays

Compounds **10**, **11**, **23** and **24** were sent to the department of pharmacy of the university of Palermo (Italy) to determine their activites on tumor cells. The antitumor activity of these compounds were tested in three different types of cancer cell lines: The human chronic myelougenous leukaemia cell line K562, the human non-small-lung cancer NCI-H460 and a human colon cancer cell line HT-29 (Table 18).
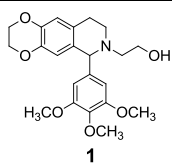
Table 18. K562, NCI-H460 and HT29 cell viability after treatment with 10 µM of test compounds

Products / Assays	K562 % inhibition [10 µM]	NCI-H460 % inhibition [10 µM]	HT29 % inhibition [10 µM]
Ellipticine	73.5	97.7	98.8
10	2.4	0	0
11	19.9	12.9	0
23	22.2	0	0
24	14.3	0	0

Footnote: Leukemia K562, human lung cancer NCI-H460 and colon HT29 cell lines.

The results show that **10**, **11**, **23** and **24** have little to no inhibition on the three studied cancer cell lines. Biological studies of **1** performed at the Biochemistry Department of the Chemistry University of Barcelona revealed that the IC_{50} of the benzodioxin-tetrahydroisoquinoline **1** on the NCI-H460 cell line is 0.2 µM in 72 h for 10 µM (Table 19).

Table 19. IC 50 of the benzodioxin-tetrahydroisoquinoline **1**

Product:	
Time (hours)	**IC$_{50}$ (μM)**
24	0.8
48	0.7
72	0.2

The S.A.R between **10** and **1** shows that the dimethoxy groups result in a decrease of biological activity compared to the benzodioxin moiety. The S.A.R between **10** and **11** reveals that a diol side chain results in an increase of antitumour activity compared to a terminal alcohol side chain.

4.2. Complementary biological assays: Diabetes, neurological disorders and other biological trials

4.2.1. Diabetes biological trials

4.2.1.1. Introduction

4.2.1.1.1. GLP-1 Secretion

Glucagon-like peptide 1 (GLP-1) is derived from transcription of the proglucagon gene followed by post-translational modifications of proglucagon of the following biologically active peptides: GLP-1 (7-37) and GLP-1 (7-36) NH2. GLP-1 secretion by ileal L cells is dependent on the presence of nutrients in the small intestine. GPL-1 is a potent anti-hyperglycemic hormone inducing glucose-dependent insulin secretion and suppressing glucagon secretion. The glucose dependency of this mechanism is particularly important because GLP-1 does not stimulate insulin secretion and cause hypoglycemia when plasma glucose concentrations are in the normal fasting range.[111]

The GLP-1 secretion diabetes phenotypic module identifies compounds that stimulate secretion of glucagon-like peptide one (GLP-1) in mouse and human cell lines derived

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[111] K. Dungan, J. B. Buse. *Clinical Diabetes* **2011**, *23*, 56-62.

from gastrointestinal tract tissue. GLP-1 secretion is measured using a Lilly proprietary ELISA assay that was specifically designed to detect the appropriate forms of GLP-1 secreted from these cells. If active in the cell-based GLP-1 assay, molecules will be further tested for selectivity in assays that measure activation of GPCRs known to stimulate GLP-1 secretion.

4.2.1.1.2. GPR 119 Receptor Agonist

GPR119 belongs to a family of G protein-coupled receptors. Activation of GPR119 produces an increase in cycline adenosine monophosphate (cAMP) levels. GPR119 has a limited tissue distribution, and is expressed only in pancreas and intestine. In pancreas, activation of GPR119 has a limited tissue distribution, and is expressed only in pancreas and intestine. In pancreas, activation of GPR119 results in a potentiation of glucose-induced insulin secretion. In gastrointestinal tract, activation of the receptor increases secretion of incretin (group of gastrointestinal hormones that increase the amount of insulin). Thus, GPR119 agonists might exert a dual control of glucose homeostasis.[112]

The GPR119 module tests for compounds that increase intracellular cAMP levels in cells expressing human GPR119 receptor. The active molecules will be further characterized for their specificity, ability to activate the mouse rodent GPR119 receptor and to increase glucagon like peptide 1 (GLP-1) secretion in murine enteroendocrine cells. Compounds of interest selectively activate GPR119 receptors and increase GLP-1 secretion. These compounds could be used as a new treatment for obesity and diabetes.[113]
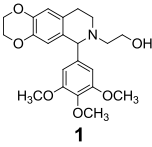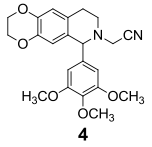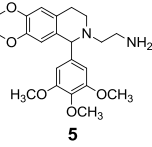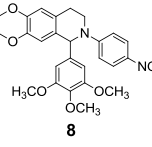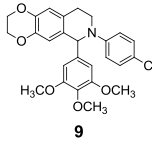
[112] H. A. Overton et al. *Cell. Metab.* **2006**, *3*, 167-175.
[113] H. A. Overton, M. C. Fyle, C. Reynet. *Br. J. Pharmacol.* **2007**, *153*, 576-581.

4.2.1.2. Diabetes biological activities of the tested compounds

4.2.1.2.1. Diabetes biological activities of the tetrahydroisoquinoline compounds (Table 20)
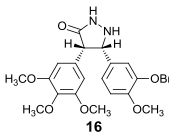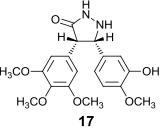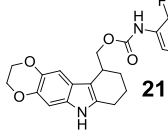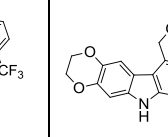
Table 20. Diabetes biological activity of the tetraisoquinoline analogues

Assays		Products	1	4	5	8	9
GLP-1 secretion	hNCl GLP-1 Sec	% Stim 2µM	0.7	2.9	0	1.2	1.5
		% Stim 20 µM	3.4	3.8	2.6	1	0
GPR 119 Receptor	hGPR119Ag	% Stim 10 µM	0	0	0	0	0
Insulin Secretion	Insulin Secretion Hi Glc	% Stim 2 µM	0.7	0	3.1	0	4.8
		% Stim 10 µM	0	1.8	3.1	0	1.0

The results show that the tested tetrahydroisoquinoline analogues have no significant diabetes activities.

4.2.1.2.2. Diabetes biological activities of the *combretastatin* and the dioxancarbazole analogues (Table 21)
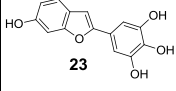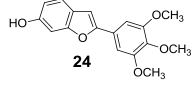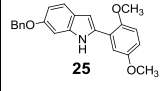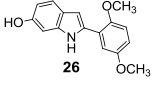
Table 21. Diabetes biological activity of the *combretastatin* and the dioxancarbazole analogues

Assays		Products	16	17	21	22
GLP-1 secretion	hNCI GLP-1 Sec	% Stim 2 µM	0	1.7	2	0
		% Stim 20 µM	0	3.2	2.1	0
GPR 119 Receptor	hGPR119Ag	% Stim 10 µM	0	0	0	0
Insulin Secretion	Insulin Secretion Hi Glc	% Stim 2 µM	0	1	0	0.8
		% Stim 10 µM	0	0.1	0	1.8

The results reveal that the tested combretastatin and dioxancarbazole analogues have no significant diabetes activities.

4.2.1.2.3. Diabetes biological activities of the *resveratrol* analogues (Table 22)

Table 22. Diabetes biological activity of the *resveratrol* analogues

Assays		Products	23	24	25	26
GLP-1 secretion	hNCI GLP-1 Sec	% Stim 2 µM	3.1	0.7	1.7	0
		% Stim 20 µM	0	1.3	2	2.2
GPR 119 Receptor	hGPR119Ag	% Stim 10 µM	0	0	0	0
Insulin Secretion	Insulin Secretion Hi Glc	% Stim 2 µM	1.7	0	0.2	0
		% Stim 10 µM	2.2	0	0	0.5

The results show that the tested *resveratrol* analogues have no significant diabetes activities.

4.2.2. Neurological disorder biological assays

4.2.2.1. Introduction

4.2.2.1.1. Calcitonin Gene-Related Peptide (CGRP) receptor Antagonist

CGRP is a 37 amino acid neuropeptide that plays a key role in the patho-physioloy of migraine. CGRP levels in venous plasma have been reported to be elevated during a migraine attack and are normalized after successful treatment of the migraine with a triptan (Goadsby & Edvinsson, 1994). Infusion of CGRP into individuals with a past history of migraine attacks can induce an attack, and CGRP receptor antagonists have successfully treated migraine attacks. As such, we are interested in the identification of novel, non-peptide CGRP receptor antagonists for the treatment of migraine attacks and other disorders.[114]

The CGRP cAMP assay tests compounds that inhibit the activation of the CGRP receptor and the resulting generation of cAMP by CGRP. The active molecules will be further characterized for their ability to inhibit the activity of the hormones calcitonin and amylin and the peptide adrenomedullin at receptors that respond to these molecules. Compounds of interest will selectively block the CGRP receptor and not have a biological effect at the calcitonin, amylin or adrenomedullin receptors.

4.2.2.1.2. MGlu2R Receptor Allosteric Antagonist

Glutamate is the major excitatory neurotransmitter in the mammalian CNS, acting at both ligand gated ion channels and G-protein coupled receptors, the latter known as metabotropic glutamate receptors (mGluR). It has been postulated that antagonists of the group II subclass of metabotropic glutamate receptors (mGlu2 and mGlu3) would be useful in certain neurological and psychiatric indications. The identification of small molecule competitive and allosteric antagonists of these receptors has been a long-standing goal toward novel therapeutic agents.[115]

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[114] A. Recobera, A. F. Russob. *Current Opinion in Neurology* **2009**, *22*, 241-246.
[115] J. M. Witkin, W. J. A. Eiler. *Drug Dev. Res.* **2007**, *5*, 187-194.
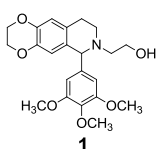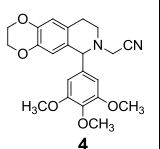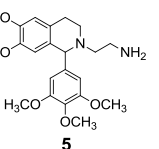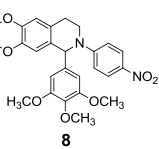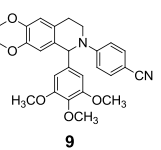
We seek to identify small molecule (MW < 500) negative allosteric modulators (NAMs, aka allostreric antagonists) for mGlu2R for the above mentioned uses. We desire compounds that are orally bioavailable with a suitable solubility.

4.2.2.2. Neurologic biological activity of the tested compounds

4.2.2.2.1. Neurologic biological activity of the tetrahydroisoquinoline analogues (Table 23)
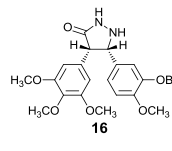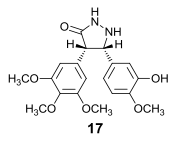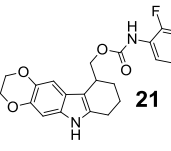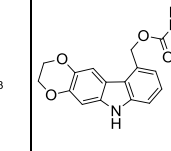
Table 23. Neurologic biological activity of the tetrahydroisoquinoline analogues

Products / Assays			1	4	5	8	9
mGluR Antagonist	hMGLUR2Antag	% inhib 50µM	4.9	5.5	11.1	14.6	2.7
CGRP Receptor	hCGRP1Antag	% inhib 30µM	0	0	0	0	9.7

The results show that the tested tetrahydroisoquinoline analogues have very little mGluR and CGRP biological activity.

4.2.2.2.2. Neurologic activity of the *combretastatin* and the dioxancarbazoles analogues (Table 24)
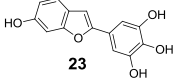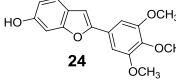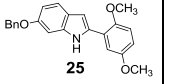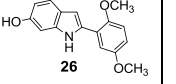
Table 24. Neurologic biological activity of the *dioxancarbazole* analogues

Assays		Products	16	17	21	22
mGluR Antagonist	hMGLUR2Antag	% inhib 50 µM	8.6	4.9	19.2	22.3
CGRP Receptor	hCGRP1Antag	%inhib 30 µM	0	0	0	0

The results reveal that the dioxancarbazole analogues **21** and **22** have a low mGluR and CGRP activity. However, the tested *combretastatin* analogues have no significant mGluR and CGRP biological activity.

4.2.2.2.3. Neurologic biological activity of the *resveratrol* analogues (table 25)

Table 25. Neurologic biological activity of the *resveratrol* analogues

		Products / Assays	23	24	25	26
mGluR Antagonist	hMGLUR2Antag	% inhib 50 µM	0	0	36.9	48.7
CGRP Receptor	hCGRP1Antag	% inhib 30 µM	0	0	16.7	0

The results show that the *resversatrol* analogues **25** and **26** present a moderate mGluR biological activity. **25** Also possesses a low CGRP activity. However, the compounds **23** and **24** present no mGluR and CGRP biological activity.

4.2.3. Complementary biological activity assays of the tetrahydroisoquinoline analogues

4.2.3.1. Introduction

4.2.3.1.1. Apelin Receptor (APJ) Agonist

The Apelin Receptor (APJ) agonist module tests for compounds that activate the G protein coupled receptors APJ. Apelin has been implicated in varied biological processes such as angiogenesis, blood pressure regulation, feeding behavior, and HIV entry. APJ activation is determined by the inhibition of forskolin stimulated cAMP in cells expressing human APJ (Forskolin is a labdane diterpene commonly used to raise levels of cAMP in the study and research of cell physiology). APJ activation is

confirmed by the lack of inhibition of forskolin stimulated cAMP in mock transfected cells.[116]

4.2.3.1.2. Hexokinase 2 inhibitors

The hexakinase is an enzyme that phorphorylates hexoses. Glucose is the most important substrate of hexokinases, and glucose-6-phosphate is the most important product formed (Scheme 72).
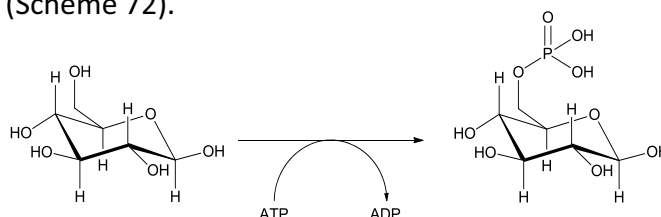
Scheme 72. Hexokinase-mediated phosphorylation of glucose.

Hexokinase II constitutes the principal isoform and is present in higher quantities in cancer diseases[117].

4.2.3.2. APJ and hexokinase biological activity of the tetrahydroisoquinoline analogues (table 26)

Table 26. APJ and hexokinase biological activity of the tetrahydroisoquinoline analogues

		Products / Assays	1	4	5	8	9
APJ Agonist	hApelin Ag	% Stim 30 µM	3.5	20.2	8.2	11.6	0
Hexokinase 2	hHK2 ADP-FP	% inhib 20 µM	0	28.7	0	23.6	23.2
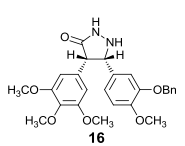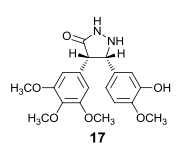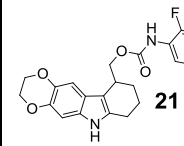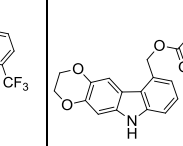
[116] K. Tatemoto, M. Hosoya, Y. Habata, Fujii. R. T. Kakegawa, M. X. Zou, Y. Kawamata, S. Fukusumi, S. Hinuma, C. Kitada, T. Kurokawa, H. Onda, M. Fujino. *Biochem. Biophys. Res. Commun*. **1998**, *251*, 471-476.
[117] H. Osawa, C. Sutherland, R. B. Robey, R. L. Printz, D. K. Granner. *J. Biol. Chem*. **1996**, *271*, 16690-16694.

The results show that the tetrahydroisoquinoline analogues present a low APJ activity, compound **4** being the most active. Moreover, compounds **4**, **8** and **9** possess a low hexokinase 2 inhibition.

4.2.3.3. APJ and hexokinase biological activity of the *combretastatin* and the dioxancarbazole analogues (Table 27)
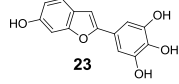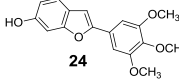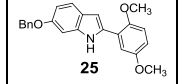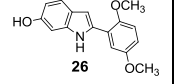
Table 27. APJ and hexokinase biological activity of the combretastatin and the dioxancarbazole analogues

Products / Assays			16	17	21	22
APJ Agonist	hApelin Ag	% Stim 30 µM	0	13.7	7.5	2.4
Hexokinase 2	hHK2 ADP-FP	% inhib 20 µM	9.5	5.2	22.5	0

The results show that only the *combretastatin* analogues presents a low APJ activity of 13.7% inhibition. Moreover, compound **21** possesses a significant hexokinase 2 inhibition of 22.5%.

4.2.3.4. APJ and hexokinase biological activity of the *resveratrol* analogues (table 28)

Table 28. APJ and hexokinase biological activity of the *resveratrol* analogues

Assays \ Products			23	24	25	26
APJ Agonist	hApelin Ag	% Stim 30 µM	0	0	0	2.4
Hexokinase 2	hHK2 ADP-FP	% inhib 20 µM	11.8	22.3	15.3	0

The results show that the *resveratrol* analogues studied don't have a significant APJ activity. presents a low APJ activity. However, compounds **23**, **24** and **25** present a low hexokinase 2 inhibition.

5. Experimental section

Safety procedures:

The experimenter has to be aware of the risks of the equipment he uses. Before any experiment, the experimenter has to write in his lab book the experiment number, the date, the reaction scheme and the quantity of reagents he will be using.

Control Measures:

All experiments are to be carried out in a fume cupboard. Gloves, coat and lab spectacles are to be worn at all times. Inhalations of chemicals and skin or eye contact are to be avoided.

Emergency procedure:

In case of skin/eye contact, rinse immediately with plenty of water and seek medical attention. If feeling unwell, seek medical attention. In case of solvent spillage clean up with paper towels and leave to evaporate at the back of the fume hood. Reagent spillage has to be cleaned with paper towels and disposed of via the safety office.

Waste disposal:

Flammable waste has to be disposed of via the flammable waste stream. Special chemical waste has to be put in separated bottle for special disposal

General experimental:

Melting points were obtained on an MFB-595010M Gallenkamp apparatus in open capillary tubes and are uncorrected. IR spectra were obtained using an FTIR Perkin-Elmer 1600 Infrared Spectrophotometer. Only noteworthy IR absorptions are listed (cm^{-1}) ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 (200 and 50.3 MHz, respectively) or Varian Gemini-300 (300 and 75.5 MHz) Instrument using CDCl$_3$ as solvent with tetramethylsilane as internal standard. Mass spectra were recorded on a Hewlett-Packard 5988-A. Column chromatography was performed with silica gel (E. Merck, 40-60 μm) or aluminium oxide (E. Merck, 90 standardized). Reactions were monitored by TLC using 0.25 mm silica gel F-254 (E. Merck). p*H* were measured with a universal p*H* paper indicator (Merck, p*H* 1-10). All reagents were of commercial quality or were purified before use according to the literature procedure. Organic solvents were of analytical grade or were purified by standard procedures. Commercial products were purchased from Sigma-Aldrich.

2-[6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolin-7-yl)] ethanol (1)

3
$C_{20}H_{23}NO_5$
357,40 g/mol

1
$C_{22}H_{27}NO_6$
401,45 g/mol
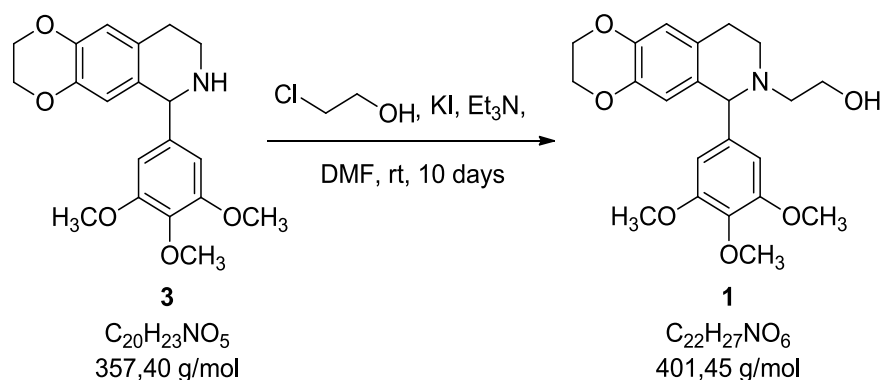
The tetrahydroisoquinoline **3** (150 mg, 0.42 mmol) was dissolved in DMF (7 mL) and put in a flame-dried round-bottom flask under argon. 2-Chloroethanol (0.11 mL, 1.66 mmol), KI (cat) and Et_3N (0.47 mL, 3.36 mmol) were added under argon. The reaction was stirred at rt and 2-Chloroethanol (0.11 mL, 1.66 mmol) and KI (cat) were added every day under argon for 7 days. Then, TLC of the crude mixture (EtOAc/MeOH 9:1) indicated formation of a product (R_f 0.50) and complete consumption of SM (R_f 0.15). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 3:7) to afford the 2-substitued ethanol **1** (54 mg, 32% yield) as a white solid.

Product aspect: white solid.

Theoretical mass: 168 mg.

Mass obtained: 54 mg.

Yield: 32%.

Analytical data:
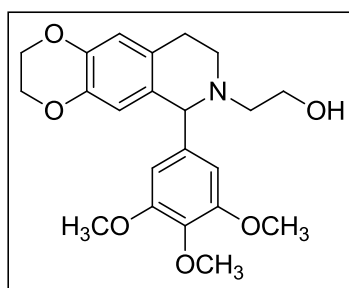
*Compound **1***

R_f = 0.50 (EtOAc/MeOH 9:1).

mp: 120-122 $^{\circ}$C (CH$_2$Cl$_2$).

IR (film) ν cm^{-1} : 3527 (OH), 1852-1920 (C-H), 1593, 1502, 1465, 1422 (Ar-H), 1302, 1127 (Ar-O), 1062 (Ar-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.36-2.43 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 2.52-2.61 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 2.71-2.85 (m, 2H, N-C$\underline{H}_2$-CH$_2$-OH), 2.95-3.05 (m, 1H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.21-3.28 (m, 1H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.40-3.47 (m, 1H, C$\underline{H}_2$-OH), 3.65-3.73 (m, 1H, C$\underline{H}_2$-OH), 3.81 (s, 6H, CH$_3$-O (x 2)), 3.84 (s, 3H, CH$_3$-O), 4.15-4.25 (m, 4H, O-CH$_2$-CH$_2$-O), 4.40 (s, 1H, H-6), 6.26 (s, 1H, H-5), 6.45 (s, 2H, H-2', H-6'), 6.63 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 28.6 (CH$_2$, Ar-$\underline{C}$H$_2$-CH$_2$-N). 47.8 (CH$_2$, Ar-CH$_2$-$\underline{C}$H$_2$-N), 55.6 (CH$_2$, N-$\underline{C}$H$_2$-CH$_2$-OH), 56.5 (CH$_3$, CH$_3$-O (x 2)), 58.5 (CH$_2$, Ar-CH$_2$-$\underline{C}$H$_2$-OH), 61.2 (CH$_3$, CH$_3$-O), 64.7 (CH$_2$, O-CH$_2$-$\underline{C}$H$_2$-O), 64.7 (CH$_2$, O-$\underline{C}$H$_2$-CH$_2$-O), 69.4 (CH, C-6), 106.7 (CH, C-2', C-6'), 116.4 (CH, C-5), 117.1 (CH, C-10), 127.7 (C, C-5a), 131.3 (C, C-9a), 139.6 (C, C-1'), 141.9 (C, C-4a), 142.4 (C, C-10a), 153.5 (C, C-3', C-4', C-5').

MS (EI) (m/z, %): 401 (M$^+$, 6), 370 (M$^+$-CH$_2$OH, 100), 234 (M$^+$-C$_9$H$_{12}$O$_3$, 78).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

(6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro-[1,4]dioxino[2,3-*g*]isoquinolin-7-yl) 2,2,2-trifluoroacetyl (2)
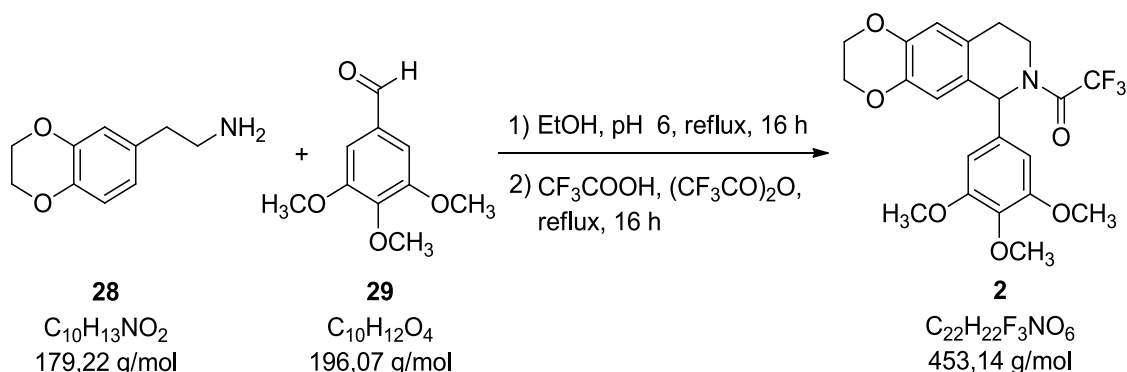
To a solution of 2-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)ethylamine **28** (250 mg, 1.39 mmol) in ethanol (20 mL) was added HCl (0,1 mL), molecular sieves (50 mg) and 3,4,5-trimethoxybenzaldehyde **29** (410 mg, 2.09 mmol) in a flame-dried round-bottom flask under argon. Et$_3$N was added until p*H* 6-6.5 was reached and the mixture was refluxed under stirring for 16 h. The mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc (20 mL), washed with NaOH (3 x 20 mL of a 2N solution), filtered and concentrated *in vacuo* to afford a brown oil. CF$_3$COOH (3 mL, excess amount) and (CF$_3$CO)$_2$O (3 mL, excess amount) were added and the crude mixture was refluxed under stirring for 16 h. Then, TLC of the crude mixture (EtOAc/hexane 1:1) indicated presence of a new compound (R$_f$ 0.75) and uncomplete consumption of the 3,4,5-trimethoxybenzaldehyde (Rf 0.80). The mixture was dissolved in EtOAc (20 mL), washed with NaOH (3 x 30 mL of a 2N solution), dried (Na$_2$SO$_4$) filtered and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 7:3) to afford the desired isoquinoline **2** (102 mg, 16% yield) as a brown oil.

Product aspect: brown oil.

Theoretical mass: 638 mg.

Mass obtained: 102 mg.

Yield: 16%.

Analytical data:
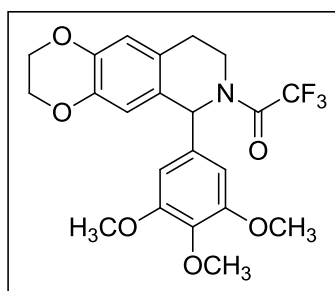
Compound 2

R$_f$ = 0.75 (hexane/EtOAc 1:1).

IR (film) ν cm^{-1} : 1686 (C=O), 1504 (Ar-H), 1299 (Ar-O), 1127 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.75-2.82 (m, 1H, CH$_2$-CH$_2$-N), 2.94-3.05 (m, 2H, CH$_2$-CH$_2$-N, CH$_2$-CH$_2$-N), 3.18-3.25, (m, 1H, CH$_2$-CH$_2$-N), 3.77 (s, 6H, CH$_3$-O (x 2)), 3.83 (s, 3H, CH$_3$-O), 4.20-4.25 (m, 4H, CH$_2$-O), 6.45 (s, 2H, H-2', H-6'), 6.57 (s, 1H, H-5), 6.62 (s, 1H, H-6), 6.70 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 28.4 (CH$_2$, CH$_2$N), 39.5 (CH$_2$, CH$_2$-Ar), 56.2 (CH, C-6), 60.8 (CH$_3$, OCH$_3$ (x 2)), 64.4 (CH$_3$, OCH$_3$), 106.2 (CH, C-2', C-6'), 116.5 (CH, C-5), 116.3 (C, *J* = 288 Hz, CF$_3$), 116.8 (CH, C-10), 126.0 (C, C-1'), 136.5 (C, C-5a), 137.8 (C, C-9a), 142.3 (C, C-10a), 142.9 (C, C-4a), 152.9 (C, C-4'), 153.0 (C, C-3', C-5'), 156.4 (C, C=O).

 MS (EI) (m/z, %): 453 (M$^+$, 71), 438 (M$^+$-CH$_3$, 100), 286 (M$^+$-C$_9$H$_{11}$O$_3$, 32).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinoline (3)
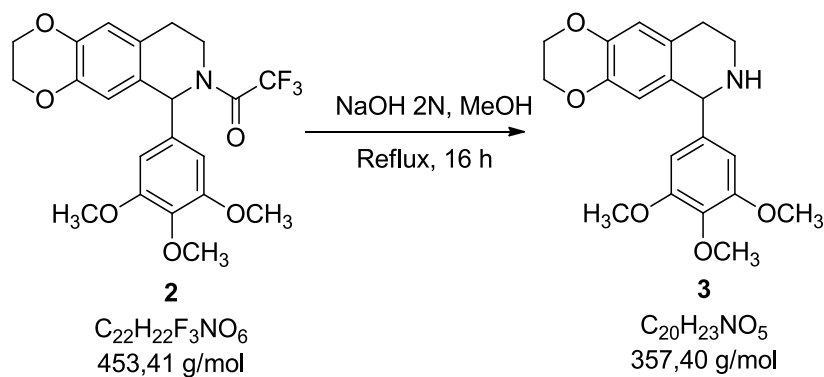
The isoquinolin-trifluoroacetate **2** (285 mg, 0.63 mmol) was dissolved in MeOH (15 mL), then NaOH 2N (45 mL) was added and the reaction was refluxed overnight under stirring. Then, TLC of the crude mixture (EtOAc/hexane 1:1) showed total consumption of SM (R_f 0.80) and formation of the desired product (R_f 0.10). The methanol was evaporated *in vacuo* and the aqueous phase was extracted with diethyl ether (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the substituted isoquinoline **3** (268 mg, 94% yield) as a brown solid.

Product aspect: brown solid.

Theoretical mass: 285 mg.

Mass obtained: 268 mg.

Yield: 94%.

Analytical data:
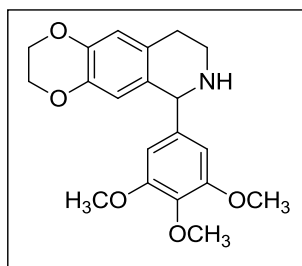
Compound 3

R$_f$ = 0.30 (EtOAc).

mp: 131-132 ^{o}C (diethyl ether).

IR (KBr) ν cm^{-1}: 3100 (NH), 1589 (C=C), 1297 (Ar-O), 1125 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.54-2.65 (m, 1H, C$\underline{H}_2$-CH$_2$-N), 2.83-3.03 (m, 2H, CH$_2$-C$\underline{H}_2$-N, C$\underline{H}_2$-CH$_2$-N), 3.18-3.25 (m, 1H, CH$_2$-C$\underline{H}_2$-N), 3.75 (s, 6H, CH$_3$-O (x 2)), 3.77 (s, 3H, CH$_3$-O), 4.13 (m, 4H, CH$_2$-O (x 2)), 4.81 (s, 1H, H-6), 6.22 (s, 1H, H-5), 6.45 (s, 2H, H-2', H-6'), 6.56 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 28.9 (CH$_2$, C-9), 43.1 (CH$_2$, CH$_2$N), 56.0 (CH$_3$, OCH$_3$ (x 2)), 60.7 (CH$_3$, OCH$_3$), 62.4 (CH, C-6), 64.3 (CH$_2$, OCH$_2$), 64.2 (CH$_2$, OCH$_2$), 105.6 (CH, C-2', C-6'), 115.9 (CH, C-5), 116.6 (CH, C-10), 128.1 (C, C-5a), 131.3 (C, C-9a), 140.1 (C, C-1'), 141.4 (C, C-4a), 141.9 (C, C-10a), 152.8 (C, C-4'), 152.9 (C, C-3', C-5').

6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinoline (3) (alternative synthesis)

28	**29**	**3**
$C_{10}H_{13}NO_2$	$C_{10}H_{12}O_4$	$C_{20}H_{23}NO_5$
179,22 g/mol	196,07 g/mol	357,40 g/mol
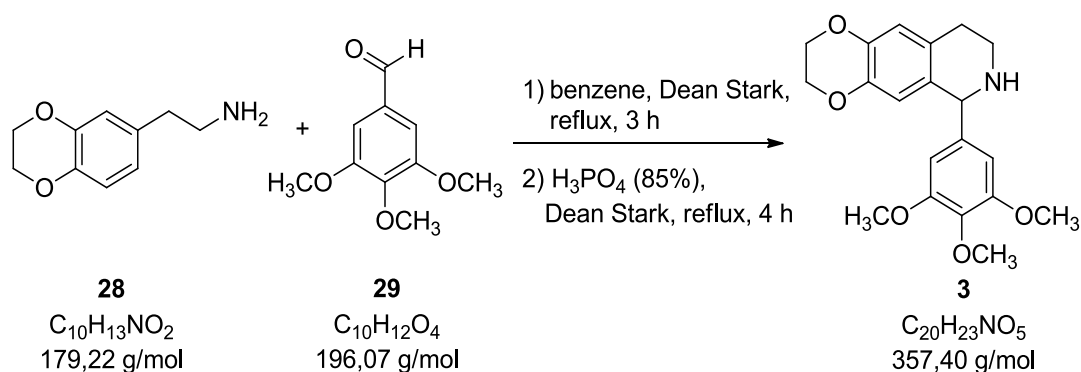
The phenethylamine **28** (228 mg, 0.84 mmol) and 3,4,5-trimethoxybenzaldehyde (**29**) (274 mg, 0.92 mmol) were dissolved in benzene (15 mL) in a flame-dried round-bottom flask under argon. The reaction mixture was refluxed under stirring in a Dean Stark reaction for 4 h. The solution was cooled at 0 ^{o}C and H_3PO_4 (2 mL of a 85% aqueous solution) was added. The reaction mixture was refluxed under stirring in a Dean Stark reaction for 3 h and TLC of the crude mixture (EtOAc) indicated presence of the tetrahydroisoquinoline desired product (R_f 0.30) and uncomplete consumption of the aldehyde (R_f 0.80). The reaction mixture was cooled at 0 ^{o}C and quenched with NaOH (5 mL of a 2N aqueous solution). The mixture was dissolved in CH_2Cl_2 (20 mL), washed with NaOH 2N (3 x 30 mL) and the organic phases were re-extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 3:7) to afford the tetrahydroisoquinoline **3** (100 mg, 20% yield) as a brown solid. The reaction was scaled up using 2 g of phenethylamine **28** to afford **3** (1.4 g, 32% yield) as a brown solid.

Product aspect: brown solid.

Theoretical mass: 4.4 g.

Mass obtained: 1.4 g.

Yield: 32%.

Analytical data was identical with the previously described compound.

2-(6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolin-7-yl)acetonitrile (4)
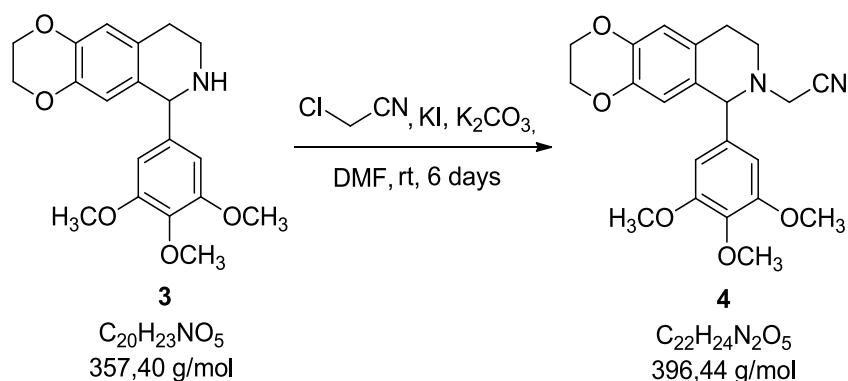
The isoquinoline **3** (150 mg, 0.42 mmol) was dissolved in DMF (10 mL) in a flame-dried round-bottom flask under argon. Chloroacetonitrile (0.08 mL, 1.26 mmol), KI (cat) and K_2CO_3 (290 mg, 2.1 mmol) were added under argon. The reaction was stirred at rt and chloroacetonitrile (0.08 mL, 1.26 mmol) and KI (cat) were added every day under argon for 6 days. Then, TLC of the crude mixture (EtOAc) showed formation of a new compound (R_f 0.85) and complete consumption of the starting compound (R_f 0.10). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 1:1) to afford the desired compound **4** (140 mg, 84% yield) as a brown solid.

Product aspect: brown solid.

Theoretical mass: 167 mg.

Mass obtained: 140 mg.

Yield: 84%.

Analytical data:
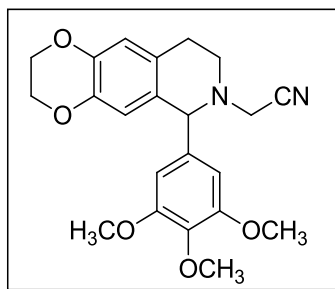
Compound 4

R_f = 0.85 (EtOAc).

mp: 144-146 °C (hexane/EtOAc).

IR (film) ν cm⁻¹: 2925 (C-H), 2363 (CN), 1588, 1503, 1455, 1417 (Ar-H), 1295, 1233 (Ar-O), 1122, 1066 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.65-2.75 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 2.85-3.00 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 3.01-3.35 (m, 2H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.43 (s, 2H, C$\underline{H}_2$-CN), 3.80 (s, 6H, OCH$_3$ (x 2)), 3.82 (s, 3H, OCH$_3$), 4.09-4-26 (m, 4H, O-CH$_2$-CH$_2$-O), 4.45 (s, 1H, H-6), 6.18 (s, 1H, H-5), 6.52 (s, 2H, H-2', H-6'), 6.58 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 29.0 (CH$_2$, Ar-$\underline{C}$H$_2$-CH$_2$-N), 44.3 (CH$_2$, CH$_2$-N), 50.5 (Ar-$\underline{C}$H$_2$-CN), 56.5 (CH$_3$, CH$_3$-O (x 2)), 61.1 (CH$_3$, CH$_3$-O), 64.6 (CH$_2$, O-CH$_2$-$\underline{C}$H$_2$-O), 64.7 (CH$_2$, O-$\underline{C}$H$_2$-CH$_2$-O), 67.6 (CH, C-6), 106.3 (CH, C-2', C-6'), 115.1 (C, CN), 116.4 (CH, C-5), 116.9 (CH, C-10), 126.8 (C, C-5a), 130.7 (C, C-9a), 137.7 (C, C-1'), 137.9 (C, C-1'), 142.0 (C, C-4a), 142.5 (C, C-10a), 153.4 (C, C-4'), 153.8 (C, C-3', C-5').

MS (EI) (m/z, %): 396 (M$^+$, 33), 229 (M$^+$-C$_9$H$_{12}$O$_3$, 100).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

2-(6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolin-7-yl)ethylamine (5)
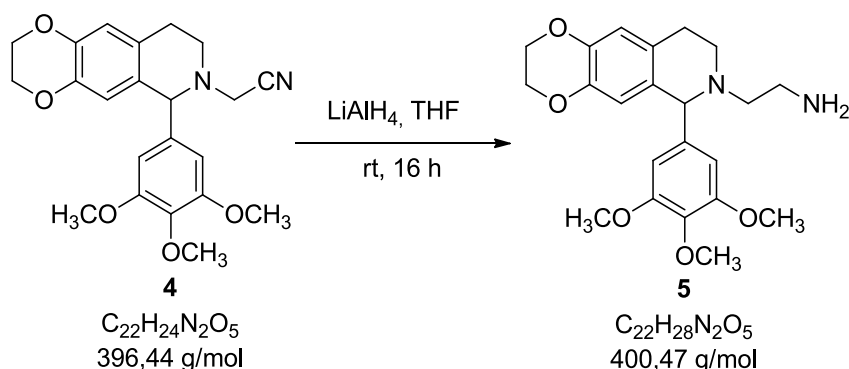
The isoquinoline **4** (115 mg, 0.29 mmol) was dissolved in THF (10 mL) in a flame-dried round-bottom flask under argon, and LiAlH$_4$ (35 mg, 0.86 mmol) was added. The reaction was stirred at rt for 16 h and TLC of the crude mixture (EtOAc/MeOH, 7:3) showed formation of a new compound (R$_f$ 0.25) and complete consumption of SM (0.95). The crude mixture was quenched dropwise with water and filtered. The solid residue was extracted with CH$_2$Cl$_2$ (3 x 20 mL) and the combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo.* The residue was purified by silica gel flash column chromatography (EtOAc/MeOH, 2:8) to afford the isoquinoline **5** (60 mg, 52% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 115 mg.

Mass obtained: 60 mg.

Yield: 52 %.

Analytical data:
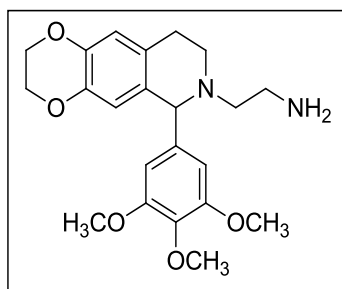
Compound 5

R$_f$ = 0.25 (EtOAc/MeOH 7:3).

mp: 55-60 °C (hexane/EtOAc).

IR (film) ν cm^{-1}: 3300-3100 (NH$_2$), 2923, 2834 (C-H), 1588, 1503, 1456, 1418 (Ar-H), 1296, 1232 (Ar-O) 1122, 1066 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.40-2.56 (m, 2H, CH$_2$-Ar), 2.59-2.85 (m, 4H, CH$_2$-N (x 2), 2.96-3.06 (m, 1H, C$\underline{H}_2$-N), 3.15-3.22 (m, 1H, C$\underline{H}_2$-N), 3.81 (s, 6H, CH$_3$-O (x 2)), 3.84 (s, 3H, CH$_3$-O), 4.14-4.20 (m, 4H, O-CH$_2$-CH$_2$-O), 4.27 (s, 1H, H-6), 6.25 (s, 1H, H-5), 6.51 (s, 2H, H-2', H-6'), 6.61 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 28.9 (CH$_2$, Ar-C$\underline{H}_2$-CH$_2$-N), 39.4 (CH$_2$, N-CH$_2$-C$\underline{H}_2$-NH$_2$), 48.4 (CH$_2$, Ar-CH$_2$-C$\underline{H}_2$-N), 56.4 (CH$_3$, OCH$_3$ (x 2)), 57.4 (CH$_2$, 2H, N-C$\underline{H}_2$-CH$_2$-NH$_2$) , 61.1 (CH$_3$, OCH$_3$), 64.6 (CH$_2$, O-CH$_2$-C$\underline{H}_2$-O), 64.7 (CH$_2$, O-C$\underline{H}_2$-CH$_2$-O), 69.9 (CH, C-6), 106.7 (CH, C-2', C-6'), 116.4 (CH, C-5), 117.0 (CH, C-10) , 127.9 (C, C-5a), 131.8 (C, C-9a), 140.2 (C, C-1'), 141.8 (C, C-4a), 142.2 (C, C-10a), 153.4 (C, C-3', C-4', C-5').

MS EI m/z (%): 400 (M$^+$, 1), 370 (M$^+$-CH$_5$N, 64) 355 (M$^+$-C$_2$H$_7$N, 57).

6-(3,4,5-Trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-*g*]isoquinolin-7-yl)

ethylamine (6)
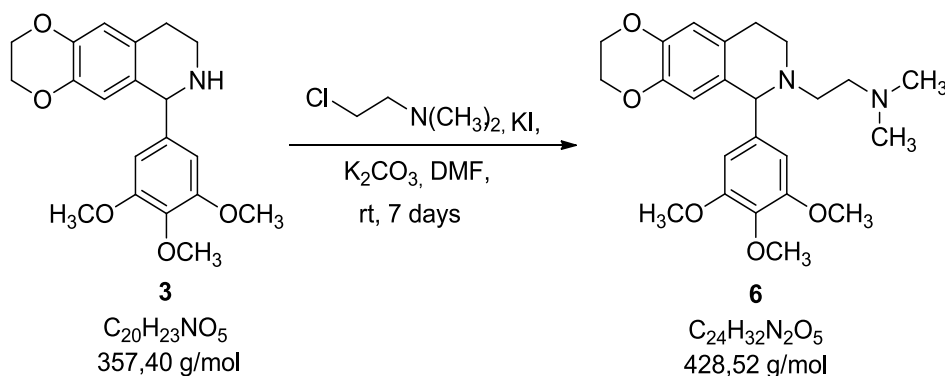
3
$C_{20}H_{23}NO_5$
357,40 g/mol

6
$C_{24}H_{32}N_2O_5$
428,52 g/mol

The tetrahydroisoquinoline **3** (100 mg, 0.28 mmol) was dissolved in DMF (10 mL) in a flame-dried round-bottom flask under argon. 2-Dimethylaminoethyl chloride (121 mg, 0.84 mmol), KI (cat) and K_2CO_3 (193 mg, 1.4 mmol) were added under argon. The reaction was stirred at rt and 2-dimethylaminoethyl chloride (121 mg, 0.84 mmol) and KI (cat) were added every day under argon for 7 days. Then, TLC of the reaction mixture (EtOAc/MeOH 7:3) showed formation of a new compound (R_f 0.25) and uncomplete consumption of the starting compound (R_f 0.75). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (EtOAc/MeOH, 8:2) to afford the substituted isoquinoline **6** (10 mg, 8% yield) as a yellow oil. The reaction was repeated with 300 mg of SM to afford the substituted isoquinoline **6** (20 mg, 5% yield) as a yellow oil.

Product aspect: yellow oil.

Theoretical mass: 120 mg.

Mass obtained: 10 mg.

Yield: 8%.

Analytical data:
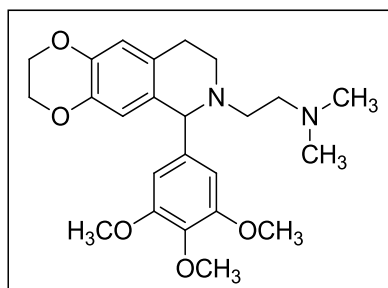
Compound 6

R_f = 0.25 (EtOAc/MeOH 8:2).

IR (film) ν cm^{-1} : 2935, 2800 (C-H), 1589, 1505, 1418 (Ar-H), 1290, 1235, 1124 (Ar-O), 1067 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.31 (s, 6H, CH$_3$-N (x 2)), 2.64 (t, J = 6 Hz, 2H, N-CH$_2$-CH$_2$-N), 2.69 (t, J = 6 Hz, 2H, N-CH$_2$-CH$_2$-N), 2.80-2.95 (m, 2H, Ar-CH$_2$-CH$_2$-N), 3.20-3.29 (m, 2H, Ar-CH$_2$-CH$_2$-N), 3.78 (s, 6H, OCH$_3$ (x 2)), 3.82 (s, 3H, OCH$_3$), 4.20-4.30 (m, 4H, O-CH$_2$-CH$_2$-O), 4.28 (s, 1H, H-6), 6.45 (s, 2H, H-2', H-6'), 6.57 (s, 1H, H-5), 6.68 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 38.7 (CH$_3$, CH$_3$-N (x 2)), 46.1 (CH$_2$, Ar-CH$_2$), 56.5 (CH$_2$, N-CH$_2$), 57.73 (CH, C-6), 57.8 (CH$_3$, O-CH$_3$), 58.5 (CH$_2$, N-CH$_2$), 61.2 (CH$_3$, OCH$_3$ (x 2)), 63.8 (CH$_2$, N-CH$_2$), 64.7 (CH$_2$, O-CH$_2$-CH$_2$-O), 64.8 (CH$_2$, O-CH$_2$-CH$_2$-O), 106.1 (CH, C-2', C-6'), 116.9 (CH, C-5), 117.1 (CH, C-10), 128.4 (C, C-5a), 137.7 (C, C-9a), 138.8 (C, C-1') 142.2 (C, C-4a), 142.9 (C, C-10a), 153.2 (C, C-3', C-4', C-5').

MS (EI) (m/z, %): 428 (M$^+$, 21).

N-(3,3-(Diethoxy)propyl)-6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-g]isoquinoline (7)
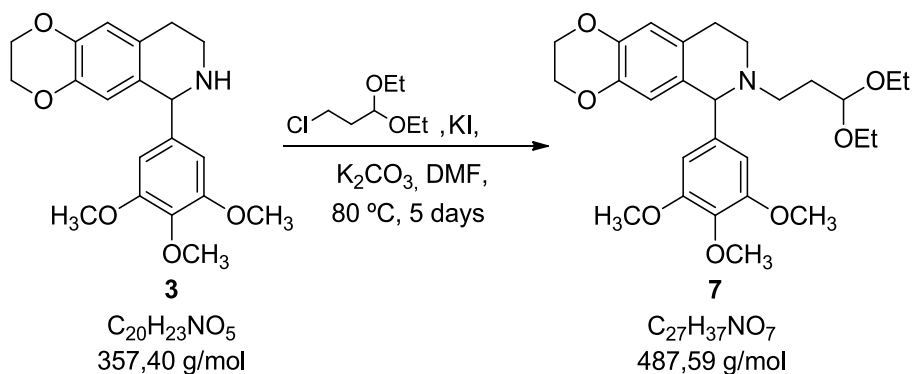
The isoquinoline **3** (200 mg, 0.56 mmol) was dissolved in DMF (5 mL) in a flame-dried round-bottom flask under argon. 3-Chloropropionaldehydediethylacetal (0.14 mL, 0.84 mmol), KI (cat) and Et$_3$N (0.31 mL, 2.24 mmol) were added under argon. The reaction was stirred at 80-90 °C and chloropropionaldehydediethylacetal (0.14 mL, 0.84 mmol) and KI (cat) were added every day under argon for 5 days. Then, TLC of the crude mixture (EtOAc) showed formation of a new product (R$_f$ 0.80) and complete consumption of the starting material (R$_f$ 0.10). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H$_2$O (5 x 30 mL), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo.* The residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired acetal **7** (40 mg, 15% yield) as a white oil.

Product aspect: white oil.

Theoretical mass: 267 mg.

**Mass obtained:** 40 mg.

Yield: 15%.

Analytical data:
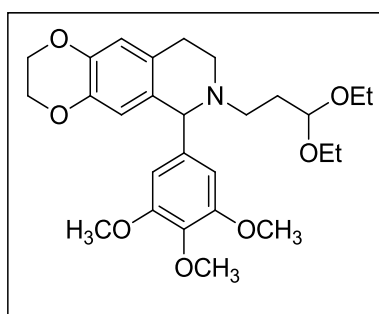
*Compound **7***

R_f = 0.80 (EtOAc).

IR (film) ν cm⁻¹ : 2949, 2929 (C-H), 1586, 1505, 1457 (Ar-H), 1300, 1122 (Ar-O), 1062 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.05 (t, J = 6.9 Hz, 3H, O-CH$_2$-C$\underline{H}_3$), 1.13 (t, J = 6.9 Hz, 3H, O-CH$_2$-C$\underline{H}_3$), 1.70-1.85 (m, 2H, N-CH$_2$-C$\underline{H}_2$-CH), 2.20-2.35 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 2.40-2.80 (m, 4H, Ar-C$\underline{H}_2$-C$\underline{H}_2$-N, N-C$\underline{H}_2$-CH$_2$-CH), 2.90-3.10 (m, 1H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.14-3.33 (m, 2H, O-C$\underline{H}_2$-CH$_3$), 3.37-3.60 (m, 2H, O-C$\underline{H}_2$-CH$_3$), 3.81 (s, 6H, OCH$_3$ (x 2)), 3.83 (s, 3H, OCH$_3$), 4.12-4.22 (m, 4H, O-CH$_2$-CH$_2$-O), 4.26 (s, 1H, H-6), 4.45 (t, J = 5.1 Hz, 1H, CH$_2$-CH$_2$-C$\underline{H}$), 6.24 (s, 1H, H-5), 6.51 (s, 2H, H-2', H-6'), 6.59 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 25.9 (CH$_3$, OCH$_3$), 28.9 (CH$_3$, O-CH$_2$-CH$_3$), 31.2, (CH$_2$, $\underline{C}$H$_2$-CH), 48.6 (CH$_2$, Ar-$\underline{C}$H$_2$), 50.5 (CH$_2$, Ar-CH$_2$-$\underline{C}$H$_2$-N), 56.4 (CH$_3$, OCH$_3$ (x 2)), 57.2 (CH$_3$, OCH$_3$), 60.7 (CH$_2$, N-$\underline{C}$H$_2$-CH$_2$-CH), 61.1 (CH$_2$, O-CH$_2$-CH$_3$), 61.6 (CH$_2$, CH$_3$-$\underline{C}$H$_2$-O), 63.1 (CH$_2$, O-CH$_2$-$\underline{C}$H$_2$-O), 64.7 (CH$_2$, O-$\underline{C}$H$_2$-CH$_2$-O), 69.4 (CH, C-6), 101.7 (CH,CHOO), 106.7 (CH, C-2', C-6'), 116.3 (CH, C-5), 116.5 (CH, C-10), 127.9 (C-5a), 132.9 (C-4a), 137.3 (C-1'), 141.7 (C, C-9a), 142.1 (C, C-10a), 153.3 (C, C-3', C-5'), 153.9 (C, C-4').

 MS (EI) (m/z, %): 487 (M$^+$, 4), 370 (M$^+$-C$_7$H$_{16}$O$_2$, 65), 356 (M$^+$-C$_6$H$_{14}$O$_2$], 94) 320 (M-C$_9$H$_{12}$O$_3$, 100).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

N-(4-Cyanophenyl)-6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-g]isoquinoline (8)
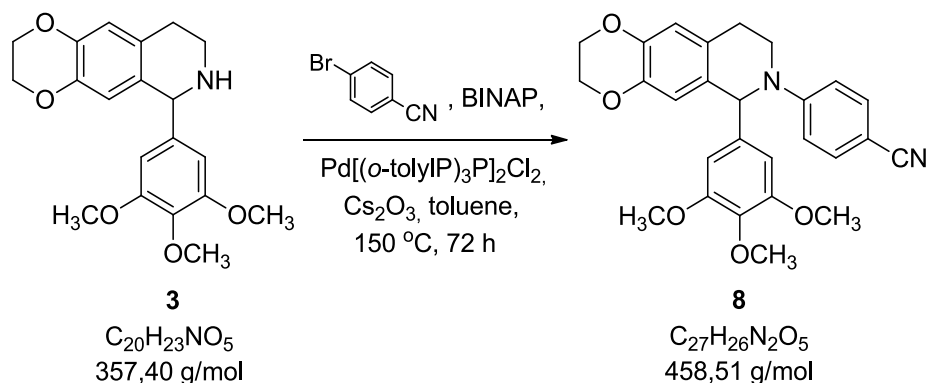
The tetrahydroisoquinoline **3** (120 mg, 0.34 mmol) and 1-bromo-4-cyanobenzene (92 mg, 0.50 mmol) were dissolved in toluene (5 mL) in a flame-dried round-bottom flask under argon. Cs_2CO_3 (2.44 mg, 0.67 mmol), (±) BINAP (cat) and Pd[(o-tolyl)$_3$P]$_2$Cl$_2$ (cat) were added under argon. The reaction was heated at 150 °C under stirring for 72 h and TLC of the reaction mixture (hexane/EtOAc 1:1) indicated formation of a yellow product (R$_f$ 0.60) and uncomplete consumption of SM. The crude mixture was concentrated *in vacuo* and purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired isoquinoline **8** (40 mg, 26% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 154 mg.

Mass obtained: 40 mg.

Yield: 26%.

Analytical data:
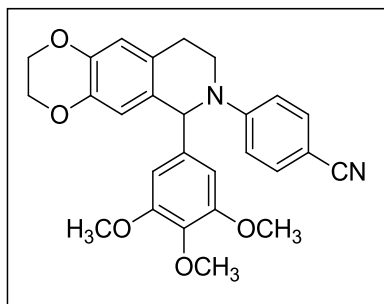
*Compound **8***

R$_f$ = 0.60 (hexane/EtOAc 1:1).

mp: 72-78 $^{\circ}$C (hexane/EtOAc).

IR (film) ν cm^{-1} : 2924, 2851 (C-H), 2212 (CN), 1603, 1503, 1461, 1413 (Ar-H), 1235 (Ar-O), 1178, 1066 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ **(ppm):** 2.84-2.91 (m, 2H, Ar-C$\underline{H}_2$-CH$_2$-N), 3.46-3.54 (m, 2H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.74 (s, 6H, OCH$_3$ (x 2)), 3.80 (s, 3H, OCH$_3$), 4.26 (s, 4H, 2 x CH$_2$-O), 5.66 (s, 1H, H-6), 6.41 (s, 2H, H-2', H-6'), 6.70 (s, 1H, H-5), 6.79 (d, 2H, H-2'', H-6''), 6.86 (s, 1H, H-10), 7.47 (d, 2H, H-3'', H-5'').

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ **(ppm):** 27.6 (CH$_2$, Ar-$\underline{C}$H$_2$-CH$_2$-N), 44.7 (CH$_2$, CH$_2$-N), 56.6 (CH$_3$, CH$_3$-O (x 2)), 61.1 (CH, C-6), 62.2, (CH$_3$, CH$_3$-O), 64.7 (CH$_2$, CH$_2$-O (x 2)), 99.0 (C, CN) 104.2 (CH, C-2', C-6'), 112.8 (CH, C-2'', C-6''), 116.4 (CH, C-5), 116.8 (CH, C-10), 120.7 (C, C-4''), 128.4 (C, C-5a), 130.3 (C, C-9a), 133.8 (CH, C-3'', C-5''), 137.6 (C, C-1'), 142.4 (C, C-4a), 143.2 (C, C-10a), 152.2 (C, C-1''), 153.6 (C, C-3', C-4', C-5').

MS (EI) (m/z, %): 458 (M$^+$, 23), 291 (M$^+$-C$_9$H$_{12}$O$_3$, 100).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

N-(4-Nitrophenyl)-6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-g]isoquinoline (9)

3
C$_{20}$H$_{23}$NO$_5$
357,40 g/mol

9
C$_{26}$H$_{26}$N$_2$O$_7$
478,49 g/mol
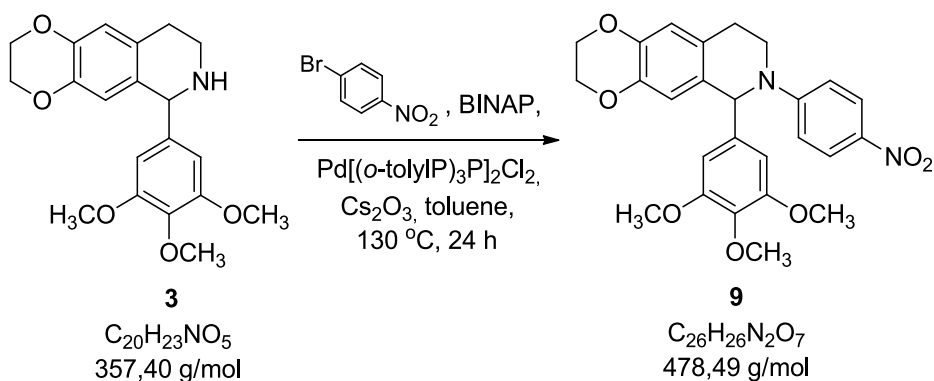
The tetrahydroisoquinoline **3** (120 mg, 0.34 mmol) and 1-bromo-4-nitrobenzene (81 mg, 0.4 mmol) were dissolved in toluene (5 mL) in a flame-dried round-bottom flask under argon. Cs$_2$CO$_3$ (219 mg, 0.67 mmol), (±) BINAP (cat) and Pd[(o-tolyl)$_3$P]$_2$Cl$_2$ (cat) were added under argon. The reaction was heated at 130 °C for 24 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a yellow product (R$_f$ 0.55) and complete consumption of SM (R$_f$ 0.10). The crude mixture was evaporated *in vacuo* and purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the *N*-arylated isoquinoline **9** (52 mg, 33% yield) as a bright yellow solid.

Product aspect: bright yellow solid.

Theoretical mass: 156 mg.

Mass obtained: 52 mg.

Yield: 33%.

Analytical data:
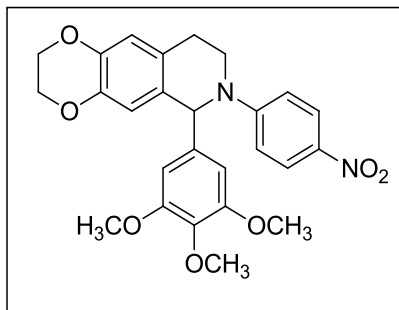
*Compound **9***

R_f **=** 0.55 (hexane/EtOAc 1:1).

mp: 78-80 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 2934 (C-H), 1591, 1502, 1460, 1414 (Ar-H), 1290, 1112 (Ar-O), 1066 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.83-2.98 (m, 2H, Ar-C$\underline{H}_2$-CH$_2$-N), 3.52-3.61 (m, 2H, Ar-CH$_2$-C$\underline{H}_2$-N), 3.74 (s, 6H, OCH$_3$ (x 2)), 3.80 (s, 3H, OCH$_3$), 4.26 (s, 4H, CH$_2$-O (x 2)), 5.74 (s, 1H, H-6), 6.41 (s, 2H, H-2', H-6'), 6.72 (s, 1H, H-5), 6.77 (d, 2H, H-2'', H-6''), 6.89 (s, 1H, H-10), 8.12 (d, 2H, H-3'', H-5'').

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 27.6 (CH$_2$, Ar-$\underline{C}$H$_2$-CH$_2$-N), 45.2 (CH$_2$, CH$_2$-N), 56.6 (CH$_3$, CH$_3$-O (x 2)), 61.1 (CH$_3$, CH$_3$-O), 62.3 (CH, C-6), 64.7 (CH$_2$, CH$_2$-O (x 2)), 104.2 (CH, C-2', C-6'), 111.6 (CH, C-3'', C-5''), 116.5 (CH, C-5), 116.9 (CH, C-10), 126.4 (CH, C-2'', C-6''), 128.2 (C, C-5a), 130.1 (C, C-9a), 137.3 (C, C-1'), 138.1 (C, C-4''), 142.5 (C, C-4a), 143.3 (C, C-10a), 153.7 (C, C-3', C-4', C-5'), 153.9 (C, C-1'').

MS (EI) (m/z, %): 479 (M$^+$, 7), 311 (M$^+$-C$_9$H$_{12}$O$_3$, 100).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

2-(6,7-Dimethoxy-1-(3,4,5-trimethoxyphenyl))ethanol (10)
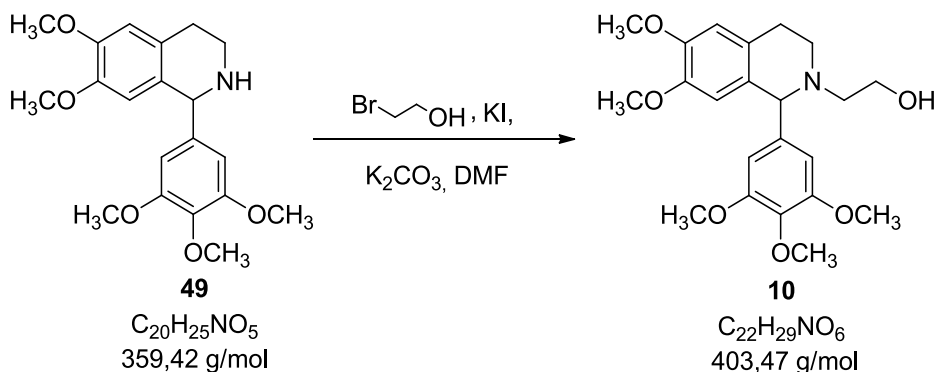
The substituted isoquinoline **49** (150 mg, 0.42 mmol) was dissolved in EtOH (25 mL). Then, 2-bromoethanol (0.06 mL, 0.83 mmol), Et_3N (0.11 mL, 0.83 mmol) and KI (cat) were added. The reaction was stirred at rt during 7 days and TLC of the reaction mixture (EtOAc/MeOH 8:2) showed formation of a new compound (R_f 0.65) and uncomplete consumption of SM (R_f 0.95). Water (20 mL) was added to the crude of reaction which was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (EtOAc/MeOH 9:1) to afford the desired alcohol **10** (55 mg, 33% yield) as a white oil.

Product aspect: white oil.

Theoretical mass: 168 mg.

Mass obtained: 55 mg.

Yield: 33%.

Analytical data:
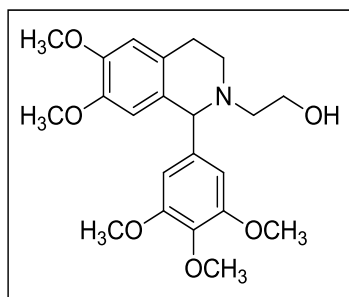
*Compound **10***

R$_f$ = 0.65 (EtOAc/MeOH 8:2).

mp: 77-80 ^{o}C (EtOAc).

IR (film) ν cm^{-1} : 3516 (OH), 2937, 2834 (C-H), 1591, 1516, 1423 (Ar-H), 1257, 1221 (Ar-O), 1121 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.40-2.52 (m, 1H, C$\underline{H}_2$-Ar), 2.52-2.65 (m, 1H, C$\underline{H}_2$-Ar), 2.70-2.85 (m, 2H, C$\underline{H}_2$-N), 2.85-3.00 (m, 1H, C$\underline{H}_2$-N), 3.10-3.25 (m, 1H, C$\underline{H}_2$-N), 3.40-3.55 (m, 1H, CH$_2$-OH), 3.61 (s, 3H, CH$_3$-O), 3.62-3.70 (m, 1H, CH$_2$-OH), 3.76 (s, 6H, CH$_3$-O (x 2)), 3.81 (s, 3H, CH$_3$-O), 3.82 (s, 3H, CH$_3$-O), 4.45 (s, 1H, H-1), 6.22 (s, 1H, H-5), 6.41 (s, 2H, H-2', H-6'), 6.58 (s, 1H, H-8).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 28.1 (CH$_2$, $\underline{C}$H$_2$-Ar), 46.9 (CH$_2$, $\underline{C}$H$_2$-N), 55.4 (CH$_2$, $\underline{C}$H$_2$-N), 56.1 (CH$_3$, OCH$_3$), 56.2 (CH$_3$, OCH$_3$), 56.5 (CH$_3$, OCH$_3$ (x 2)), 58.5 (CH$_2$, $\underline{C}$H$_2$-OH), 61.2 (CH$_3$, OCH$_3$), 68.6 (CH, C-1), 106.7 (CH, C-2', C-6'), 111.1 (CH, C-5), 111.8 (CH, C-8), 126.8 (C, C-4a), 129.4 (C, C-8a), 139.6 (C, C-1'), 147.4 (C, C-7), 147.9 (C, C-6), 153.4 (C, C-3', C-4', C-5').

MS EI m/z (%): 403 (M$^+$, 11), 372 (M$^+$-C$_2$H$_8$, 100), 236 (M$^+$-C$_9$H$_{12}$O$_3$, 77).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

3-(6,7-Dimethoxy-1-(3,4,5-trimethoxyphenyl)isoquinolin-2-yl)-1,2-propanediol (11)

52
$C_{23}H_{29}NO_6$
415,48 g/mol

1,4 dioxan, NaOH (2N)
rt, 48 h

11
$C_{23}H_{31}NO_7$
433,49 g/mol
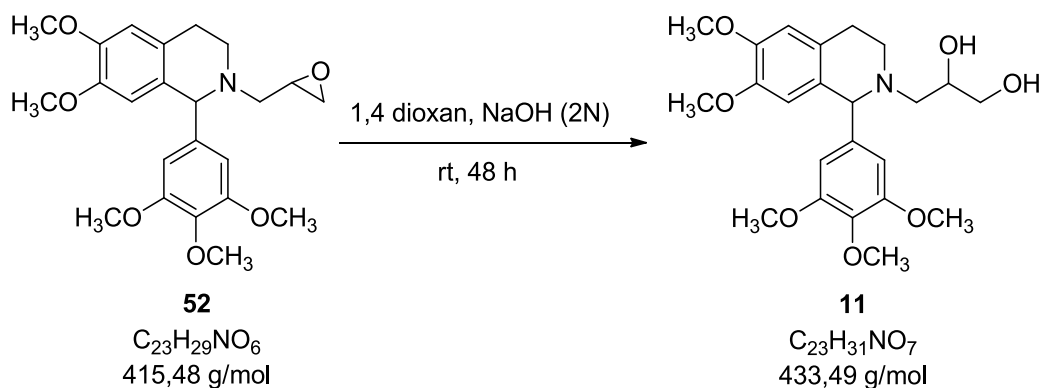
The substituted isoquinoline **52** (100 mg, 0.24 mmol) was dissolved in 1,4 dioxane (10 mL). NaOH 2N (25 mL) was added and the reaction was stirred for 2 days. TLC of the reaction mixture (EtOAc/MeOH 8:2) showed complete consumption of SM (R_f 0.95) and formation of a new compound (R_f 0.60). The crude mixture was evaporated *in vacuo*. The residue was dissolved in a mixture of diethyl ether (20 mL) and EtOAc (20 mL) and washed with water (3 x 20 mL). The organic phase was dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired diol **11** (12 mg, 11% yield) as a brown solid. The reaction was repeated using 69 mg of isoquinoline **52** to afford the desired diol **11** (36 mg, 48% yield) as a brown solid.

Product aspect: brown solid.

Theoretical mass: 75 mg.

Mass obtained: 36 mg.

Yield: 48%.

Analytical data:
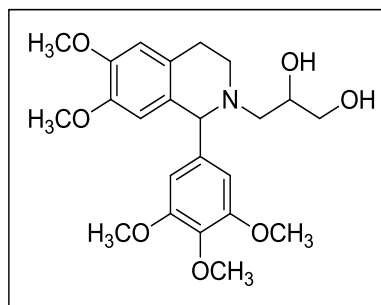
*Compound **11***

R$_f$ = 0.60 (EtOAc/MeOH 8:2).

mp: 70-73 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3700-3050 (OH), 2919, 2849 (C-H), 1505, 1461, 1452, 1415 (Ar-H), 1215 (Ar-O), 1120 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.60-3.25 (m, 9H, C$\underline{H}$-OH, C$\underline{H}_2$-OH, C$\underline{H}_2$-N (x 2), Ar-C$\underline{H}_2$-CH$_2$-N). 3.73 (s, 3H, CH$_3$-O), 3.81 (s, 3H, CH$_3$-O), 3.83 (s, 3H, CH$_3$-O (x 2)), 3.84 (s, 3H, CH$_3$-O), 4.18 (s, 1H, H-1), 6.58 (s, 1H, H-5), 6.80 (s, 2H, H-2', H-6'), 6.85 (s, 1H, H-8).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 30.0 (CH$_2$, $\underline{C}$H$_2$-4), 47.9 (CH$_2$, $\underline{C}$H$_2$-3), 54.3 (CH$_2$, N-$\underline{C}$H$_2$-CH$_2$-OH), 56.1 (CH$_3$, OCH$_3$), 56.4 (CH$_3$, OCH$_3$), 56.5 (CH$_3$, OCH$_3$ (x 2)), 61.1 (CH$_3$, OCH$_3$), 65.0 (CH$_2$, $\underline{C}$H$_2$-OH), 75.8 (CH, C-1), 76.7 (CH, $\underline{C}$H-OH), 104.2 (CH, C-2', C-6'), 110.5 (CH, C-8), 111.6 (C, C-5), 126.9 (C, C-8a), 128.7 (C, C-4a), 140.7 (C, C-1'), 147.8 (C, C-7), 148.7 (C, C-6), 153.0 (C, C-4'), 153.3 (C, C-3', C-5').

MS EI m/z (%): 433 (M$^+$, 0.1), 432 (M$^+$-1, 0.4), 431 (M$^+$-2, 1.6), 400 (M$^+$-H$_2$O$_2$, 18.8), 264 (M$^+$-C$_9$H$_{13}$O$_3$, 100).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl)

3-(4-(Benzyloxy)-3-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2,5-dione (14)

76
$C_{27}H_{24}O_8$
476,47 g/mol

14
$C_{27}H_{25}NO_7$
475,49 g/mol
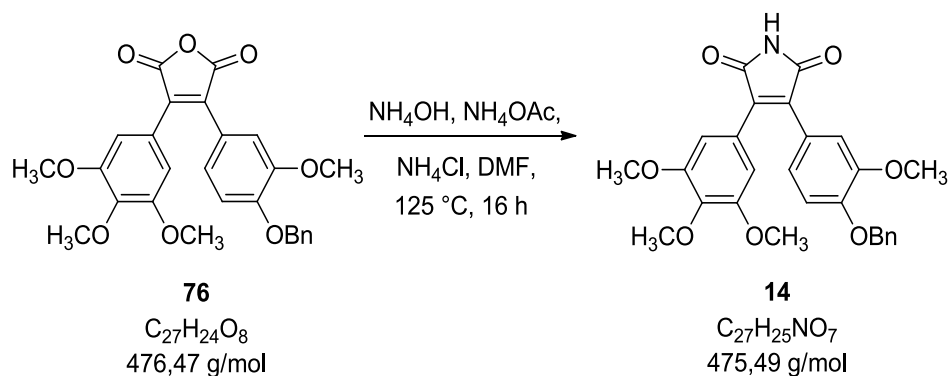
The anhydride **76** (260 mg, 0.55 mmol) was dissolved in DMF (5 mL) in a flame-dried round-bottom flask under argon. NH_4OH (0.8 mL of a 25% aqueous solution, 11 mmol), NH_4Cl (290 mg, 5.5 mmol) and NH_4OAc (419 mg, 5.5 mmol) were added to the solution. The reaction was heated at 125 °C for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a bright yellow compound (R_f 0.50) and complete consumption of starting material (R_f 0.60). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (hexane/EtOAc 2:8) to afford the desired imide **14** (108 mg, 42% yield) as an orange solid.

Product aspect: orange solid.

Theoretical mass: 260 mg.

Mass obtained: 108 mg.

Yield: 42%.

Analytical data:
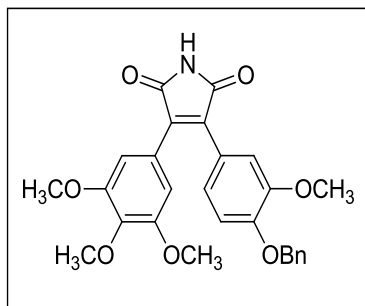
*Compound **14***

R_f **=** 0.50 (hexane/EtOAc 1:1).

mp: 198-200 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3290 (NH, OH)**,** 2994, 2935 (C-H), 1707 (CO), 1597, 1574, 1506, 1458 (Ar-H), 1251, 1229 (Ar-O), 1129, 1121 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ **(ppm):** 3.69 (s, 6H, OCH$_3$ (x 2), 3.74 (s, 3H, OCH$_3$), 3.87 (s, 3H, OCH$_3$), 5.19 (s, 2H, O-CH$_2$), 6.75 (s, 2H, H-2, H-6), 6.87 (d, *J* = 8.4 Hz, 1H, H-5'), 7.06 (d, *J* = 2.1 Hz, 1H, H-2'), 7.15 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H, H-6'), 7.25-7.45 (m, 5H, OBn).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ **(ppm):** 56.2 (CH$_3$, OCH$_3$), 56.4 (CH$_3$, OCH$_3$ (x 2)), 61.27 (CH$_3$, OCH$_3$), 71.0 (CH$_2$, C̲H$_2$-O-Ar), 107.5 (CH, C-2, C-6), 113.5 (CH, C-5'), 113.6 (CH, C-2'), 121.6 (C, C=C̲), 123.9 (C, C̲=C), 124.2 (CH, C-6'), 127.5 (CH, C-2'', C-6''), 128.4 (CH, C-4''), 128.9 (CH, C-3'', C-5''), 135.2 (C, C-1'), 136.7 (C, C-1), 139.8 (C, C-4'), 149.6 (C, C-3'), 150.0 (C, C-4), 153.5 (C, C-3, C-5), 170.8 (C, C=O (x 2)).

 MS (EI) m/z, (%): 475 (M$^+$, 37.13).

3-(4-(Hydroxy-3-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2,5-dione (15)
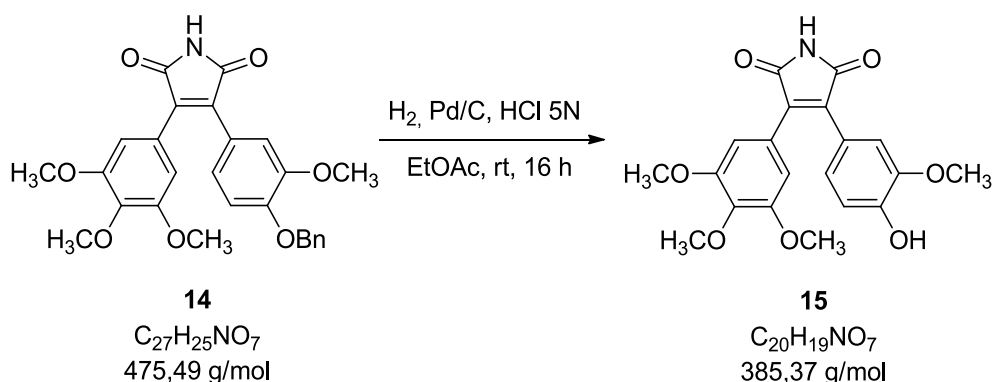
The imide **14** (88 mg, 0.21 mmol) was dissolved in EtOAc (20 mL). Pd/C (9 mg, 10% w/w) and HCl 5N (60 μL, cat) were added and the resulting mixture was put under hydrogen atmosphere for 24 h. Then, TLC of the crude mixture (EtOAc/hexane 1:1) indicated formation of a new compound (R_f 0.25) and complete consumption of starting material (R_f 0.50). The mixture was filtered and evaporated *in vacuo* to afford the debenzylated imide **15** (66 mg, 92% yield) as a bright yellow solid.

Product aspect: yellow solid.

Theoretical mass: 71 mg.

Mass obtained: 66 mg.

Yield: 92%.

Analytical data:
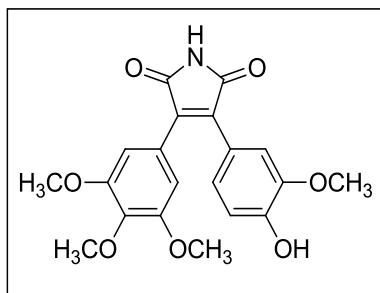
*Compound **15***

R$_f$ = 0.25 (hexane/EtOAc 1:1).

mp: 224-226 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3490, 3311 (NH, OH) 2931 (C-H), 1713 (CO), 1581, 1506, 1459, 1413 (Ar-H, C=C), 1281, 1234, 1201 (Ar-O), 1120 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.73 (s, 6H, 2 x OCH$_3$), 3.77 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 5.90 (s, 1H, OH), 6.76 (s, 2H, H-2, H-6), 6.91 (d, *J* = 8.4 Hz, 1H, H-5'), 7.07 (d, *J* = 1.5 Hz, 1H, H-2'), 7.16 (dd, *J* = 1.5 Hz, *J* = 8.4 Hz, 1H, H-6'), 7.71 (s, 1H, NH).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 56.3 (CH$_3$, CH$_3$-O), 56.5 (CH$_3$, 2 x CH$_3$-O), 61.3 (CH$_3$, C̲H$_3$-O-Ar), 107.5 (CH, C-2, C-6), 112.7 (CH, C-2'), 114.9 (CH, C-5'), 120.7 (C, C=C̲), 124.23 (CH, C-6'), 124.7 (C, C̲=C), 135.0 (C, C-1'), 136.5 (C, C-1), 139.8 (C, C-4'), 146.6 (C, C-3'), 147.9 (C, C-4), 153.5 (C, C-3, C-5), 170.8 (C, C=O), 170.9 (C, C=O).

MS (EI) (m/z, %): 385 (M$^+$, 100).

5-(3-(Benzyloxy)-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-3(2*H*)-one (16)

54
$C_{27}H_{28}O_7$
464,51 g/mol

16
$C_{26}H_{28}N_2O_6$
464,51 g/mol
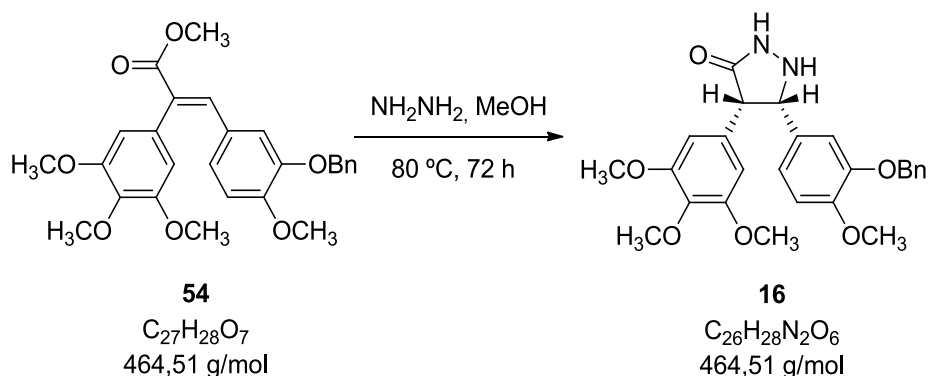
The methyl ester **54** (90 mg, 0.85 mmol) was dissolved in MeOH (2 mL) and hydrazine hydrate (4 mL) in a flame-dried round-bottom flask under argon. The reaction was heated at 80 $^{\circ}$C under stirring for 3 days and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.20) and complete consumption of starting material (R_f 0.65). The crude mixture was dissolved in diethyl ether (20 mL) and EtOAc (20 mL). The organic phase was washed with water (3 x 30 mL), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo.* The residue was purified by silica gel flash column chromatography (hexane/EtOAc 1:1) to afford the desired pyrazolone **16** (45 mg, 53% yield) as a white solid.

Product aspect: white solid.
Theoretical mass: 85 mg.
Mass obtained: 45 mg.
Yield: 53%.

Analytical data:
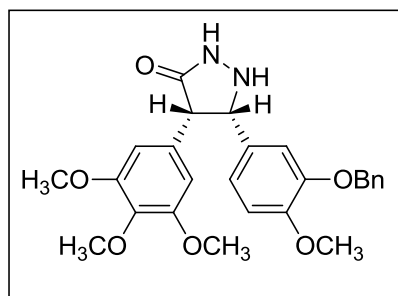
Compound 16

R_f = 0.40 (EtOAc/MeOH 8:2).

mp: 48-52 °C (hexane/EtOAc).

IR (film) ν cm^{-1}: 3218 (NH), 2921, 2850 (C-H), 1660 (C=O), 1588, 1509, 1454, 1422 (Ar-H), 1256 (Ar-O), 1235 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.88 (dd, *J* = 5.8 Hz, *J* = 12.8Hz, 1H, CO-C$\underline{H}$). 3.20-3.40 (m, 1H, C$\underline{H}$-NH), 3.81 (s, 3H, OCH$_3$), 3.82 (s, 6H, OCH$_3$ (x 2)), 3.83 (s, 3H, OCH$_3$), 5.01 (s, 1H, C$\underline{H}_2$-OBn), 6.47 (s, 2H, H-2, H-6), 6.55 (d, *J* = 1.8 Hz, H-2'), 6.66 (dd, *J* = 1.8 Hz, 7.8 Hz, 1H, 2H, H-5'), 6.76 (d, *J* = 7.8 Hz, H-6'), 7.25-7.50 (m, 5H, OBn).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 49.5 (CH, $\underline{C}$H-CO), 54.4 (CH, $\underline{C}$H-NH), 56.4 (CH$_3$, O-$\underline{C}$H$_3$ (x 2)), 56.5 (CH$_3$, O-$\underline{C}$H$_3$), 61.1 (CH$_3$, O-$\underline{C}$H$_3$), 71.3 (CH$_2$, $\underline{C}$H$_2$-OBn), 105.3 (CH, C-5), 105.9 (CH, C-2), 112.1 (CH, C-2'), 115.6 (CH, C-5'), 122.1 (CH, C-6'), 127.7 (CH, C-2'', C-6''), 128.1 (CH, C-4''), 128.7 (CH, C-3'', C-5''), 132.7 (C, C-1'), 135.5, (C, C-1), 137.6 (C, C-1''), 145.7 (C, C-3'), 148.1 (C, C-4'), 153.3 (C, C-4), 156.6 (C, C-3, C-5), 174.6 (C, $\underline{C}$ONH).

MS EI m/z (%): 464.2 (M$^+$, 5), 463 (M$^+$-1, 25), 462 (M$^+$-2, 87).

5(3-Hydroxy-4-methoxy-phenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-3(2*H*)-one (17)

16
C$_{26}$H$_{28}$N$_2$O$_6$
464,51 g/mol

17
C$_{19}$H$_{22}$N$_2$O$_6$
374,39 g/mol
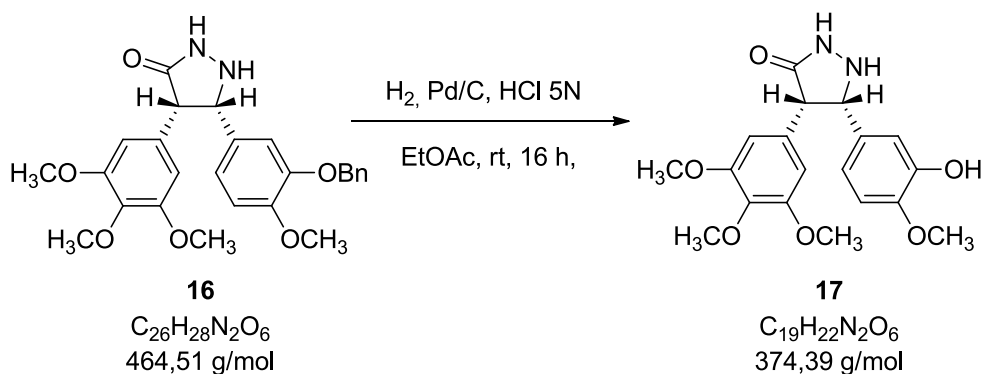
The pyrazolone **16** (150 mg, 0.32 mmol) was dissolved in EtOAc (10 mL) and MeOH (10 mL) and HCl 5N (40 µL) was added. Then, 10% palladium on charcoal catalyst (268 mg, 10% w/w) in ethyl acetate (5 mL) was added and the mixture was put under hydrogen atmosphere for one day. Then, TLC of the crude mixture (EtOAc) indicated formation of a new compound (R$_f$ 0.20) and complete consumption of starting material (R$_f$ 0.65). The mixture was filtered, concentrated *in vacuo* and purified by silica gel flash column chromatography (EtOAc/MeOH 8:2) to afford the desired compound **17** (37 mg, 33% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 111 mg.

Mass obtained: 37 mg.

Yield: 33%.
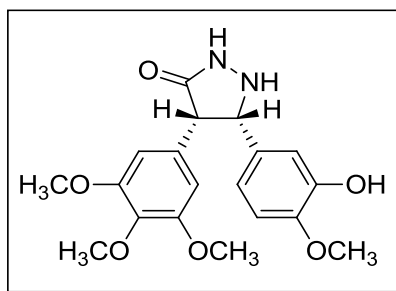
*Compound **17***

R_f = 0.60 (EtOAc).

mp: 65-70 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3243 (NH, OH), 2921, 2850 (C-H), 1739 (C=O), 1589, 1508, 1458 (Ar-H), 1238, 1123 (C-O).

RMN ^{1}H (CDCl₃, 300 MHz) δ (ppm): 2.92 (dd, *J* = 5.8 Hz, *J* = 12.8 Hz, 1H, CO-C<u>H</u>), 3.50-3.30 (m, 1H, C<u>H</u>-NH), 3.81 (s, 3H, OCH₃), 3.82 (s, 9H, OCH₃ (x 3)), 4.72 (m, NH), 6.55 (d, *J* = 3 Hz, 1H, H-2'), 6.63 (s, 2H, H-2, H-6), 6.66 (dd, *J* = 3 Hz, *J* = 9 Hz, 1H, H-6'), 6.78 (d, *J* = 9 Hz, 1H, H-6'), 8.24 (s, OH).

RMN ^{13}C (CDCl₃, 75.5 MHz) δ (ppm): 38.8 (CH-CO), 49.4 (CH, <u>C</u>H-NH), 56.4 (CH₃, O-CH₃ (x 3)), 61.1 (CH₃, O-<u>C</u>H₃), 105.9, (CH, C-2, C-6), 110.8 (CH, C-2'), 115.5 (CH, C-5'), 120.9 (CH, C-6'), 133.4, (C, C-1'), 135.6 (C, C-1), 145.7 (C, C-3'), 145.9 (C, C-4'), 153.7 (C, C-4), 155.9 (C, C-3, C-5), 174.7 (C, <u>C</u>ONH).

MS EI m/z (%): 359 (M$^+$-CH₃, 3.4) 344 (M$^+$-C₂H₆, 9.8), 317 (M$^+$-C₃H₉, 21.5).

(3,6,7,8,9,10-Hexahydro-2*H*-[1,4]dioxino[2,3-*b*]carbazol-10-yl)methyl   *N*-((2-fluoro-5-trifluoromethyl)phenyl)carbamate (21)
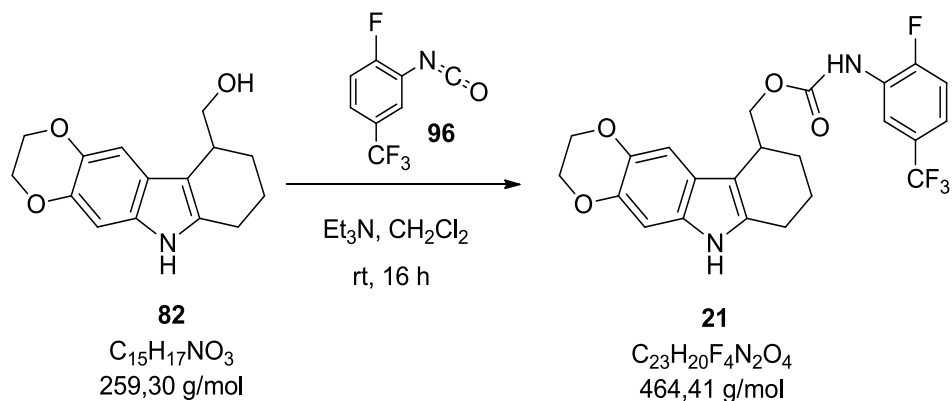
To the alcohol **82** (80 mg, 0.31 mmol) dissolved in CH_2Cl_2 (20 mL) was added 2-fluoro-5-(trifluoromethyl)aniline **96** (63 mg, 0.31 mmol) and Et_3N (43 µL, 0.31 mmol) in a flame-dried round-bottom flask under argon. The reaction was stirred at rt for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.90) and complete consumption of starting materials (R_f 0.65, R_f 0.95). The reaction crude mixture was washed with water (2 x 20 mL) and HCl 1N (2 x 20 mL). The aqueous phases were re-extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by aluminium oxide flash column chromatography (hexane/EtOAc 35:17) to afford the desired compound **21** (20 mg, 14% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 143 mg.

Mass obtained: 20 mg.

Yield: 14%.

Analytical data:
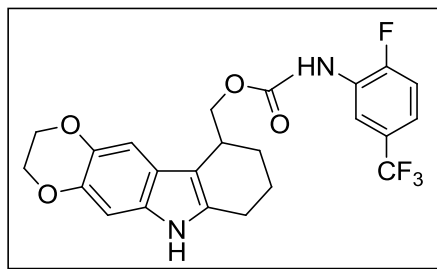
*Compound **21***

R$_f$ = 0.20 (hexane/EtOAc 2:1).

mp: 53-56 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3347 (NH), 2926 (C-H), 1735 (C=O), 1546, 1469, 1444 (Ar-H), 1211, 1163, 1118, 1065 (C-O).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 1.60-1.80 (m, 2H, CH$_2$-8) 1.90-2.10 (m, 2H, CH$_2$-9) 2.50-2-70 (m, 2H, CH$_2$-7), 3.15-3.30 (m, 1H, CH-CH$_2$-O), 4.25 (s, 4H, CH$_2$-O (x 2)), 4.27-4.35 (m, 1H, CH-CH$_2$-O), 4.40-4.50 (m, 1H, CH-CH$_2$-O), 6.79 (s, 1H , H-5) 6.91 (s, 1H, H-11), 6.90-7.35 (m, 3H, H-3', H-4', H-6'), 7.85 (s, 1H, NH), 8.47 (s, 1H, NH).

RMN ^{13}C (Acetone-*d6*, 75.5 MHz) δ (ppm): 19.8 (CH$_2$, CH$_2$-9), 31.9 (CH$_2$, CH$_2$-8), 38.8 (CH, CH-CH$_2$-O), 38.9 (CH$_2$, CH$_2$-7), 63.7 (CH$_2$, CH$_2$-O (x 2)), 65.4 (CH$_2$, CH$_2$-O), 108.8 (CH, C-5), 110.8 (CH, C-11), 112.47 (C, C-10a), 115.4 (CH, *J* = 21 Hz, C-3'), 117.7 (CH, C-6'), 120.2 (CH, C-4'), 123.54 (C, *J* = 261 Hz, CF$_3$) 123.7 (C, *J* = 32 Hz, C-5'), 127.6 (C, *J* = 12 Hz, C-1'), 134.8 (C, C-10b), 141.1 (C-6a), 143.3 (C-5a), 146.9 (C, C-4a), 153.2 (C, C-11a), 156.4 (C, *J* = 240 Hz, C-2'), 184.43 (C, C=O).

MS EI m/z (%): 464 (M$^+$, 18.03), 463 (M$^+$-1, 18), 462 (M$^+$-2, 60), 228 (M$^+$-C$_9$H$_6$NO$_2$F$_4$), 100.

3,6-Dihydro-2*H*-[1,4]dioxino[2,3-*b*]carbazol-10-yl)methyl(3-nitrophenyl)carbamate (22)

80
$C_{15}H_{13}NO_3$
255,27 g/mol

22
$C_{22}H_{17}N_3O_6$
419,38 g/mol
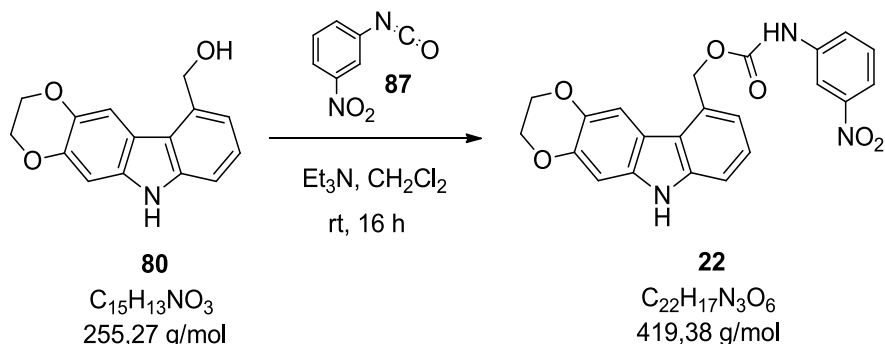
To the alcohol **80** (67 mg, 0.26 mmol) dissolved in CH_2Cl_2 (20 mL) was added 3-nitroaniline **87** (43 mg, 0.26 mmol) and Et_3N (36 µL, 0.26 mmol) in a flame-dried round-bottom flask under argon. The reaction was stirred at rt for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.80) and complete consumption of the starting material **80** (R_f 0.40). The reaction mixture was washed with water (2 x 20 mL) and HCl 1N (2 x 20 mL) and the aqueous phases were re-extracted with CH_2Cl_2 (2 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by aluminium oxide flash column chromatography (hexane/EtOAc 7:3) to afford the desired compound **22** (90 mg, 78% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 115 mg.

Mass obtained: 90 mg.

Yield: 78%.

Analytical data:
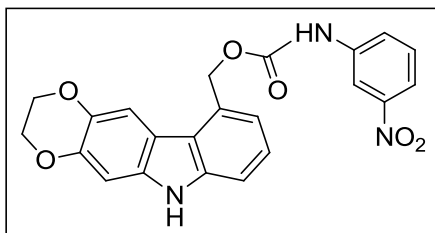
Compound 22

R_f = 0.80 (hexane/EtOAc 1:1).

mp: 170-172 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3385 (NH), 2923 (C-H), 1712 (C=O), 1536, 1522, 1469, 1427 (Ar-H), 1314, 1212 (Ar-O), 1182, 1067 (C-O).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 4.26-4.33 (m, 4H, CH$_2$-O (x 2), 5.51 (s, 2H, CH$_2$-OCONH), 6.96 (s, 1H, H-11), 7.12 (t, *J* = 7.5 Hz, 1H, H-8), 7.38 (d, *J* = 7.5 Hz, 1H, H-9), 7.55 (s, 1H, H-5), 7.59 (t, *J* = 8.1 Hz, 1H, H-5'), 7.88 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 2H, H-4', H-6'), 7.98 (d, *J* = 7.5 Hz, 1H, H-7), 8.62 (t, *J* = 2.1 Hz, 1H, H-2').

RMN ^{13}C (Acetone-*d6*, 75.5 MHz) δ (ppm): 64.5 (CH$_2$, CH$_2$-O), 64.9 (CH$_2$, CH$_2$-O), 65.0 (CH$_2$, CH$_2$-O), 98.8 (CH, C-5), 107.6 (CH, C-11), 112.8 (CH, C-2'), 117.4 (CH, C-10a), 117.5 (CH, C-7), 118.5 (C, C-10b), 118.8 (CH, C-9), 120.5 (CH, C-4'), 124.2 (C, C-5a), 124.4 (CH, C-8), 125.9 (CH, C-6'), 130.4 (CH, C-5'), 135.9 (C, C-10), 139.1 (C, C-3'), 139.8 (C, C-6a), 141.1 (C, C-4a), 144.2 (C, C-11a), 149.3 (C, C-1'), 154.2 (C, C=O).

MS EI m/z (%): 419 (M$^+$, 1.3), 375 (M$^+$-NO$_2$, 8.2), 255 (M$^+$-C$_7$H$_6$N$_2$O$_3$, 8.04).

(C$_7$H$_6$N$_2$O$_3$ = 3-nitrophenylcarbamate).

2-(3,4,5-Trihydroxyphenyl)-6-hydroxybenzofuran (23)
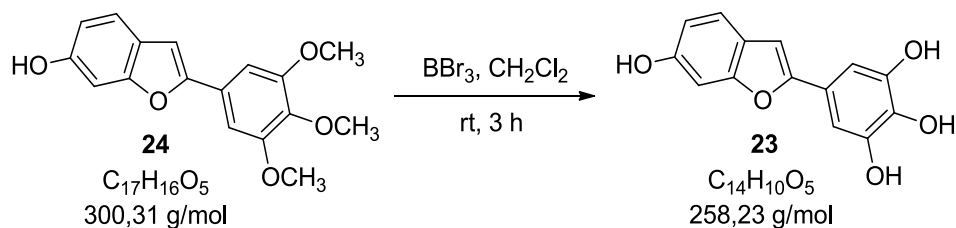
6-Hydroxy-2-(3,4,5-trimethoxyphenyl) isobenzofuran (**24**) (60 mg, 0.2 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) in a flame-dried round-bottom flask under argon. The reaction was cooled at -30 °C and BBr_3 (0.4 mL of a 99% solution, 4.2 mmol) was added. The reaction was stirred for 10 min at -30 °C and allowed to warm at rt for 3 h. Then, TLC of the reaction mixture (hexane/EtOAc 2:1) indicated formation of a new compound (R_f 0.10) and complete consumption of starting material (R_f 0.35). The crude mixture was cooled down to 0 °C and water (30 mL) was added dropwise. The solution was let to stir at rt for 10 min. Then, NaOH 5N was added until pH 10 and washed with CH_2Cl_2 (3 x 20 mL). Finally, HCl 5N was added until pH 1 and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the desired compound **23** (32 mg, 62% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 52 mg.

Mass obtained: 32 mg.

Yield: 62%.

Analytical data:
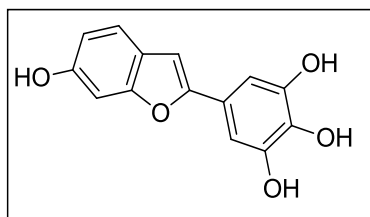
*Compound **23***

R_f = 0.10 (hexane/EtOAc 2:1).

mp: 292-295 $^{\circ}$C (hexane/EtOAc).

IR (film) ν cm^{-1}: 3389, 3339, 3197 (Ar-OH), 2922 (Ar-H), 2852 (Ar-H), 1237 (Ar-O).

RMN ^{1}H (Acetone-*d6*, 200 MHz) δ (ppm): 6.72 (dd, *J* = 2 Hz, *J* = 8 Hz, 1H, H-5), 6.73 (s, 2H, H-2', H-6'), 6.74 (d, *J* = 2 Hz, 1H, H-7), 7.43 (d, *J* = 8.0 Hz, 1H, H-4), 7.77 (s, 1H, H-3).

RMN ^{13}C (Acetone-*d6*, 100 MHz) δ (ppm): 102.8 (CH, C-3), 108.7 (CH, C-2', C-6'), 113.9 (CH, C-7), 124.4 (C, C-3a), 130.39 (CH, C-5), 134.3 (C, C-1'), 139.7 (CH, C-4), 146.3 (C, C-3', C-4', C-5'), 156.0 (C, C-6), 160.9 (C, C-2), 161.5 (C, C-7a).

HRMS -ESI m/z (%): Calculated for $C_{14}H_{10}O_5$-H (M-H)$^{-}$: 257.0528. Found: 257.0468.

6-Hydroxy-2-(3,4,5-trimethoxyphenyl) isobenzofuran (24)

105
$C_{18}H_{16}O_6$
328,32 g/mol

24
$C_{17}H_{16}O_5$
300,31 g/mol

HCl 2N, H_2O,
reflux, 16 h
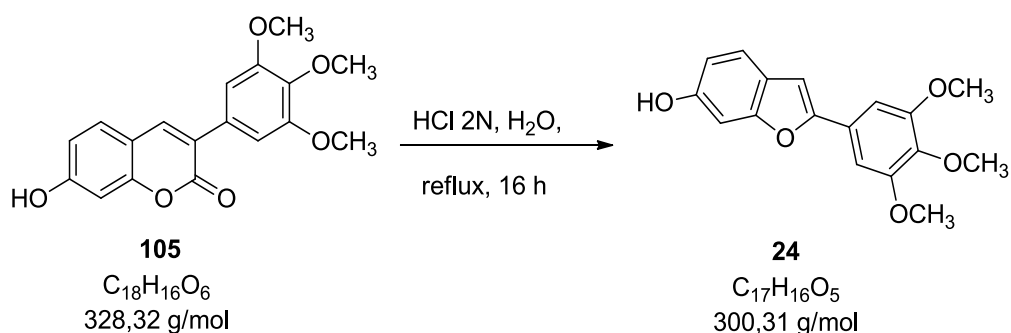
7-Hydroxy-3-(3,4,5-trimethoxyphenyl)chromen-2-one (**105**) (150 mg, 0.46 mmol) was dissolved in ethanol (30 mL) and HCl 2N (120 mL) was added. The reaction was refluxed under stirring for 16 h and TLC of the crude mixture (hexane/EtOAc 2:1) indicated formation of a new compound (R_f 0.35) and complete consumption of starting material (R_f 0.20). The ethanol was evaporated *in vacuo* and the aqueous phase was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 2:1) to afford the desired compound **24** (25 mg, 18% yield) as a white solid. The reaction was scaled up using 660 mg of 7-hydroxy-3-(3,4,5-trimethoxyphenyl)-chromen-2-one (**105**) to obtain **24** (174 mg, 29% yield) as a white solid.

Product aspect: white solid.

Theoretical mass: 601 mg.

Mass obtained: 174 mg.

Yield: 29%.

Analytical data:
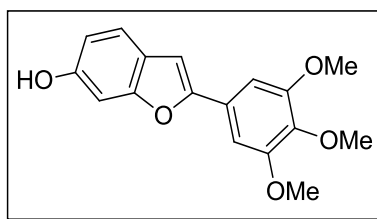
*Compound **24***

R_f = 0.35 (hexane/EtOAc 2:1).

mp: 230-232 $^{\circ}$C (hexane/EtOAc).

IR (film) ν cm^{-1}: 3208 (Ar-OH), 1607, 1510, 1446, 1415 (=C-H), 1218 (Ar-O), 1121 (C-O).

RMN ^{1}H (CDCl$_3$, 200 MHz) δ (ppm): 3.89 (s, 3H, O-CH$_3$), 3.92 (s, 6H, O-CH$_3$ (x 2)), 6.85 (dd, J = 2 Hz, J = 8 Hz, 1H, H-5), 6.91 (s, 2H, H-2', H-6'), 6.93 (d, J = 2 Hz, 1H, H-7), 7.42 (d, J = 8.0 Hz, 1H, H-4), 7.77 (s, 1H, H-3).

RMN ^{13}C (CDCl$_3$, 50 MHz) δ (ppm): 56.4 (CH$_3$, OCH$_3$ (x 2)), 61.2 (CH$_3$, OCH$_3$), 103.1 (CH, C-3), 106.4 (CH, C-2', C-6'), 114.1 (C, C-4a), 129.4 (CH, C-7), 130.9 (CH, C-5), 138.8 (C, C-1'), 140.8 (CH, C-4), 153.4 (C, C-3', C-4', C-5'), 155.3 (C, C-6), 160.7 (C,C-2), 161.9 (C, C-7a).

MS (ESI (+)) m/z (%): 300 (M$^+$, 3.12), 285 (M$^+$-15, 21), 255 (M$^+$-45, 11.73), 181 (M$^+$-C$_{10}$H$_{13}$O$_3$, 27.61).

6-(Benzyl)-2-(2,5-dimethoxyphenyl)indole (25)

113
$C_{13}H_{13}NO$
199,25 g/mol

123
$C_{10}H_{11}BrO_3$
259,10 g/mol

25
$C_{23}H_{21}NO_3$
359,42 g/mol
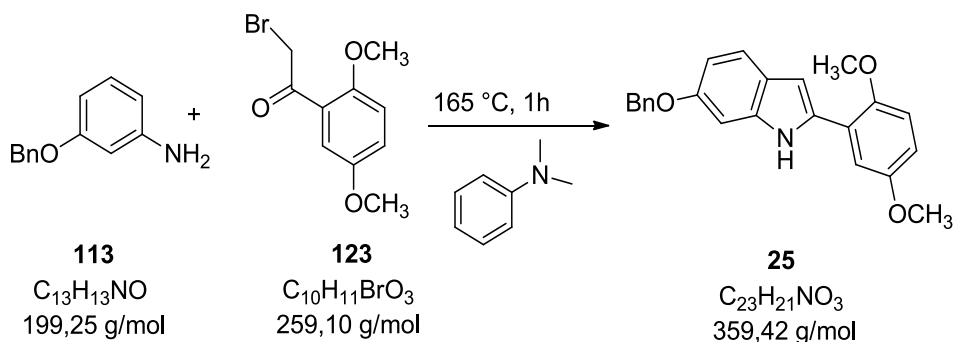
3-Benzoxyaniline **113** (2.3 g, 6.95 mmol) was dissolved in *N,N*-dimethylaniline (25 mL) in a flame-dried round-bottom flask under argon. The reaction was heated to 150 °C and 2'-bromo-2,5-dimethoxyacetophenone (**113**) (1 g, 2.32 mmol) was added and the reaction was heated at 165 °C for 1 h. Then, TLC of the crude mixture (hexane/EtOAc 8:2) indicated formation of a new compound (R_f 0.45) and complete consumption of starting materials (R_f 0.25, R_f 0.10). The crude mixture was dissolved in EtOAc (50 mL), washed with HCl 2N (4 x 50 mL) and the aqueous phase were re-extracted with EtOAc (3 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo.* The residue was purified by aluminium oxide flash column chromatography (hexane/EtOAc 8:2). The residue was recristalised (hexane/EtOAc 8:2) to afford (6-benzyl)-2-(2,5-dimethoxyphenyl)indole (**25**) (740 mg, 53% yield) as a white solid. The product is unstable and must be put in the fridge and protected from the light.

Product aspect: white solid.

Theoretical mass: 1.39 g.

Mass obtained: 740 mg.

Yield: 53%.

Analytical data:
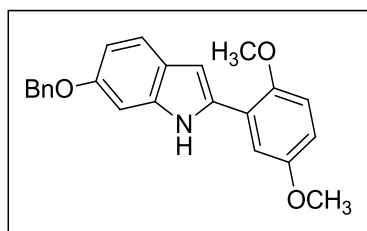
*Compound **25***

R$_f$ = 0.45 (hexane/EtOAc 8:2).

mp: 114-117 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3436 (NH), 2923 (C-H), 1624, 1484, 1461 (Ar-H, C=C-H), 1213, 1182 (Ar-O), 1040, 1023 (C-O).

RMN ^{1}H (CDCl$_3$, 400 MHz) δ (ppm): 3.81 (s, 3H, CH$_3$-O, 3.91 (s, 3H, CH$_3$-O), 5.10 (s, 2H, CH$_2$-O), 6.75 (dd, *J* = 3.2 Hz, *J* = 9.2 Hz, 1H, H-4'), 6.81 (d, *J* = 2 Hz, 0.8 Hz, 1H, H-6'), 6.86 (dd, *J* = 2 Hz *J* = 8.4 Hz, 1H, H-5), 6.91 (d, *J* = 8.4 Hz, 1H, H-4), 6.94 (d, *J* = 2.4 Hz, 1H, H-7), 7.34-7.37 (m, 2H, H-3, H-3'), 7.39-7.44 (t, *J* = 6.8 Hz, 2H, H-3'', H-5''), 7.48-7.55 (m, 3H, H-2'', H-4'', H-6'').

RMN ^{13}C (CDCl$_3$, 100 MHz) δ (ppm): 56.2 (CH$_3$, OCH$_3$), 56.8 (CH$_3$, OCH$_3$), 70.9 (CH$_2$, CH$_2$-OBn), 96.0 (CH, C-7), 100.3 (CH, C-3), 111.3 (CH, C-6'), 113.3 (CH, C-5), 113.5 (CH, C-3'), 113.6 (CH, C-4'), 121.3 (CH, C-4), 121.9 (C, C-3a), 123.0 (C, C-1'), 127.8 (CH, C-2'', C-6''), 128.1 (CH, C-3'', C-5''), 128.9 (CH, C-4''), 135.2 (C, C-7a), 137.2 (C, C-1'')*, 137.9 (C, C-2)*, 150.4 (C, C-6), 154.5 (C, C-2'), 155.9 (C, C-5').

*Interchangeable.

MS EI m/z (%): 359 (M$^+$, 24), 268 (100).

6-Hydroxy-2-(2,5-dimethoxyphenyl)indole (26)
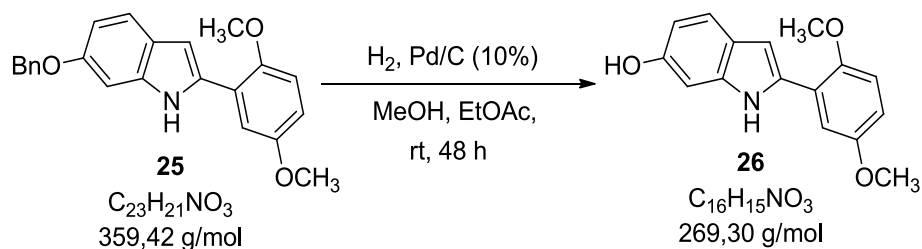
6-(Benzyl)-2-(2,5-dimethoxyphenyl)indole (**25**) (80 mg, 0.22 mmol) was dissolved in methanol (30 mL) and EtOAc (5 mL). 10% palladium on charcoal catalyst (8 mg, 10% w/w) in EtOAc (5 mL) was added and the reaction mixture was put under hydrogen atmosphere under stirring for 2 days. Then, TLC of the crude mixture (hexane/EtOAc 8:2) indicated formation of a new compound (R_f 0.10) and complete consumption of starting material (R_f 0.45). The crude mixture was filtered and concentrated *in vacuo* to afford 81 mg of a white solid. The residue was purified by aluminium oxide flash column chromatography (hexane/EtOAc 6:4) to afford the desired compound **26** (33 mg, 55% yield) as a white solid. The product was unstable and must be put in the fridge and protected from the light.

Product aspect: white solid.

Theoretical mass: 60 mg.

Mass obtained: 33 mg.

Yield: 55%.

Analytical data:
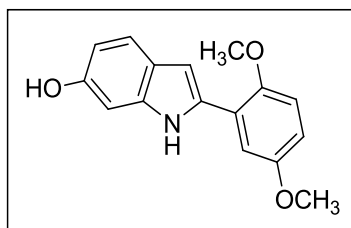
*Compound **26***

R$_f$ = 0.10 (hexane/EtOAc 8:2).

mp: 50-52 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3433 (Ar-OH, NH), 2922 (C-H), 1624, 1498, 1450 (Ar-H, C=C-H), 1211, 1164 (Ar-O), 1041, 1020 (C-O).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 3.83 (s, 3H, OCH$_3$), 3.94 (s, 3H, OCH$_3$), 5.19 (bs, 1H, OH), 6.69 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H, H-5), 6.79 (s, 1H, H-3), 6.80 (dd, *J* = 3 Hz, *J* = 9 Hz, 1H, H-6'), 6.85 (d, *J* = 2.2 Hz, 1H, H-7), 6.92 (d, *J* = 9 Hz, 1H, H-4) 7.31 (d, *J* = 3 Hz, 1H, H-4'), 7.45 (d, *J* = 9 Hz 1H, H-3').

RMN ^{13}C (Acetone-*d6*, 75.5 MHz) δ (ppm): 55.6 (CH$_3$, CH$_3$-O), 56.1 (CH$_3$, CH$_3$-O), 96.9 (CH, C-7), 101.5 (CH, C-3), 110.5 (CH, C-6'), 113.2 (CH, C-5), 113.6 (CH, C-3'), 113.7 (CH, C-4'), 120.9 (CH, C-4), 122.5 (C, C-3a), 122.8 (C, C-1'), 134.2 (C, C-7a), 138.4 (C, C-2), 150.7 (C, C-6), 154.1 (C, C-2'), 154.5 (C, C-5').

MS EI m/z (%): 269 (M$^+$, 100), 254 (M$^+$-CH$_3$, 77), 239 (M$^+$-C$_2$H$_6$, 45).

2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)ethylamine (28)

36
C$_{10}$H$_9$NO$_4$
207,18 g/mol

28
C$_{10}$H$_{13}$NO$_2$
179,22 g/mol
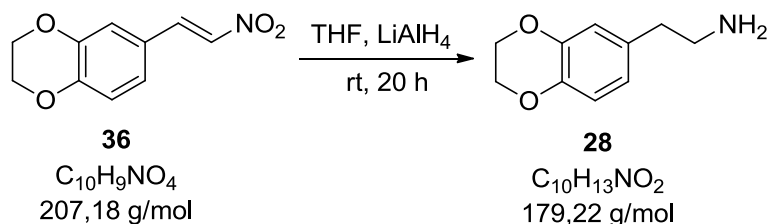
(E)-6-(2-Nitrovinyl)-2,3-dihydro[1,4]benzodioxine (**36**) (1 g, 4.82 mmol) was dissolved in THF (20 mL). LiAlH$_4$ (740 mg, 19.5 mmol) was added portionwise. The reaction was stirred at rt for 20 h and TLC of the reaction mixture (hexane/EtOAc 1:1) indicated formation of a new compound and complete consumption of starting material (R$_f$ 0.60). The crude mixture was quenched dropwise with water (1 mL) and filtered. The solid residue was extracted with CH$_2$Cl$_2$ (3 x 20 mL) and the combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford 2-(2,3-dihydro[1,4]benzodioxin-6-yl)ethylamine (**28**) (530 mg, 61% yield) as a pale brown oil. This material was identical in all respects with that previously described.

Theoretical mass: 869 mg.

Mass obtained: 530 mg.

Yield: 61%.

Analytical data:

R$_f$ = 0.55 (hexane/EtOAc 2:8).

IR (film) ν cm^{-1}: 3100 (NH), 2910 (CH), 1200 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.63 (t, *J* = 7.6 Hz, Ar-CH$_2$-CH$_2$). 2.91 (t, *J* = 7.6 Hz, 2H, CH$_2$-CH$_2$-NH$_2$), 4.24 (s, 4H, CH$_2$-O (x 2)), 6.66 (dd, *J* = 2 Hz, 8.2 Hz, 1H, H-7), 6.70 (d, *J* = 2 Hz, 1H, H-5), 6.80 (d, *J* = 8.2 Hz, 1H, H-8).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 38.7 (CH$_2$, CH$_2$-Ar), 43.1 (CH$_2$, CH$_2$-N), 63.8 (CH$_2$, CH$_2$-O), 63.9 (CH$_2$, CH$_2$-O), 116.7 (CH, C-5), 116.9 (CH, C-8), 121.2 (CH, C-7), 132.5 (C, C-6), 141.4 (C, C-4a), 142.9 (C, C-8a).

2-(2,3-Dihydro[1,4]benzodioxin-6-yl)ethylamine (28) (alternative synthesis)

36
$C_{10}H_9NO_4$
207,18 g/mol

28
$C_{10}H_{13}NO_2$
179,22 g/mol
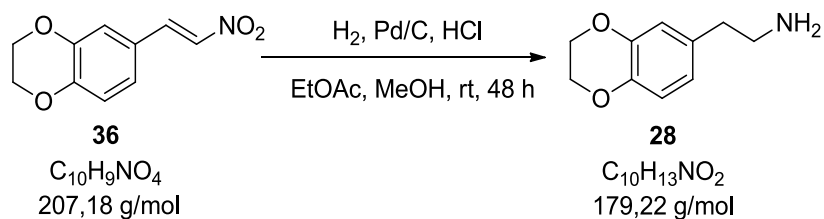
(E)-6-(2-Nitrovinyl)-2,3-dihydro[1,4]benzodioxine (**36**) (1.6 g, 7.68 mmol) was dissolved in EtOAc (80 mL) and MeOH (5 mL). 10% Palladium on charcoal catalyst (268 mg, 10% w/w) was added and the mixture was put under a 7 atm hydrogen atmosphere under stirring for 2 days. After 16 h, TLC of the crude mixture (hexane/EtOAc) indicated formation of a new compound and complete consumption of starting material (R_f 0.80). The mixture was filtered and concentrated *in vacuo* to afford **28** (852 mg, 53% yield) as a brown oil.

Product aspect: Brown oil.

Theoretical mass: 1.60 g.

Mass obtained: 852 mg.

Yield: 53%.

Analytical data was identical with the previously described compound.

2-(2,3-Dihydro[1,4]benzodioxin-6-yl)ethylamine (28) (alternative synthesis)

33
C$_{17}$H$_{19}$NO$_2$
269,34 g/mol

28
C$_{10}$H$_{13}$NO$_2$
179,22 g/mol
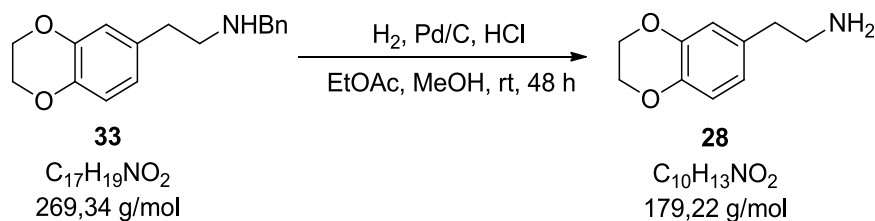
6-(2-Chloroethyl)-2,3-dihydrobenzo[1,4]dioxine (**33**) (200 mg, 1.01 mmol) was dissolved in EtOAc (80 mL) and MeOH (5 mL). 10% Palladium on charcoal catalyst (268 mg) and HCl (30 μL) were added and the mixture was put under hydrogen atmosphere under stirring for 2 days. Then, TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound and complete consumption of the starting material (R$_f$ 0.60). The crude mixture was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to afford 2-(2,3-dihydro[1,4]benzodioxin-6-yl)ethylamine (**28**) (71 mg, 53% yield) as a brown oil. This material was identical in all respects with that previously described.

Product aspect: brown oil.

Theoretical mass: 133 mg.

Mass obtained: 71 mg.

Yield: 53%.

Analytical data was identical with the previously described compound.

2-(2,3-Dihydro[1,4]benzodioxin-6-yl)ethylamine (28) (alternative synthesis)

31
$C_{10}H_{11}NO_5$
225,20 g/mol

28
$C_{10}H_{13}NO_2$
179,22 g/mol
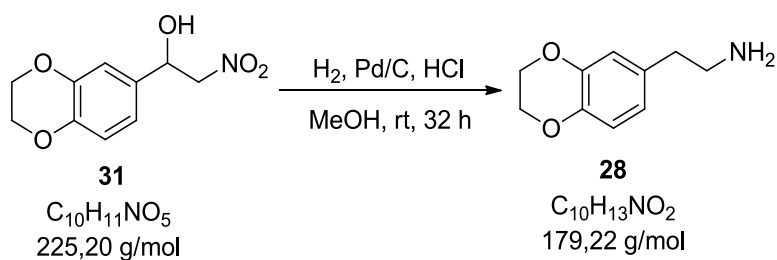
1-(2,3-Dihydro[1,4]benzodioxin-6-yl)-2-nitroethanol (**31**) (554 mg, 2.46 mmol) was dissolved in methanol (20 mL). HCl (30 µL) and 10% Palladium on charcoal catalyst (110 mg, 10% w/w) in EtOAc (5 mL) were added. The reaction mixture was put under hydrogen atmosphere under stirring for 32 h and TLC of the crude mixture (EtOAc/hexane 7:3) indicated formation of a new compound and complete consumption of starting material (R_f 0.45). The crude mixture was filtered and concentrated *in vacuo* to afford 2-(2,3-dihydro[1,4]benzodioxin-6-yl)ethylamine (**28**) (242 mg, 55% yield) as a brown oil. This material was identical in all respects with that previously described.

Product aspect: brown oil.

Theoretical mass: 440 mg.

Mass obtained: 242 mg.

Yield: 55%.

Analytical data was identical with the previously described compound.

2,3-Dihydro[1,4]benzodioxin-6-carbaldehyde (30)

37
C$_8$H$_7$BrO$_2$
215,04 g/mol

30
C$_9$H$_8$O$_3$
164,16 g/mol
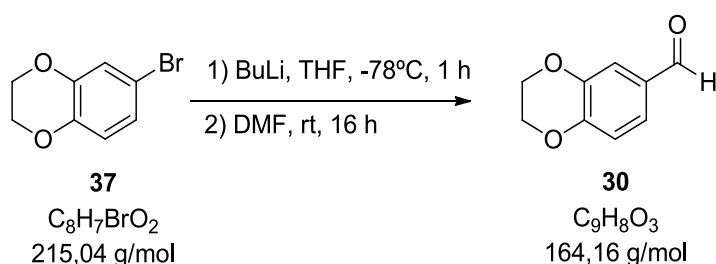
6-Bromo-2,3-dihydro-[1,4]-benzodioxin (**37**) (500 mg, 2.33 mmol) was dissolved in anhydrous THF (10 mL) and put in a flame-dried two-neck round-bottom flask. The mixture was cooled at -78 °C and BuLi (0.33 mL of a 1.6 M solution in hexane, 3.49 mmol) was added portionwise. After 1h, DMF (0.36 mL, 4.65 mmol) was added. The reaction was allowed to cool at rt and stirred for 16 h. Then, TLC of the crude mixture (hexane/EtOAc 7:3) indicated formation of a major product (R$_f$ 0.50) and uncomplete consumption of starting material (R$_f$ 0.60). The reaction mixture was quenched with NH$_4$Cl (2 mL). Water (20 mL) was added and the mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H$_2$O (5 x 30 mL), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford 2,3-dihydro-[1,4]-benzodioxin-6-carbaldehyde (**30**) (232 mg, 60% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 387 mg.

Mass obtained: 232 mg.

Yield: 60%.

Analytical data:

mp: 52-54 °C (MeOH).

R$_f$ = 0.50 (hexane/EtOAc 7:3), 0.80 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 4.25-4.40 (m, 4H, CH$_2$-O (x 2)), 6.98 (d, 1H, *J* = 8.8 Hz, H-8), 7.38-7.42 (m, 2H, H-5, H-7), 9.82 (s, 1H, Ar-CO<u>H</u>).

2,3-Dihydro[1,4]benzodioxin-6-carbaldehyde (30) (alternative synthesis)
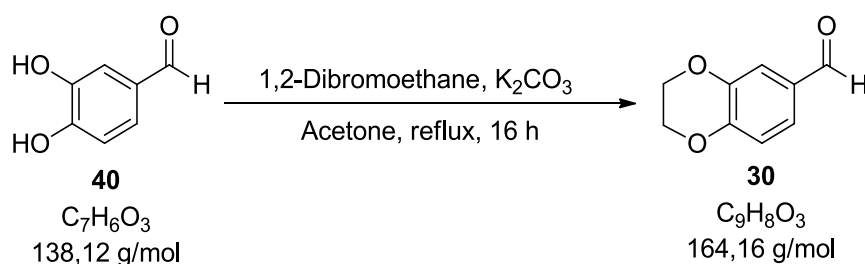
To 3,4-dihydroxybenzaldehyde **40** (2 g, 14.5 mmol) dissolved in acetone (30 mL) was added K_2CO_3 (10 g, 72.4 mmol) and 1,2-dibromoethane (1.5 mL, 17.38 mmol) in a flame-dried round-bottom flask under argon. The reaction was refluxed under stirring for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated presence of a new compound (0.80) and uncomplete consumption of starting material (R_f 0.50). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in diethyl ether (20 mL), washed with NaOH 2N (3 x 20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to afford 2,3-dihydro[1,4]benzodioxin-6-carbaldehyde (**30**) (1 g, 42% yield) as a yellow oil. HCl 5N was added to the aqueous phase until p*H* 1 and extracted with EtOAc (3 x 30 mL). The combined organic phases were dried and concentrated *in vacuo* to afford 3, 4-dihydroxybenzaldehyde (**40**) (280 mg, 14% yield) as a dark blue solid.

Product aspect: yellow oil.

Theoretical mass: 2.38 g.

Mass obtained: 1.00 g.

Yield: 42%.

Recuperation of starting material: 14%.

Analytical data was identical with the previously described compound.

2,3-Dihydro[1,4]benzodioxin-6-carbaldehyde (30) (alternative synthesis)

40
$C_7H_6O_3$
138,12 g/mol

30
$C_9H_8O_3$
164,16 g/mol
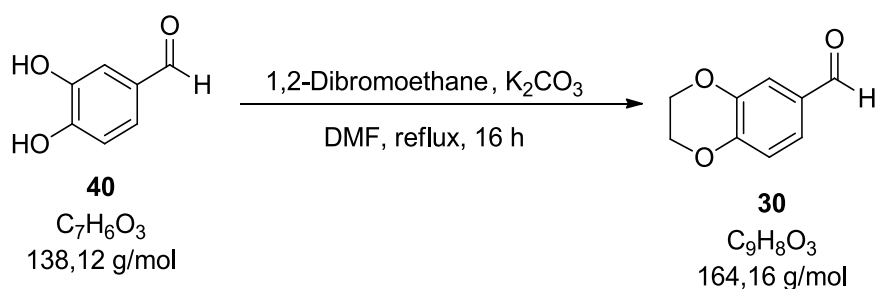
To 3, 4-dihydroxybenzaldehyde (**40**) (2 g, 14.5 mmol) dissolved in DMF (30 mL) was added K_2CO_3 (4 g, 29 mmol) and 1,2-dibromoethane (1.5 mL, 17.38 mmol) in a flame-dried round-bottom flask under argon. The reaction was refluxed under stirring for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated presence of a new compound (R_f 0.80) and partial consumption of starting material (R_f 0.50). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford 2,3-dihydro[1,4]benzodioxin-6-carbaldehyde (**30**) (1.54 g, 81% yield) as a yellow oil.

Product aspect: yellow oil.

Theoretical mass: 2.38 g.

Mass obtained: 1.54 g.

Yield: 65%.

Analytical data was identical with the previously described compound.

1-(2,3-Dihydro[1,4]benzodioxin-6-yl)-2-nitroethanol (31)

30
$C_9H_8O_3$
164,16 g/mol

31
$C_{10}H_{11}NO_5$
225,20 g/mol
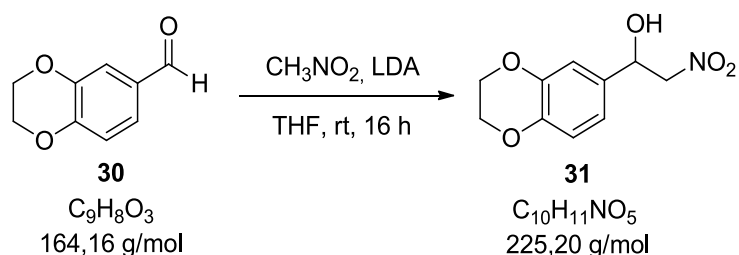
Anhydrous THF (5 mL) was put in a flame-dried two-necked round-bottom flask under argon. The mixture was cooled at -78 °C. CH_3NO_2 (0.245 mL, 4.57 mmol) and LDA (0.619 mL, 4.57 mmol) were added and the mixture was stirred at -78 °C for 3 h. 2,3-Dihydro[1,4]benzodioxin-6-carbaldehyde (**30**) (500 mg, 3.05 mmol) was dissolved in anhydrous THF (5 mL) in a flame-dried round-bottom flask under argon and added to the solution. The reaction was allowed to warm up at rt and stirred for 6 h. Then, TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a major product (R_f 0.35) and incomplete consumption of starting material (R_f 0.80). The crude mixture was quenched with ammonium chloride (3 mL). Diethyl ether (20 mL) was added. The organic phase was washed with water (3 x 10 mL) and the combined aqueous phases were re-extracted with diethyl ether (3 x 10 mL). The combined organic phases were dried (MgSO$_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 1:1) to afford 1-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-2-nitroethanol (**31**) (119 mg, 18% yield) as a yellow solid and starting material **30** (280 mg, 56% yield). The reaction was repeated using with 500 mg of **30** to afford **31** (674 mg, 21% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 661 mg.

Mass obtained: 119 mg.

Yield: 18%.

Analytical data:

mp: 74-78 $^{\circ}$C (EtOAc).

R$_f$ = 0.35 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 200 MHz) δ (ppm): 4.26 (s, 4H, CH$_2$-O (x 2)), 4.40-4.62 (m, 2H, C$\underline{H}_2$-NO$_2$), 5.34 (dd, J = 3.6 Hz, J = 9.2 Hz, 1H, C$\underline{H}$-OH), 6.83-6.88 (m, 2H, H-5, H-7), 6.90-6.93 (m, 1H, H-8).

Ethyl 2-(2,3-dihydro[1,4]benzodioxin-6-yl)-2-oxoacetate (32)
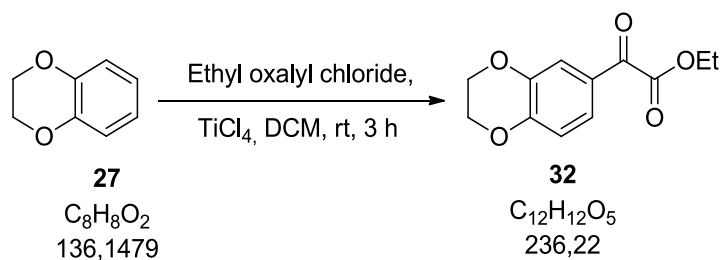
2,3-Dihydro-1,4-benzodioxin (**27**) (1 g, 7.34 mmol) was dissolved in CH_2Cl_2 (10 mL) in a flame-dried round-bottom flask. The mixture was cooled to 0 °C and ethyl oxalyl chloride (0.9 mL, 8.08 mmol) was added portionwise. $TiCl_4$ (1 mL) was added. The reaction was stirred at rt for 3 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a major product (R_f 0.65) and complete consumption of starting material (R_f 0.75). The reaction was quenched with ice and stirred for 15 min. H_2O (20 mL) was added. The organic phase was washed with NaOH 5N (3 x 20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford ethyl 2-(2,3-dihydro[1,4]benzodioxin-6-yl)-2-oxoacetate (**32**) (1.71 g, 98% yield) as a bright yellow solid

.

Product aspect: bright yellow solid.

Theoretical mass: 1.74 g.

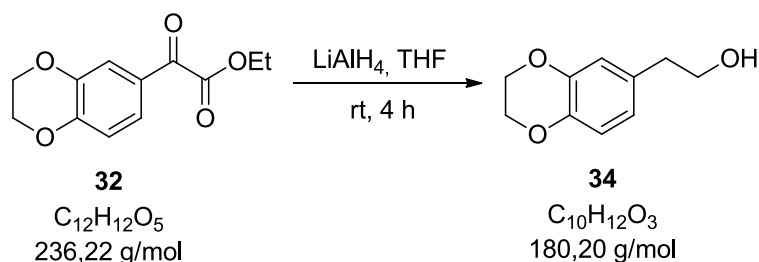**Mass obtained:** 1.71 g.

Yield: 98%.

Analytical data:

mp: 60-63 °C (EtOAc).

R_f = 0.65 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.41 (t, *J* = 7.2 Hz, 3H, O-CH$_2$-C$\underline{H}_3$), 4.39-4.20 (m, 4H, CH$_2$-O (x 2)), 4.42 (q, *J* = 7.2 Hz, 2H, O-C$\underline{H}_2$-CH$_3$), 6.95 (dd, *J* = 1.8 Hz, *J* = 7.5 Hz, 1H, H-8), 7.53-7.58 (m, 2H, H-5, H-7).

2-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)ethanol (34)

32
$C_{12}H_{12}O_5$
236,22 g/mol

34
$C_{10}H_{12}O_3$
180,20 g/mol

Ethyl 2-(2,3-dihydro[1,4]benzodioxin-6-yl)-2-oxoacetate (**32**) (200 mg, 0.85 mmol) was dissolved in THF (20 mL) in a flame-dried round-bottom flask under argon. LiAlH$_4$ (128.54 mg, 3.34 mmol) was added. The reaction was stirred at rt for 4 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a major product and complete consumption of starting material (R$_f$ 0.65). The reaction mixture was quenched with water portionwise and let to stir for 15 minutes. Diethyl ether (20 mL) and water (20 mL) were added. The organic phase was extracted with water (3 x 10 mL) and the combined aqueous phases were re-extracted with diethyl ether (3 x 10 mL). The combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford (2,3-dihydro[1,4]benzodioxin-6-yl)ethanol **34** (85 mg, 57% yield) as a colorless oil. The reaction was repeated using 600 mg of **32** to afford 2-(2,3-dihydrobenzo[1,4]dioxin-6-yl)ethanol (**34**) (340 mg, 76% yield) as a colourless oil.

Product aspect: colorless oil.

Theoretical mass: 447 mg.

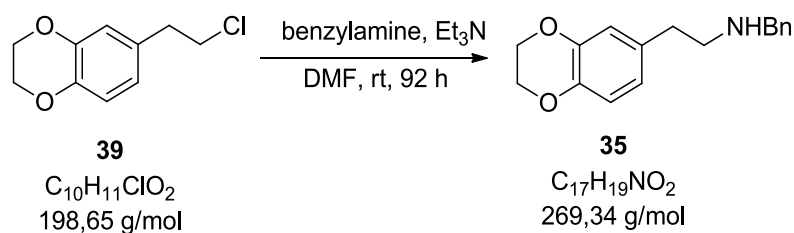**Mass obtained:** 340 g.

Yield: 76%.

Analytical data:

R$_f$ = 0.35 (hexane/EtOAc 2:8).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.70-3.52 (m, 2H, Ar-CH$_2$-CH$_2$-OH), 4.21 (s, 6H, CH$_2$-O (x 3), 6.93-6.79 (m, 3H, H-5, H-7, H-8).

_N_-Benzyl-2-(2,3-dihydro[1,4]benzodioxin-6-yl)ethylamine (35)

39
$C_{10}H_{11}ClO_2$
198,65 g/mol

35
$C_{17}H_{19}NO_2$
269,34 g/mol

6-(2-Chloroethyl)-2,3-dihydro[1,4]benzodioxine (**39**) (200 mg, 1.01 mmol) was dissolved in DMF (50 mL). Benzylamine (0.17 mL, 1.51 mmol) and triethylamine (0.21 mL, 1.51 mmol) were added. The reaction was heated at 90 °C under stirring for 92 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.50) and uncomplete consumption of **39** (R_f 0.60). The crude mixture was quenched with ice (20 mL), HCl 1N (20 mL) was added and the mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was microdistilled to afford _N_-benzyl-2-(2,3)-dihydro[1,4]benzodioxin-6-yl)ethylamine **35** (178 mg, 66% yield) as a brown oil.

Product aspect: brown oil.

Theoretical mass: 270 mg.

Mass obtained: 178 mg.

Yield: 66%.

Analytical data:

R_f = 0.50 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.18-2.50 (m, 2H, CH$_2$-Ar), 3.51-3.73 (m, 4H, CH$_2$-CH$_2$-NH, NH-CH$_2$-Ar), 4.22- 4.26 (m, 4H, CH$_2$-O (x 2)), 6.70-6.85 (m, 3H, H-5, H-7, H-8), 7.18-7.40 (m, 5H, NBn).

(*E*)-6-(2-Nitrovinyl)-2,3-dihydro[1,4]benzodioxine (36)

30
C$_9$H$_8$O$_3$
164,16 g/mol

36
C$_{10}$H$_9$NO$_4$
207,18 g/mol
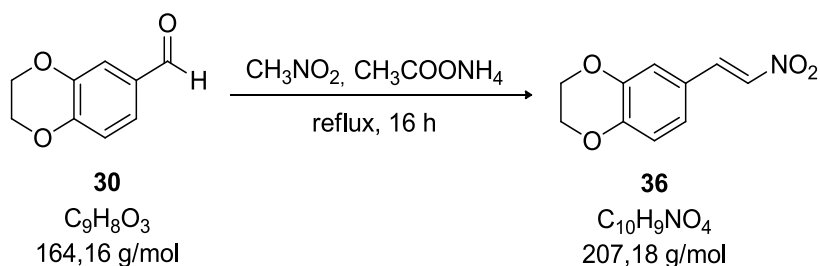
2,3-Dihydro[1,4]benzodioxin-6-carbaldehyde (**30**) (1 g, 6.1 mmol) and ammonium acetate (123 mg, 1.57 mmol) were dissolved in nitromethane (10 mL) in a flame-dried round-bottom flask under argon and refluxed under stirring for 16 h. Then, TLC of the crude mixture (CH$_2$Cl$_2$ / hexane 7:3) indicated formation of a bright yellow compound (R$_f$ 0.55) and complete consumption of the starting material (R$_f$ 0.35). The crude mixture was filtered and concentrated *in vacuo* to afford *(E)*-6-(2-nitrovinyl)-2,3-dihydro[1,4]benzodioxine (**36**) (1.25 g, 99% yield) as a bright yellow solid.

Product aspect: bright yellow solid.

Theoretical mass: 1.26 g.

Mass obtained: 1.25 g.

Yield: 99%.

Analytical data:

mp: 148-150 °C (EtOAc).

R$_f$ = 0.55 (CH$_2$Cl$_2$/hexane 7:3).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 4.30 (s, 4H, C$\underline{H}_2$-O (x 2)), 6.91 (d, *J* = 6.0 Hz, 1H, H-8), 7.05 (dd, *J* = 3.0 Hz, *J* = 6.0 Hz, 1H, H-7), 7.07 (d, *J* = 3.0 Hz, 1H, H-5), 7.47 (d, *J* = 13.5 Hz, 1H, C$\underline{H}$=CH-NO$_2$), 7.90 (d, *J* = 13.5 Hz, 1H, CH=C$\underline{H}$-NO$_2$).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 64.6 (CH$_2$ (x 2)), 117.7 (CH, C-5), 117.9 (CH, C-8), 122.0 (C, C-6), 122.0 (CH, C-7), 130.2 (CH, CH-NO$_2$), 142.7 (C, C-8a), 143.9 (C, C-4a), 150.9 (CH, $\underline{C}$H=CH-NO), 151.1 (CH, CH=$\underline{C}$H-NO$_2$).

6-Bromo-2,3-dihydro-[1,4]-benzodioxin (37)
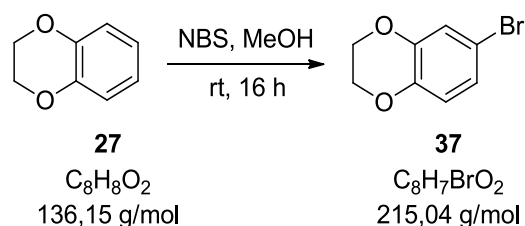
2,3-Dihydro-1,4 benzodioxin (**27**) (1 g, 7.34 mmol) was dissolved in methanol (10 mL) and put in a flame-dried round-bottom flask under argon. The crude mixture was cooled to 0°C and *N*-Bromosuccinimide (1.58 g, 7.34 mmol) was added. The reaction mixture was stirred at rt for 16 h and TLC of the crude mixture (hexane/EtOAc 7:3) indicated formation of a major product (R_f 0.60) and complete consumption of starting material (R_f 0.65). The reaction was concentrated *in vacuo*, dissolved in CH_2Cl_2 and washed with NaOH (3 x 20 mL of a 5N solution). The aqueous phases were re-extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford 6-bromo-2,3-dihydro-[1,4]-benzodioxin (**37**) (1.3 g, 97% yield) as a yellow solid.

Product aspect: yellow solid.

Theoretical mass: 1.34 g.

Mass obtained: 1.30 g.

Yield: 97%.

Analytical data:

mp: 128-132 $^\circ$C (EtOAc).

R_f = 0.55 (hexane/EtOAc 7:3).

RMN ^{1}H (CDCl$_3$, 200 MHz) δ (ppm): 4.23 (s, 4H, CH_2-O (x 2)), 6.73 (d, *J* = 8.4 Hz, 1H, H-8), 6.93 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H, H-7), 7.00 (d, *J* = 2.2 Hz, 1H, H-5).

RMN ^{13}C (CDCl$_3$, 50.3 MHz) δ (ppm): 64.1 (CH_2, CH_2-O), 64.2 (CH_2, CH_2-O), 112.7 (C, C-6), 118.5 (CH, C-5), 120.2 (CH, C-8), 124.1 (CH, C-7), 142.8 (C, C-8a), 144.3 (C, C-4a).

6-(2-Chloroethyl)-2,3-dihydro[1,4]benzodioxine (39)

34
$C_{10}H_{12}O_3$
180,20 g/mol

39
$C_{10}H_{11}ClO_2$
198,65 g/mol
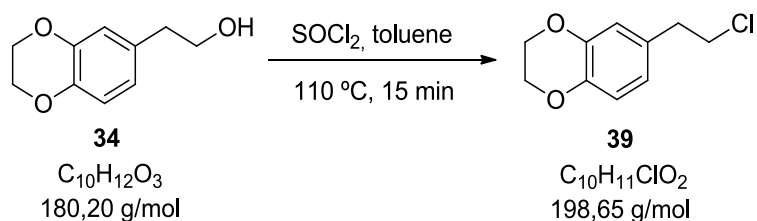
(2,3-Dihydro[1,4]benzodioxin-6-yl)ethanol (**34**) (300 mg, 2.22 mmol) was dissolved in $SOCl_2$ (1.5 mL) in a flame-dried round-bottom flask under argon, heated at 110 °C and stirred for 15 min. Then, TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.60) and complete consumption of SM. The crude mixture was quenched with ice (20 mL) and NaOH was added until pH 14. The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 6-(2-chloroethyl)-2,3-dihydro[1,4]benzodioxine (**39**) (338 mg, 77% yield) as a brown oil.

Product aspect: brown oil.

Theoretical mass: 439 mg.

Mass obtained: 338 mg.

Yield: 77%.

Analytical data:

R_f = 0.6 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.44 (m, 2H, Ar-C$\underline{H}_2$-CH$_2$-Cl) 3.77 (m, 2H, Ar-CH$_2$-C$\underline{H}_2$-Cl), 4.43 (m, 4H, CH$_2$-O (x 2)), 6.83-7.35 (m, 3H, H-5, H-7, H-8).

_N_-Ethoxycarbonyl-6-(3,4,5-trimethoxyphenyl)-2,3,6,7,8,9-hexahydro[1,4]dioxino[2,3-_g_]isoquinoline (45)

3
$C_{20}H_{23}NO_5$
357,40 g/mol

45
$C_{23}H_{27}NO_7$
429,46 g/mol
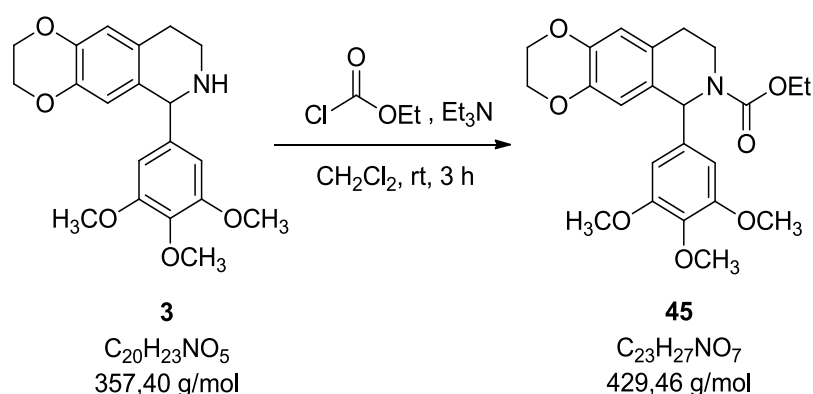
The isoquinoline **3** (100 mg, 0.28 mmol) was dissolved in CH_2Cl_2 (10 mL) in a flame-dried round-bottom flask under argon. Et_3N (0.06 mL, 0.42 mmol) was added and the reaction mixture was stirred for 15 min. The reaction was cooled to 0 °C and ethyl chloroformate (0.04 mL, 0.42 mmol) was added. The reaction was stirred at rt for 3 h and TLC of the crude reaction (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.60) and complete consumption of the starting material (R_f 0.05). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (hexane/EtOAc 7:3) to afford the desired chloroformiate **45** (87 mg, 73% yield) as a yellow oil.
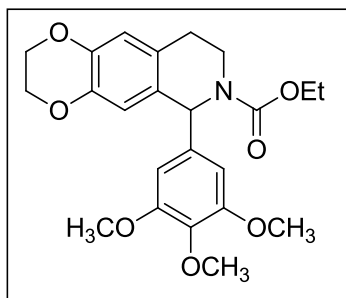
Product aspect: yellow oil.

Theoretical mass: 120 mg.

Mass obtained: 87 mg.

Yield: 73%.

Analytical data:

*Compound **45***

R$_f$ = 0.60 (hexane/EtOAc 1:1).

IR (film) ν cm^{-1} : 2981-2837 (C-H), 1687 (C=O), 1589, 1503, 1460, 1417 (Ar-H), 1289, 1220, 1123 (Ar-O), 1100, 1067 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.29 (t, *J* = 9.0 Hz, C$\underline{H}_3$-CH$_2$), 2.61-2.70 (m, 2H, C$\underline{H}_2$-Ar), 2.79-2.95 (m, 1H, C$\underline{H}_2$-N), 3.17-3.27 (m, 1H, C$\underline{H}_2$-N), 3.77 (s, 6H, CH$_3$-O (x 2)), 3.82 (s, 3H, CH$_3$-O), 4.19 (s, 1H, H-6), 4.20-4.26 (m, 6H, CH$_3$-C$\underline{H}_2$-O, CH$_2$-O (x 2) 6.57 (s, 2H, H-2', H-6'), 6.68 (s, 1H, H-5), 6.79 (s, 1H, H-10).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 15.1 (CH$_2$-$\underline{C}$H$_3$), 28.1 (CH$_2$-Ar), 38.5 (CH$_2$-N), 56.4 (CH$_3$, OCH$_3$ (x 2)), 57.5 (CH, C-6), 61.0 (CH$_3$, OCH$_3$), 61.8 (CH$_2$, CH$_2$-O), 64.6 (CH$_2$, O-CH$_2$-$\underline{C}$H$_2$-O), 64.7 (CH$_2$, O-$\underline{C}$H$_2$-CH$_2$-O), 106.0 (CH, C-2', C-6'), 116.9 (CH, C-5, C-10), 128.3 (C, C-5a), 137.6 (C, C-9a), 138.9 (C, C-1'), 142.2 (C, C-4a), 142.9 (C, C-10a), 153.2 (C, C-3', C-4', C-5'), 155.7 (C, N$\underline{C}$O$_2$Et).

MS (EI) (m/z, %): 429 (M$^+$, 52), 356 (M$^+$-CO$_2$CH$_2$CH$_3$, 100), 262 (M$^+$-C$_9$H$_{11}$O$_3$, 43). (C$_9$H$_{11}$O$_3$ = (3,4,5-trimethoxyphenyl-H)$^+$).

6,7-Dimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (49)

51
$C_{22}H_{24}F_3NO_6$
455,42 g/mol

49
$C_{20}H_{25}NO_5$
359,42 g/mol
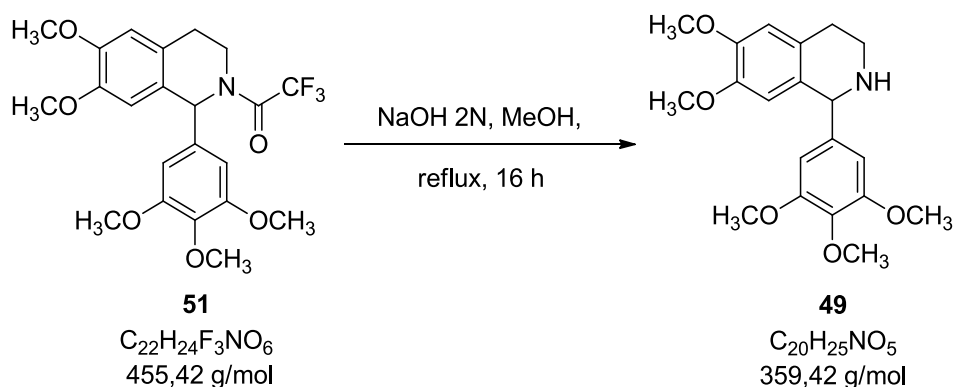
The substitued isoquinoline **51** (870 mg, 1.91 mmol) was dissolved in MeOH (15 mL). NaOH 2N (45 mL) was added and the reaction was heated to reflux for 16 h. Then, TLC of the crude mixture (hexane/EtOAc 1:1) showed total consumption of SM (R_f 0.75) and formation of a new compound (R_f 0.15). The methanol was evaporated *in vacuo*. The aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo.* The crude residue was purified by flash column chromatography (hexane/EtOAc 1:1) to afford the desired isoquinoline **49** (480 mg, 70% yield) as a white solid.
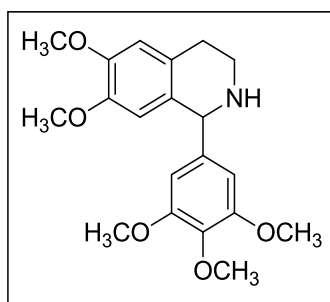
Product aspect: white solid.

Theoretical mass: 683 mg.

Mass obtained: 480 mg.

Yield: 70%.

Analytical data:

*Compound **49***

R$_f$ = 0.15 (hexane/EtOAc 1:1).

**mp:** 95-97 °C (EtOAc).

IR (film) ν cm^{-1} : 3310 (NH), 3002-2828 (C-H), 1736 (C=O), 1590, 1515, 1504, 1461, 1420 (Ar-H), 1251, 1226, 1214 (Ar-O), 1123, 1111 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.65-2.78 (m, 1H, CH$_2$-CH$_2$-N), 2.90-3.10 (m, 2H, H-C-4, H-C-3) 3.20-3.30 (m, 1H, CH$_2$-N), 3.68 (s, 3H, CH$_3$-O), 3.81 (s, 6H, CH$_3$-O (x 2)), 3.85 (s, 3H, CH$_3$-O), 3.88 (s, 3H, CH$_3$-O), 4.97 (s, 1H, H-1), 6.31 (s, 1H, H-5), 6.49 (s, 2H, H-2', H-6'), 6.63 (s, 1H, H-8).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 29.0 (CH$_2$, Ar-CH$_2$), 39.7 (CH$_2$, CH$_2$-N), 56.2 (CH$_3$, CH$_3$-O), 56.3 (CH$_3$, OCH$_3$), 56.5 (CH$_3$, OCH$_3$ (x 2)), 56.8 (CH$_3$, OCH$_3$), 61.1 (CH, C-1), 106.6 (CH, C-2', C-6'), 111.3 (CH, C-5), 111.4 (CH, C-8), 125.2 (C, C-4a), 126.1 (C, C-8a), 136.8 (C, C-1'), 148.2 (C, C-7), 148.9 (C, C-6), 153.5 (C, C-3', C-4', C-5').

HRMS +ESI m/z (%): Calculated for C$_{20}$H$_{25}$NO$_5$ (M+H)$^+$: 359.1733. Found: 359.1851.

(6,7-Dimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinolin-2-yl) 2,2,2-trifluoroacetate (51)
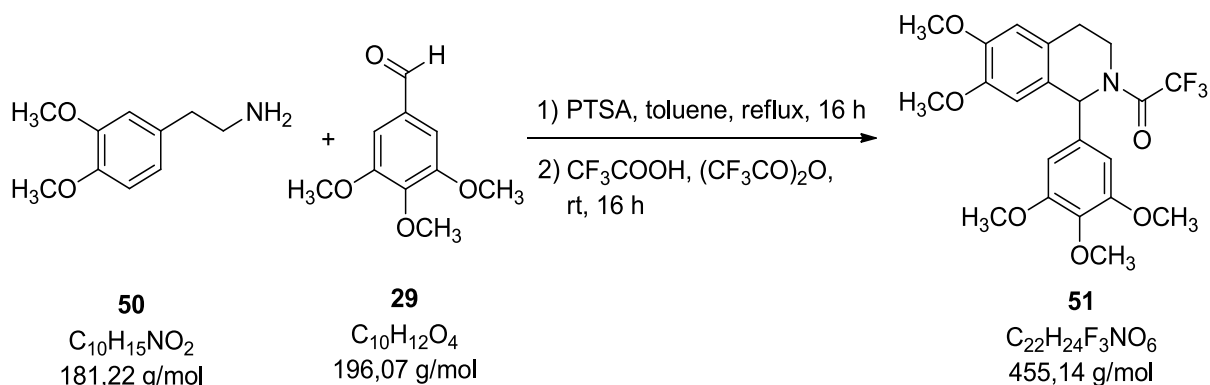
The veratrolethylamine **50** (2g, 11 mmol) and 3,4,5-trimethoxybenzaldehyde (**29**) (2.38 g, 12.1 mmol) were dissolved in toluene (30 mL) in a flame-dried round-bottom flask under argon. APTS (catalytic amount) and 4 Å molecular sieves (50 mg) were added. The reaction mixture was refluxed under stirring for 16 h and TLC of the reaction mixture (EtOAc) indicated the presence of the aldehyde (R_f 0.80) and the imine (R_f 0.85). The crude mixture was filtered to afford 5 g of a brown oil. CF_3COOH (7 mL) and $(CF_3CO)_2O$ (7 mL) were added and the mixture was stirred for 24 h. TLC of the crude mixture (EtOAc/hexane 1:1) indicated the presence of the desired compound (Rf 0.75) and complete consumption of the imine (R_f 0.85). The crude reaction was dissolved in EtOAc (20 mL), washed with NaOH 2N (3 x 30 mL) and the combined aqueous phases were re-extracted with EtOAc (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 1:1) to afford the desired compound **51** (1.67 g, 33% yield) as a pale brown oil.
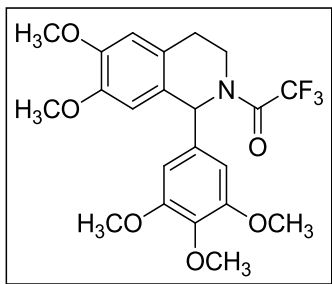
Product aspect: pale brown oil.

Theoretical mass: 5.01 g.

**Mass obtained:** 1.67 g.

Yield: 33%.

Analytical data:

Compound 51

R$_f$ = 0.75 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ **(ppm):** 2.70-2.85 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 2.90-3.10 (m, 1H, Ar-C$\underline{H}_2$-CH$_2$-N), 3.35-3.50 (m, 1H, CH$_2$-N), 3.68 (s, 3H, CH$_3$-O (x 2)), 3.71 (s, 3H, CH$_3$-O), 3.77 (s, 3H, CH$_3$-O), 3.83 (s, 3H, CH$_3$-O), 3.85-3.95 (m, 1H, CH$_2$-N), 6.39 (s, 2H, H-2', H-6'), 6.46 (s, 1H, H-5), 6.61 (s, 1H, H-1) 6.62 (s, 1H, H-8).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ **(ppm):** 28.9 (CH$_2$, CH$_2$-Ar), 39.6 (CH$_2$, CH$_2$-N), 56.1 (CH$_3$, OCH$_3$), 56.3 (CH$_3$, OCH$_3$), 56.4 (CH$_3$, OCH$_3$ (x 2)), 56.8 (CH$_3$, OCH$_3$), 61.0 (CH, C-1), 106.5 (CH, C-2', C-6'), 111.2 (CH, C-8), 111.3 (CH, C-5), 116.5 (C, J = 288 Hz, CF$_3$), 125.1 (C, C-4a), 125.9 (C, C-8a), 136.8 (C, C-1'), 148.1 (C, C-7), 148.8 (C, C-6), 153.5 (C, C-3', C-4', C-5'), 156.0 (C, J = 36 Hz, C=O).

6,7-Dimethoxy-*N*-(oxirane-2-yl-methyl)-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetra hydro-isoquinoline (52)

49	**52**
$C_{20}H_{25}NO_5$	$C_{23}H_{29}NO_6$
359,42 g/mol	415,48 g/mol
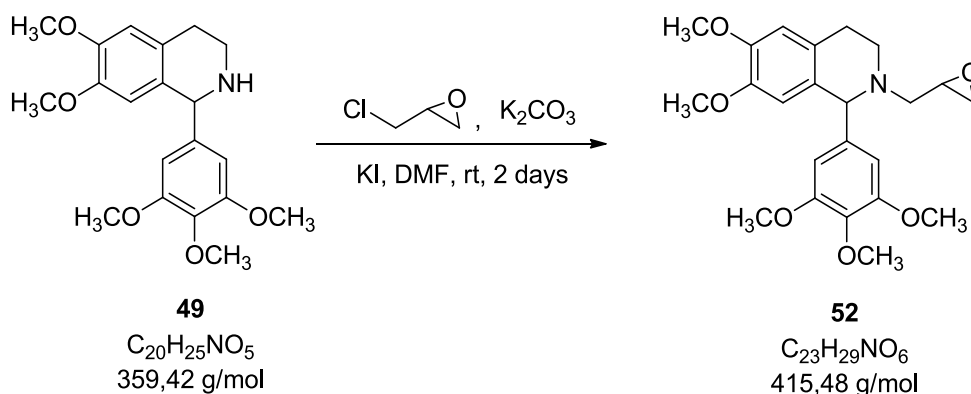
The isoquinoline (**49**) (100 mg, 0.28 mmol) was dissolved in DMF (20 mL) in a flame-dried round-bottom flask under argon. Epichlorhydrin (0.2 mL, 2.55 mmol), K_2CO_3 (350 mg, 2.5 mmol) and KI (catalyst) were added. The reaction was stirred during 48 h and TLC of the crude mixture (hexane/EtOAc 2:8) showed formation of a new compound (R_f 0.30) and uncomplete consumption of SM (R_f 0.35). Water (20 mL) was added and the crude reaction was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H_2O (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the desired isoquinoline **52** (52 mg, 45% yield) as an orange solid.
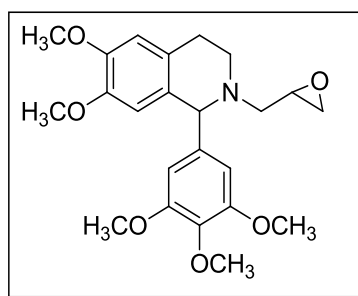
Product aspect: orange solid.

Theoretical mass: 116 mg.

Mass obtained: 52 mg.

Yield: 45%.

Analytical data:

*Compound **52***

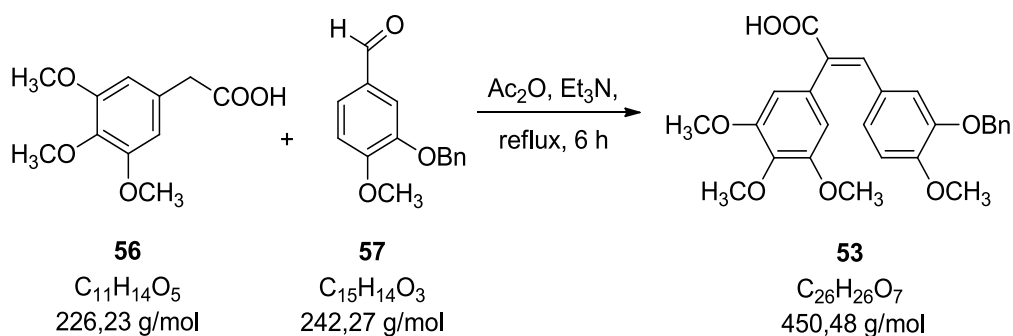**R_f = 0.30** (hexane/EtOAc 2:8).

IR (film) ν cm^{-1} : 2920, 2849 (C-H), 1586, 1503, 1450, 1410 (Ar-H), 1221 (Ar-O), 1120, 1002 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 2.50-3.10 (m, 9H, C<u>H$_2$</u>-CH$_2$-N, C<u>H$_2$</u>-N (x 2), C<u>H</u>-O (x 2), CH$_2$-O), 3.65 (s, 3H, CH$_3$-O), 3.81 (s, 6H, CH$_3$-O (x 2)), 3.85 (s, 3H, CH$_3$-O), 3.87 (s, 3H, CH$_3$-O), 4.60 (s, 1H, H-1), 6.25 (s, 2H, H-2', H-6'), 6.48 (s, 1H, H-5), 6.63 (s, 1H, H-8).

(*E*)-3-(3-(Benzyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylic acid (53)

56	**57**	**53**
C$_{11}$H$_{14}$O$_5$	C$_{15}$H$_{14}$O$_3$	C$_{26}$H$_{26}$O$_7$
226,23 g/mol	242,27 g/mol	450,48 g/mol

3,4,5-Trimethoxyphenylacetic acid (**56**) (773 mg, 3.42 mmol) and 3-benzyloxy-4-methoxybenzaldehyde (**57**) (827 mg, 3.42 mmol) were dissolved in acetic anhydride (5 mL) in a flame-dried round-bottom flask under argon. Et$_3$N (2,5 mL, excess amount) was added under argon. The reaction was refluxed under stirring for 6 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R$_f$ 0.20) and uncomplete consumption of SM (R$_f$ 0.80). The reaction mixture was cooled down to 0 °C and quenched with an excess of HCl 5N (30 mL). The residue formed was

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washed with HCl 5N (3 x 30 mL) and diethyl ether (3 x 30 mL) and filtered to afford the desired compound **53** (400 mg, 27% yield) as a yellow solid.
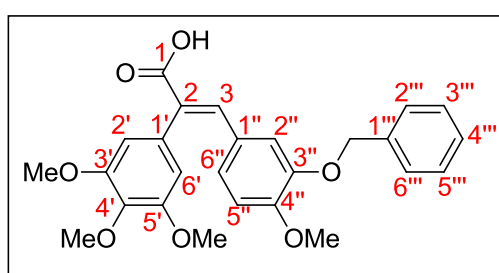
Product aspect: yellow solid.

Theoretical mass: 1.48 g.

Mass obtained: 400 mg.

Yield: 27%.

Analytical data:

*Compound **53***

mp: 192-195 $^\circ$C (hexane/EtOAc).

R$_f$ = 0.20 (hexane/EtOAc 1:1).

IR (film) ν cm^{-1} : 3200-2500 (OH), 2990-2910 (C-H), 1662 (C=O), 1597, 1581, 1505 (Ar-H), 1259, 1241 (Ar-O), 1122 (C-O).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 3.91 (s, 3H, OCH$_3$), 3.96 (s, 6H, OCH$_3$ (x 2)), 3.99 (s, 3H, OCH$_3$), 4.86 (s, 2H, C$\underline{H}_2$-OBn), 6.74 (s, 2H, H-2, H-6), 6.91 (d, *J* = 2.1 Hz, 1H, H-2''), 7.08 (d, *J* = 8.4 Hz, 1H, H-5''), 7.12 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H, H-6''), 7.58-7.44 (m, 5H, OBn), 7.91 (s, 1H, C=C-$\underline{H}$).

RMN ^{13}C (Acetone-*d6*, 75.5 MHz) δ (ppm): 47.0 (CH$_3$, OCH$_3$). 47.8 (CH$_3$, OCH$_3$ (x 2)), 51.55 (CH$_3$, OCH$_3$), 60.78 (CH$_2$, CH$_2$-OBn), 98.24 (CH, C-2', C-6'), 103.0 (CH, C-2''), 105.5 (CH, C-5''), 116.9 (CH, C-6''), 118.2 (C, C-1''), 119.0 (CH, C-2''', C-6'''), 119.4 (CH, C-4'''), 119.9 (CH, C-3''', C-5'''), 122.4 (C, C-1'), 123.9 (C, $\underline{C}$=CH), 127.9 (C, C-1'''), 128.4 (C, C-3''), 130.2 (CH, C=$\underline{C}$H), 138.3 (C, C-4''), 141.6 (C, C-4'), 144.8 (C, C-3', C-5'), 159.8 (C, C=O).

MS EI m/z (%): 450 (M$^+$, 62).

(*E*)-Methyl 3-(3-(benzyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl) acrylate (54)

53
C$_{26}$H$_{26}$O$_7$
450,48 g/mol

54
C$_{27}$H$_{28}$O$_7$
464,51 g/mol
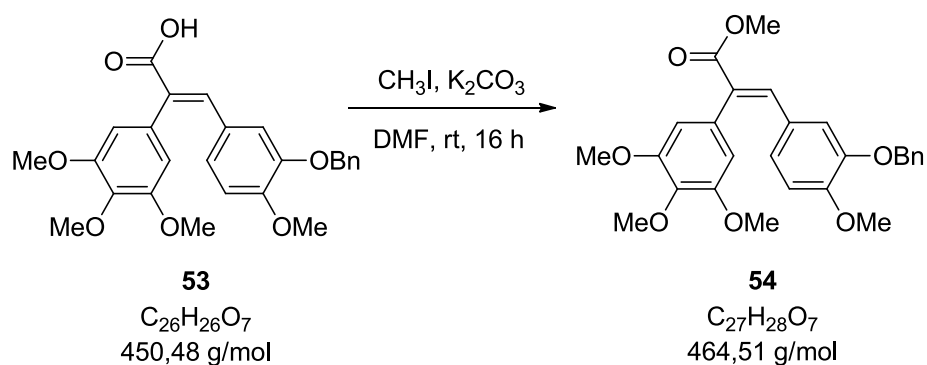
The carboxylic acid **53** (385 mg, 0.85 mmol) was dissolved in DMF (10 mL) in a flame-dried round-bottom flask under argon. Then, K$_2$CO$_3$ (236 mg, 1.71 mmol) and CH$_3$I (0.266 mg, 2.28 mmol) were added dropwise to the solution. The reaction was stirred at rt for 16 h and TLC of the crude mixture (EtOAc) indicated formation of a new compound (R$_f$ 0.90) and complete consumption of the starting material (R$_f$ 0.70). Water (20 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with H$_2$O (5 x 30 mL), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford the desired compound **54** (380 mg, 94% yield) as a yellow solid.
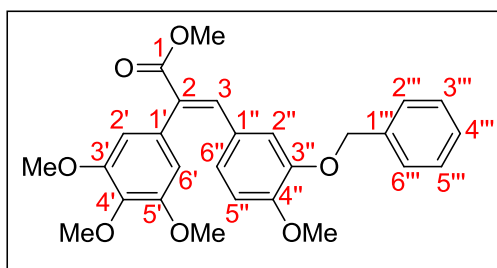
Product aspect: yellow solid.
Theoretical mass: 404 mg.
Mass obtained: 380 mg
Yield: 94%.

Analytical data:

*Compound **54***

R_f = 0.90 (EtOAc).

mp: 90-92 °C (hexane/EtOAc).

IR (film) ν cm⁻¹ : 3000 (C=CH), 2953, 2829 (C-H), 1708 (C=O), 1582, 1508, 1467 (Ar-H), 1241 (Ar-O), 1122, 1009 (C-O).

RMN ¹H (CDCl₃, 300 MHz) δ (ppm): 3.78 (s, 6H, OCH₃ (x 2)), 3.79 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 4.74 (s, 2H, C$\underline{H}$₂-OBn), 6.45 (s, 2H, H-2, H-6), 6.61 (d, J = 2.1 Hz, 1H, H-2''), 6.75 (d, J = 8.7 Hz, 1H, H-5''), 6.84 (dd, J = 2.1 Hz J = 8.7 Hz, 1H, H-6''), 7.20-7.40 (m, 5H, OBn), 7.71 (s, 1H, C=C$\underline{H}$).

RMN ¹³C (CDCl₃, 75.5 MHz) δ (ppm): 52.6 (CH₃, COO$\underline{C}$H₃), 56.2 (CH₃, Ar-O$\underline{C}$H₃), 56.5 (CH₃, Ar-O$\underline{C}$H₃ (x 2)), 61.2 (CH₃, Ar-O$\underline{C}$H₃), 70.5 (CH₂, CH₂-O), 107.1 (CH, C-2', C-6'), 111.3 (CH, C-2''), 115.0 (CH, C-5''), 125.8 (CH, C-6''), 126.1 (C, C-1''), 127.3 (CH,C-2''', C-6'''), 127.4 (CH,C-4'''), 128.01 (CH,C-3''', C-5'''), 128.8 (C, C-1'), 131.9 (C, C-1'''), 136.8 (C, MeOOC-$\underline{C}$=CH), 137.9 (C, C-3''), 140.6 (CH, MeOOC-C=$\underline{C}$H), 147.7 (C, C-4''), 151.0 (C, C-4'), 153.9 (C, C-3', C-5'), 168.6 (C, C=O).

MS EI m/z (%): 464 (M⁺, 91).

(Z)-5-(3-Benzyloxy)-4-methoxystyryl)-1,2,3-trimethoxybenzene (55a)

53
$C_{26}H_{26}O_7$
450,48 g/mol

55a
$C_{25}H_{26}O_5$
406,47 g/mol
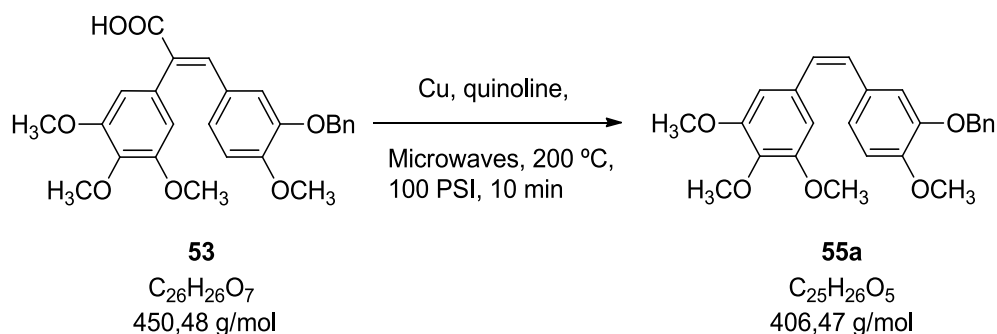
The carboxylic acid **53** (100 mg, 0.22 mmol) was dissolved in quinoline (5 mL). Cu (catalytic amount) was added. The reaction was heated under microwave oven at 200 °C under stirring at 100 PSI for 5 min. Then, TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of two new compounds (R_f 0.80, 0.60) and complete consumption of starting material (R_f 0.20). The reaction mixture was dissolved in diethyl ether (20 mL) and washed with H_2O (3 x 20 mL), NaOH 2N (3 x 20 mL) and HCl 2N (5 x 20 mL). The combined aqueous phases were re-extracted with diethyl ether (20 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo.* The residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired compound **55a** (67 mg, 75% yield) as a pale brown solid and **55b** (20 mg, 21% yield) as a pale brown solid.

Product aspect: pale brown solid.

Theoretical mass: 89 mg.

Mass obtained: 67 mg.

Yield: 75%.

Analytical data:
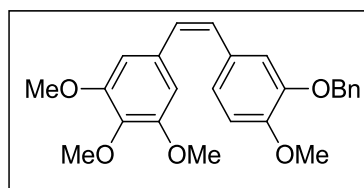
Compound 55a

R$_f$ = 0.80 (hexane/EtOAc 1:1).

IR (film) ν cm^{-1} : 3002 (C=C), 2933, 2834 (C-H), 1581, 1508, 1453, 1427 (Ar-H), 1262 (Ar-O), 1238 (Ar-O), 1126 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.69 (s, 6H, OCH$_3$ (x 2)), 3.83 (s, 3H, OCH$_3$), 3.85 (s, 3H, OCH$_3$), 4.93 (s, 2H, CH$_2$-OBn), 6.41 (d, *J* = 12 Hz, 1H, C=C-H), 6.46 (d, *J* = 12 Hz, 1H, C=C-H), 6.49 (s, 1H, H-2, H-6), 6.78 (d, *J* = 9 Hz, 1H, H-5'), 6.86 (dd, *J* = 1.7 Hz, *J* = 9 Hz, 1H, H-6'), 7.28 (d, *J* = 1.7 Hz, 1H, H-2'), 7.28-7.32 (m, 5H, OBn).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 56.3 (CH$_3$, OCH$_3$ (x 2)), 56.3 (CH$_3$, OCH$_3$), 61.2 (CH$_3$, OCH$_3$), 71.2 (CH$_2$, CH$_2$O), 106.3 (CH, C-2, C-6), 111.8 (CH, C-2'), 114.8 (CH, C-5'), 122.8 (CH, C-6'), 127.5 (CH, C-2'', C-6''), 128.1 (CH, C-4''), 128.8 (CH, C-3'', C-5''), 129.1 (CH, Ar-CH=CH-Ar), 129.9 (CH, Ar-CH=CH-Ar), 130.1 (C, C-1'), 133.3 (C, C-1), 137.2 (C, C-1''), 137.4 (C, C-3'), 148.0 (C, C-4'), 149.2 (C, C-4), 153.2 (C, C-3, C-5).

MS EI m/z (%): 406 (M$^+$, 100), 391 (M$^+$-CH$_3$, 7.44), 376 (M$^+$-C$_2$H$_6$, 11.02), 361 (M$^+$-C$_3$H$_9$, 4.39), 252 (M$^+$-C$_9$H$_{11}$O$_3$, 25.94).

(C$_9$H$_{11}$O$_3$ = 3,4,5-trimethoxyphenyl).

(Z)-5-(3-Benzyloxy)-4-methoxystyryl)-1,2,3-trimethoxybenzene (55a) (alternative method)
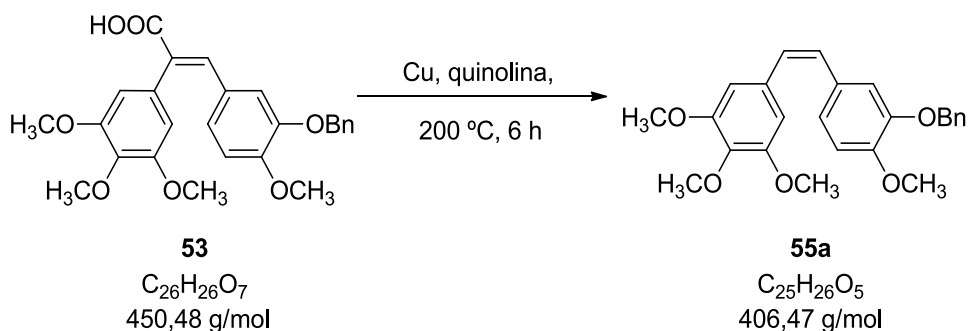
The carboxylic acid **53** (100 mg, 0.22 mmol) was dissolved in quinoline (5 mL) in a flame-dried round-bottom flask under argon and Cu (cat) was added under argon. The reaction was heated at 200 °C for 6 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of two new compounds (R_f 0.80, 0.60) and complete consumption of starting material (R_f 0.20). The reaction mixture was dissolved in diethyl ether (20 mL), washed with H_2O (3 x 20 mL), NaOH 2N (3 x 20 mL) and HCl 2N (5 x 20 mL). The combined aqueous phases were re-extracted with diethyl ether (20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo.* The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford **55a** (54 mg, 60% yield) as a pale brown solid and **55b** (22 mg, 24% yield) as a pale brown solid.

Product aspect: pale brown solid.

Theoretical mass: 89 mg.

Mass obtained: 54 mg.

Yield: 60%.

Analytical data was identical with the previously described compound.

(*Z*)-5-(3-Benzyloxy)-4-methoxystyryl)-1,2,3-trimethoxybenzene (55a) from 55b

55b
$C_{25}H_{26}O_5$
406,47 g/mol

55a
$C_{25}H_{26}O_5$
406,47 g/mol
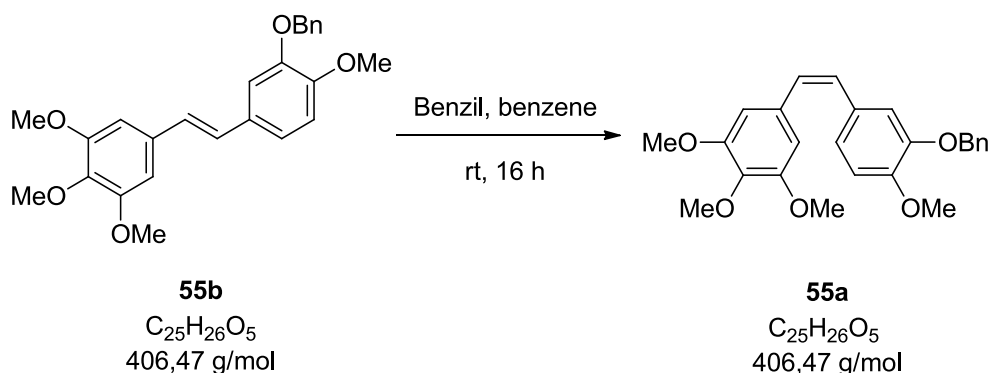
The olefin **55b** (40 mg, 0.13 mmol) was dissolved in benzene (5 mL) in a flame-dried round-bottom flask under argon. Benzil (114 mg, 0.64 mmol) was added. The reaction was let to stir at rt under U.V. radiation (254 nm) for 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.80) and uncomplete consumption of starting material **55b** (R_f 0.60). The crude reaction was concentrated *in vacuo.* The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired compound **55a** (24 mg, 60% yield) as a pale brown solid and the starting material **55b** (16 mg, 40% yield) as a pale brown solid.

Product aspect: pale brown solid.

Theoretical mass: 40 mg.

Mass obtained: 24 mg.

Yield: 60%.

Analytical data was identical with the previously described compound.

3-Benzyloxy-4-methoxybenzaldehyde (57)
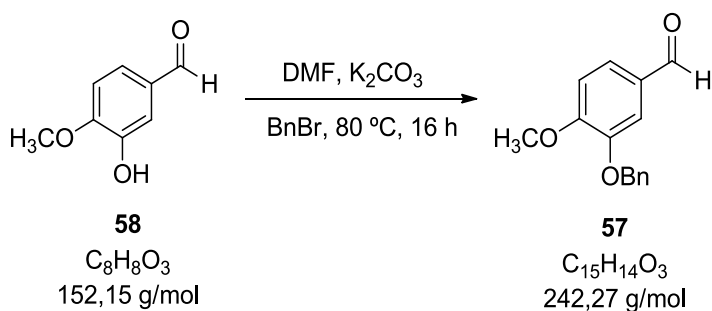
3-Hydroxy-4-methoxybenzaldehyde (**58**) (1 g, 6.57 mmol) was dissolved in DMF (10 mL) in a flame-dried round-bottom flask under argon. K_2CO_3 (1.27 g, 9.2 mmol) and benzyl bromide (1.09 mL, 9.2 mmol) were added under argon. The reaction was heated at 80 °C during 16 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.75) and total consumption of the starting material (R_f 0.40). Diethyl ether (15 mL) was added, the solution was washed with NaOH 2N (3 x 15 mL) and water (4 x 15 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford 3-benzyloxy-4-methoxybenzaldehyde (**57**) (1.5 g, 94% yield) as a yellow solid.

Product aspect: Yellow solid.

Theoretical mass: 1.59 g.

Mass obtained: 1.50 g.

Yield: 94%.

<u>**Analytical data:**</u>

R_f = 0.75 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.96 (s, 3H, OCH$_3$), 5.18 (s, CH$_2$, CH$_2$-O), 6.99 (d, J = 9 Hz, 1H, H-5), 7-30 (m, 2H, H-2, H-6), 7.50-7.43 (m, 5H, OBn), 9.82 (s, 1H, C<u>H</u>O).

(*E*)-3-(3-(Benzyloxy)-4-methoxyphenyl-2-(4-methylsulfonyl)phenyl)acrylic acid (59a) and (59b)

60
$C_9H_{10}O_4S$
214,24 g/mol

57
$C_{15}H_{14}O_3$
242,27 g/mol

59a
$C_{24}H_{22}O_6S$
438,49 g/mol

59b
$C_{24}H_{22}O_6S$
438,49 g/mol
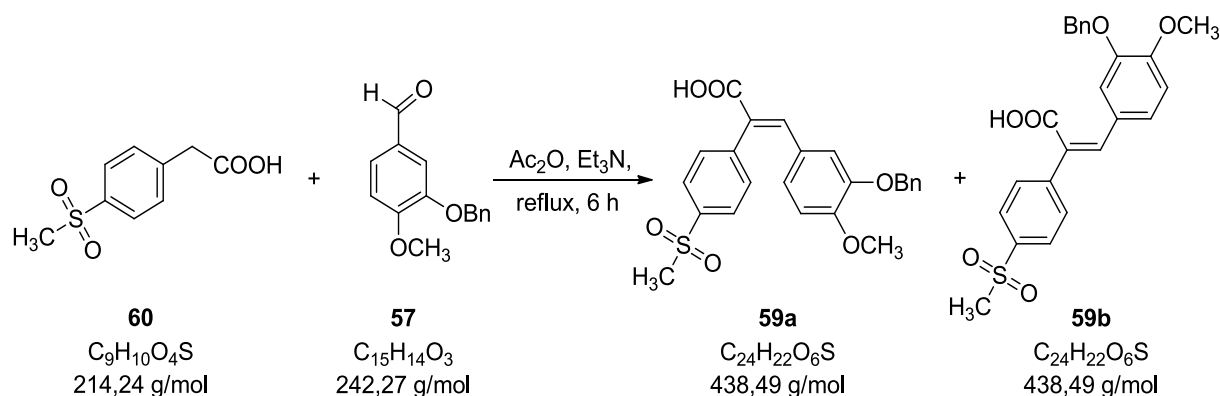
(4-Methylsulfonyl)phenylacetic acid (**60**) (300 mg, 1.40 mmol) and 3-benzyloxy-4-methoxybenzaldehyde (**57**) (339 mg, 1.4 mmol) were dissolved in acetic anhydride (5 mL) in a flame-dried round-bottom flask under argon. Et$_3$N (2,5 mL, excess amount) was added under argon. The reaction was refluxed under stirring for 6 h and TLC of the crude mixture (EtOAc) indicated formation of a new compound (R$_f$ 0.40) and uncomplete consumption of SM (R$_f$ 0.80, 0.15). The crude mixture was cooled down to 0 °C and quenched with HCl 5N (20 mL). NaOH 30% was added until p*H* 14 and the aqueous phase was washed with diethyl ether (3 x 20 mL). HCl 5N was added until p*H* 1 and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic phases were dried (NaSO$_4$), filtered and evaporated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 4:6) to afford the carboxylic acids **59a** and **59b** (32 mg, 5% yield) as a white solid, starting material **57** (304 mg) and starting material **60** (251 mg).

Product aspect: white solid.

Theoretical mass: 614 g.

Mass obtained: 32 mg.

Yield: 5%.
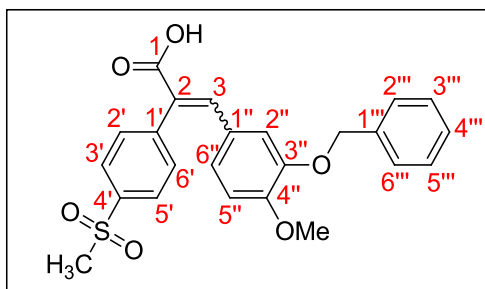
*Compound **59a** and **59b***

R_f = 0.40 (EtOAc).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): *Mixture of Cis and Trans*. 3.29 (s, 3H, CH$_3$-SO$_2$ *cis*), 3.31 (s, 3H, CH$_3$-SO$_2$ *trans*), 3.94 (s, 3H, OCH$_3$ *cis*), 3.99 (s, 3H, OCH$_3$ *trans*), 4.15 (s, 3H, OCH$_3$), 4.82 (s, 2H, C$\underline{H}_2$-OBn), 6.71 (d, *J* = 3 Hz, H-2''), 6.79 (s, 1H, C$\underline{H}$=C *cis*), 6.94 (d, *J* = 8.4 Hz, 1H, H-5''), 7.03 (dd, *J* = 3 Hz, *J* = 8.4 Hz, 1H, H-6''), 7.08 (s, 1H, C$\underline{H}$=C *trans*), 7.52-7.57 (m, 5H, C-2''', C-3''', C-4''', C-5''', C-6'''), 7.64 (d, *J* = 8.4 Hz, 2H, H-2', H-6' *trans*), 7.73 (d, *J* = 8.4 Hz, 2H, H-2', H-6' *cis*), 8.07 (d, *J* = 8.4 Hz, 2H, H-3', H-5' *cis*), 8.22 (d, *J* = 8.1 Hz, 2H, H-3', H-5' *trans*).

RMN ^{1}H (DMSO-*d6*, 75.5 MHz) δ (ppm): *Mixture of Cis and Trans*. δ 45.93 (CH$_3$, $\underline{C}$H$_3$-SO$_2$), 56.52 (CH$_3$, O$\underline{C}$H$_3$), 69.75 (CH$_2$, CH$_2$-OBn), 107.18 (CH, C-2''), 112.01 (CH, C-5''), 114.50 (CH, C-6''), 125.94 (CH, C-2', C-6'), 125.96 (CH, C-3', C-5'), 127.25 (C, C-1''), 128.05 (CH, C-2''', C-6'''), 128.44 (CH, C-4'''), 128.92 (CH, C-3''', C-5'''), 131.07 (C, C-1'), 132.94 (C, C-1'''), 136.91 (C, $\underline{C}$-COOH *trans*) 137.40 (C, $\underline{C}$-COOH *cis*), 139.42 (CH, C=$\underline{C}$H), 147.36 (C, C-4'), 150.65 (C, C-3''), 153.85 (C, C-4''), 168.90 (C, $\underline{C}$OOH).

MS EI m/z (%): 438 (M$^+$, 9.19).

2-(4-Methylthio)phenylacetic acid (61)
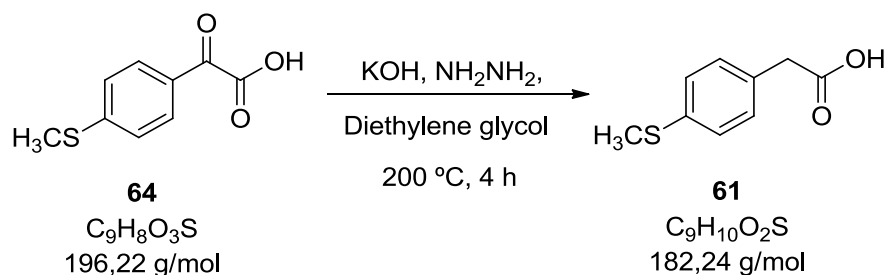
To the carboxylic acid (**64**) (150 mg, 0.765 mmol) and KOH (107 mg, 1.91 mmol) in diethylene glycol (2 mL) was added $NH_2NH_2.H_2O$ (0.087 mL, 2.3 mmol). The reaction mixture was heated at 200 ^{o}C for 4 h and TLC of the crude mixture (EtOAc/methanol 8:2) indicated formation of a new compound (R_f 0.65) and uncomplete consumption of starting material (R_f 0.30). The aqueous phase was washed with diethyl ether (3 x 20 mL). HCl 2N was added until pH 1 and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the desired compound **61** (109 mg, 78% yield) as an orange solid.

Product aspect: orange solid.

Theoretical mass: 140 mg.

Mass obtained: 109 mg.

Yield: 78%.

<u>**Analytical data:**</u>

mp: 96-98 ^{o}C (ethanol).

$\mathbf{R_f}$ **=** 0.30 (EtOAc/MeOH 8:2).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 2.65 (s, 3H, C$\underline{H}_3$-S), 3.77 (s, 2H, C$\underline{H}_2$-COOH), 7.50-7.30 (m, 4H, H-2, H-3, H-5, H-6).

Ethyl 2-(4-(methylthiophenyl)-2-oxoacetate (63)
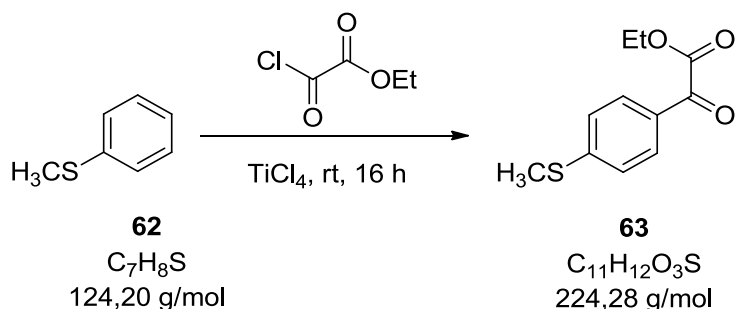
Ethyl chlorooxoacetate (0.296 mL, 2.60 mmol) and thioanisole (**62**) (300 mg, 2.42 mmol) were dissolved in CH_2Cl_2 (10 mL) in a flame-dried round-bottom flask under argon. The mixture was cooled at 0 °C and $TiCl_4$ (3.5 mL) was added. The reaction was stirred at rt for 16 h and TLC of the crude mixture (hexane/EtOAc 8:2) indicated formation of a major compound (R_f 0.55) and uncomplete consumption of starting materials (R_f 0.80). The reaction mixture was washed with water (3 x 20 mL) and the aqueous phases were re-extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo.* The crude residue was purified by silica gel flash column chromatography (hexane/EtOAc 9:1) to afford the desired compound **63** (200 mg, 37% yield) as a yellow solid. The reaction was scaled up using 1 g of thioanisole (**62**) to afford the ketoester **63** (1.6 g, 50% yield) as a yellow oil.

Product aspect: yellow oil.

Theoretical mass: 3.2 g.

Mass obtained: 1.6 g.

Yield: 50%.

Analytical data:

mp: 82-84 °C (EtOAc).

R$_f$ = 0.55 (hexane/EtOAc 8:2).

IR (film) ν cm^{-1}: 2918, 2848 (C-H), 1731, 1672 (C=O), 1555, 1460, 1406 (Ar-H), 1207 (S-Ar), 1177 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.41 (t, *J* = 7 Hz, 3H, C$\underline{\text{H}}_3$-CH$_2$-O), 2.53 (s, 3H, C$\underline{\text{H}}_3$-S), 4.44 (q, *J* = 7 Hz, 2H, C$\underline{\text{H}}_2$-O), 7.29 (d, *J* = 9 Hz, 2H, H-2, H-6), 7.92 (d, *J* = 9 Hz, 2H, H-3, H-5).

MS EI m/z (%): 224 (35.95, M$^+$), 151 (100, M$^+$-73), 123 (22.18, M$^+$).

2-(4-Methylthio)phenyl)-2-oxoacetic acid (64)
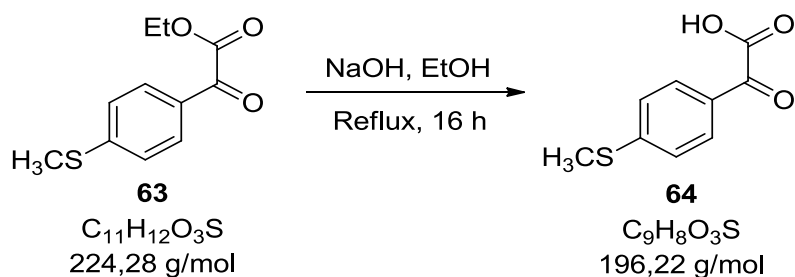
The ethyl ester **63** (100 mg, 0.45 mmol) was dissolved in ethanol (10 mL) and NaOH 2N (25 ml) was added. The reaction was heated to reflux for 16 h and TLC of the crude mixture (EtOAc) indicated formation of a new compound (R_f 0.0) and complete consumption of starting material (R_f 0.95). The ethanol was evaporated *in vacuo* and the aqueous phase was washed with diethyl ether (3 x 20 mL). HCl 2N was added until p*H* 1 and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo* to afford the desired ketoacid (73 mg, 84% yield) as a brown solid. The reaction was scaled up using 1.6 g of the starting material to obtain the desired compound **64** (1.09 g, 78% yield) as a brown solid.

Product aspect: brown solid.

Theoretical mass: 87 g.

Mass obtained: 73 mg.

Yield: 84%.

Analytical data:

mp: 110-112 °C (EtOAc).

R_f = 0.10 (EtOAc/MeOH 9:1).

RMN ^{1}H (Acetone-*d6*, 300 MHz) δ (ppm): 2.77 (s, 3H, C$\underline{H}_3$-S), 7.62 (d, *J* = 9 Hz, 2H, H-3, H-5), 8.15 (d, *J* = 9 Hz, 2H, H-2, H-6).

3-(4-(Benzyloxy)-3-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)furan-2,5-dione (79)

80
C$_{11}$H$_{12}$O$_6$
240,21 g/mol

81
C$_{16}$H$_{16}$O$_4$
272,30 g/mol

79
C$_{27}$H$_{24}$O$_8$
476,47 g/mol
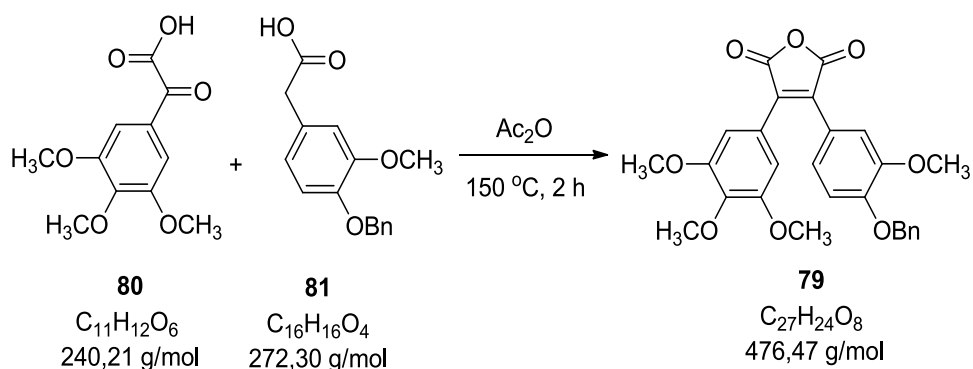
2-Oxo(3,4,5-trimethoxyphenyl)acetic acid (**80**) (96 mg, 0.40 mmol) and 4-benzyloxy-3-methoxyphenylacetic acid (**81**) (218 mg, 0.80 mmol) were dissolved in acetic anhydride (5 mL) in a flame-dried round-bottom flask under argon. The reaction was heated at 150 $^{\circ}$C under stirring for 2 h and TLC of the crude mixture (hexane/EtOAc 2:8) indicated formation of a bright yellow compound (R$_f$ 0.90) and complete consumption of starting materials (R$_f$ 0.60, R$_f$ 0.05). The crude mixture was microdistilled (135 °C) and the resulting residue was dissolved in CH$_2$Cl$_2$ (20 mL) and washed with NaOH 0.1N (3 x 20 mL). The combined aqueous phases were re-extracted with CH$_2$Cl$_2$ (3 x 20 mL) and the combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford the desired compound **79** (260 mg) as an orange solid. Due to stability problems of the anhydride group, the product was used as crude without any further purification.

Product aspect: orange solid.

Theoretical mass: 190 mg.

Mass obtained: 260 mg.
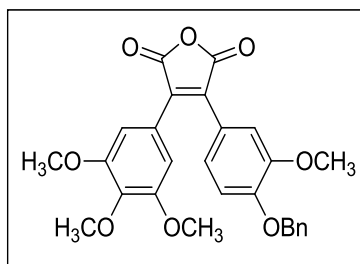
Compound **79**

R$_f$ = 0.60 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.70 (s, 6H, CH$_3$-O (x 2)), 3.75 (s, 3H, OCH$_3$), 3.89 (s, 3H, OCH$_3$), 5.13 (s, 2H, C$\underline{H}_2$-O), 6.74 (dd, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H, H-6'), 6.82 (s, 2H, H-2, H-6), 6.89 (d, *J* = 8.4 Hz, 1H, H-5'), 7.14 (d, *J* = 1.8 Hz, 1H, H-2'), 7.10-7.50 (m, 5H, OBn).

(3,6,7,8,9,10-Hexahydro-2H-[1,4]dioxino[2,3-b]carbazol-10-yl)methanol (82)

84
C$_{17}$H$_{19}$NO$_4$
301,34 g/mol

82
C$_{15}$H$_{17}$NO$_3$
259,30 g/mol
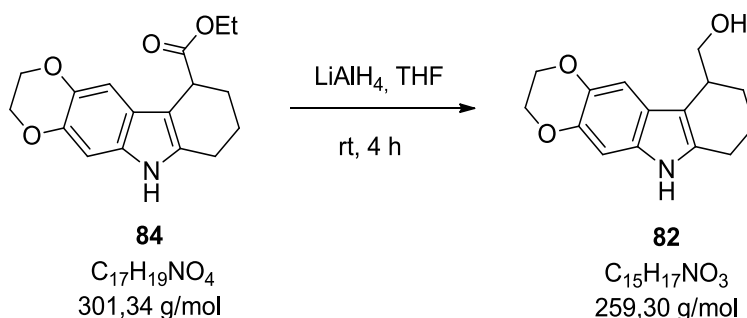
To the ethyl ester **84** (327 mg, 1.09 mmol) dissolved in THF (20 mL) was added LiAlH$_4$ (164 mg, 4.32 mmol) in a flame-dried round-bottom flask under argon. The reaction was stirred at rt for 4 h and TLC of the crude mixture (hexane/EtOAc 8:2) indicated formation of a new compound and complete consumption of starting materials (R$_f$ 0.25). Water (30 mL) was added and the crude mixture was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with water (20 mL), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 6:4) to afford the desired alcohol **82** (207 mg, 74% yield) as a colourless liquid.

Product aspect: colourless liquid.

Theoretical mass: 280 mg.

Mass obtained: 207 mg.

Yield: 74%.

Analytical data:

R_f **=** 0.05 (hexane/EtOAc 8:2).

IR (film) ν cm^{-1}: 3355 (OH, NH), 2918 (C-H), 1499, 1467 (Ar-H), 1200, 1172 (Ar-O), 1063 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.60-2.10 (m, 4H, C$\underline{H}_2$-9, C$\underline{H}_2$-8), 2.56 (dd, J = 2.1 Hz, J = 5.4 Hz, 1H, CH-7), 2.59 (dd, J = 2.1 Hz, J = 5.4 Hz, 1H, CH-7), 2.64 (t, J = 4.5 Hz, 1H, CH-10), 3.06 (bs, 1H, OH), 3.73 (d, J = 4.5 Hz, 1H, CH-OH), 3.90 (dd, J = 4.5 Hz, J = 9.0 Hz, 1H, CH-OH). 4.25 (m, 4H, CH$_2$-O (x 2)), 6.79 (s, 1H, H-5), 6.91 (s, 1H, H-11).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 21.3 (CH$_2$, CH$_2$-9), 22.6 (CH$_2$, CH$_2$-8), 26.3 (CH$_2$, CH$_2$-7), 37.1 (CH, $\underline{C}$H-CH$_2$-OH), 64.6 (CH$_2$, $\underline{C}$H$_2$-O), 64.9 (CH$_2$, $\underline{C}$H$_2$-O), 67.8 (CH$_2$, $\underline{C}$H$_2$-OH), 98.5 (CH, C-5), 104.7 (CH, C-11), 109.8 (C, C-10a), 122.0 (C, C-6a), 131.2 (C, C-10b), 136.4 (C, C-5a), 138.7 (C, C-4a), 140.4 (C, C-11a).

MS EI m/z (%): 259 (M$^+$, 29), 228 (M$^+$-CH$_2$OH, 100).

3,6-Dihydro-2*H*-[1,4]dioxino[2,3-*b*]carbazol-10-yl)methanol (83)
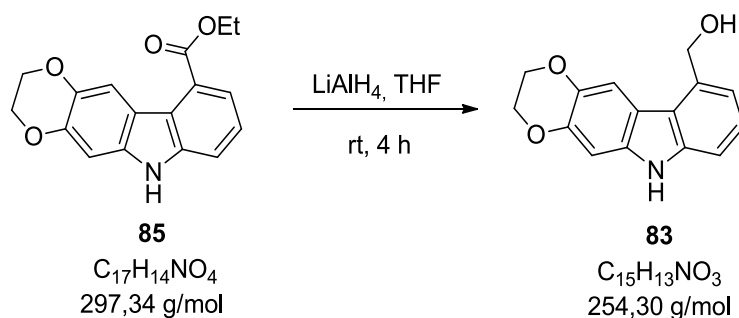
To the ester **85** (351 mg, 1.18 mmol) dissolved in THF (20 mL) was added LiAlH$_4$ (164 mg, 4.32 mmol) in a flame-dried round-bottom flask under argon. The reaction was stirred at rt for 4 h and TLC of the reaction mixture (hexane/EtOAc 8:2) indicated formation of a new compound and complete consumption of starting materials (R$_f$ 0.25). Water (30 mL) was added and the crude mixture was extracted with diethyl ether (2 x 20 mL). The combined organic phases were washed with water (20 mL), dried (Na$_2$SO$_4$) and concentrated *in vacuo.* The residue was purified by silica gel flash column chromatography (hexane/EtOAc 6:4) to afford the desired compound **83** (250 mg, 83% yield) as a colourless liquid.

Product aspect: colourless liquid.

Theoretical mass: 301 mg.

Mass obtained: 250 mg

Yield: 83%.

Analytical data:

R$_f$ = 0.20 (hexane/EtOAc 6:4).

IR (film) ν cm^{-1} : 3356 (OH, NH), 2918 (C-H), 1499, 1466 (Ar-H), 1203, 1172 (Ar-O), 1063 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.58 (bs, 1H, OH), 4.31 (s, 4H, OC$\underline{H}_2$ (x 2)), 5.02 (s, C$\underline{H}_2$-OH), 6.91 (s, 1H, H-5), 7.09 (t, *J* = 7.5 Hz, H-8), 7.15 (d, *J* = 7.5 Hz, 1H, H-7), 7.50 (s, 1H, H-11), 7.85 (d, *J* = 7.5 Hz, 1H, H-9).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 64.5 (CH$_2$, CH$_2$-O), 64.6 (CH$_2$, CH$_2$-O), 65.0 (CH$_2$, CH$_2$-O), 98.7 (CH, C-5), 107.7 (CH, C-11), 115.8 (C, C-10a), 118.8 (CH, C-7), 119.6 (CH, C-9), 122.4 (C, C-10b), 122.8 (C-10) 123.3 (CH, C-8), 123.7 (C, C-10) 135.3 (C, C-5a), 138.6 (C, C-6a), 139.3 (C, C-4a), 143.6 (C, C-11a).

Ethyl 3,6,7,8,9,10-hexahydro-2H-[1,4]dioxino[2,3-b]carbazole-10-carboxylate (84) and ethyl 3,6-dihydro-2H-[1,4]dioxino[2,3-b]carbazole-10-carboxylate (85)
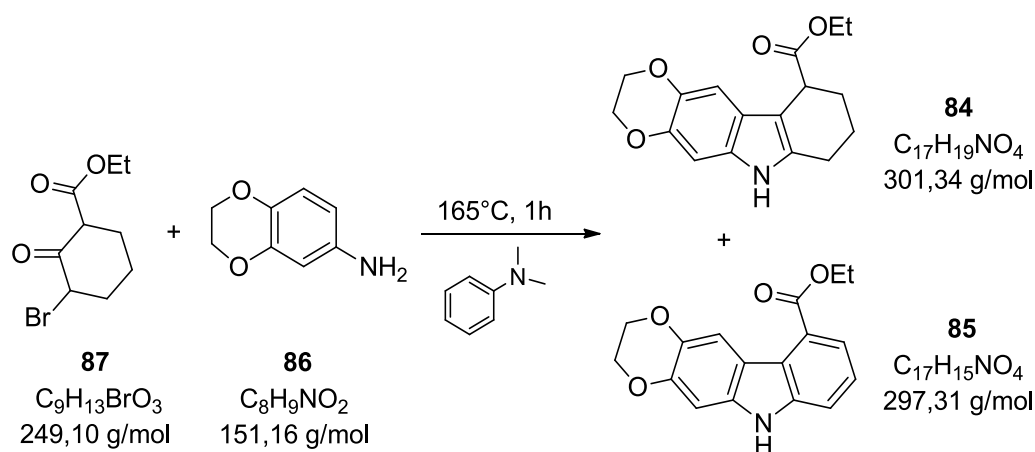
1,4-Benzodioxan-6-amine (**86**) (1,39 g, 9.176 mmol) was dissolved in *N,N*-dimethylaniline (25 mL) in a flame-dried round-bottom flask under argon and heated to 150 °C. Ethyl 3-Bromo-2-oxocyclohexanecarboxylate (**87**) (762 mg, 3.06 mmol) was slowly added and the reaction was heated to 165 °C for 1 h. Then, TLC of the crude mixture (hexane/EtOAc 7:3) indicated formation of two new compounds (R$_f$ 0.65, R$_f$ 0.55) and complete consumption of the starting materials (R$_f$ 0.20, 0.80). The crude reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with HCl 3N (4 x 50 mL). The combined aqueous phases were re-extracted with EtOAc (3 x 30 mL) and the combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 17:3) to afford the aliphatic ethyl ester **84** (206 mg, 22% yield) as a yellow oil and the aromatic ester **85** (109 mg, 12% yield) as a yellow oil. The reaction was scaled up using 1.25 g of ethyl 3-bromo-2-oxocyclohexanecarboxylate (**87**) to afford **84** (295 mg, 20% yield) as a yellow oil and **85** (185 mg, 13% yield) as a yellow oil.

Product aspect: yellow oil.

Theoretical mass: 1.46 g.

Mass obtained: 206 mg (**84**), 109 mg (**85**).

Yield: 22% (**84**), 12% (**85**).

Analytical data:

Compound 84:

R_f = 0.65 (hexane/EtOAc 7:3).

IR (film) ν cm^{-1} : 3352 (NH), 2918 (C-H), 1665 (C=O), 1595, 1496, 1467 (Ar-H), 1350-1028 (Ar-O, C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 1.30 (m, 3H, CH$_2$-CH$_3$), 1.60-2.20 (m, 4H, CH$_2$-9, CH$_2$-8), 2.62 (m, 2H, CH$_2$-7), 3.78 (m, 1H, H-10), 4.22 (q, *J* = 6 Hz, 2H, O-CH$_2$-CH$_3$), 4.25 (s, 4H, O-CH$_2$-CH$_2$-O), 6.81 (s, 1H, H-5), 6.91 (s, 1H, H-11), 8.05 (bs, 1H, NH).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 14.8 (CH$_3$, CH$_3$-CH$_2$-O), 21.6 (CH$_2$, CH$_2$-9), 22.1 (CH$_2$, CH$_2$-8), 26.2 (CH$_2$, CH$_2$-7), 40.9 (CH, CH-CO$_2$Et), 62.3 (CH$_2$, CH$_3$-CH$_2$-O), 63.8 (CH$_2$, CH$_2$-O), 64.6 (CH$_2$, CH$_2$-O), 99.0 (CH, C-5), 107.8 (CH, C-11), 111.1 (C, C-10a), 122.8 (C, C-6a), 129.9 (C, C-10b), 131.7 (C, C-5a), 139.5 (C, C-4a), 141.2 (C, C-11a), 173.4 (C, CO$_2$Et).

Compound 85:

R_f = 0.55 (hexane/EtOAc 7:3).

IR (film) ν cm^{-1} : 3420 (NH), 2900-2858 (C-H), 1682 (C=O), 1496, 1463 (Ar-H), 1273 (Ar-O), 1183, 1143, 1167 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 4.25-4.35 (s, 4H, 2 x OCH$_2$). 4.46 (q, *J* = 7.2 Hz, 2H, OCH$_2$-CH$_3$), 6.96 (s, 1H, H-5), 7.16 (t, *J* = 7.5 Hz, H-8), 7.51 (s, 1H, H-11), 7.98 (d, *J* = 7.5 Hz, 1H, H-7), 8.07 (d, *J* = 7.5 Hz, 1H, H-9).

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 14.9 (CH$_3$, OCH$_3$), 61.1 (CH$_2$, OCH$_2$), 64.5 (CH$_2$, OCH$_2$), 65.0 (CH$_2$, OCH$_2$), 99.0 (CH, H-5), 107.9 (CH, H-11), 111.8 (C, C-10b), 116.7 (C, C-5a), 118.2 (CH, H-7), 124.8 (CH, H-9), 124.9 (C, C-10), 126.4 (CH, C-8), 135.3 (C, C-5a), 139.0 (C, C-6a), 141.0 (C, C-4a), 144.1 (C, C-11a), 167.8 (C, CO$_2$Et).

Ethyl 3-bromo-2-oxocyclohexanecarboxylate (87)

88
$C_9H_{14}O_3$
170,21 g/mol

87
$C_9H_{13}BrO_3$
249,10 g/mol
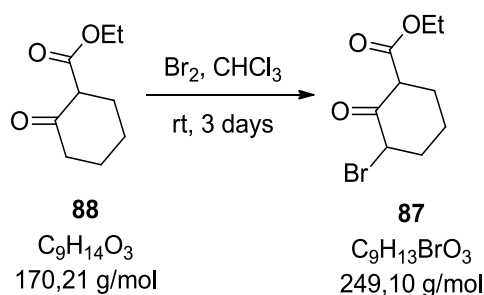
Ethyl 2-oxocyclohexanecarboxylate (**88**) (0.94 mL, 5.87 mmol) was dissolved in $CHCl_3$ (30 mL) in a flame-dried round-bottom flask under argon and cooled to 0 °C with extern bath. Br_2 (0.50 mL, 2 eq) was dissolved in $CHCl_3$ (10 mL) and added to the solution. The reaction was stirred for 72 h. TLC of the crude mixture (hexane/EtOAc 9:1) indicated no R_f modification of the new product (R_f 0.30). The crude reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL), quenched with NaOH 1N (20 mL) and washed with water (3 x 20 mL). The aqueous phase was re-extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 9:1) to afford ethyl 3-bromo-2-oxocyclohexane carboxylate (**87**) (1.45 g, 99% yield) as a colourless liquid. This material was identical in all respects with that previously described.[118]

Product aspect: colourless liquid.

Theoretical mass: 1.46 g.

Mass obtained: 1.45 g.

Yield: 99%.

Analytical data:

R_f = 0.30 (hexane/EtOAc 9:1).

RMN ^{1}H ($CDCl_3$, 300 MHz) δ (ppm): 1.29 (t, *J* = 6 Hz, 3H, C$\underline{H}_3$-CH_2-O). 1.70-2.60 (m, 6H, CH_2-4, CH_2-5, CH_2-6), 2.91 (m, 1H, H-1), 4.25 (q, *J* = 7.2 Hz 2H, CH_2-O), 4.69 (m, 1H, H-3).

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[118] A. D. Napper, J. Hixon, T. Mcdonagh, J. O. Saunders, P. S. Distefano, R. Curtis. *J. Med. Chem.* **2005**, *48*, 8045-8054.

7-Hydroxy-3-(3,4,5-trimethoxyphenyl)-chromen-2-one (105)

56
$C_{11}H_{14}O_5$
226,23 g/mol

106
$C_7H_6O_3$
138,12 g/mol

105
$C_{18}H_{16}O_6$
328,32 g/mol
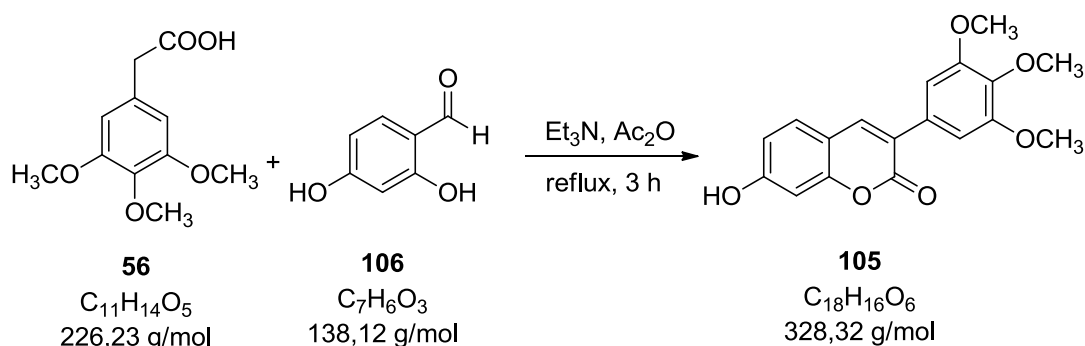
2,4-Dihydroxybenzaldehyde (**106**) (305 mg, 2.21 mmol), 2-(3,4,5-trimethoxyphenyl)-acetic acid (**56**) (500 mg, 2.21 mmol) and Et₃N (1.7 mL, 12.1 mmol) were dissolved in acetic anhydride (3 mL, 32 mmol) in a flame-dried round-bottom flask under argon. The reaction was refluxed under stirring for 3 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a green fluorescent compound (R_f 0.65) and complete consumption of starting material (R_f 0.80). CH₂Cl₂ (20 mL) was added and the crude mixture was quenched with water (30 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 30 mL) and the combined organic phases were dried (Na₂SO₄) and concentrated *in vacuo.* The crude of reaction was microdistilled at 150 °C and purified by silica gel flash column chromatography (hexane/EtOAc 8:2) to afford 7-hydroxy-3-(3,4,5-trimethoxyphenyl)-chromen-2-one (**105**) (360 mg, 47% yield) as a white solid.

Product aspect: white solid.

Theoretical mass: 767 mg.

Mass obtained: 360 mg.

Yield: 47%.

Analytical data:
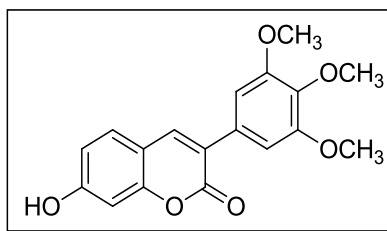
*Compound **105***

R$_f$ = 0.80 (hexane/EtOAc 8:2).

mp: 141-143 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1} : 3210 (Ar-OH), 2919 (CH), 2848 (=C-H), 1719 (C=O), 1225 (Ar-O), 1103 (C-O).

RMN ^{1}H (CDCl$_3$, 200 MHz) δ (ppm): 3.86 (s, 3H, O-CH$_3$), 3.89 (s, 6H, O-C$\underline{H}_3$ (x 2)), 6.93 (s, 2H, H-2', H-5'), 7.06 (dd, *J* = 2 Hz, *J* = 8 Hz, 1H, H-6), 7.14 (d, *J* = 2 Hz, 1H, H-8), 7.54 (d, *J* = 8.0 Hz, 1H, H-5), 7.78 (s, 1H, H-4).

RMN ^{13}C (CDCl$_3$, 50.3 MHz) δ (ppm): 57.3 (CH$_3$, OCH$_3$ (x 2)), 61.5 (CH$_3$, OCH$_3$), 103.8 (CH, C-2', C-6'), 107.5 (CH, C-8), 110.0 (CH, C-6), 126.1 (C, C-4a), 129.3 (CH, C-5), 130.94 (C, C-3), 139.1 (CH, C-4), 152.4 (C, C-4'), 153.0 (C, C-3', C-5'), 154.6 (C, C-7), 160.1 (C, C-8a), 169.3 (C, C-2).

MS EI m/z (%): 327 (M$^+$, 92).

2,4-Dihydroxybenzaldehyde (106)
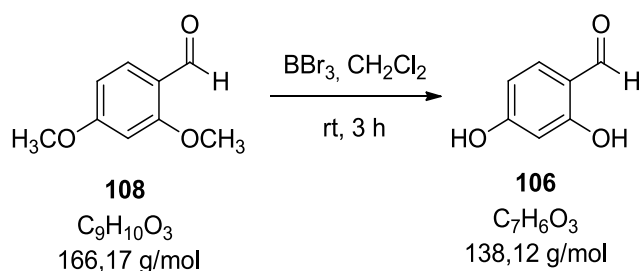
2,4-Dimethoxybenzaldehyde (**108**) (500 mg, 3.00 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) in a flame-dried round-bottom flask under argon. The reaction was cooled at -30 °C and BBr_3 (1.4 mL, 15 mmol) was added. The reaction was stirred for 10 min at -30 °C and at rt for 3 h. Then, TLC of the crude mixture (hexane/EtOAc 1:1) indicated no R_f modification of the product (R_f 0.75). The crude mixture was cooled down to 0 °C and water (30 mL) was added dropwise. The solution was let to stir at rt for 10 min. The mixture was extracted with NaOH 2N (3 x 20 mL) and the combined aqueous phases were washed with CH_2Cl_2 (3 x 20 mL). HCl 5N was added until p*H* 1 and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford 2,4-dihydroxybenzaldehyde (**106**) (397 mg, 96% yield) as a purple solid.

Product aspect: purple solid.

Theoretical mass: 414 mg.

Mass obtained: 397 mg.

Yield: 96%.

Analytical data:

R_f = 0.75 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 6.35 (d, *J* = 1.8 Hz, 1H, H-3), 6.54 (dd, *J* = 2.2 Hz, *J* = 8.6 Hz, 1H, H-5), 7.58 (d, *J* = 8.4 Hz, 1H, H-6), 9.75 (s, 1H, C<u>H</u>O).

3,4,5,2',4'-Pentamethoxystyrene (107)

109
C$_{19}$H$_{22}$O$_5$
330,37 g/mol

107
C$_{14}$H$_{12}$O$_5$
260,24 g/mol
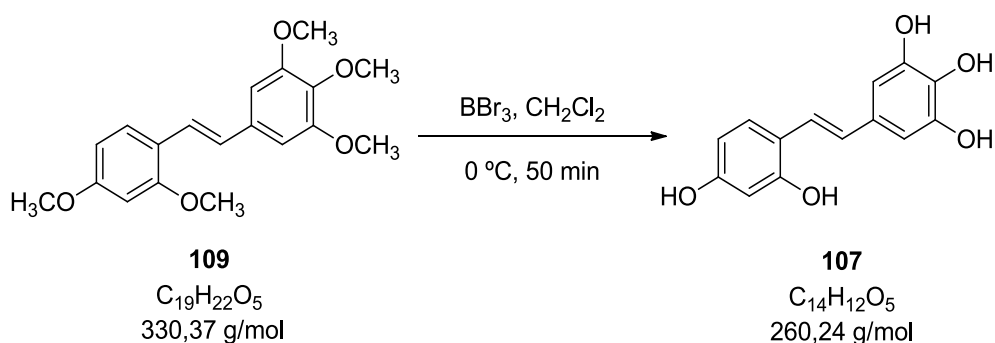
(*E*)-[1-(3,4,5-trimethoxyphenyl)-2-(2',4'dimethoxyphenyl)]ethane (**109**) (200 mg, 0.6 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (5 mL) in a flame-dried round-bottom flask under argon. The reaction was cooled at -30 °C and BBr$_3$ (0.70 mL of a 99% solution, 7.5 mmol) was added. The reaction was stirred at -30 °C for 10 min and at 0 °C for 50 min and TLC of the reaction mixture (hexane/EtOAc 2:1) indicated formation of a major compound and complete consumption of starting material **109** (R$_f$ 0.40). The crude mixture was cooled down to 0 °C, water (30 mL) was added dropwise and the solution was let to stir at rt for 10 min. Then, NaOH 5N was added until p*H* 14 and the crude mixture was washed with CH$_2$Cl$_2$ (3 x 20 mL). HCl 5N was added until p*H* 1 and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford 3,4,5,2',4'-pentamethoxystyrene (**107**) (188 mg, 98% yield) as a white solid.

Product aspect: white solid.

Theoretical mass: 795 mg.

Mass obtained: 188 mg.

Yield: 98%.

Analytical data:
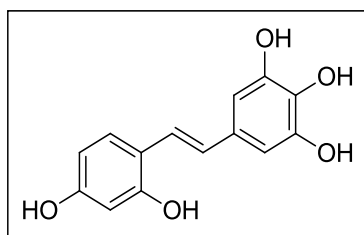
*Compound **107***

R$_f$ = 0.25 (hexane/EtOAc 1:1).

IR (film) ν cm^{-1} : 2933 (Ar-H), 2836 (=C-H), 1205 (Ar-O), 1128 (C-O).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 6.40-6.70 (m, 2H, H-5', H-3'). 6.73 (s, 2H, H-2, H-6), 7.62 (d, *J* = 12 Hz, 1H, Ar-CH=C<u>H</u>-Ar), 7.78 (d, *J* = 12 Hz, 1H, Ar-C<u>H</u>=CH-Ar), 8.37 (s, 1H, H-6').

RMN ^{13}C (CDCl$_3$, 75.5 MHz) δ (ppm): 101.7 (CH, C-3'), 106.4 (CH, C-5'), 118.2 (CH, Ar-CH=C<u>H</u>-Ar), 123.4 (CH, Ar-C<u>H</u>=CH-Ar), 125.6 (CH, C-2, C-6), 126.8 (CH, C-6'), 128.7 (C, C-OH), 134.0 (C, C-1), 137.7 (C, C-OH), 139.3 (C, C-1'), 146.5 (C, 2 x C-OH), 152.1 (C, C-OH).

HRMS -ESI m/z (%): Calculated for C$_{14}$H$_{12}$O$_5$ (M-H)$^-$: 259.0685. Found: 259.0663.

.

(*E*)-[1-(3,4,5-Trimethoxyphenyl)-2-(2,4-dimethoxyphenyl)]ethane (109)

108
C₉H₁₀O₃
166,17 g/mol

29
C₁₀H₁₂O₄
196,20 g/mol

109
C₁₉H₂₂O₅
330,37 g/mol
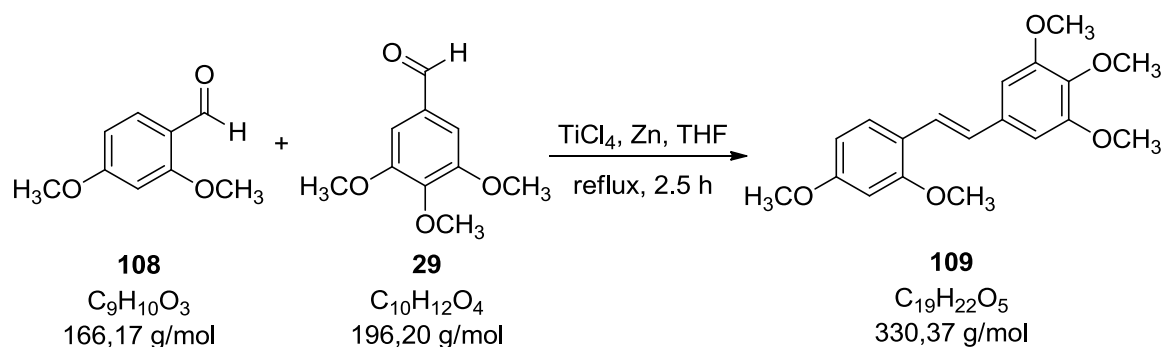
Zn (0.8 g, 12 mmol) and anhydrous THF (30 mL) were put in a three neck round-bottom flask under argon. The reaction was cooled to 0 °C and TiCl₄ (0.65 mL, 6 mmol) was added portionwise. The reaction mixture was allowed to warm up at rt for 30 min and refluxed under stirring for 2.5 h. The solution was cooled down to 0 °C. 3,4,5-trimethoxybenzaldehyde (**29**) (471 mg, 2.4 mmol) and 2,4-dimethoxybenzaldehyde (**108**) (400 mg, 2.4 mmol) were dissolved in anhydrous THF (30 mL) in a flame-dried round-bottom flask and added to the solution. The mixture was heated to reflux under stirring for 3 days and TLC of the crude reaction (EtOAc/hexane, 1:1) indicated formation of a major compound (R$_f$ 0.65) and complete consumption of SM (R$_f$ 0.85, R$_f$ 0.75). The crude mixture was quenched with NaHCO₃ (25 mL of a 10% aqueous solution) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic phases were dried (Na₂SO₄) filtered and concentrated *in* vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 7:3) to afford (*E*)-[1-(3,4,5-trimethoxyphenyl)-2-(2',4') dimethoxyphenyl)]ethene (**109**) (302 mg, 38% yield) as a white solid.

Product aspect: white solid.

Theoretical mass: 795 mg.

Mass obtained: 302 mg.

Yield: 38%.

Analytical data:
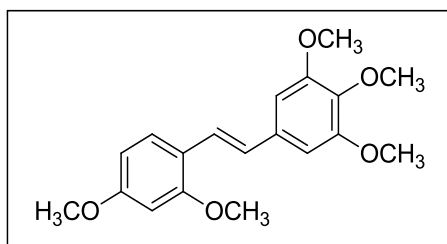
*Compound **109***

R$_f$ = 0.75 (hexane/EtOAc 1:1).

mp: 115-117 ^{o}C (hexane/EtOAc).

IR (film) ν cm^{-1}: 2933 (Ar-H), 2836 (=C-H), 1205 (Ar-O), 1128 (C-O).

RMN ^{1}H (Acetone-*d6*, 200 MHz) δ **(ppm):** 3.84 (s, 3H, C<u>H</u>$_3$-O), 3.88 (s, 3H, C<u>H</u>$_3$-O), 3.88 (s, 3H, C<u>H</u>$_3$-O), 3.91 (s, 6H, CH, C<u>H</u>$_3$-O x 2), 6.49 (d, *J* = 2.4 Hz, 1H, H-3), 6.51 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H, H-5), 6.73 (s, 2H, H-2', H-6'), 6.94 (d, *J* = 16.4 Hz, 1H, Ar-CH=C<u>H</u>-Ar), 7.28 (d, *J* = 16.4 Hz, 1H, Ar-C<u>H</u>=CH-Ar), 7.49 (d, *J* = 8.4 Hz, 1H, H-6).

RMN ^{13}C (Acetone-*d6*, 50 MHz) δ **(ppm):** 55.8 (CH$_3$, OCH$_3$ (x 2)), 56.4 (CH$_3$, OCH$_3$ (x 2)), 61.2 (CH$_3$, OCH$_3$), 98.8 (CH, C-3), 103.7 (CH, C-2', C-6'), 105.3 (CH, C-5), 119.7 (C, C-1), 123.2 (CH, Ar-CH=<u>C</u>H-Ar), 127.3 (CH, Ar-<u>C</u>H=CH-Ar), 127.6 (CH, C-6), 134.5 (C, C-1'), 137.8 (C, C-4'), 153.6 (C, C-3', C-5'), 158.3 (C, C-2), 160.9 (C, C-4).

2-Chloro-1-(3,4-dimethoxyphenyl)ethanone (120)
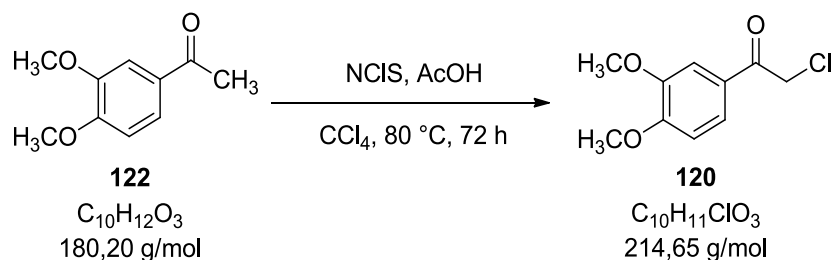
To 3,4-dimethoxy-acetophenone (**122**) (200 mg, 1.1 mmol) dissolved in CCl₄ (10 mL) was added NClS (225 mg, 1.65 mmol) and AcOH (63 µL, 1.1 mmol) in a flame-dried round-bottom flask under argon. The reaction was refluxed under stirring for 92 h and TLC of the crude mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R_f 0.75) and complete consumption of starting materials (R_f 0.65). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL), washed with a saturated solution of $NaHCO_3$ (3 x 50 mL) and the aqueous phases were re-extracted with CH_2Cl_2 (30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 7:3) affording the desired compound **120** (230 mg, 99% yield) as a pale brown solid.

Product aspect: pale brown solid.

Theoretical mass: 232 mg.

Mass obtained: 230 mg.

Yield: 99%.

Analytical data:

R_f = 0.75 (hexane/EtOAc 1:1).

RMN ¹H (CDCl₃, 300 MHz) δ (ppm): 3.95 (s, 3H, O-CH₃), 3.97 (s, 3H, O-CH₃), 4.67 (s, 2H, CO-C$\underline{H}_2$-Cl), 6.91 (d, *J* = 8.4 Hz, 1H, H-5), 7.53 (d, *J* = 2.1 Hz, 1H, H-2), 7.58 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H, H-6).

2-Bromo-1-(3,4-dimethoxyphenyl)ethanone (121)

122
$C_{10}H_{12}O_3$
180,20 g/mol

121
$C_{10}H_{11}BrO_3$
259,10 g/mol
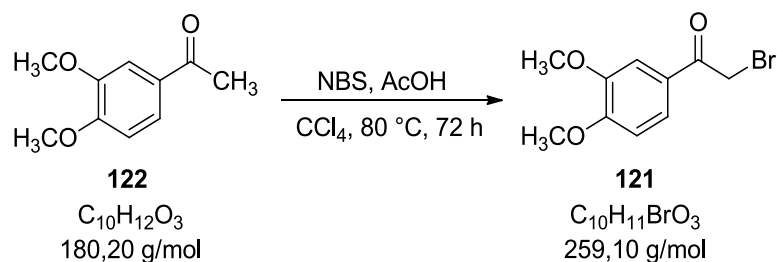
To 3,4-dimethoxyacetophenone (**122**) (2 g, 11 mmol) dissolved in CCl$_4$ (30 mL) was added NBS (7,2 g, 40 mmol) and AcOH (2.28 mL, 40 mmol) in a flame-dried round-bottom flask under argon. The reaction was refluxed under stirring for 72 h and TLC of the reaction mixture (hexane/EtOAc 1:1) indicated formation of a new compound (R$_f$ 0.70) and uncomplete consumption of the starting material (R$_f$ 0.65). The crude mixture was concentrated *in vacuo*. The residue was dissolved in CH$_2$Cl$_2$ (50 mL), washed with a saturated solution of NaHCO$_3$ (3 x 50 mL) and the aqueous phases were re-extracted with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (hexane/EtOAc 7:3) affording the desired compound **121** (1.15 g, 40% yield) as a pale brown solid.

Product aspect: pale brown solid.

Theoretical mass: 2.88 g.

Mass obtained: 1.15 g.

Yield: 40%.

Analytical data:

R$_f$ = 0.70 (hexane/EtOAc 1:1).

RMN ^{1}H (CDCl$_3$, 300 MHz) δ (ppm): 3.94 (s, 3H, OCH$_3$), 3.96 (s, 3H, OCH$_3$), 4.67 (s, 2H, CH$_2$-Br), 6.90 (d, *J* = 8.4 Hz, 1H, H-5), 7.53 (d, *J* = 2.0, 1H, H-2), 7.56 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H, H-6).

1. Of the 3 studied methods for the preparation of the arylethylamine **28**, the more practical and efficient consists in the double alkylation of the 2,3-dihydro-[1,4]-benzodioxin-6-carbaldehyde (**40**) with 1,2-dibromoethane and K_2CO_3, followed by the treatment with nitromethane in the presence of ammonium acetate and reduction with $LiAlH_4$ of the nitro group. The overall yield is 34%. The best method for the preparation of the isoquinoline **3** begins with amination of the 3,4,5-trimethoxibenzaldehyde (**29**) with the arylethylamine **28** in refluxing benzene, followed by the treatment with H_3PO_4 in 32% yield. The tetrahydroisoquinolines **1, 4, 5, 6, 7** were obtained by alkylation of the tetrahydroisoquinoline **3** with the corresponding alkyle halide in moderate yield, while **8** and **9** were obtained by treatment of the corresponding arylbromide in *cross-coupling* conditions in acceptable yields.

2. The dimethoxytetrahydroisoquinoline intermediate **49** was prepared by amination of the 3,4,5-trimethoxybenzaldehyde (**29**) by treatment with the 2-(3,4-dimethoxyphenyl)ethylamine (**50**) in the presence of PTSA, followed by cyclization of the resulting imine using a trifluoroacetic acid and trifluoroacetic anhydride mixture and hydrolysis of the trifluoroacetamide **51** with NaOH (overall yield 32%). The dimethoxytetrahydroisoquinoline **10** was obtained by alkylation of the tetrahydroisoquinoline **49** with 2-chloroethanol, while the dimethoxytetrahydroisoquinoline **11** was obtained by alkylation of **49** with (±)-epichlorhydrin, followed by hydrolysis with NaOH in moderate yield.

3. The intermediate vinyl ester **54** was synthesized by benzylation of the 3-hydroxy-4-methoxybenzaldehyde (**58**) with benzyl bromide followed by treatment with 3,4,5-trimethoxyphenylacetic acid (**56**) under *Perkin* conditions, and finally the alkylation of the carboxylic acid **53** with iodomethane and K_2CO_3 in 24% overall yield of 3 steps. The same reaction using 4-(methylsulfonyl)phenylacetic acid (**60**) or 4-(thiomethyl)phenylacetic acid (**61**) instead of 3,4,5-trimetoxyphenylacetic acid (**29**) was attempted. The difficulties encountered in this synthesis suggest that *para* substituted sulfone or methylsufonyl groups decrease dramatically the reactivity of the corresponding carboxylic acid.

5. The estilbene derivative **55a** was prepared by decarboxylation of the carboxylic acid **53** in high yield. Various reaction conditions were investigated for the cyclopropanation and epoxidation of the estilbene derivative **55a**. The negative results in most cases emphasize the difficulty of functionalization of the corresponding olefin. The lack of stability of the desired products is underlined in the case of the epoxides.

6. The pyrazolone **16** was obtained by treatment of the carboxylic acid **53** or the ester **54** with hydrazine hydrate at reflux temperature. The catalytic hydrogenation of **16** in atmospheric pressure yielded the *O*-debenzylated pyrazolone **17** in low yields. The azoledione **15**, structuraly related to the *combretastatin* A-4, was prepared by condensation of the glyoxylic aryl acid **80** with the substituted benzaldehyde **81** in presence of acetic anhydride, followed by the formation of the imine intermediate **14** by treatment of the corresponding anhydride with ammonium acetate and the debenzylation of **14** by hydrogenation over palladized charcoal in dry ethyl acetate.

7. The dioxancarbazoles **21** and **22** were synthesized from the aniline **86** and the bromoketoester **87** under *Bishler* reaction conditions, followed by the reduction of the dioxancarbazole ester **84** and **85** respectively with LiAlH$_4$ in good yields. The alcohol **82** obtained reacted with the isocyanate **96** in presence of Et$_3$N forming **21** in low yield. Similary, the reaction between the dioxancarbazole-2-ethanol **83** and the isocyanate **95** led to **22** in good yield. The substitution of the alcohol group of the dioxancarbazoles **82** and **83** with a bromine, chlorine or a triflate resulted in the elimination of the corresponding leaving group into the unsaturated by-products **100** or **104**.

8. The furan **23** was synthesized by condensation of 3,4,5-trimethoxyphenylacetic acid (**56**) with 2,4-dihydroxybenzaldehyde (**106**), Ac$_2$O and Et$_3$N followed by the hydrolysis of the corresponding lactone **105** with HCl 2N and treatment with BBr$_3$ in moderate yield. The indole **21** was prepared by a modified *Bischler* reaction conditions from the 3-benzyloxyaniline **112** and 2-bromo-1-(2,5-dimethoxyphenyl)ethanone **123**, followed by a hydrogenation reaction catalyzed by Pd/C in moderate yield. The same modified *Bischler* reaction conditions using 2-chloro-1-(3,4-dimethoxyphenyl)ethanone (**120**) or

2-bromo-1-(3,4-dimethoxyphenyl)ethanone (**121**) was attempted. The negative results emphasize the fact that this reaction does not occur with hindered α-halogenated ketones.

9. The compound with the major cytotoxic activity was the pyrazolone **17**. This compound shows a high anti-angiogenesis and anti-osteoporosis activity both related with the K-Ras inhibition (HCT-KRASSL 100% inhib. At 20 μM). The *O*-benzylated pyrazolone **16** presents less K-Ras inhibitory effect than the pyrazolone **17** (a >50% decrease) and its anti-angiogenesis properties were reduced more than 50 times (IC_{50} (16) > 10 μM; IC_{50} (17) = 0.507 μM). Compound **17** showed a significant G_2M cell cycle arrest concretely at the subphase 4N, as expected for anti-angiogenesis agents. Compound **17** is a suitable scaffold to optimize in order to achieve the desired profile. It displayed favorable *in vitro* pharmacological profiles that warrant further investigation.

10. A short series of isoquinolines was readily prepared by our improved synthetic route. Most of the *N*-substituted isoquinolines displayed K-Ras inhibition, but the aminoethyl and the aryl substituents (compounds **5**, **8** and **9**) led to a novel class of K-Ras inhibitors possessing high inhibitory potency. Compound **9** showed the most potent enzyme activity (RKO KRASSL 95.8% inhib. At 0.2 μM).

7. References

[1] http://www.cancerresearchuk.org/cancer-help/about-cancer/.

[2] http://www.cancer.gov/cancertopics/types/alphalist.

[3] P. Anand, A. B. Kunnumakkara. *Pharm. Res*. **2008**, *25*, 2097-2116.

[4] L. H. Kushi, T. Byers, C. Doyle. *Cancer. J. Clin.* **2006**, *56*, 254-281.

[5] H. Brenner, D. Rothenbacher, V. Arndt. *Epidemiology of stomach cancer*. Edited by Mukesh Verna, Humana press. **2009**, *472*, 467-477.

[6] I. Lee, Y. Oguma, D. Schottenfeld, J. F. Fraumeni. *Cancer Epidemiology and Prevention*. 3rd ed. New York Oxford University Press **2006**. Chap. 1.

[7] F. Bianchini, R. Kaaks, H. Vainio. *IARC Handbooks of Cancer Prevention* **2002**, *6*, 5-8.

[8] H. K. Biesalski, B. Bueno de Mesquita, A. Chesson. *Cancer J. Clin.* **1998**, *3*, 167-176.

[9] S. Dubey, C. A. Powell. *Am. J. Respir. Crit. Care Med*. **2007**, *9*, 941-946.

[10] H. Kuper, P. Boffetta, H. Adami. *J. Int. Med.* **2002**, *3*, 206-224.

[11] H. Kuper, P. Boffetta, H. Adami. *J. Int. Med.* **2002**, *6*, 455-466.

[12] A. Benedetti, M. Parent, J. Siemiatycki. *Canc. Det. Prev.* **2009**, *32*, 352-362.

[13] P. Boffetta, M. Hashibe, C. La Vecchia, W. Zatonski, J. Rehm. *Int. J. Cancer* **2006***, 4*, 884-887.

[14] *WHO calls for prevention of cancer through healthy workplaces*. World Health Organization **2007**.

[15] H. Zur Hausen. *Science* **1991**, *5035*, 1167-1173.

[16] S. Peter, C. Beglinger. *Digestion* **2007**, *1*, 25-35.

[17] C. Wang, Y. Yuan, R. Hunt. *Gastroenterol.* **2007**, *8*, 1789-1798.

[18] R. C. Bast, D. W. Kufe, R. E. Pollock. *Cancer Med.* **2000**.

[19] R. C. Bast, D. W. Kufe, R. Weichselbaum, J. F. Holland, E. Frei. *Holland-Frei Cancer. Med*. Edited by BC Decker. 5th edition. **2003**. Chap 14.

[20] http://www.ehrs.upenn.edu/programs/radiation/nonionizing_faq.html.

[21] http://www.mdanderson.org/patient-and-cancer-information/cancer-information/cancer-topics/prevention-and-screening/hereditary-cancer-syndromes/index.html.

[22] D. H. Roukos. *Expert. Rev. Anticanc. Therap.* **2009**, *4*, 389-392.

[23]http://www.sekmchd.org/cms/Services/ChronicDiseaseControl/Cancer/PancreaticCancerFAQs/tabid/1340/Default.aspx

[24] A. G. Knudson. *Nat. Rev.* **2001**, *2*, 157-162.

[25] C. M. Croce. *New. Eng. J. Med.* **2008**, *5*, 502-511.

[26] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, D. Forman. *A Cancer. J. Clinic.* **2011**, 61-65.

[27] A. Jemal, R. Siegel, E. Ward. *Cancer. J. Clin.* **2008**, *2*, 71-96.

[28] P. Kaatsch. *Cancer Treat. Rev.* **2010**, *4*, 277-285.

[29] W. Kufe, M. D. Raphael, E. Pollock, R. Weichselbaum, M. D. R. Bast, M. D. Gansler, J. Holland, E. Frei. Holland-Frei Cancer Med, 6th edition. **2003**, Chap.40.

[30] W. Kufe, M. D. Raphael, E. Pollock, R. Weichselbaum, M. D. R. Bast, M. D. Gansler, J. Holland, E. Frei. Holland-Frei Cancer Med, 6th edition. **2003**, Chap.41.

[31] http://www.cancerresearchuk.org/cancer-help/about-cancer/ 28/04/2013.

[32] H. J. Droogendijk, H. J. Klin-Nelemons, A. P. Orange. *Cancer* **2000**, *24*, 935-942.

[33] A. D. Richards, A. Rodgers. *Chem. Soc. Rev*. **2007**, *36*, 471-483.

[34] M. Stiborova, J. Sejbal, L. Borek-Dohalska, D. Aimova, J. Poljakova, K. Forsterova, M. Rupertova, J. Wiesner, J. Hudecek, M. Wiessler, E. Frei. *Canc. Res.* **2004**, *64*, 8374-8380.

[35] http://helicase.pbworks.com/w/page/17605643/Justin-Neese (28-04-2013).

[36] J. M. Berger, S. J. Gamlin, S. C. Harrison, J. C. Wong. *Nature* **1996**, *379*, 225-232.

[37] J. M. Berger. *Curr. Opin. Struct. Biol*. **1998**, *8*, 26-32.

[38] K. R. Hande. *Eur. J. Cancer* **1998***, 34*, 1514-1521.

[39] J. Lei, H. Jiege. *J. Mol. Onc.* **2012**, *20*, 643-645.

[40] Y. Wang, L. Zhu, C. Liu, L. Liu, S. Ye, M. Wu, Y. Liu, D. Zhongshan, K. Yixue. *J. Sun Yat-sen Univ*. **2012**, *33*, 8-15. DOI: 158:234030.

[41] A. M. Petros, A. Medek. *Proc. Natl. Acad. Sci.* **2001**, *98*, 3012-3017.

[42] N. Liu, H. Jiang, A. Ben-Shlomo, K. Wawrowsky, X. Fan, S. Lin, S. Melmed. *Natl. Acad. Sci. USA*. **2011**, *108*, 8414-8419.

[43] C. W. Reuter, M. A. Morgan, L. Bergmann. *Blood* **1996**, *5*, 1655-1669.

[44] G. Anuj, R. Agrawal, R. Rakesh. *Farnesyltransferase Inhibitor in Cancer Treatment, Current Cancer Treatment*. Edited by Öner Özdemir. **2011**, 150-160.

[45] J. A. Sparano, S. Moulder, A. Kazi. *Clin. Cancer. Res.* **2009**, *15*, 2942-2948.

[46] R. Airley. *Cancer Chemotherapy, Basic Science to the Clinic*. Edited by Wiley-Blackwell. **2009**, 199-209.

[47] E. C. Hayden. *Nature* **2009**, *458,* 686-687.

[48] H. S. Kim. *Cytogenet. Cell Genet.* **1998**, *83*, 1-2.

[49] S. M. Wilhelm, L. Adnane, P. Newell, A. Villanueva, J. M. Llovet, M. Lynch. *Mol. Canc. Ther.* **2008**, *10*, 3129-3140.

[50] R. J. Amato, M. S. Loughnan, E. Flynn, J. Folhman. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 4082-4085.

[51] *deaoptcancer.ptt.* presentation from M. D. Pujol group **2007**.

[52] A. S. Capilla, M. Romero, M. D. Pujol, D. H. Caignard and P. Renard. *Tetrahedron* **2001**, *57*, 8297-8303.

[53] K. Ohsumi, T. Hatanaka, K. Fujita, R. Nakagawa, Y. Fukuda, Y. Nihei, Y. Suga, Y. Morinaga, Y. Akiyama, T. Tsuji. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153-3158.

[54] J. Lei, H. Jiege. *J. Mod. Oncol*. **2012**, *20*, 643-645.

[55] D. Simoni, R. Romagnoli, R. Baruchello, R. Rondanin, M. Rizzi, M. G. Pavani, D. Alloatti, G. Giannini, M. Marcellini, T. Riccioni, M. Castorina, M. B. Guglielmi, F. Bucci, P. Carminati, C. Pisano. *J. Med. Chem.* **2006**, *49*, 3143-3152.

[56] Sergi Capilla Mies. *Estudi sintètíc i estructural de nous sistemes policíclics derivats de la podofil.lotoxina que contenen el nucli de la 2,3-dihidro-1,4-benzodioxina. Agents amb potencial activitat antitumoral.* Tesi doctoral, **1998**, Facultad de Farmacia. Universidad de Barcelona.

[57] María Teresa Vázquez Fernández, *Estudio de estrategias sintéticas para la preparación de nuevos compuestos antiinflamatorios y antitumorales que contienen el nucleo de 2,3-dihidro-2,4-benzodioxino como subestructura*. Tesis doctoral, **1997**, Facultad de Farmacia. Universidad de Barcelona.

[58] Y. Tachibana, H. Kikuzaki, N. H. Lafis, N. Naketami. *J. Agric. Food Chem*. **2001**, *49*, 5589-5594.

[59] Núria Mur Blanch. *Disseny I síntesis de nous compostos de naturalesa heterocíclica amb potencial activitat anticancerígena. Síntesi de nous inhibidors de CDKS.* Tesis doctoral, **2011**, Facultad de Farmacia. Universidad de Barcelona.

[60] H. C. Hansen, F. S. Chiacchia, R. Patel, N. C. W. Wong, V. Khlebnikov, R. Jankowska, K. Patel, M. M. Reddy. *J. Org. Chem*. **2010**, *45*, 2018-2023.

[61] J. Lu, C. T. Ho, G. Ghai, K. Y. Chen. *Carcinogenesis* **2001**, *22,* 321-328.

[62] G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J, M, Schmidt, F. Hogan, *J. Med. Chem*. **1995**, *38*, 1666-1672.

[63] G. R. Pettit, A. Thornhill, N. Melody, J. C. Knight. *J. Nat. Prod*. **2009**, *72*, 380-388.

[64] A. Katritzky, C. Ramsden, J. Joule, V. Zhdankin. *Handbook of heterocyclic Chemistry*. 3rd edition. Edited by Elsevier. **2010**.

[65] M. G. Valverde, T. Torroba. *Molecules* **2005**, *10*, 318-320.

[66] R. Faust, P. J. Garratt, M. A. T. Pérez, V. J. D. Piccio. C, Madsen, A. Stenstrom, B. Frolund, K. Davidson, K. Teh, D. Sugden. *Bioorg. Med. Chem.* **2007**, *15*, 4543-4551.

[67] Taylor, W. I. *Helv. Chim. Acta*. **1950**, *33*, 164-168.

[68] J. S. Carey, D. Laflan, C. Thomson, M. T. Williams. *Org. Biomol. Chem.* **2006**, *4*, 2337-2347.

[69] J. J. Lee, Name reactions: A collection of Detailled mechanisms and Synthetic Applications. 4th edition. Edited by Springer. **2007**, 48-49.

[70] Manel Romero i Balaguer, *Preparació de nous agents antitumorals. Síntesi I avaluació citotoxica de sistemes políciclics que contenen el nucli d'1,4-benzodioxina.* Tesis doctoral, **2001**, Facultad de Farmacia. Universidad de Barcelona.

[71] Y. Harrak, M. Romero, P. Constans, M. D. Pujol. *Lett. Org. Chem*. **2006**, *3*, 29-35.

[72] M. Romero, Y. Harrak, J. Basset, L. Ginet, P. Constans, M. D. Pujol. *Tetrahedron* **2006**, *60*, 9010-9016.

[73] K. C. Sanko, L. Illes, K. Felfoeldi, J. Kiss, P. Sipos, I. Palinko. *J. Mol. Struct.* **2011**, *993*, 259-263.

[74] A. Khalaf, I. M. Awad, I. M. El-Emary, T. I. Abd El-Aal. *J. Ind. Chem. Soc.* **2010**, *87*, 595-600.

[75] G. R. Pettit, C. R. Anderson, D. L. Herald, M. K. Jung, D. J. Lee. *J. Med. Chem.* **2003**, *46*, 525-531.

[76] H. E. Simmons, D. S. Ronald. *J. Chem. Soc*. **1959**, *81*, 4256-4264.

[77] G. Chen, S. J. Cho, X. Huang, N. H. Jensen, A. Svennebring, M. F. Sassano, B. L. Roth, A. P. Kozikowski. *ACS Med. Chem. Lett*. **2011**, 929-932.

[78] A. G. Whittaker, D. M. P. Mingos. *J. Chem. Soc*. **2002**, 3967-3970.

[79] C. Leslie, D. J. Salmon, W. Donald. *J. Chem. Soc*. **1971**, *2*, 304-312.

[80] F. D. Ozdermirhan, M. Celik, S. Ath, C. Tanyeli. *Tetrahedron* **2006**, *17*, 287-291.

[81] M. M. Diaz-Requejo, A. Caballero, T. R. Belderrain, M. C. Nicasio, S. Trofimenko, P. J. Pérez. *J. Am. Chem. Soc*. **2002**, *124*, 978-983.

[82] K. Shanmugan, T. Balakrishan. *Ind. J. Org. Chem*. **2007**, *7*, 1069-1074.

[83] J. D. Clark, A. S. Shah, J. C. Peterson. *Thermochim. Acta* **2002**. 177-186.

[84] C. Dallanoce, P. Magrone, P. Bazza, G. Grazioso, L. Rizzi, L. Riganti, C. Gotti, F. Clementi, K. Frydenvang, M. Amici. *Chem. Biodiversity* **2009**, *6*, 244-259.

[85] J. L. Garcia Ruano, C. Fajardo, A. Fraile, M. Rosario Martin. *J. Org. Chem*. **2005**, *70*, 4300-4306.

[86] M. F. Braña, A. Gradillas, A. G. Ovalles, B. Lopez, N. Acero, F. Llinares, D. M. Mingarro. *Bioorg. Med. Chem.* **2011**, *14*, 9-16.

[87] B. Burja, M. Kocevar, S. Polanc. *Tetrahedron* **2009**, *65*, 8690-8696.

[88] A. A. B. Robertson, N. P. Botting. *Tetrahedron* **1999**, 13269-13284.

[89] M. Gucma, W. M. Golebiewski, B. Morytz, H. Charville, A. Whitting. *Lett. Org. Chem.* **2010**, *7*, 502-507.

[90] A. Lapworth. *J. Chem. Soc.* **1904**, *85*, 1206-14.

[91] J. H. Biel, E. P. Sprengeler, H. A. Leiser, J. Horner, A. Drukker, H. L. Friedman, *J. Am. Chem. Soc*, **1955,** *77*, 2250-2256.

[92] Ruben Francisco Castillo. *Síntesis de maleimidas disubstituidas con potencial actividad antiangiogénica.* Tesis doctoral en curso*,* **2013**, Facultad de Farmacia. Universidad de Barcelona.

[93] G. Fodor, S. Nagubendi. *Tetrahedron* **1980**, *36*, 1279-1300.

[94] E. Campaigne, R. D. Lake. *J. Org. Chem*. **1959**, *24*, 478-487

[95] M. Romero, M. D. Pujol. **2013**. Unpublished results.

[96] W. H. Perkin. *J. Chem. Soc.* **1868**, *21*, 181-184.

[97] J. E. Mc. Murry. *J. Am. Chem. Soc*. **1974**, *96*, 4708-4709.

[98] A. F. Crowther, F. G. Mann, D. Purdie. *J. Chem. Soc.* **1943**, *4*, 28-31

[99] D. A. Chan, A. J. Giaccia. *Nat. Rev. Drug. Discov*. **2011**, *10*, 351-364.

[100] Cetuximab (Erbitux) and Ponitumab (Vectibix). U.S. *Food and Drug Administration* **2010-01-11**

[101] https://openinnovation.lilly.com/dd/about-open-innovation/resources-links.html.

[102] D. Hanahan, R. A. Weinberg. *Cell* **2011**, *144*, 646-674.

[103] D. Lu, J. Brol. *Chem*. **2003**, 278, 43496-43507.

[104] H. Youssoufian. *Clin. Canc. Res*. **2007**, *13*, 55445-55485.

[105] K. M. Cook, W. D. Figg. *Cancer. J. Clin.* **2010,** *60*, 222-243.

[106] Y. Matsuo, P. M. Campbell, R. A. Breckken, B. Sung, M. M. Ouellette, J. B. Flemming, B. B. Aggarwal, C. J. Der, S. Guha. *Mol. Canc. Res.* **2009**, *7*, 799-808.

[107] Y. Chen, B. A. Alman. *J. Cell. Biochem*. **2009**, *106*, 353-362.

[108] H. L. Lin, S. H. Chiou, C. W. Wu, W. B. Lin, L. H. Shen, Y. P. Yang, M. L. Tsai, Y. H. Uen, J. P. Liou, C. W. Chi. *J.P.E.T*. **2007**, *323*, 365-373.

[109] Arturo Vinuesa Hernando. *Diseño y Síntesis de Nuevas Purinas 6,9-disustituidas con potencial actividad antitumoral por inhibición de CDKs*. Master experimental, **2012**, Facultad de Farmacia. Universidad de Barcelona.

[110] A. Bishayee. *Cancer Prev. Res*. **2009**, *2*, 409-418.

[111] K. Dungan, J. B. Buse. *Clinical Diabetes* **2011**, *23*, 56-62.

[112] H. A. Overton. *Cell. Metab*. **2006**, *3*, 167-175.

[113] H. A. Overton, M. C. Fyle, C. Reynet. *Br. J. Pharmacol*. **2007**, *153*, 576-581.

[114] A. Recobera, A. F. Russob. *Curr. Opin. Neurol.* **2009**, *22*, 241-246.

[115] J. M. Witkin, W. J. A. Eiler. *Drug Dev. Res.* **2007**, *5*, 187-194.

[116] K. Tatemoto, M. Hosoya, Y. Habata, Fujii. R. T. Kakegawa, M. X. Zou, Y. Kawamata, S. Fukusumi, S. Hinuma, C. Kitada, T. Kurokawa, H. Onda, M. Fujino. *Biochem. Biophys. Res. Commun*. **1998**, *251*, 471-476.

[117] H. Osawa, C. Sutherland, R. B. Robey, R. L. Printz, D. K. Granner. *J. Biol. Chem*. **1996**, *271*, 16690-16694.

[118] A. D. Napper, J. Hixon, T. Mcdonagh, J. O. Saunders, P. S. Distefano, R. Curtis. *J. Med. Chem.* **2005**, *48*, 8045-8054

8. Appendix

RMN ^{1}H (300 MHz, CDCl$_3$) of compound 1
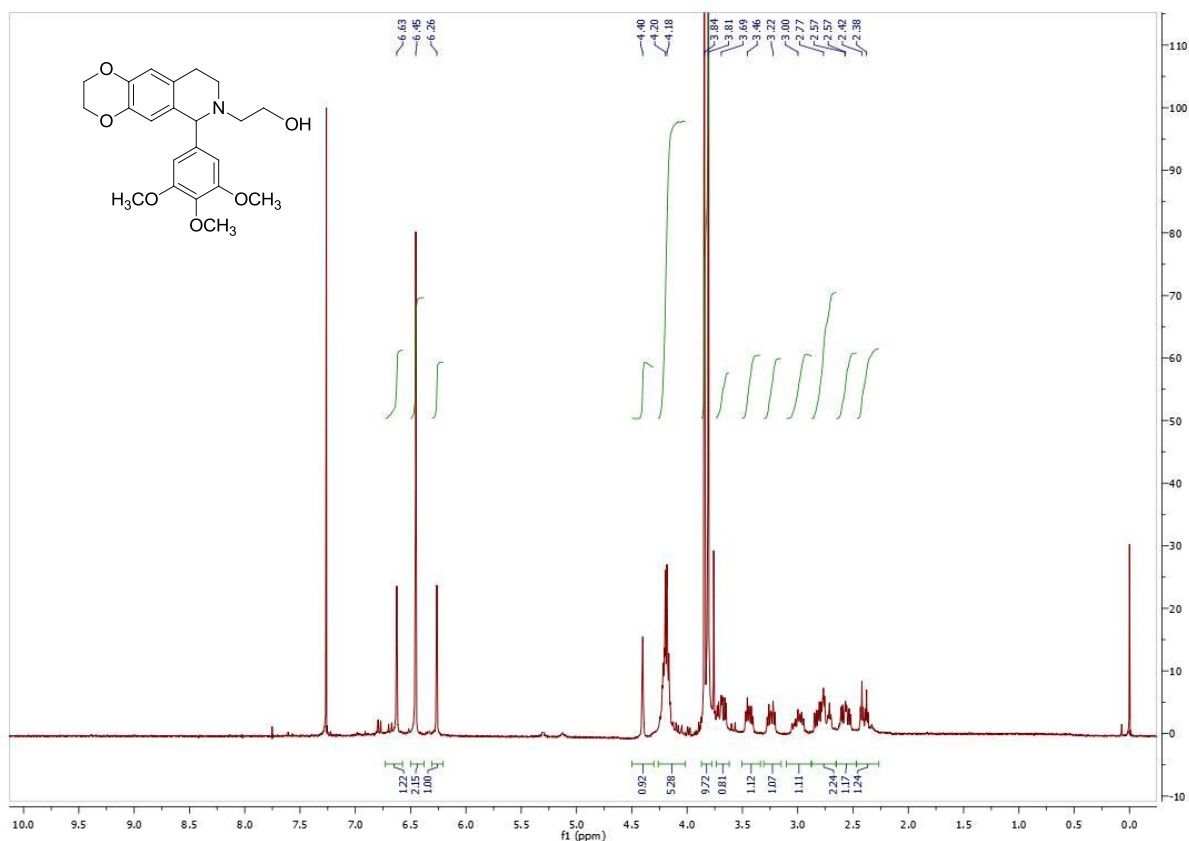
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 1
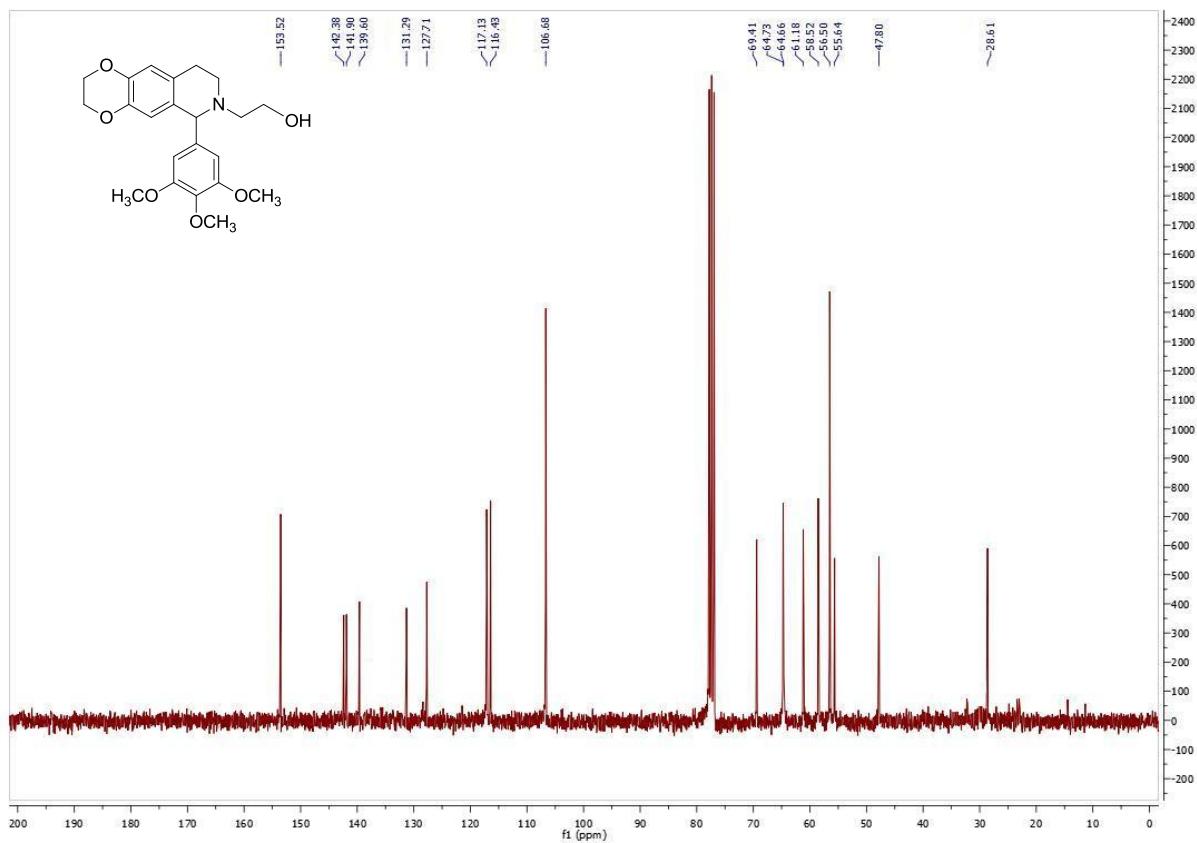
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 4
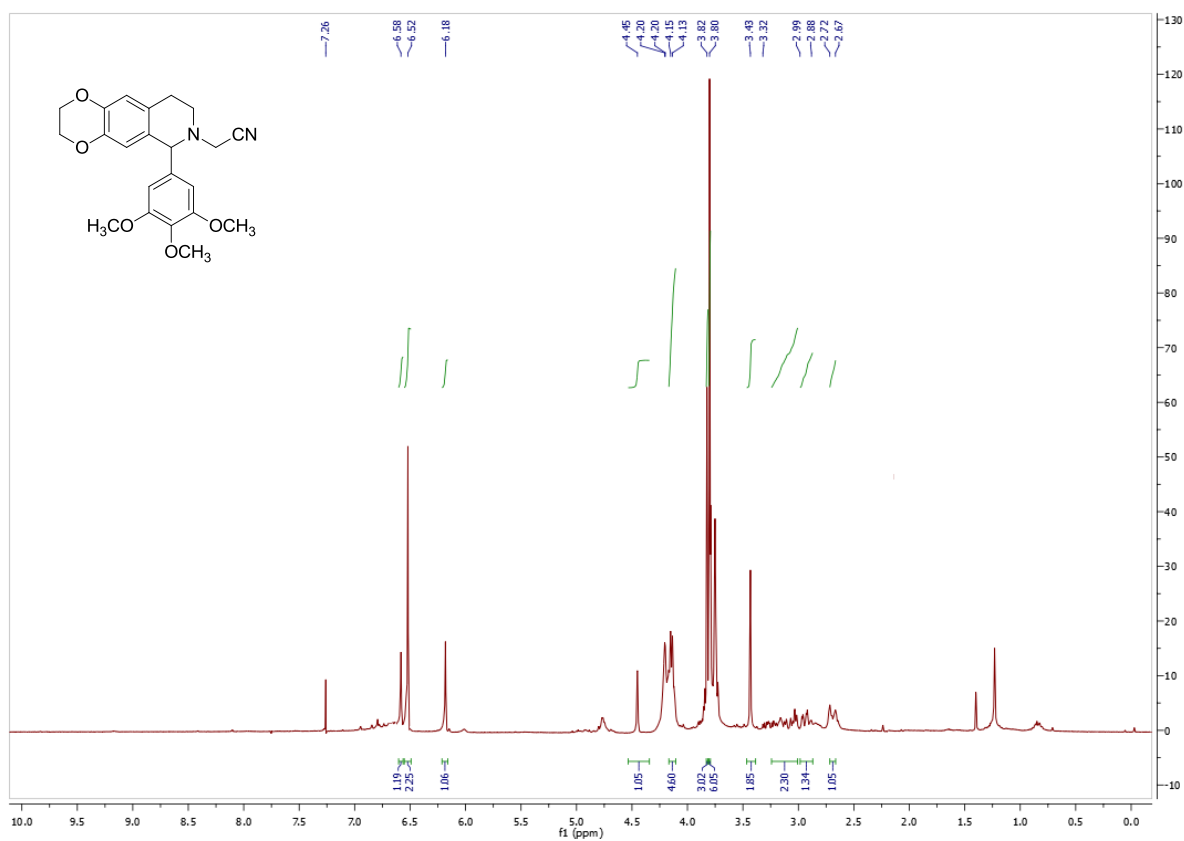
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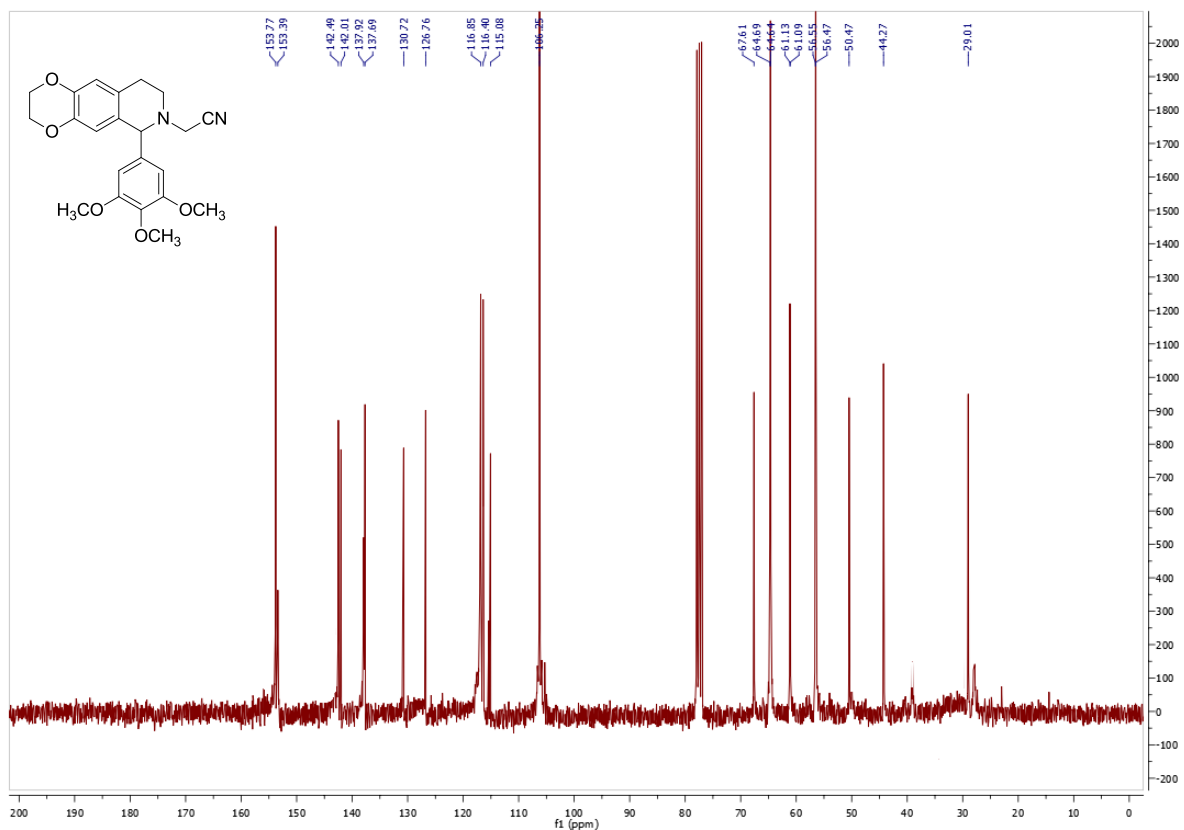
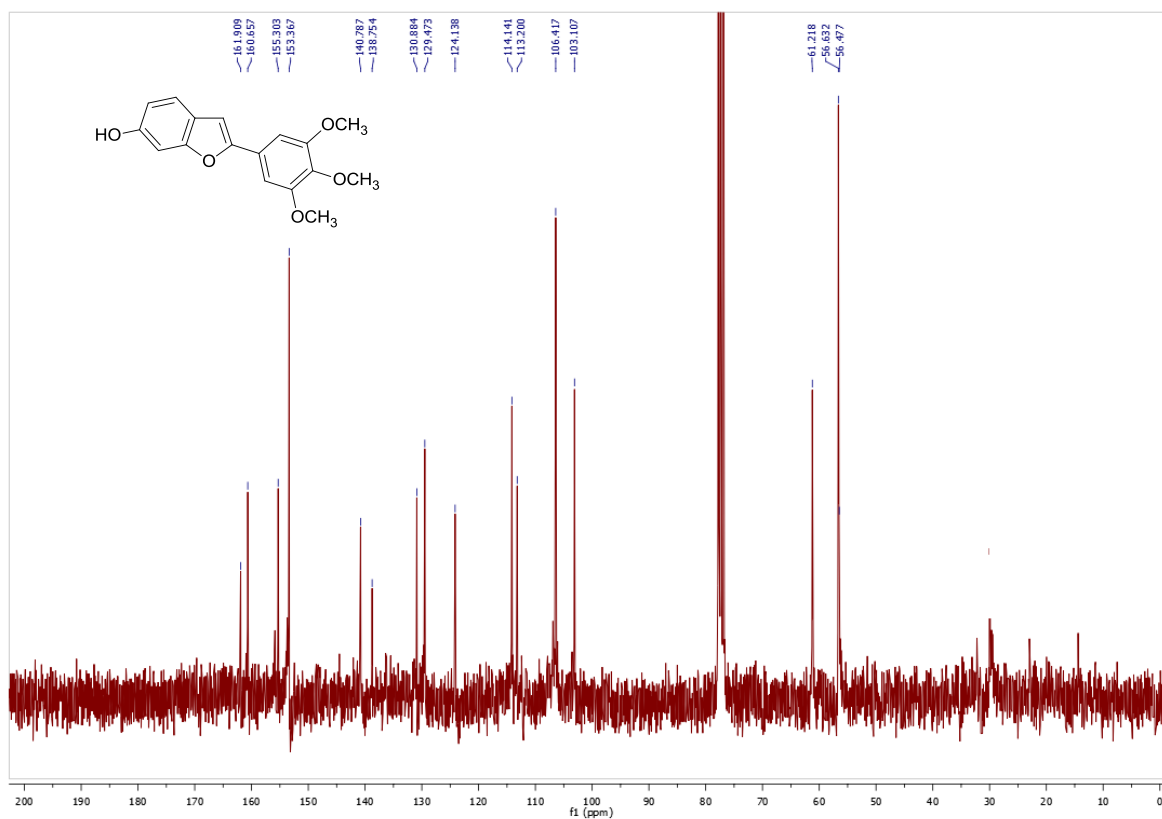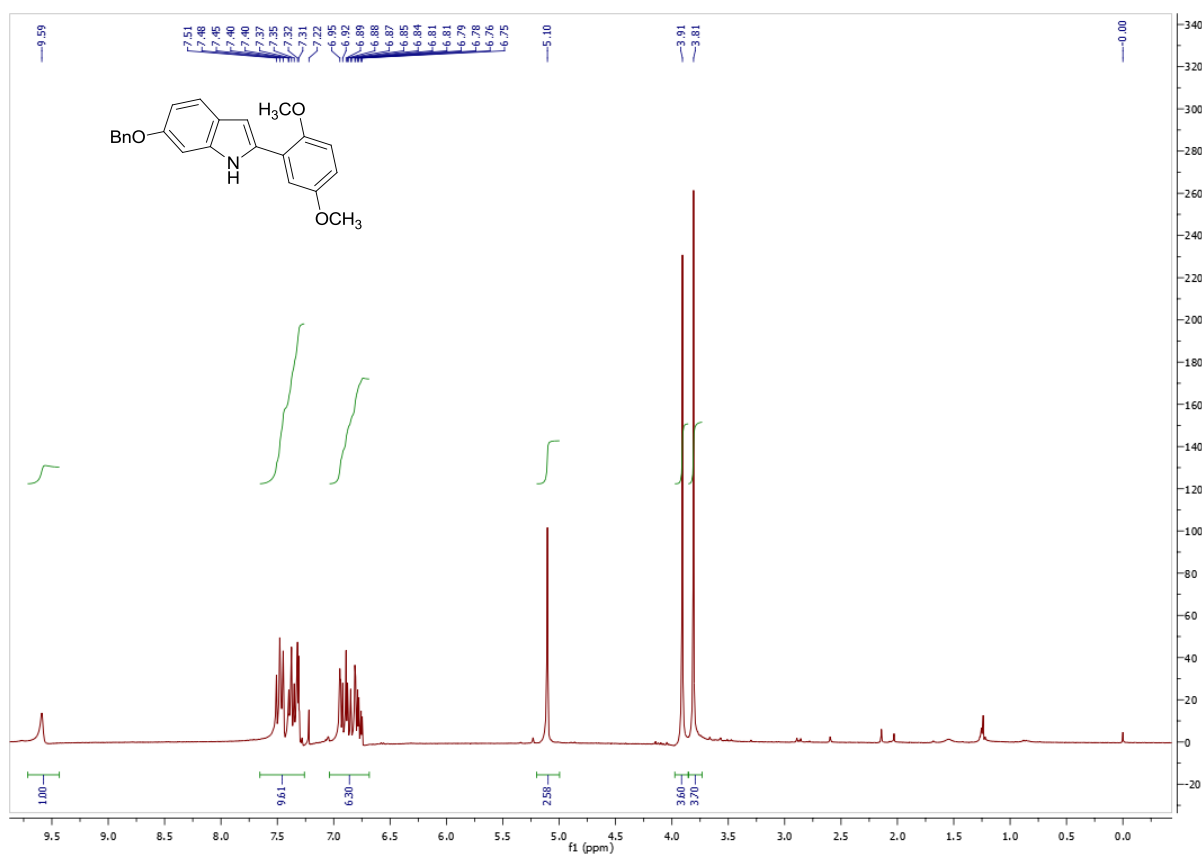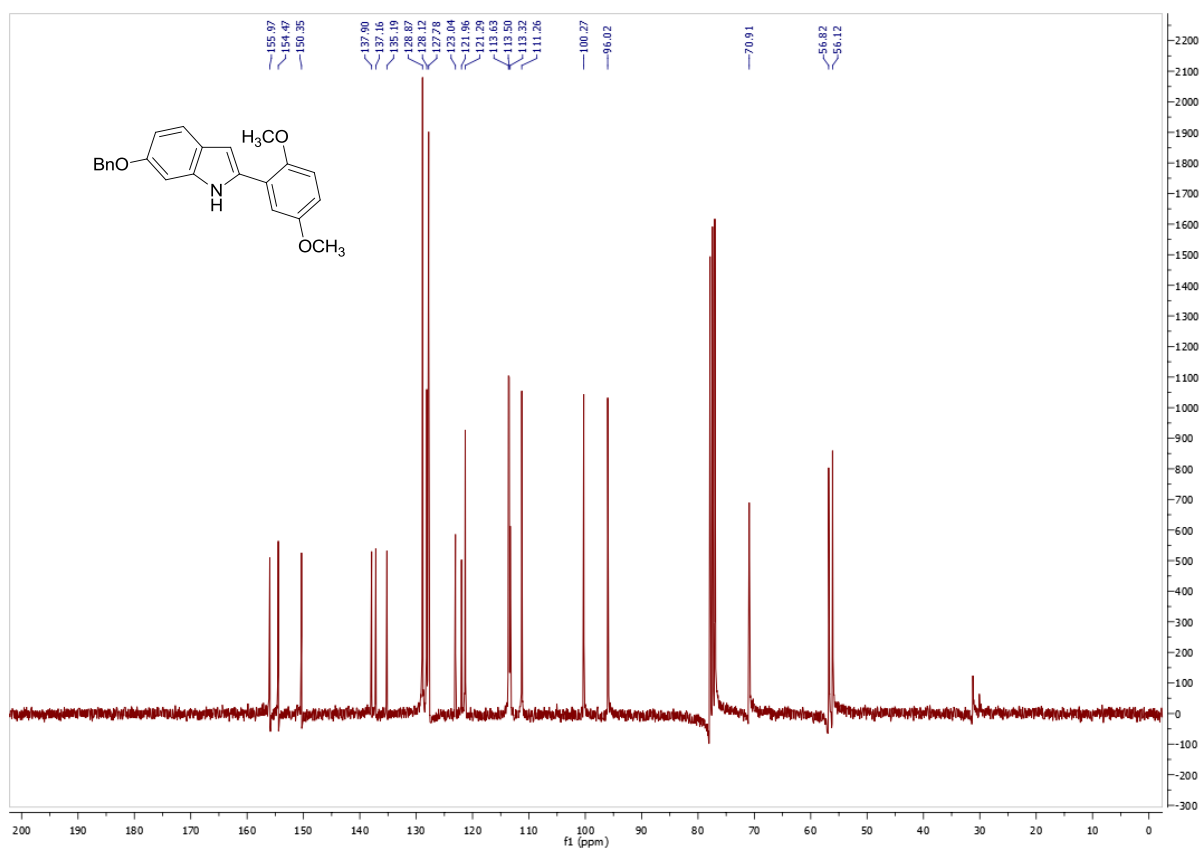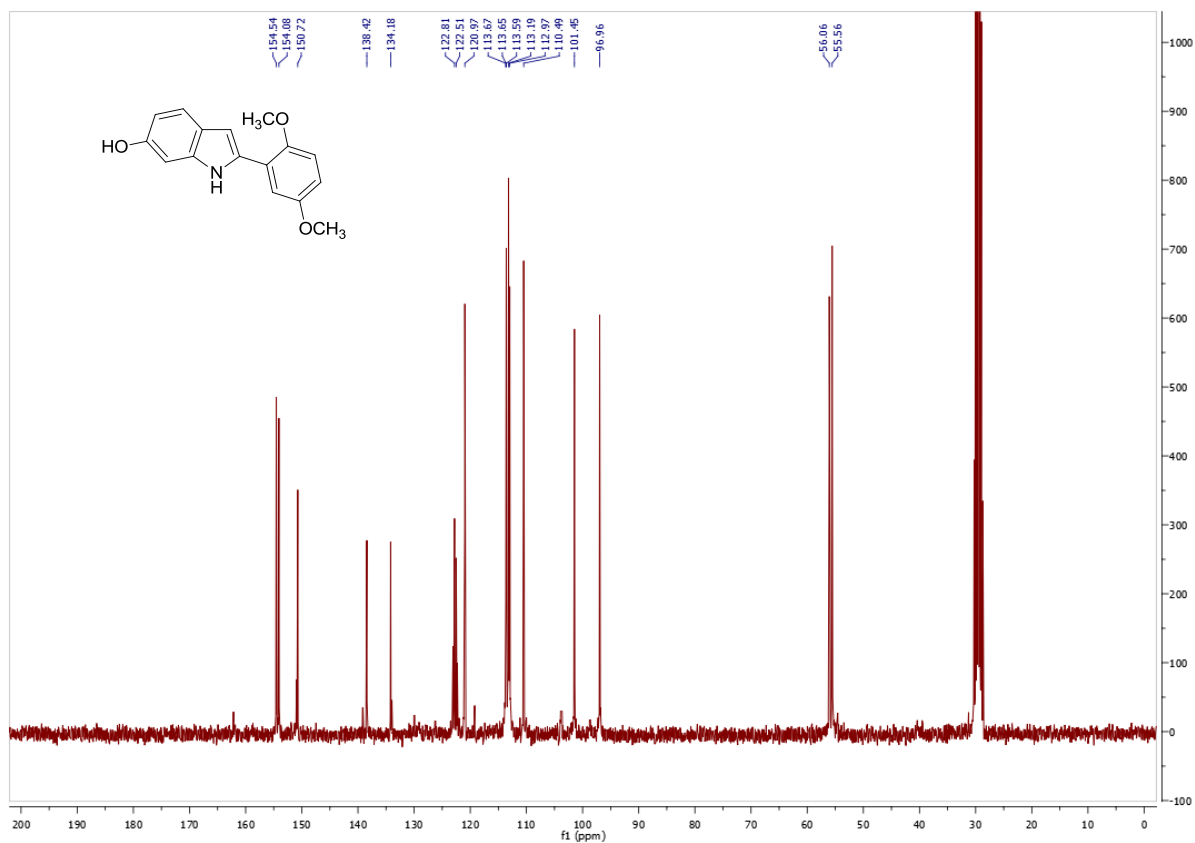# RMN ^{1}H (300 MHz, CDCl$_3$) of compound 5
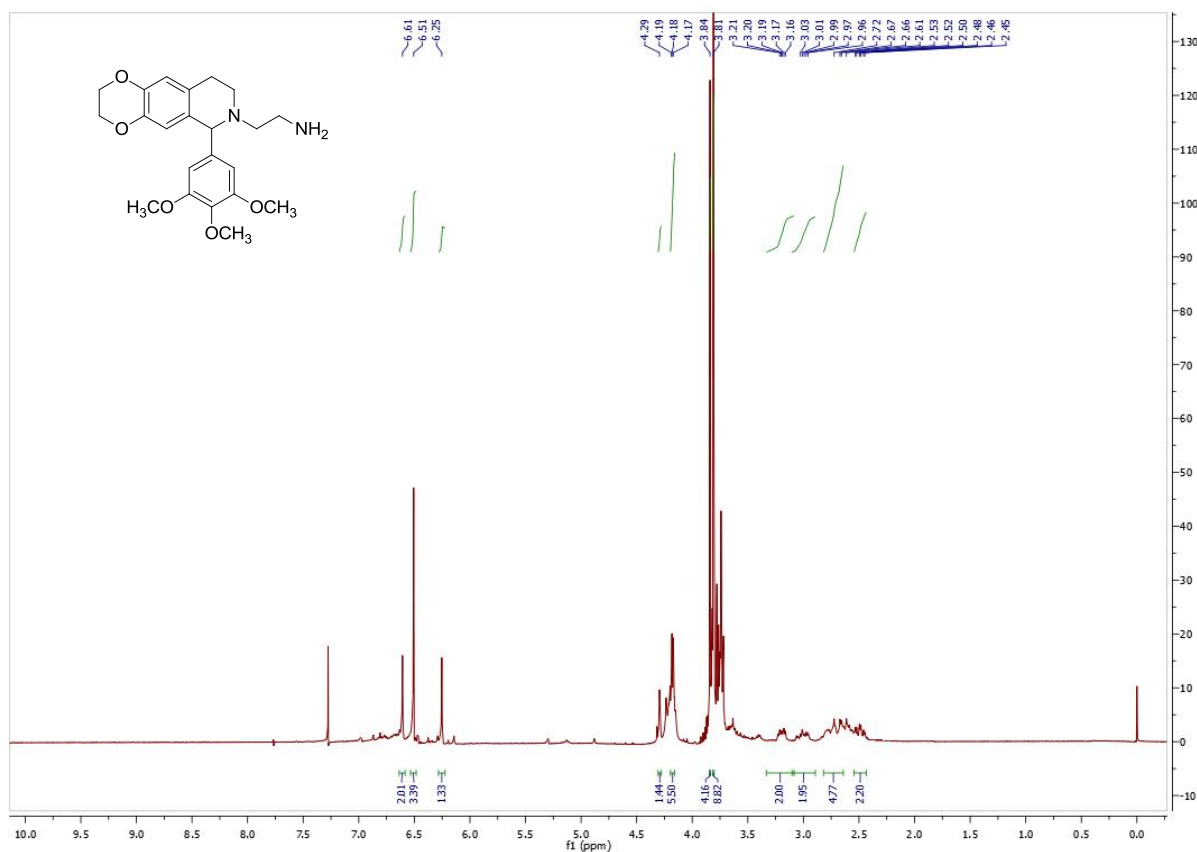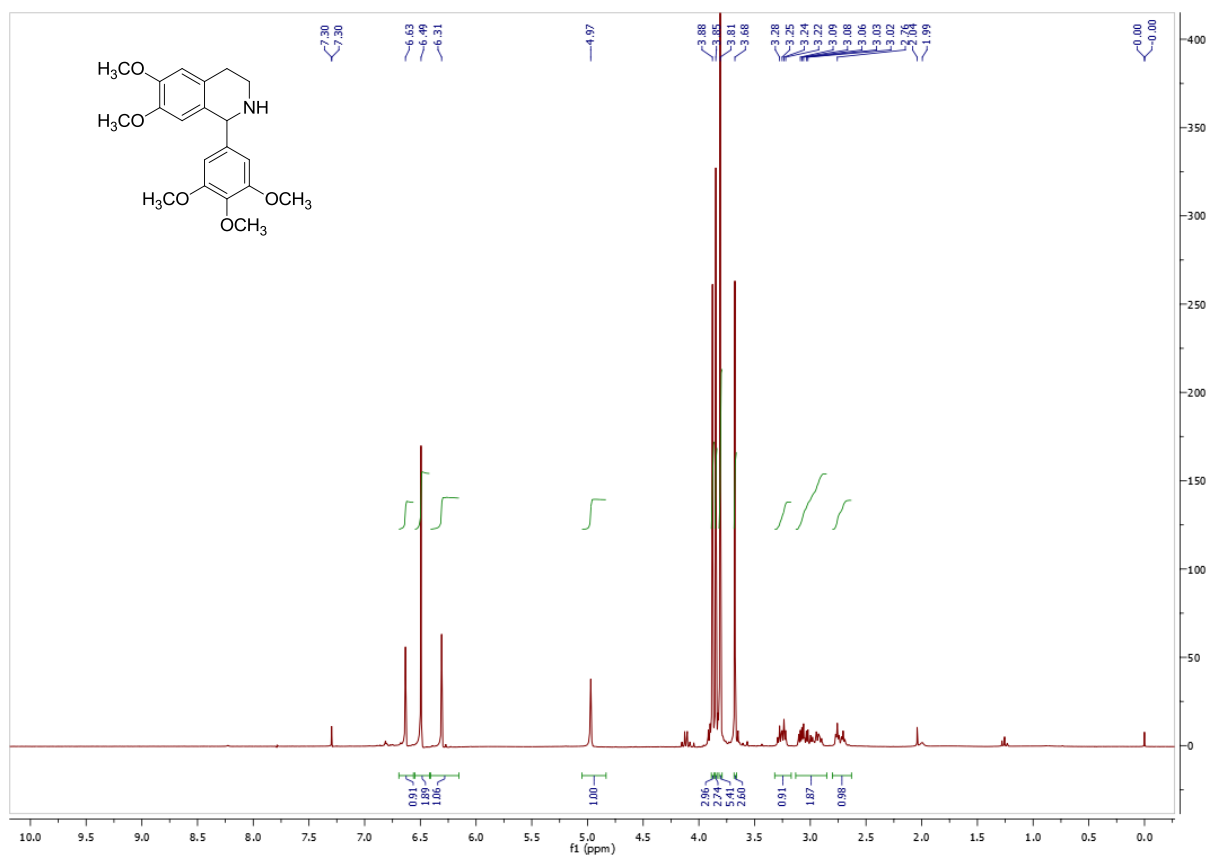

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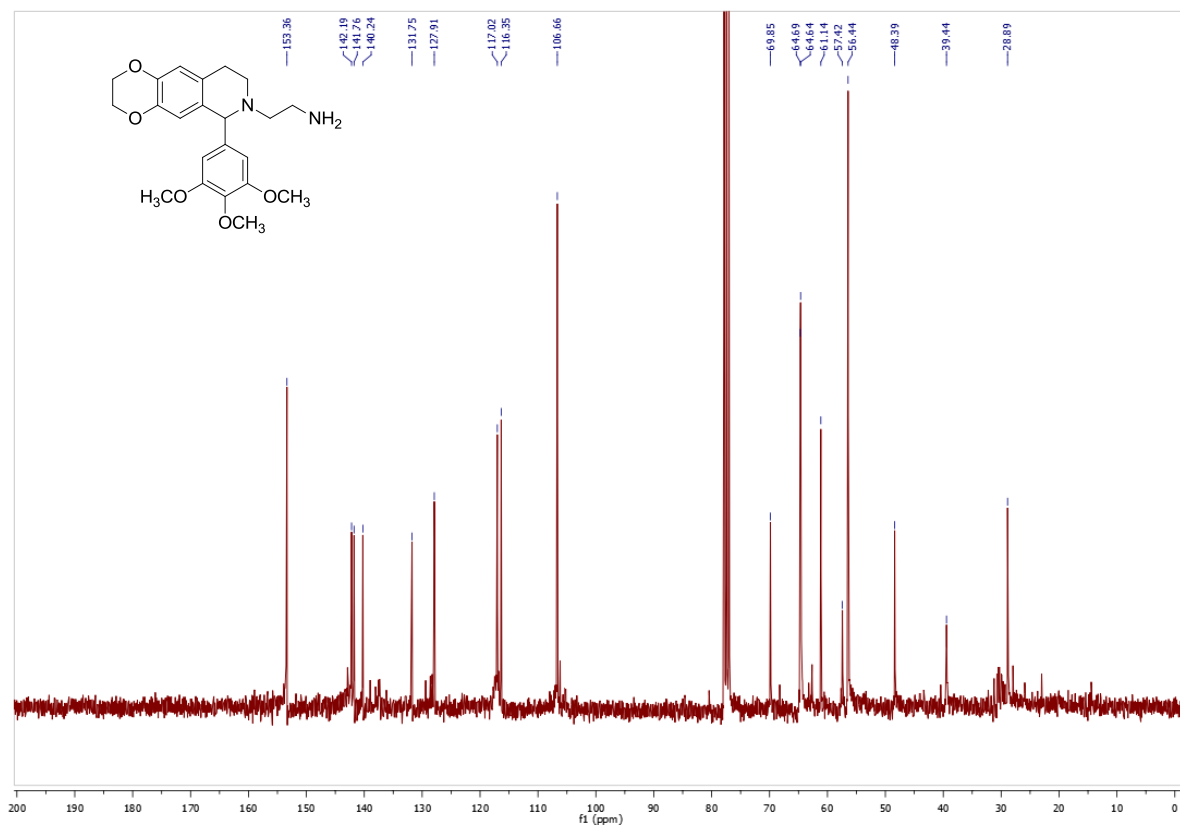
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 6
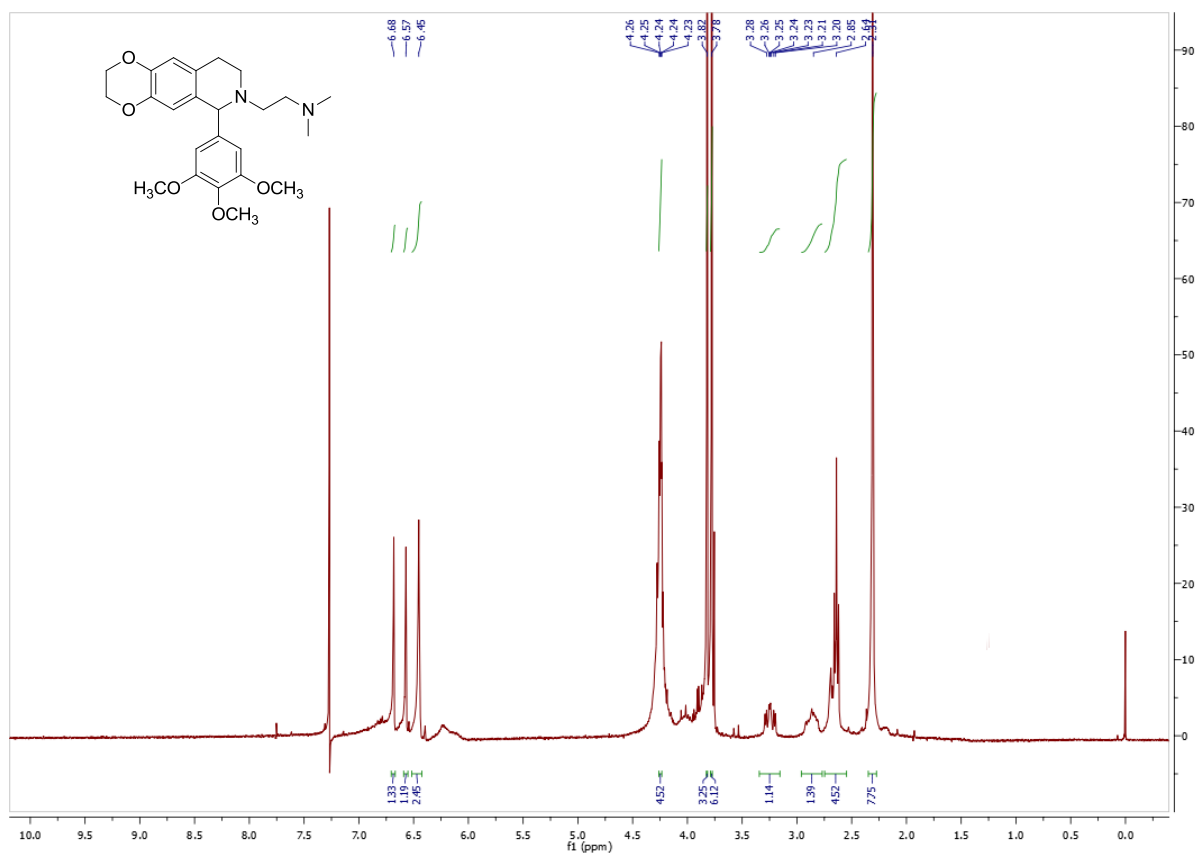
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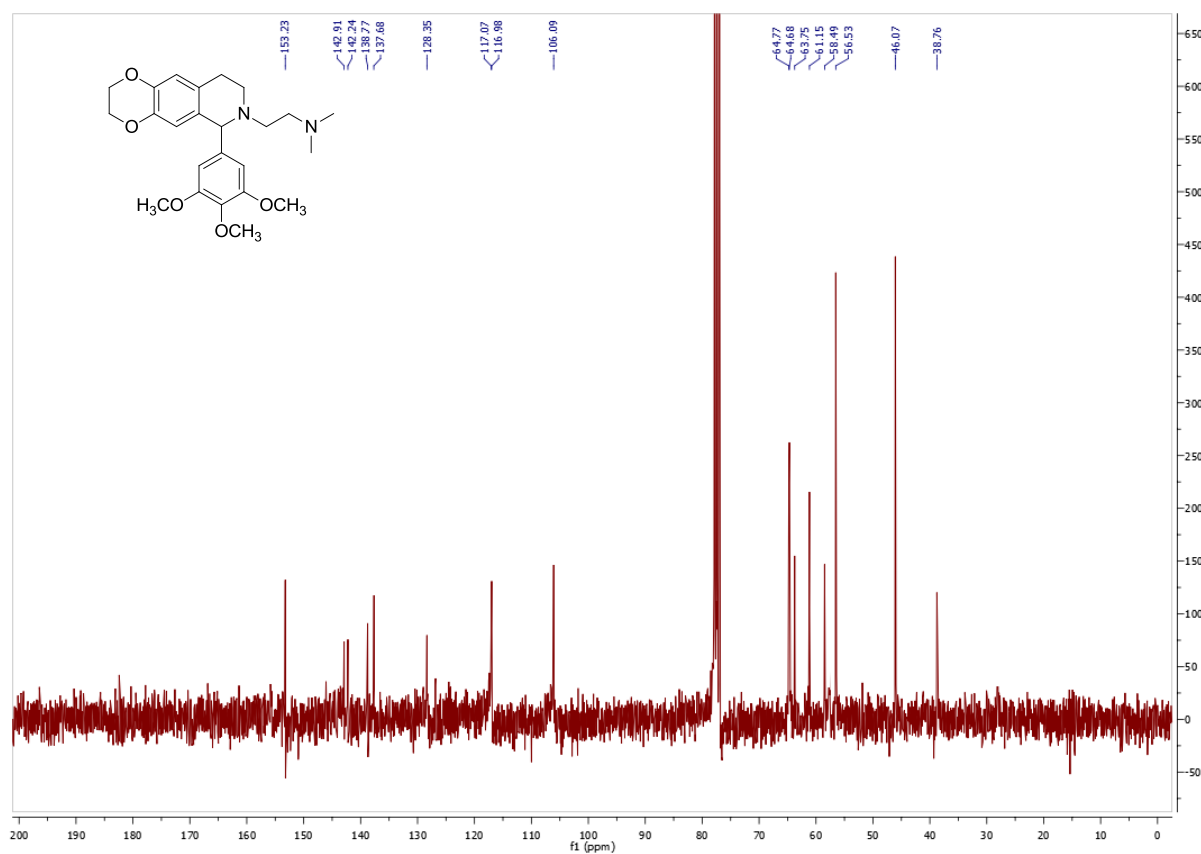
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 7
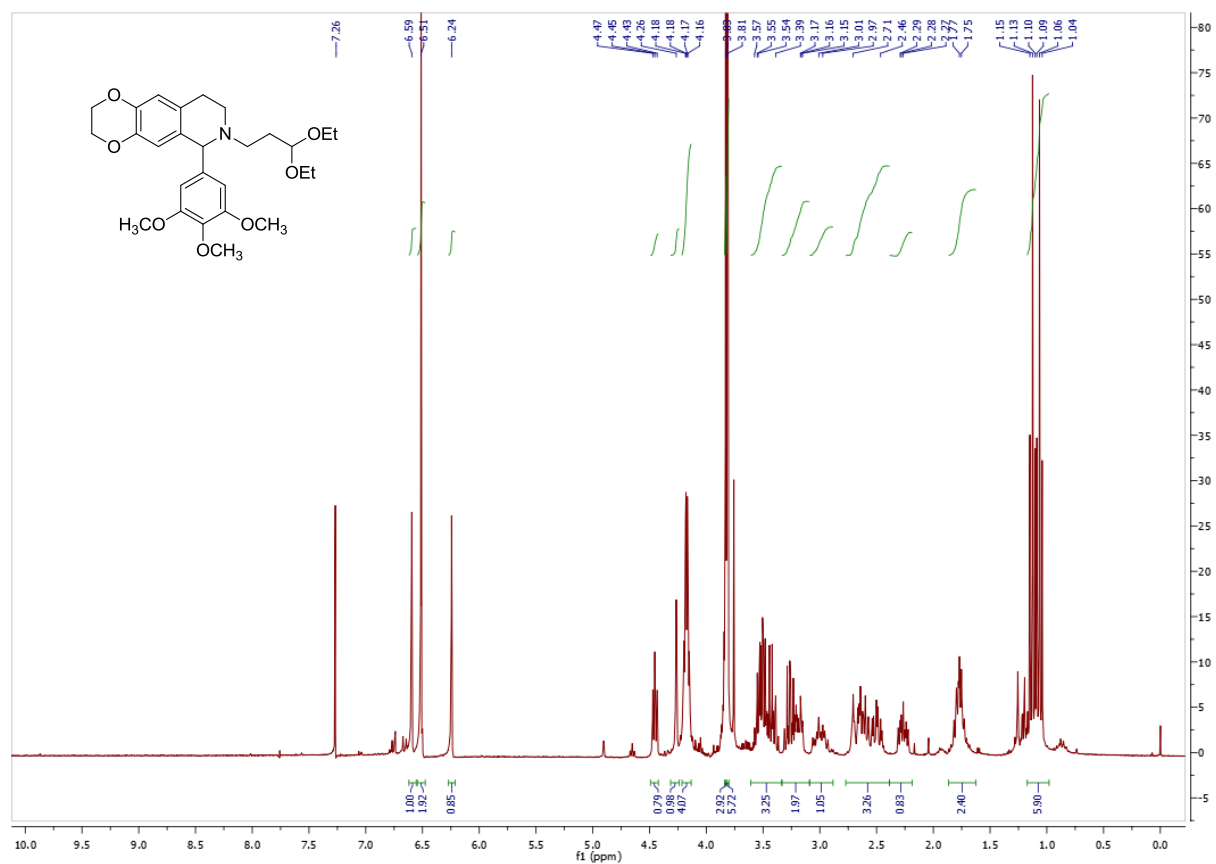
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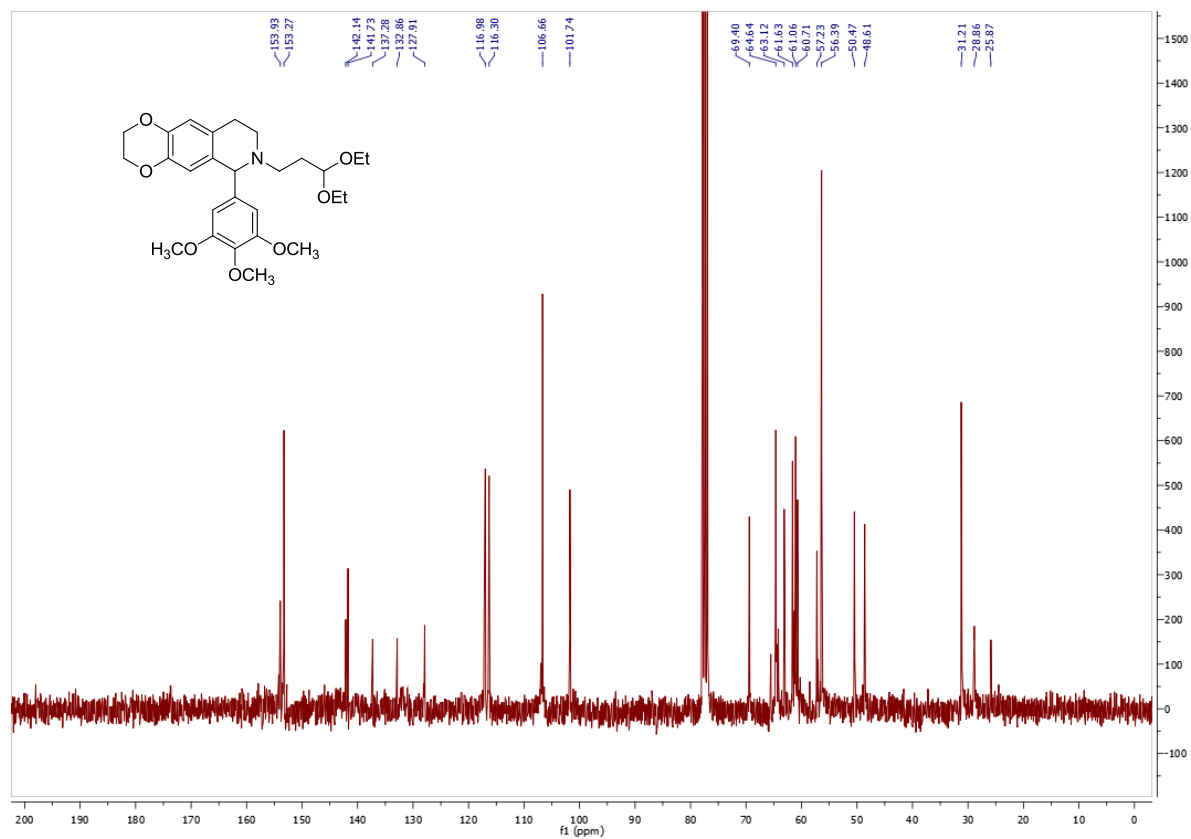
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 8
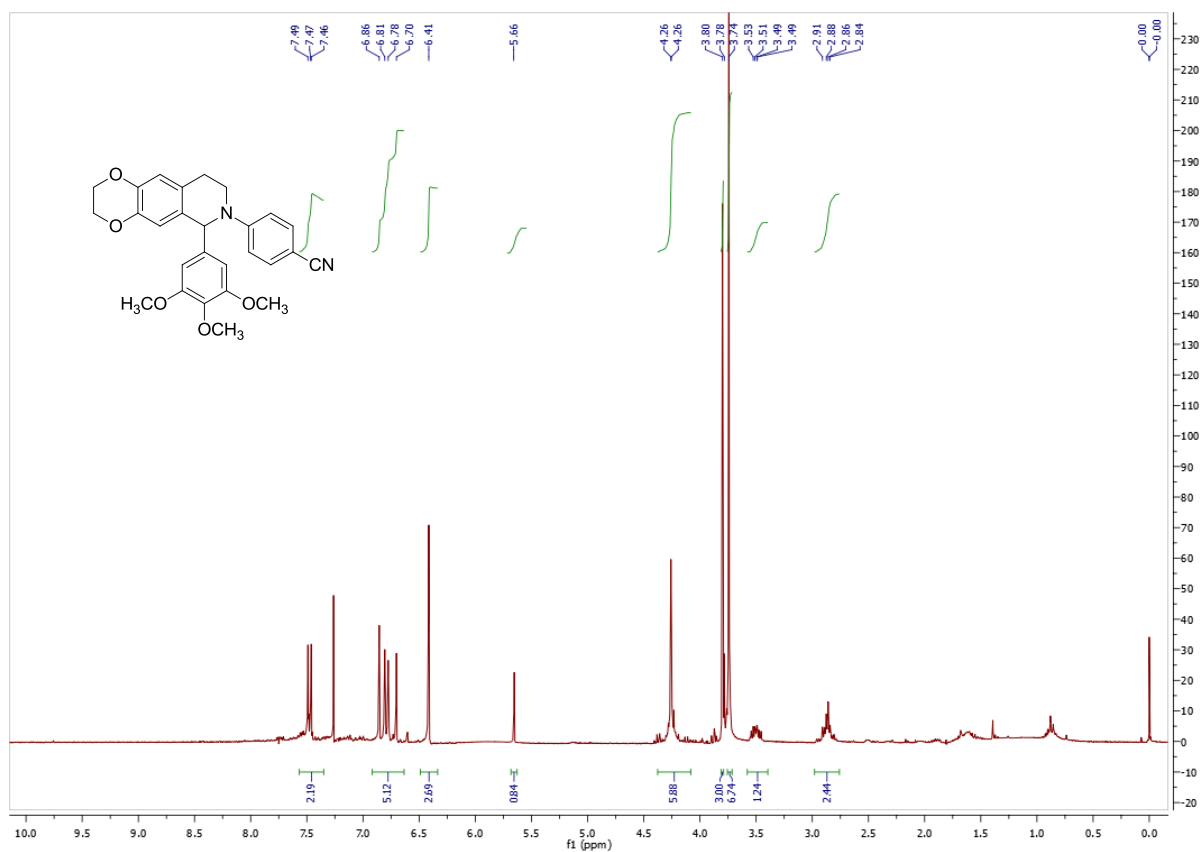
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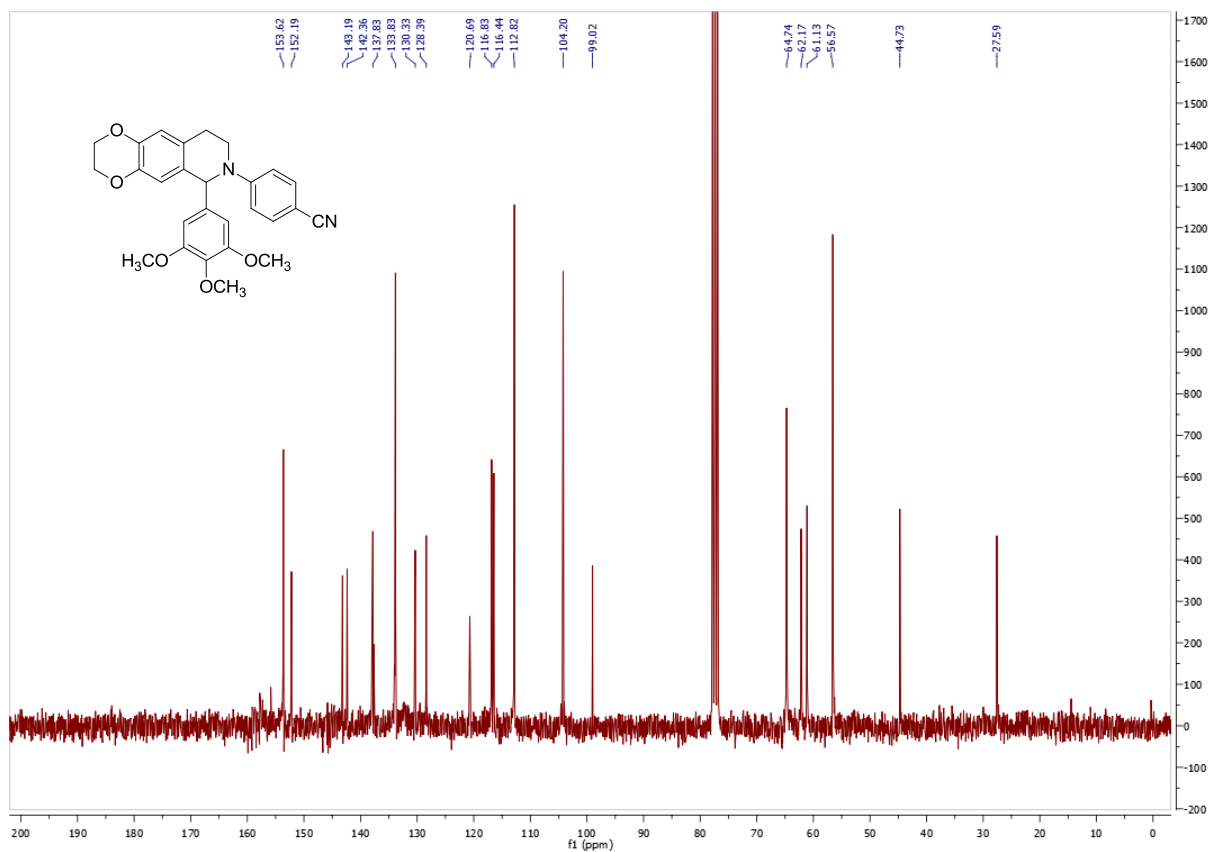
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 9
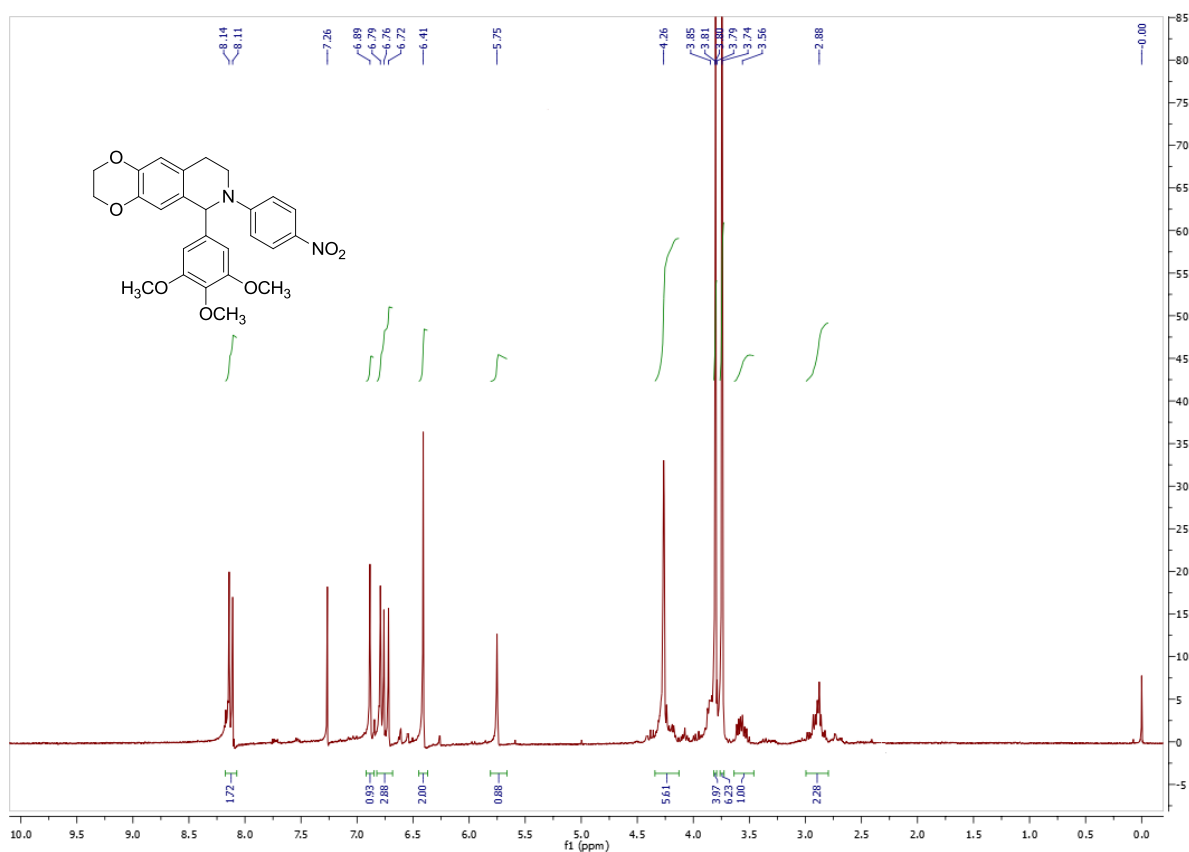
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 9
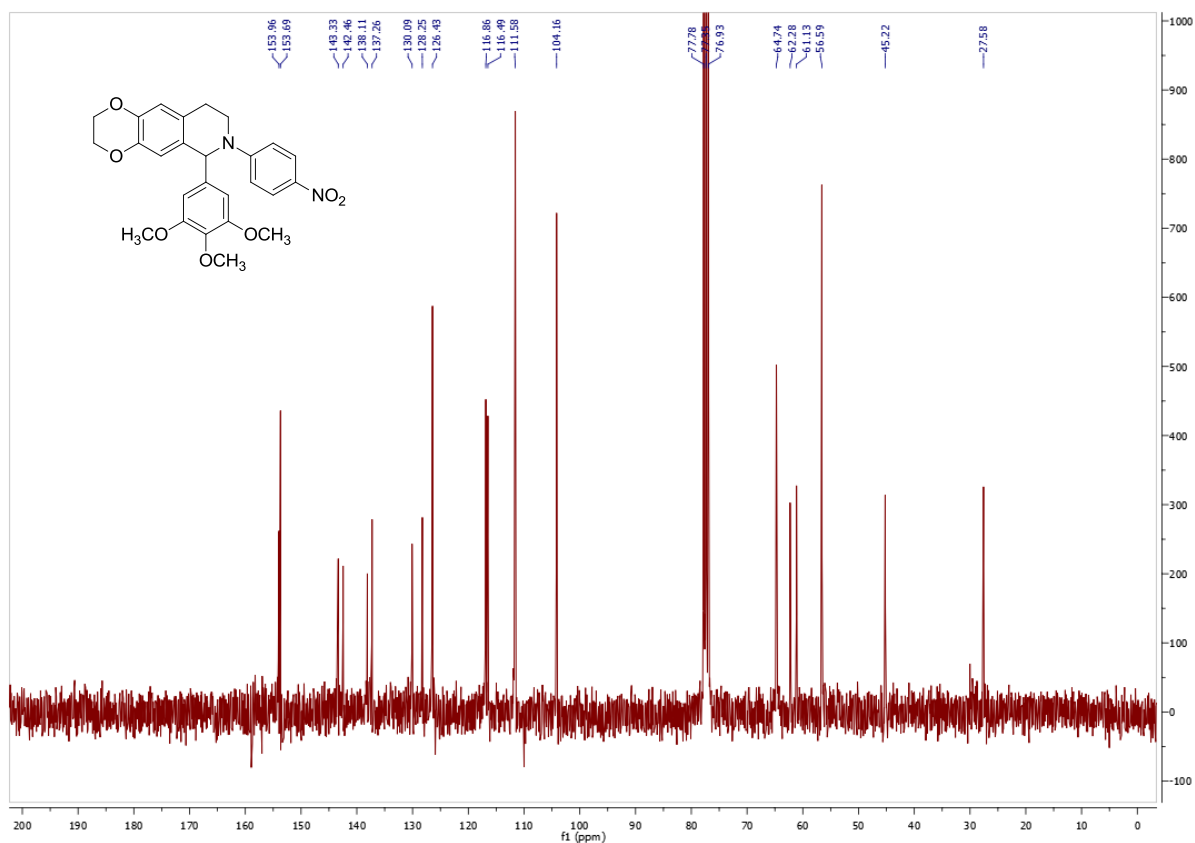
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 10
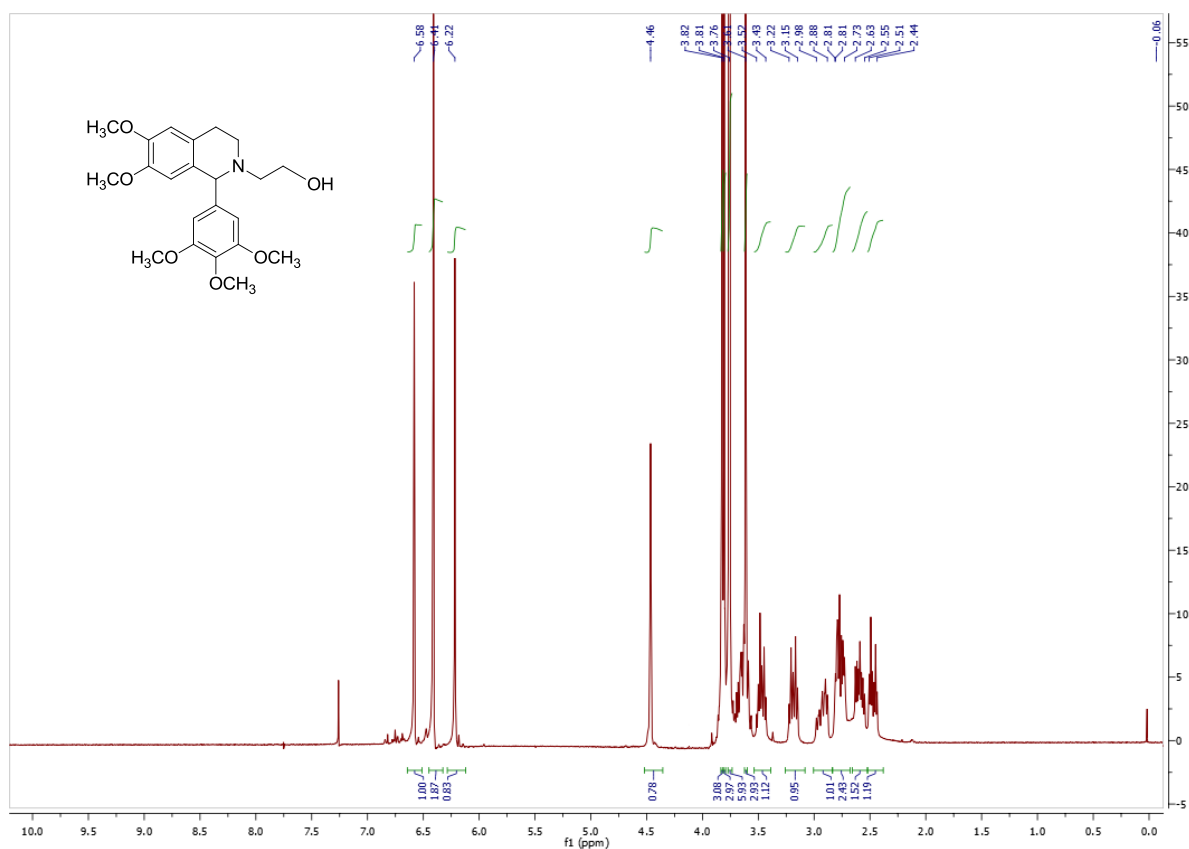
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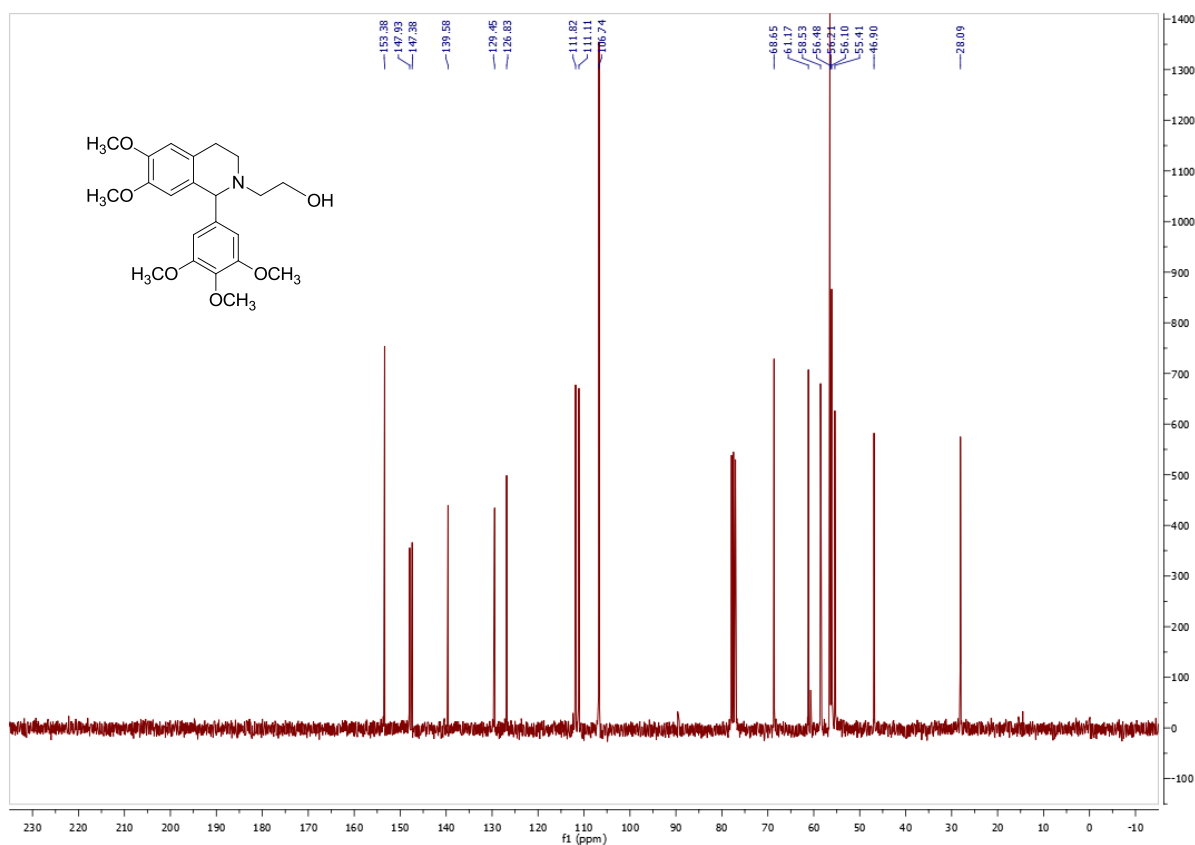
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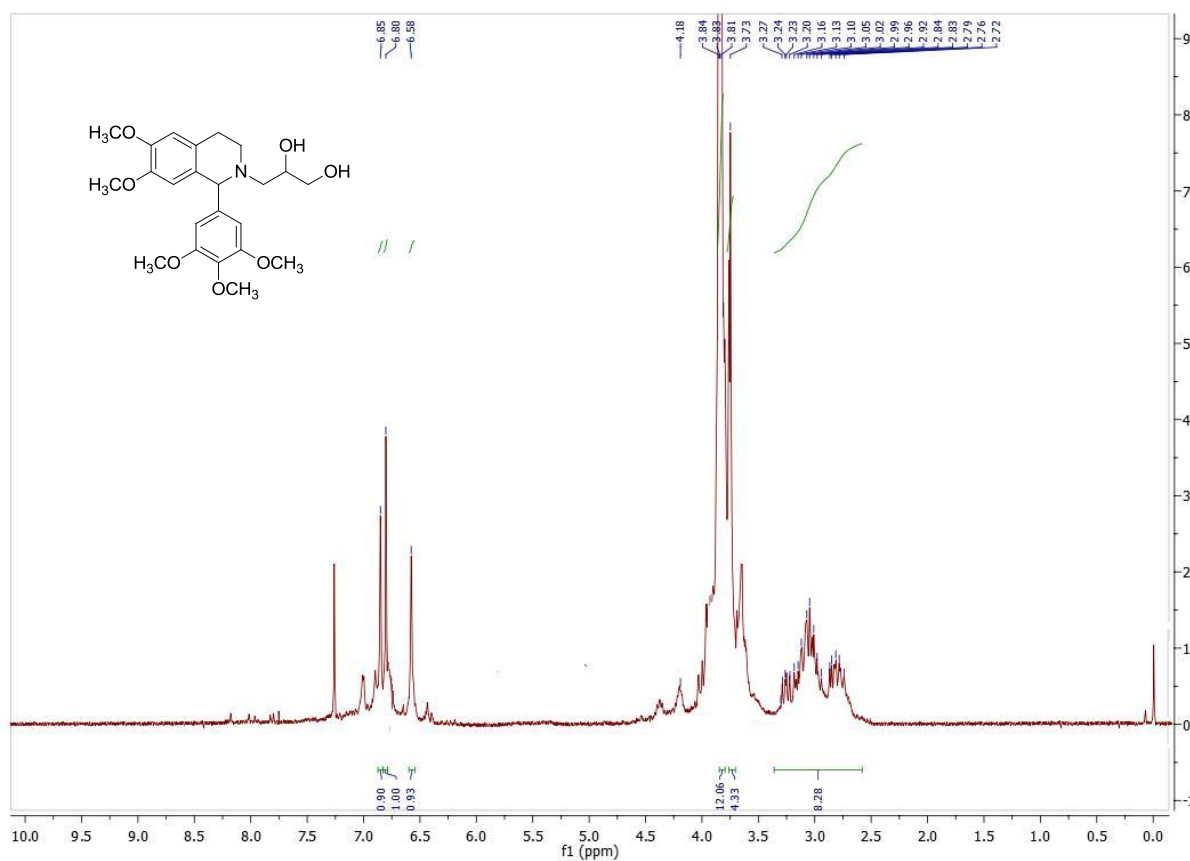
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 11
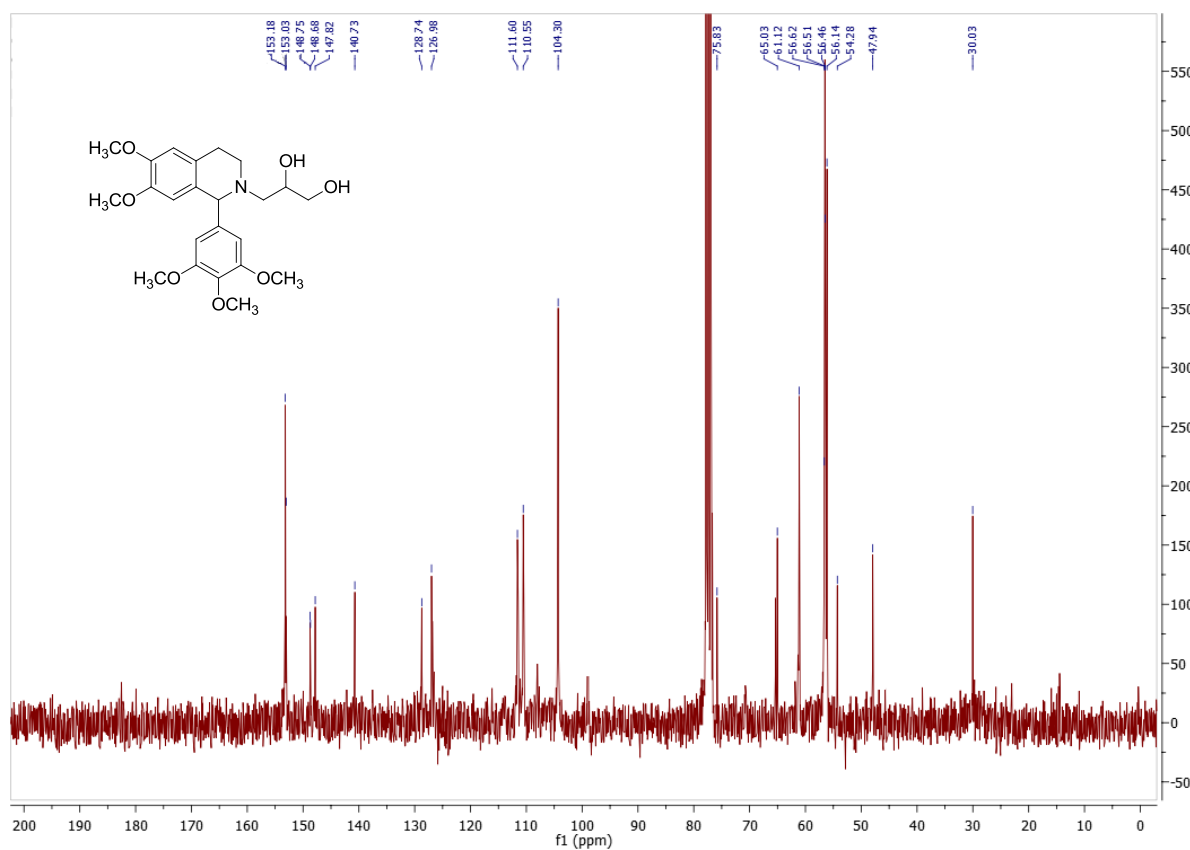
RMN ¹H (300 MHz, CDCl₃) of compound 14
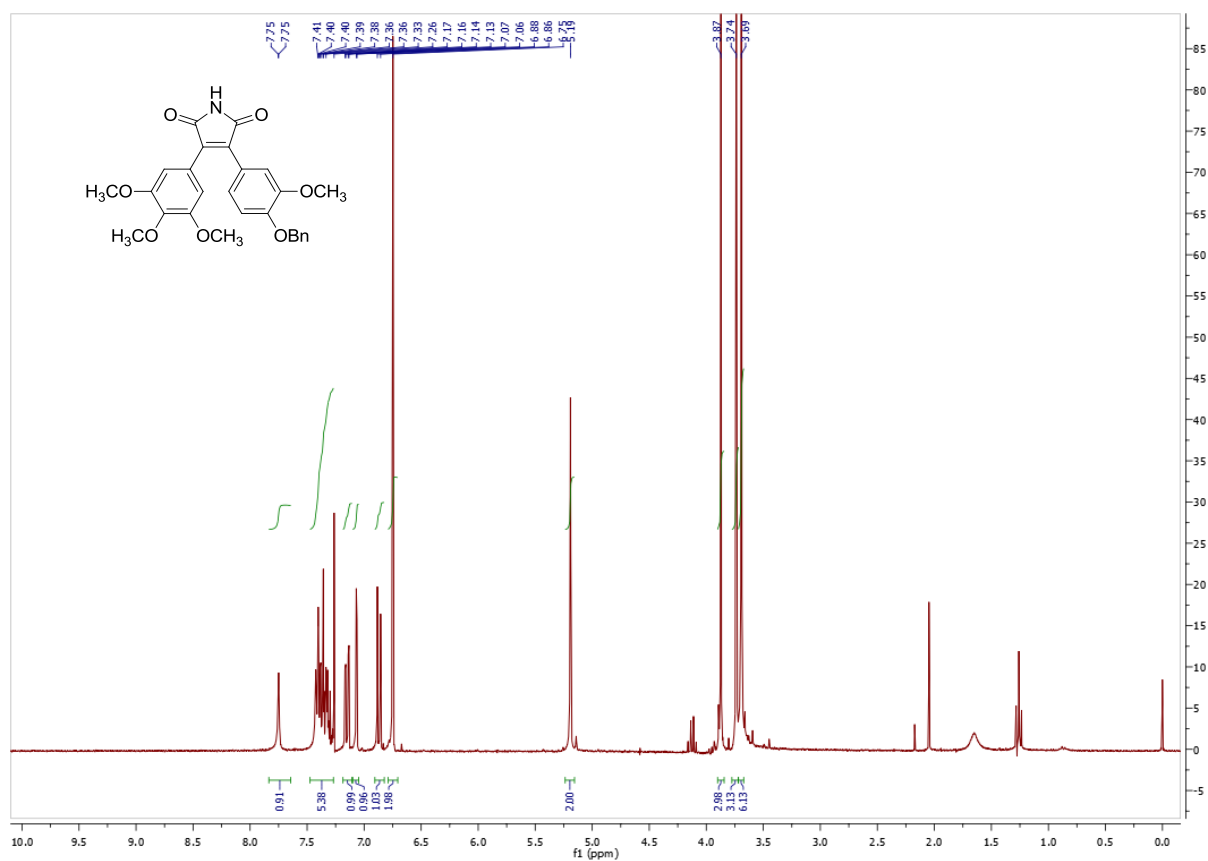
RMN ¹³C (75.5 MHz, CDCl₃) of compound 14
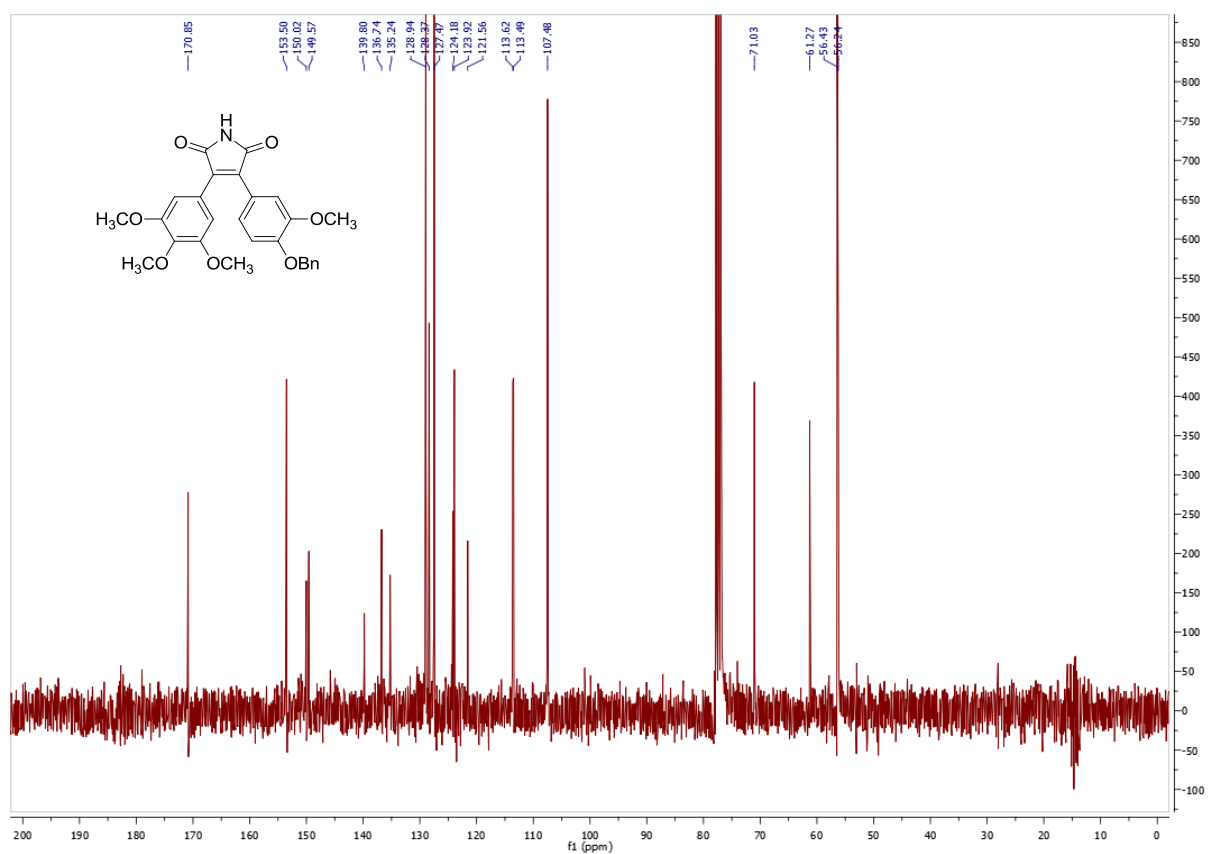
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 15
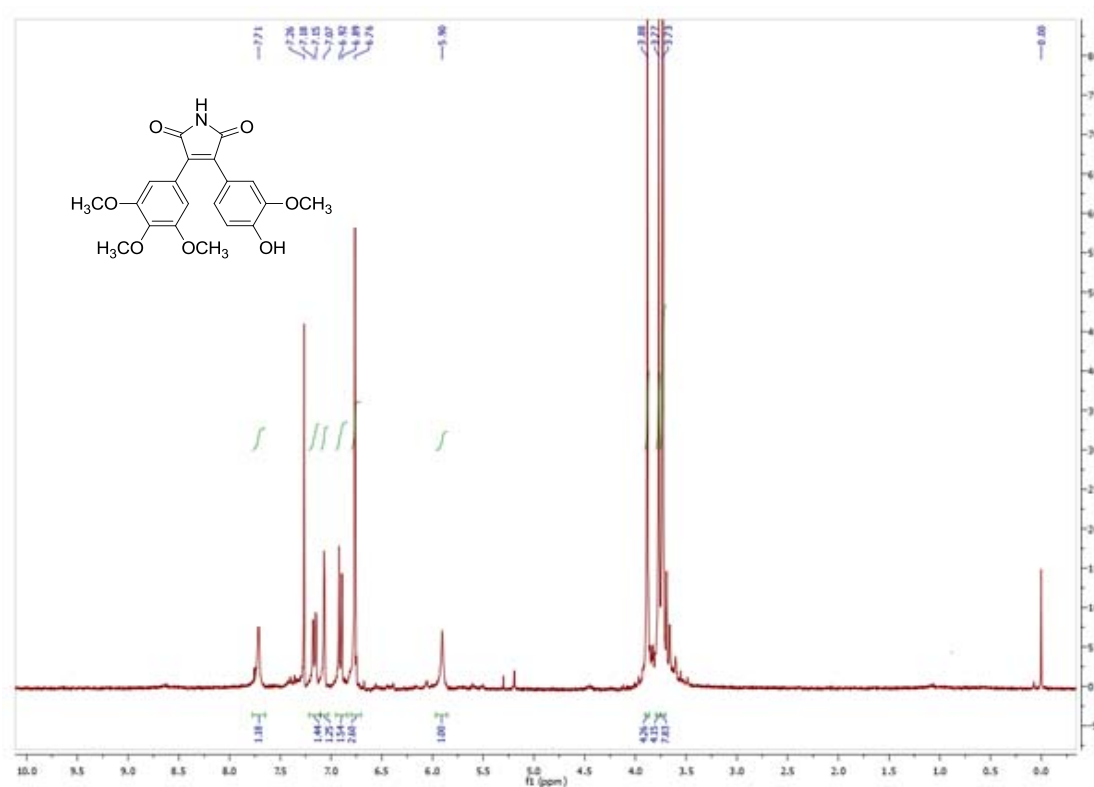
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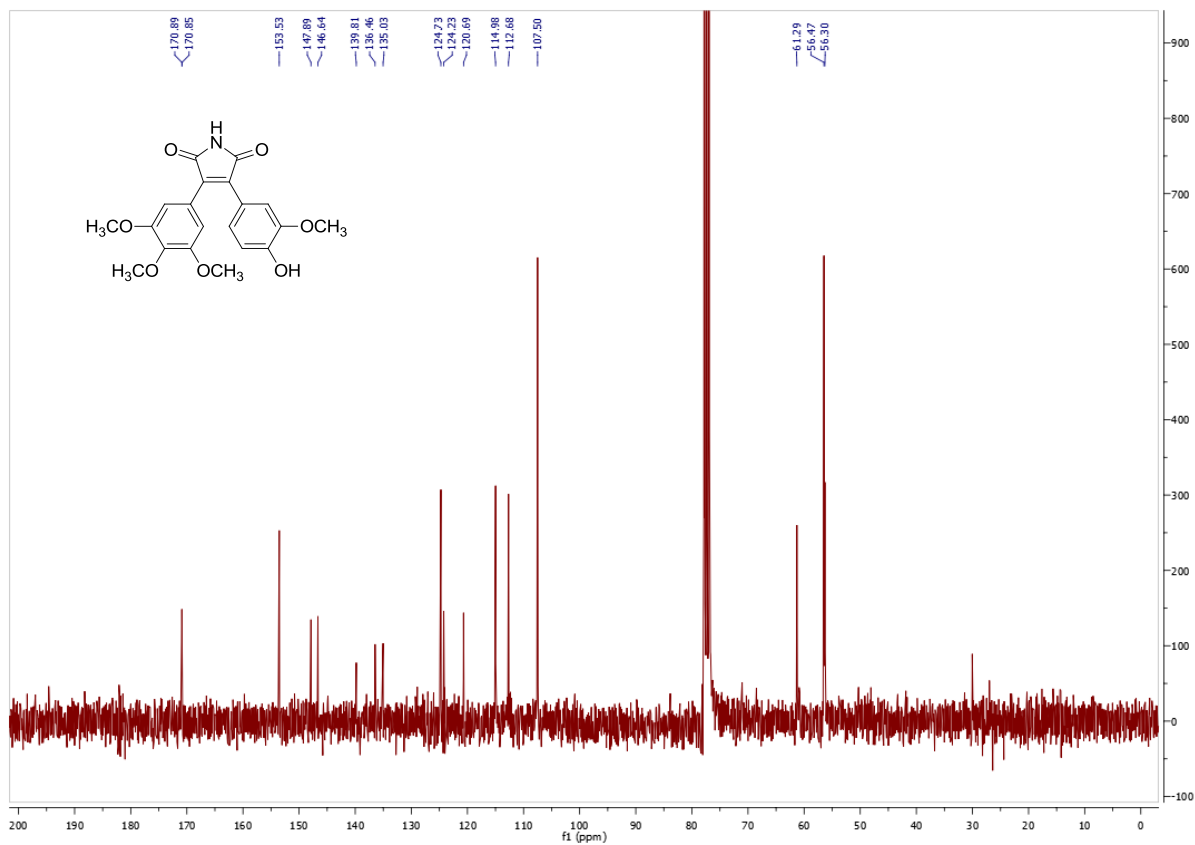
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 16
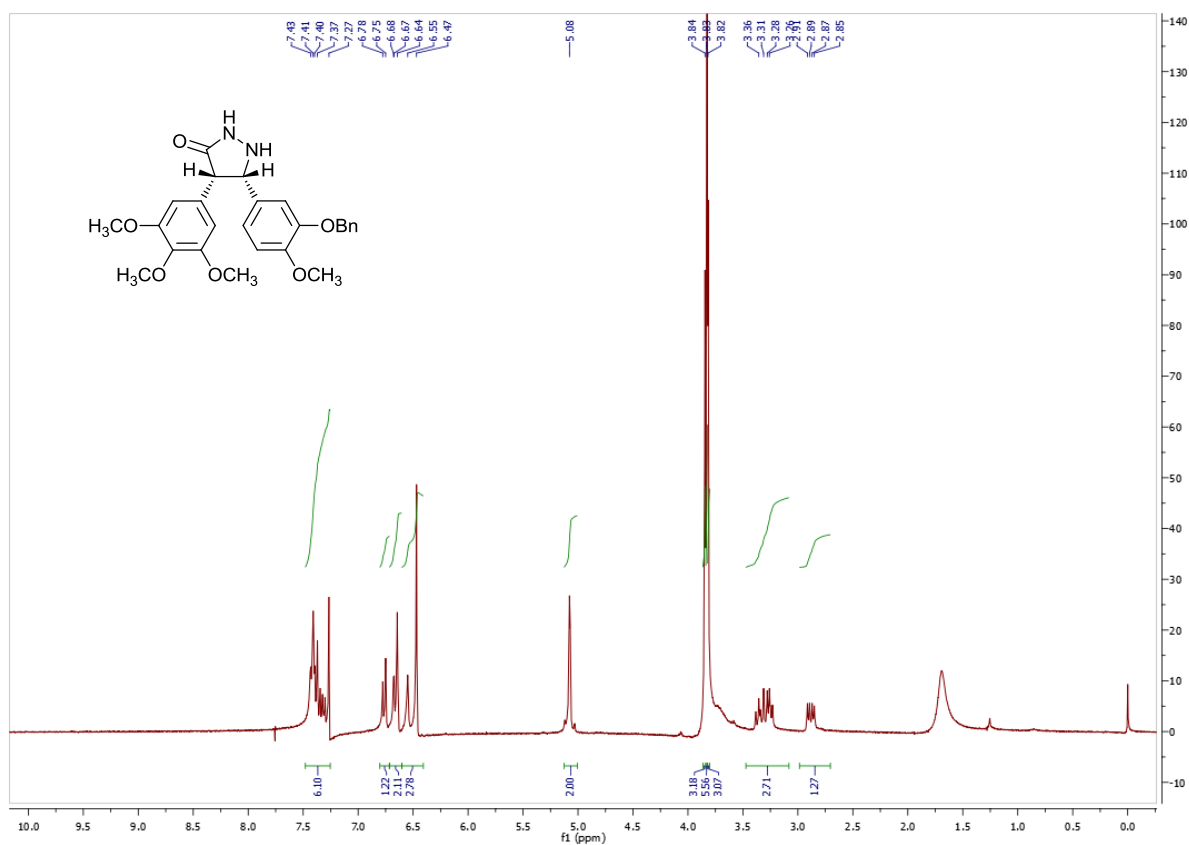
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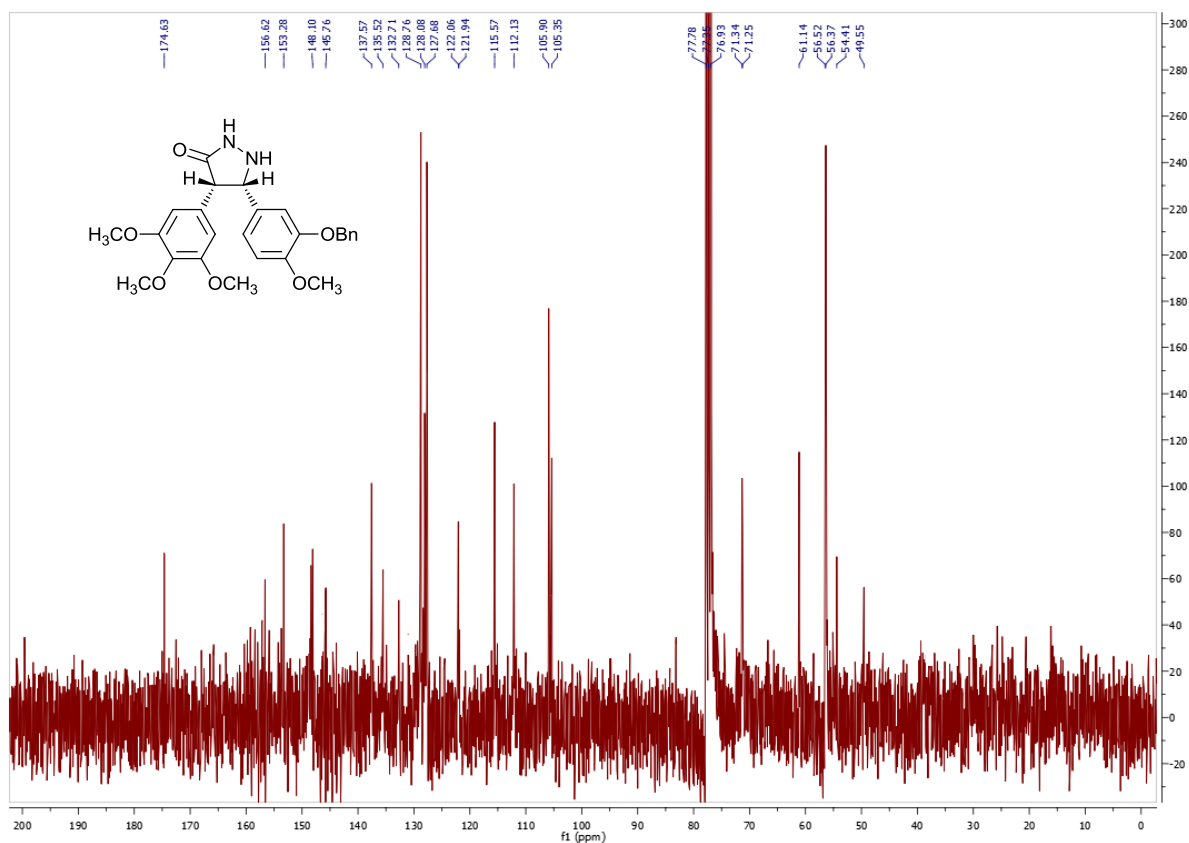
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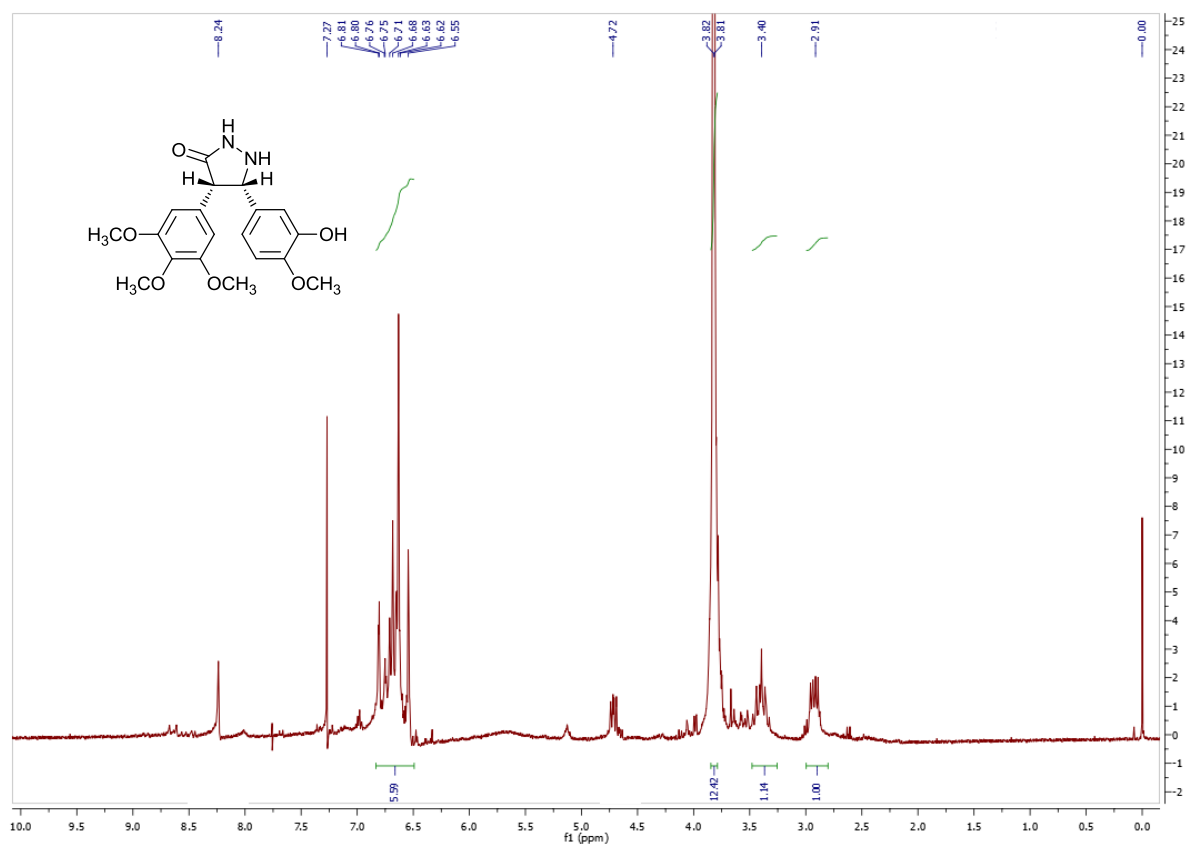
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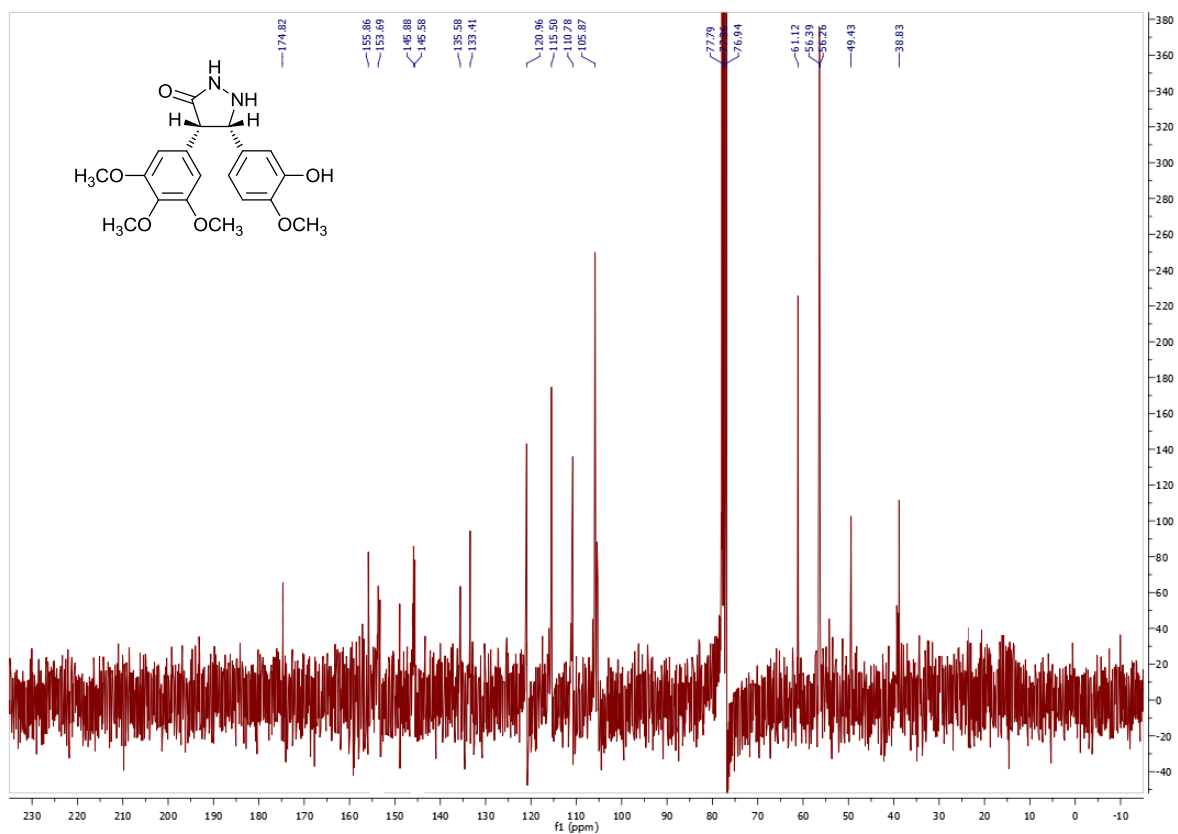
RMN ^{1}H (300 MHz, acetone-*d6*) of compound 21
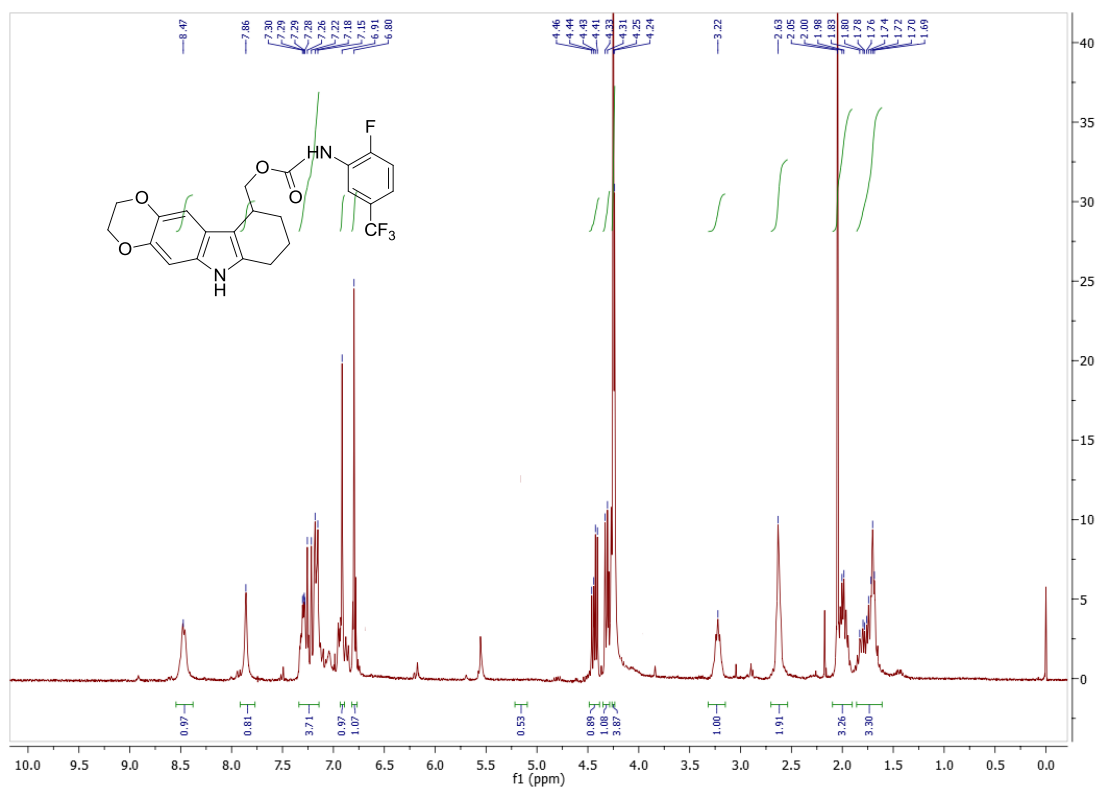
RMN ^{13}C (100 MHz, acetone-*d6*) of compound 21
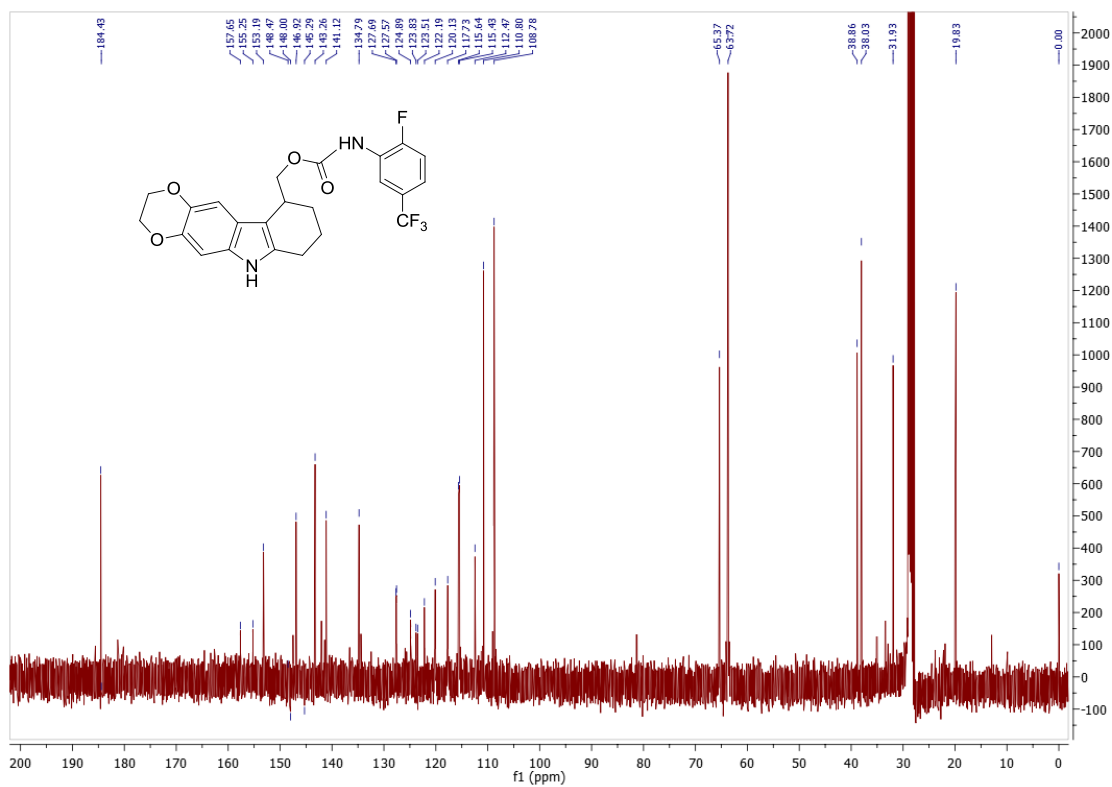
RMN ^{1}H (300 MHz, acetone-*d6*) of compound 22
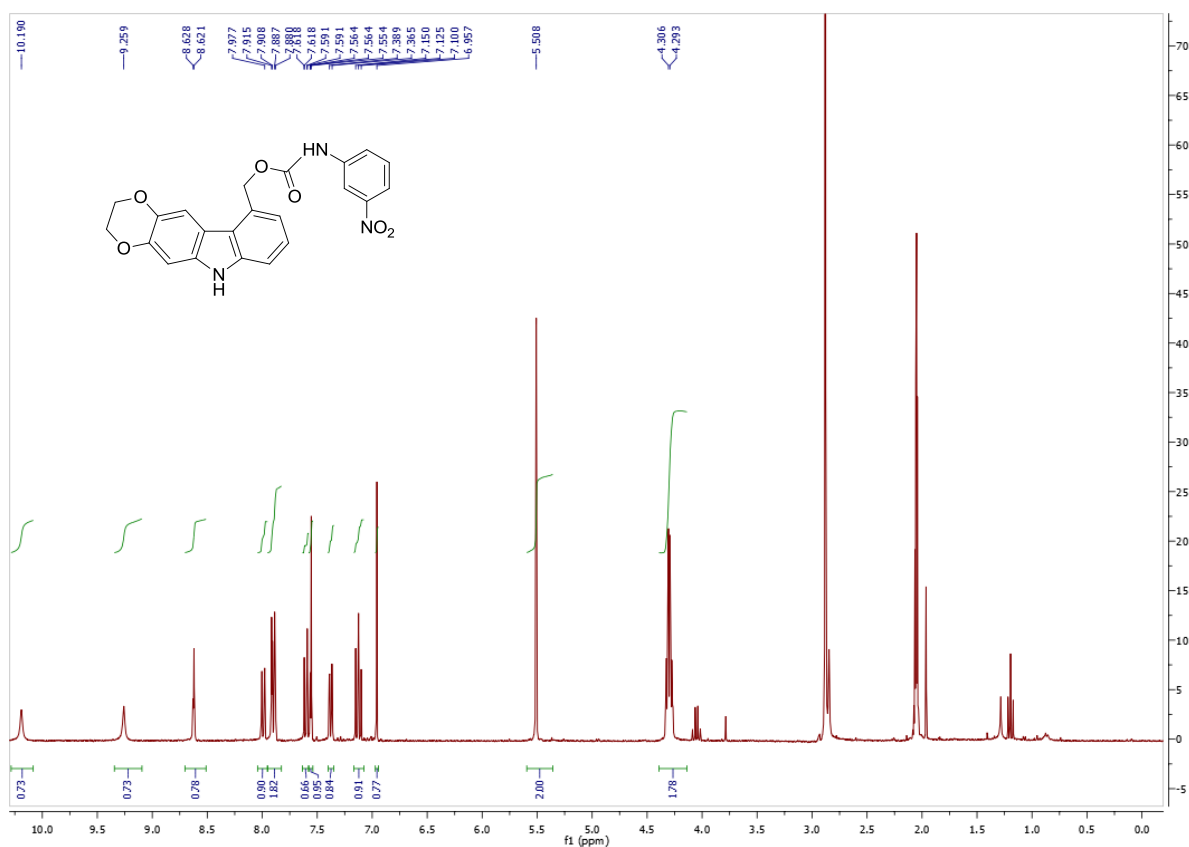
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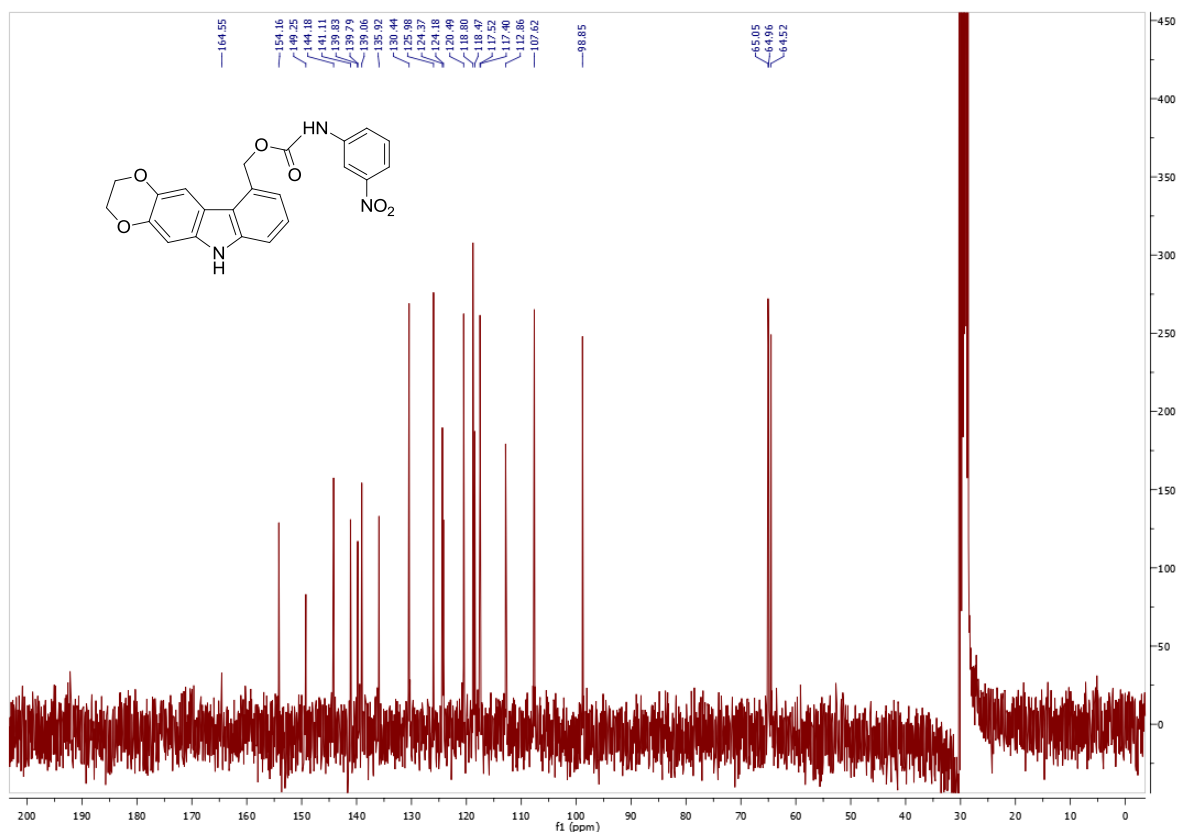
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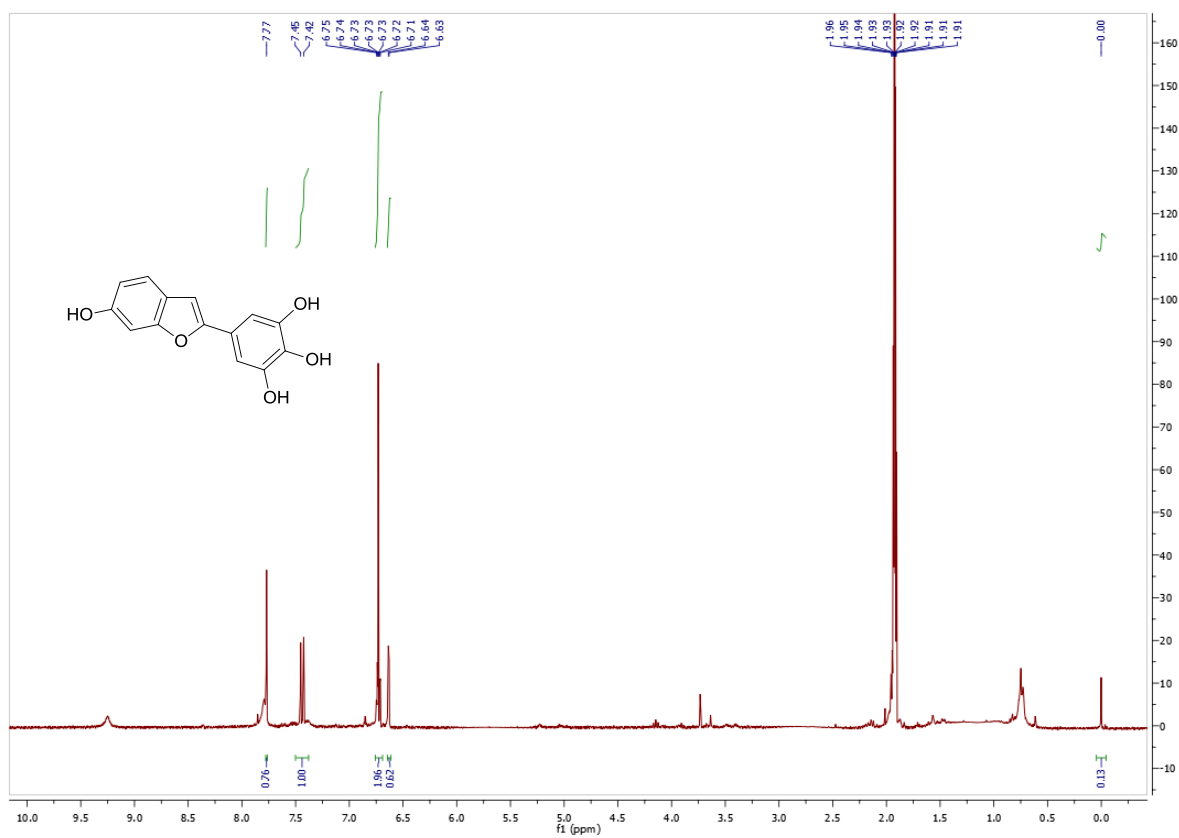
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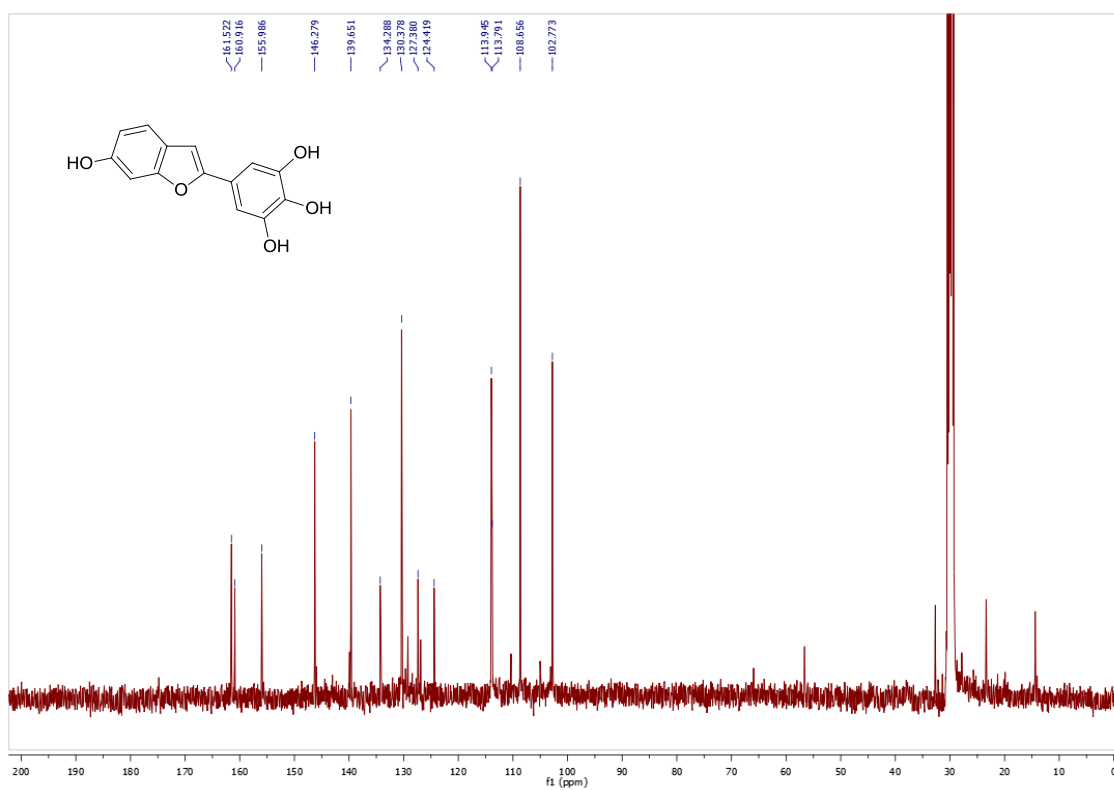
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 24
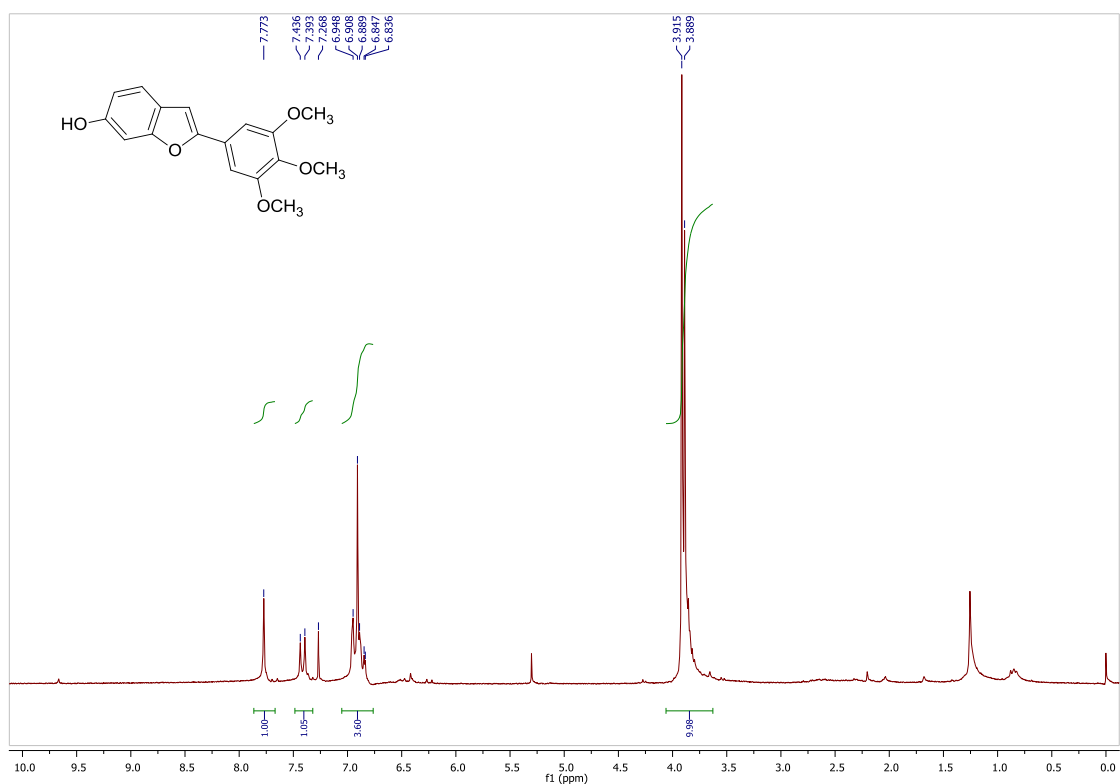
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 24

RMN ^{1}H (300 MHz, CDCl$_3$) of compound 25

RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 25

RMN ^{1}H (300 MHz, acetone-$d6$) of compound 26
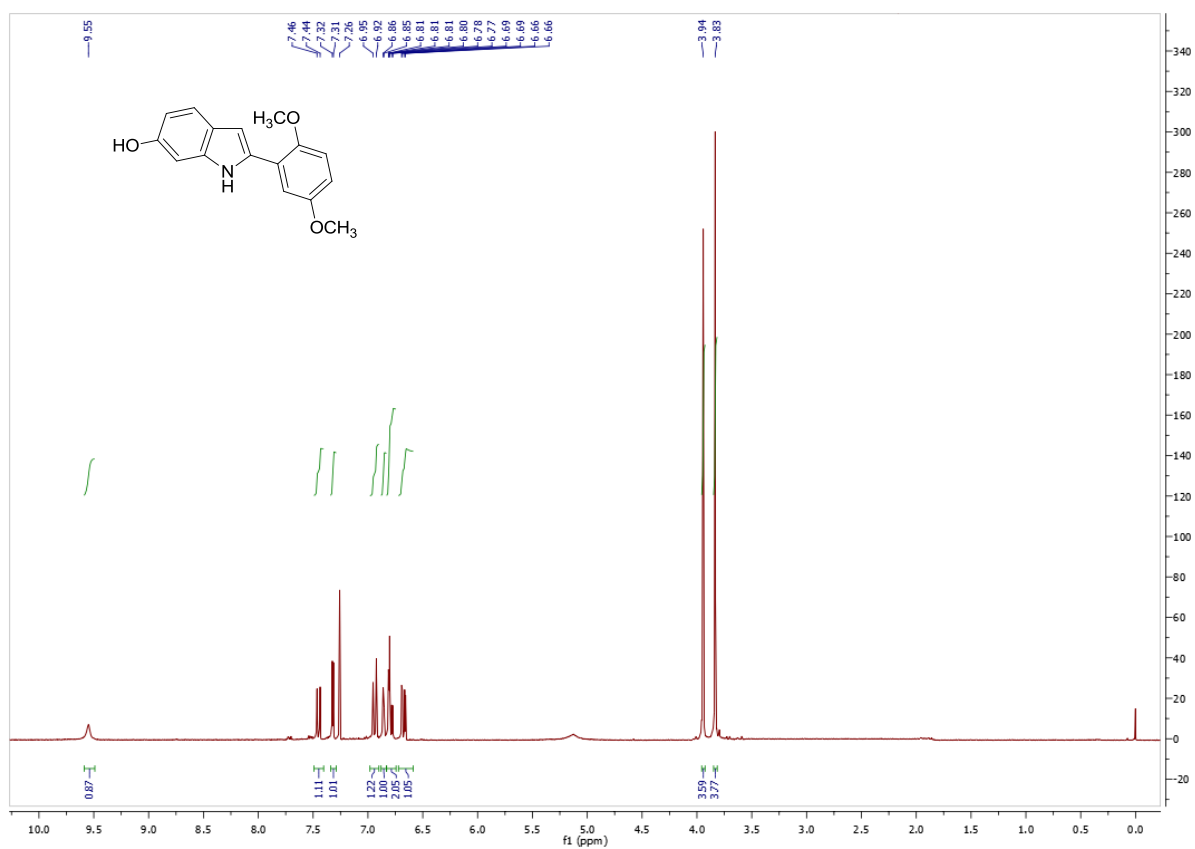
RMN ^{13}C (75.5 MHz, acetone-$d6$) of compound 26

RMN ^{1}H (300 MHz, CDCl$_3$) of compound 49

RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 49
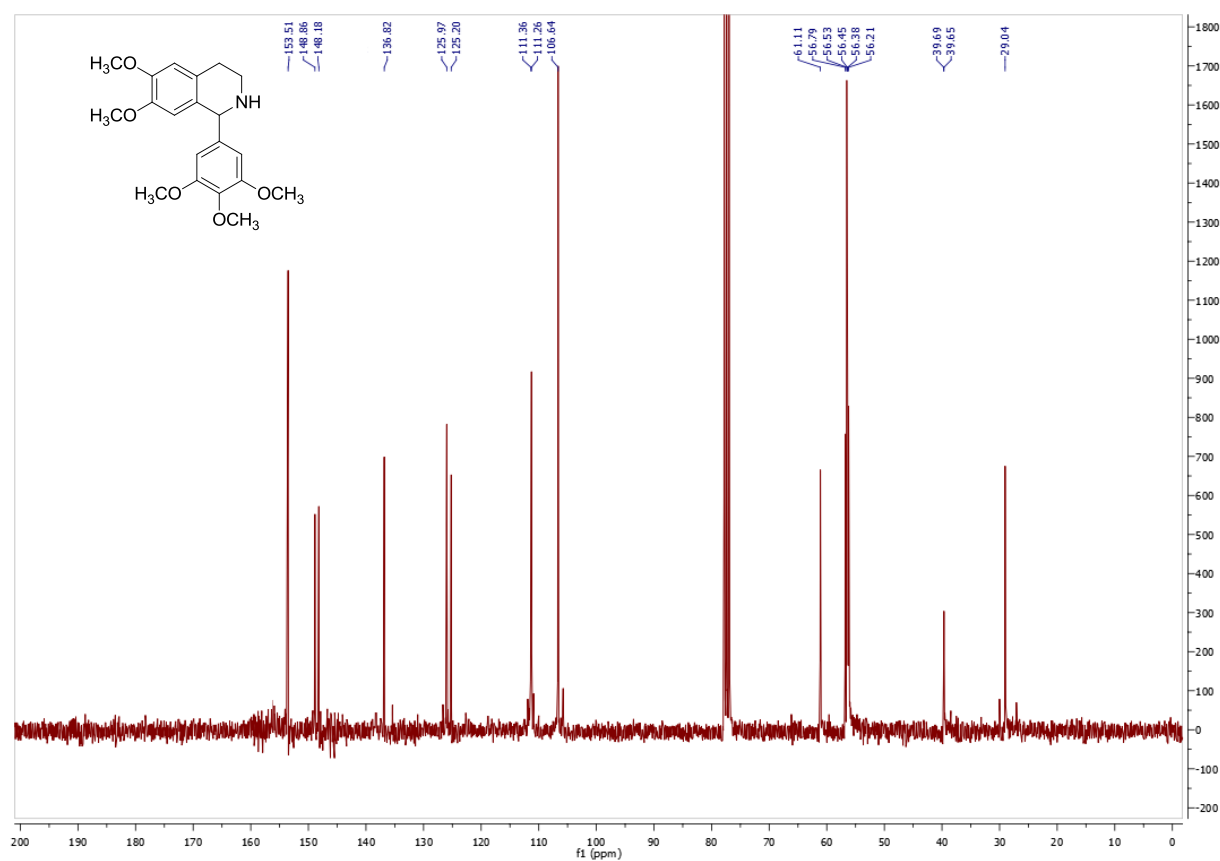
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 51
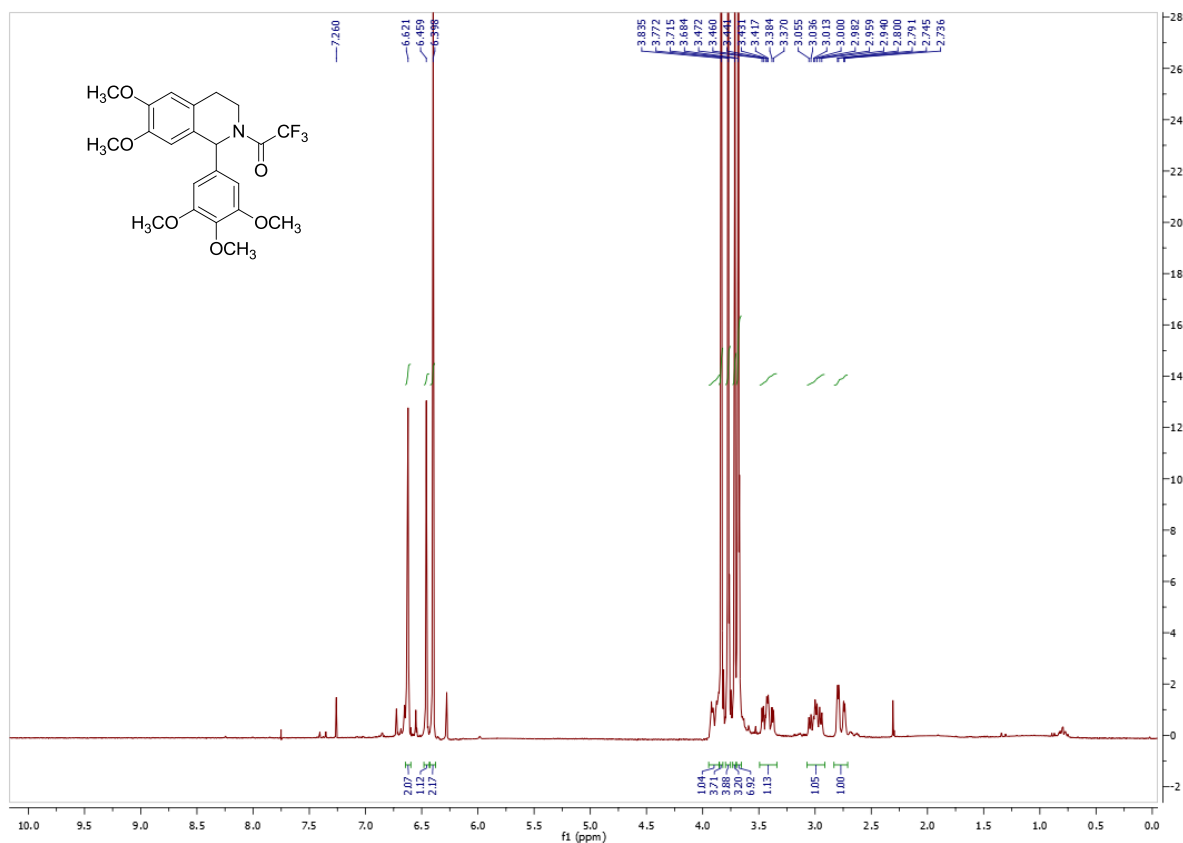
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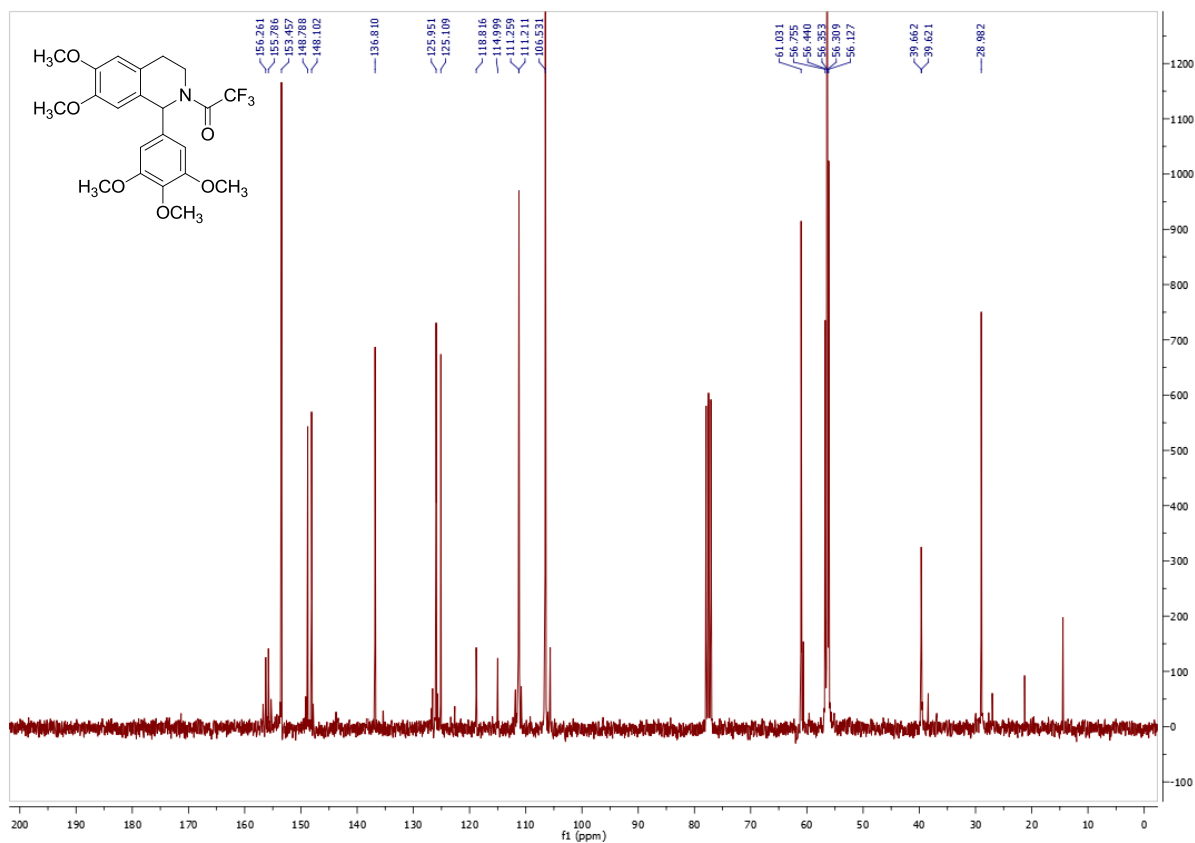
RMN ^{1}H (300 MHz, Acetone-*d6*) of compound 53
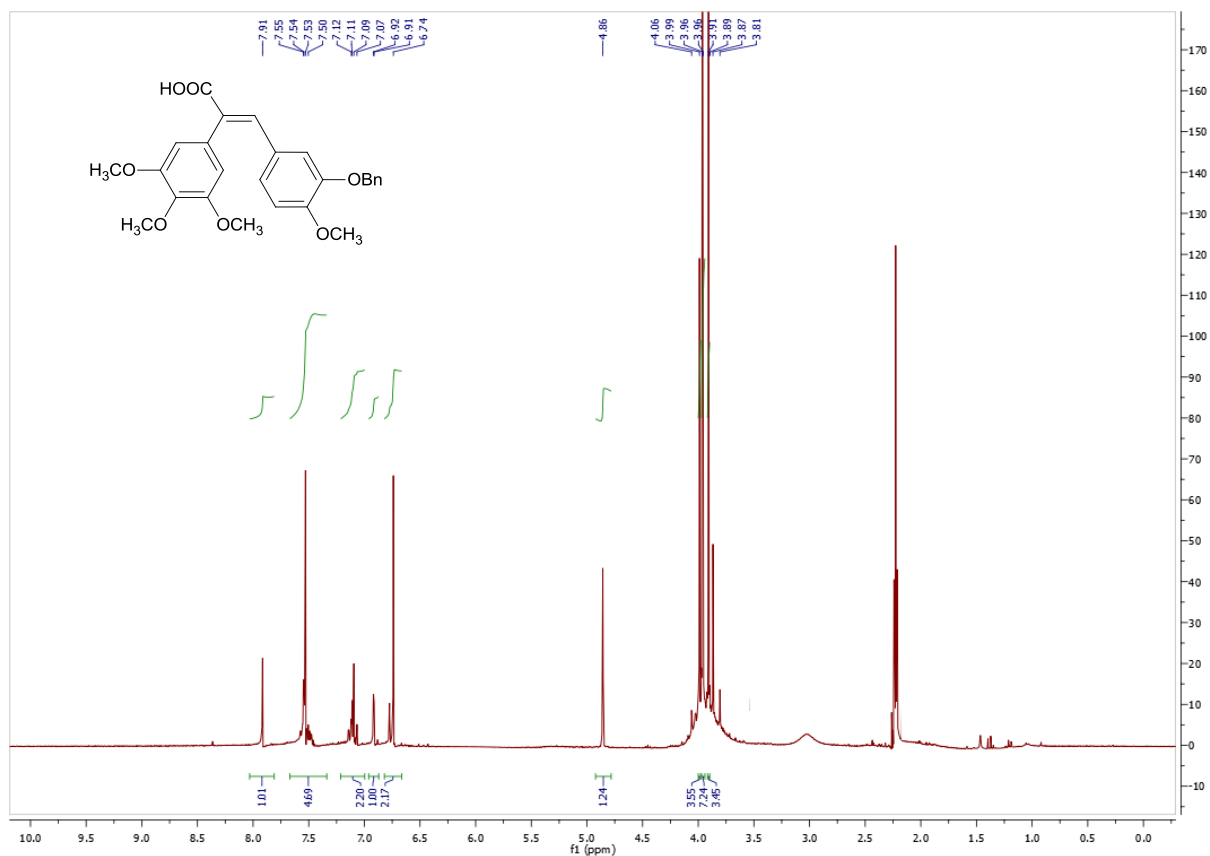
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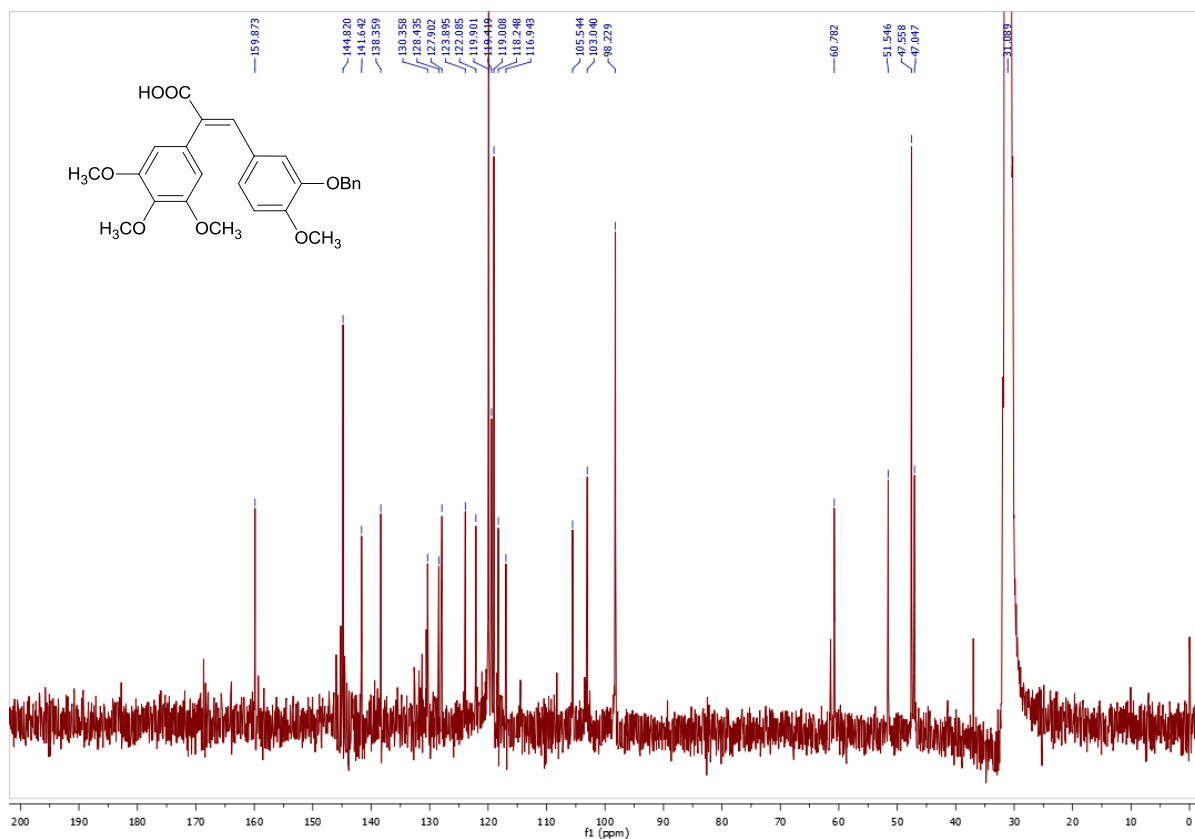
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 54
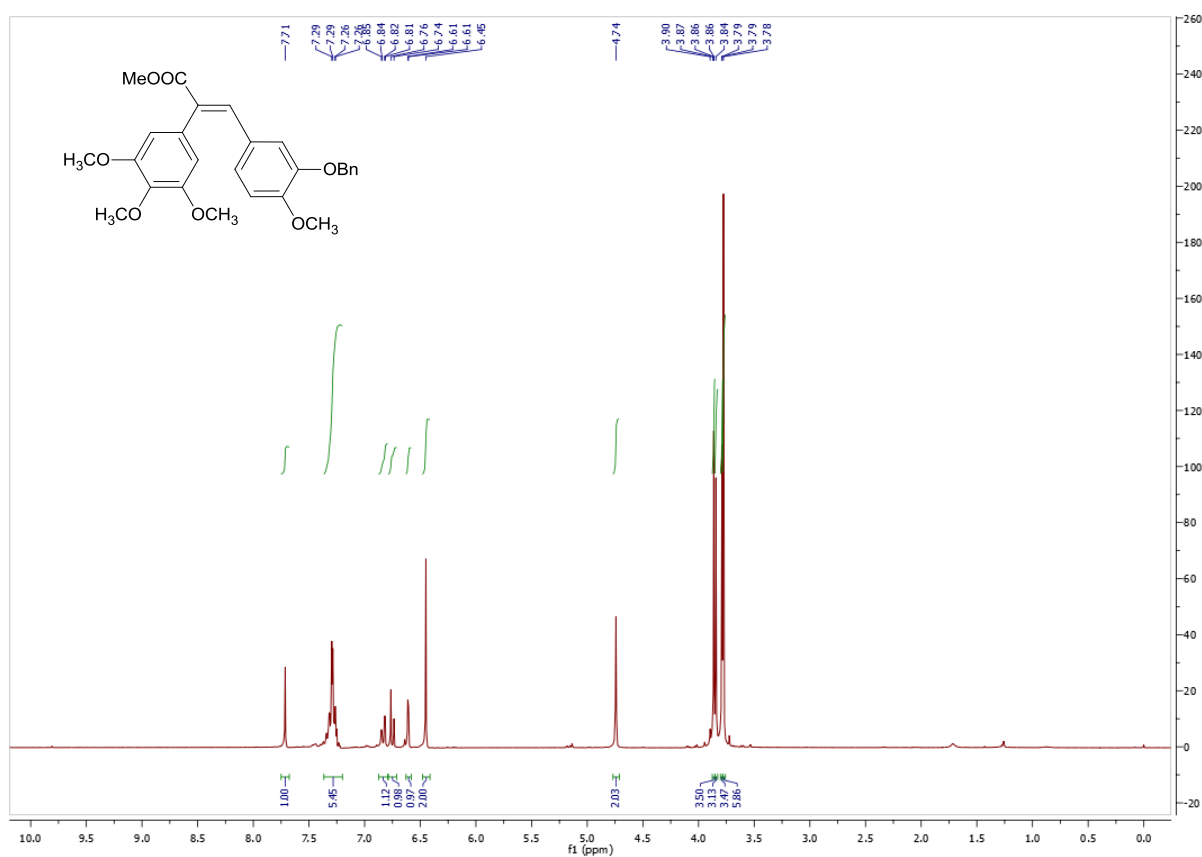
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 54
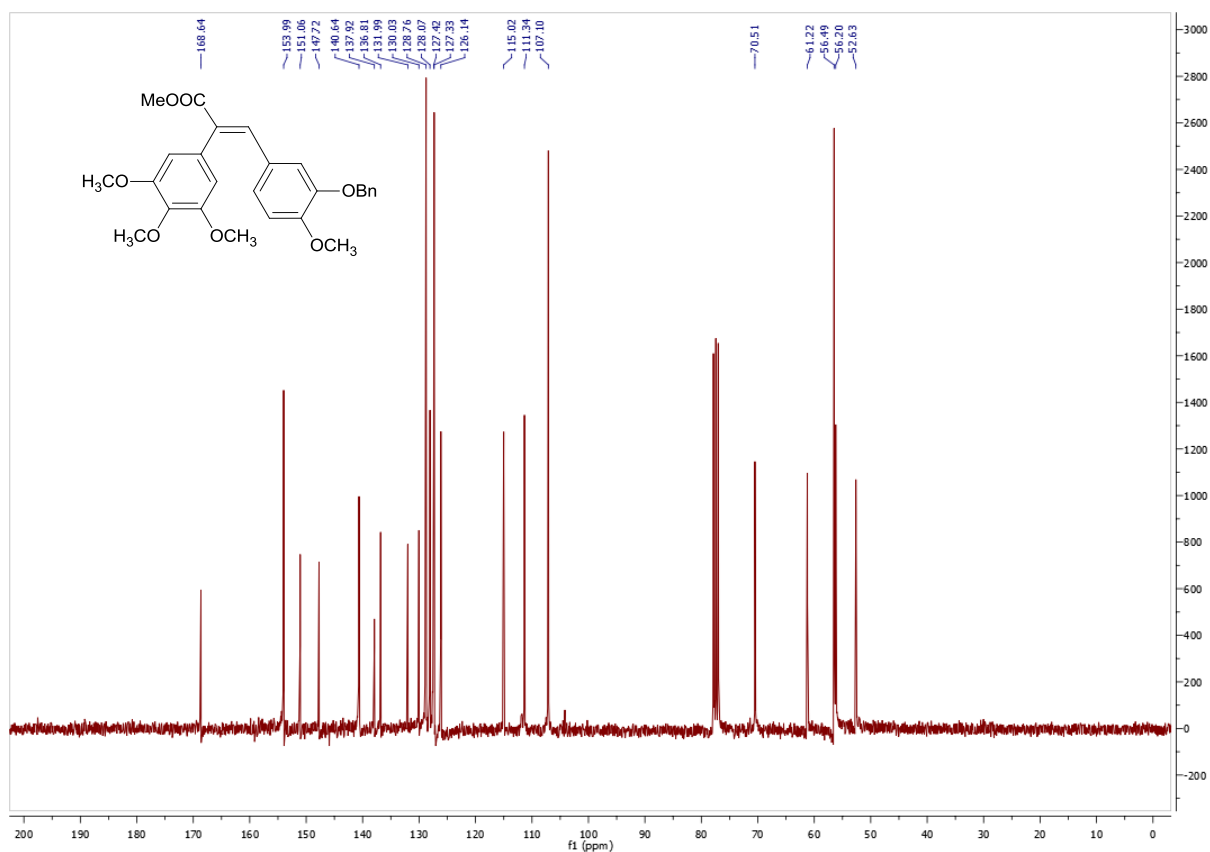
RMN ^{1}H (300 MHz, CDCl$_3$) of compound 55a
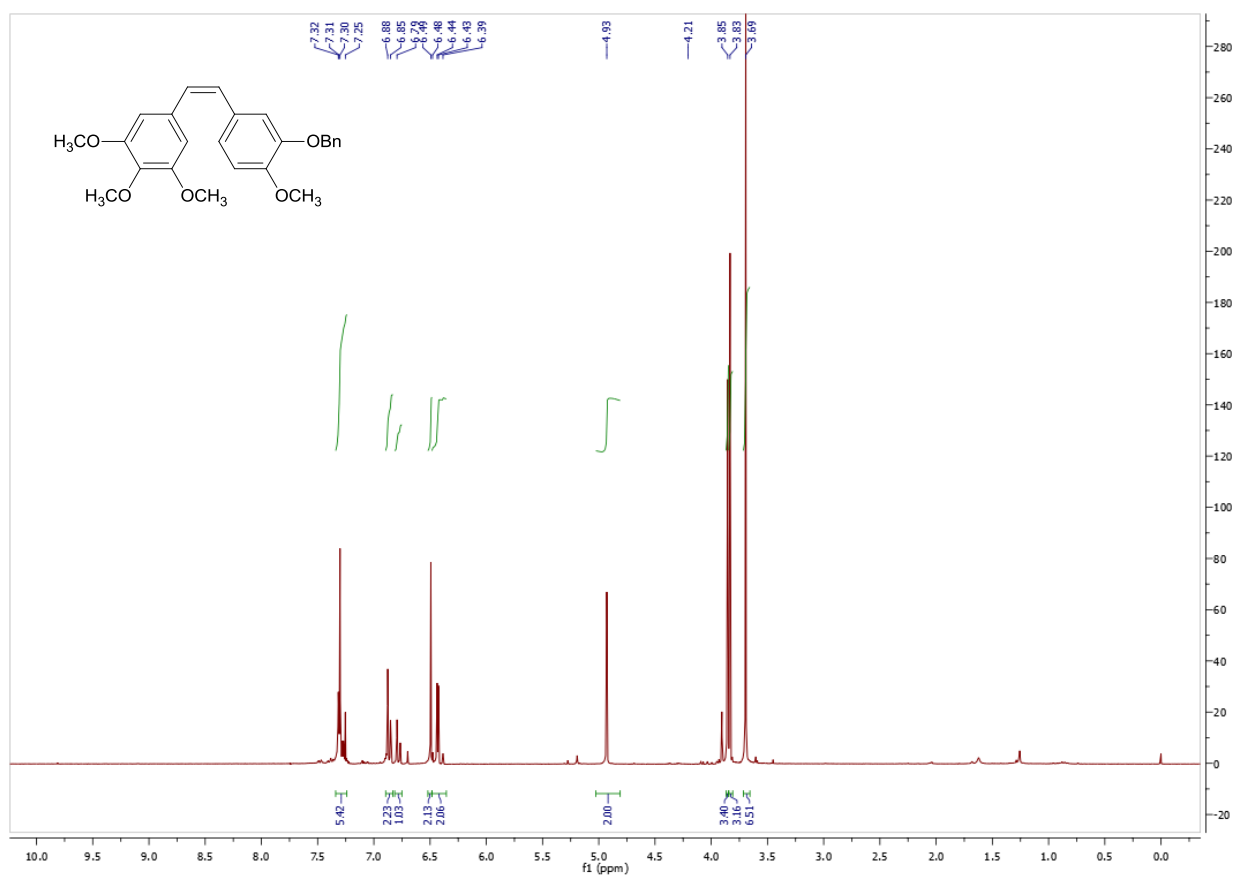
RMN ^{13}C (75.5 MHz, CDCl$_3$) of compound 55a
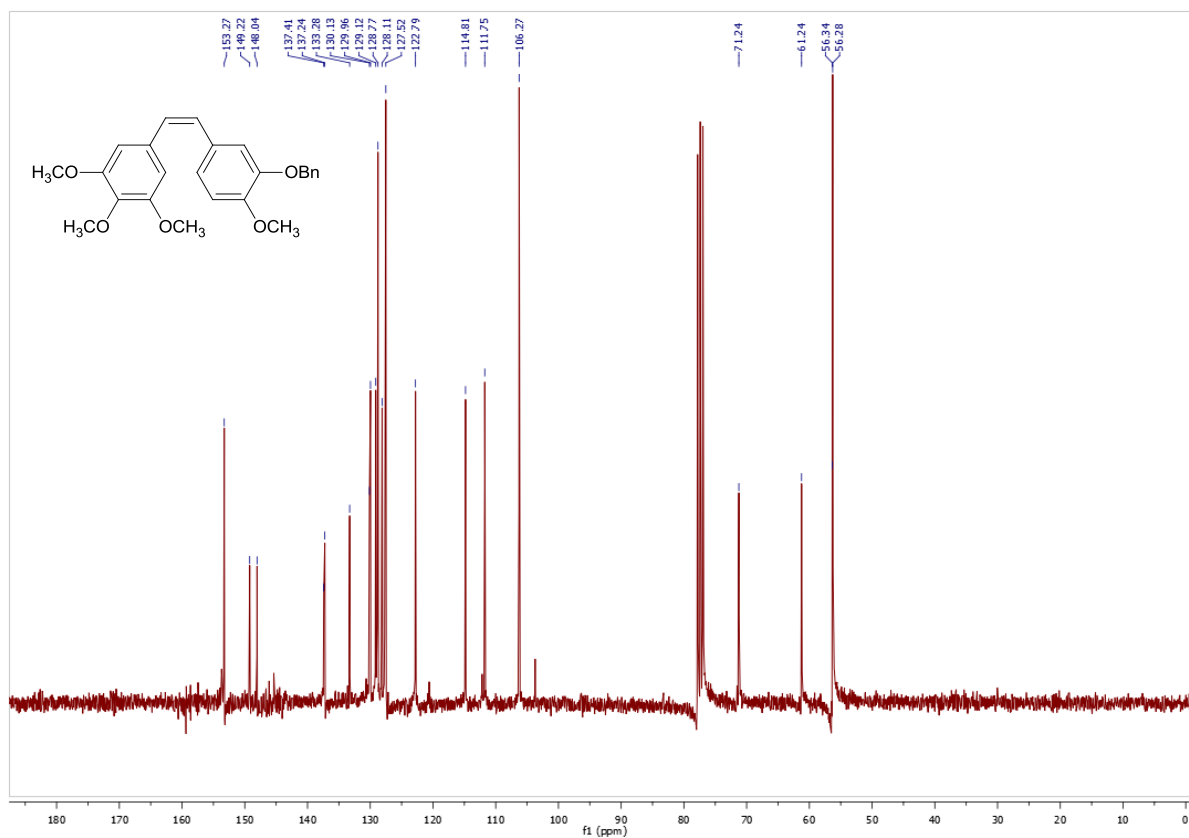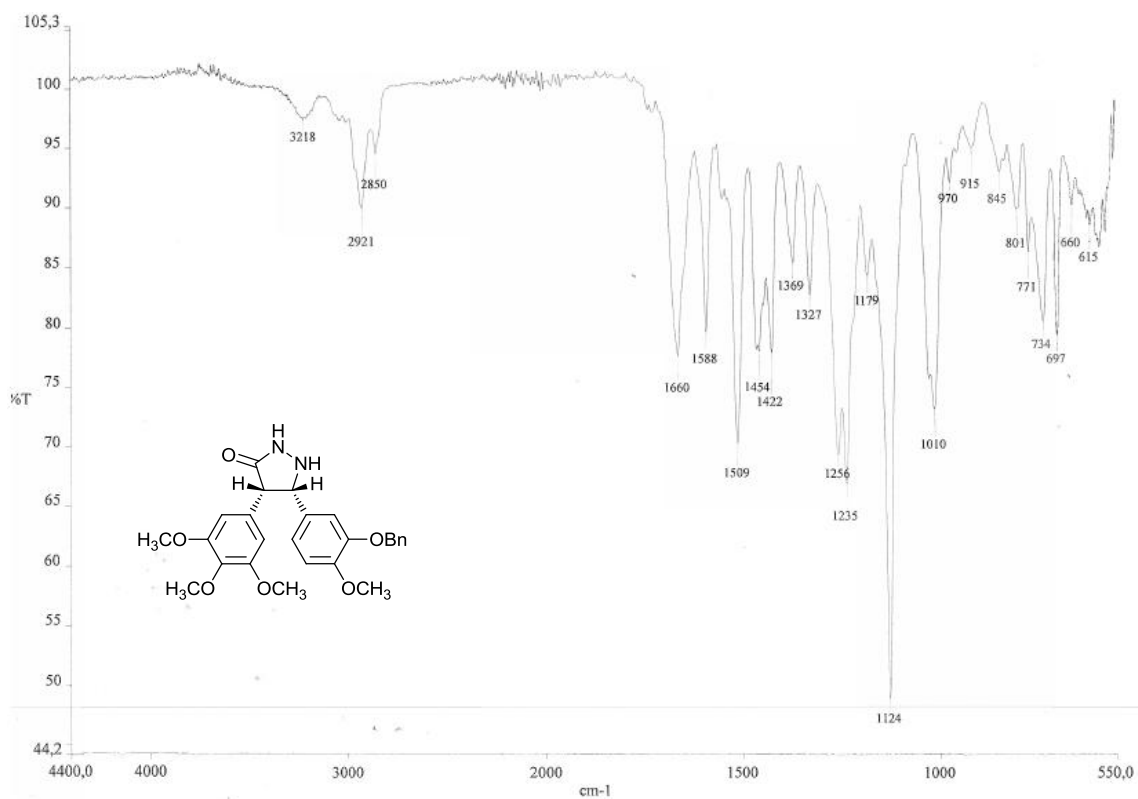
IR (film) of compound 1
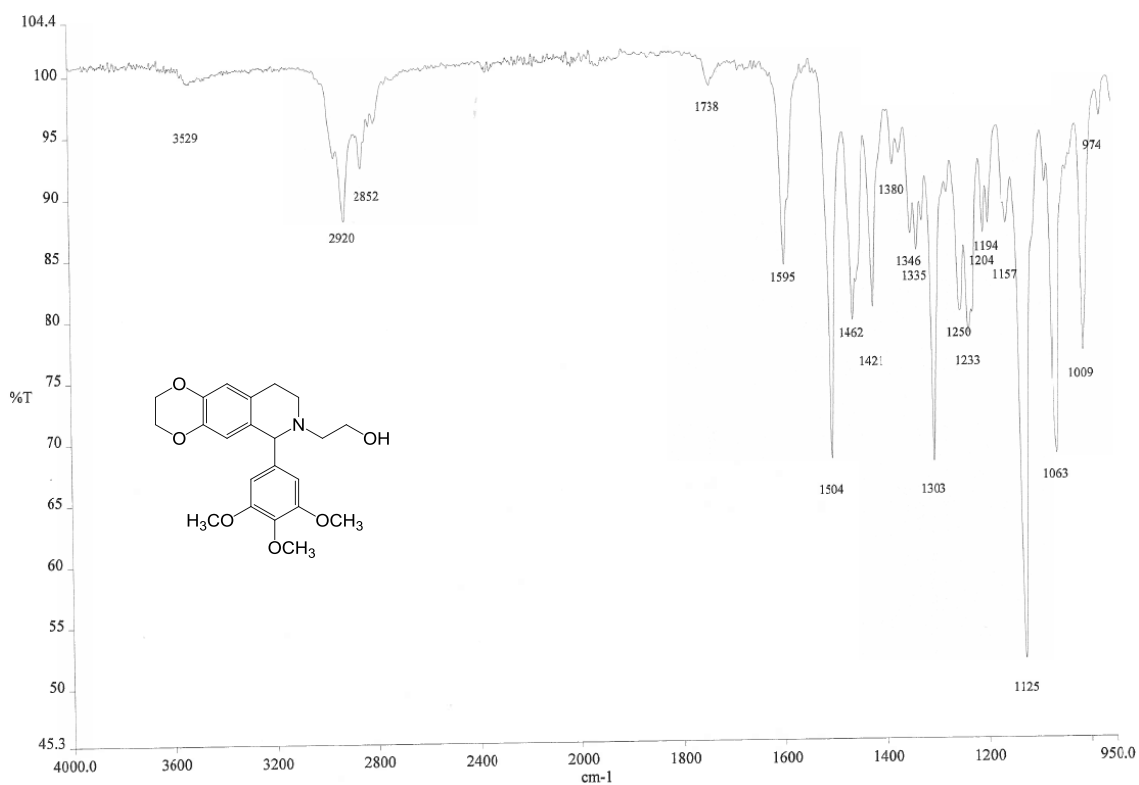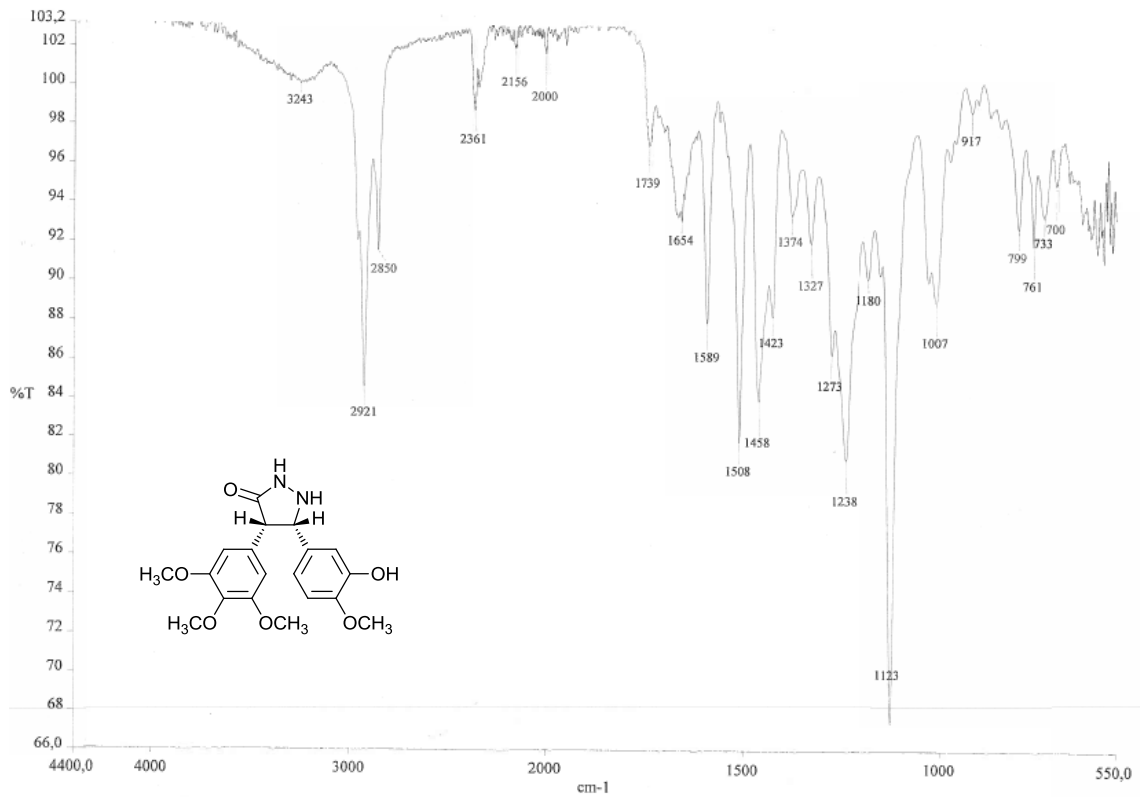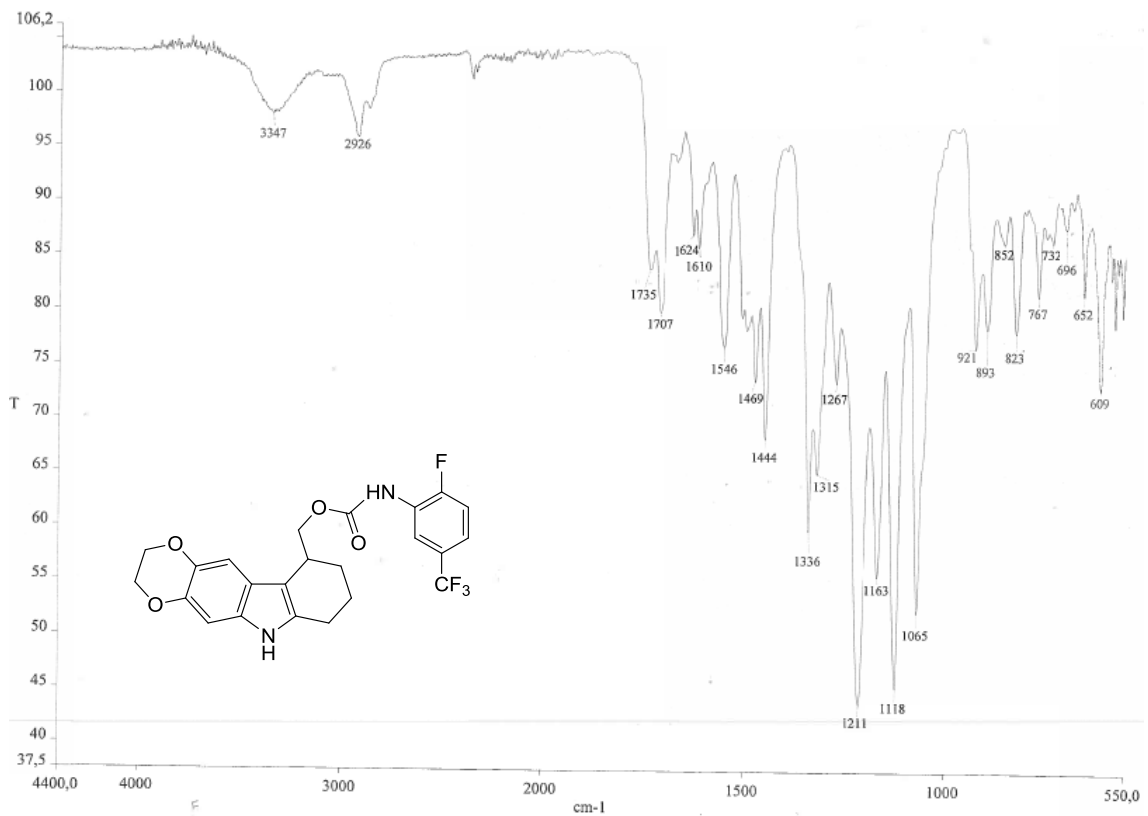

IR (film) of compound 4
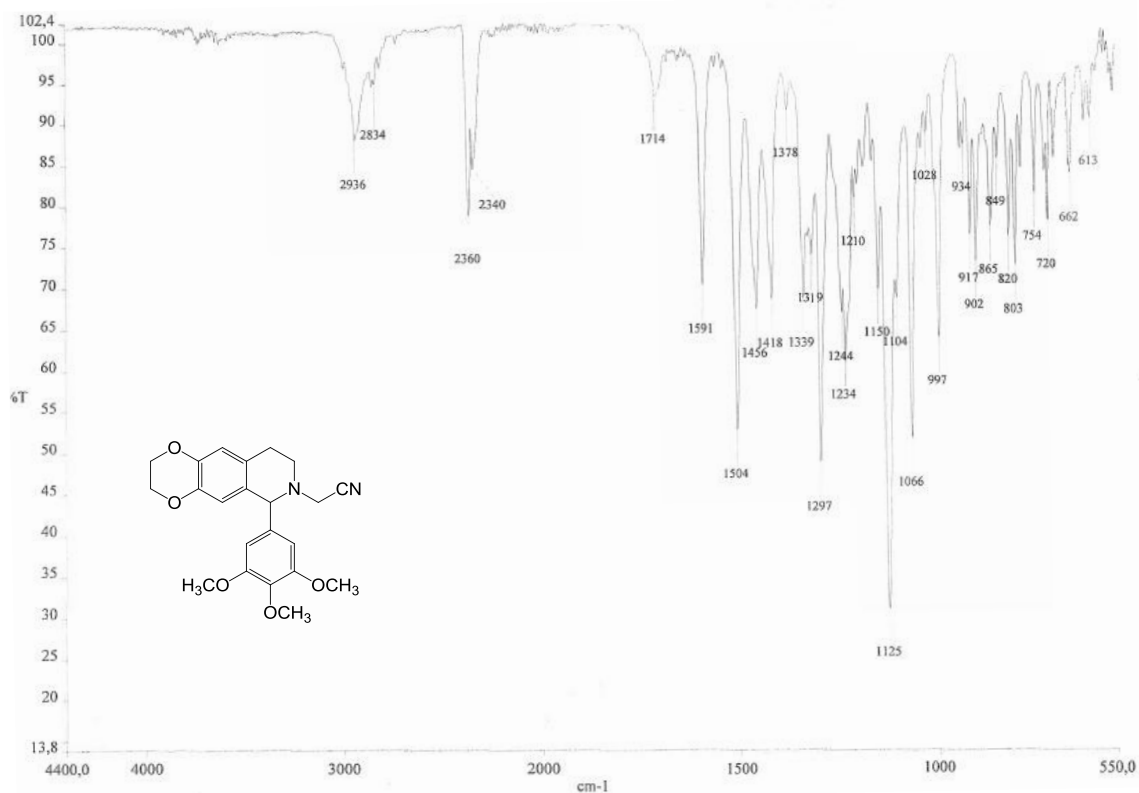
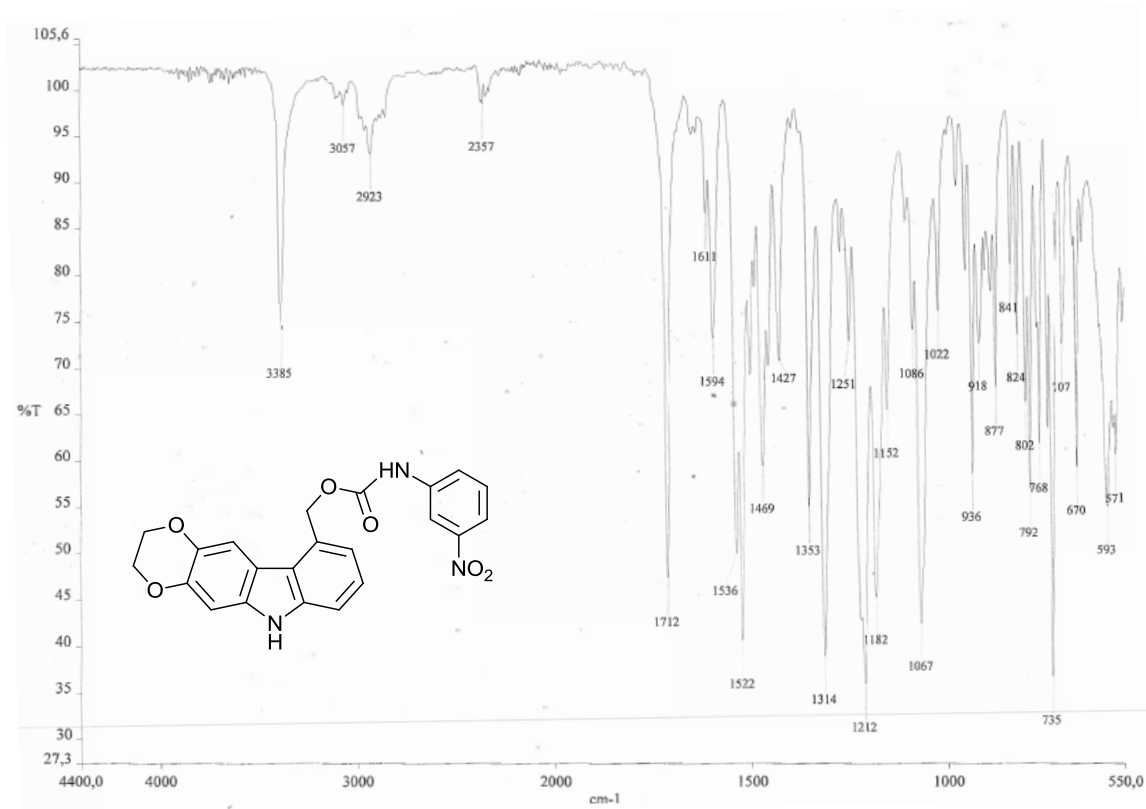# IR (film) of compound 5
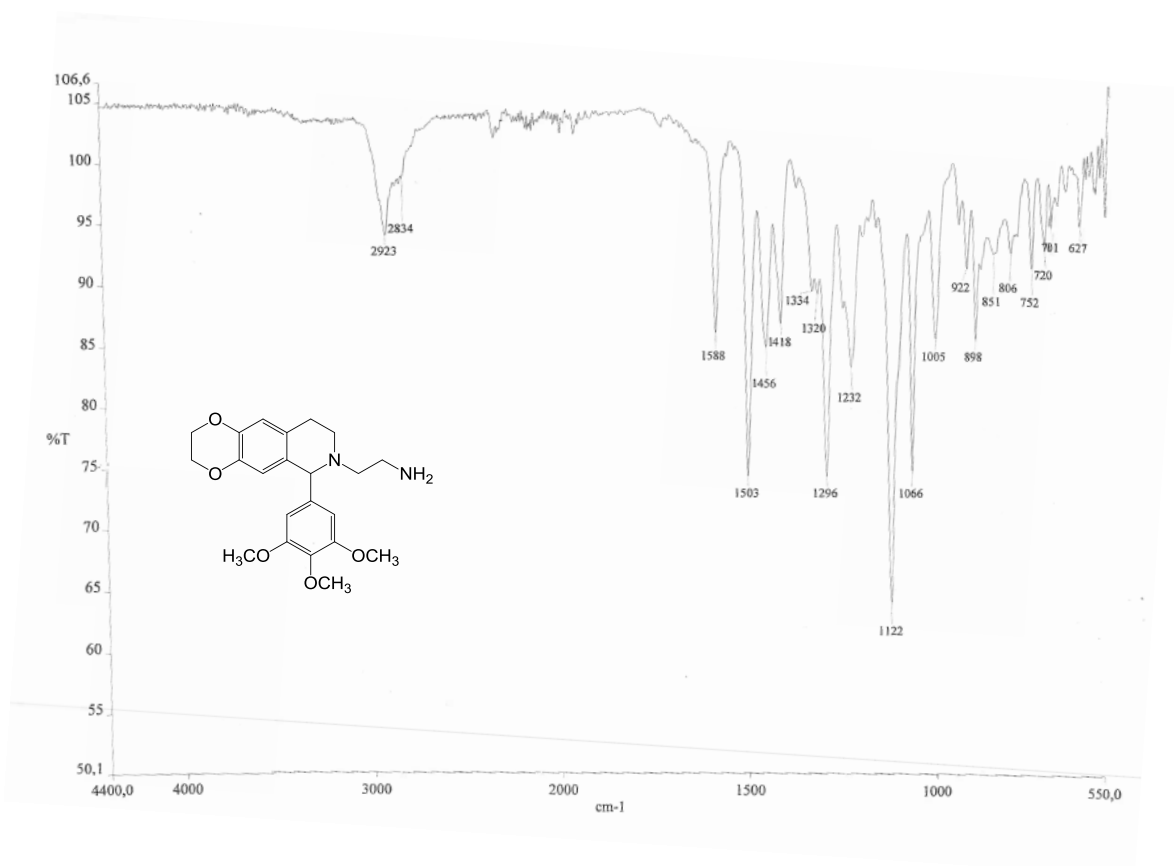
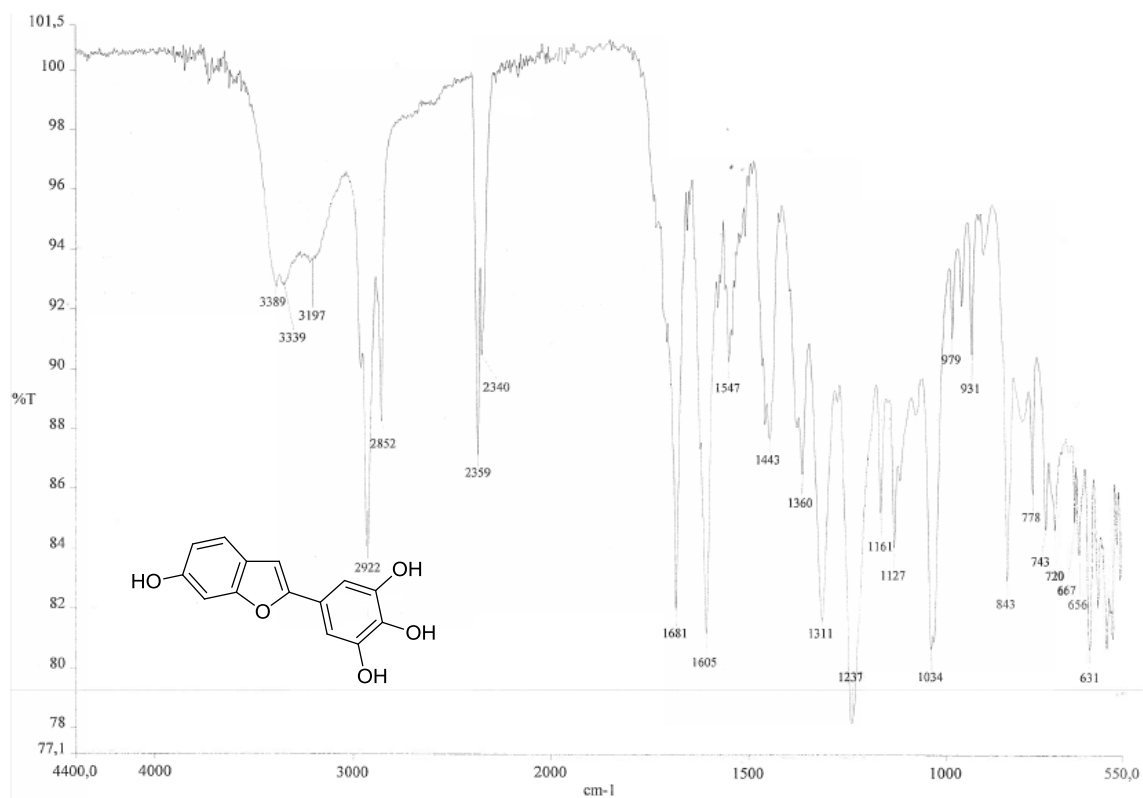
IR (film) of compound 6
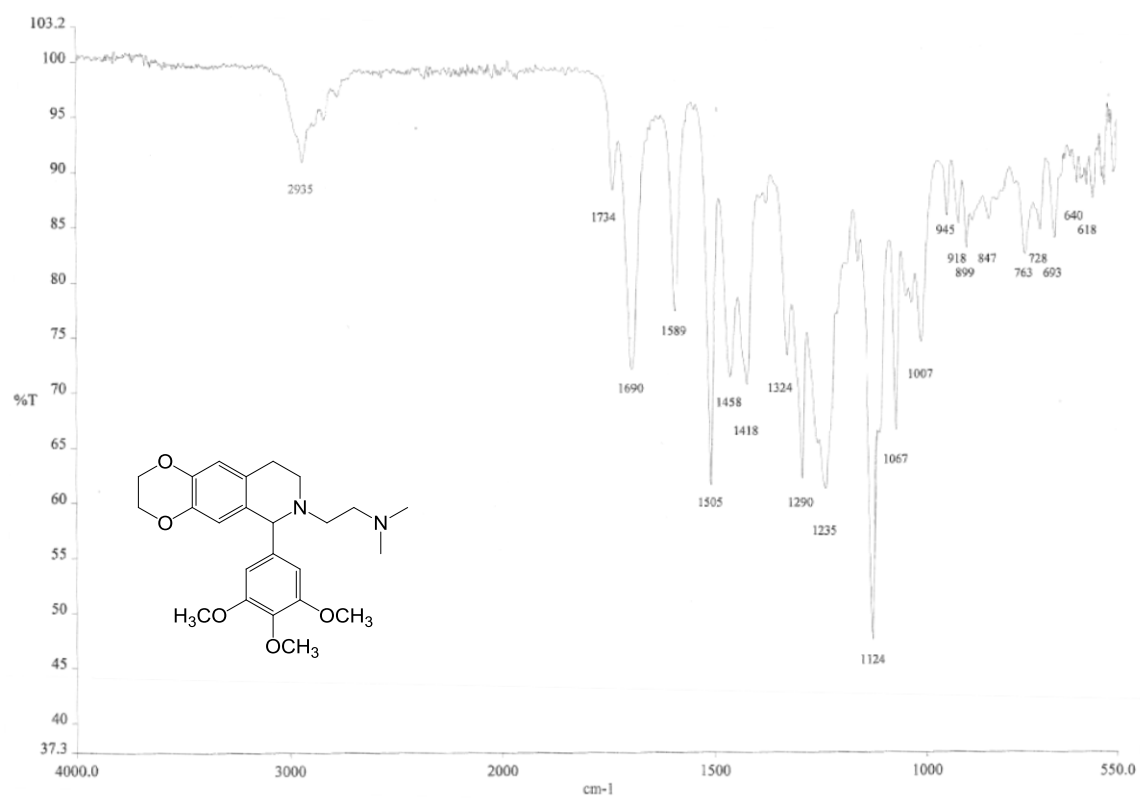
IR (film) of compound 7
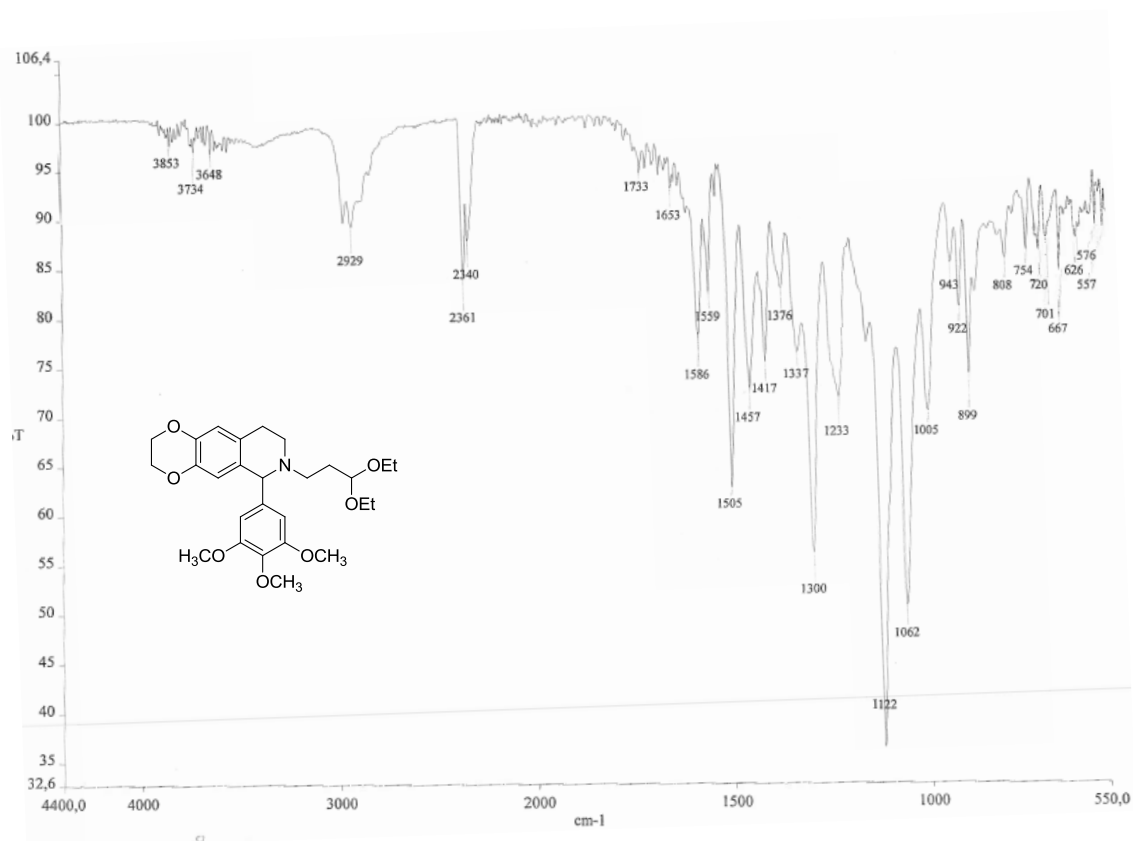
IR (film) of compound 8
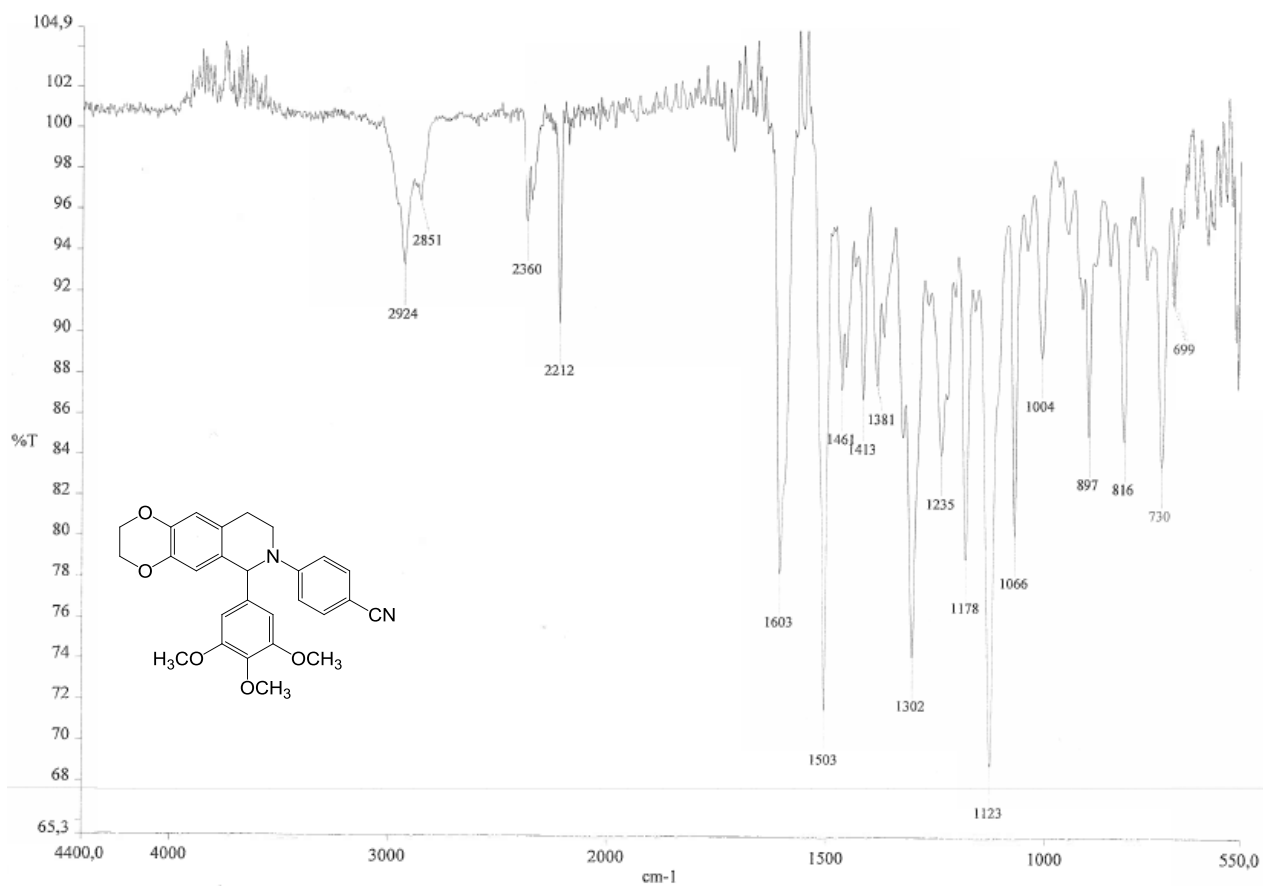
IR (film) of compound 9
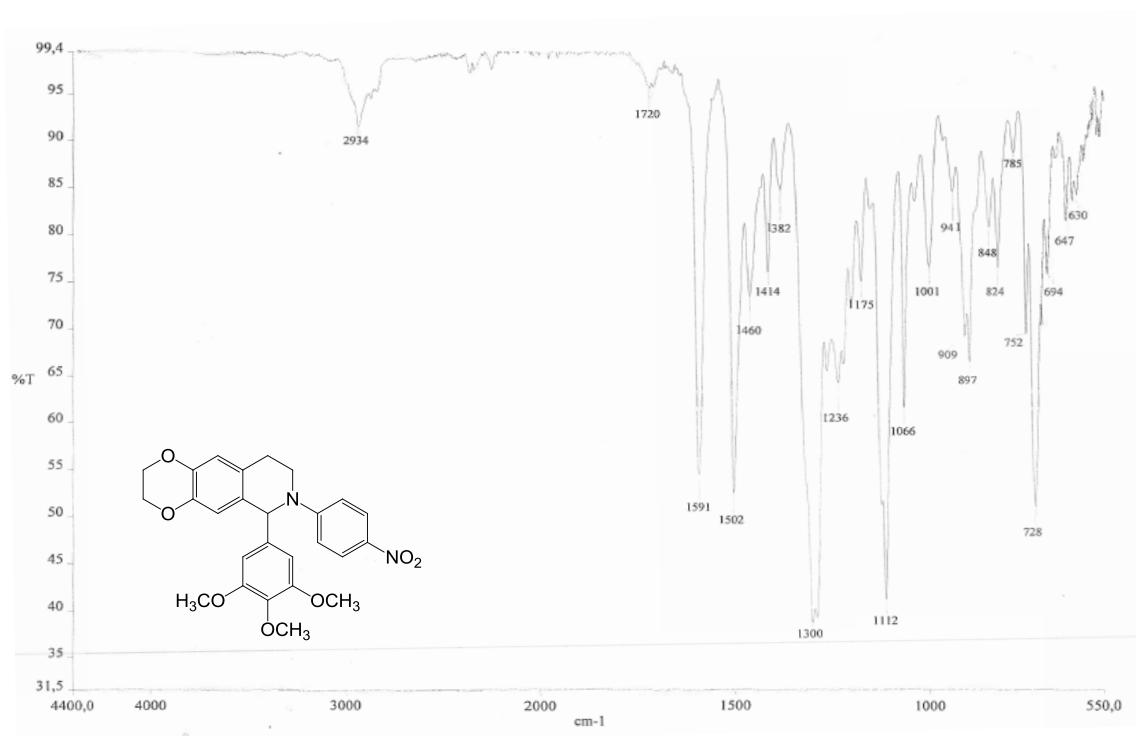
IR (film) of compound 10

IR (film) of compound 11

IR spectrum showing peaks at 3279.0, 2919.3, 2849.5, 1641.5, 1582.6, 1556.6, 1505.1, 1461.3, 1415.2, 1383.7, 1336.3, 1288.7, 1247.0, 1229.8, 1215.5, 1187.7, 1170.0, 1150.8, 1120.3, 1081.3, 998.8, 968.8, 908.8, 856.5, 765.6, 766.0, 727.5, 719.5, 658.9, 645.4, 624.5, 604.2 cm-1

IR (film) of compound 14

IR spectrum showing peaks at 3289.55, 2996.02, 2935.82, 2353.73, 1765.40, 1707.70, 1597.84, 1574.83, 1506.17, 1458.71, 1416.39, 1350.34, 1289.22, 1254, 1229.43, 1121.19, 1029.24, 991.55, 911.06, 856.58, 790.77, 750.30, 702.05, 606.75 cm-1

IR (film) of compound 15

IR (film) of compound 16

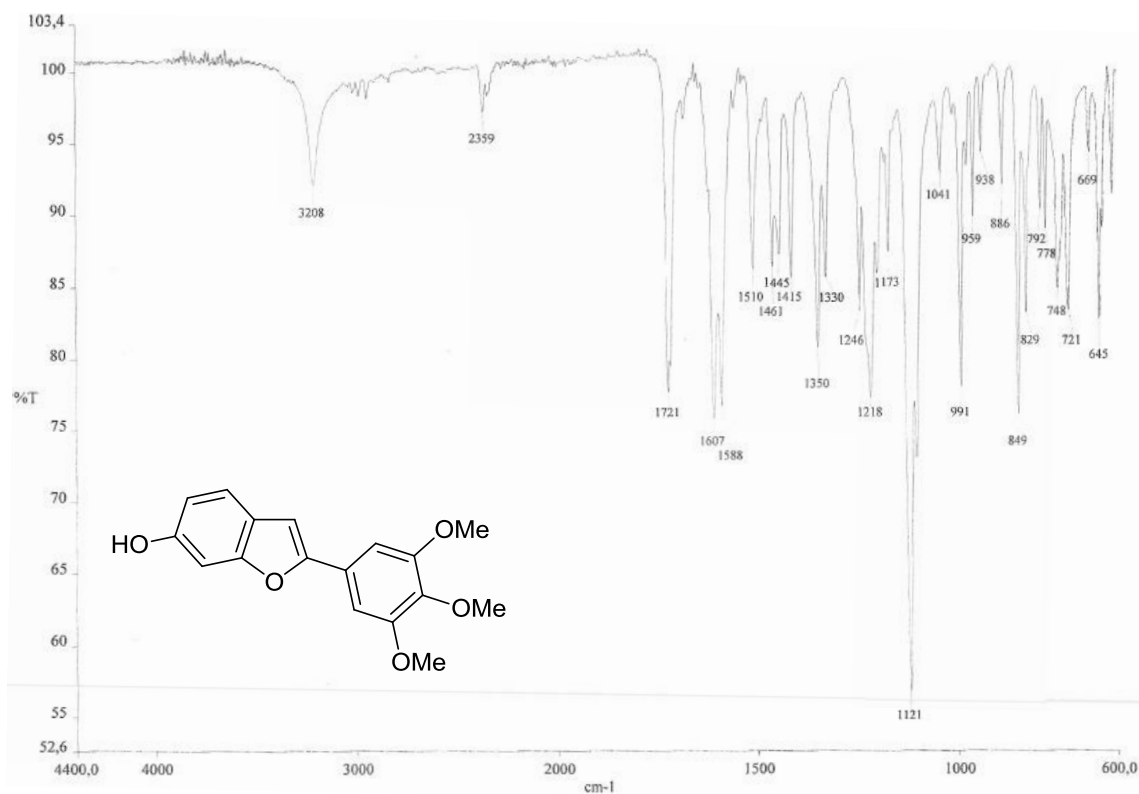## IR (film) of compound 17

IR (film) of compound 21
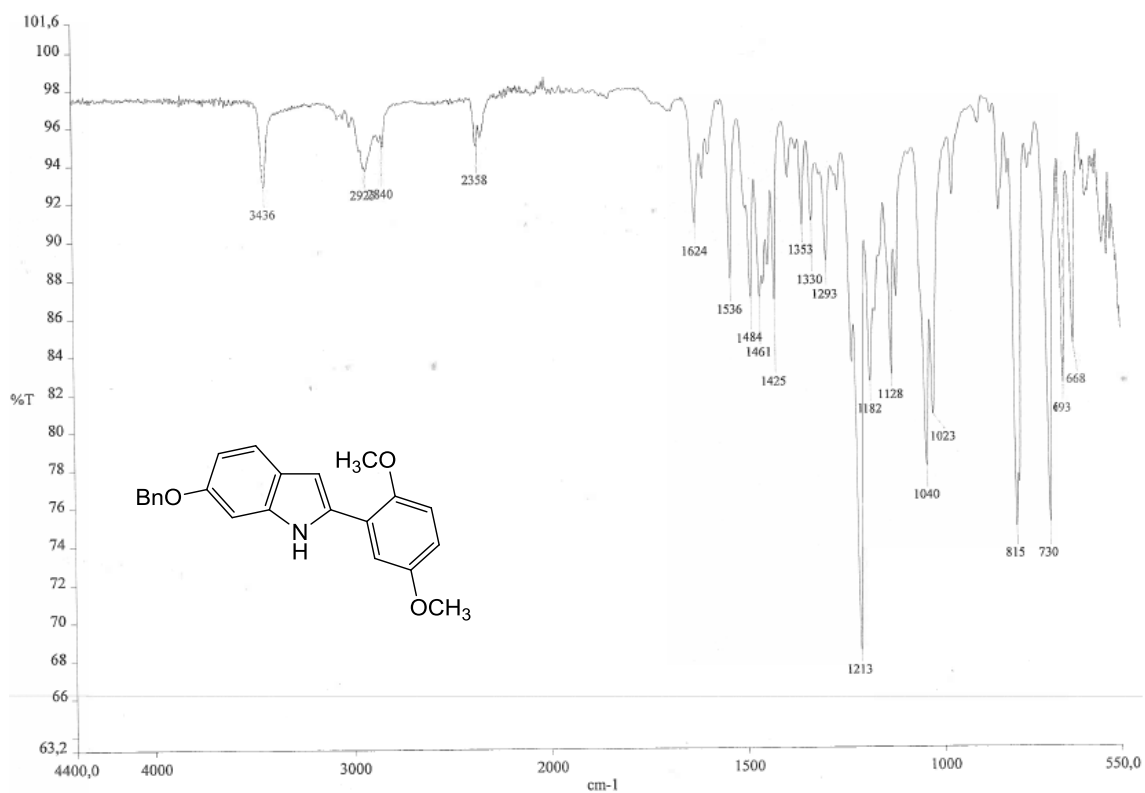
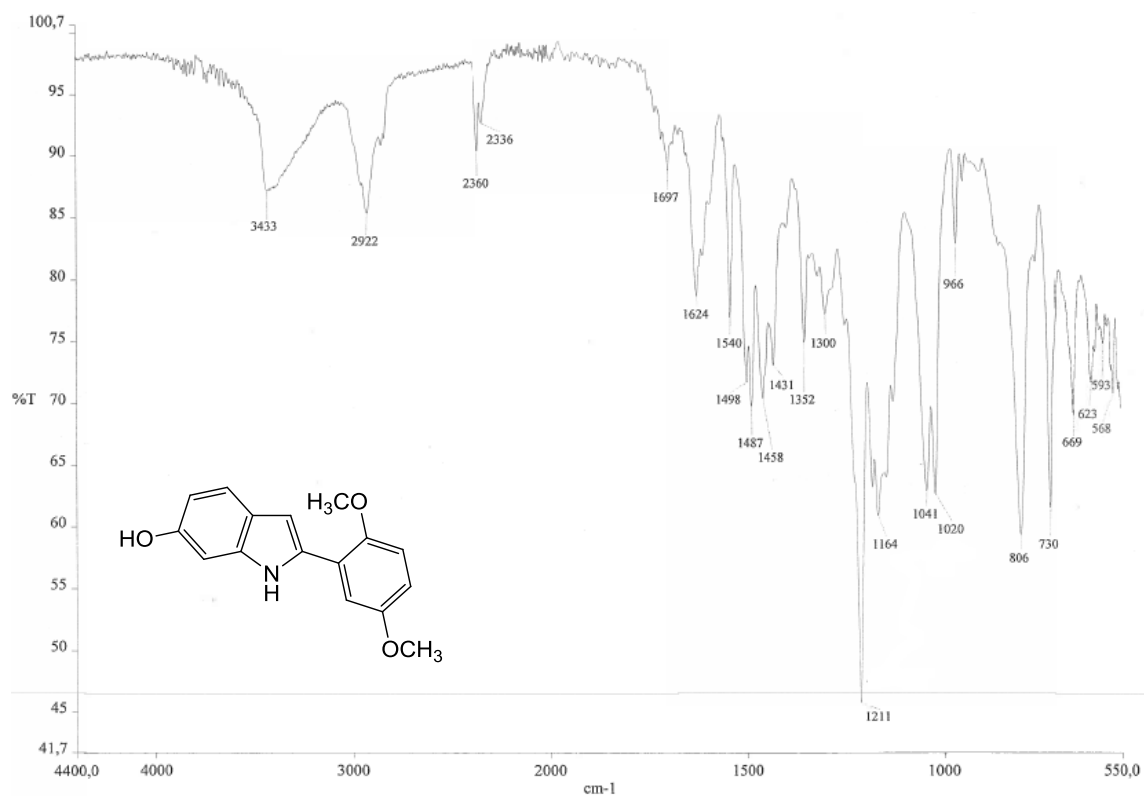## IR (film) of compound 22

IR (film) of compound 23
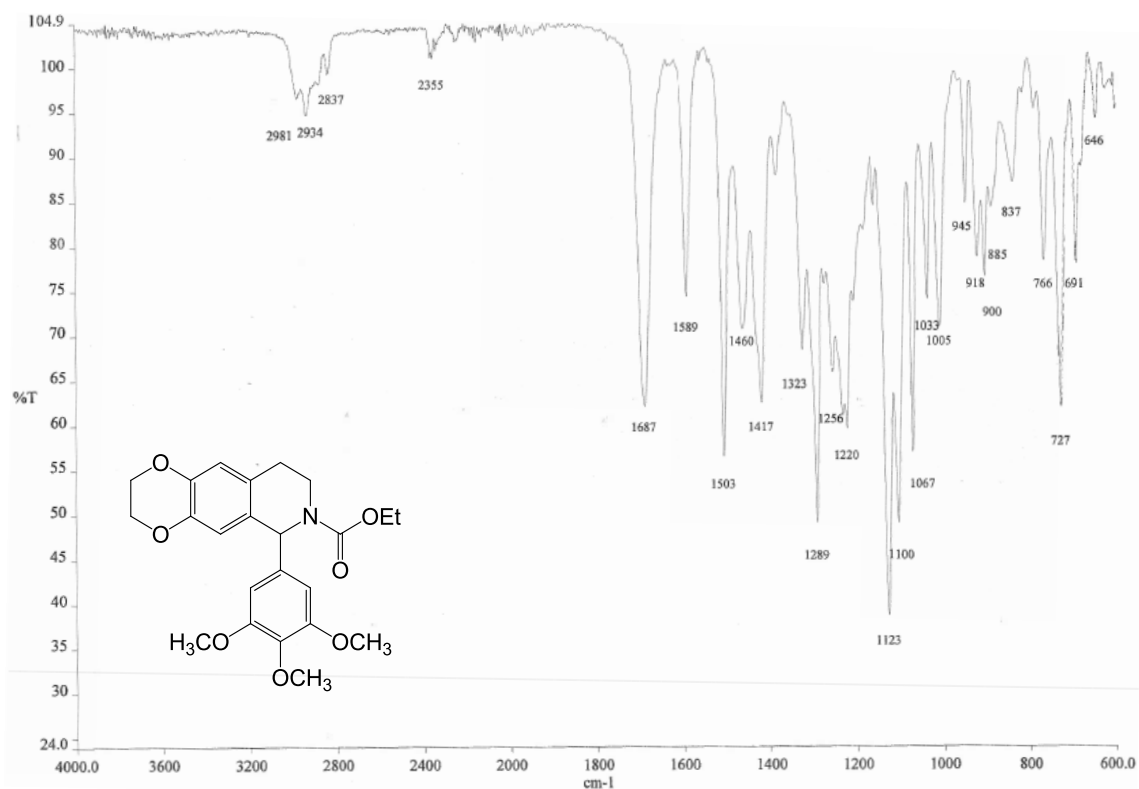
IR (film) of compound 24
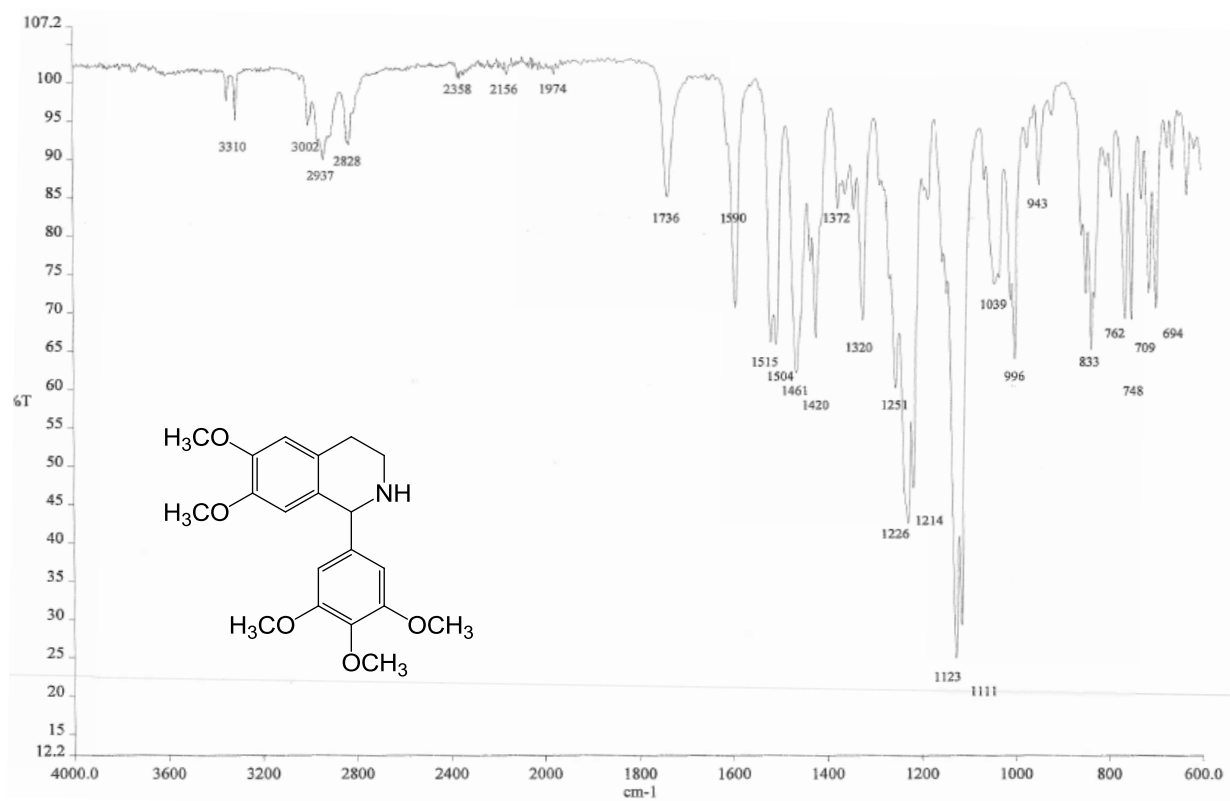

IR (film) of compound 25

IR (film) of compound 26

IR (film) of compound 45

IR (film) of compound 51

Abstract

The study deals with the synthesis and biological activity of 4 different antitumours: Tetrahydro(1,4)-dioxanisoquinolines, *combretastatin* A-4, dioxancarbazoles and *resveratrol* analogues. This study describes the synthesis strategy and multi-step synthesis of these antitumour compounds. Various tetrahydroisoquinolines were synthesized, five of which were biologically tested and have a promising K-ras inhibition activity. Two of them show a high antiangiogenesis activity, one of which also presents antiosteoporosis properties. Two pirazolone derivatives were synthetized and biologically tested, one of which shows a very high K-Ras inhibition, antiangiogenesis and antiosteoporosis activity and inhibits the G_2 cell cycle phases and subphases. Two Azoledione *combretastatin* A-4 derivatives were also synthesized and will be biologically tested. Two dioxancarbazoles were prepared and biologically tested, one of which shows a high K-ras and angiogenesis inhibition. Finally, four *resveratrol* analogues were synthesized and biologically tested. Biological results show a moderate and selective K-ras inhibition on the studied cell lines. In the future, the rest of the synthesized compounds are to be biologically tested. Further CDK and topoisomerase II inhibition trials as well as toxicity studies will be carried on.