UNIVERSITÉ DU QUÉBEC À MONTRÉAL

# ISOLATION AND CHEMICAL MODIFICATIONS OF NATURAL PRODUCTS FROM MEDICINAL PLANTS

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# ISOLATION ET MODIFICATIONS CHIMIQUES DES PRODUITS NATURELS ISSUS DES PLANTES MÉDICINALES

MÉMOIRE
PRÉSENTÉ
COMME EXIGENCE PARTIELLE
DE LA MAÎTRISE EN CHIMIE

PAR
MELISSA BARRERA TOMAS

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| | |
|---|---|
| Å | Angstrom |
| α | Alpha |
| Abs | Absorbance |
| Ac | Acetyle |
| arom. | Aromatic |
| β | Beta |
| B | Base |
| Bz | Benzene |
| *c* | Concentration (g/100 mL) |
| C | Carbone |
| °C | Temperature |
| $CDCl_3$ | Deuterated chloroform |
| $(CD_3)_2SO$ | Deuterated dimethyl sulfoxide |
| $CH_2Cl_2$ | Dichloromethane |
| cm | Centimeter |
| COSY | **CO**rrelation **S**pectroscop**Y** |
| d | Doublet |
| dd | Doublet of doublets |
| DCM | Dichloromethane |
| DIC | Diisopropylcarbodiimide |
| DIPEA | *N,N'*-diisopropylethylamine |
| DMF | *N,N*-Dimethylformamide |

| | |
|---|---|
| DMSO | Dimethyl sulfoxide |
| *e* | Electron |
| *E. coli* | *Escherichia coli* |
| eq. | Equivalent |
| ESI | Electrospray ionization |
| EtOAc | Ethyle acetate |
| $Et_2O$ | Diethyl ether |
| $Et_3N$ | Triethylamine |
| $F_{254}$ | Silica gel technical grade |
| g | Gram |
| H | Hydrogen |
| $H_2$ | Dihydrogen |
| $H_2SO_4$ | Sulfuric acid |
| h | Hour |
| HCl | Acide Chloride |
| Hex | Hexane |
| HIV | Human immunodeficiency virus |
| HMBC | **H**eteronuclear **M**ultiple **B**ond **C**orrelation |
| HOAt | 1-Hydroxy-7-azabenzotriazole |
| HOBt | 1-Hydroxybenzotriazole |
| HODhbt | 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one |
| Hz | Hertz |
| $IC_{50}$ | Half maximal inhibitory concentration ($IC_{50}$) |

| | |
|---|---|
| IR | Infrared |
| *J* | Coupling constant |
| Km | Kilometers |
| $KHCO_3$ | Potassium bicarbonate |
| $LC_{50}$ | Median lethal dose |
| M | Molar, concentration (g/mol) |
| m | Multiplet |
| Me | Methyle |
| MHz | MegaHertz |
| MICs | Minimun inhibitory concentrations |
| min | Minute |
| mL | Milliliter |
| mmol | Millimole |
| mm | millimeter |
| *m/z* | Mass/charge ratio |
| NaCl | Sodium chloride |
| $NaHCO_3$ | Sodium bicarbonate |
| NOE | Nuclear overhauser effect |
| NOESY | **N**uclear **O**verhauser **E**ffect **S**pectroscopy |
| $N_2$ | Nitrogen gas |

| | |
|---|---|
| $Pd^{\circ}$ | Palladium zero |
| Pd/C | Palladium on carbon |
| $Pd(OAc)_2$ | Palladium(II) acetate |
| ppm | Parts per million |
| pyr | Pyridine |
| | |
| *Rf* | Retention factor |
| RMN | **R**ésonance **M**agnétique **N**ucléaire |
| | |
| s | Singlet |
| | |
| TBAB | Tetrabutylammonium bromide. |
| TBTU | 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate |
| TLC | Thin layer chromatography |
| TMS | Tetramethylsilane |
| | |
| µ | Micro |
| µL | Microliter |
| µM | Micromolar |

# RÉSUMÉ

Les organismes végétaux et animaux offrent une large gamme de produits naturels pouvant servir de médicaments ou source d'inspiration dans le développement de nouveaux agents thérapeutiques. En particulier, la synthèse de leurs analogues peut conduire à l'amélioration de leurs propriétés essentielles telles que la solubilité et la biodisponibilité dans le corps humain. Dans ce contexte, ce projet vise à valoriser deux produits naturels connus selon leurs activités biologiques: l'acide usnique, isolé du lichen *Evernia prunastri* du Pérou et la *trans*-fagaramide, isolée de la plante *Fagara heitzii* d'origine camerounaise. Parallèlement, en raison de leur disponibilité naturelle limitée, des méthodologies de synthèse donnant accès à de plus grandes quantités de ce dernier ont été développées au laboratoire. Dans ce contexte, la synthèse de la *trans*-fagaramide a été effectuée en utilisant une condensation de type Knoevenagel faisant intervenir l'acide maléique et le piperonal, suivie d'un couplage peptidique en présence d'isobutylamine, avec un rendement global de 56%. Une librairie de 61 analogues dérivés de la *trans*-fagaramide a été développée à partir de réactions d'estérification, d'amidation et de couplage organométallique de type Heck. De plus, cette plateforme a aussi été un précurseur pour la synthèse du régioisomère d'autre produit naturel connu comme l'Heitziamide A (65 %) en utilisant une cycloaddition de Diels-Alder. Le projet sera par la suite complété par l'élaboration d'analogues de l'acide usnique nouvellement isolé dans le but d'obtenir des candidats synthétiques efficaces ayant des propriétés pharmacologiques optimisés visant particulièrement les cellules cancéreuses.

**Mots-clés**: *trans*-Fagaramide, Heitziamide A, *Fagara heitzii,* acide usnique, *Evernia prunastri,* condensation de Knoevenagel, couplage peptidique, réactions d'estérification, couplage organométallique de type Heck, cycloaddition de Diels-Alder, agents thérapeutiques, cellules cancéreuses.

## SUMMARY

Natural substances extracted from plants or animal sources represent a wide field in pharmacological research, showing a great potential for the development of new drugs. In particular, the synthesis of their analogs can improve their properties such as solubility and bioavailability in the human body. In this context, the present work focuses on the study of two compounds: "usnic acid„ isolated from lichen "*Evernia prunastri*„ (Peru) and "*trans*-fagaramide„ isolated from the plant *Fagara heitzii* (Cameroon). Their isolation was performed using standard protocols of extraction and chromatographic purification. Considering the range of their known biological activities, including inhibition of carcinogenic processes, the next part of the project concerned their synthesis and chemical modifications in order to improve their biopharmaceutical properties. As the natural occurrence of these compounds is quite limited, synthetic methodology giving access to larger quantities of *trans*-fagaramide has been developed. In this context, the synthesis of *trans*-fagaramide was achieved by Knoevenagel condensation using maleic acid and piperonal, followed by peptide coupling with isobutyl amine giving an overall yield of 56%. This synthetic platform has been used as starting material for the elaboration of a chemical library of 61 analogs derivatives of *trans*-fagaramide generated from Fischer esterifications, palladium-mediated Heck couplings, etc... Furthermore, the platform was also a precursor for the synthesis of a regioisomer of a natural product, Heitziamide A using a Diels-Alder cycloaddition, giving a yield of 65%. The project will be subsequently completed with the synthesis of analogs of usnic acid in order to obtain effective synthetic candidates towards the determination of optimized and efficient drug candidates against cancer cells.



**Keys words:** *trans*-Fagaramide, Heitziamide A, *Fagara heitzii,* usnic acid, *Evernia prunastri,* Knoevenagel condensation, peptide coupling, Fischer esterification, palladium-mediated Heck couplings, Diels-Alder cycloadditions, drug candidates, cancer cells.

# CHAPTER I

## INTRODUCTION

Throughout our evolution, natural substances extracted from plants or animal sources have represented a wide field in medicine development. Since our earliest ancestors chewed on certain herbs to relieve pain, or wrapped leaves around wounds to improve healing, natural products have often been the basis for the treatment of injuries and diseases. In fact, it has only been during the past decades that natural products have played a basic role in drug development, including drug design and combinatorial chemistry, which make possible the rational design of chemical compounds to target specific molecules.

Modern chemistry has ushered in a new era for the study and use of natural products. Analytical chemistry and organic structural spectroscopy have provided the tools to purify various compounds and to determine their structures. Subsequently, a vast number of well-known natural compounds were identified, analysed, synthesized and functionalized to furnish optimized drug candidates against devastating diseases such as cancer. In this context, the first part of the project is focused on the study of two compounds known for their biological activities: **"usnic acid"**, isolated from lichen *Evernia prunastri* (Peru) and ***"trans*-fagaramide"**, isolated from the plant *Fagara heitzii* (Cameroon), as well as their synthesis and chemical modifications.

Lupenone, lupeol, *trans*-fagaramide and usnic acid were isolated. Their chemical structures were elucidated using 1D and 2D NMR spectroscopy and mass spectrometry, thus corroborating spectroscopic data reported in the literature. Based on the fact that the natural occurrence of these compounds is quite limited, the next aim was the achievement of synthetic *trans*-fagaramide. Finally synthetic *trans*-fagaramide has been used as starting material for the elaboration of a chemical library containing 61 analogs derivatives of *trans*-

fagaramide generated from Fischer esterification, palladium-mediated Heck coupling, Diels-Alder, etc...

As a future goal, this library will be submitted to biological test, in order to analyse if it presents biological activity against certain cancer cells.

## 1.1. FAGARA HEITZII

*Fagara heitzii or Zanthoxylum heitzii,* commonly known as "olon„, is a medicinal plant belonging to the Rutaceae family. This species rises under the humid rainforests, within southern Cameroon and the Central African Republic to Gabon and Bas-Congo. Its range also includes warm temperate and subtropical areas worldwide.

*Fagara heitzii* is characterized for being a deciduous long tree reaching up to 35 m tall, with branchless bole, usually straight and cylindrical for up to 20 m and a diameter up to 150 cm. The outer bark is grey to greenish grey and the inner bark is granular to fibrous, yellowish brown, often mottled with orange. The leaves are 100 cm long, imparipinnate. The flowers of *Fagara heitzii* are unisexual and tend to be small. The male flowers present 5 stamens, a conical thick disk and a rudimentary ovary; the female, a superior globose ovary of 1-1.5 mm long and rudimentary stamens. The fruit is distinct by a globose follicle (4 mm in diameter), glandular pitted, dehiscent, 1-seeded. The seed is black and shiny; it presents a globose form with a 2.5 mm in diameter **(Figure 1.1)**.[1]

The use of *F. heitizii* is reflected in different commercial areas. For example: *Fagara heitzii* plays important roles in construction process, perfumery and food industry as well as in "traditional medicine„. The obtention of volatile oils, which is a common feature among the plants belonging to this family, allows this plant to be considered as a source for the elaboration of new fragrances and essences.[2]

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[1] http:// database.prota.org/PROTAhtml/Zanthoxylum%20heitzii_En.htm. Accessed 20 July 2014.
[2] Adesina, S. K. The Nigerian *Zanthoxylum*; chemical and biological values. *Afr. J. Tradit Complem.* **2005**, *2*, 282-301.

In Cameroon, their fruits are used as a spice for the traditional dishes such as "nkui„ and "Nah poh„. The seeds are also used to promote digestion.[3]

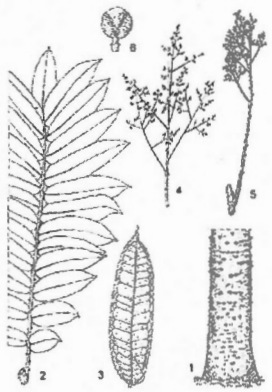

**Figure 1.1** Parts of the plant *Fagara heitzii:* 1) bole base, 2) leaf, 3) leaflet, 4) male inflorescence, 5) female inflorescence, 6) fruit.[3]

The most remarkable use of *Fagara heitzii* lies on the medicinal field. Its use dates back to the 19th century, when indigenous tribes used the steam bark to treat numerous diseases such as malaria, syphilis, gonorrhoea, stiffness, painful joints and male sexual impotence. The bark is also used as an analgesic, especially to relieve toothache. Recent studies of *Fagara heitzii* reveal some activities against cardiac palpitations, urogenital affections and certain types of cancer cell lines such as PC-3 (prostate cancer) and THP-1 (leukemia cancer).[4]

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[3] Pauline, N.; Prosper, B.; Constant, P.; Moor, A.; Jocelyne, V.; Bruno, M. and Yonkeu, Ngogang. The in vitro antisickling and antioxidant effects of aqueous extracts *Zanthoxyllum heitzii* on siekle cell disorder. *BMC Complem. Altern. M.* **2013**, *13*, 1-7.

[4] Dzoyem, J. P.; Kumar, S.; Anatole, C.; Kuete, V.; Sharma, A.; Ali, I.; Kumar, A. and Anuj, R. Cytotoxic and antimicrobial activity of selected Cameroonian edible plants. *BMC Complem. Altern. M.* **2013**, *13*, 1-6.

### 1.1.1. PHYTOCHEMICAL ANALISIS OF GENUS *FAGARA HEITZII*

*Fagara heitzii* presents a variety of secondary metabolites among which highlights: alkaloids, terpenes, coumarins, lignans, amides polyphenols and flavonoids (**Figure 1.2**).[5] According to the article on "Lignans and other constituents of *Zanthoxylum heitzii*„[6] and "Constituents of *Zanthoxylum heitzii*„[7], *Fagara heitzii* is rich particularly in alkaloids, terpenes, and coumarins.

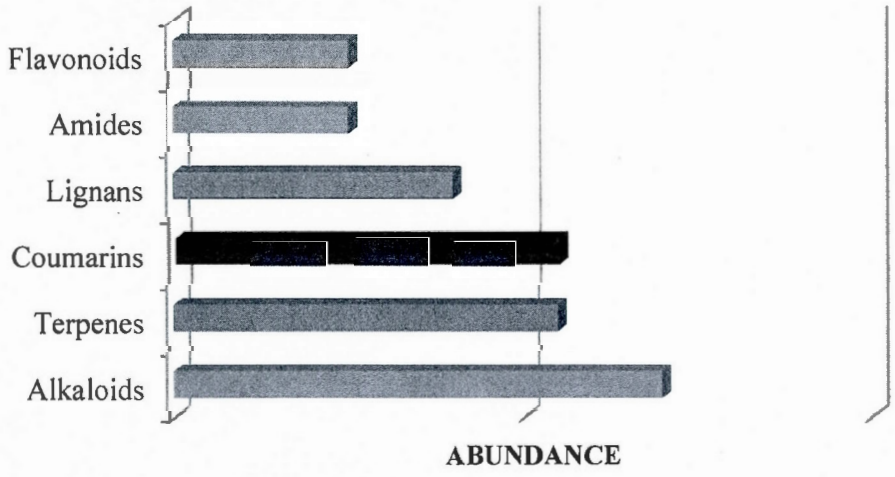


**Figure 1.2** Phytochemical Screening of *Fagara heitzii.*[5]

Most of the secondary metaboltites are classified based on their byosinthetic origin. The following paragraphs describe some of these metabolites, giving some examples according to their family classification.

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[5] Mbaze, L.; Lado, J.; Duplex, J.; Chieh, T.; Dako, D.; Ahmed, M.; Iqbal, M.; Lacaille-Dubois, M.; Wandji, J.; Roy, R. and Sewald, N. Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark. *Phytochemistry*. **2009**, *70*, 1442-1447.

[6] Ngouela, S.; Tsamo, E. and Connolly, J. Lignans and other constituents of *Zanthoxylum heitzii*. *Phytochemistry*. **1994**, *37*, 867-869.

[7] Bongui, J.; Blanckaert, A.; Elomri, A. and Seguin, E. Constituents of *Zanthoxylum heitzii*. *Biochem.Syst. and Ecol*. **2005**, *33*, 845-847.

1.1.1.1. ALKALOIDS

Alkaloids are a large class of organic molecules derived from amino acids or from transamination process. They cover natural compounds similar in behavior to alkalis, or basic compounds. They are basically produced by plants but also occur in the animal kingdom.

Alkaloids are classified according to the amino acids that provide their nitrogen atom as part of their skeleton. Recent reports show that there are more than 10 000 natural known alkaloids. These natural compounds can be sub-divided according to their chemical structures into the following groups: heterocyclic alkaloids, alkaloids with an exocyclic nitrogen atom, polyamines, peptide alkaloids and terpene alkaloids (**Figure 1.3**).[8,9]

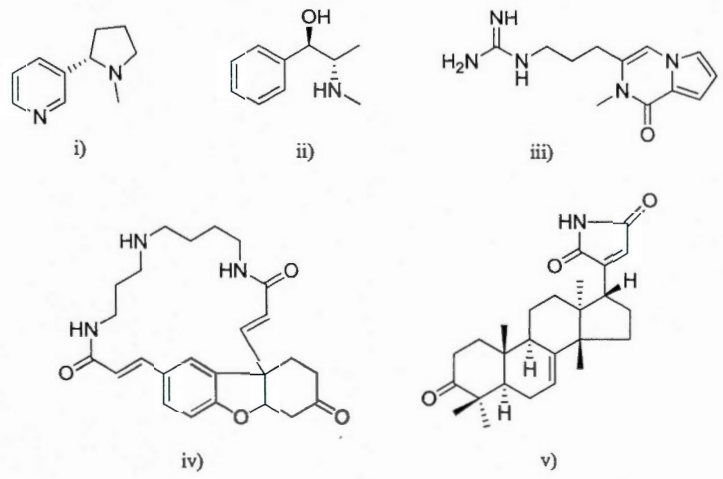


**Figure 1.3.** Examples of alkaloids according to their chemical structures: heterocyclic alkaloids: nicotine (i), alkaloids with an exocyclic nitrogen atom: ephedrine (ii), polyamines: peramine (iii), peptide alkaloids: lunarine (iv) and terpene alkaloids: laxiracemosin (v).

[8] Aniszewski, T. Alkaloids-Secrets of Life: Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. **2007**. Elsevier. 334 pages.
[9] Koskinen, M. P. Asymmetric Synthesis of Natural Products. **2012**. John Wiley & Sons Inc. 332 pages.

Alkaloid applications can be found in different areas of economy, industry, trade and services. They have been used throughout history in folk medicine, being specially exploited in phytotherapy. Some alkaloids are used in modern medicine as natural or modified compounds, having powerful effects in the synthesis and semi synthesis of drugs.[1]

The Rutaceae family is characterized for containing high levels of alkaloids, which have been isolated from species that have been phytochemically studied, being the most abundant class of benzophenanthridinic.[10]

The following scheme shows some representative alkaloids that have been isolated within the genus *Zanthoxylum* (**Table 1.1**).

[10] Santiago, L. Etude Phytochimique de Plantes Médicinales des Andes Vénézuéliennes: *Zanthoxylum rhoifolium* LAM (Rutaceae) et *Bulnesia arborea* Cl. Gay (Zygophyllaceae). **2011**. Thesis. Université Bordeaux 1. France.

**Table 1.1** Representative alkaloids identified within the genus *Zanthoxylum*.[10]

| Genus | Representative alkaloids | Genus | Representative alkaloids |
|---|---|---|---|
| *Fagara mayu* (*Z. mayu*) | Dictamine, γ-fagarine, magnoflorine, skimmianine, chelerytrine, (-)-edulinine, ribaline, bocconoline, tembetarine | *Zanthoxylum myriacanthum* | Dihydropyridine, magnoflorine, tembetarine |
| *Fagara coco* (*Z. coco*) | 8-metoxidictamine, (γ - fagarine) skimmianine, (β-fagarine) α-allocriptopine (α-fagarine) | *Zanthoxylum avicennae* | (-)-culantramine, (-)-culantraminol, avicennamine, (-)-culantraramine-*N*-oxide, (-) culantraminol-*N*-oxide |
| *Fagara rhetsa* *(Z. rhetsa)* | (±)-evodiamina, 8-methoxy-*N*-methyflindersine | *Zanthoxylum rubescens* | Magnoflorine, chloronitidine |
| *Zanthoxylum conspersipunctatum* | Protopine | *Zanthoxylum decaryi* | Dictamine, skimmianine, decarine |
| *Fagara vitiensis* (*Z. vitiensis*) | Chelerythrine, nitidine | *Zanthoxylum fagara* | Candicine, tembetarine, magnoflorine |

| Genus | Representative alkaloids | Genus | Representative alkaloids |
|---|---|---|---|
| *Fagara flava* (*Z. flavum*) | Dihydrorutaecarpine | *Zanthoxylum oxyphyllum* | Coridine, Zanoxyline |
| *Zanthoxylum monophyllum* | 8-methoxyflindersine | *Zanthoxylum coriaceum*, | Dihydrochelerythrine, *N-methylisocoridine*, alfileramine |
| *Zanthoxylum budrunga* | Rutaecarpine, lunacridine, *N*-methyflindersine, zanthobungeanine, γ-fagarine, dictamine, *canthin-6-one, rutaecarpine, (±)-evodiamine,* skimmianine | *Zanthoxylum culantrillo* | *N-methylisocoridine,* candicine, synephrine, magnoflorine, tembetarine |
| *Zanthoxylum microcarpum* | Magnoflorine, haplopine, hordenine, decarine, *N*-methylamine | *Zanthoxylum spinosum* | Norchelerithrine, dihydrochelerythrine, decarine, oxychelerythrine |
| *Zanthoxylum thomense* | Decarine, norchelerithrine, angoline | *Zanthoxylum sarasinii* | Tembetarine, colletine, skimmianine |
| *Zanthoxylum macrophylla* | Chloronitidine, 6-oxynitidine | *Zanthoxylum punctatum* | *N-methylisocoridine,* magnoflorine, punctatine, alfileramine |

1.1.1.2. TERPENES

Terpenes are compounds formed by $C_5$ units called isopentenyl or isoprene units. They are linked together from head to tail, following the isoprene rule. They are widely distributed in the plant kingdom and their abundance is associated with climate change and genetic factors. Many terpenes are aromatic hydrocarbons and thus, may have had a protective function. When terpenes are modified chemically, either by oxidation or rearrangement of the carbon skeleton, the resulting compounds are known as terpenoids.

Terpenes are classified according to the number of pairs of these $C_5$ units present in the molecule. They exist in the nature as monoterpenes, $C_{10}$; sesquiterpenes, $C_{15}$; diterpenes, $C_{20}$; sesterterpenes, $C_{25}$; triterpenes, $C_{30}$; and so on (**Figure 1.4**).[9]

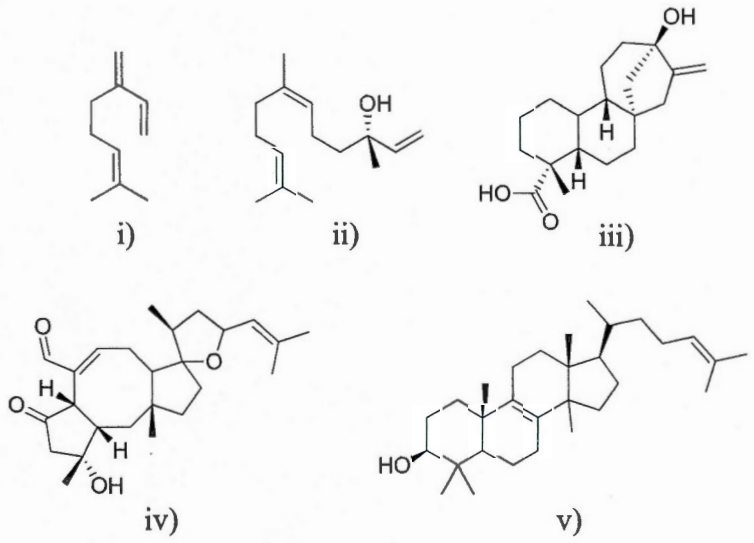


**Figure 1.4.** Examples of terpenes: Monoterpene: myrcene (i), sesquiterpene: nerolidol (ii), diterpene: steviol (iii), sesterterpene: ophiobolin A (iv) and triperpene: lanosteol (v).

Most of terpenes present in the genus *Zanthoxylum* are found in essential oils (leaves) or fruits. Essential oils are used extensively as natural additives for food; they are also used as fragrances and in traditional medical treatments in form of aromatherapy.[11]

Some terpenes among the genus *Zanthoxylum* are shown in **Table 1.2.**

**Table 1.2** Representative terpenes identified within the genus *Zanthoxylum*.[9]

| **Genus** | **Representative terpenes** | **Genus** | **Representative terpenes** |
|---|---|---|---|
| *Zanthoxylum rhetza* | Xanthoxylon, mullilamdiol | *Zanthoxyllum. gilletii* | Myrcene, limonene, camphene, α-pinene, β-pinene, geranyol, ocimene, sabinene, linalool, fenchol |
| *Zanthoxylum procerum* | β-phellandrene, α-cubeban, humulene, aromadendrene | *Zanthoxylum ailanthoides* | β-caryophyllene, α-humelene |
| *Zanthoxylum heitzii* | Lupeol, lupeone, β-sitosterol | *Zanthoxylum sarasiinii* | Lupeol, β-sitosterol |

[11] Angulo, A. Búsqueda de sustancias bioactivas en la flora cordobesa. Convocatoria Interna de Proyectos de Investigación y Extensión. **2006**. Universidad de Córdoba. España.

1.1.1.3. COUMARINS

Coumarins owe their class name to 'Coumarou', the vernacular name of the tonka bean (*Dipteryx odorata*), from which coumarin was first isolated in 1820. It is a natural component of soya, which has been intensively investigated as a chemopreventive agent against hormonally regulated breast and prostate cancer in animal models.

Coumarins consist of a benzene ring joined to a pyrone ring (benzopyrone). They comprise essential oils such as cinnamon bark oil, cassia leaf oil and lavender oil. They are also present in green tea and in some fruits. Coumarins are although distributed throughout all parts of the plant. They are divided in four main sub-types: Simple coumarin (i), furanocoumarin (ii) pyranocoumarin (iii) and pyrone-substituted coumarin (iv) **(Figure 1.5)**.[12]

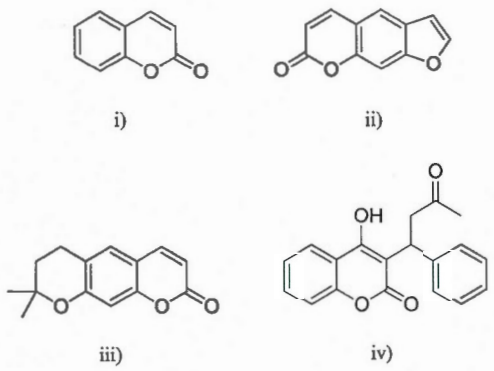


**Figure 1.5** Examples of coumarins: Simple coumarin (i), furanocoumarin (ii) pyranocoumarin (iii), pyrone-substituted coumarin (iv).[13]

Biologically, coumarins are very useful since they exhibited antibacterial, antitumor and anticoagulant activities. It was noted that most coumarins are free from toxic side effects; however, overdose can cause haemorrhages.[13]

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[12] Lacy, A. and O'Kennedy, R. Studies on Coumarins and Coumarin-Related Compounds to determine their Therapeutic Role in the Treatment of Cancer. *Curr. Pharm. Design.* **2004**, *10*, 3797-3811.
[13] http://www.intechopen.com/books/bioactive-compounds-in-phytomedicine/zanthoxylum-genus-as-

Most coumarins occur in higher plants, being the richest sources the families Rutaceae and Umbelliferae. **Table 1.3** shows some coumarins found in the genus *Zanthoxylum.*

**Table 1.3** Representative coumarins identified within the genus *Zanthoxylum.*[9]

| **Genus** | **Representative coumarins** | **Genus** | **Representative coumarins** |
|---|---|---|---|
| *Zanthoxylum Schinifolium* | Scopoletin, anisocoumarin H, methylschinilenol, schinitrienin, schininallylone | *Zanthoxylum avicennae* | 5'-methoxyauraptene, 6,5'-dimethoxyaurapten e, 5'-methoxycollinin |
| *Zanthoxylum americanum* | Dipetaline, allloxanthoxyletin, xanthoxyletin, xanthyletin | *Zanthoxylum dimorphophyllum* | Scoparone, dimoxylin |
| *Zanthoxylum limonella* | Rutaecarpine, xanthoxyletin, osthol, scopoletin | *Zanthoxylum elephantiasis* | Braylin, xanthoxyline |
| *Zanthoxylum tingoassuiba* | Xanthotoxin, isopimpinellin | *Zanthoxylum flavum* | Suberosin, psoralene |
| *Zanthoxylum dominianum* | Suberosin, Isopimpinellin, suberenol | *Zanthoxylum sarasinii* | Xanthoxyline, *cis*-avicennol, avicennol |
| *Zanthoxylum dipelatum* | Dipelactona, dipetaline | *Zanthoxylum fagara* | Castanaguyone |

potential-source-of-bioactive-compounds. Accessed 20 July 2014.

1.1.1.4. LIGNANS

"Lignans„ was first introduced by Haworth to describe a complex aromatic biopolymer. Lignans are a large class of natural products, which can be found in more than 60 families of vascular plants. Their tissues are found rich in lignin. They can be found in a variety of plant materials including flaxseed, pumpkin seed, sesame seed, soybean, broccoli, and some berries.[14]

Lignin structure is formed by two coupled units of phenylpropanoids, where two $C_6$-$C_3$ are attached by its central carbon ($C_8$), as it is shown in **Figure 1.6.**[15]

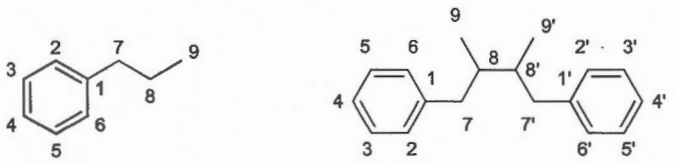


**Figure 1.6** Phenylpropanoid unit (i) and lignin structure (ii).

Among the several secondary metabolites existing in the entire world, lignans are recognized as a class of natural products with a wide spectrum of important biological activities such as antiviral, anticancer, anti-inflammatory, antimicrobial, antioxidant, immunosuppressive and osteoporosis prevention, etc.[13]

**Table 1.4** shows some lignans particularly found in the genus *Zanthoxyllum.*

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[14] Touré, A. and Xueming, X. Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant, Activity, Bio-Active, Components, and Health Benefits. *Compr. Rev. Food Sci F.* **2010**, *9*, 261-269.
[15] http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/lignans-chemical-and-biological-properties. Accessed 20 July 2014.

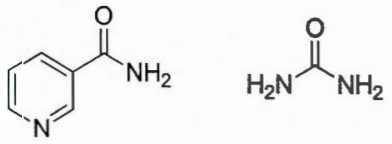
**Table 1.4** Representative lignans identified within the genus *Zanthoxylum.*[9]

| Genus | Representative lignans | Genus | Representative lignans |
|---|---|---|---|
| *Zanthoxylum pluviatile* | Pluviatolide, pluviatilol, hinokinin, isohinokinin, pluviatide | *Zanthoxylum piperitum* | Xanthoxylol, piperitol, (-)-asarinin, sanshodiol, (+)-sesamin, pluviatilol |
| *Zanthoxylum culantrillo* | (-)-eudesmin, (+)-epieudesmin | *Zanthoxylum tessmannii* | (+)-sesamin, savinin, arctigenin |
| *Zanthoxyllum fagara* | (+)-eudesmin, (+)-epieudesmin | *Zanthoxylum valens* | (-)-kobusin, (-)-asarine, (+)-sesamin |
| *Zanthoxyllum setulosum* | (+)-sesamin, (+/-)-siringaresinol | *Zanthoxyllum kellermanii* | (-)-pinoresinol, mataresinol |
| *Zanthoxylum petiolare* | (+)-sesamin, piperitol, furofurano | *Zanthoxylum simulans* | (-)-simulanol |
| *Zanthoxylum usambarense* | (+)-pinoresinol, (+)-sesamin | *Zanthoxylum budruga* | Sesamin, (-)-siringaresinol |
| *Zanthoxylum davyi* | mesosesamin | *Zanthoxylum ailanthoides* | ailanthoidol |
| *Zanthoxylum lemaire* | Savinin, hinokinin, arctigenin, guayadenin, (+)-sesamin, methyltrachelogenin | *Zanthoxylum heitzii* | meso-2,3-bis(3,4,5-trimethoxybenzyl)-1,4-butanediol, 4-acetoxy-2,3-bis(3,4,5-trimethoxybenzyl)-1-butanol, (+)-sesamina, arctigenin |

1.1.1.5. AMIDES

Many natural products (extracting from living systems) contain amide groups. Amides are derived from carboxylic acids, where the group -OH (-COOH) is replaced by an -$NH_2$ group. The hydrogen bonding in these molecules is the strongest bond observed.

The arrangements of amides constitute the formation of proteins, which are considered the most common examples of amides in the natural world. A naturally occurring amide is nicotinamide (i), one of the B vitamins; another example of natural amide is "urea„ (carbamide) (ii), which is excreted from mammalian bodies (**Figure 1.7**).[16, 17]




i) ii)

**Figure 1.7.** Examples of amides found in the nature: Nicotinamide (i) and urea (ii).

Besides the presence of alkaloids, the amide group has been also reported in the genus *Zanthoxylum*. They are structurally related to alkaloids and mostly found in fruits, bark and essential oils. Some isolated amides from the genus *Zanthoxylum* are shown in **Table 1.5**.

[16] Krishnaswamy, N. Learning Organic Chemistry Through Natural Products. *Springer India.* **1996**, *1*, 56-62.
[17] http://science.jrank.org/pages/285/Amides.html. Accessed 20 July 2014.

**Table 1.5** Representative amides identified within the genus *Zanthoxylum.*[9]

| Genus | Representative amides | Genus | Representative amides |
|---|---|---|---|
| *Zanthoxylum clava-herculis* | Herculin, *neo*-herculin | *Zanthoxylum ocumarence* | Tembamide, aegeline |
| *Zanthoxylum arnottianum* | Arnottiamide, isoarnottiamide | *Zanthoxylum thomense* | Zanthomamide |
| *Zanthoxylum alanthoides* | γ-sanshoöl, hydroxy- γ-sanshoöl | *Zanthoxylum piperitum* | γ-sanshoö, hydroxy- γ-sanshoöl, α-sanshoöl, hydroxy-α –sanshoöl. |
| *Zanthoxylum piperitum* | ZP-amide A, ZP-amide B, ZP-amide C, ZP-amide D, ZP-amide E, ZP-amide F | *Zanthoxylum bungeanum* | hydroxy- γ-sanshoöl, hydroxy-α –sanshoöl, bungeanool, isobungeanool, tetrahydrobungeanool, dihydrobungeanool |
| *Zanthoxylum rubescens* | Rubescenamide, rubescenamine, rubemamine, rubemamide, dioxamine, dioxamide, rubesamide | *Zanthoxylum lemairie* | γ-sanshoöl, fagaramide, piperlongumine, herclavine, zanthomamide, arnottianamide, zanthosinamide, lemairamide |
| *Budo-Zanthoxylum* | γ-sanshoöl, hydroxy- γ-sanshoöl, α –sanshoöl, hydroxy- α –sanshoöl | *Zanthoxylum armatum* | Armatamide |
| *Zanthoxylum integrifolium* | Lanyuamide I, lanyuamide II, lanyuamide III | *Zanthoxylum Heitzii* | *trans*-fagaramide, arnottianamide |

1.1.1.6. FLAVONOIDS

Flavonoids are a group of polyphenolic compounds, which present a common skeleton of flavan nucleus.[18] They are ubiquitous in nature and are categorized according to their chemical structure into: flavonols, flavones, catechins, anthocyanidins and chalcones (**Figure 8**).

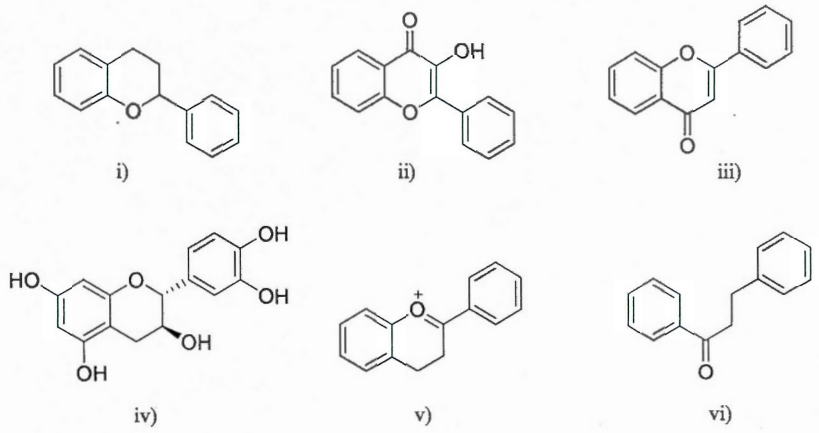



**Figure 8.** Chemical structures of flavonoids: Flavan nucleus (i), flavonol (ii), flavones (iii), (+)-catechins (iv), anthocyanidins (v) and chalcones (vi).

Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages such as tea, coffee, beer, wine and fruit drinks. In plants, they fulfill many functions; the most important is being pigments for flowers coloration. They are also involved in UV filtration.[19]

Besides their importance as components in human diet, flavonoids have aroused

[18] Pietta, T. and Mauri, P. Analysis of flavonoids in medicinal plants. *Method. Enzymol.* **2001**, *335*, 26-45.
[19] Pal, D. and Verma, P. Flavonoids a powerful and abundant source of antioxidants. *Inter. J. Pharm. Sci.* **2013**, *5*, 95-98.

Besides their importance as components in human diet, flavonoids have aroused considerable interest because of their pharmacological activity. They are known for their diverse biological properties, such as antioxidants, anti-inflammatory, antithrombotic, antibacterial, antihepatotoxic, antitumor, antihypertensive, antiviral, antiallergic and estrogenic.[20]

In *Zanthoxylum* genus, flavonoids are mainly represented by flavonols, and flavones. Flavonoids found in the genus *Zanthoxylum* and those isolated in other genera of Rutacea family are characterized for be polymethoxylated.[9] Examples are shown in **Table 1.6**.

**Table 1.6** Representative flavonoids identified within the genus *Zanthoxylum*.

| **Genus** | **Representative flavonoids** | **Genus** | **Representative flavonoids** |
|---|---|---|---|
| *Zanthoxylum acanthopodium* | Tambulin, tambulol, tambuletin | *Zanthoxylum alatum* | Zanthoxylflavon |
| *Zanthoxylum rhoifolium* | Vitexine, isovitexine, hesperidin | *Zanthoxylum bungeanum* | Quercetin, quercitrine, foeniculin, rutin, tamarixetin |
| *Zanthoxylum integrifolium* | 3,5- diacetyltambuline | *Zanthoxyllum heitzii* | Stigmasterol-3-O-β -D-glucopyranoside |

[20] http://lpi.oregonstate.edu/f-w00/flavonoid.html. Accessed 20 July 2014.

## 1.1.2. ISOLATED COMPOUNDS FROM *FAGARA HEITZII* AND THEIR BIOLOGICAL ACTIVITIES

*Fagara heitzii* is known for its chemical diversity and ethnobotanical properties. These characteristics have been the fundamental reason for developing several biological activity studies that have helped to find new bioactive extracts and compounds.

Among the studies already made on *Fagara heitzii*; potential cytotoxic, anti-inflammatory, antioxidant and antimalarial agents were discovered. Traditionally, *Fagara heitzii* was used as a medicinal plant against syphilis, cardiac palpitations, rheumatism, malaria and urogenital infections.[5]

### 1.1.2.1. COMPOUNDS ISOLATED FROM FAGARA HEITZII

The compounds already isolated, elucidated and characterized are currently known as: flindersine (**1**), meso-2,3-bis(3,4,trimethoxybenzyl)-1,4-butanediol (**2**), 4-acetoxy-2,3-bis(3,4,5-trimethoxybenzyl)-1-butanol (**3**), (+)-sesamin (**4**), (+)-eudesmin (**5**), fagaramide (**6**), Heitziamide B (**7**), nitidine (**8**), lupeol (**9**), lupeone (**10**), campesterol (**11**), β-sitosterol (**12**), stigmasterol (**13**), skimmianine (**14**), iso-γ-fagarine (**15**), arctigenin methyl ether (**16**), savinin (**17**), arnottianamide (**18**), stigmasterol-3-O-β-D-glucopyranoside (**19**), Heitziamide A (**20**), Heitziethanoid A (**21**) and Heitziethanoid (**22**) (**Figure 1.9**).[5,6,7]

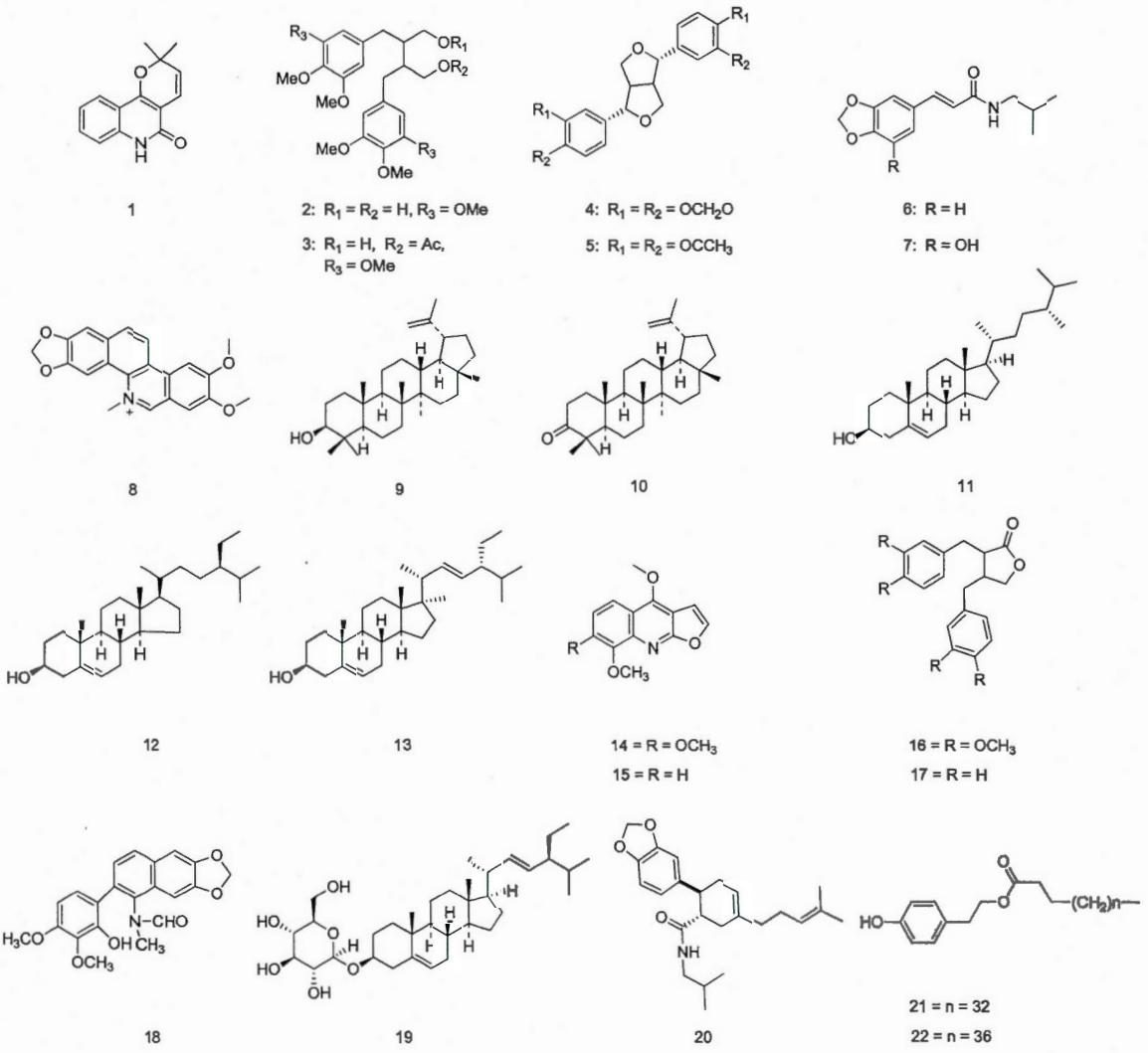



**Figure 1.9.** Compounds isolated from *Fagara heitzii.*[5,6]

1.1.2.1.1 FAGARAMIDE

Fagaramide, also known as *N*-isobutyl-3,4-(methylenedioxy)-cinnamamide (IUPAC name)[21] (**Figure 1.10**), is specially found in Zanthoxylum and Piper genus. It was first isolated from the leaves *of Zanthoxylum rubescen.*[22] Fundamentally, fagaramide is formed by a methylenedioxyphenyl group and a α,β-unsaturated-amide.

*trans*-Fagaramide (6)

**Figure 1.10** Structure in 2D and 3D of *trans*-fagaramide (**6**).

Over many years, fagaramide has been the subject for several studies and investigations. Earlier biological activity studies of fagaramide showed cytotoxicity against selected cancer cell lines and pathogenic bacteria. Some of these studies are shown in **Table 1.7**.

[21] Schobert, R., *et al.* Three-component synthesis of (E)-α,β-unsaturated amides of the piperine family. *J. Chem. Soc.* **2001**, *1*, 2393-2397.
[22] Moody, J. and Sofowora, A. Leaf alkaloids of *Zanthoxylum rubescens*. *Planta Med.* **1984**, *1*, 101-103.

**Table 1.7** Biological studies of *trans*-fagaramide.

| **Biological studies of *trans*-fagaramide in cancer cell lines, pathogenic bacteria and as an insecticidal.** |
|---|
| 1. Evaluated against prostate cancer cell (PC-3). No cytotoxic effect was observed ($IC_{50} > 100$ μM).[5] |
| 2. Showed antifungal activity, determined by direct bioautography against *Cladosporium sphaerospermum* (10 μg)[a].[23] |
| 3. Evaluated against epimastigote forms of *Trypanosoma cruzi,* a protozoan parasite (causative agent of Chagas disease). Shown to be inactive ($IC_{50} > 250$ μM).[24] |
| 4. Showed toxicity to the house fly, *Musca domestica* (alone or added to pyrethrum sprays).[25] |
| 5. Showed strong ovicidal activity against the beetle *Leptinotarsa decemlineata.*[26] |
| 6. Showed larvicidal activity against the tropical mosquito *Culex quinquefasciatus* ($LC_{50} = 7.92 \pm 1.22$ ppm).[27] |

[a] Minimum amount required for the inhibition of fungal growth on thin-layer chromatography (TLC) plates.

[23] Vasquez, R., *et al.* Antifungal amides from *Piper arboreum* and *Piper tuberculatum. Phytochemistry*. **2002**, *59*, 521-527.
[24] Continguiba, F., *et al.* Piperamides and their derivatives as potencial anti-trypanosomal agents. *Med. Chem. Res*. **2009**, *18*, 521-527.
[25] Synerholm, M. E.; Hartzell, A. Compounds containing the 3,4-methylenedioxyphenyl group and their toxicities toward houseflies. Contributions from Boyce Thompson Institute. **1945**, *14*, 79-89.
[26] Ginesta, E., *et al.* Compounds with ovicidal effect isolated from *Fagara xanthoxyloides* Lam. *Biosci, Biothech and Bioch*. **1994**, *5*, 936-937.
[27] Navarrete, A., *et al.* Análisis isobolográfico de la interacción entre α-sanshool, sesamina, asarinina, fagaramida y piperina sobre la actividad larvicida en *Culex quinquefasciatus* Say. *Revista de la Sociedad Química de México*. **2003**, *47*, 178-185.

1.1.2.1.2 HEITZIAMIDE A

Heitziamide A (**Figure 1.11**) is a novel phenylamide, first isolated as a racemate from the stem bark of *Fagara heitzii.* There exist few studies of Heitziamide A (**20**), the most recently reveals significant effects on the oxidative burst of whole blood. ($IC_{50}$ = 2.6 ±0.4 μM).[5]

Synthetically, Heitziamide A arises from Diels-Alder cycloaddition reaction between *trans*-fagaramide (also obtained from *Fagara heitzii*) and the monoterpene myrcene (**Figure 1.12**).[28]

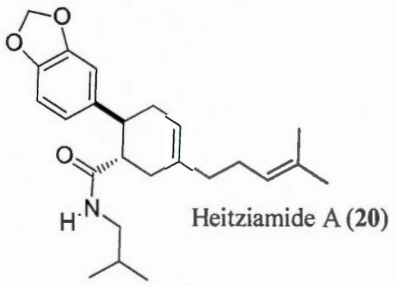



**Figure 1.11** Structure of Heitziamide A.

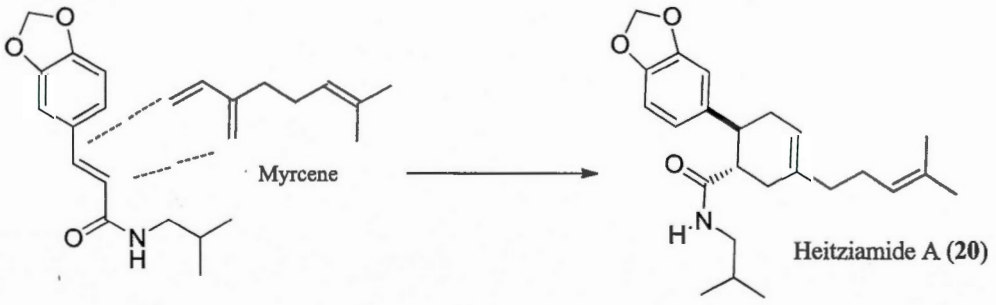



**Figure 1.12** Diels-Alder cycloaddition between *trans*-fagaramide and myrcene.

[28]Shishi, L., *et al.* Radical Cation Diels-Alder Cycloadditions by Visible Light Photocatalysis. *J. Am. Chem. Soc.* **2011**, *133*, 19350-19353.

## 1.2 EVERNIA PRUNASTRI

The second plant analysed as a part of this project was *Evernia prunastri*, a lichen which is a symbiotic organism of fungi (mycobiont) and algae (photobiont), belonging to the family Usneaceae.[29] This species, also known as "oakmoss„ grows up throughout the Northern hemisphere. It has been extensively studied based on the fact that their extracts and major compounds exert a wide variety of biological actions such as antimycotic antiviral, antibiotic, analgesic, anti-inflammatory, antiproliferative and cytotoxic effects.[30]

Traditionally, *Evernia prunastri* is found in mountainous temperate regions within North America and Central Europe.[31] It grows on the branches and trunk of oak trees. It also appears on the bark of deciduous trees and conifers. Nowadays it can be also found in high altitude levels where there is abundant fog such as the Andes of Peru.

*Evernia prunastri* possesses a short and bushy thallus (3-6 cm in length), composed by flat and flexible straps usually pendulous. They are highly branched, similar in appearance to the form of deer antlers, ending in pointed tips. Apothecia rarely appears in *Evernia prunastri*, it is shortly and stoutly; it contains red-brown discs with 2-5 mm in diameter. It also presents simple elliptical colorless pores.[32,33]

The color of oakmoss ranges from green to greenish-yellow (when it is dry) and dark green to yellow-green (when it is wet)[34] **(Figure 1.13)**.

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[29] Heide, R., *et al.* Qualitative Analysis of Odoriferous Fraction of Oakmoss (*Evernia prunastri* (L.) Ach.). *J. Agric. Food Chem.* **1975,** *23*, 950-957.

[30] KosaniĆ, M. *et al. Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents. *Food Chem Toxicol.* **2013**, *53*, 112-118.

[31] http://treparriscosfieldnotebook.blogspot.ca/1992/05/evernia-prunastri.html. Accessed 22 July 2014.

[32] http://www.lichens.lastdragon.org/Evernia_prunastri.html. Accessed 22 July 2014.

[33] http://www.britannica.com.proxy.bibliotheques.uqam.ca:2048/EBchecked/topic/423437/oak-moss. Accessed 22 July 2014.

[34] http://treparriscosfieldnotebook.blogspot.ca/1992/05/evernia-prunastri.html. Accessed 22 July 2014.

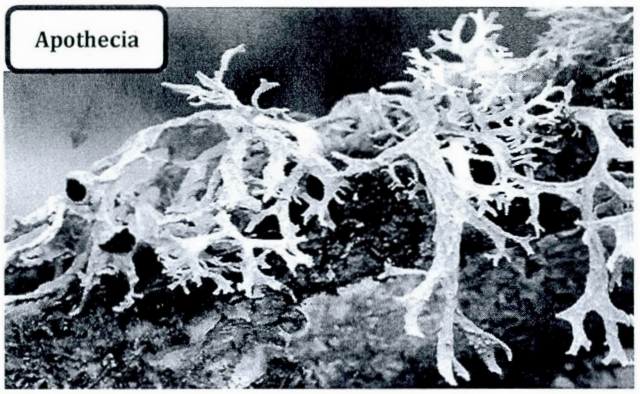


**Figure 1.13** Lichen *Evernia prunastri.*

*Evernia prunastri* is also known for being used in medicine, perfumery, cosmetics and ecology in many countries. Their metabolites have a manifold biological activity, which has been the principal reason to continue exploring this species. Historically, it has been used as dyes. In ancient times "oaksmoss„ was imported from Greece and Cyprus to Egypt and used in wrapping of embalmed mummies. It also plays an important role in perfumery industry since the 16$^{th}$ century. As a raw material, *Evernia prunastri* is widely used as perfume fixatives and forms the basis notes of many fragrances. It is also consider as an useful tool for mesure the degree of air pollution.

Lichen *Evernia prunastri* has been also used in traditional medicine over a considerable period of time. Formerly, their extracts were used in drugs for treating external wounds and infections diseases. The essential oil has antiseptic, emollient and expectorant properties. There exist some references that corroborate its use in aromatherapy and in the treatment of sinusitis as well.[35]

Presently *Evernia prunastri* has been reported to have several biological activities such as antiviral, antibiotic, allergenic, antiherbivore and enzyme inhibitory.[36]

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[35] http://www.herbal-supplement-resource.com/oakmoss.html. Accessed 22 July 2014.

[36] Melgarejo, M. *et al.* More investigations in potent activity and relationship structure of the lichen antibiotic (+)-Usnic Acid and its derivate dibenzylusnic acid. *Revista Boliviana de Química.* **2008**, *25*,

## 1.2.1. PHYTOCHEMICAL ANALYSIS OF EVERNIA PRUNASTRI

Secondary metabolites from *Evernia prunastri* are derived from mycobiont metabolism. They are classified as depsides, depsidones, dibenzofurans, terpenes, etc (**Figure 1.14**).[37,38]

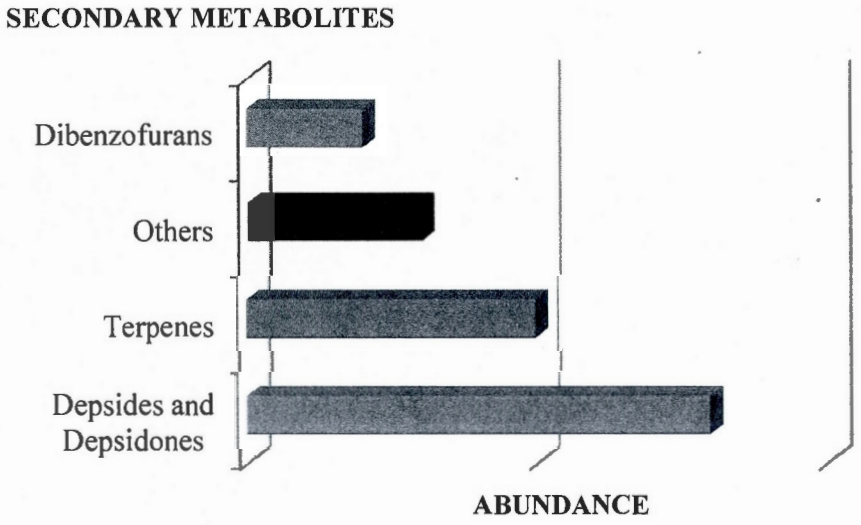


**Figure 1.14** Phytochemical screening of *Evernia prunastrI.* [37,38]

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24-29.

[37] Nicollier, G., *et al.* Identification et synthèse de nouveaux depsides isolées de la mousse de chêne (*Evernia Prunastri* (L.) ACH). *Helv Chim Acta.* **1979**, *62*, 711-717.

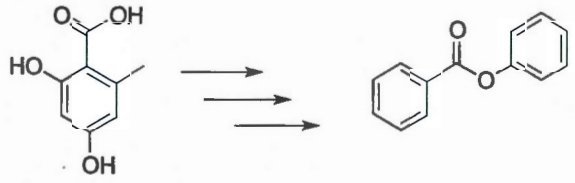
[38] Lokeman, A., *et* al. Antigenotoxic potencies of a lichen species, *Evernia Prunastri. Toxicol Ind Health.* **2012**. 1-9.

## 1.2.2. LICHEN SUBSTANCES

The term "lichen substances" applies to all compounds synthesized exclusively by lichens.[39] Most of the secondary metabolites present in *Evernia prunastri* are aromatic compounds; within them the more abundant are depsides, depsidones, dibenzofurans and usnic acids.

### 1.2.2.1. DEPSIDES

Depsides are polyphenolic compounds derived from orsellinic acid. They are constituted by two or more monocyclic aromatic units, which are linked by an ester bond (**Figure 1.15**).[40]




Orsellinic acid Depside

**Figure 1.15** Structures of orsellinic acid and depside.

Depsides present antiproliferative, antioxidant, anti-HIV, and antiobiotic properties. They are also used as potent anti-inflammatory.[41]

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[39] Robles, C.; Pastor, A. and Morales, P. Líquenes y sustancias liquénicas. *Revista Química-PUCP*. **1992**, *6*, 65-76.

[40] Sebastián, B., *et al.* Oxidation reactions are required to produce atranorin from acetate by alginate-immobilized cells of Cladonia verticillaris. *Tropical Bryology*. **2000**, *19*, 73-80.

[41] Reynertson, K. *et al.* Bioactive depsides and anthocyanins from jacoticaba (*Myrciaria cauliflora*). *J. Nat. Prod.* **2006**, *69*, 1228-1230.

Some of the depsides already elucidated from the *Evernia prunastri,* are currently known as lecanoric acid (**23**), evernic acid (**24**), divaricatic acid (**25**), barbatic acid (**26**), thamnolic acid (**27**), atranorin (**28**), chloroatranorin (**29**) (**Figure 1.16**).[30,31]

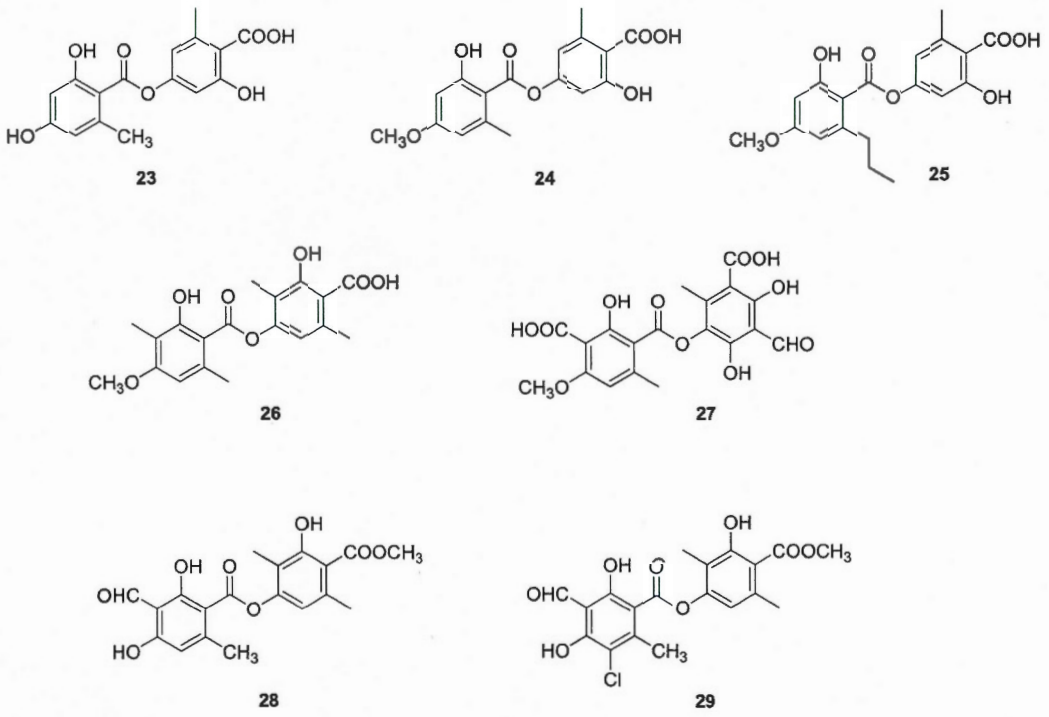
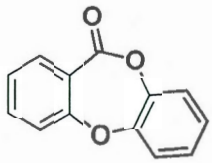



**Figure 1.16** Depsides isolated from *Evernia prunastri.*

1.2.2.2. DEPSIDONES

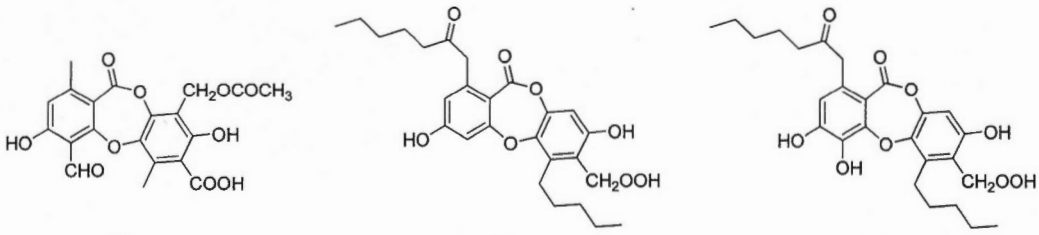
Depsidones (**Figure 1.17**) are one of the most abundant metabolites produced by lichen; they consist of esters such as depsides, but are also cyclic ethers.



Depsidone

**Figure 1.17** Structure of depsidone.

Lichen depsidones have been reported to possess many biological activities, such as antitumor and antimicrobial activities.[42] Some depsidones from *Evernia prunastri,* are currently known as physodalic acid (**30**), physodic acid (**31**), 3-hydroxyphysodalic acid (**32**) (**Figure 1.18**).[30]



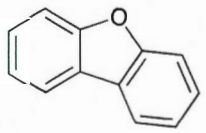
**30** **31** **32**

**Figure 1.18** Depsidones isolated from *Evernia prunastri.*

[42] Stojanovic, G. *et al.* Lichen Depsidones as Potencial Novel Pharmacologically Active Compounds. *Mini-Rev. Org. Chem.* **2012**, *9*, 178-184.

1.2.2.3. DIBENZOFURAN

Dibenzofuran is a heterocyclic organic compound (**Figure 1.19**), which contains two benzenes rings fused to one furan ring in the middle. It serves as a precursor for many plastic additives, fragances, sedatives and pharmaceuticals products.

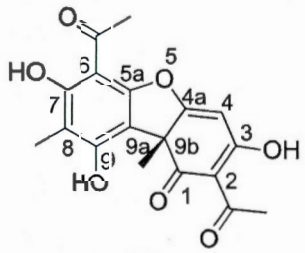



Dibenzofuran

**Figure 1.19** Structure of dibenzofuran.

1.2.2.4. USNIC ACID

Usnic acid (**33**) (**Figure 1.20**), also known as 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3[2H,9bH]-dibenzofurandione (IUPAC name), is a yellowish highly functionalized dibenzofuran metabolite found in various lichen genera. It was first identified by Knop in 1884.[43]




**33**

**Figure 1.20** Chemical structure of usnic acid (**33**).

[43] Segatore, B., *et al.* In vitro interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant *Staphylococcus aureus* clinical isolates determined by FICI and ΔE model methods. *Phytomedicine.* **2012**, *19*, 341-347.

Usnic acid is widely distributed in species of *Cladonia*, *Usnea*, *Lecanora*, *Ramalina*, *Evernia* and *Parmotrema*. It can exist as (+) and (-) enantiomers, but most of its biological activity is attributed to the (+) enantiomer.[44]

Usnic acid has been extensively used for centuries in popular medicine for the treatment of pulmonary tuberculosis, pain relief, fever control, mycoses, wounds, toothache, sore throat and several skin infections. It has been also reported that Usnic acid has multiple biological effects, including anti-bacterial, anti-parasitic, anti-viral, anti-mycotic, anti-protozoal, anti-proliferative, anti-inflammatory, anti-pyretic, and citotoxicity against human cancer cell lines.[45,46]

### 1.2.2.4.1. BIOSYNTHESIS OF USNIC ACID

Early works by Shibata *et al.*[47] confirmed that usnic acid has been derived from acetic acid, presumably via a polyketide pathway. The proposed biosynthetic pathway showed in **Scheme 1.1** suggests that the enzyme involved in the production of usnic acid is polyketide synthase (PKS), which is responsible for the biosynthesis of the key intermediate on the pathway methylphloracetophenone. The final step in the biosynthesis of usnic acid is an oxidative homocoupling of two molecules of methylphloracetophenone.

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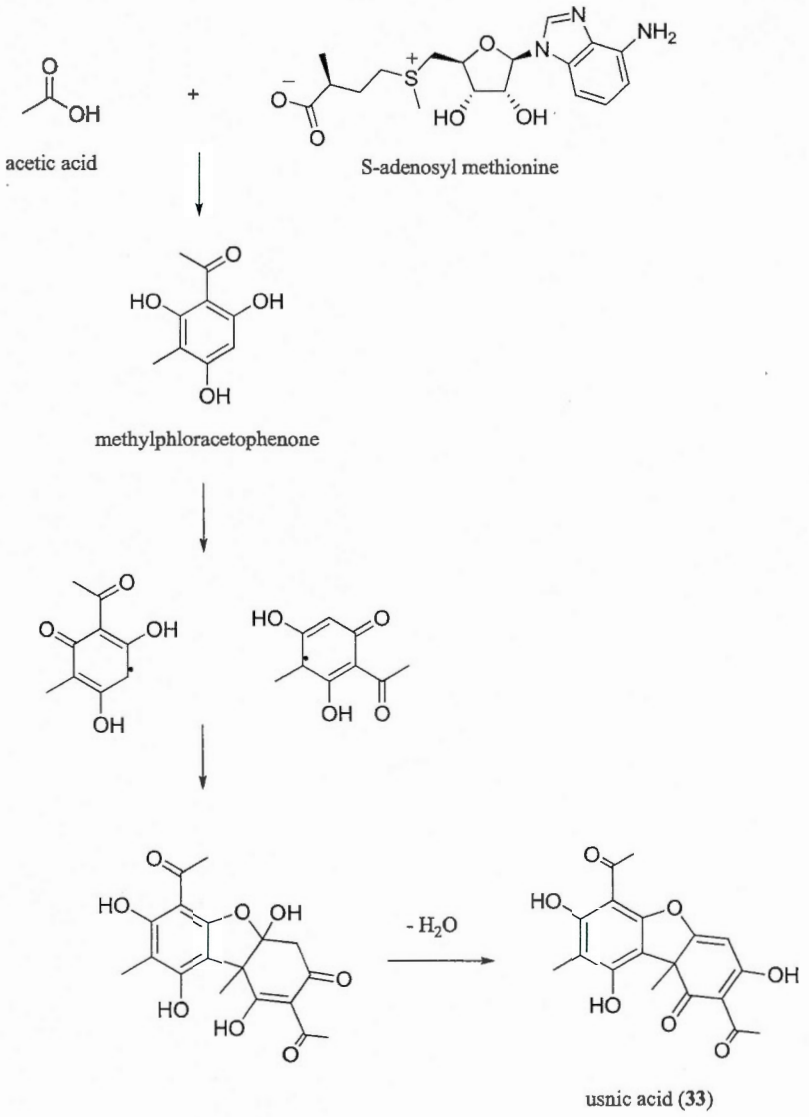
[44] Lira, M., *et al.* Inclusion complex of usnic acid with β-cyclodextrin characterization and nanoencapsulation into liposomes. *J. Inclu. Phenom. Macrocycl. Chem.* **2009**, *64*, 215-224.

[45] Bačkor, M., *et al.* Comparison of the Phytotoxic Effects of Usnic Acid on Cultures of Free-Living Alga *Scenedesmus quadricauda* and Aposymbiotically Grown Lichen Photobiont *Trebouxia erici. J. Chem. Ecol.* **2010**, *36*, 405-411.

[46] Brisdelli, F., *et al.* Citotoxic Activity and Antioxidant Capacity of Purified Lichen Metabolites: An In Vitro Study. *Phytother. Res.* **2013**, *27*, 431-437.

[47] Shibata, S., Ukita, T.,Tamura, T. and Miura, Y. Relation between chemical constitutions and antibacterial effects of usnic acid andderivatives. *Jap. Med.* **1948**, *1*, 152-155.




**Scheme 1.1** Proposed biosynthesis of usnic acid. Methylphloracetophenone is produced by a polyketide synthase. It is first oxidized, followed by elimination of $H_2O$ to produce usnic acid.[48]

[48] Hawranik, D., *et al.* The chemoenzymatic synthesis of usnic acid. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2383-2385.

### 1.2.2.4.2. USES AND PROPERTIES OF USNIC ACID

Isolated usnic acid is endowed with several chemical and biological properties (see page 30) that give usnic acid-producing lichens an advantage over competing plants and microbial species.[49]

Due to its antioxidant and bacteriostatic activities, usnic acid has been used in the elaboration of dermatological and cosmetic products such as hair shampoos, toothpastes, deodorants, etc.[50] Furthermore, it has been used in the elaboration of collagen-based films containing usnic acid as a wound dressing for dermal burn healing.[51]

It has also been promoted as a dietary supplement for weight loss, however cases of hepatotoxicity have been observed in some individuals, which has warned on the use of LipoKinetix (usnic acid-containing product), which was withdrawn from the market in 2001.[52]

Despite its recognized features, usnic acid therapeutic application has not yet been introduced due to its low water solubility and high hepatotoxicity.[53]

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[49] Sokolov, D., *et al.* Anti-viral activity of (-)- and (+)-usnic acids and their derivatives against influenza virus A (H1N1). *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7060-7064.
[50] Bruno, M., *et al.* (+)-Usnic acid enamines with remarkable cicatrizing properties. *Bioorg. Med. Chem.* **2013**, *21*, 1834-1843.
[51] Nunes, P., *et al.* Collagen-Based Films Containing Liposome-Loaded Usnic Acid as Dressing for Dermal Burn Healing. *J Biomed Biotechnol.* **2010**.
[52] Shi, Q., *et al.* Inhibition of cytothrome P450s enhances (+)-usnic acid cytotocxicity in primary cultured rat hepatocytes. *J. Appl. Toxicol.* **2013.**
[53] Francolini, I. *et al.* Water Soluble Usnic Acid-Polyacrylamide Complexes with Enhanced Antimicrobial Activity against *Staphylococcus epidermidis*. *Int. J. Mol. Sci.* **2013**, *14*, 7356-7369.

**Table 1.8** Biological studies of usnic acid

| **Biological studies of usnic acid in cancer cell lines, and pathogenic bacteria.** |
|---|
| 1. Active against B subtilis, B cereus, *Staphylococcus aureus*, and *Escherichia coli* with MICs of 8, 8, 31, and 31 mg/mL respectively.[54]<br>2. Evaluated against *Mycobacterium avium* (ATCC 15769), *Mycobacterium tuberculosis* (ATCC 27294) and *Mycobacterium kansasii* (ATCC 12478) with 16, 8 and 8 μg/mL respectively.[55]<br>3. Inhibits growth of eight fungal and *Omycota* species in the genera *Pythium*, *Phytophthora*, *Rhizoctonia*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Stagonospora*, and *Ustilago*.[48]<br>4. Increases P-450 activity and show toxicity against oxidative stress in human hepatoblastoma cells. (HepG2), breast cancer T-47D, pancreatic cancer Capan-2 cell lines.<br>5. Inhibits growth of human prostate carcinoma DU-145 and melanoma M-14 cells, inducing apoptotic cell death.[56]<br>6. Considered as a natural antimicrobial agent effective against some phatogenic fungi and gram positive bacteria, including *Staphylococcus*, *Streptococcus*, and *Pneumococcus*.[57] |

[54] Sultana, N., *et al.* A new depsidone and antibacterial activities of compounds from Usnea undulate Stirton. *Asian Nat Prod Res.* **2011**, *13*, 1158-1164.
[55] Lucarini, R., *et al.* Antimycobacterial activity of Usnea steineri and its major constituent (+)- usnic acid. *African Journal of Biotechnology.* **2012**, *11*, 4636-4639.
[56] Kowalski, M., *et al.* Bioactiviy of secondary metabolites and thallus extracts from lichen fungi. *Mycoscience.* **2011**, *52*, 413-418.
[57] Kim, S., *et al.* Effects, antimicrobial efficacy and cytotoxicity of usnic acid as a biofilm prophylaxis in PMMA. *J. Mater Med.* **2011**, *22*, 2773-2780.

# CHAPTER II

# EXTRACTION AND ISOLATION OF COMPOUNDS FROM FAGARA HEITZII AND EVERNIA PRUNASTRI

The first part of this work is focused on the study of 2 species: *Evernia prunastri* (Peru) and *Fagara heitzii* (Cameroon). For this purpose, a comprehensive review of the phytochemical of both species was done based on the studies reported in recent years.

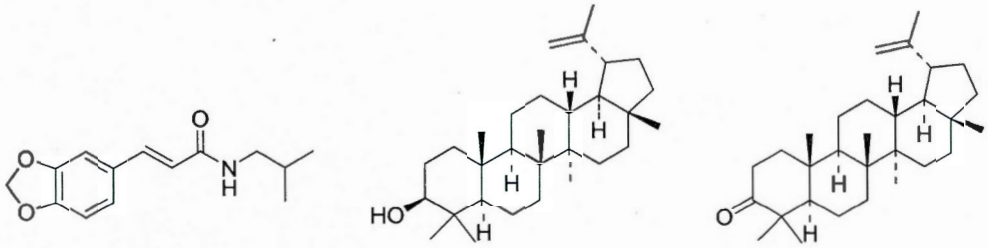
## 2.1. PLANT RESOURCE

The stem bark of *Fagara heitzii* was collected at Mbalmayo-Cameroon in June 2006 and identified by Mr. Nana Victor of National Herbarium, Yaounde, Cameroon, while branched thallus of *Evernia prunastri* were collected at Porcon farm located 30 km from the city of Cajamarca-Peru in July 2009 and identified by Dr Luis Dávila of National University of Cajamarca, Cajamarca, Peru.

## 2.2. RESULTS AND DISCUSSION

First of all, the stems bark of *Fagara heitzii* (994 g) were dried, powdered and soaked in solvents with an increasing polarity order: hexane (24 h (3x)), ethyl ether (24 h (x2)), chloroform (24 h (3x)), ethyl acetate (24 h (x2)) and methanol (24 h (3x)) at room temperature, respectively. After removal of the solvents by evaporation under reduced pressure, the crude products obtained from the chloroform extract were separated by repeated flash column chromatography on silica gel with hexane/dichloromethane (75:25-0:100) and dichloromethane/ethyl acetate (99:1-96:4) systems. Eleven fractions: A, B, C, D, E, F, G, H, I, J and K were collected and separated on the basis of TLC analysis. Fraction B and C were separated by preparative TLC and repeated columns chromatography, from which three

compounds were isolated and identified as lupeol (**9**), lupenone (**10**) and *trans*-fagaramide (**6**) (**Figure 2.1**). They chemical structure were confirmed by $^{1}$H-NMR and $^{13}$C-NMR spectrums and compared with reported data.[58,59,60]




*trans*-Fagaramide (**6**) Lupeol (**9**) Lupenone (**10**)

**Figure 2.1** Chemical structure of identified compounds in the extract of *Fagara heitzii*. *trans*-Fagaramide (**6**), lupeol (**9**) and lupenone (**10**).

Compound **6** (***trans*-fagaramide**) (12 mg) was obtained as a white solid. The IR spectrum shows bands at 3294 and 1650 corresponding to an amide fonction, and at 1253, 1038 and 931 cm$^{-1}$ corroborating the presence of double bond. The $^{1}$H-NMR spectrum shows chemical shifts at δ 7.53 ppm (d, 1H, $^{3}J_{CHCH}$ = 15.7 Hz, benzene-C*H*) and δ 6.21 ppm (d, 1H, $^{3}J_{CHCH}$ = 15.6 Hz, C*H*CO) corresponding to the protons at the β and α positions respectively. Chemical shifts at δ 5.99 and δ 5.56 ppm correspond to the methylenedioxy and N*H*, respectively (**Figure 2.2**).

---

[58] Gauthier, C., Legault, J., Lebrun, M., Dufour, P. and Pichette, A., Glycosidation of lupane-type triterpenoids as potent *in vitro* cytotoxic agents. *Bioorg. Med. Chem. Lett.* **2006**, *14*, 6713-6725.

[59] Fotie, J., Bohle, D., Leimanis, M., Georges, E., Rukunga, G. and Nkengfack, A. Lupeol Long-Chain Fatty Acid Esters with Antimalarial Activity from *Holarrhena floribunda*. *J. Nat. Prod.* **2006**, *69*, 62-67.

[60] Prakash, Ch. and Prakash, I. Isolation and Structural Characterization of Lupane Triterpenes from *Polypodium Vulgare*. *Res. J. Pharmaceutical Sci.* **2012**, *1*, 23-27.

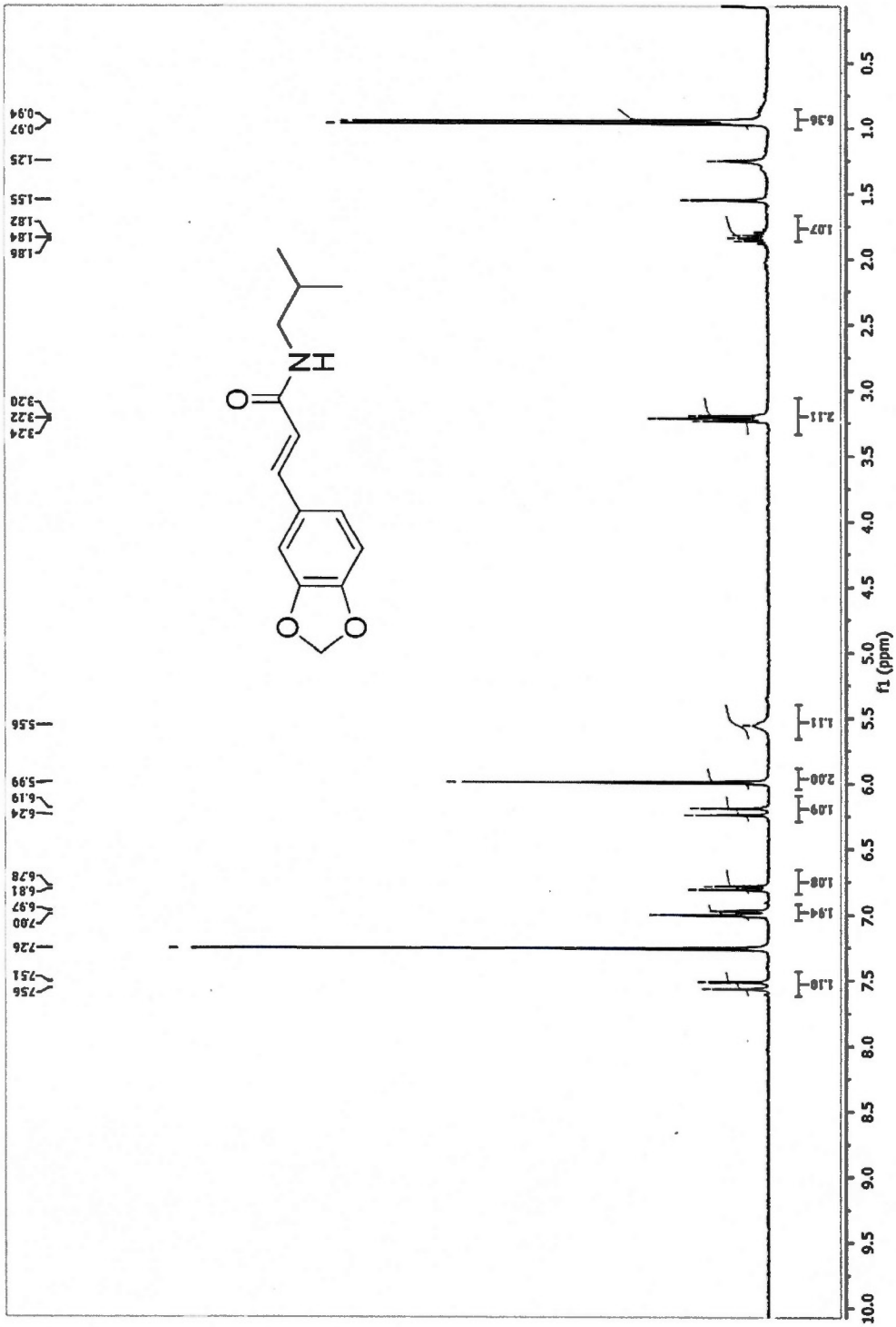



**Figure 2.2 $^{1}$H-NMR (300 MHz, $CDCl_3$) spectrum of *trans*-fagaramide isolated from *Fagara heitzii* (6).**

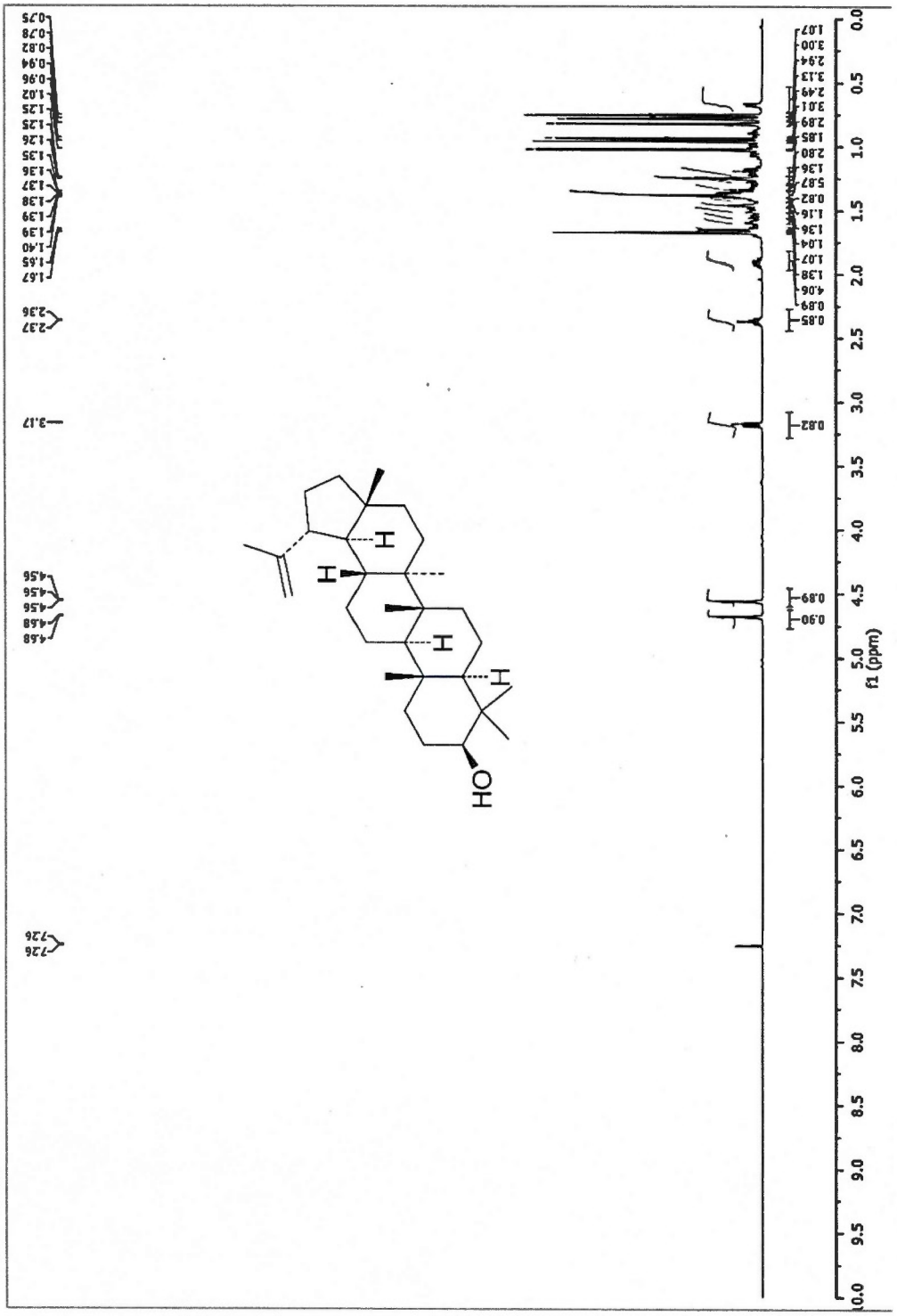
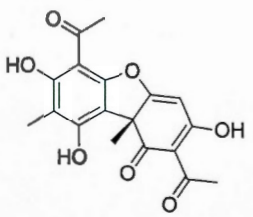



**Figure 2.3 $^{1}$H NMR (600 MHz, $CDCl_3$) spectrum of lupeol isolated from *Fagara heitzii* (9).**

Compound **9 (lupeol)** (26 mg) was obtained as a white solid. The IR spectrum shows the presence of the OH stretching with a value of 3311 cm-1. The 1H-NMR spectrum exhibits signals for the 6 methyl substituents at δ 0.80-0.99 ppm, signals at δ 4.57 and δ 4.69 ppm correspond to the methylene group. It also shows characteristic signals of H-3 at δ 3.19 ppm (dd, $^{3}J_{CHCH}$ = 4.98 and 11.72 Hz, CHOH) **(Figure 2.3)**.

Compound **10 (lupenone)** (10 mg) was obtained as a yellow solid. The determination of its chemical structure was made by comparing its $^{1}$H-NMR spectrum with the spectrum of compound **9 (lupeol)**, showing the absence of signal at δ 3.19 ppm, which confirms the presence of carbonyl instead of the hydroxyl group.

Then, the same procedure was followed for the isolation of compounds from *Evernia prunastri*. The branched dried thallus of *Evernia prunastri* (100 g) was crushed into a powder and extracted with an increasing polarity order of solvents using hexane (72 h (3x)), ethyl ether (72 h (x2)), chloroform (72 h (2x)), ethyl acetate (72 h (x2)), methanol (72 h (3x)) and water (72 h (3x)) at room temperature, respectively. The ethyl ether extract (4.8 g) was separated using repeatedly flash column chromatography with hexane/ethyl acetate (7:3) and hexane/toluene/ethyl acetate (85:5:15), obtaining 4 fractions A, B, C and D. Fraction D was cooled to room temperature then filtered whereby a precipitate was obtained as a greenish yellow solid. This precipitate was separated out and recrystallized in ethanol 99%, from which the majority compound was obtained as yellow crystals and identified as usnic acid **(33)** **(Figure 2.4)**, confirmed by $^{1}$H-NMR and $^{13}$C-NMR spectrums and by comparison with reported data.[61]




Usnic acid **(33)**

**Figure 2.4** Chemical structure of compound **33**

[61] Ingólfsdóttir, K. Usnic Acid. *Phytochemistry*. **2002**, *61*, 729-736.

Compound **33 (usnic acid)** (2.8 g) was obtained as yellow crystals. The IR spectrum showed bands at 2929 (intramolecular OH-stretching) 1687, 1628, 1540, 1455, 1421 $cm^{-1}$ (**Figure 2.5**). The $^{1}$H-NMR spectrum shows characteristic signals at δ 13.31 and δ 11.02 ppm corresponding to the hydroxyl groups; δ 5.97 ppm corresponding to the H from the double bond and δ 2.67, 2.66, 2.10, and 1.75 ppm corresponding to the methyl substituents (**Figure 2.6**).[62]

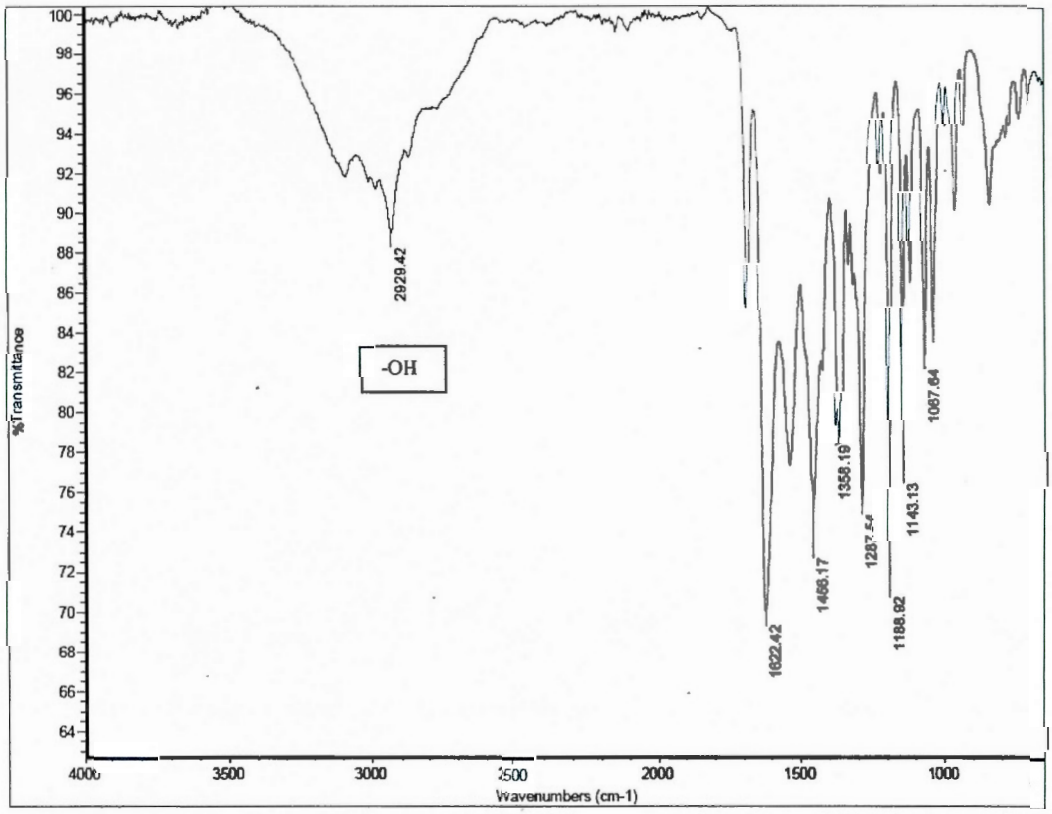



**Figure 2.5** IR spectrum of compound **33**.

[62] Melgarejo, M., Sterner, O., Vila, J. and Mollinedo P. More investigations in potent activity and relationship structure of the lichen antibiotic (+)-Usnic Acid and its derivate Dibenzoylusnic acid. *Revista Boliviana de Química.* **2008**, *25*, 24-29.

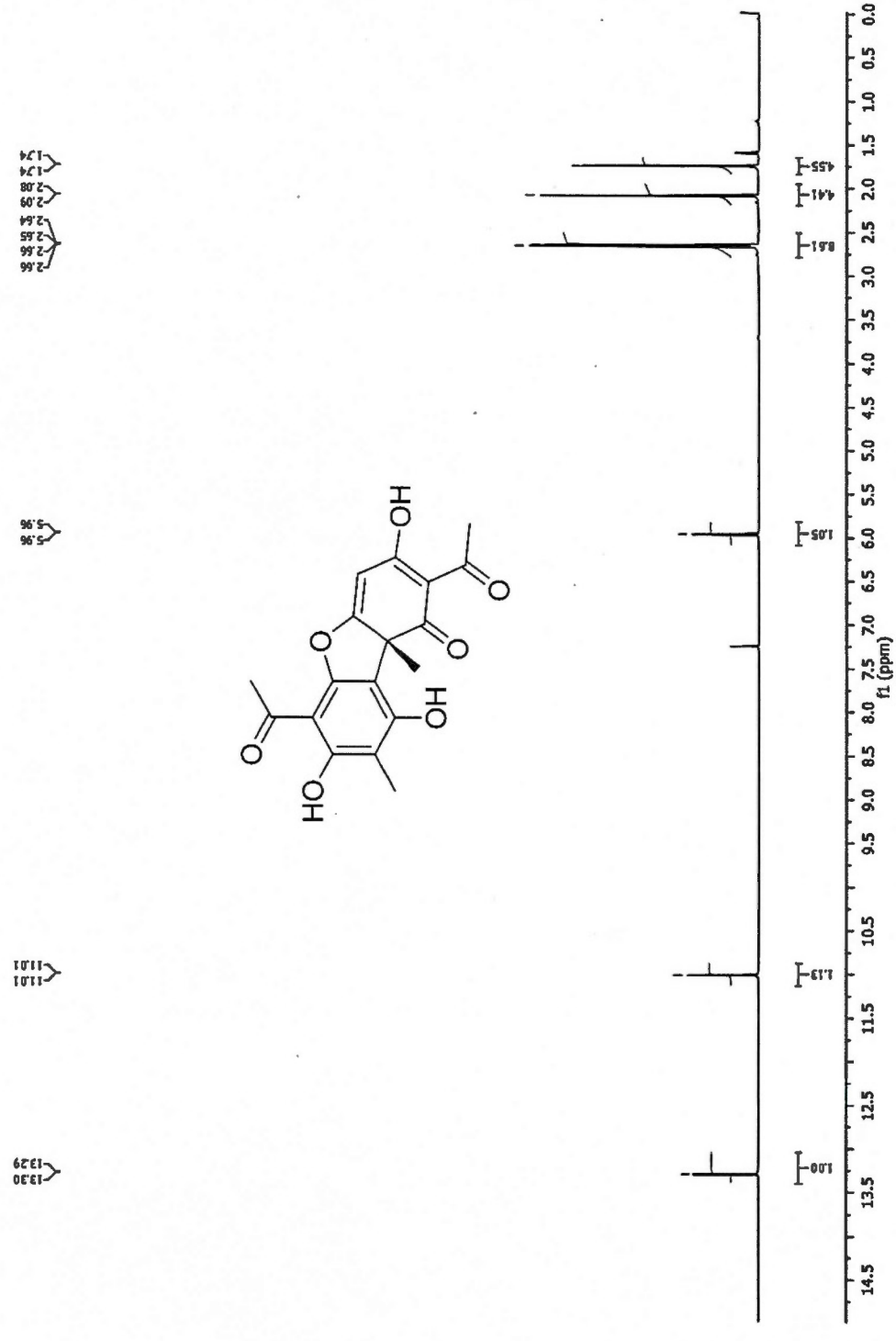



**Figure 2.6 $^{1}$H NMR (600 Hz, CDCl $_{3}$) spectrum of usnic acid isolated from *Evernia prunastri* (33).**

## 2.3 GENERAL CONCLUSIONS

*trans*-Fagaramide (26 mg) and (+)-usnic acid (2.8 g) were isolated from the plant *Fagara heitzii* (Cameroon) and the lichen *Evernia prunastri* (Peru), respectively (**Figure 2.7**). Their isolation was performed using standard protocols of extraction and chromatographic purification. Their chemical structures were elucidated using 1D and 2D NMR spectroscopy and mass spectrometry, thus corroborating spectroscopic data reported in the literature.[61,65]

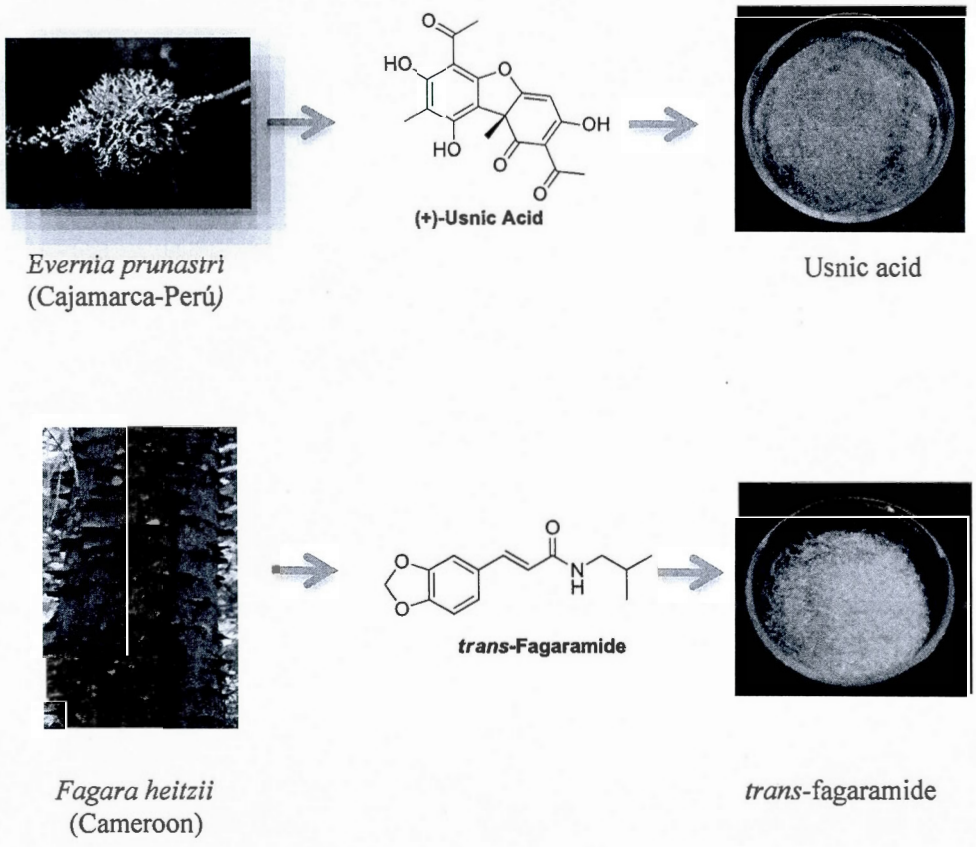



**Figure 2.7** Structures and crystals of usnic acid from *E. prunastri* (Peru) and *trans*-fagaramide from *F. heitzii* (Cameroon).

# CHAPTER III

## SYNTHESIS OF TRANS-FAGARAMIDE AND COMPOUNDS 34-39

trans-Fagaramide, isolated from the plant Fagara heitzii (Cameroon) was studied and elucidated using 1D and 2D NMR spectroscopy and mass spectrometry. Considering the range of its known biological activities, the next part of the project concerned the synthesis of *trans*-fagaramide and other compounds with similar chemical structures.

The synthetic platform formed by these compounds has served as starting material for subsequent reactions.

The reactions exploited for the elaboration of this platform are described in the following paragraphs.

### 3.1. KNOEVENAGEL CONDENSATION

Knoevenagel condensation of malonic acid with aromatic aldehydes is an important route for substituted α,β-unsaturated acids. This involves heating aromatic aldehydes and malonic acid in presence of excess basic solvents such as pyridine and piperidine to finally afford cinnamic acids (**Scheme 3.1**).[63, 64, 65, 66]

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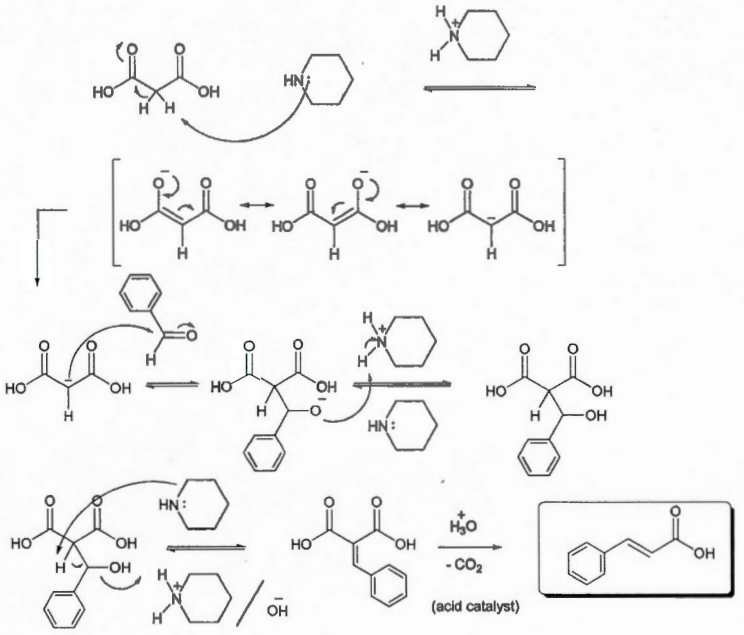
[63] Kumar, S., *et al.* Non solvent reaction ammonium acetate catalyzed highly convenient preparation of *trans*-cinnamic acids. *Synthetic Commun.* **1998**, *28*, 3811-3815.

[64] Mogilaiah, K., *et al.* Microwave-Assisted Solvent-Free Synthesis of trans-Cinnamic Acids Using Lithium Chloride as Catalyst. *Synthetic Commun.* **2004**, *34*, 205-210.

[65] Van Veldhoven, J. P. D., *et al.* Structure-acitivity relationships of *trans*-substituted-propenoic acid derivatives on the nicotinic acid receptor HCA2 (GRPR109A). *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2736-2739.

[66] Guan, L., Wei, C., Deng, X., Sui, X., Piao, H. and Quan, Z. Synthesis and anticonvulsant activity of N-(2-hydroxyethyl) cinnamamide derivatives. *Eur. J. Med. Chem.* **2009**, *44*, 3654-3657.




**Scheme 3.1** Knoevenagel condensation of malonic acid with aromatic aldehydes.

## 3.2. PEPTIDE COUPLING

There are different ways for the activation of carboxylic acid groups; one of them is using carbodiimide reagents which have been used in peptide synthesis because of their moderate activity and cheap price. Carbodiimides have expanded their scope with the aid of some additives such HOAt, HODhbt, HOBt, etc. These additives complement the weakness of coupling reagents by enhancing the reaction rate (**Scheme 3.2**).[67, 68]

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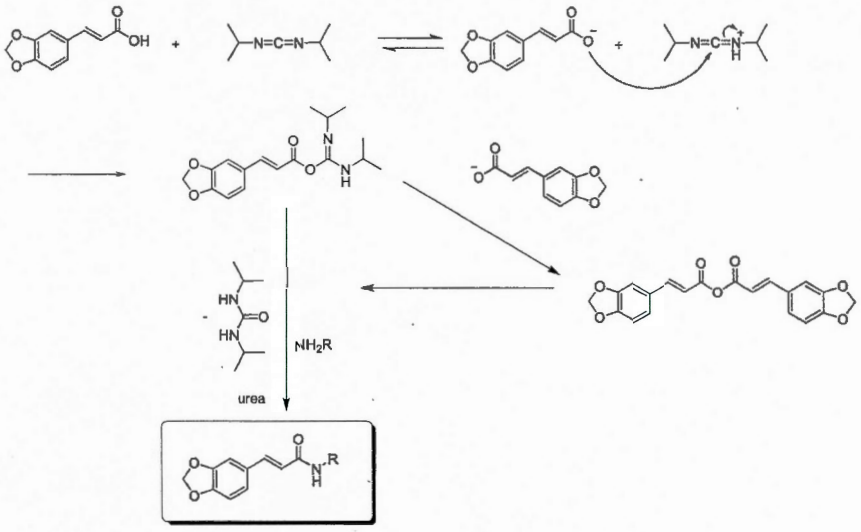
[67] Han, S., *et al.* Recent development of peptide coupling reagents in organic synthesis. *Tetrahedron.* **2004,** *60*, 2447-2467.

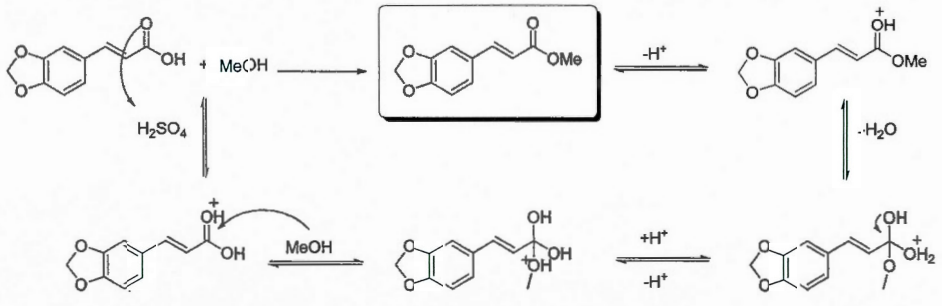
[68] Montalbetti, C., *et al.* Amide bond formation and peptide coupling. *Tetrahedron.* **2005**, *61*, 10827-10852.




**Scheme 3.2** Peptide coupling using DIC.

## 3.3. FISCHER ESTERIFICATION

Fischer esterification is considered one of the most important reactions of carboxylic acids. The reaction between a carboxylic acid and an alcohol in the presence of an acid catalyst (normally, $H_2SO_4$ or HCl) allows the formation of esters by elimination of a molecule of water (**Scheme 3.3**).[69]

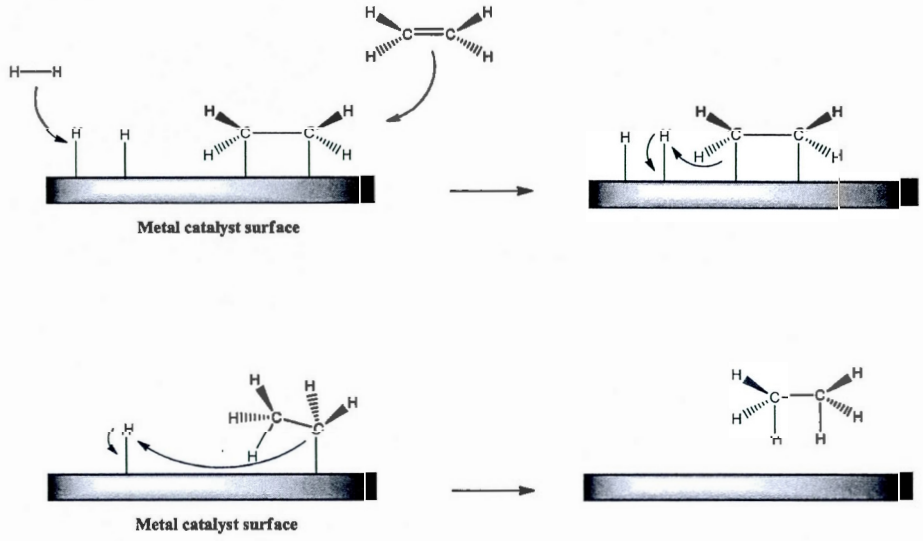



**Scheme 3.3** Fischer Condensation between carboxylic acid and methanol.

[69] http://open.bu.edu/bitstream/handle/2144/2686/Fisher%20Esterification.pdf?sequence=4.Accessed 30 July 2014.

## 3.4. HYDROGENATION REACTION

Basically, hydrogenation consists in the addition of pairs of hydrogen atoms to a multiple bond, usually in the presence of a catalyst. The process is commonly employed to reduce or saturate organic compounds. Platinum, palladium, rhodium and ruthenium are considered as highly active catalyst (**Scheme 3.4**).[70]




**Scheme 3.4** Example of catalytic hydrogenation of alkenes.

## 3.5. RESULTS AND DISCUSSION

The synthesis of *trans*-fagaramide (compound **35**) is performed in two steps with an overall yield of 56 %. First, compound **34** (75 %) is obtained by Knoevenagel condensation between malonic acid and piperonal, then *trans*-fagaramide (75 %) is obtained by coupling peptide employing isobutylamine and compound **34**, DIC and HOBt were used as coupling agents (**Scheme 3.5**).

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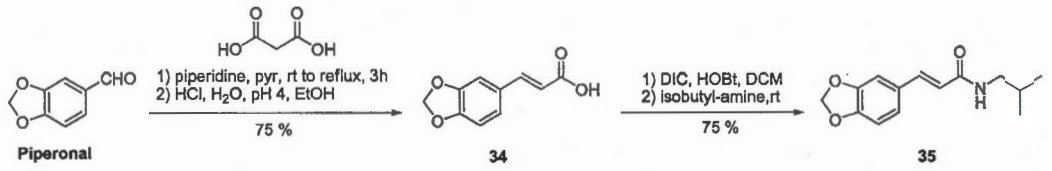
[70] http://www.cyclopaedia.de/wiki/Catalytic_addition_of_hydrogen. Accessed 30 July 2014.




**Scheme 3.5** Synthesis of *trans*-fagaramide (**35**)

$^1$H-NMR spectrums of compound **34** and **35** were analyzed and compared. The $^1$H-NMR spectrum of compound **35** shows the characteristic signals of isobutylamine appearing upfield, which confirms the obtention of the desired molecule without the presence of the starting material (**Figure 3.1**).

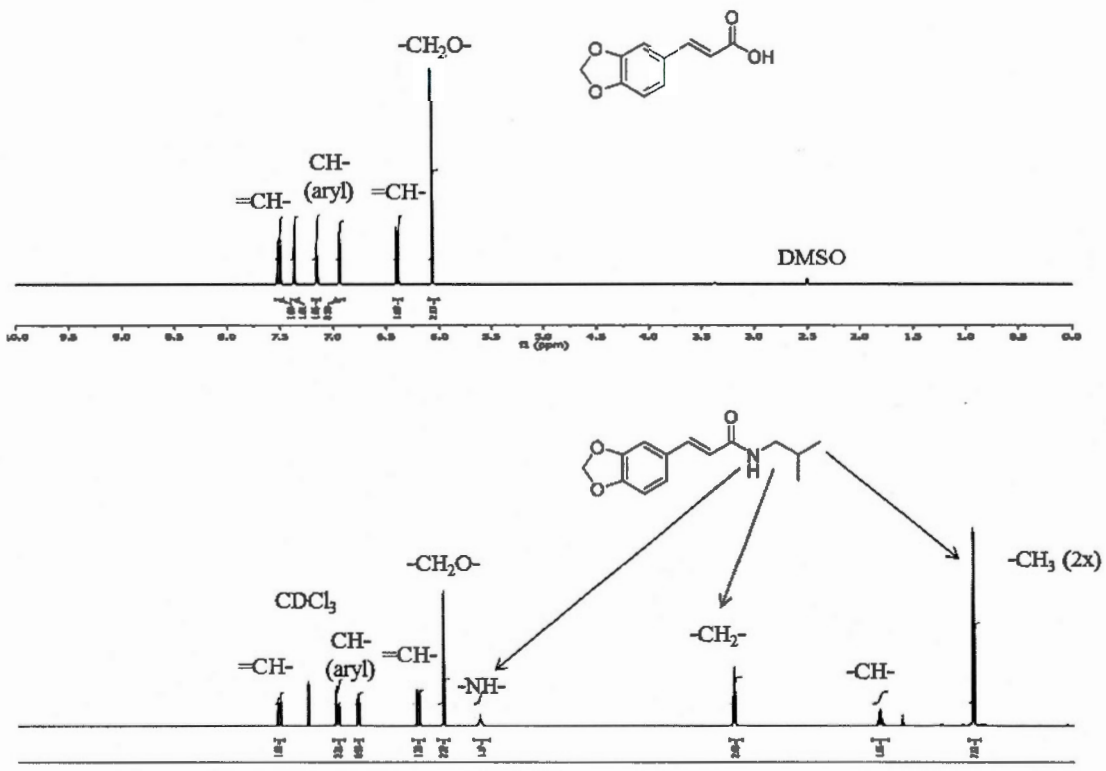



**Figure 3.1** $^1$H-NMR spectrums of compound **34** and **35**.

The $^{1}$H-NMR spectrum of isolated *trans*-fagaramide was also compared with the $^{1}$H-NMR spectrum of the synthetic *trans*-fagaramide showing signals with similar chemical shifts (**Figure 3.2**).

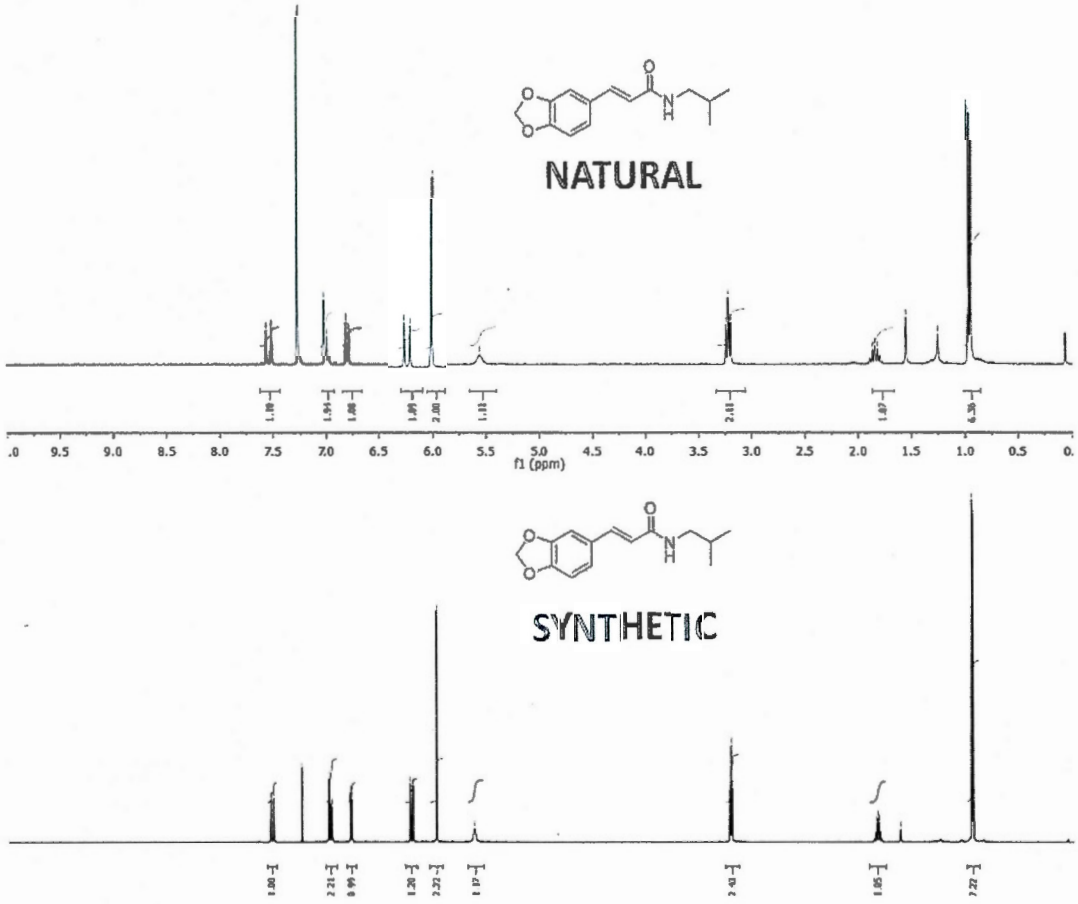
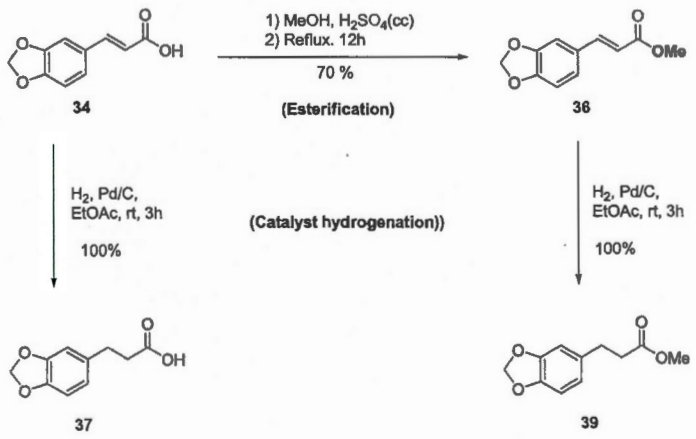



**Figure 3.2.** Comparison between $^{1}$H-NMR (600 Hz, $CDCl_3$) spectrums of natural and synthetic *trans*-fagaramide.

Compound **36** (70 %) was obtained by Fischer esterification, being the first synthetized analog for the elaboration of the chemical library. Compounds **37, 38** and **39** were obtained with yields of 100% by using catalyst hydrogenation with compounds **34, 35** and **36** as reagents, respectively (**Scheme 3.6**).

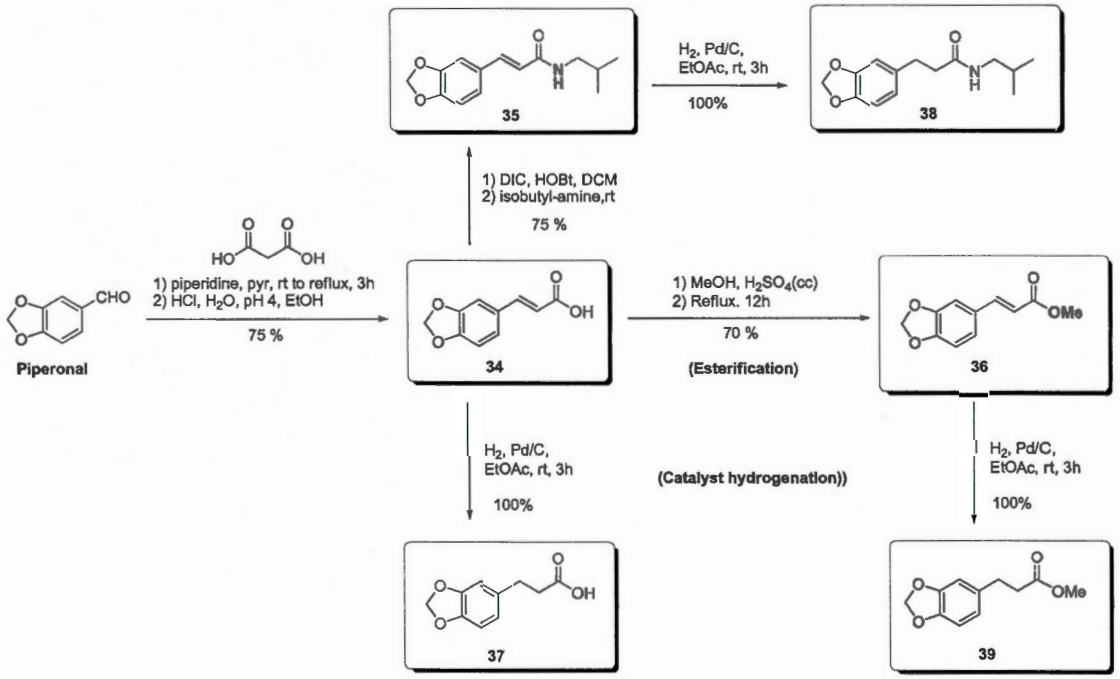



**Scheme 3.6** Synthesis of compounds **36, 37** and **39.**

$^1$H-NMR spectrums of compounds **34, 35, 36, 37, 38** and **39** are shown in **figures**: **3.3, 3.4, 3.5, 3.6, 3.7** and **3.8** respectively.

## 3.6 GENERAL CONCLUSIONS

*trans*-Fagaramide (compound **35** ) and 5 more compounds: Compounds **34, 36, 37, 38** and **39** were successfully synthesized in high yields through Knoevenagel condensation and peptide coupling, Fischer esterification and hydrogenation over catalyst (**Scheme 3.7**). All synthetics compounds were elucidated using 1D and 2D NMR spectroscopy and mass spectrometry, thus corroborating spectroscopic data reported in the literature.




**Scheme 3.7** Synthesis of *trans*-fagaramide (**35**) and compounds **34**, **36**, **37**, **38** and **39**.

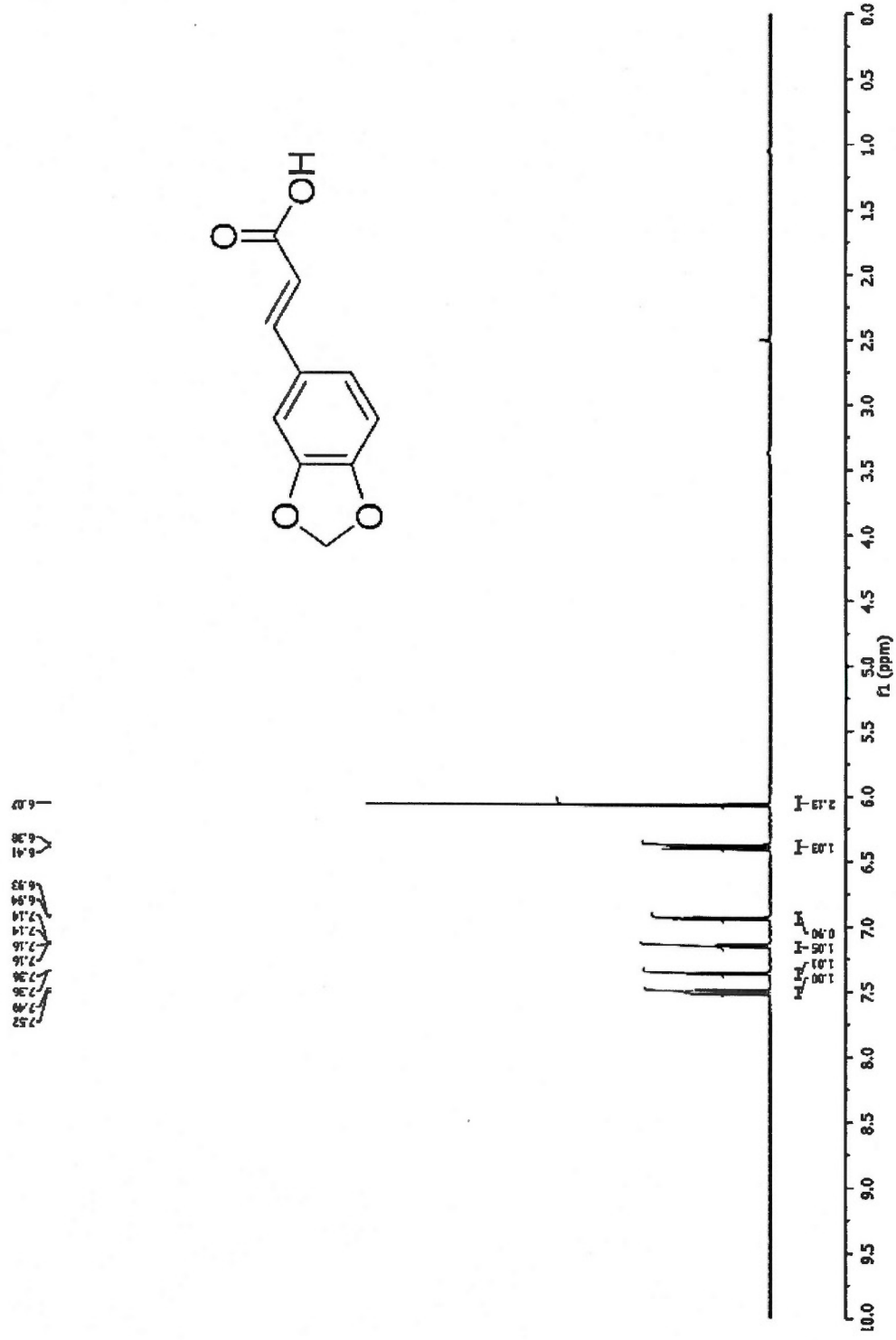



**Figure 3.3 $^1$H NMR ( 600 MHz, DMSO) spectrum of compound 34.**

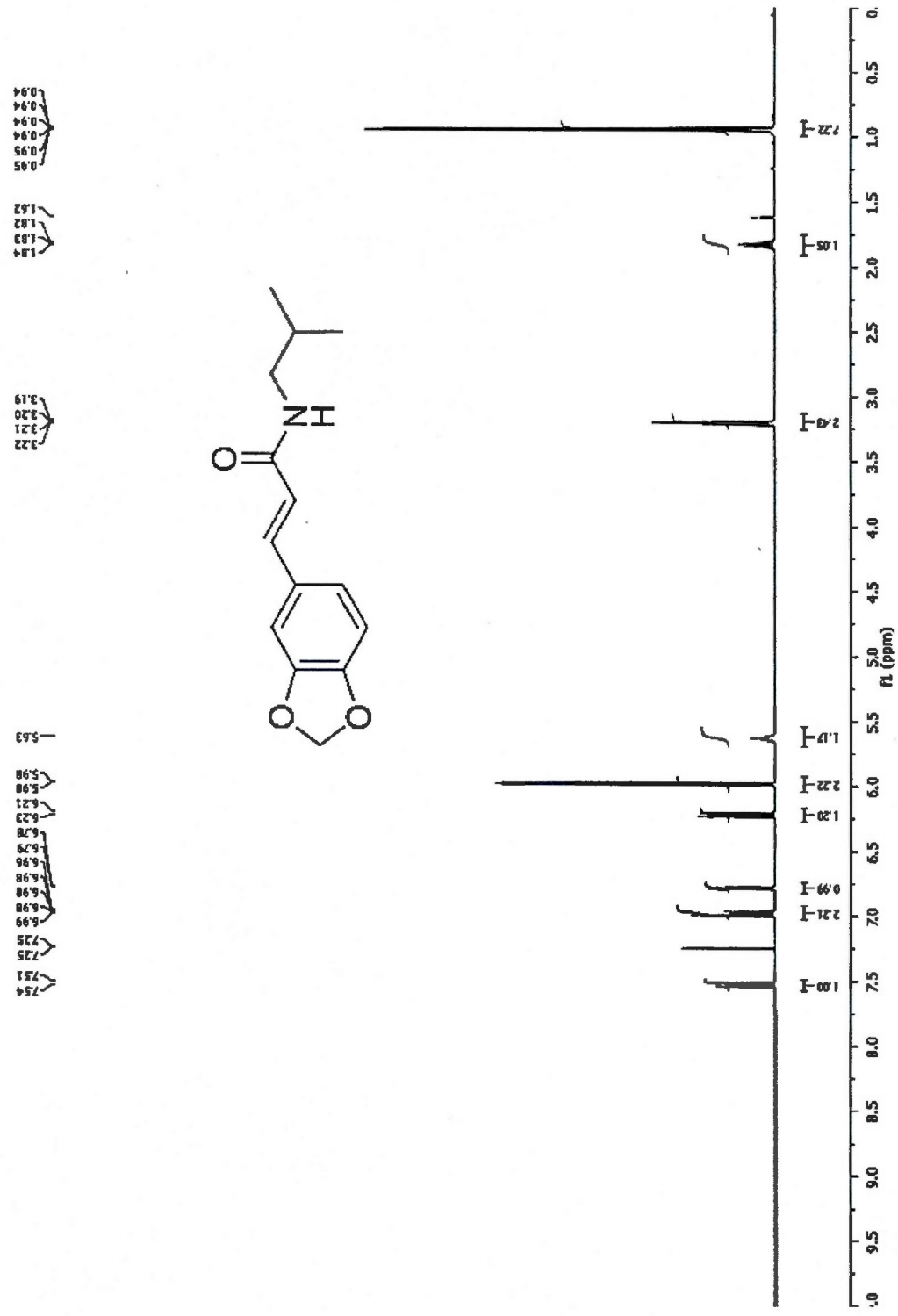



**Figure 3.4 $^{1}$H NMR (600 MHz, CDCl $_3$) spectrum of compound 35.**

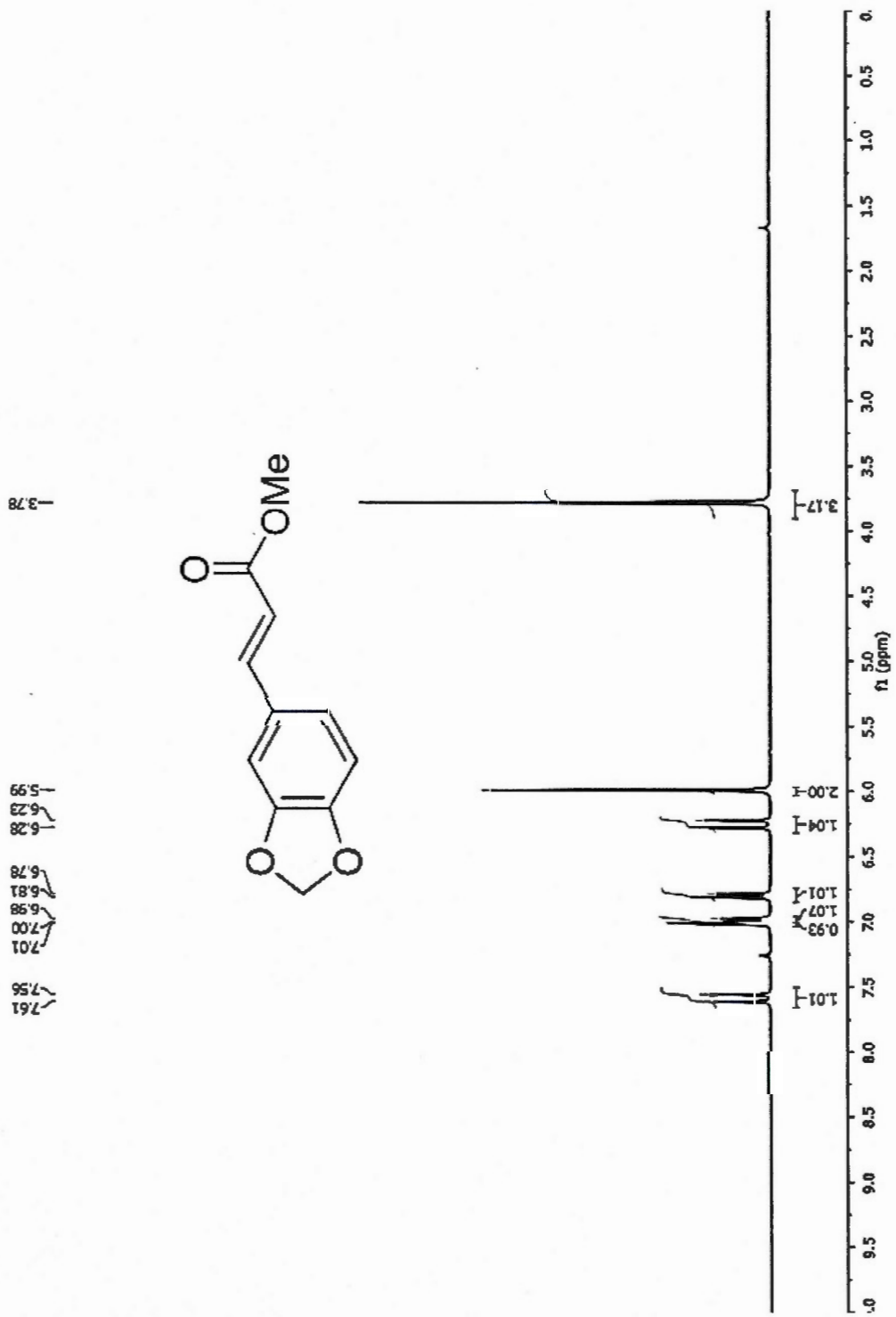



**Figure 3.5 $^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of compound 36.**

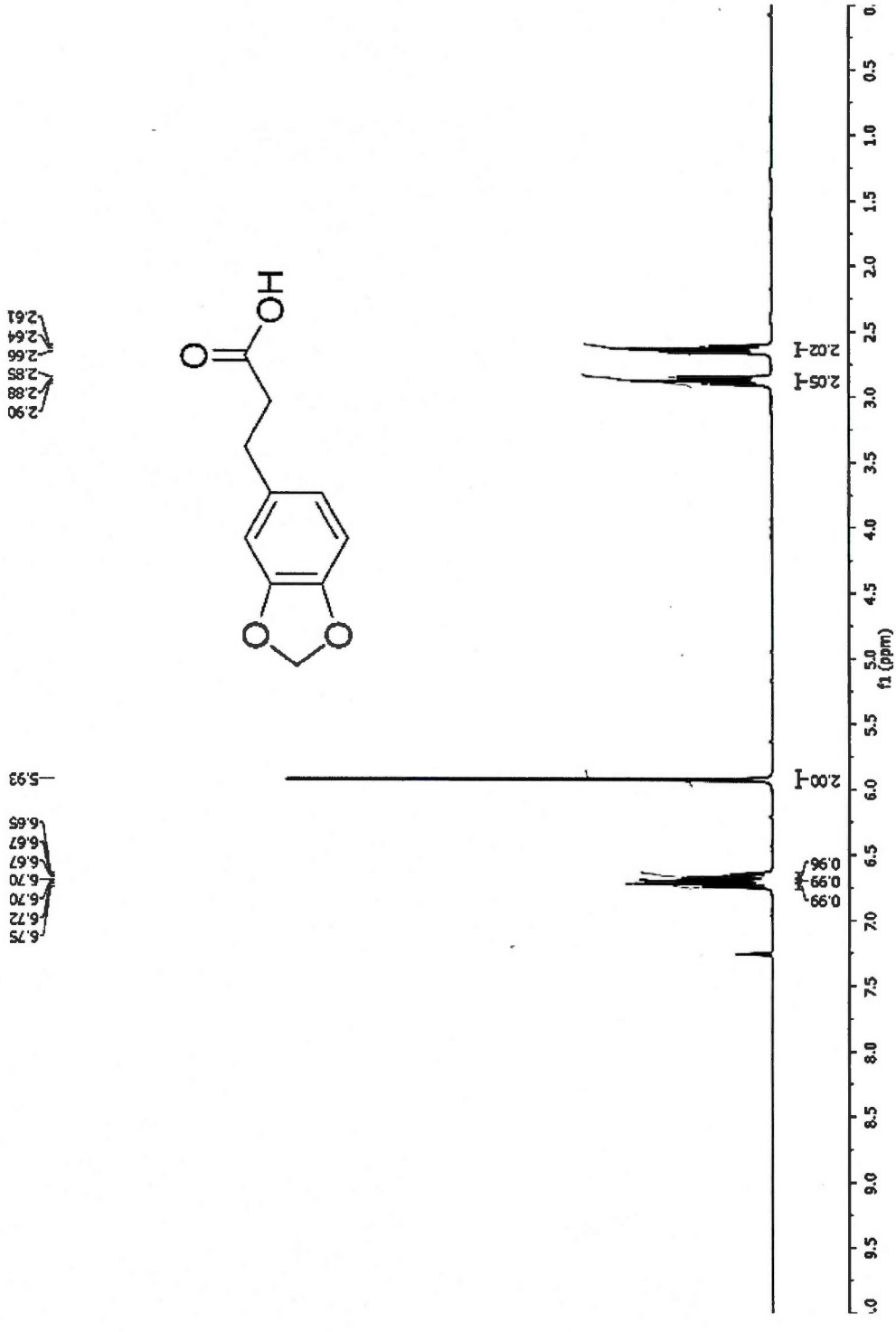



**Figure 3.6 $^1$H NMR (300 MHz, $CDCl_3$) spectrum of compound 37.**

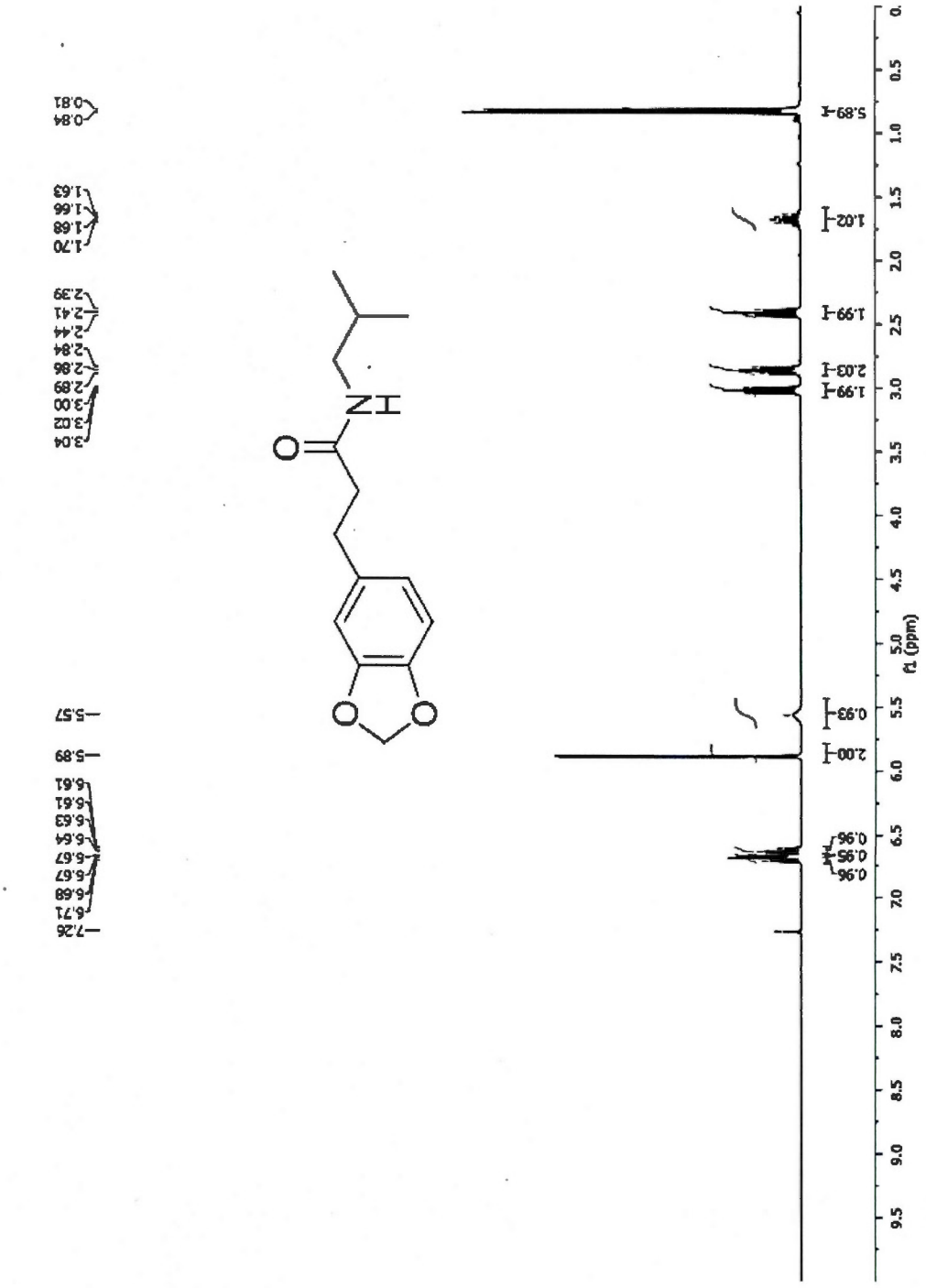



**Figure 3.7 $^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of compound 38.**

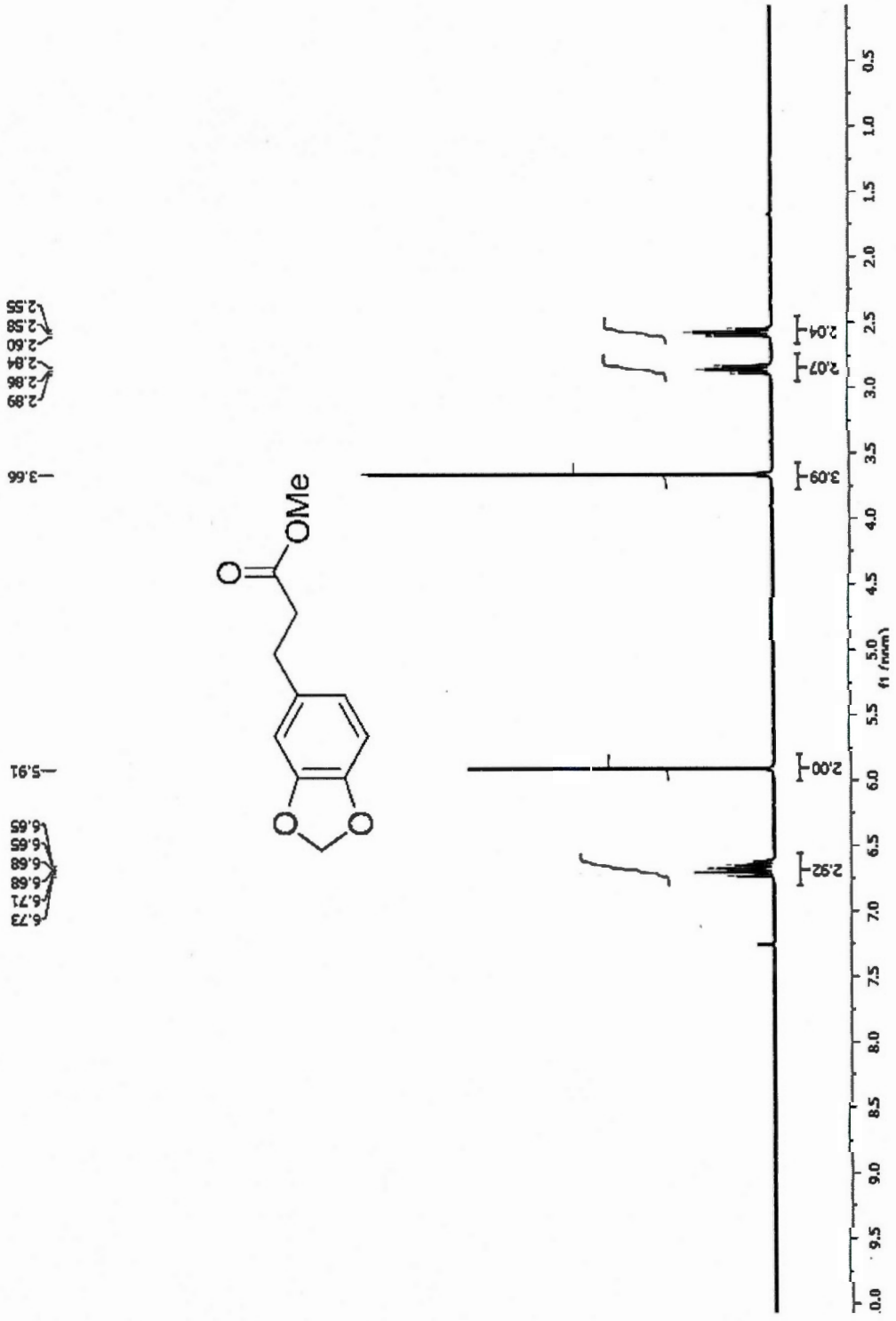



**Figure 3.8 $^1$H NMR ( 300 MHz, $CDCl_3$ ) spectrum of compound 39.**

CHAPTER IV

## SYNTHESIS OF REGIOISOMER OF HEITZIAMIDE A

As a second part of the project, the synthesis of a regioisomer of Heitziamide A was obtained based on the previous literature reports.[28]

Heitziamide A, a natural compound found in *Fagara heitzii,* is regarded as being derived from Diels-Alder reaction between *trans*-fagaramide and the monoterpene, myrcene (**Figure 4.1**). The first interest was to synthesize heitziamide A and then synthesize some other analogs based on the same type of reaction. (See page 23).[3,5,71]

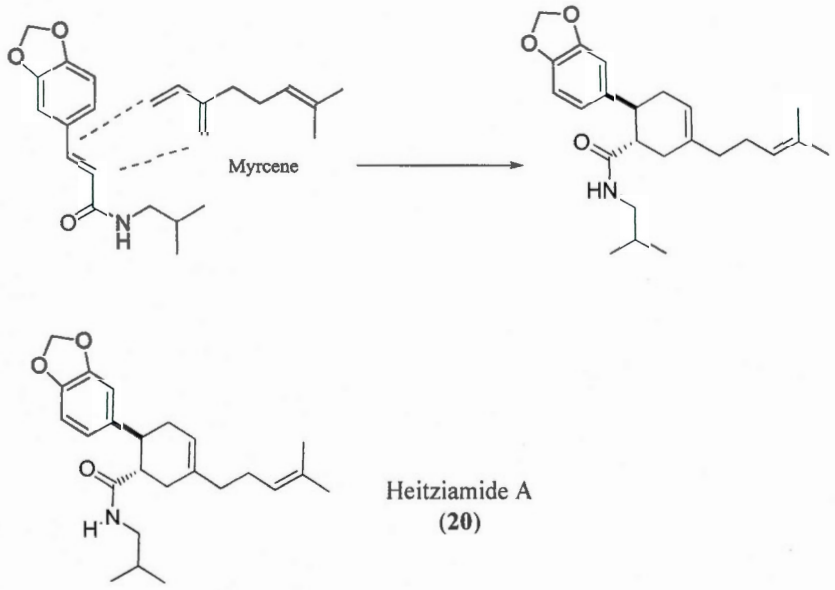



**Figure 4.1** Proposed synthesis of Heitziamide A.

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[71] Corbett, J. and Weavers, R. Aluminosilicate Catalysis of Chalcone Diels-Alder Reactions. *Synthetic Commun.* **2008**, *38*, 489-498.

## 4.1. DIELS ALDER CYCLOADDITIONS

The Diels-Alder reaction is a concerted ($4\pi + 2\pi$) cycloaddition reaction of a conjugated diene on a dienophile (**Scheme 4.1**). This reaction provides access to C-C bond-forming reactions. Diels-Alder cycloaddition is a powerful tool in organic synthesis since it gives rise to several pathways towards the construction of substituted cyclohexenes with a regioselectivity, diastereoselectivity, and enantioselectivity.[72, 73, 74]


**Scheme 4.1** Diels-Alder cycloadditions.

## 4.2. RESULTS AND DISCUSSION

As it was expected, the polarization of the diene (myrcene) and dienophile (*trans*-fagaramide) π bonds gave compound **40**, which is a regioisomer of Heitziamide A, obtained by thermal Diels-Alder cycloaddition between myrcene and *trans*-fagaramide (compound **35**) with a yield of 65 % (100 mg). Therefore a racemic mixture was obtained by the analysis of $^1$H-NMR spectrum of the raw product. Isomers *cis/trans* showed ratios of 1:2 respectively, which were determinated by comparing proton integrations of the given signals in the $^1$H-NMR spectrum (**Figure 4.2**).

---

[72] Oskooie, H. Diels-Alder reaction of myrcene with carbonyl containing dienophiles supported on silica gel under microwave irradiation. *Phosphorus Sulfur Silicon Relat. Elem.* **2004**, *179*, 1165-1167.
[73] Yin, D., Yin, D., Fu, Z. and Li. Q. The regioselectivity of Diels Alder reaction of myrene with carbonyl-containing dienophiles catalysed by Lewis acids. *J.Mol. Cata. A: Chem.* **1999**, *148*, 87-95.
[74] Lauchli, R., Whitney, J., Zhu, L. and Shea, K. Synthesis and Chemistry of Bridgehead Allylsilanes. Steroselective Reactions with Aldehydes. *Org. Lett.* **2005**, 7, 3913-3916.

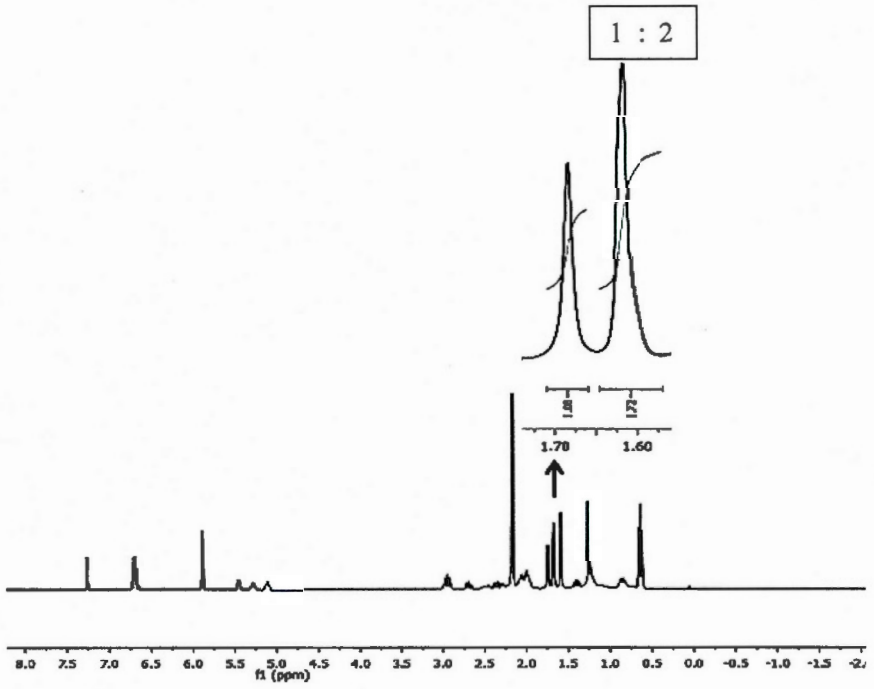
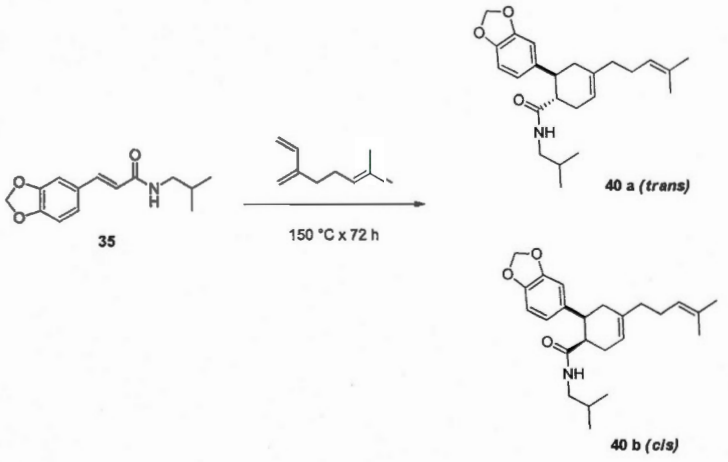



**Figure 4.2** $^{1}$H-NMR spectrum of raw product obtained in the synthesis of compound **40.**

The mixture was sucessfully separated into its two stereoisomers by column chromatography with hexane/ethyl acetate (50:0-40:10). The isomers were named compound **40 a (*trans*)** and **40 b (*cis*),** respectively. **(Scheme 4.2).**




**Scheme 4.2** Synthesis of compound **40.**

To assign the correct configuration (*cis/trans*) to each of the 2 stereoisomers, the techniques of 2D-COSY, HMBC and NOESY were employed. For this purpose both isomers (compound **40 a** and **40 b**) were analyzed by $^{1}$H-NMR (**Figure 4.3** and **4.4**), $^{13}$C-NMR (**Figure 4.6** and **4.7**), COSY (**Figure 4.9**) and HMQC in order to establish the connections between its strucural units.

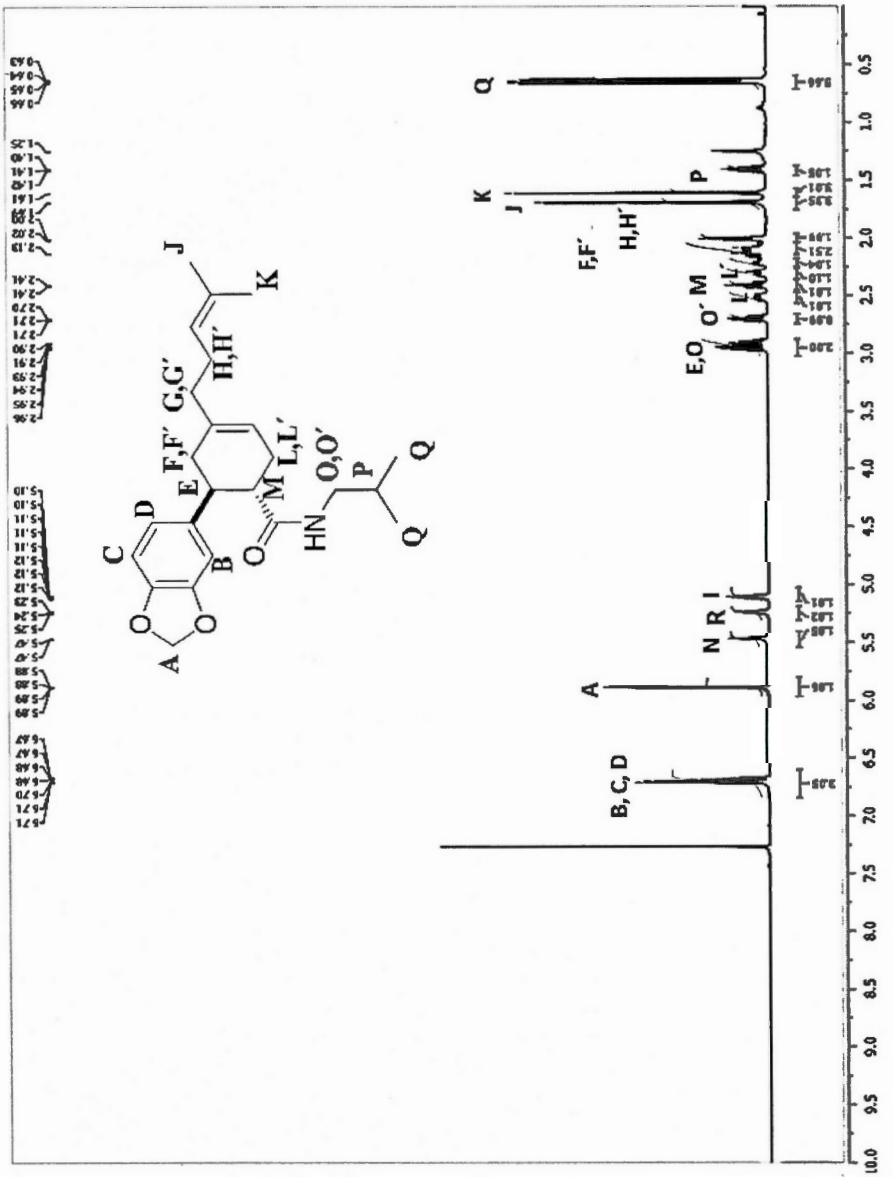



**Figure 4.3 $^{1}$H NMR (600 MHz, $CDCl_3$) spectrum of compound 40 a (*trans*).**

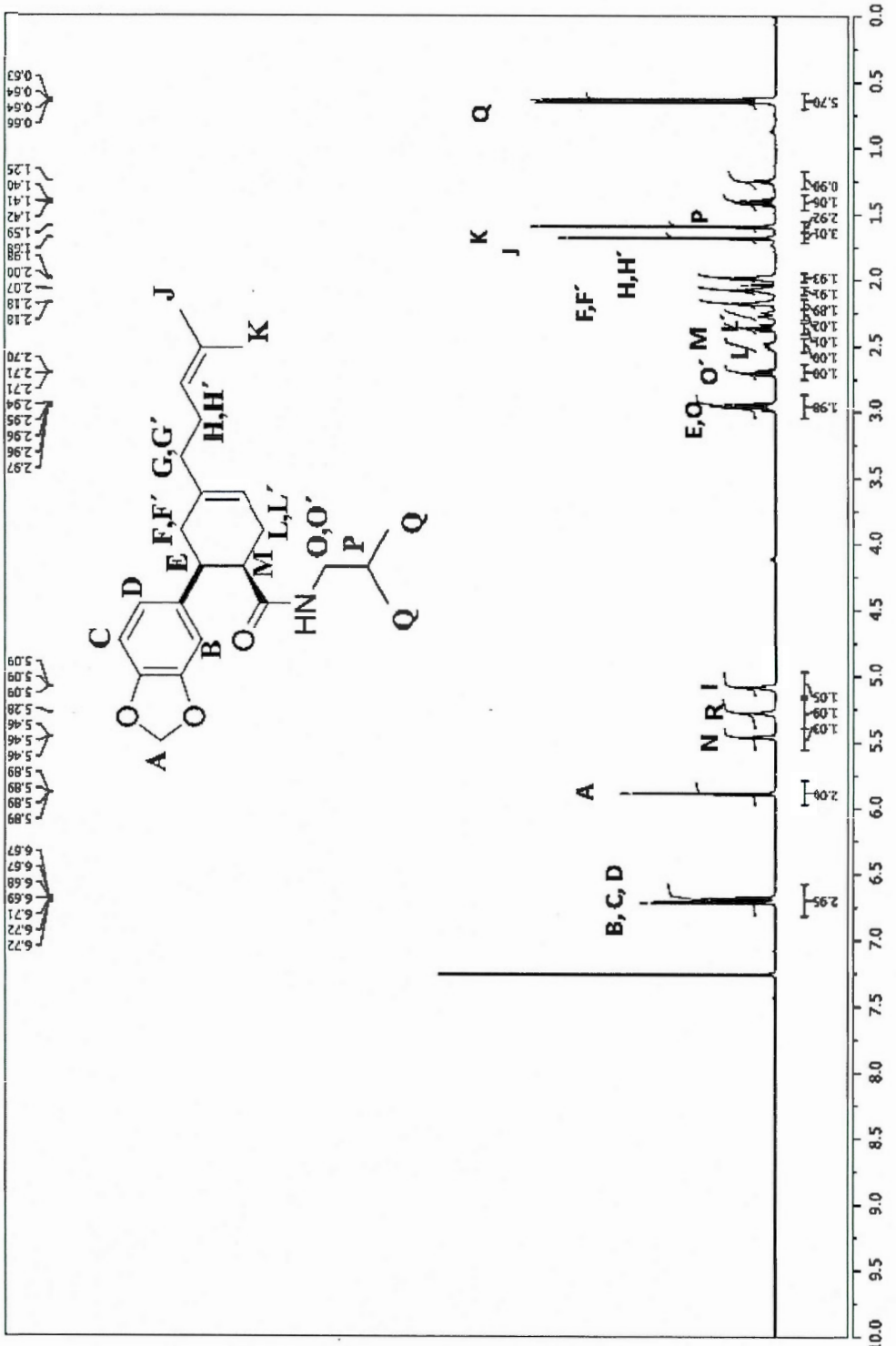



**Figure 4.4 $^{1}$H NMR (600 MHz, $CDCl_3$) spectrum of compound 40 b (*cis*).**

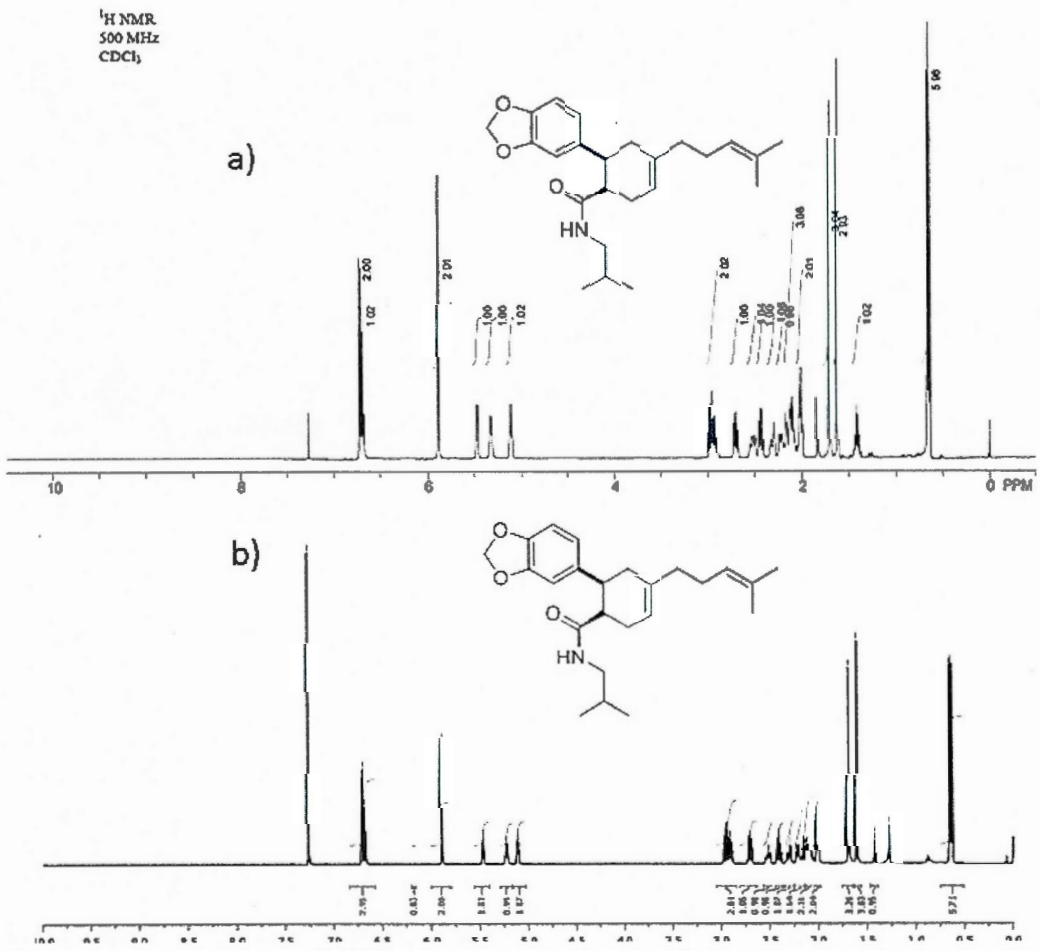



**Figure 4.5** Comparison between $^{1}$H-NMR spectrum of compound 40 b (*cis*) (600 MHz, $CDCl_3$) (b) and $^{1}$H-NMR spectrum from the article by Lin et *al.* [28](a)

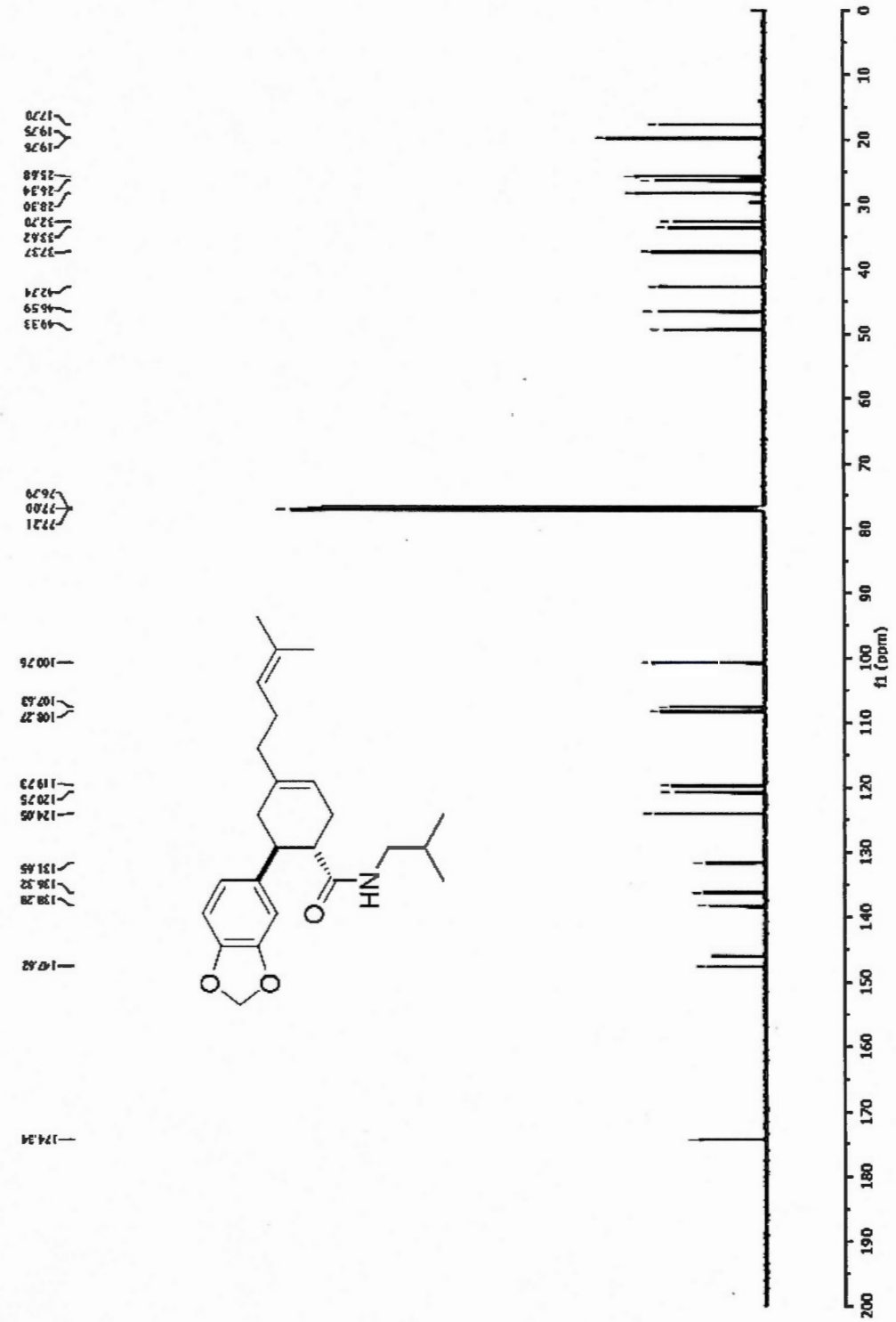



**Figure 4.6 $^{13}$C NMR (600 MHz, $CDCl_3$) spectrum of compound 40 a (*trans*).**

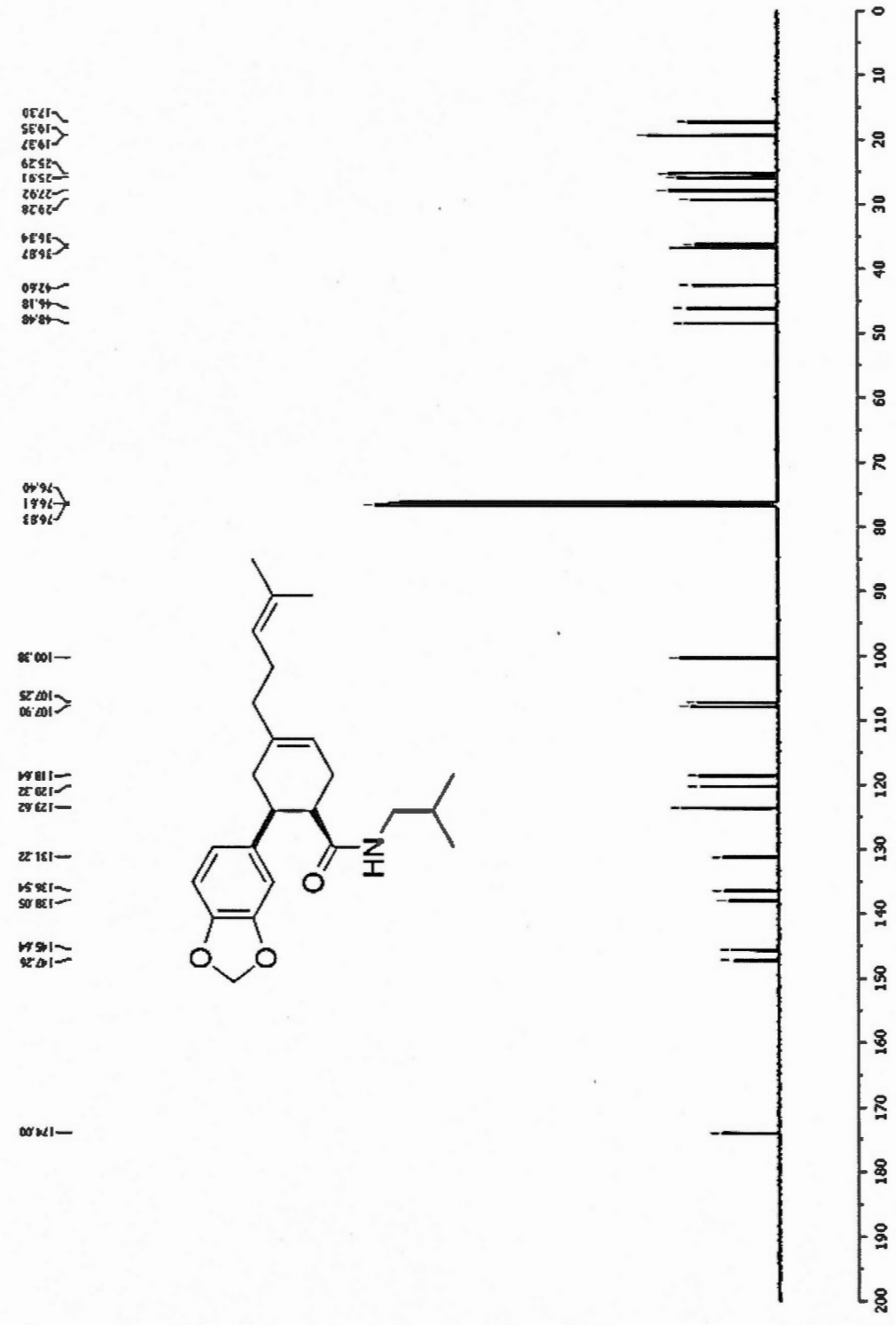



**Figure 4.7** $^{13}$C NMR (600 MHz, $CDCl_3$) spectrum of compound 40 b (*cis*).

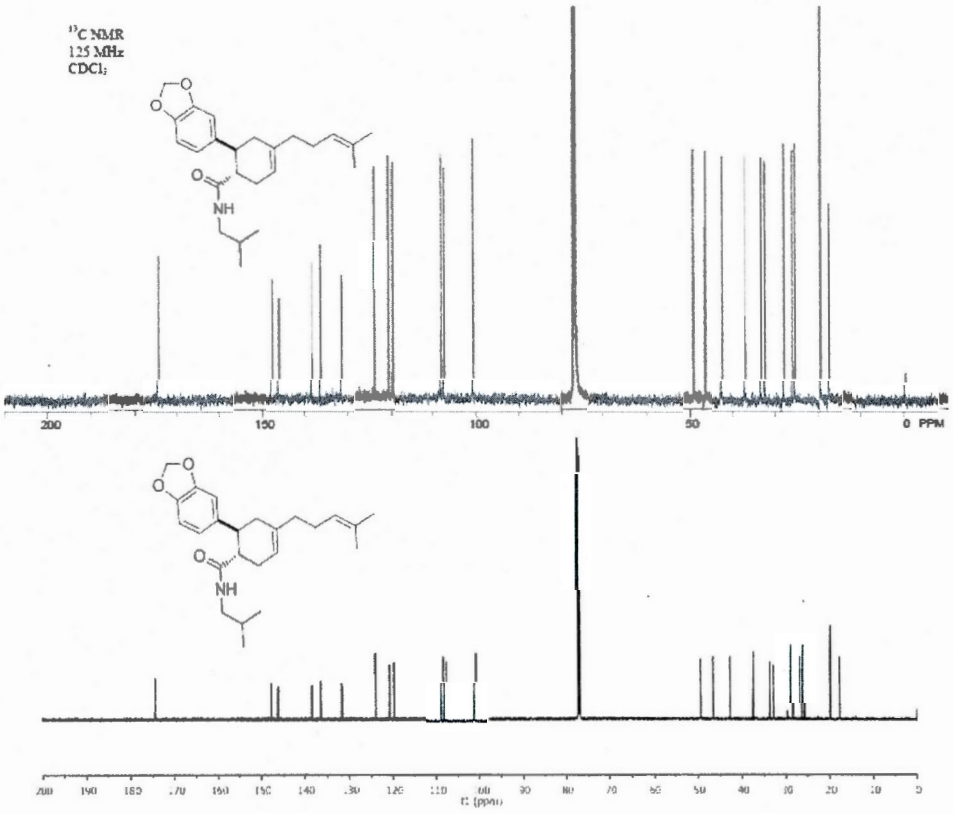



**Figure 4.8** Comparison between $^{13}$C-NMR spectrum of compound 40 a (*trans*) (150 MHz, $CDCl_3$) (b) and $^{13}$C-NMR spectrum from the article by Lin *et al* [28] (a)

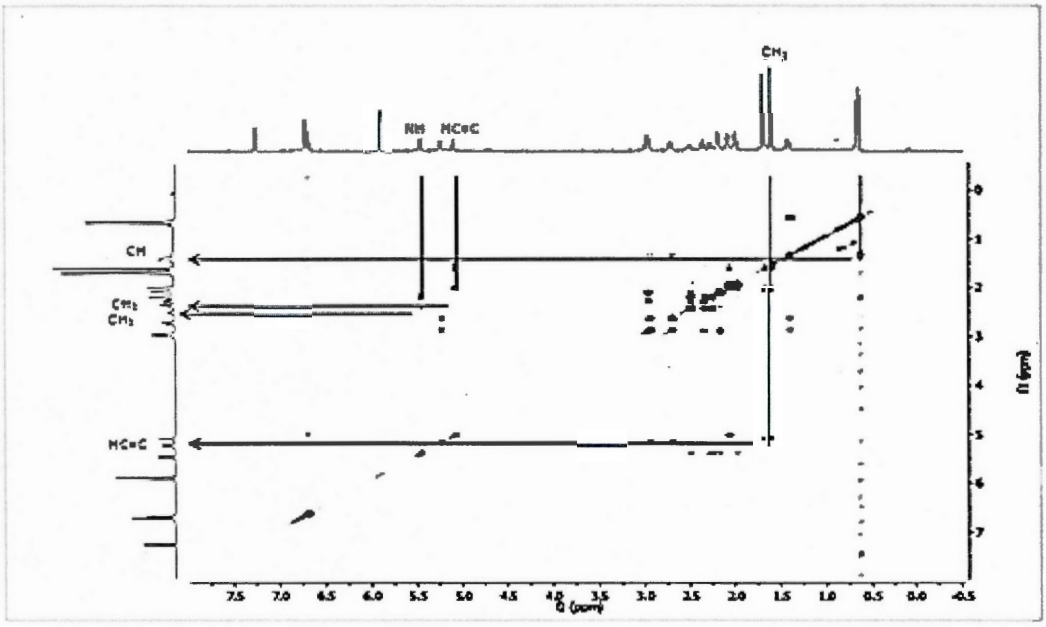



**Figure 4.9** 2D-COSY spectrum of compound **40 b** (***cis***).

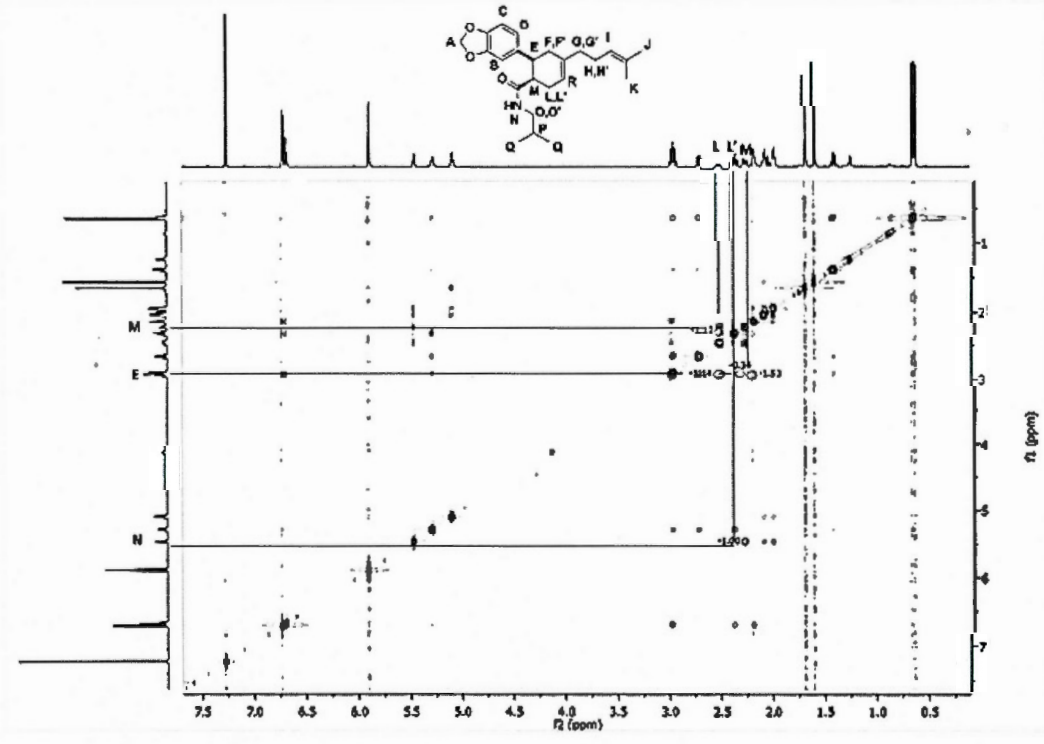



**Figure 4.10** NOESY spectrum of compound **40 b** (***cis***).

The assignment of the signals was attributed according to the 1D-$^{1}$H NMR, $^{1}$H-$^{1}$H COSY and $^{1}$H-$^{13}$C HMBC spectrums. Subquently, the spectrum of $^{1}$H-$^{1}$H NOESY was analyzed. The relatives NOE values were stablished and allowed the estimation of the connectivity between the protons: $H_{L'}$ / $H_N$ = 1, $H_{L'}$ / $H_E$ = 0.34, $H_L$ / $H_M$ = 1.12, $H_E$ / $H_L$ = 1.14 and , **$H_E$ / $H_M$ = 1.53**. Furthermore, the high value of the correlation **$H_E$ / $H_M$ = 1.53** (***$J_{E-M}$*** **= 10.8)** indicates that **$H_E$** and **$H_M$** are close, which means that the racemate **40 b** have the ***cis*** configuration. (**Figure 4.10** and **4.11**).

The ratio of 1:2 corresponding to *cis/trans* (**Figure 4.2**), confirms that the *trans* regioisomer is preferred due to steric hindrance which is corroborated by the NOESY experiment (**Figure 4.10** and **4.14**).

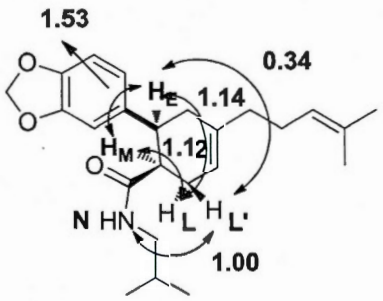



**Figure 4.11:** Representative NOESY contacts of compound **40 b (*cis*).**

According to the article by Lin *et al*,[77] the mayor product was assigned as *trans* consequently, the minor was *cis*. In order to compare compound **40** with the molecule described in the article; $^{1}$H-RMN, $^{13}$C-RMN and $^{1}$H-$^{1}$H NOESY were made by using benzene as a solvent, as it was mentioned in the article.

1H-RMN (600 MHz, $C_6D_6$) spectrums were analysed and interpreted (See page 98) (**Figure 47** and **48)** showing a considerably different chemical shift for $H_I$ in compound 40 a (δ 4.75) and **40 b** (δ 5.13), which also confirms the obtention of isomers *cis* and *trans*.

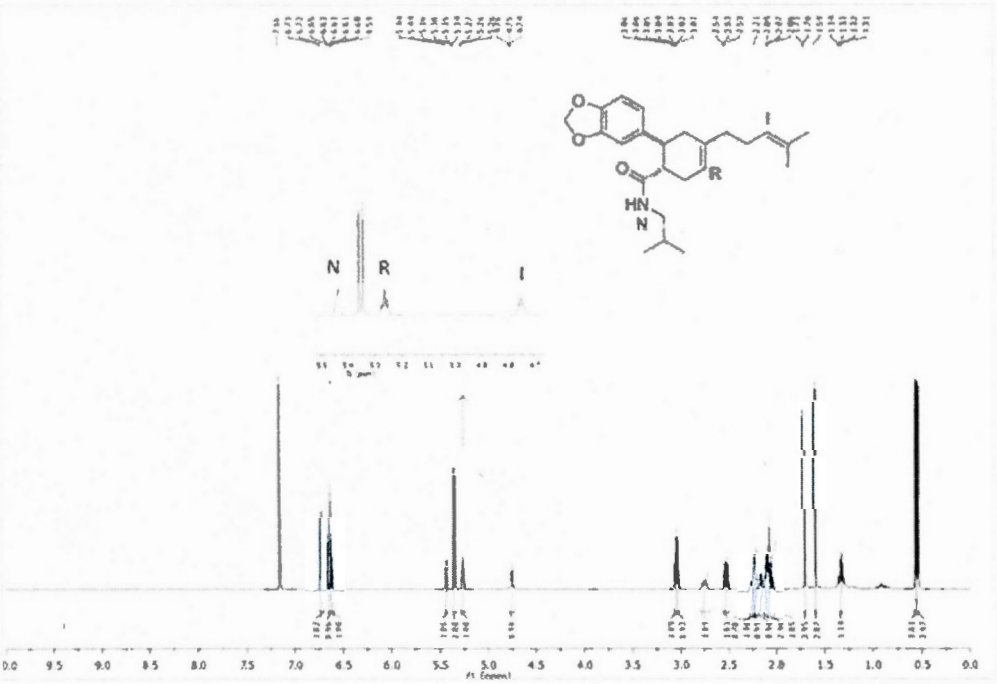



**Figure 4.12.** $^{1}H$ NMR (600 MHz, $C_6D_6$) spectrum of compound **40 a** ***(trans)***.

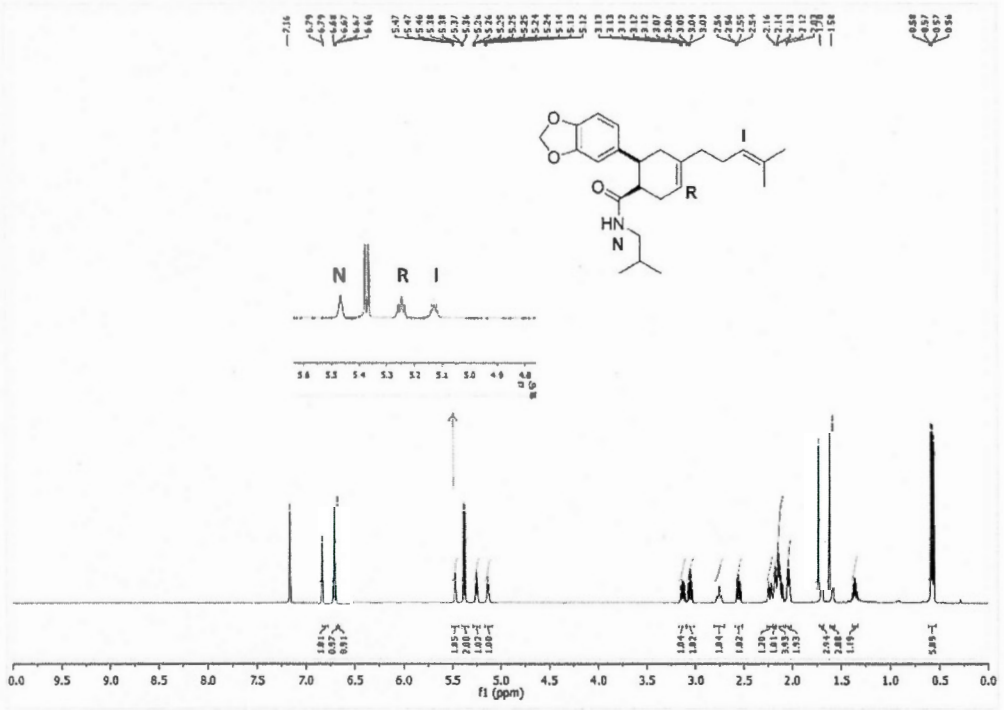



**Figure 4.13** $^{1}H$ NMR (600 MHz, $C_6D_6$) spectrum of compound **40 b** ***(cis)***.

Eventually, the $^{1}H$-$^{1}H$ NOESY ($C_6D_6$) analyse of compound **40 a** was made in order to compare with the one from the article. **(Figure 4.14)**

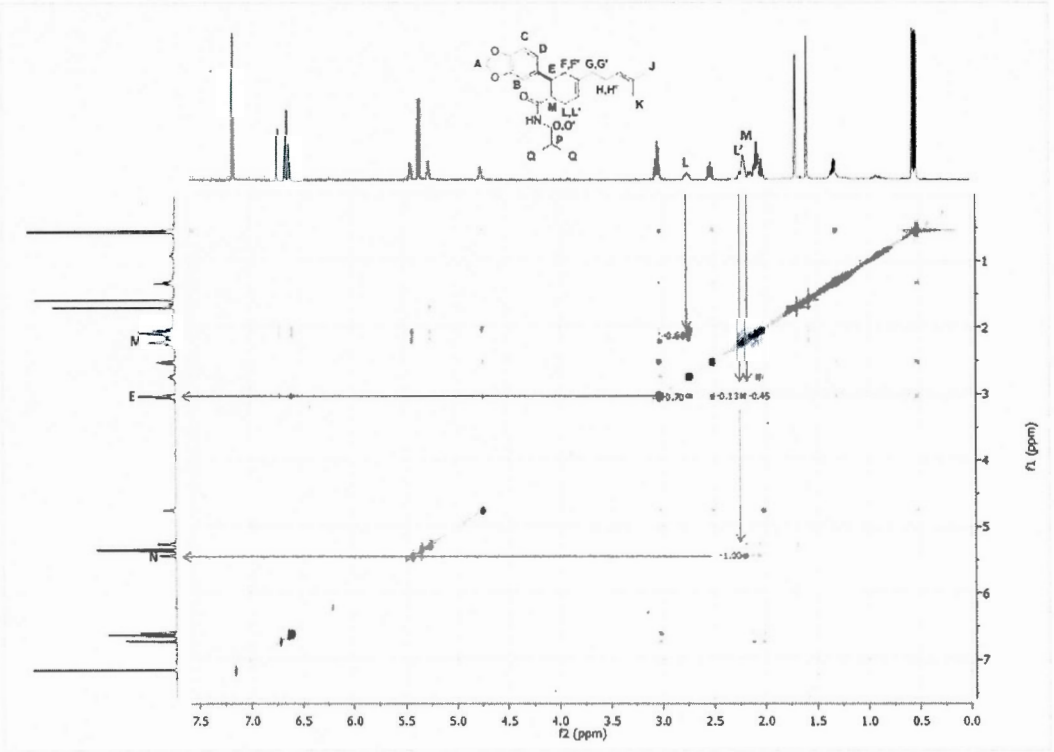



**Figure 4.14:** NOESY spectrum of compound **40 a** (***trans***).

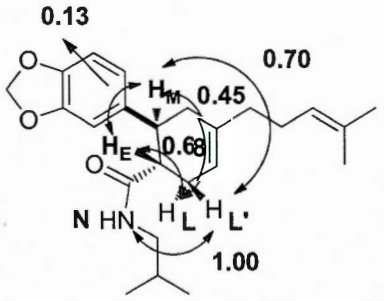



**Figure 4.15:** Representative NOESY of compound **40 a** (***trans***).

The relatives NOE values allow the estimation of the distance between the protons: $H_{L'}$ / $H_N$ = 1, $H_{L'}$ / $H_E$ = 0.45, $H_L$ / $H_M$ = 0.68, $H_E$ / $H_L$ = 0.70 and , **$H_E$ / $H_M$ = 0.13.** Furthermore, the low value of the correlation **$H_E$ / $H_M$ = 0.13** indicates that **$H_E$** and **$H_M$** are far (**$J_{E-M}$ = 8 Hz**), which means that the racemate **40 a** have the configuration ***trans.*** **(Figure 49** and **50)**.

## 4.3 GENERAL CONCLUSIONS

A regioisomer of *Heitziamide A* (compound **40**) was obtained by thermal Diels-Alder cycloaddition between myrcene and *trans*-fagaramide (compound **35**). Compound **40** was obtained as a mixture of *cis* (**$J_{E-M}$ = 10.8**) and *trans* (**$J_{E-M}$ = 8 Hz**) stereoisormers, both of them were sucessfully separated and elucidated by using 1D and 2D NMR spectroscopy. The values and results obtained were corroborated with the data reported in the literature.[28]

CHAPTER V

# CHEMICAL LIBRARY OF ANALOGS OF *TRANS*-FAGARAMIDE

Natural substances extracted from plants or animal sources represent a wide field in development of new drugs. The isolation and characterization of these compounds have a great importance since the knowledge of their structure let us understand their interactions with other molecules in the human body. Furthermore, these compounds serve as models for the preparation of new bioactive substances. The modern tools of chemistry and biology now allow scientists to detail the exact nature of the biological effects of a molecule, as well as supress or enhance certain characteristics such as solubility, efficiency or stability in the human body, which holds much promise for the development of new therapies against devastating diseases. Among them, cancer is considered the most widespread and feared diseases in the world. Toxicity and adverse side effects of synthetic drugs made a back up to folk medicine in order to improve the fulliment of what health needs. Natural compounds play a special role in the treatment of cancer. It is estimated that plant-derived compounds constitute more than 50 % of anticancer agents, and about 74 % of anticancer compounds are either natural or natural-derived products (**Figure 5.1**). [75,76]

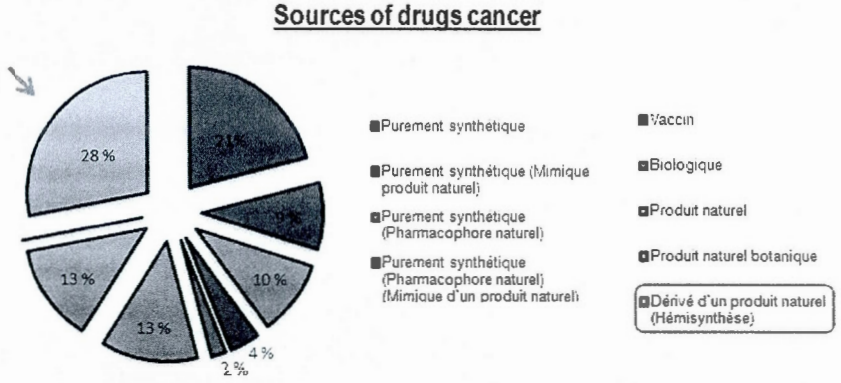



**Figure 5.1** Sources of anticancer drugs.[76]

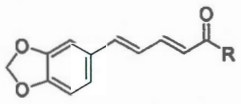
[75] Newman, D. and Cragg, G. Natural Products as sources of new drugs over the 30 years from 1981-2010. *J. Nat. Prod.* **2012**, *75*, 311-335.

[76] http://www.naturalhealth365.com/stop_cancer/anti_cancer.html. Accessed 18 August 2014.

## 5.1. ANALOGS OF TRANS-FAGARAMIDE

*trans*-Fagaramide, mostly obtained from the family Rutacea, is known as a medicinal source against gingival bleeding, infections, cancer, rheumatism, and complications related to HIV. Previous reports showed that there are a considerable numbers of compounds which have shown activities against certain cancer cells that contains structural units of *trans*-fagaramide. Some examples are described below:

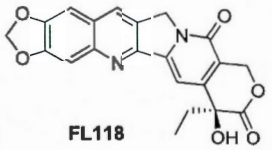
A series of piperidine analogs (**i-v**), having the same skeleton structure (**Figure 5.2**), were synthesized and evaluated for their anticancer activity against human cancer cell lines lines (MCF-7, breast cancer cell line and Hela cervix cell line), analog **i** shows significant activity against breast cancer cell line and analog **iii** shows significant activity against Hela cervix cell line.[77]




| analogs | R: |
|---|---|
| i) | Histidine |
| ii) | Phenylalanine |
| iii) | Tryptophan |
| iv) | p-ChloroAniline |
| v) | p-BromoAniline |

**Figure 5.2** Analogs from piperidine.

The identification and characterization of **FL118** was reported as an active compound which selectively inhibits multiple cancer survival and proliferation-associated antiapoptotic proteins (survivin, Mcl-1, XIAP, clAP2) (**Figure 5.3**).

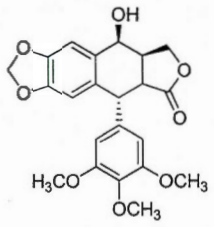



**FL118**

**Figure 5.3** Chemical structure of **FL118**.

[77] Umadevi, P., Deepti, K. and Venugopal, Durvasula. Synthesis, anticancer and antibacterial activities of piperine analogs. *Med. Chem. Res.* **2013**, *22*, 5466-5471.

The lignan podophyllotoxin, a potent spindle poison, has been used to treat warts and keratosis on the outside skin of the genital areas (**Figure 5.4**).




Podophyllotoxin

**Figure 5.4** Chemical structure of Podophyllotoxin.

The exact mechanism of action is not well understood, it is presumed that it may bind and inhibit topoisomerase II during the late S and early G2 stage. An example of podophyllotoxin bound to the active site of tubulin is shown below (**Figure 5.5**).[78,79]

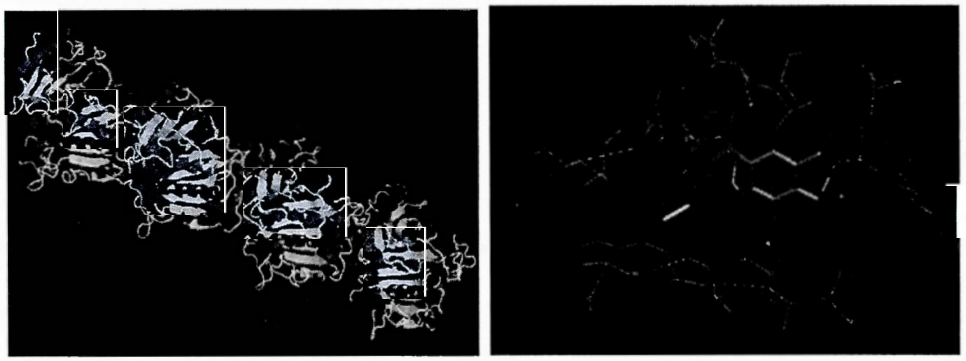
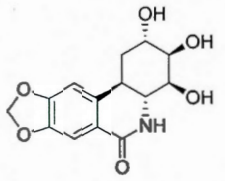


**Figure 5.5** Podophyllotoxin bounding to the active site of tubulin.

[78] http://www.rcsb.org/pdb/ligand/ligandsummary.do?hetId=POD&sid=1SA. Accessed 21 August 2014.

[79] Kelleher, J. Tubulin Binding Affinities of Podophyllotoxin and Colchicine Analogues. *Mol Pharmacol.* **1977**, *13*, 232-241.

Another example is the natural product (+)-*trans*-Dihydrolycoricidine (**Figure 5.6**), which displays biological activities, including potent anticancer and antiviral activities.[80]



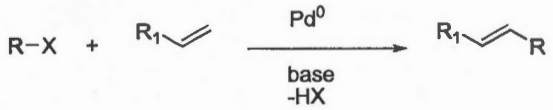

**(+)-*trans*-Duhydrolycoricidine**

**Figure 5.6** Chemical structure of (+)-*trans*-Dihydrolycoricidine.

In this context, the next part of the project will be completed with the synthesis of analogs of *trans*-fagaramide, based on the fact that this molecule shows suitable properties. Thus its chemical structure modification can give better combination of properties to make a most effective anti-cancer drug.

## 5.2. HECK REACTION

Heck reaction is a metal-catalyzed cross-coupling reaction, which contributes in the formation of carbon-carbon and carbon-heteroatom bonds. Basically, Heck reaction is referred to the formation of and aryl-, benzyl-, or styril-substitued olefins when an aryl, benzyl or styryl halides react with olefinic compounds with elevated temperatures in the presence of an amine base and catalytic amount of $Pd^0$ (**Scheme 5.1**).[81]




**Scheme 5.1** Palladium-mediated Heck coupling.

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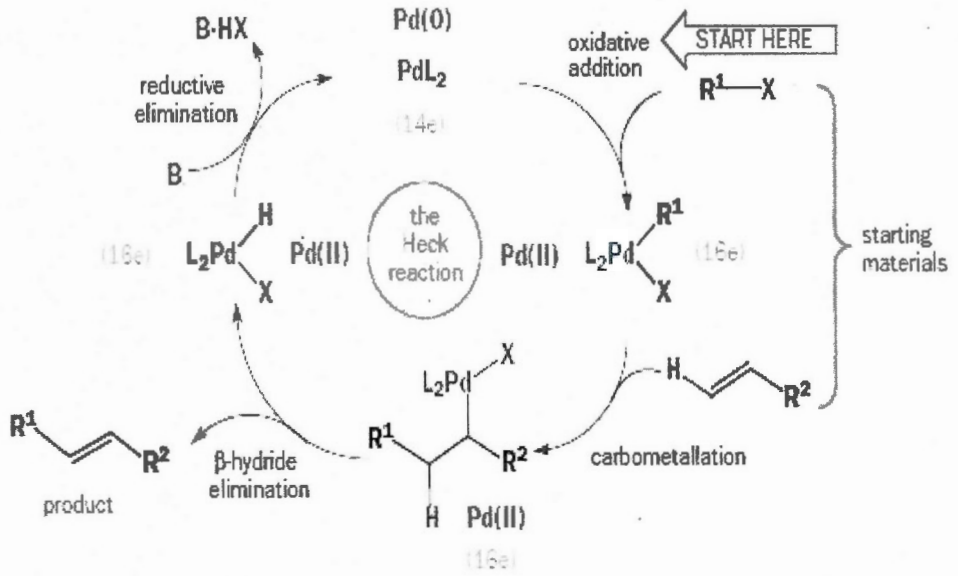
[80] McNulty, J. and Zepeda-Velázquez, C. Enantioselective Organocatalytic Michael/Aldol Sequence: Anticancer Natural Product (+)-*trans*-Dihydrolycoricidine. *Angew. Chem. Int. Ed.* **2014**, *53*, 8450-8454.

[81] Li, P.; Wang, L.; Zhang, L. and Wang, G. Magnetic Nanoparticules-Supported Palladium: A Highly Efficient and Reusable Catalyst for the Suzuki, Sonogashira, and Heck Reactions. *Adv. Synth. Catal.* **2012**, *354*, 1307-1318.

The Heck reaction consists fundamentally in 4 steps: i) oxidative addition, ii) migratory insertion or carbometallation, iii) β-hydride elimination and iv) reductive elimination.[82]




**Scheme 5.2** Proposed mechanism of palladium-mediated Heck coupling.[83]

## 5.3. RESULTS AND DISCUSSION

The synthesis of compound **41**, yield 55% (100 mg) was obtained by Diels-Alder cycloaddition between myrcene and compound **36. (Scheme 5.3)**.

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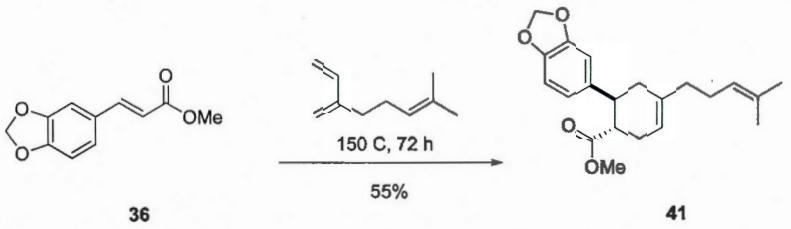
[82] Kürti, L. and Czakó, B.Strategic Applications of Named Reactions in Organic Synthesis. *Elsevier Inc.* **2005**, 196-197.

[83] Clayden, Greeves, Warren and Wothers. Organic Chemistry. *Oxford University Press.* **2001**, 1321.




**Scheme 5.3** Synthesis of compound **41**.

For the correct assigment of the configuration (*cis/trans*) to compound **40**, the techniques of 2D-COSY (**Figure 5.7**), HMBC and NOESY (**Figure 5.8**) were employed in order to establish the connections between the strucural units.

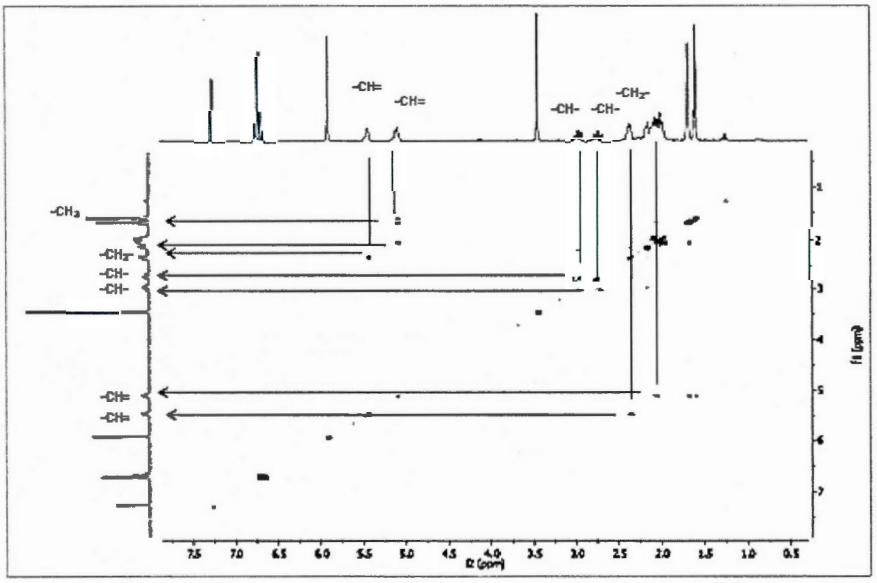



**Figure 5.7** 2D-COSY of compound **41**.

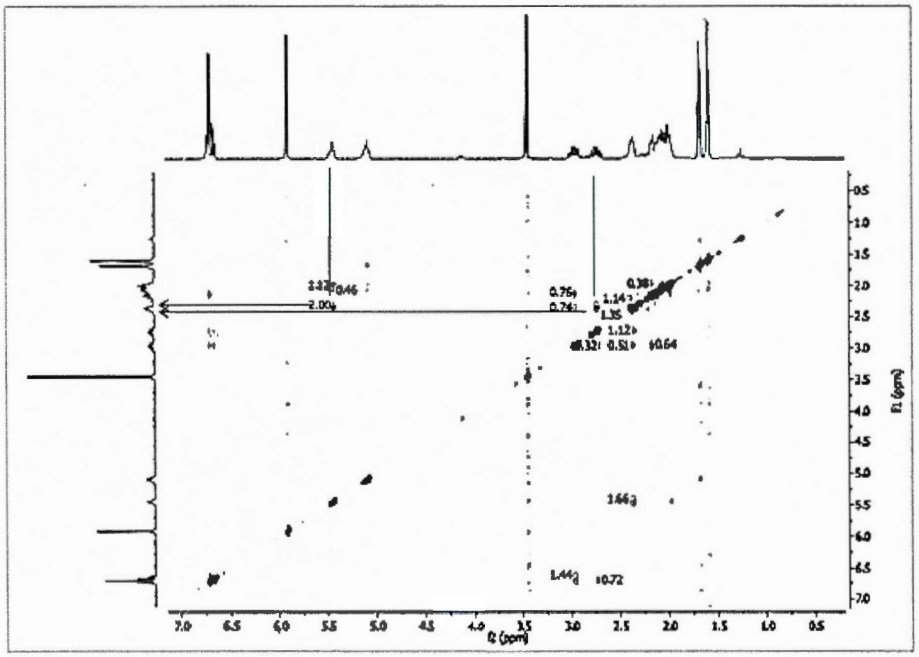


**Figure 5.8** 2D-NOESY of compound **41**.

The chemical shifts for **compound 41** were assigned acording to the $^{1}$H-NMR (**Figure 5.10**), $^{1}$H-$^{1}$H COSY and $^{1}$H-$^{13}$C HMBC spectrums. Subquently, the spectrum of $^{1}$H-$^{1}$H NOESY was analyzed. The relatives NOE values allow the estimation of the distance between the protons: $H_{L'}$/ $H_N$= 1, $H_{L'}$/ $H_E$ = 0.46, $H_L$/ $H_M$= 0.64, $H_E$/ $H_L$= 0.74 and , $\mathbf{H_E}$/ $\mathbf{H_M}$ = **0.15**. Furthermore, the low value of the correlation $\mathbf{H_E}$ / $\mathbf{H_M}$ = **0.13** indicates that $\mathbf{H_E}$ and $\mathbf{H_M}$ are far ($J_{E\text{-}M}$ = **10.2 Hz**), which means that the racemate **40 a** have the configuration ***trans.*** (**Figures 5.8** and **5.9**)

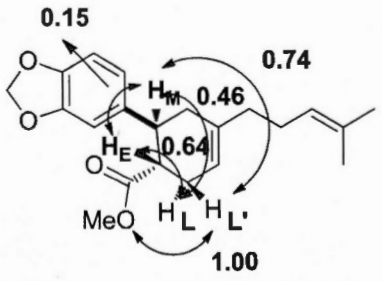



**Figure 5.9** Representative NOESY contacts of compound **41**.

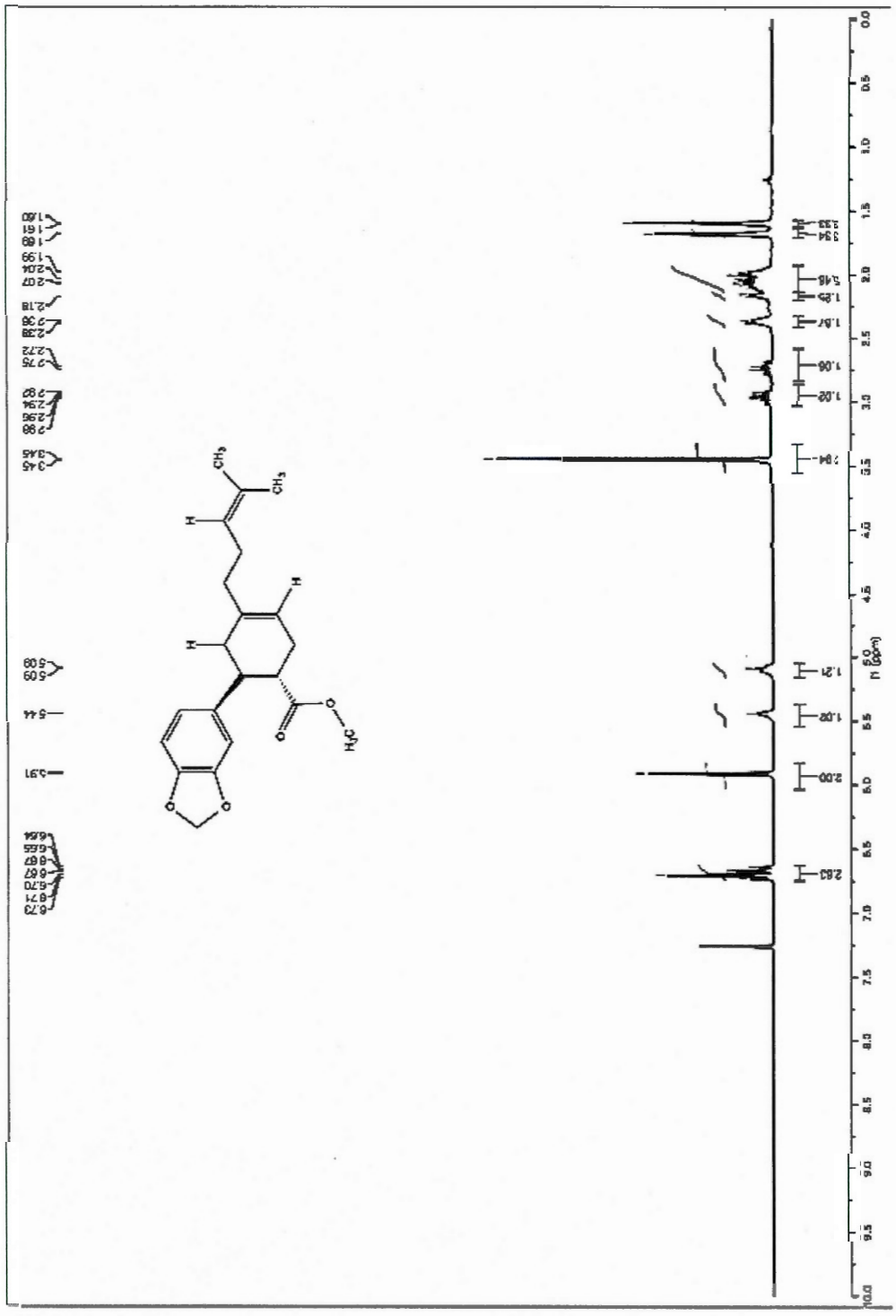
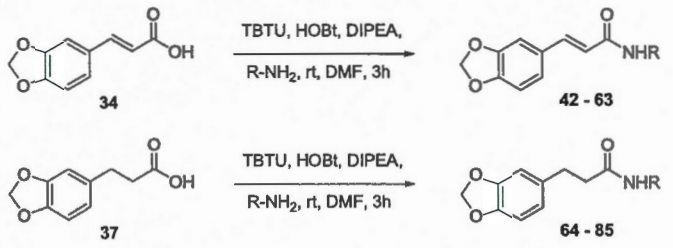


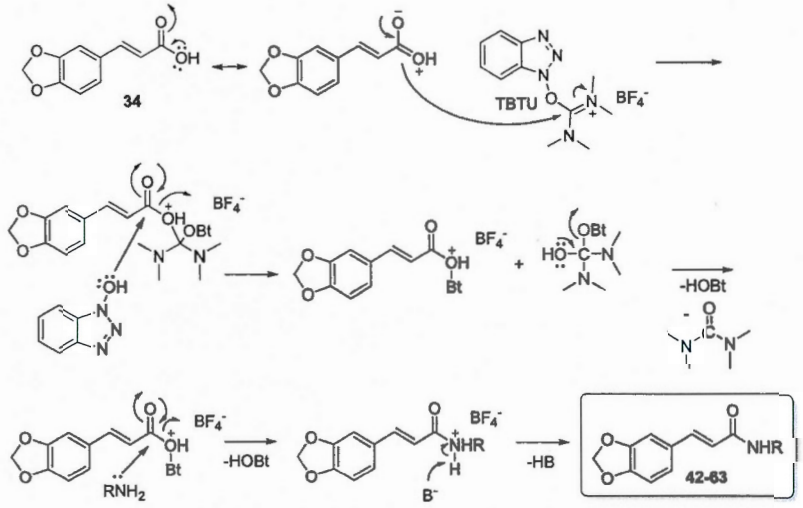
**Figure 5.10 $^{1}$H NMR (600 MHz, $CDl_3$) spectrum of compound 41.**

Compounds **42-85** were synthetized by coupling peptide employing compounds **34** and **37** as synthetic precursors, using TBTU and HOBt as coupling agents (**Scheme 5.4**).




**Scheme 5.4** Synthesis of compounds **42-85.**

The proposed mechanism for the peptide coupling used for the synthesis of compounds **42-63** is shown below (**Scheme 5.5**).




**Scheme 5.5** Proposed mechanism for coupling peptide with TBTU.

Analogs of *trans*-fagaramide by coupling peptide were obtained with an average yield of 62 % (**Figure 5.11** and **5.12**).

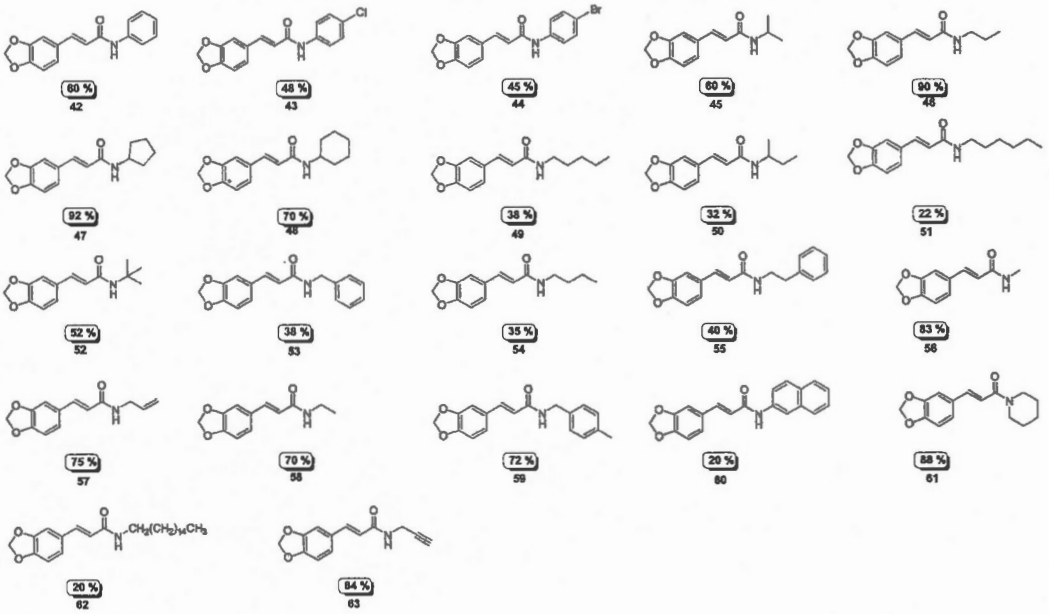



**Figure 5.11** Chemical structures and yields for compounds **42-63.**

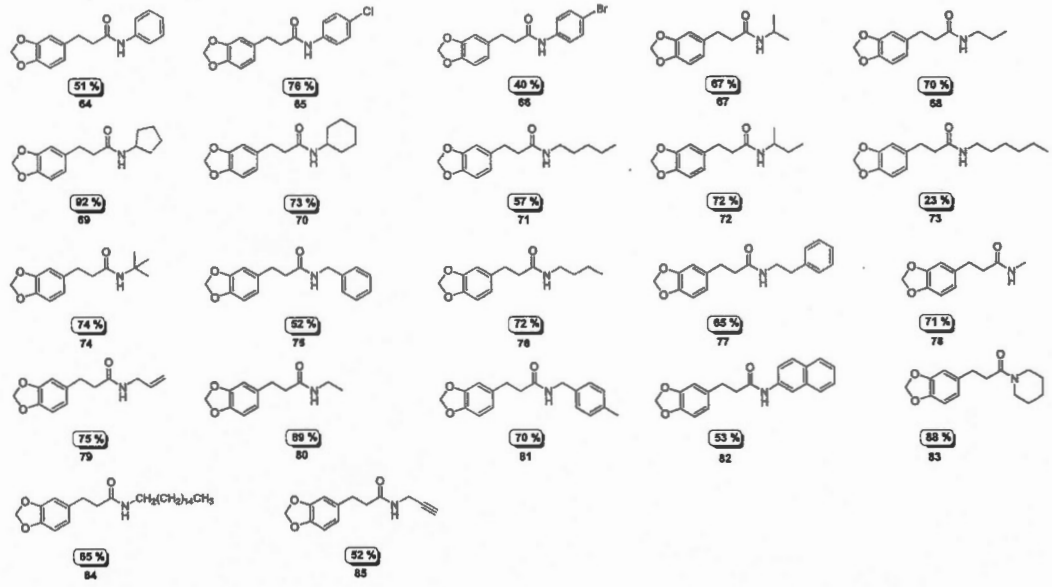
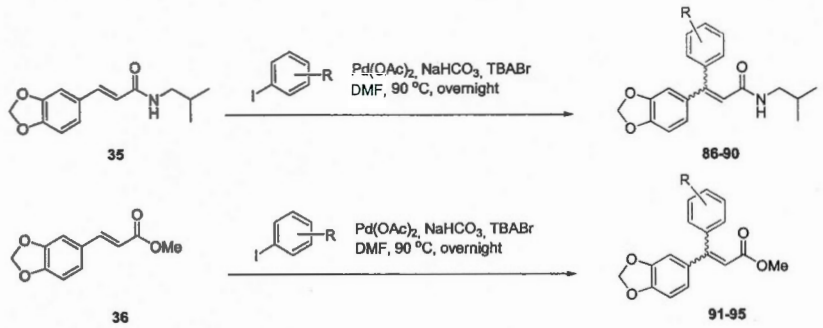



**Figure 5.12** Chemical structures and yields of compounds **64-85.**

The synthesis of Compounds **86-95** were achieved by palladium-mediated Heck coupling (**Scheme 5.6**).




**Scheme 5.6** Synthesis of compounds **86-95.**

In general, the synthesis of compounds **86-95** contains the starting material (25 %) (compounds **35** or **36)** as byproducts. Compound **90** (35 %) was obtained as a white solid (**Figure 5.14**).

In constrast to the other compounds, compound **90** contains a coumarin unit in its chemical structure, which was formed by the attack of the hydroxyl group from the benzene ring to the carbonyl group. The rest of the compounds were obtained as a mixture of isomers with an average yield of 70 % (**Figure 5.13**).

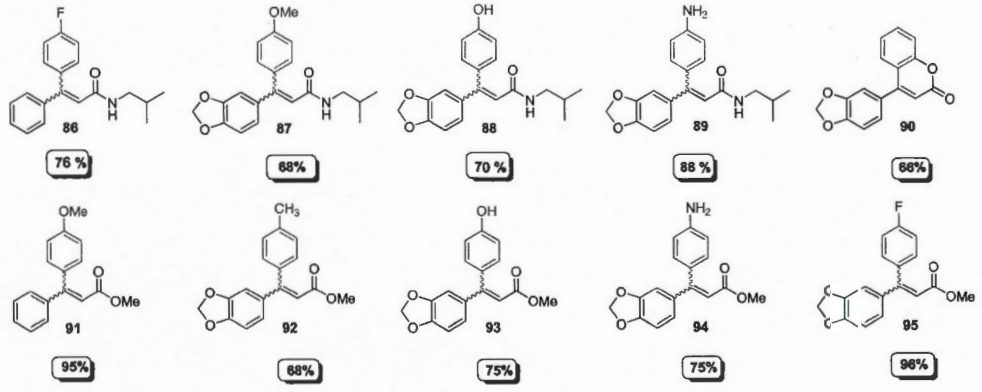



**Figure 5.13.** Chemical structure and yield of compounds **86-95.**

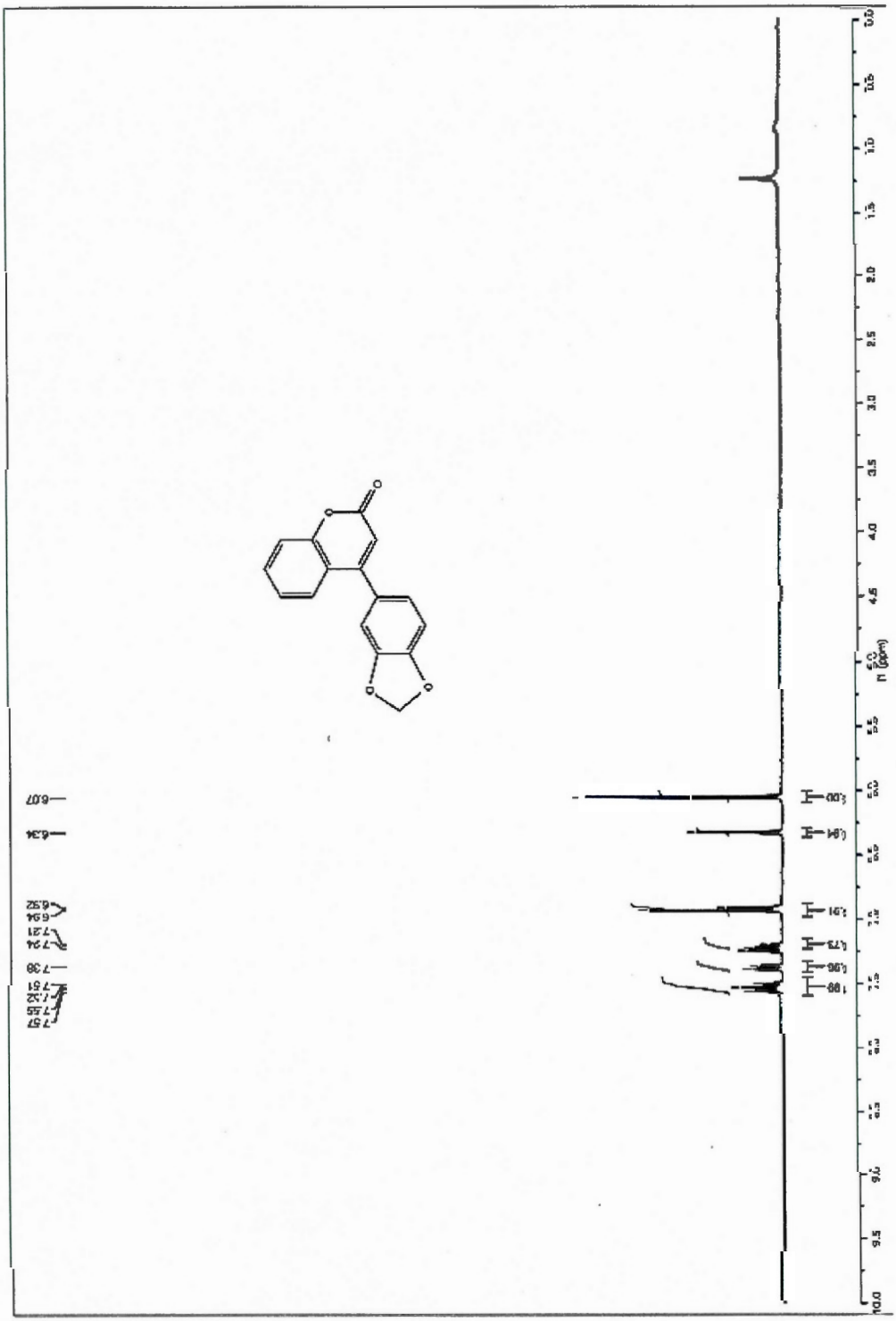



**Figure 5.14 $^1$H NMR ( 600 MHz, $CDCl_3$ ) spectrum of compound 90.**

Compound **93** was chosen as an example for the analysis of regiosomers.

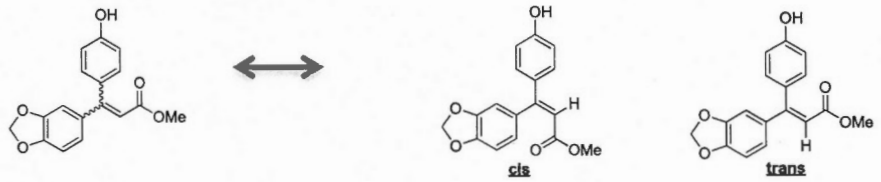
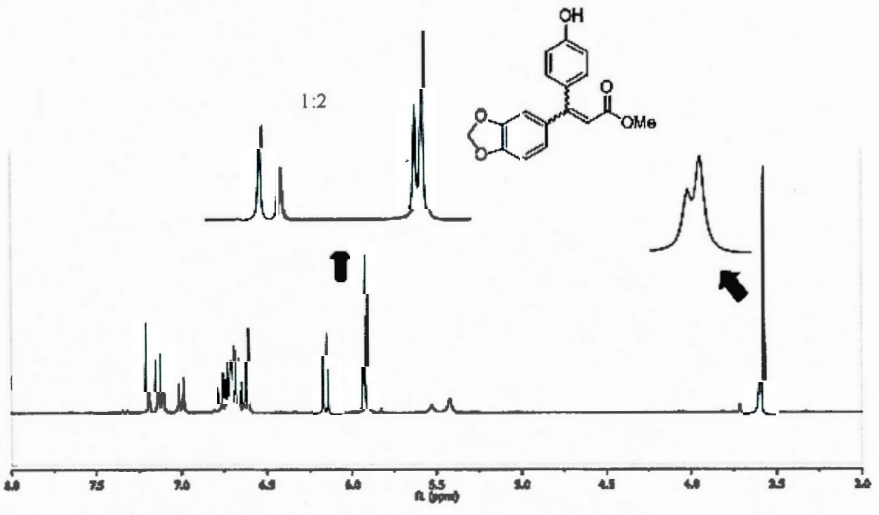



**Figure 5.15** Isomers *cis* and *trans* of compound **93.**

To obtain the correct assignment of steroisomers (**Figure 5.15**), "Nuclear Overhauser Effect,, (NOE) technique was exploited, based on its capacity to detect intra-(and even inter) molecular contacts and then distances. The resulting white mixture *cis* and *trans* was analyzed by $^{1}$H-NMR and the spectrum obtained confirmed the presence of both isomers, showing different ratios for each of them, which were determined by comparing proton integrations (**Figure 5.16**).

**$^{1}$H-NMR ($CDCl_3$, 300 MHz):**



**$^1$H-NMR ($CDCl_3$, 600 MHz):**

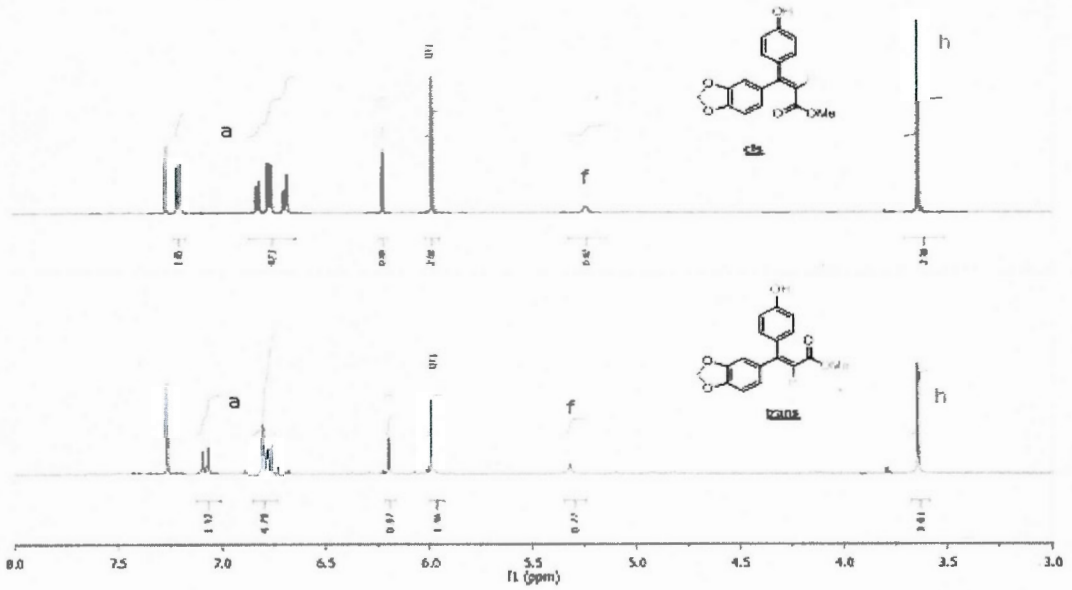



**Figure 5.16** $^1$H-NMR Spectrums of compound **93** and stereoisomers.

The mixture was successfully separated into its two isomers. The isomer which was more abundant (**93 b**) was then analyzed by $^1$H-NMR (**Figure 5.17**).

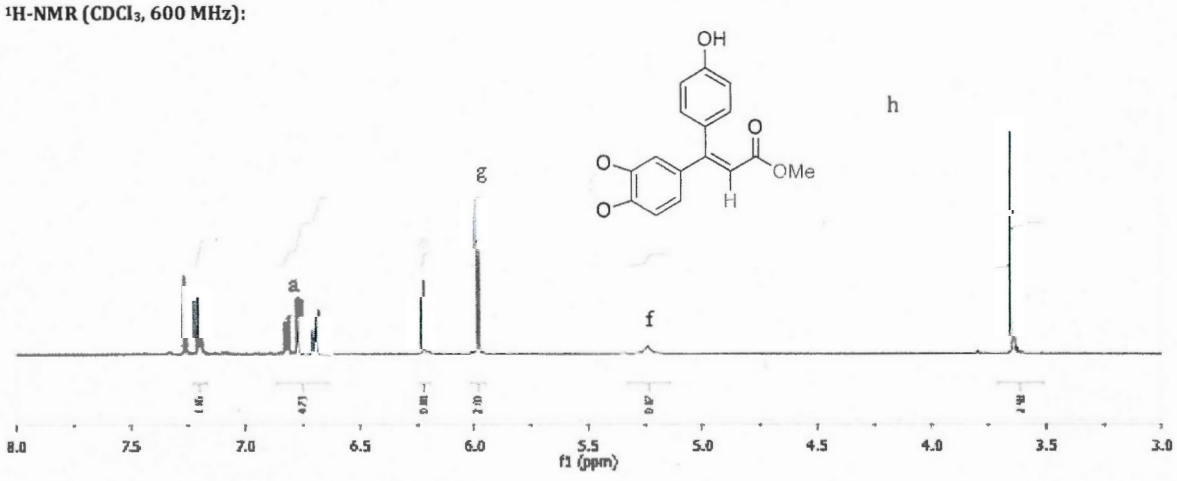
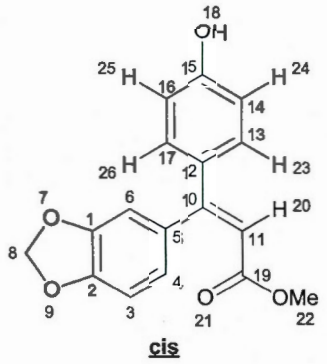



**Figure 5.17.** $^1$H NMR (600 MHz, $CDCl_3$) spectrum of compound **93 b.**

According to the $^1$H-NMR spectrum the assignment of the signals was attributed as it is shown in table 5.1.

**Table 5.1** Attribution of the chemical shift of compound **93b**

| **Assignment** | **$^1$H (ppm) (Figure 58)** |
|---|---|
| 7.21- 6.68 | $\Phi_1,\Phi_2$ |
| 6.23 | 20 |
| 5.98 | 8 |
| 5.24 | 18 |
| 3.64 | 21 |




cis

**Figure 5.18** Structure of compound **93 b**

According to the signal assignment shown in **Table 5.1 (Figure 5.18)**, the signal (OH) at δ 5.24 ppm was saturated, affecting the signal at δ 6.77 ppm which corresponds to H-24, 25. Subsequently this signal was also saturated giving as variation of the intensity of the signal at δ 7.21 ppm (H-23). Finally the proton corresponding to the signal at δ 6.23 ppm (H-20) was irradiated and consequently the intensity of signal δ 7.21 ppm was affected (H-20), which confirms that the isomer analyzed corresponds to the *cis*-isomer (**Figure 5.19**).

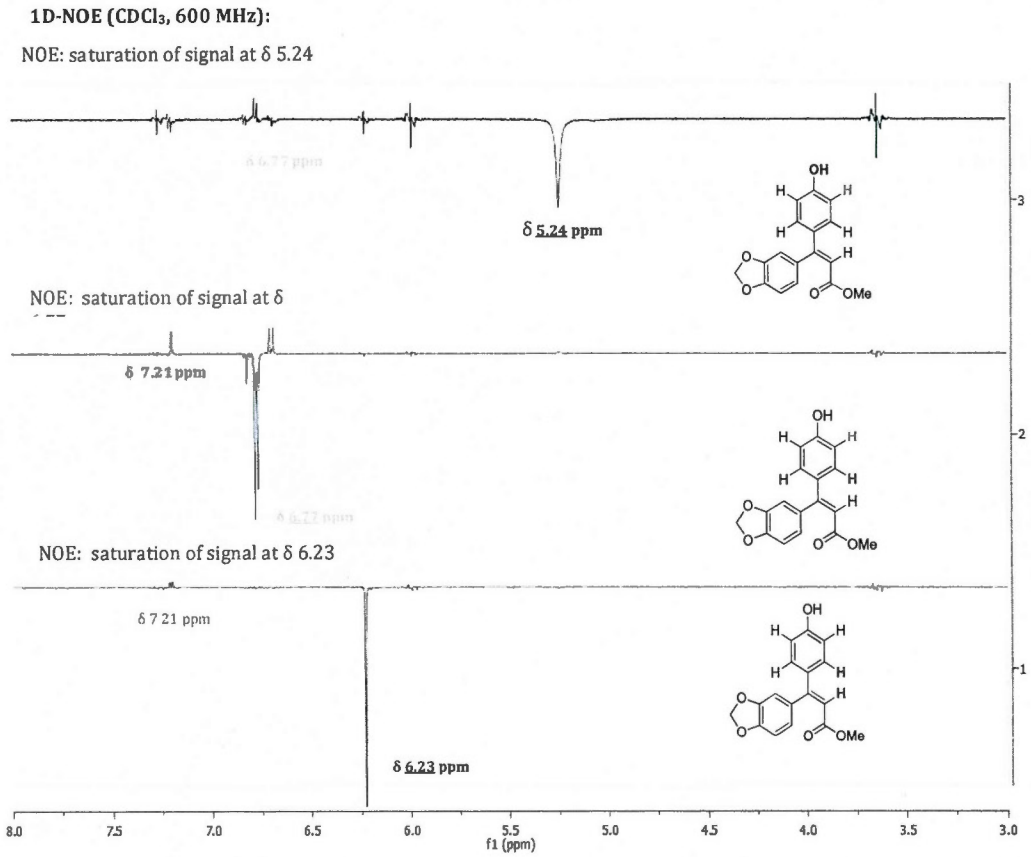



**Figure 5.19** 1D-NOE spectrum of compound **93 b.**

## 5.4 GENERAL CONCLUSIONS

Compound **41** was obtained as a pure racemate (55 %). It was sucessfully elucidated by using 1D and 2D NMR spectroscopy. Specifically, its *trans* configuration was determinated by using the technique of $^{1}H$-$^{1}H$ NOESY. Compounds **42-85** (44 compounds) were synthesized by peptide coupling obtaining an average yield of 62 %. The synthesis of compounds **86-95** contains the starting material (25 %). Compound **90** was obtained in a yield of 35 % as a white solid. The rest of the compounds were obtained as mixture of isomers with an average yield of 77 %. All compounds were elucidated using 1D ($^{1}H$-NMR and NOE) and 2D spectroscopy (COSY, HMQC, NOESY) and mass spectrometry.

# CHAPTER VI

## CONCLUSIONS AND PERSPECTIVES

*trans*-Fagaramide and (+)- usnic acid were isolated from the plant *Fagara heitzii* (Cameroon) and the lichen *Evernia prunastri* (Peru) respectively. Their isolation was performed usind standard protocols of extractions and chromatographic purification. (**Figure 6.1**) Their chemical structures were elucidated using 1D and 2D NMR spectroscopy and mass spectrometry, thus corroborating spectroscopic data reported in the literature.

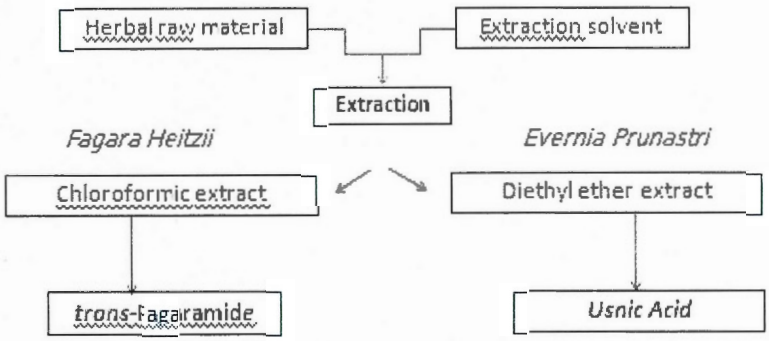



**Figure 6.1** Extraction of *trans*-fagaramide and usnic acid.

Synthetic *trans*-fagaramide and five other precursors were used as starting material for the elaboration of a chemical library containing functionalized derivatives of *trans*-fagaramide generated from Fisher esterifications, palladium-mediated Heck couplings, Diels-Alder cycloaddition and catalytic hydrogenation. The compounds are being screened in cancer cell and toxicity experiments. Results are still in treatment. The goal is toward the determination of optimized and efficient drug candidates againts cancer cells (**Figure 6.2**).

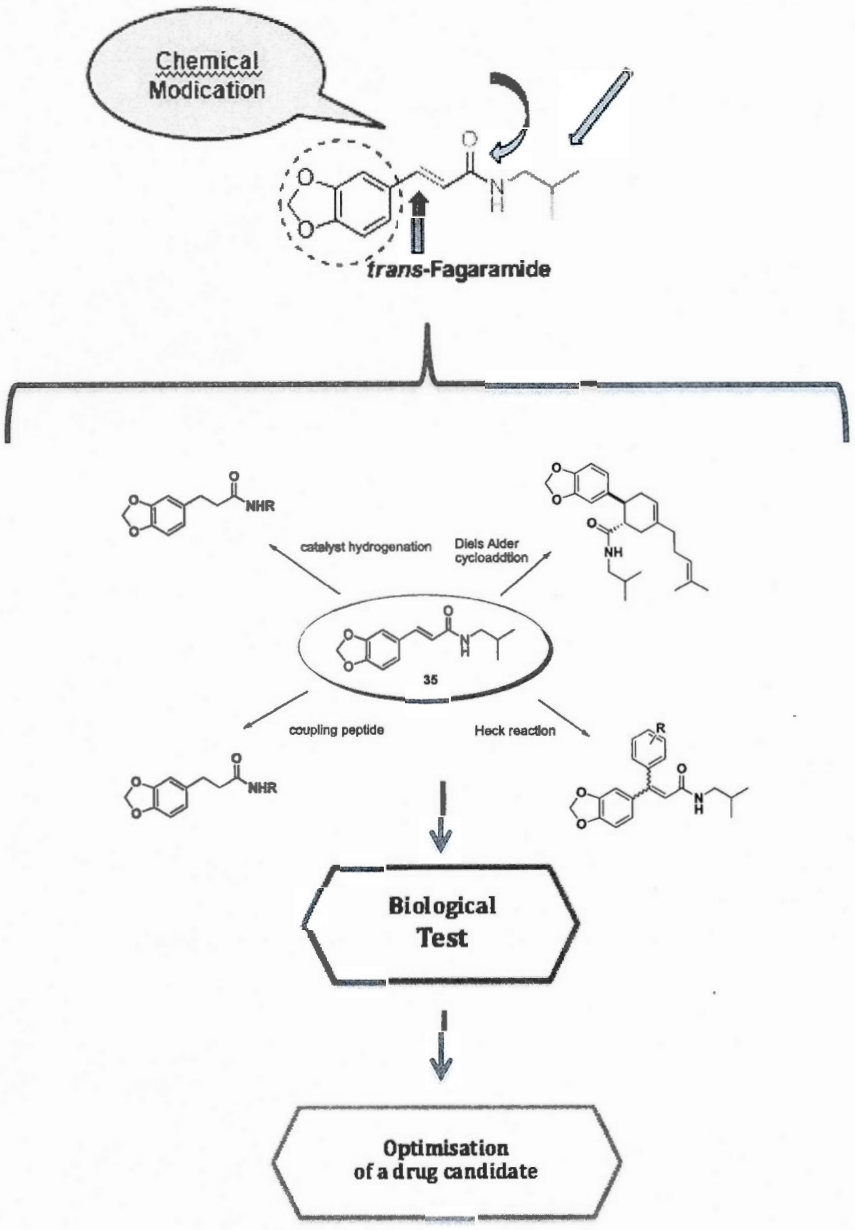



**Figure 6.2** Perspective of the project.

# CHAPTER VII

# EXPERIMENTAL PART

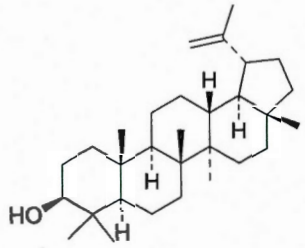
## 7.1. MATERIALS AND METHODS

In general, all solvents (Acros Organics or Aldrich) were used without prior purification. The isolation and purification were monitored by thin layer chromatography (TLC) on silica gel plate (Merck 60 $F_{254}$), preparative chromatography (Merck 60 $F_{254}$) and by column chromatography using silica gel (Gel 60 silica particle size 0.063-0.200 mm).

The compounds obtained by extraction were analyzed and characterized in $CDCl_3$ by $^1$H-NMR 300 MHz, $^1$H-NMR 600 MHz and $^{13}$C-NMR 150 MHz with a Varian Inova AS600 spectrometer. The molecular ions were protonated $[M+H]^+$ and/or $[M-H]^-$ for the confirmation of their empirical formula. The notations used for the spectral analysis are: s (singlet), d (doublet), dd (doublet of doublets), m (multiplet). Chemical shifts are reported in δ (ppm) using TMS as the internal standard; coupling constants (*J*) were measured in Hz. Infrared spectra were recorded on a JASCO FT/IR-410 spectrophotometer.

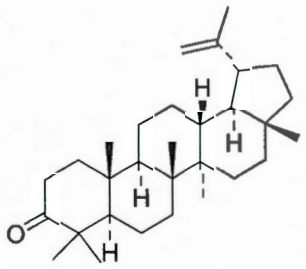
## 7.2. CHARACTERIZATION OF COMPOUNDS ISOLATED FROM FAGARA HEITZII AND EVERNIA PRUNASTRI

**(1*R*,3a*R*,5a*R*,5b*R*,7a*R*,9*S*,11a*R*,11b*R*,13a*R*,13b*R*)-3a,5a,5b,8,8,11a-Hexamethyl-1-(prop-1-en-2-yl)icosahydro-1*H*-cyclopenta[*a*]chrysen-9-ol. Lupeol (9).**



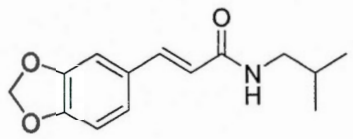

Compound **9** (26 mg) was obtained as a white solid. **$^1$H RMN (600 MHz, $CDCl_3$):** δ = 0.78, 0.80, 0.83, 0.95, 0.97, 0.99, 1.68 (s, 3H, C*H*$_3$), 2,38 (m,1H, C*H*), 3.19 (dd, $^3J_{CHCH}$ = 4.98 and11.72 Hz, H-3), 4.57 (s, 1H, C*H*$_2$), 4.69 ppm (s, 1H, C*H*$_2$). **$^{13}$C RMN (150 MHz, $CDCl_3$):** δ =150.9 (*C*CH$_2$), 109.4 (C*C*H$_2$), 78.9 (*C*OH), 48.3 (*C*HC), 27.4 (*C*H$_3$), 20.9 (*C*H$_3$), 19.3 (*C*H$_3$), 18.3 (*C*H$_3$), 15.3 (*C*H$_3$), 10.1 ppm (*C*H$_3$).

**(1*R*,3a*R*,5a*R*,5b*R*,7a*R*,11a*R*,11b*R*,13a*R*,13b*R*)-3a,5a,5b,8,8,11a-Hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one. Lupenone (10)**



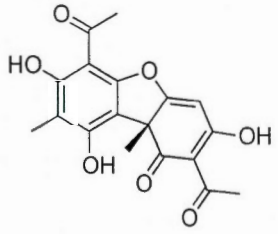

Compound **10** (10 mg) was obtained as a yellow solid. **$^1$H RMN (600 MHz, $CDCl_3$):** δ = 0.81, 0.93, 0.95, 1.02, 1.07, 1.68 (s, 3H, C*H*$_3$), 1.89 (m, 2H, C*H*$_2$), 2.39 (m,1H, C*H*), 2.48 (m, 1H, C*H*), 4.55 (s, 1H, C*H*$_2$), 4.69 ppm (s, 1H, C*H*$_2$). **$^{13}$C RMN (150 MHz, $CDCl_3$):** 218.2 (*C*O), 150.9 (*C*CH$_2$), 109.4 (C*C*H$_2$), 54.9 (*C*H), 49.8 (*C*H$_3$), 48.3 (*C*H), 48.0 (*C*H), 47.3 (CO*C*), 43.0 (*C*C), 40.8 (C*C*H$_3$), 40.0 (*C*H$_2$), 38.2 (*C*H$_2$), 36.9 (*C*CH), 35.5 (*C*H$_2$), 34.2 (*C*H$_2$), 33.6 (*C*H$_2$), 29.8 (*C*H$_2$), 27.4 (*C*H$_2$), 26.7 (*C*H$_2$), 25.2 (*C*H$_2$), 21.5 (*C*H$_3$), 21.0 (*C*H$_2$), 19.7 (*C*H$_2$), 19.3 (*C*H$_3$), 18.0 ppm (*C*H).

**(*E*)-3-(benzo[*d*][1,3]dioxol-5-yl)-*N*-isobutylacrylamide. *trans*-Fagaramide (6)**



Compound **6** (12 mg) was obtained as a white solid. **$^{1}$H RMN (600 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^{3}J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 7.00 (s, 1H, $H_a$), 6.97 (d, 1H, $^{3}J_{CHCH}$ = 8.5 Hz, $H_b$), 6.80 (d, 1H, $^{3}J_{CHCH}$ = 8.1 Hz, $H_c$), 6.21 (d, 1H, $^{3}J_{CHCH}$ = 15.6 Hz, C*H*CO), 5.99 (s, 2H, OC$H_2$O), 5.56 (s, 1H, N*H*), 3.22 (t, 2H, C$H_2$), 1.84 (m, 1H, C*H*), 0.95 ppm (d, 6H, $CH_3$). **$^{13}$C RMN (150 MHz, $CDCl_3$):** δ = 166.0 (*C*ONH), 149.0, 148.2, 129.3, 123.8, 108.5, 106.3 ($C_{arom}$), 140.6 (*C*HCH), 118.8 (*C*HCO), 101.4 (O*C*$H_2$O), 47.01(*C*$H_2$), 28.4 (*C*H), 20.1 ppm (*C*$H_3$).

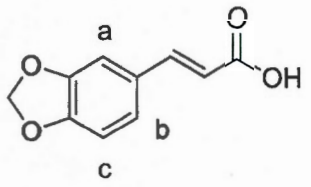
**(*R*)-1,1'-(3,7,9-trihydroxy-8,9b-dimethyl-1-oxo-1,9b-dihydrodibenzo[*b*,*d*]furan-2,6-diyl)diethanone. Usnic acid (33)**



Compound **33** (2.8 g) was obtained as yellow crystals. Mp (°C) = 205-208. $[\alpha]_D^{25}$ (*c* = 0.7, $CH_3Cl$) = +451. **$^{1}$H RMN (600 MHz, $CDCl_3$):** δ = 13.31 (s, 1H, O*H*), 11.02 (s, 1H, O*H*), 5.97 (s, 1H, CC*H*), 2.67 (s, 3H, C$H_3$), 2.66 (s, 3H, C$H_3$), 2.10 (s, 3H,C$H_3$), 1.75 ppm (s, 3H,C$H_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 201.9 (*C*O), 200.4 (*C*O), 198.2 (*C*O), 191.8 (*C*OH), 179.5, 164.0, 157.6, 155.3, 109.4, 105.3, 104.1, 101.6, 98.5, 59.2 ($C_{dibenzofuran}$), 32.3 (CO*C*$H_3$), 31.4 (CO*C*$H_3$), 28.1 (*C*$H_3$), 7.7 ppm (*C*$H_3$).

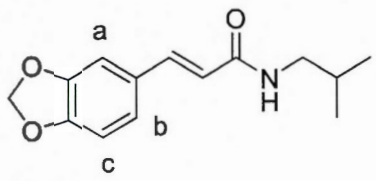
## 7.3. CHARACTERIZATION OF SYNTHETIC TRANS-FAGARAMIDE AND PRECURSORS

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)acrylic acid (34)**



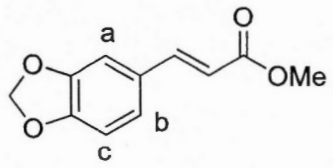

To a solution of piperonal (2.3 g, 15 mmol, 1 eq), malonic acid (3.1 g, 30 mmol, 2 eq) and pyridine (5 mL, 61 mmol, 4 eq), piperidine (50 µmL, 0.48 mmol, 0,032 eq) was slowly added drop by drop and the final solution was stirred at room temperature for 45 min. Then the reaction was heated at 100 °C for 3h. The mixture was cooled and the excess of pyridine was neutralized by the addition of HCl 1 M (20 mL), a white precipitated appeared between pH 4-5. The precipitated acid is filtered and washed with cold EtOAc and recrystallized from saturated hot ethanol. The filtrate was dried under vacuum in a desiccant container for 48 h to afford 11.55 mmol (75 %) of **compound 34** as white crystals. $R_f = 0.28$ (Hex/EtOAc 7:3). **$^1$H RMN (600 MHz, $(CD_3)_2SO$)):** δ = 7.51 (d, 1H, $^3J_{CHCH}$ = 16.0 Hz, benzene-C*H*), 7.36 (d, 1H, $^4J_{CHCCH}$ = 1.6 Hz, $H_a$), 7.15 (dd, 1H, $^4J_{CHCH}$ = 1.6 Hz, $^3J_{CHCH}$ = 8.2 Hz, $H_b$), 6.94 (d, 1H, $^3J_{CHCH}$ = 8.2 Hz, $H_c$), 6.40 (d, 1H, $J_{CHCH}$ = 16.0 Hz, C*H*CO), 6.07 (s, 2H, O$CH_2$O). **$^{13}$C RMN (150 MHz,$(CD_3)_2SO$)):** δ = 167.8 (*C*OOH), 149.1, 148.0, 128.7, 124.6, 108.5, 106.7 ($C_{arom}$), 143.9 (*C*HCH), 117.1 (*C*HCO), 101.6 ppm (O*C*$H_2$O). **ESI$^+$-HRMS:** *m/z* calculated for $C_{10}H_9O_4$ $[M + H]^+$: 193.0495 ; found: 193.0497 .

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-isobutylacrylamide. Synthetic *trans*-Fagaramide (35)**




Compound **34** (2.5 g, 13 mmol, 1 eq) in dry DCM (130 mL) was purged with $N_2$. N,N'-Diisopropylcarbodiimide (DIC) (2.0 mL, 13 mmol, 1 eq) was added followed by Hydroxybenzotriazole (HOBt) (175.7 mg, 1.3 mmol, 0,1 eq) and isobutylamine (1.3 mL, 13 mmol, 1 eq) respectively. The mixture was stirred at room temperature for 24h (monitored by TLC) and then concentrated under vaccum to remove most of the $CH_2Cl_2$. The crude material was diluted with EtOAc, and washed successively with distilled water in the presence of hexane (1/4 of the volume of EtOAc), then washed with saturated potassium bisulfate and bicarbonate de sodium. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. The crude material was purified by column chromatography on silicagel (eluent: hexane /EtOAc (100:0 to 75:25) to afford 9.75 mmol (75 %) of product **35** as white crystals. $R_f$ = 0.30 (Hex/ EtOAc 6:3). **$^1$H RMN (600 MHz, $CDCl_3$):** $\delta$ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.9 Hz, benzene-C*H*), 7.0 (s, 1H, $H_a$), 6.97 (d, 1H, $^3J_{CHCH}$ = 8.2 Hz, $H_b$), 6.78 (d, 1H, $^3J_{CHCH}$ = 8.2 Hz, $H_c$), 6.22 (d, 1H, $^3J_{CHCH}$ = 15.9 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.63 (s, 1H, N*H*), 3.20 (t, 2H, C$H_2$), 1.13 (m, 1H, C*H*), 0.94 ppm (d, 6H, $CH_3$). **$^{13}$C RMN (150 MHz, $CDCl_3$):** $\delta$ = 166.0 (*C*ONH), 149.0, 148.2, 129.3, 123.8, 108.5, 106.3 ($C_{arom}$), 140.6 (*C*HCH), 118.8 (*C*HCO), 101.4 (O*C*$H_2$O), 47.0 (*C*$H_2$), 28.6 (*C*H), 20.1 ppm (*C*$H_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{18}NO_3$ $[M + H]^+$: 248.1281; found: 248.1281.

**(E)-Methyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (36)**




Compound **34** (2.0 g, 10.4 mmol, 1 eq) was dissolved in dry methanol (25 mL). $H_2SO_{4(cc)}$ was then added dropwise and allowed to stir for some minutes. The mixture was heated a reflux for 12 h. The solution was cooled to room temperature and then concentrated under pressure. The crude material was diluted with EtOAc, and washed with aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous sodium sulfate, concentrated and recrystallized from saturated hot Hex/EtOAc (1/4) to afford 7.37 mmol (70 %) of product **37** as white crystals. $R_f$ = 0.25 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.58 (d, 1H, $^3J_{CHCH}$ = 16.3 Hz, benzene-C*H*), 7.01 (s, 1H, $H_a$), 6.99 (d, 1H, $^3J_{CHCH}$ = 8.0 Hz, $H_b$), 6.80 (d, 1H, $^3J_{CHCH}$ = 8.0 Hz, $H_c$), 6.26 (d, 1H, $^3J_{CHCH}$ = 16.3 Hz, C*H*CO), 5.99 (s, 2H, OC$H_2$O), 3.78 ppm (s, 3H, C$H_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 167.7 (*C*ONH), 149.7, 148.5, 128.9, 124.5, 108.7, 106.6 ($C_{arom}$), 144.7 (*C*HCH), 115.8 (*C*HCO), 101.4 (O*C*$H_2$O), 51.7 ppm (*C*$H_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{11}H_{11}O_4$ [M + H]$^+$: 208.0686 ; found: 208.0675 .

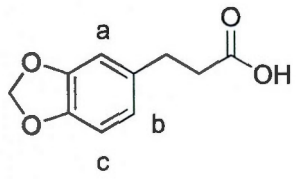
**GENERAL PROCEDURE OF CATALYST HIDROGENATION**

The procedure showed below was followed for the synthesis of compound **37**, **38** and **39**, using compound **34**, **35** and **36** as starting materials respectively.

Compound **34**, **35** or **36** (1 eq), was weighed into a dry flask and dissolved in dry EtOAc. The solution was purged with $N_2$ and stirring for 5 min. Catalytic amount of Pd/C

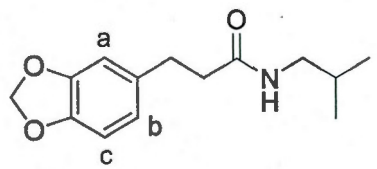
then was added to the solution under an inert atmosphere. (Making sure to wash down any Pd/C stuck to the flask walls). A flow of hydrogen was introduced through the flask and the solution was stirring during 3 hours. The reaction mixture was filtered, washed and concentrated under pressure.

**3-(Benzo[d][1,3]dioxol-5-yl)propanoic acid (37)**



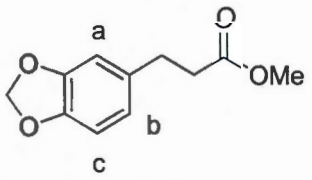

Compound **34** (2.0 g, 10.3 mmol, 1 eq), was used as reagent for the hydrogenation reaction to afford 10.3 mmol (100 %) of compound **37** as white solid. $R_f$ = 0.70 (EtOAc/MeOH 95:5). **$^1$H RMN (300 MHz, ($CDCl_3$):** δ = 6.73 (d, 1H, $^3J_{CHCH}$ = 7.8 Hz, $H_c$), 6.70 (d, 1H, $^4J_{CHCCH}$ = 1.5 Hz, $H_a$), 6.66 (dd, 1H, $^4J_{CHCH}$ = 1.5 Hz, $^3J_{CHCH}$ = 7.8 Hz, $H_b$), 5.93 (s, 2H, $OCH_2O$), 2.88 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, benzene-C*H*), 2.64 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, C*H*CO). (Figure 27) **$^{13}$C RMN (75 MHz, ($CDCl_3$):** δ = 179.2 (*C*OOH), 147.8, 146.2, 134.1, 121.3, 108.9, 108.5 ($C_{arom}$), 101.0 ($OCH_2O$), 36.1 (*C*HCH), 30.5 (*C*HCO). **ESI$^+$-HRMS :** *m/z* calculated for $C_{10}H_{11}O_4$ $[M + H]^+$: 250.1438; found: 250.1437. **ESI$^+$-HRMS:** *m/z* calculated for $C_{10}H_{11}O_4$ $[M + H]^+$: 195.0686 ; found: 195.0654 .

**3-(Benzo[d][1,3]dioxol-5-yl)-N-isobutylpropanamide (38)**



Compound **35** (100 mg, 0.52 mmol, 1 eq) was used as reagent for the hydrogenation reaction to afford 0.37 mmol (71 %) of product Compound **38** as white solid. $R_f$ = 0.12 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.69 (d, 1H, $^3J_{CHCH}$ = 7.8 Hz, $H_c$), 6.67 (d, 1H, $^4J_{CHCCH}$ = 1.6 Hz, $H_a$), 6.62 (dd, 1H, $^4J_{CHCH}$ = 1.6 Hz, $^3J_{CHCH}$ = 7.8 Hz, $H_b$), 5.89 (s, 2H, $OCH_2O$), 5.57 (s, 1H, N*H*), 3.02 (t, 2H, $CH_2$), 2.86 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, benzene-$CH_2$), 2.41 (t, 2H, $^3J_{CH2CH2}$= 7.4 Hz, $CH_2CO$), 1.68 (m, 1H, C*H*), 0.82 ppm (d, 6H, $CH_3$ (2x)). (Figure 28) **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 172.1 (*C*ONH), 147.7, 146.0, 134.8, 121.2, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 46.9 (NH*C*H), 38.9 ($CH_2CH_2$) 31.6 ($CH_2CO$), 28.5 ($C(CH_3)_2$) 20.1 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{20}NO_3$ $[M + H]^+$: 251.147; found: 251.1472.

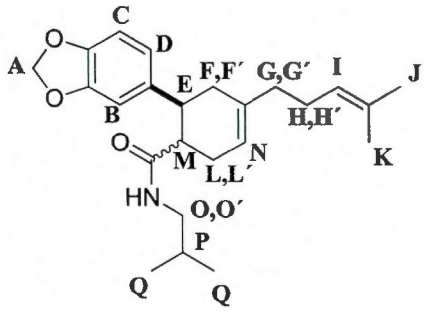
**Methyl 3-(benzo[*d*][1,3]dioxol-5-yl)propanoate (39)**




Compound **36** (1.5g, 7.3 mmol, 1 eq) was used as reagent for the hydrogenation reaction to afford 7.3 mmol (100 %) of product compound **39** as white solid. $R_f$ = 0.38 (Hex/EtOAc 9:1). **$^1$H RMN (300 MHz, ($CDCl_3$):** δ = 6.72 (d, 1H, $^3J_{CHCH}$ = 7.8 Hz, $H_c$), 6.68 (d, 1H, $^4J_{CHCCH}$ = 1.6 Hz, $H_a$), 6.64 (dd, 1H, $^4J_{CHCH}$ = 1.6 Hz, $^3J_{CHCH}$ = 7.8 Hz, $H_b$), 5.91 (s, 2H, $OCH_2O$), 3.67 (s, 3H, $CH_3$), 2.86 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, benzene-C*H*), 2.58 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, C*H*CO). (Figure 29) **$^{13}$C RMN (75 MHz, ($CDCl_3$):** δ = 173.4 (*C*OOH), 147.8, 146.1, 134.4, 121.2, 108.9, 108.4 ($C_{arom}$), 101.0 ($OCH_2O$), 51.7 ($CH_3$), 36.1 ($CH_2CH_2$), 30.8 ppm ($CH_2CO$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{11}H_{13}O_4$ $[M + H]^+$: 209.0808 ; found: 209.0809.

## 7.4. CHARACTERIZATION OF REGIOISOMER OF HEITZIAMIDE A (40) AND COMPOUND 41

**6-(benzo[d][1,3]dioxol-5-yl)-N-isobutyl-4-(4-methylpent-3-enyl)cyclohex-3-enecarboxamide. (40)**



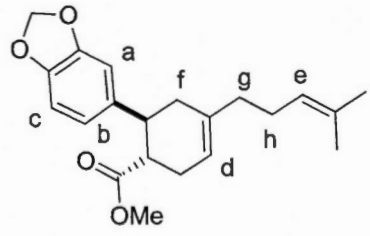

Compound **35** (100 mg, 0.4 mmol, 1 eq) was stirred with myrcene (1.4 mL, 8 mmol, 20 eq) for 72 hours at 130 C. The reaction was cooled to room temperature and then passed through a plug of $SiO_2$ eluting with 10:1 hexanes: ethyl acetate. The solvent was removed by rotary evaporation. The crude material was purified by column chromatography on silicagel (eluent: hexane/EtOAc (gradient: 50:0 to 35:15) to afford 0.26 mmol (65 %) of a mixture of (2:1) *trans* (a)*: cis* (b) diastereomers as white solids.

***trans* Diastereomer 40 a*:*** $R_f$ = 0.71 (Hex/ EtOAc 6:4). **$^1$H RMN (600 MHz, $CDCl_3$):** δ = 6.72 (d, 1H, $^3J_{CHCH}$ = 7.8 Hz, $H_D$), 6.71 (s, 1H, $H_B$), 6.67 (d, 1H, $^3J_{CHCH}$ = 7.8 Hz, $H_C$), 5.89 (s, 2H, $OCH_2O$), 5.47 (s, 1H, N*H*), 5.18 (t, 1H, $^3J_{CHCH}$ = 5.5 Hz, $H_N$), 5.11 (t, 1H, $^3J_{CHCH}$ = 6.7 Hz, $H_I$), 2.94 (m, 2H, NHC*H*, COCHC*H*), 2.71 (m, 1H, *H*CCH), 2.52 (m, 1H, *H*CCH), 2.40 (td, 1H, $^3J_{CHCH}$ = 5.0 Hz, $^3J_{CHCH}$ = 10.9 Hz, C*H*CO), 2.32 (m,1H, NHC*H*), 2.19 (m, 2H, $H_{F,F'}$), 2.10 (m, 2H, $H_{H,H'}$), 2.02 (m, 2H, $H_{G,G'}$), 1.69 (s, 3H, $CH_3$), 1.61 (s, 3H, $CH_3$), 1.41 (m, 1H, $CH(CH_3)_2$), 0.64 ppm (m, 6H, $((CH_3)_2)$ **(figure 38). $^{13}$C RMN (150 MHz, $CDCl_3$):** δ = 174.3, (*C*ONH), 147.6, 138.5, 120.8, 108.3, 107.6 ($C_{arom}$), 136.3 (*C*CH), 131.7 (CH*C*), 124.1 (*C*HC), 119.7 (C*C*H), 100.8 ($OCH_2O$), 49,3 (*C*HCO), 46.6 ($NHCH_2$), 42.3 (*C*HCHCO), 37.4 ($CHCH_2$), 33.6 ($CHCH_2$), 32.7 ($CH_2CH$), 28.4 ($CH(CH_3)_2$), 26.3 ($CH_2CH$),

25.7 ($CCH_3$), 19.4 ($CH(CH_3)_2$), 17.7 ppm ($CCH_3$). **$ESI^+$-HRMS:** *m/z* calculated for $C_{24}H_{34}NO_3$ $[M + H]^+$: 385.2566 ; found: 385.2549.

***cis* Diastereomer 40 *b*:** $R_f$ = 0.66 (Hex/ EtOAc 6:4). **$^1H$ RMN (600 MHz, $CDCl_3$):** δ = 6.72 - 6.68 ($H_{arom}$), 5.90 (s, 2H, $OCH_2O$), 5.46 (s, 1H, N*H*), 5.24 (t, 1H, $^3J_{CHCH}$ = 5.0 Hz, $H_N$), 5.09 (t, 1H, $^3J_{CHCH}$ = 6.4 Hz, $H_{G,G'}$), 2.96 (m, 2H, NHC*H*, COCHC*H*), 2.71 (m, 1H, *H*CCH), 2.50 (m, 1H, *H*CCH), 2.36 (td, 1H, $^3J_{CHCH}$ = 5.1 Hz, $^3J_{CHCH}$ = 10.7 Hz, C*H*CO), 2.26 (m,1H, NHC*H*), 2.19 (m, 2H, $H_{F,F'}$), 2.08 (m, 2H, $H_{H,H'}$), 2.00 (m, 2H, $H_{G,G'}$), 1.69 (s, 3H, $CH_3$), 1.61 (s, 3H, $CH_3$), 1.41 (m, 1H, $CH(CH_3)_2$), 0.64 ppm (m, 6H, $((CH_3)_2)$) **(figure 39).** **$^{13}C$ RMN (150 MHz, $CDCl_3$):** δ = 174.0, (*C*ONH), 147.3, 145.6, 138.1, 120.3, 107.9, 107.3 ($C_{arom}$), 138.1 (*C*CH), 131.2 (CH*C*), 123.6 (*C*HC), 118.6 (C*C*H), 100.4 ($OCH_2O$), 48.5 (*C*HCO), 46.2 ($NHCH_2$), 42.6 (*C*HCHCO), 37.0 ($CHCH_2$), 36.3 ($CHCH_2$), 29.3 ($CH_2CH$), 27.9 ($CH(CH_3)_2$), 25.9 ($CH_2CH$), 25.3 ($CCH_3$), 19.4 ($CH(CH_3)_2$), 17.3 ppm ($CCH_3$ **$ESI^+$-HRMS:** *m/z* calculated for $C_{24}H_{34}NO_3$ $[M + H]^+$: 385.2566 ; found: 385.2565.

**(1S,6R)-Methyl 6-(benzo[d][1,3]dioxol-5-yl)-4-(4-methylpent-3-enyl)cyclohex-3-enecarboxylate (41)**




Compound **36** (100 mg, 0.48 mmol, 1 eq) was stirred with myrcene (1.7 mL, 9.6 mmol, 20 eq) for 72 hours at 130 °C. The reaction was cooled to room temperature and then passed through a plug of $SiO_2$ eluting with 10:1 hexanes: ethyl acetate. The solvent was removed by rotary evaporation. The crude material was purified by column chromatography on silicagel (eluent: hexane/EtOAc (50:0 to 45:5)) to afford 0.27 mmol (55 %) of white solid racemate *trans*.

***trans* Diastereomer 41** $R_f$ = 0.35 (Hex/ EtOAc 9:1). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.73 - 6.64 ($H_{arom}$), 5.91 (s, 2H, $OCH_2O$), 5.44 (m, 1H, $H_d$), 5.09 (m, 1H, $H_e$), 3.45 (s, 3H, $OCH_3$), 2.95 (m, 1H, COCHCH), 2.75 (m, 1H, COCH), 2.37 (m, 2H, $COCHCH_2$), 2.16 (m, 2H, $H_f$), 2.05 (m, 4H, $H_h$, $H_g$), 1.69 (s, 3H, $CH_3$), 1.60 ppm (s, 3H, $CH_3$). **$^{13}$C RMN (150 MHz, $CDCl_3$):** δ = 175.8, (CONH), 147.7, 147.6, 138.2, 120.6, 108.3, 107.8 ($C_{arom}$), 137.4 (CCH), 131.8 (CHC), 124.1 (CHC), 118.6 (CCH), 100.9 ($OCH_2O$), 51.5 ($OCH_3$) 46.5 (COCH), 43.1 (COCHCH), 37.4 ($CHCH_2$), 37.1 ($CHCH_2$), 29.5 ($CH_2CH$), 26.4 ($CH_2CH$), 25.8 ($CCH_3$), 17.8 ppm ($CCH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{21}H_{27}O_4$ $[M + H]^+$: 343.1938 ; found: 343.1901.

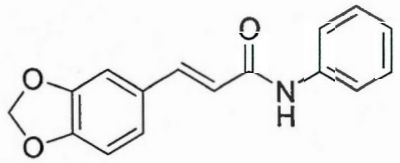
## 7.5. CHARACTERIZATION OF COMPOUNDS 42-85.

### GENERAL PROCEDURE OF COUPLING PEPTIDE

The procedure below was followed for the synthesis of compounds **42-85**. Except for the compounds **56, 58, 78** and **80**. Compounds **34** or **37** were used as starting materials.

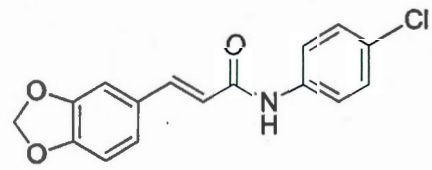
To a solution of either **34** or **37** (1 eq) in dry DMF (5 mL) at room temperature, N,N,N',N'-Tetramethyl-O-(benzotriazol-1-yl) uronium tetrafluoroborate (TBTU) (167.0 mg, 0.52 mmol, 1 eq) and Hydroxybenzotriazole (HOBt) (35.16 mg, 0.26 mmol, 0.5 eq) were added while the solution was stirred for 30 min. The correspond amine (0.78 mmol, 1.5 eq) and N,N-Diisopropylethylamine (DIPEA) (0.52 mL, 3.12 mmol, 6 eq) were added to the solution respectively. The reaction was stirred at room temperature for 2,5 hours and concentrated under reduced pressure. The crude product was dissolved in EtOAc and washed subsequently with distilled water in the presence of hexane (1/4 of the volume of EtOAc), then washed with saturated $KHSO_4$ and $NHCO_3$. The organic phase was dried over anhydrous sodium sulfate and the filtrate was evaporated to dryness in vacuo.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-phenylacrylamide (42)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with aniline (89 µL, 0.78 mmol, 1.5 eq) to afford 0.31 mmol (60 %) of compound **41** as white solid. $R_f$ = 0.53 (Hex/EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.93 (d, 1H, $^3J_{CHCH}$ = 16 Hz, benzene-C*H*), 7.67-7.58 (m, 5H, $H_{arom}$), 7.53-7.06 (m,3H, $H_{arom}$), 6.65 (d, 1H, $J_{CHCH}$ = 16 Hz, C*H*CO), 6.27 (s, 2H, OC$H_2$O). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.0 (*C*ONH), 149.5, 148.4, 138.2, 120.1, 108.7, 106.6 ($C_{arom}$), 142.3 (*C*HCH) 118.2 ppm ($CH_2$CO) 101.1 (O$CH_2$O), 137.8, 129.2, 124.4, 120.1 ppm ($C_{arom}$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{14}NO_3$ $[M + H]^+$: 268.0968; found: 268.096.

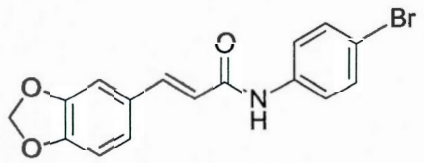
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-chlorophenyl)acrylamide (43)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide 4-Chloroaniline (99.5 mg, 0.78 mmol, 1.5 eq) to afford 0.29 mmol (56 %) of compound **42** as white solid. $R_f$ = 0.19 (Hex/ EtOAc 8:2). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.66 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, benzene-C*H*), 7.58-7.29 (m, 4H, $H_{arom}$), 7.33 (s, 1H, N*H*), 7.03-6.80 (m, 3H, $H_{arom}$), 6.35 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, C*H*CO), 6.01 ppm (s, 2H, OC$H_2$O). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 174.6 (*C*ONH), 148.5, 148.0, 129.2, 121.3, 108.8, 106.5,

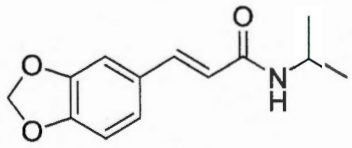
($C_{arom}$), 142.8 (*C*HCH), 136.8, 133.3, 129.0, 121.3 ($C_{arom}$), 117.8 (*C*HCO), 101.7 ppm ($OCH_2O$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{13}ClNO_3$ $[M + H]^+$ : 302.0578; found: 302.0586.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-bromophenyl)acrylamide (44)**



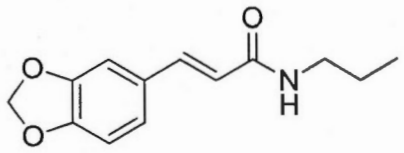

Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with 4-Bromoaniline (134.2 mg, 0.78 mmol, 1.5 eq) to afford 0.23 mmol (45 %) of compound **44** as white solid. $R_f$ = 0.23 (Hex/EtOAc 8:2). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.66 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 7.53-7.43 (m, 4H, $H_{arom}$), 7.33 (s, 1H, N*H*), 7.03-6.80 (m, 3H, $H_{arom}$), 6.35 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, *CH*CO), 6.01 ppm (s, 2H, $OCH_2O$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.5 (*C*ONH), 148.5, 148.0, 128.9. 122.9, 108.8, 106.5 ($C_{arom}$), 142.2 (*C*HCH), 132.2, 130.3, 124.6, 121.6 ($C_{arom}$), 118.9 (*C*HCO), 101.7 ppm ($OCH_2O$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{13}BrNO_3$ $[M + H]^+$: 346.0073; found: 346.0078.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-isopropylacrylamide (45)**



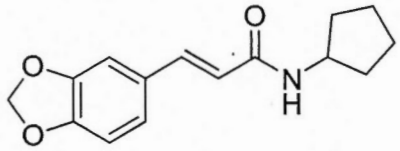
Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with isopropylamine (64 μL, 0.78 mmol, 1.5 eq) to afford 0.31 mmol (60 %) of compound **45** as white solid. $R_f$ = 0.25 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.51 (d, 1H, $J_{CHCH}$ = 16.2 Hz, benzene-C*H*), 6.98-6.77 (m, 3H, $H_{arom}$), 6.18 (d, 1H, $J_{CHCH}$ = 16.2 Hz, *CH*CO), 5.98 ppm (s, 2H, OC$H_2$O), 5.45 (s, 1H, N*H*), 4.21 (m, 1H, C*H*), 1.22 ppm (d, 6H, C$H_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.3 (*C*ONH), 140.6 (*C*HCH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 119.2 (*C*HCO), 101.5 (O*C*$H_2$O), 41.7 (NH*C*H), 23.0 ppm (*C*$H_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{16}NO_3$ $[M + H]^+$: 234.1125; found: 234.1136.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-propylacrylamide (46)**



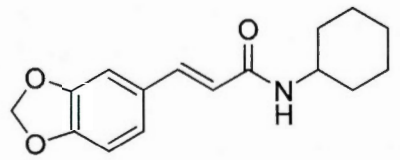

Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with propylamine (64 μL, 0.78 mmol, 1.5 eq) to afford 0.47 mmol (90 %) of compound **46** as white solid. $R_f$ = 0.27 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 6.98-6.77 (m,3H, $H_{arom}$), 6.23 (d, 1H, $J_{CHCH}$ = 15.7 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.70 (s, 1H, N*H*), 3.33 (m, 2H, NHC$H_2$), 1.60 (m, 2H, NHCH$_2$C$H_2$), 0.95 (m, 3H, C$H_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.2 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.6, 106.4 ($C_{aroma}$), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.5 (O*C*$H_2$O), 41.6 (NH*C*$H_2$), 23.1 (NHCH$_2$*C*$H_2$) 11.6 ppm (*C*$H_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{16}NO_3$ $[M + H]^+$: 233.1285; found: 233.1266.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclopentylacrylamide (47)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with cyclopentylamine (80 μL, 0.78 mmol, 1.5 eq) to afford 0.48 mmol (92 %) of compound **47** as white solid. $R_f$ = 0.32 (Hex/EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.52 (d, 1H, $J_{CHCH}$ = 15.3 Hz, benzene-C*H*), 6.98-6.77 (m, 3H, $H_{arom}$), 6.18 (d, 1H, $J_{CHCH}$ = 15.3 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.66 (s, 1H, N*H*), 4.33 (m, 1H, NHC*H*), 2.07 ( m, 2H, C$H_2$CH$_2$CH$_2$), 1.66 (m, 4H, CH$_2$C$H_2$C$H_2$), 1.44 ppm (m, 2H, C$H_2$CH$_2$CH$_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.7 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 140.6 (*C*HCH), 119.1 (*C*HCO), 101.5 (OC$H_2$O), 51.5 (NH*C*H), 33.4 (*C*H$_2$CH$_2$CH$_2$*C*H$_2$), 23.7 ppm (CH$_2$*C*H$_2$*C*H$_2$CH$_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{18}NO_3$ $[M + H]^+$: 260.1281; found: 260.1293.

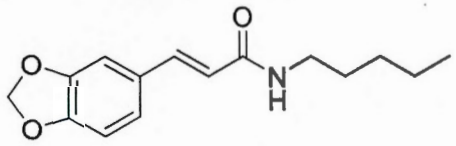
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclohexylacrylamide (48)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with cyclohexylamine (90 μL, 0.78 mmol, 1.5 eq) to afford 0.36 mmol (70 %) of compound **48** as white solid. $R_f$ = 0.22 (Hex/EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.51 (d, 1H, $^3J_{CHCH}$ = 15.6 Hz, benzene-C*H*), 6.99-6.77 (m,3H, $H_{arom}$), 6.18 (d, 1H, $^3J_{CHCH}$ = 15.6 Hz, *CH*CO), 5.98 (s, 2H, OC$H_2$O), 5.46 (s, 1H, N*H*), 3.90 (m, 1H, NHC*H*), 2.01-1.15

ppm (m, 10H, cyclohexyl). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 140.5 (*C*HCH), 119.3 (*C*HCO), 101.5 (O*C*$H_2$O), 48.4 (NH*C*H), 33.4 ($CH_2CH_2CH_2CH_2CH_2$), 25.7 ($CH_2CH_2CH_2CH_2CH_2$), 25.0 ppm ($CH_2CH_2CH_2CH_2CH_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{20}NO_3$ $[M + H]^+$: 274.1438; found: 274.1438.

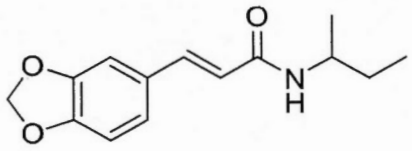
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-pentylacrylamide (49)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with n-amylamine (90 μL, 0.78 mmol, 1.5 eq) to afford 0.2 mmol (38 %) of compound

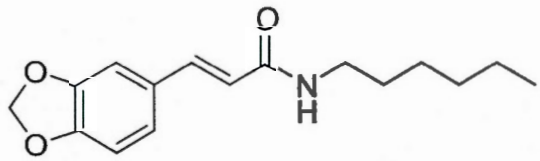
**49** as white solid. $R_f$ = 0.29 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 6.94-6.77 (m,3H, $H_{arom}$), 6.20 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, *CH*CO), 5.98 (s, 2H, OC$H_2$O), 5.63 (s, 1H, N*H*), 3.37 (m, 1H, NHC$H_2$), 1.56 (m, 2H, $NHCH_2CH_2$), 1.34 (m, 4H, $NHCH_2CH_2CH_2CH_2$), 0.90 (m, 3H, C$H_3$). **$^3$C RMN (75 MHz $CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.5 ($C_{arom}$), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.6 (O*C*$H_2$O), 39.9 (NH*C*$H_2$), 29.5 ($NHCH_2CH_2$), 29.3 ($CH_2CH_2CH_3$), 22.5 ($CH_2CH_3$), 14.1 ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{20}NO_3$ $[M + H]^+$: 262.1438; found: 262.1426.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-sec-butylacrylamide (50)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with sec-butylamine (80 µL, 0.78 mmol, 1.5 eq) to afford 0.17 mmol (32 %) of compound **50** as white solid. $R_f$ = 0.40 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.52 (d, 1H, $^3J_{CHCH}$ = 15.5 Hz, benzene-C*H*), 6.98-6.77 (m, 3H, $H_{arom}$), 6.20 (d, 1H, $^3J_{CHCH}$ = 15.5 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.43 (s, 1H, N*H*), 4.05 (m, 1H, NHC*H*), 1.51 (m, 2H, NHCHC$H_2$), 1.18 (m, 3H, NHCHC$H_3$), 0.93 ppm (m, 3H, NHCHCH$_2$C$H_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.5 (*C*ONH), 149.0, 148.3, 129.5, 123.8, 108.6, 106.4 ($C_{aroma}$), 140.6 (*C*HCHCO), 119.3 (*C*HCO), 101.5 (O*C*H$_2$O), 45.9 (NH*C*H), 29.9 (NHCH*C*H2), 20.7 (NHCH*C*H$_3$), 10.5 ppm (CHCH$_2$*C*H$_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{18}NO_3$ [M + H]$^+$: 248.1281; found: 248.1273.

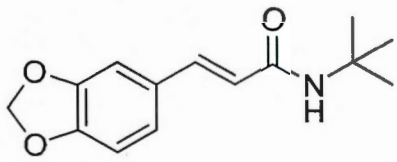
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-hexylacrylamide (51)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with hexylamine (110 µL, 0.78 mmol, 1.5 eq) to afford 0.17 mmol (33 %) of compound **51** as white solid. $R_f$ = 0.35 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ

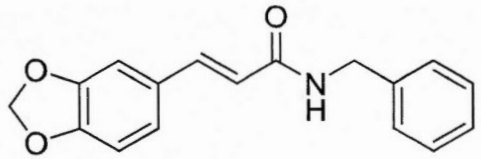
= 7.53 (d, 1H, $^3J_{CHCH}$ = 15.3 Hz, benzene-C*H*), 6.99-6.77 (m,3H, $H_{arom}$), 6.20 (d, 1H, $^3J_{CHCH}$ = 15.3 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.62 (s, 1H, N*H*), 3.37 (m, 2H, NHC$H_2$), 1.52 (m, 2H, $NH_2CH_2CH_2$), 1.31 (m,6H, $NH_2CH_2CH_2$ $CH_2CH_2CH_2$), 0.88 ppm (m, 3H, $CH_2CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.6 ($OCH_2O$), 39.9 (m, 2H, NH*C*$H_2$), 31.6 ($NHCH_2CH_2$), 29.8 ($NHCH_2CH_2CH_2$), 26.8 ($CH_2CH_2CH_3$), 22.7 ($CH_2CH_3$), 14.2 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{22}NO_3$ $[M + H]^+$: 276.1594; found: 276.1602.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-tert-butylacrylamide (52)**



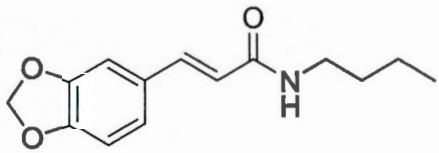
Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with t-butylamine (82 μL, 0.78 mmol, 1.5 eq) to afford 0.27 mmol (52 %) of compound **52** as white solid. $R_f$ = 0.23 (Hex/ EtOAc 8:2). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.47 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, benzene-C*H*), 6.97-6.76 (m,3H, $H_{arom}$), 6.15 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, *CH*CO), 5.98 (s, 2H, OC$H_2$O), 5.44 (s, 1H, N*H*), 1.42 pm (s, 9H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.5 (*C*ONH), 149.0, 148.3, 129.5, 123.8, 108.7, 106.4 ($C_{arom}$), 140.1 (*C*HCH), 120.2 (*C*HCO), 101.5 ($OCH_2O$), 51.6 ($C(CH_3)_3$), 29.0 ppm ($C(CH_3)_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{18}NO_3$ $[M + H]^+$: 248.1281; found: 248.1285.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-benzylacrylamide (53)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with benzylamine (90 µL, 0.78 mmol, 1.5 eq) to afford 0.18 mmol (38%) of compound **53** as white solid. $R_f$ = 0.33 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.58 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 7.35-7.26 (m, 5H, $H_{arom}$), 6.99-6.78 (m, 3H, $H_{arom}$), 6.24 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, *CH*CO), 5.99 (s, 2H, OC$H_2$O), 5.87 (s, 1H, N*H*), 4.57 ppm (d, 2H, NHC$H_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.0 (*C*ONH), 149.2, 148.4, 129.3, 124.0, 108.7, 106.5 ($C_{arom}$), 138.4, 128.9, 128.1, 127.7 ($C_{arom}$), 141.3 (*C*HCH), 118.5 (*C*HCO), 101.6 (O*C*$H_2$O), 44.0 ppm (NH*C*$H_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{17}H_{16}NO_3$ $[M + H]^+$:282.1125 ; found: 282.1141.

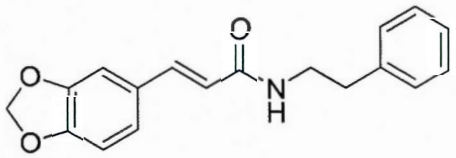
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-butylacrylamide (54)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with n-butylamine (80 µL, 0.78 mmol, 1.5 eq) to afford 0.18 mmol (35%) of compound **54** as white solid. $R_f$ = 0.32 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, benzene-C*H*), 6.99-6.77 (m, 3H, $H_{arom}$), 6.21 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.61 (s, 1H, N*H*), 3.38 (m, 2H, NHC$H_2$), 1.52 (m, 2H, NHCH$_2$C$H_2$), 1.35 (m, 2H, C$H_2$CH$_3$), 0.88 ppm (t, 3H, CH$_2$C$H_3$). **$^{13}$C RMN (75 MHz**

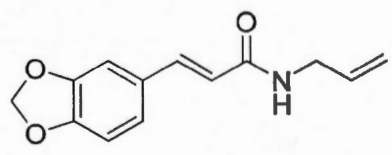
**$CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.5 (O*C*$H_2$O), 39.6 (NH*C*$H_2$), 31.9 (*C*$H_2$CH$_2$CH$_3$), 20.3 (*C*$H_2$CH$_3$), 13.9 (*C*$H_3$), **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{18}NO_3$ $[M + H]^+$: 248.1281; found: 248.1269.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-phenethylacrylamide (55)**



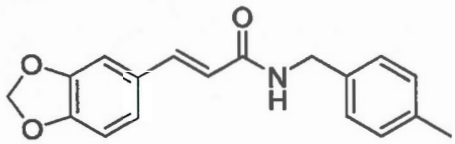
Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with phenetylamine (100 µL, 0.78 mmol, 1.5 eq) to afford 0.21 mmol (40 %) of compound **55** as white solid. $R_f$ = 0.30 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 7.36-7.21 (m,5H, $H_{arom}$), 6.97-6.77 (m, 3H, $H_{arom}$), 6.14 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, C*H*CO), 5.98 (s, 2H, OC$H_2$O), 5.57 (s, 1H, N*H*), 3.65 (q, 2H, NHC$H_2$), 2.89 ppm (t, 2H, C$H_2$-benzene). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.4, 129.3, 124.0, 108.7, 106.5 ($C_{arom}$), 140.9 (*C*HCH), 139.0, 129.0, 128.8, 126.7 ($C_{arom}$) 118.8 (*C*HCO), 101.6 (O*C*$H_2$O), 40.9 (NH*C*$H_2$), 35.8 ppm (*C*$H_2$-benzene). **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{18}NO_3$ $[M + H]^+$: 296.1281; found: 296.1294.

**(E)-N-allyl-3-(Benzo[d][1,3]dioxol-5-yl)acrylamide (57)**



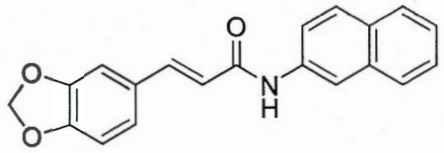
Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with allylamine (60 µL, 0.78 mmol, 1.5 eq) to afford 0.39 mmol (75 %) of compound **57** as white solid. $R_f$ = 0.48 (Hex/ EtOAc 1:1). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.55 (d, 1H, $^3J_{CHCH}$ = 15.5 Hz, benzene-C*H*), 6.99-6.77 (m, 3H, $H_{arom}$), 6.25 (d, 1H, $^3J_{CHCH}$ = 15.5 Hz, *CH*CO), 5.98 (s, 2H, OC$H_2$O), 5.90 (m, 1H, *CH*$CH_2$), 5.85 (s, 1H, N*H*), 5.20 (m, 2H, CHC$H_2$), 4.01 ppm (m, 2H, NHC$H_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.0 (*C*ONH), 149.2, 148.4, 129.3, 124.0, 108.6, 106.5 ($C_{arom}$), 141.1 (*C*HCH), 134.3 (*C*H$CH_2$),118.6 (*C*HCO), 116.7 (CH*C*$H_2$), 101.6 (O*C*$H_2$O), 42.3 ppm (NH*C*$H_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{14}NO_3$ $[M + H]^+$: 232.0968; found: 232.0974.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-methylbenzyl)acrylamide (59)**



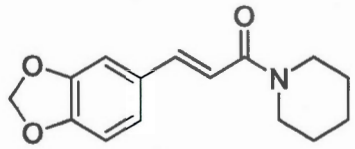
Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with methylbenzylamine (100 µL, 0.78 mmol, 1.5 eq) to afford 0.37 mmol (72%) of compound **59** as white solid. $R_f$ = 0.37 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.58 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, benzene-C*H*), 7.23-7.14 (m, 4H, $H_{arom}$), 6.99-6.78 (m, 3H, $H_{arom}$), 6.21 (d, 1H, $^3J_{CHCH}$ = 15.8 Hz, C*H*CO), 5.99 (s, 2H, OC$H_2$O), 5.79 (s, H, N*H*), 4.52 (d, 2H, NHC$H_2$), 2.34 ppm (s, 3H, C$H_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.9 (*C*ONH), 149.2, 148.4, 129.6, 124.0, 108.7, 106.5 ($C_{arom}$), 141.2 (*C*HCH), 137.5, 135.4, 129.3, 128.1 ($C_{arom}$), 118.6 (*C*HCO), 101.6 (O*C*$H_2$O), 43.8 (NH*C*$H_2$), 21.2 (*C*$H_3$) ppm. **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{18}NO_3$ $[M + H]^+$: 296.1281; found: 296.1281.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(naphthalen-2-yl)acrylamide (60)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide with 2-Naphtylamine (112 mg, 0.78 mmol, 1.5 eq) to afford 0.18 mmol (35 %) of compound **60** as white solid. $R_f$ = 0.33 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 8.32 (s, 1H, N*H*), 7.79-7.39 (m, 7H, $H_{arom}$), 7.70 (d, 1H, $^3J_{CHCH}$ = 15.3 Hz, benzene-C*H*), 7.01-6.76 (m, 3H, $H_{arom}$), 6.45 (d, 1H, $^3J_{CHCH}$ = 15.3 Hz, C*H*CO), 5.99 ppm (s, 2H, OC$H_2$O). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 164.5 (*C*ONH), 149.5, 148.8, 129.1, 124.4, 108.7, 106.6 ($C_{arom}$), 142.4 (*C*HCH), 135.7, 134.0, 130.8, 128.9, 127.9, 127.7, 126.6, 125.2, 120.3, 117.3 ($C_{napthalene}$), 119.0 (*C*HCO), 101.6 ppm (O*C*$H_2$O). **ESI$^+$-HRMS:** *m/z* calculated for $C_{20}H_{17}NO_3$ $[M + H]^+$: 318.1125; found: 318.1114.

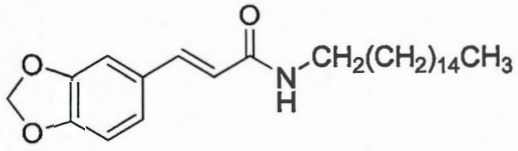
**(*E*)-3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(prop-2-yn-1-yl)acrylamide (61)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with piperidine (100 μL, 0.781 mmol, 1.5 eq) to afford 0.46 mmol (88%) of compound **61** as white solid. $R_f$ = 0.21 (Hex/ EtOAc 8:2). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.55 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-C*H*), 6.99-6.76 (m, 3H, $H_{arom}$), 6.72 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, C*H*CO), 5.96 (s, 2H, $OCH_2O$), 3.59 (m, 4H, $CH_2NHCH_2$), 1.62 ppm (m, 6H, $CH_2CH_2CH_2$).

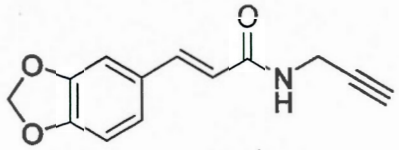
**$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.5 (*C*ONH), 148.9, 148.3, 130.0, 123.7, 108.5, 106.4 ($C_{arom}$), 142.0 (*C*HCH), 115.8 (*C*HCO), 101.5 ($OCH_2O$), 47.0, 43.4, 26.7, 26.8, 25.7, 24.7 ppm.($C_{piperidine}$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{18}NO_3$ $[M + H]^+$: 260.1281; found: 260.1284.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-hexadecylacrylamide (62)**



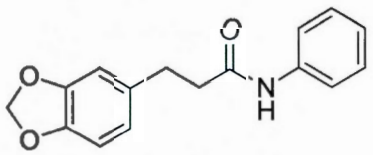

Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide hexadecylamine (189 mg, 0.78 mmol, 1.5 eq) to afford 0.16 mmol (30 %) of compound **62** as white solid. $R_f$ = 0.32 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.6 Hz, benzene-C*H*), 6.69-6.78 (m, 3H, $H_{arom}$), 6.22 (d, 1H, $^3J_{CHCH}$ = 15.6 Hz, C*H*CO), 5.99 (s, 2H, $OCH_2O$), 5.60 (s, 1H, N*H*), 3.37 (m, 2H, $NHCH_2$), 1.55 (m, 2H, $NHCH_2CH_2$), 1.30 (m, 26H, $(CH_2)_3$), 0.87 ppm (m, 3H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.4, 129.5, 123.9, 108.7, 106.4 ($C_{arom}$), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.6 ($OCH_2O$), 39.9 ($NHCH_2$), 32.1, 29.9, 29.8, 29.7, 29.5, 27.1, 22.8 ($(CH_2)_{14}$), 14.2 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{26}H_{42}NO_3$ $[M + H]^+$: 416.3159; found: 416.3166.

**(*E*)-3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(prop-2-yn-1-yl)acrylamide (63)**



Compound **34** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling peptide propargylamine (50 µg, 0.78 mmol, 1.5 eq) to afford 0.41 mmol (80 %) of compound **63** as white solid. $R_f$ = 0.23 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.57 (d, 1H, $^3J_{CHCH}$ = 15.2 Hz, benzene-C*H*), 6.99-6.78 (m, 3H, $H_{arom}$), 6.23 (d, 1H, $^3J_{CHCH}$ = 15.2 Hz, C*H*CO), 5.99 (s, 2H, OC$H_2$O), 5.85 (s, 1H, N*H*), 4.18 (m, 2H, NHC$H_2$), 2.26 ppm (m, 1H, C$H_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 165.8 (*C*ONH), 149.4, 148.4, 129.1, 124.2, 108.7, 106.5 ($C_{arom}$), 141.8 (*C*HCH), 117.8 (*C*HCO), 101.6 (O*C*H$_2$O), 79.7 (*C*CH), 71.9 (C*C*H) 29.6 (NH*C*H$_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{12}NO_3$ $[M + H]^+$: 230.0812; found: 230.0808.

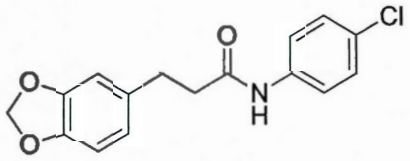
**(E)-3-(b3-(Benzo[d][1,3]dioxol-5-yl)-N-phenylpropanamide (64)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with aniline (60 µL, 0.77 mmol, 1.5 eq) to dryness in vacuo, to afford 0.26 mmol (51%) of compound **64** as white solid. $R_f$ = 0.30 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.38-7.01 (m, 5H, $H_{arom}$), 6.67-6.57 (m, 3H, $H_{arom}$), 5.83 (s, 2H, OC$H_2$O), 2.87 (t, 2H, $^3J_{CH2CH2}$ = 7.24 Hz, C$H_2$CO), 2.52 (t, 2H, $^3J_{CH2CH2}$ = 7.24 Hz, benzene-C$H_2$). **$^{13}$C RMN (75**

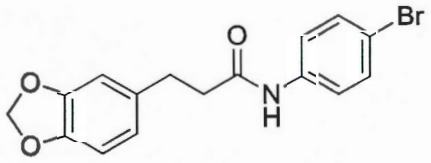
**MHz $CDCl_3$):** δ = 170.4 (*C*ONH), 147.9, 146.1, 134.5, 121.4, 109.0, 108.5 ($C_{aroma}$), 101.1 ($OCH_2O$), 137.9, 129.1, 124.4, 120.1 ($C_{arom}$), 39.8 ($CH_2CH_2$), 31.4 ppm ($CH_2CO$). **$ESI^+$-HRMS:** *m/z* calculated for $C_{16}H_{16}NO_3$ $[M + H]^+$: 270.1125; found: 270.1133.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-chlorophenyl)propanamide (65)**




Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with chloroaniline (98 mg, 0.77 mmol, 1.5 eq) to afford 0.39 mmol (76 %) of compound **65** as white solid. $R_f$ = 0.30 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.40-7.21 (m, 4H, $H_{arom}$), 6.74-6.64 (m, 3H, $H_{arom}$) 5.92 (s, 2H, $OCH_2O$), 2.95 (t, 2H, $^3J_{CH2CH2}$ = 7.32 Hz, $CH_2CO$), 2.60 ppm (t, 2H, $^3J_{CH2CH2}$ = 7.32 Hz, benzene-$CH_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 170.0 (*C*ONH), 147.9, 146.2, 134.3, 121.3, 108.9, 108.5 ($C_{aroma}$), 136.4, 129.4, 129.1, 121.2 ($C_{arom}$), 101.0 ($OCH_2O$), 39.7 ($CH_2CH_2$) 31.3 ppm ($CH_2CO$). **$ESI^+$-HRMS:** *m/z* calculated for $C_{16}H_{15}ClNO_3$ $[M + H]^+$: 304.0735 ; found: 304.0726.

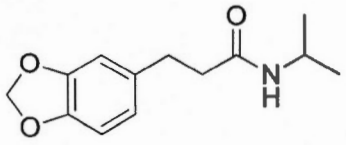
**3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-bromophenyl)propanamide (66)**




Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with bromoaniline (133 mg, 0.77 mmol, 1.5 eq) to afford 0.2 mmol (40 %) of compound **66** as

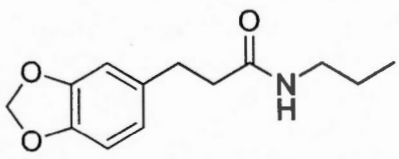
white solid. $R_f$ = 0.19 (Hex/ EtOAc 8:2). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.42-7.33 (m, 4H, $H_{arom}$), 7.05 (s, 1H, N*H*), 6.75-6.69 (m, 3H, $H_{arom}$), 5.92 (s, 2H, O$CH_2$O), 2.95 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, $CH_2$CO), 2.60 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, benzene-$CH_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 170.5 (*C*ONH), 147.9, 146.2, 134.3, 121.3, 108.9, 108.5 ($C_{aroma}$), 101.0 (O$CH_2$O), 136.9, 129.4, 121.6, 117.0 ($C_{aroma}$), 39.8 ($CH_2CH_2$) 31.3 ppm ($CH_2$CO). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{15}BrNO_3$ $[M + H]^+$: 348.023 ; found: 348.023

**3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-bromophenyl)propanamide (67)**



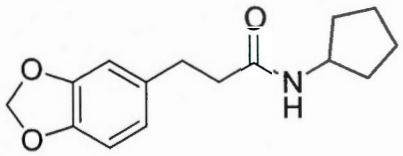
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with isopropylamine (46 mg, 0.77 mmol, 1.5 eq) to afford 0.34 mmol (67 %) of compound **67** as white solid. $R_f$ = 0.25 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.62 (m,3H, $H_{arom}$) 5.90 (s, 2H, O$CH_2$O), 5.20 (s, 1H, N*H*), 4.04 (m, 1H, C*H*), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.3 Hz, $CH_2$CO), 2.35 (t, 2H, $^3J_{CH2CH2}$ = 7.3 Hz, benzene-$CH_2$), 1.07 ppm (d, 6H, $CH_3$ (2x)). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 171.2 (*C*ONH), 147.7, 146.0, 134.9, 121.3, 108.9, 108.5 ($C_{arom}$), 100.9 (O$CH_2$O), 41.4 (NH*C*H), 39.1 ($CH_2CH_2$) 31.7 ($CH_2$CO), 22.9 ($CH_3$) . **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{18}NO_3$ $[M + H]^+$: 236.1281 ; found: 236.1273

**3-(Benzo[d][1,3]dioxol-5-yl)-N-propylpropanamide (68)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with propylamine (46 mg, 0.77 mmol, 1.5 eq) to afford 0.36 mmol (70 %) of compound **68** as white solid. $R_f$ = 0.32 (Hex/EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.61 (m, 3H, $H_{arom}$) 5.90 (s, 2H, $OCH_2O$), 5.45 (s, 1H, N*H*), 3.16 (m, 2H, $NHCH_2$), 2.87 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, $CH_2CO$), 2.40 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, benzene-$CH_2$), 1.43 (m, 2H, $CH_2CH_3$), 0.85 ppm (t, 3H, $CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.9, 121.3, 108.9, 108.4 ($C_{aroma}$), 100.9 ($OCH_2O$), 41.3 (NH*C*H), 38.9 ($CH_2CH_2$), 31.7 ($CH_2CO$), 22.9 ($CH_2CH_3$) 11.4 ppm ($CH_3$) . **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{18}NO_3$ $[M + H]^+$: 236.1281 ; found: 236.12181.

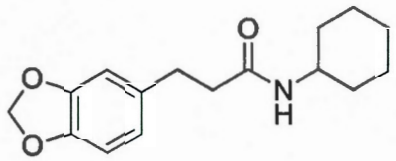
**3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclopentylpropanamide (69)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with cyclopentylamine (66 mg, 0.77 mmol, 1.5 eq) to afford 0.47 mmol (92 %) of compound **69** as white solid. $R_f$ = 0.21 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.60 (m, 3H, $H_{arom}$) 5.89

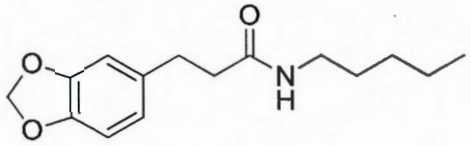
(s, 2H, $OCH_2O$), 5.41 (s, 1H, N*H*), 4.15 (m, 1H, NHC*H*), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, $CH_2CO$), 2.35 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, benzene-$CH_2$), 1.92 (m, 2H, $CH_2CH_2CH_2$), 1.57 (m, 4H, $CH_2CH_2CH_2$), 1.26 ppm (m, 2H, $CH_2CH_2CH_2$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 171.6 (*C*ONH), 146.7, 146.0, 134.9, 121.3, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 51.2 (NH*C*H) 39.0 ($CH_2CH_2$), 33.1($CH_2CH_2CH_2CH_2$), 31.7 ($CH_2CO$), 23.7 ppm ($CH_2CH_2CH_2CH_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{20}NO_3$ $[M + H]^+$: 262.1438; found : 262.1431.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclopentylpropanamide (70)**



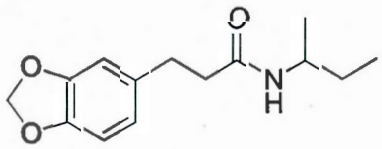
Compound **35** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with cyclohexylamine (77 mg, 0.77 mmol, 1.5 eq) to afford 0.38 mmol (73 %) of compound **70** as white solid. $R_f$ = 0.29 (Hex/AcOEt 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.60 (m, 3H, $H_{arom}$) 5.89 (s, 2H, $OCH_2O$), 5.33 (s, 1H, N*H*), 3.72 (m, 1H, NHC*H*), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.7 Hz, $CH_2CO$), 2.36 (t, 2H, $^3J_{CH2CH2}$ = 7.7 Hz, benzene-$CH_2$), 1.85-0.95 ppm (m, 10H, cyclohexyl). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 171.1 (*C*ONH), 147.7, 146.0, 134.9, 121.3, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 48.1 (NH*C*H), 39.1 ($CH_2CH_2$), 33.2 ($CH_2CH_2CH_2CH_2CH_2$), 31.7 ($CH_2CO$), 25.6 ($CH_2CH_2CH_2CH_2CH_2$), 24.9 ppm ($CH_2CH_2CH_2CH_2CH_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{22}NO_3$ $[M + H]^+$: 276.594; found: 276.1588.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-pentylpropanamide (71)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with n-amylamine (67 mg, 0.77 mmol, 1.5 eq) to afford 0.3 mmol (58 %) of compound **71** as white solid. $R_f$ = 0.26 (Hex/ EtOAc 6:4), **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.61 (m, 3H, $H_{arom}$ ) 5.89 (s, 2H, $OCH_2O$), 5.52 (s, 1H, N*H*), 3.18 (m, 2H, $NHCH_2$), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.2 Hz, $CH_2CO$), 2.39 (t, 2H, $^3J_{CH2CH2}$ = 7.2 Hz, benzene-$CH_2$), 1.39 (m, 2H, $NHCH_2CH_2$), 1.23 (m, 4H, $NHCH_2CH_2CH_2CH_2$), 0.86 (m, 3H, $CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.8, 121.3, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 39.6 ($NHCH_2$), 38.9 ($CH_2CH_2$), 31.6 ($CH_2CO$), 29.4 (NHCH2*C*H2), 29.1 ($CH_2CH_2CH_3$), 22.4 ($CH_2CH_3$), 14.0 ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{21}NO_3$ [M + H]$^+$: 264.1594; found: 264.1589.

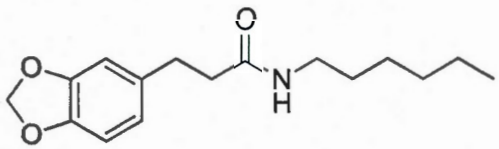
**3-(Benzo[d][1,3]dioxol-5-yl)-N-pentylpropanamide (72)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with to afford 0.37 mmol (72 %) of compound **72** as white solid. $R_f$ = 0.23 (Hex/EtOAc 65:35), **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.61 (m, 3H, $H_{arom}$ ) 5.89 (s, 2H, $OCH_2O$), 5.26 (s, 1H, N*H*), 3.87 (m, 1H, NHC*H*), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, $CH_2CO$), 2.37 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, benzene-$CH_2$), 1.35 (m, 2H, $NHCHCH_2$), 1.03 (m, 3H, $NHCHCH_3$), 0.81 (m, 3H,

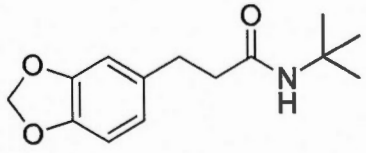
$NHCHCH_2CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 171.4 (*C*ONH), 147.7, 146.0, 134.9, 121.3, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 45.6 (NH*C*H), 39.1 ($CH_2CH_2$), 31.7 ($CH_2CO$), 29.7 (NHCH*C*H2), 20.5 ($NHCHCH_3$), 10.3 ($CHCH_2CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{20}NO_3$ $[M + H]^+$: 250.1428; found: 250.1434.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-hexylpropanamide (73)**



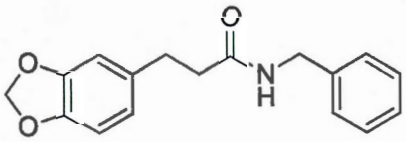
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling hexylamine (60 µL, 0.77 mmol, 1.5 eq) to afford 0.12 mmol (23 %) of compound **73** as white solide. $R_f$ = 0.21 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.60 (m, 3H, $H_{arom}$), 5.89 (s, 2H, $OCH_2O$), 5.52 (s, 1H, N*H*), 3.18 (m, 2H, $NHCH_2$), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, $CH_2CO$), 2.39 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, benzene-$CH_2$), 1.4 (m, 2H, $NH_2CH_2CH_2$), 1.23 (m, 6H, $NH_2CH_2CH_2$ $CH_2CH_2CH_2$), 0.86 ppm (m, 3H, $CH_2CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.8, 121.2, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 39.6 (NH*C*H), 38.9 (benzene-$CH_2$), 31.6 ($CH_2CO$), 31.5 ($NHCH_2CH_2$), 29.6 ($NHCH_2$ $CH_2CH_2$), 26.6 ($CH_2CH_2CH_3$), 22.6 ($CH_2CH_3$), 14.1 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{24}NO_3$ $[M + H]^+$: 278.1751; found: 278.1756.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-tert-butylacrylamide (74)**



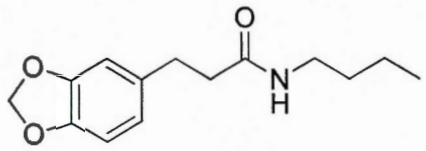
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling t-butylamine (60 μL, 0.77 mmol, 1.5 eq) to afford 0.38 mmol (74 %) of compound **74** as white solid. $R_f$ = 0.33 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.61 (m, 3H, $H_{arom}$), 5.90 (s, 2H, $OCH_2O$), 5.2 (s, 1H, N*H*), 2.83 (t, 2H, $^3J_{CH2CH2}$ = 7.1 Hz, $CH_2CO$), 2.31 (t, 2H, $^3J_{CH2CH2}$ = 7.1 Hz, benzene-$CH_2$), 1.27 pm (s, 9H,$CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 171.4 (*C*ONH), 147.7, 145.9, 135.0, 121.3, 109.4, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 51.2 ($C(CH_3)_3$), 39.8 ($CH_2CH_2$) 31.7 ($CH_2CO$), 28.9 ppm ($C(CH_3)_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{20}NO_3$ $[M + H]^+$: 250.1438; found: 250.1437.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-benzylpropanamide (75)**



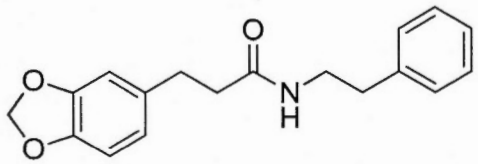
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling benzylamine (85 μL, 0.77 mmol, 1.5 eq) to afford 0.27 mmol (52 %) of compound **74** as white solid. $R_f$ =0.21 (Hex/EtOAct 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.23-7.05 (m, 5H, $H_{arom}$), 6.63-6.53 (m, 3H, $H_{arom}$), 5.81 (s, 2H, $OCH_2O$), 5.79 (s, 1H, N*H*), 4.29 (d, 2H, $NHCH_2$), 2.81 (t, 2H, $^3J_{CH2CH2}$ = 7.6 Hz, ($CH_2CH_2$), 2.37 ppm (t, 2H, $^3J_{CH2CH2}$ = 7.6 Hz, benzene-$CH_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 171.9 (*C*ONH), 147.7, 146.0, 134.7, 121.3, 108.9, 108.4 ($C_{arom}$), 138.3, 128.7, 127.8, 127.5 ($C_{arom}$), 100.9 ($OCH_2O$), 43.6 ($NHCH_2$), 38.8 ($CH_2CO$), 31.5 ppm (benzene-$CH_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{17}H_{18}NO_3$ $[M + H]^+$: 284.1281; found: 284.1276.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-butylpropanamide (76)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling benzylamine (85 μL, 0.77 mmol, 1.5 eq) n-butylamine (77 μL, 0.7725 mmol, 1.5 eq) to afford 0.37 mmol (72 %) of compound **76** as white solid. $R_f$ = 0.5 (Hex/ EtOAc 55:45). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71- 6.61 (m, 3H, $H_{arom}$), 5.89 (s, 2H, $OCH_2O$), 5.26 (s, 1H, N*H*), 3.86 (m, 2H, $NHCH_2$), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, $CH_2CO$), 2.37 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, $CH_2CH_2$), 1.37 (m, 2H, $NHCH_2CH_2$), 1.03 (m, 2H, $CH_2CH_3$), 0.81 ppm (t, 3H, $CH_2CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 171.3 (*C*ONH), 147.9, 145.9, 134.9, 121.3, 108.9, 108.3 ($C_{aroma}$), 100.9 ($OCH_2O$), 46.5 ($NHCH_2$), 39.1 ($CH_2CH_2$), 31.7 ($CH_2CO$), 29.7 ($CH_2CH_2CH_3$), 20.5 ($CH_2CH_3$), 10.3 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{14}H_{20}NO_3$ $[M + H]^+$: 250.1438; found: 250.1439.

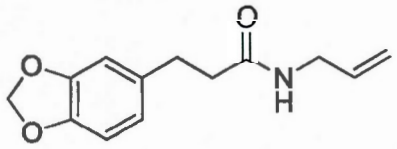
**3-(Benzo[d][1,3]dioxol-5-yl)-N-butylpropanamide (77)**



Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with phenetylamine (97 μL, 0.77 mmol, 1.5 eq) to afford 0.34 mmol (65 %) of compound **77** as white solid. $R_f$ = 0.29 (Hex/ EtOAc 55:45). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.23-7.01 (m, 5H, $H_{arom}$), 6.64-6.52 (m, 3H, $H_{arom}$), 5.82 (s, 2H, $OCH_2O$), 5.41 (s, 1H, N*H*), 3.39 (q, 2H, $^3J_{NHCH2}$ = 6.9 Hz, $NHCH_2$), 2.77 (t, 2H, $^3J_{CH2CH2}$ = 7.9 Hz, $CH_2CO$), 2.67 (t, 2H, $^3J_{CH2CH2}$ = 6.9

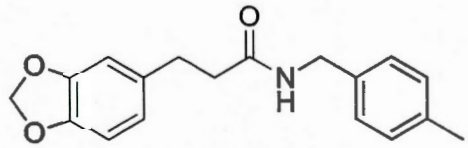
Hz, C$H_2$-benzene), 2.29 ppm (t, 2H, $^3J_{CH2CH2}$ = 7.9 Hz, benzene-C$H_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.7, 121.3, 108.9, 108.3 ($C_{arom}$), 139.0, 128.8, 128.7, 126.6 ($C_{arom}$), 100.9 (O*C*$H_2$O), 40.6 (NH*C*$H_2$), 38.8 (*C*$H_2$CO), 35.7 (*C*$H_2$-benzene), 31.5 ppm (*C*HCH). **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{18}NO_3$ $[M + H]^+$: 298.1438; found: 298.1434.

**N-allyl-3-(benzo[d][1,3]dioxol-5-yl)propanamide (79)**



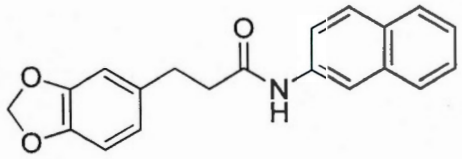
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with allyllamine (60 µL, 0.7725 mmol, 1.5 eq), to afford 0.39 mmol (75 %) of compound **79** as white crystals. $R_f$ = 0.33 (Hex/ EtOAc 1:1). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.61 (m, 3H, $H_{arom}$), 5.90 (s, 2H, OC$H_2$O), 5.72 (m, 1H, *C*HCH$_2$), 5.70 (s, 1H, N*H*), 5.07 (m, 2H, CHC$H_2$), 3.83 (m, 2H, NHC$H_2$), 2.87 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, C$H_2$CO), 2.43 (t, 2H, $^3J_{CH2CH2}$ = 7.8 Hz, benzene-C$H_2$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.7, 121.2, 108.9, 108.3 ($C_{arom}$), 134.3 (*C*HCH$_2$), 116.4 (CH*C*H$_2$), 100.9 (O*C*H$_2$O), 42.0 (NH*C*H$_2$), 38.8 (*C*H$_2$CO), 31.5 (*C*H$_2$CH$_2$). **ESI$^+$-HRMS :** *m/z* calculated for $C_{13}H_{16}NO_3$ $[M + H]^+$: 234.1125; found : 234.1123

**3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-methylbenzyl)propanamide (81)**



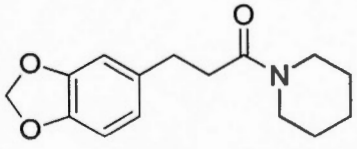
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with methylbenzylamine (94 mg, 0.77 mmol, 1.5 eq) to afford 0.36 mmol (70 %) of compound **81** as white crystals. $R_f$ = 0.32 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.09-7.03 (m, 4H, $H_{arom}$) 6.72-6.64 (m, 3H, $H_{arom}$), 5.90 (s, 2H, $OCH_2O$), 5,79 (s, 1H, N*H*), 4.33 (d, 2H, $NHCH_2$), 2.88 (t, 2H, $^3J_{CH2CH2}$ = 7.65 Hz, $CH_2CO$), 2.41 (t, 2H, $^3J_{CH2CH2}$ = 7.65 Hz, benzene-$CH_2$), 2.32 ppm (s, 3H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 171.9 (*C*ONH), 147.7, 146.0, 134.7, 121.3, 108.9, 108.3 ( $C_{arom}$), 137.2, 135.2, 129.4, 127.9, ($C_{arom}$), 100.9 ($OCH_2O$), 43.4 ($NHCH_2$), 38.5 ($CH_2CO$), 31.5 ($CH_2CH_2$), 21.2 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{20}NO_3$ $[M + H]^+$: 298.1438; found: 298.1443

**3-(Benzo[d][1,3]dioxol-5-yl)-N-(naphthalen-2-yl)propanamide (82)**



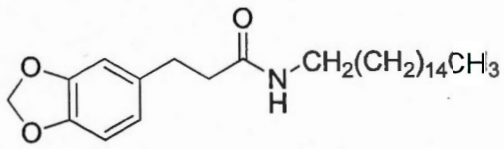
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with 2-naphtylamine (111 mg, 0.77 mmol, 1.5 eq) to afford 0.27 mmol (53 %) of compound **82** as white crystals. $R_f$ = 0.36 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 8.16 (s, 1H, N*H*), 7.75-7.37 (m, 7H, $H_{arom}$), 6.74-6.70 (m, 3H, $H_{arom}$), 5.92 (s, 2H, $OCH_2O$), 3,0 (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, $CH_2CO$), 2.66 ppm (t, 2H, $^3J_{CH2CH2}$ = 7.4 Hz, benzene-$CH_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 170.6 (*C*ONH), 147.9, 146.2, 134.5, 121.4, 109.0, 108.5 ($C_{arom}$), 135.7, 133.9, 130.8, 128.9, 127.8, 127.7, 126.6, 125.1, 120.0, 116.9 ($C_{napthalene}$), 101.0 ($OCH_2O$), 39.9 ($CH_2CH_2$), 31.4 ppm ($CH_2CO$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{20}H_{18}NO_3$ $[M + H]^+$: 320.1281; found: 320.1291.

**3-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)propan-1-one (83)**



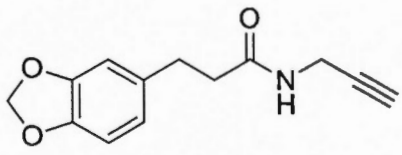
Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with piperidine (80 μL, 0.77 mmol, 1.5 eq) to afford 0.45 mmol (88 %) of compound **83** as white solid. $R_f$ = 0.32 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.63 (m, 3H, $H_{arom}$), 5.89 (s, 2H, $OCH_2O$), 3.54 (t, 2H, $^3J_{CH2CH2}$ = 5.57 Hz, $CH_2CO$), 3.32 (t, 2H, $^3J_{CH2CH2}$ = 5.57 Hz, benzene-$CH_2$), 2.86 (m, 4H, $CH_2NHCH_2$), 1.60 ppm (m, 6H, $CH_2CH_2CH_2$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 170.6 (*C*ONH), 147.9, 146.1, 135.6, 121.4, 109.2, 108.5 ($C_{arom}$), 101.1 ($OCH_2O$), 35.7 ($CH_2CO$), 31.6 ( $CH_2CH_2$), 46.9, 43.0, 26.7, 25.8, 24.8 ppm.($C_{piperidine}$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{15}H_{20}NO_3$ $[M + H]^+$: 162.1438; found: 262.1434.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-hexadecylpropanamide (84)**




Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with hexadecylamine (186 mg, 0.77 mmol, 1,5 eq) to afford 0.34 mmol (65 %) of compound **84** as white solid. $R_f$ = 0.22 (Hex/ EtOAc 7:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.62 (m, 3H, $H_{arom}$), 5.90 (s, 2H, $OCH_2O$), 5.4 (s, 1H, N*H*), 3.19 (m , 2H, $NHCH_2$), 2.87 (t, 2H, $^3J_{CH2CH2}$ = 7.6 Hz, $CH_2CO$), 2.39 (t, 2H, $^3J_{CH2CH2}$ = 7.6 Hz, benzene-$CH_2$), 1.24 (m, 28H, $(CH_2)_{14}$), 0.87 pm (m, 3H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 172.0 (*C*ONH), 147.8, 146.0, 134.9, 121.3, 108.9, 108.4 ($C_{arom}$), 100.9 ($OCH_2O$), 39.7 ($NHCH_2$), 39.0 ($CH_2CH_2CO$) 31.1 ($CH_2CO$), 29.8-29.5 (($CH_2)_{14}$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{26}H_{44}NO_3$ $[M + H]^+$: 418.3316; found: 418.3298

**3-(Benzo[d][1,3]dioxol-5-yl)-N-(prop-2-ynyl)propanamide (85)**

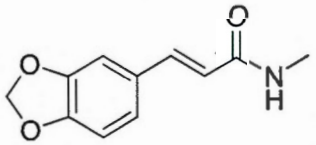


Compound **37** (100 mg, 0.52 mmol, 1 eq), was used as reagent for the coupling with propargylamine (50 µL, 0.77 mmol, 1.5 eq) to afford 0.26 mmol (52 %) of compound **85** as white crystals. $R_f$ = 0.33 (Hex/ EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.72-6.61 (m, 3H, $H_{arom}$), 5.90 (s, 2H, $OCH_2O$), 5.79 (s, 1H, N*H*), 4.01(m, 2H, $NCH_2$), 2.87 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, $CH_2CO$), 2.44 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, benzene-$CH_2$), 2.21 ppm (m, 1H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 171.8 (*C*ONH), 147.8, 146.1, 134.5, 121.3, 108.9, 108.4 ($C_{arom}$), 101.0 ($OCH_2O$), 79.3 (*C*CH), 71.7 (C*C*H), 38.5 ($CH_2CO$), 31.4 ($CH_2CH_2$), 29.2 ppm ($NHCH_2$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{13}H_{14}NO_3$ $[M + H]^+$: 232.0968; found: 232.0969.

The procedure below was followed for the synthesis of compounds **56**, **58**, **78** and **80**. Compounds **36** or **39** were used as starting materials.

Compound X (100 mg, 0.48 mmol, 1 eq) is dissolved in the respective amine (5 mL). The solution is stirred at reflux for 12 h. The mixture was cooled and the solvent is removed by rotary evaporation. The crude product was dissolved in EtOAc and washed subsequently with distilled water in the presence of hexane (1/4 of the volume of EtOAc), then washed with saturated potassium bisulfate and bicarbonate de sodium. The organic phase was dried over anhydrous sodium sulfate and the filtrate was evaporated to dryness in vacuo.

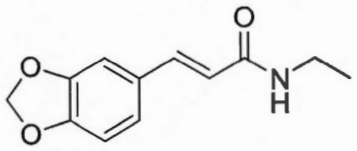
**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-methylacrylamide (56)**



Compound **36** (100 mg, 0.48 mmol, 1 eq) was used as reagent for the coupling with methylamine/$H_2O$ (30 %) to afford 0.4 mmol (83%) of compound **78** as white crystals. $R_f$ = 0.68 (Hex/ EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.60 (d, 1H, $^3J_{CHCH}$ = 16.7 Hz, benzene-C*H*), 7.03-6.80 ( (m, 3H, $H_{arom}$ ), 6.25 (d, 1H, $^3J_{CHCH}$ = 16.7 Hz, C*H*CO), 6.0 (s, 2H,

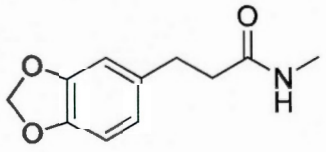
$OCH_2O$), 3.79 ppm (s, 3H, $CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 167.8 (*C*ONH), 149.8, 148.5, 129.0, 124.6, 108.7, 106.6 ($C_{arom}$), 144.7 (*C*HCH), 115.9 (*C*HCO), 101.7 ($OCH_2O$), 51.8 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{11}H_{12}NO_3$ $[M + H]^+$: 206.2002; found: 206.2104.

**(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-ethylacrylamide (58)**



Compound **36** (100 mg, 0.48 mmol, 1 eq), was used as reagent for the coupling with ethylamine/$H_2O$ (70 %) to afford 0.34 mmol (70 %) of compound **58** white crystals. $R_f$ = 0.19 (Hex/ EtOAc 65:35). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.53 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, benzene-CH), 6.98-6.76 (m, 3H, $H_{arom}$), 6.20 (d, 1H, $^3J_{CHCH}$ = 15.7 Hz, C*H*CO), 5.98 (s, 2H, $OCH_2O$), 5.69 (s, 1H, N*H*), 3.42 (m, 2H, $NHCH_2$), 1.21 ppm (t, 3H, $CH_2CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 166.1 (*C*ONH), 149.1, 148.3, 129.4, 123.9, 108.6, 106.4 ($C_{arom}$), 140.6 (*C*HCH), 119.0 (*C*HCO), 101.5 ($OCH_2O$), 34.7 ($NHCH_2$), 15.0 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{12}H_{14}NO_3$ [M + H] : 220.0968; found: 220.0969.

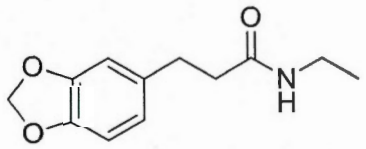
**3-(Benzo[d][1,3]dioxol-5-yl)-N-methylpropanamide (78)**



Compound **39** (100 mg, 0.48 mmol, 1 eq), was used as reagent for the coupling with methylamine/$H_2O$ (30 %) to afford 0.34 mmol (70 %) of compound **78** as white crystals. $R_f$

= 0.18 (Hex/ EtOAc 6:4. **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.70-6.59 (m, 3H, $H_{arom}$), 5.89 (s, 2H, $OCH_2O$), 5.71 (s, 1H, N*H*), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, $CH_2CO$), 2.75 (d, 3H, $CH_3$), 2.40 ppm (t, 2H, $^3J_{CH2CH2}$ = 7.5 Hz, benzene-$CH_2$); **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 172.0 (*C*ONH), 147.8, 146.0, 134.9, 121.3, 108.9, 108.4 ($C_{arom}$), 101.0 ($OCH_2O$), 38.7 ($CH_2CO$), 31.6 ($CH_2CH_2$), 26.4 ppm ($NHCH_3$). **ESI$^+$-HRM :** *m/z* calculated for $C_{11}H_{14}NO_3$ $[M + H]^+$: 208.0968; found: 208.0961.

**3-(Benzo[d][1,3]dioxol-5-yl)-N-ethylpropanamide (80)**



Compound **39** (100 mg, 0.48 mmol, 1 eq), was used as reagent for the coupling with ethylamine/$H_2O$ (70 %) to afford 0.42 mmol (89 %) of compound **80** as white crystals. $R_f$ = 0.22 (Hex/ EtOAc 1:1). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 6.71-6.60 (m, 3H, $H_{arom}$), 5.89 (s, 2H, $OCH_2O$), 5,58 (s, 1H, N*H*), 3.23 (m, 2H, $^3J_{CH2CH3}$ = 7.23 Hz, $CH_2CH_3$), 2.85 (t, 2H, $^3J_{CH2CH2}$ = 7.62 Hz, $CH_2CO$), 2.38 (t, 2H, $^3J_{CH2CH2}$ = 7.62 Hz, benzene-$CH_2$), 1.06 ppm (t, 3H, $^3J_{CH2CH3}$ = 7.23 Hz, $CH_2CH_3$). **$^{13}$C RMN (75 MHz $CDCl_3$):** δ = 172.0 (*C*ONH), 147.7, 146.0, 134.9, 121.2, 108.9, 108.3 ($C_{arom}$), 100.9 ($OCH_2O$), 38.9 ($CH_2CO$), 34.4 ($NHCH_2$), 31.6 ($CH_2CH_2$), 14.9 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{12}H_{15}NO_3$ $[M + H]^+$: 222.1125; found: 222.1126.

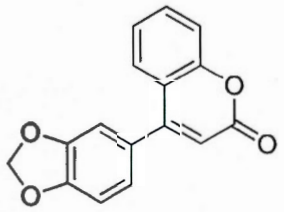
## 7.6. CHARACTERIZATION OF COMPOUNDS 85-95

Compounds **35** or **36** were used as starting materials.

**GENERAL PROCEDURE OF HECK REACTION.**

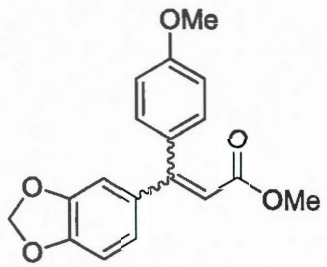
To a solution of either **35** or **36** (100 mg, 1 eq) dissolved in anhydrous DMF (3 mL) under nitrogen atmosphere, $Pd(AcO)_2$ (catalytic amount), 2-iodophenol (213.4 mg, 0.97 mmol, 2 eq), tetrabutylammonium bromide (TBABr) (161.2 mg, 0.485 mmol, 1 eq), and $NaHCO_3$ (122.2 mg, 1.455 mmol, 3 eq) were added respectively. The reaction mixture was stirred at room temperature for 15 min under nitrogen atmosphere and then heat at 105 °C overnight (monitored by TLC). After cooling to room temperature and concentrated under reduced pressure, the crude product was dissolved in EtOAc and washed subsequently with distilled water in the presence of hexane (1/4 of the volume of EtOAc), then. The organic layer was washed successively with saturated potassium bisulphate (10 mL), bicarbonate de sodium (10 mL) and brine (10 mL), dried over $Na_2SO_4$, filtered, and concentrated to dryness under reduce pressure.

**4-(Benzo[d][1,3]dioxol-5-yl)-2H-chromen-2-one (90)**



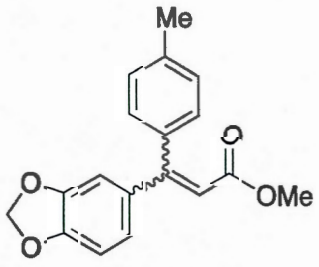
The crude material was purified by column chromatography on silicagel using the eluting mixture Hexane /$Et_2O$/ EtOAc (100:0:0 to 70:10:5), to afford 0.19 mmol (35 %) of compound **90** as white solid. $R_f$ = 0.15 (Hex/$Et_2O$/ EtOAc 7:1:2.5). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.57-7.21 (m, 7H, $H_{arom}$), 6.34 (s, 1H, CC*H*), 6.03 ppm (s, 2H, OC$H_2$O. **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 160.9 (*C*OO), 154.3, 149.1, 148.2, 132.0, 129.0, 127.1, 124.3, 122.8, 119.1, 117.5, 109.0, 108.9 ($C_{arom}$), 155.3 (*C*CHCO) 115.0 (C*C*HCO), 101.8 ppm (O$CH_2$O). **ESI$^+$-HRMS:** *m/z* calculated for $C_{16}H_{11}O_4$ $[M + H]^+$ : 267.0652; found: 267.0658.

**Methyl 3-(benzo[*d*][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)acrylate (91)**




The crude material was purified by column chromatography on silica gel using the eluting mixture Hexane/Tol/EtOAc (50:0:0 to 40:5:5), to afford 142 mg (95 %) of a mixture of isomers *cis/trans* (3:1) **91** as white solid. $R_f$ = 0.29 (Hex/Tol/ EtOAc 8:1:1). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.16 -6.69 (m, 7H, $H_{arom}$), 6.24 (s, 1H, CC*H*), 6.00 (s, 2H, O$CH_2$O), 3.86 (s, 3H, $OCH_3$), 3,63 (s, 3H, $OCH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 163.9 (*C*OO), 158.3, 148.2, 148.1, 133.6, 129.6, , 124.7, 121.9, 120.1, 116.5, 109.0, ($C_{arom}$), 153.1 (*C*CHCO) 111.3 (C*C*HCO), 101.4 ppm (O$CH_2$O), 55, 2 (O$CH_3$), 52,9 ppm (O$CH_3$) . **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{17}O_5$ $[M + H]^+$: 313.1071; found: 313.1083.

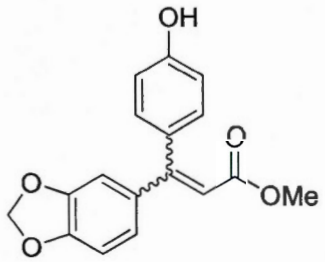
**Methyl 3-(benzo[*d*][1,3]dioxol-5-yl)-3-(*p*-tolyl)acrylate (92)**




The crude material was purified by column chromatography on silicagel using the eluting mixture Hexane/DCM (50:0 to 30:20), to afford 0.31 mmol (66 %) of a mixture of isomers

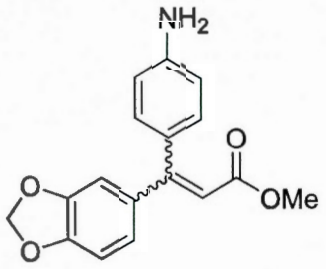
*cis/trans* of compound **92** as white solid. $R_f$ = 0.18 (Hex/DCM 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.31-6.75 (m, 7H, $H_{arom}$), 6.41 (s, 1H, CC*H*), 6.03 (s, 2H, O$CH_2$O), 3.91 (s, 3H, $OCH_3$), 2,34 (s, 3H, $CH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 165.1 (*C*OO), 148.0, 148.1, 139.2, 137.3, 129.8, 128,7, 127.9, 120.5 ($C_{arom}$), 162.1 (*C*CHCO) 110.7 (C*C*HCO), 101.2 (O$CH_2$O), 54.3 (O$CH_3$), 22,3 ppm ($CH_3$). . **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{17}O_4$ [M + H]$^+$: 297.3102; found: 297.3111.

**Methyl 3-(benzo[*d*][1,3]dioxol-5-yl)-3-(4-hydroxyphenyl)acrylate (93)**



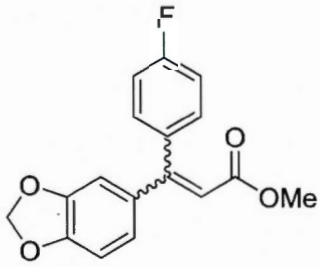

The crude material was purified by column chromatography on silicagel using the eluting mixture Hexane/EtOAc (50:0 to 1:1), to afford 0.36 mmol (75 %) of a mixture of isomers *cis/trans* (2:1) of compound **93** as white solid. $R_f$ = 0.22 (Hex/EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.22-6,68 (m, 7H, $H_{arom}$), 6.22 (s, 1H, CC*H*), 5.98 (s, 2H, O$CH_2$O), 5.17 (s, 1H, OH), 3.64 ppm (s, 3H, $OCH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.9 (*C*OO), 157.2, 147.8, 133.7, 132.7, 130.3, 123.3, 115.4, 114.7, 100.1 ($C_{arom}$), 147.8 (*C*CHCO) 108.1 (C*C*HCO), 101.3 (O$CH_2$O), 54,4 ppm (O$CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{18}H_{17}O_4$ [M + H]$^+$:299.2903; found: 299.2901.

**Methyl 3-(4-aminophenyl)-3-(benzo[*d*][1,3]dioxol-5-yl)acrylate (94)**




The crude material was purified by column chromatography on silicagel using the eluting mixture Hexane/EtOAc (50:0 to 35:15), to afford 0.36 mmol (75 %) of a mixture of isomers *cis/trans* (2:1) of compound **94** as white solid. $R_f$ = 0.22 (Hex/EtOAc 75:25). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.26-6.58 (m, 7H, $H_{arom}$), 6.21 (s, 1H, CC*H*), 5.99 (s, 2H, O$CH_2$O), 3.86 (s, 2H, NH), 3.63 ppm (s, 3H, $OCH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.5 (*C*OO), 148.7, 147.8, 133.5, 132.7, 128.9, 123.3, 121.3, 114.3, ($C_{arom}$), 152.8 (*C*CHCO) 108.3 (C*C*HCO), 101.3 (O$CH_2$O), 54,4 ppm (O$CH_3$). . **ESI$^+$-HRMS:** *m/z* calculated for $C_{17}H_{16}NO_4$ $[M + H]^+$: 298.1074; found: 298.1086.

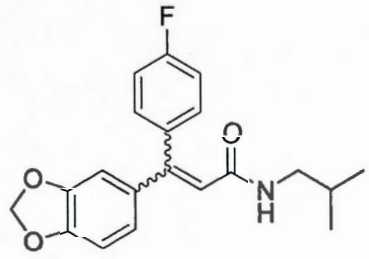
**Methyl 3-(benzo[*d*][1,3]dioxol-5-yl)-3-(4-fluorophenyl)acrylate (95)**




The crude material was purified by column chromatography on silicagel using the eluting mixture Hex/$Et_2O$/EtOAc (40:0:0 to 40:5:2), to afford 0.46 mmol (96 %) of a mixture

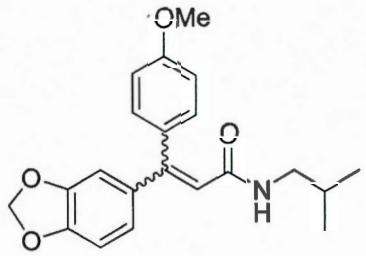
of isomers *cis/trans* of compound **94** as white solid. $R_f$ = 0.29 (Hex/$Et_2O$/EtOAc 8:1:0.5). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.26-6.75 (m, 7H, $H_{arom}$), 6.26 (s, 1H, CC*H*), 5.99 (s, 2H, $OCH_2O$), 3.61 ppm (s, 3H, $OCH_3$). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.5 (*C*OO), 162.1, 148.0, 148.1, 139.0, 131.5, 129.8 137.3, 129.8, 121.9, 115.8 ($C_{arom}$), 130.2 (*C*CHCO) 108.6 (C*C*HCO), 101.2 ($OCH_2O$), 52.3 ppm ($OCH_3$). . **ESI$^+$-HRMS:** *m/z* calculated for $C_{17}H_{14}FO_4$ $[M + H]^+$: 301.0871; found: 301.0878.

**3-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-fluorophenyl)-*N*-isobutylacrylamide (86)**



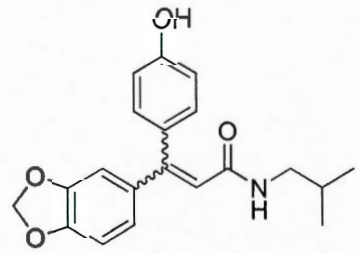

The crude material was purified by column chromatography on silicagel using the eluting mixture Hex/$Et_2O$/EtOAc (10:0:0 to 6:1:3), to afford 0.31 mmol (76 %) of a mixture of isomers *cis/trans* (3:1) of compound **86** as a white solid. $R_f$ = 0.25 (Hex/$Et_2O$/EtOAc 6:1:3). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.28-6.88 (m, 7H, $H_{arom}$), 6.54 (s, 1H, CC*H*), 6.08 (s, 2H, $OCH_2O$), 4.22 (s, 1H, N*H*), 3.02 (m, 2H, $CH_2$), 2.08 (m, 1H, C*H*), 0.87 ppm (d, 6H, $CH_3$ (2x)). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.7 (*C*OO), 162.1, 148.5, 148.1, 138.7, 131.3, 129.6, 121.9, 121.1, 115.8, ($C_{arom}$), 152.2 (*C*CHCO) 108.3 (C*C*HCO), 101.2 ($OCH_2O$), 48,1 ($CH_2$), 29.2 (*C*H) and 20.1 ppm ($CH_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{17}H_{15}O_5$ $[M + H]^+$: 299.3001; found: 299.2999.

**3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-isobutyl-3-(4-methoxyphenyl)acrylamide (87)**



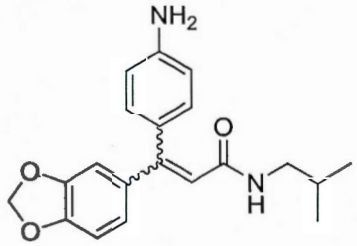

The crude material was purified by column chromatography on silicagel using the eluting mixture Hex/EtOAc (gradient: 5:1 to 1:1), to afford 0.24 mmol (60 %) of a mixture of isomers *cis/trans* (1:1) of compound **87** as a white solid. $R_f$ = 0.30 (Hex/EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.19-6.73 (m, 7H, $H_{arom}$), 6.25 (s, 1H, CC*H*), 5.99 (s, 2H, OC$H_2$O), 5.35 (s, 1H, N*H*), 2.97 (m, 2H, C$H_2$), 2.04 (m, 1H, C*H*), 0.85 ppm (d, 6H, C$H_3$ (2x)). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 164.2 (*C*OO), 160.1, 148.6, 148.0, 135.2, 131.3, 129.6, 121.1, 115.8, 108.5 ($C_{arom}$), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (O$CH_2$O), 55,6 (O$CH_3$), 47.9 (C$H_2$), 28.9 (*C*H) and 20.1 ppm (*C*$H_3$). **ESI$^+$-HRMS:** *m/z* calculated for $C_{21}H_{24}NO_4$ $[M + H]^+$: 354.17; found: 354.17.

**3-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-hydroxyphenyl)-*N*-isobutylacrylamide (88)**



The crude material was purified by column chromatography on silicagel using the eluting mixture Hex/Tol/EtOAc (5:1:1 to 5:1:4), to afford 0.28 mmol (70 %) of a mixture of isomers *cis/trans* of compound **88** as a white solid. $R_f$ = 0,17 (Hex/Tol/EtOAc 5:1:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.26-6.71 (m, 7H, $H_{arom}$), 6.23 (s, 1H, CC*H*), 5.97 (s, 2H, OC$H_2$O), 5.48 (s, 1H, N*H*), 2.98 (m, 2H, C$H_2$), 1.58 (m, 1H, C*H*), 0.78 ppm (d, 6H, C$H_3$ (2x)). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.7 (*C*OO), 157.0, 148.2, 134.1, 132.2, 131.3, 124.6, 121.1, 115.8, 109.8 ($C_{arom}$), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (O*C*H$_2$O), 55,6 (O*C*H$_3$), 47.9 (C$H_2$), 28.9 (*C*H) and 20.1 ppm (*C*H$_3$. **ESI$^+$-HRMS:** *m/z* calculated for $C_{20}H_{22}NO_4$ $[M + H]^+$: 340.1543; found: 340.1541.

**3-(4-Aminophenyl)-3-(benzo[*d*][1,3]dioxol-5-yl)-*N*-isobutylacrylamide (89)**




The crude material was purified by column chromatography on silicagel using the eluting mixture Hex/EtOAc (50:0 to 1:1), to afford 0.35 mmol (88 %) of a mixture of isomers *cis/trans* of compound **89** as a white solid. $R_f$ = 0.22 (Hex/EtOAc 6:4). **$^1$H RMN (300 MHz, $CDCl_3$):** δ = 7.08-6,59 (m, 7H, $H_{arom}$), 6.20 (s, 1H, CC*H*), 5.97 (s, 2H, OC$H_2$O), 5.36 (s, 1H, N*H*), 2.96 (m, 2H, C$H_2$), 1.52 (m, 1H, C*H*), 0.76 ppm (d, 6H, C$H_3$ (2x)). **$^{13}$C RMN (75 MHz, $CDCl_3$):** δ = 166.7 (*C*OO), 149.1, 148.2, 134.2, 132.2, 131.3, 124.6, 121.1, 115.8, 109.8 ($C_{arom}$), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (O*C*H$_2$O), 55,6 (O*C*H$_3$), 47.9 (C$H_2$), 28.9 (*C*H) and 20.1 ppm (*C*H$_3$). . **ESI$^+$-HRMS:** *m/z* calculated for $C_{20}H_{24}N_2O_3$ $[M + H]^+$:170.0888; found: 170.0887.

ANNEXES

Poster. Composés biologiquement actifs issus des plantes.

# COMPOSÉS BIOLOGIQUEMENT ACTIFS ISSUS DES PLANTES

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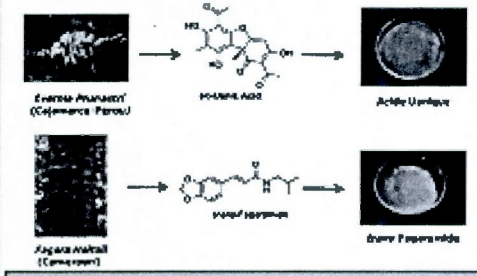
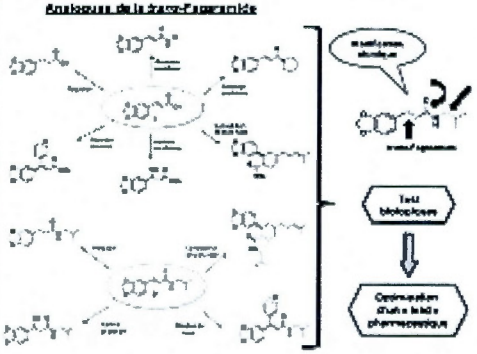
## Introduction

Les organismes végétaux et animaux offrent une large gamme de produits naturels, pouvant servir comme médicaments ou source d'inspiration dans le développement de nouveaux agents thérapeutiques. La synthèse d'analogues peut améliorer ces propriétés essentielles telles que la solubilité et la biodisponibilité dans le corps humain. Dans ce contexte, un grand nombre de composés naturels ont été identifiés, analysés et synthétisés vers l'optimisation d'un lead pharmaceutique.

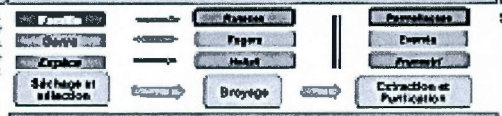
Ce projet vise à valoriser deux produits naturels connus pour leurs activités biologiques, l'"**Acide Usnique**", isolé à partir du lichen *Cladonia Pocillum* (Pérou) et la "***trans*-Fagaramide**", isolé de la plante *Fagara Heitzii* (Cameroun), afin d'en réaliser leur synthèse et faire des modifications chimiques pour la détermination de futurs médicaments contre les cellules cancéreuses.




## Objectifs




## Classification biologique, extraction et isolation




## Caractérisation de l'Acide Usnique

Propriétés Physiques:

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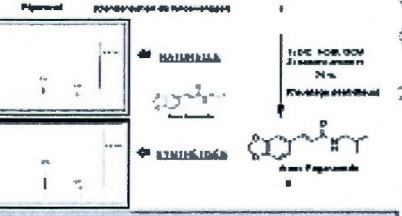
## Synthèse de la *trans*-Fagaramide

Schéma 1:




## Conclusions et Perspectives

La *trans*-Fagaramide est l'acide usnique ont été isolés de la plante "Fagara Heitzii" (Cameroun) et du lichen "Cladonia Pocillum" (Pérou) respectivement. Les deux molécules ont été caractérisées à l'aide de RMN 1D et 2D, ainsi que la spectrométrie de masse, confirmant les données spectroscopiques décrites dans la littérature. Une plateforme synthétique a été conçue afin d'établir une librairie chimique pour la détermination de médicaments candidats pour le traitement de cellules cancéreuses.

## Références




## Modification chimique de la *trans*-Fagaramide

Schéma 2: Estérification de Fischer

Schéma 3: Cycloaddition de Diels Alder

Schéma 4: Couplage peptidique

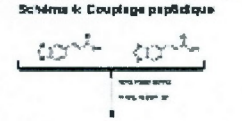
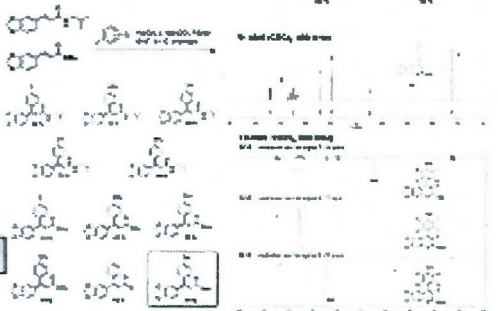


Schéma 5: Réaction de Heck:



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