Università della Calabria

PhD Course:

"Methodologies for the Development of Molecules of Pharmacological Interest"

(MDMP, *cycle XXII*)

Synthesis of Substituted Heterocycles by Metal Catalyzed Reactions

(CHIM/06)

Supervisors

Prof. Bartolo GABRIELE

Prof. Giuseppe SALERNO

PhD Student

Elvira LUPINACCI

PhD Coordinator

Prof. Bartolo GABRIELE

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Sommario

Il presente lavoro di tesi è stato suddiviso in due parti che trattano rispettivamente della sintesi di derivati eterociclici, mediante reazioni di eterociclizzazione catalizzate da metalli o da specie radicaliche, in solventi tradizionali o liquidi ionici, e delle proprietà antiinfiammatorie di composti presenti in estratti di luppolo.

In particolare, i principali contributi della tesi sono i seguenti:

1. sintesi di derivati chinolinici e indolici mediante reazioni di carbonilazione palladio-catalizzata di 1-(2-aminoaril)-2-in-1-oli;
2. sintesi di derivati chinolinici e benzofuranici mediante reazioni di eterociclizzazione rame-catalizzata di 1-(2-aminoaril)-2-in-1-oli e 1-(2-allilossifenil)-2-in-1-oli, rispettivamente, condotte in liquidi ionici;
3. sintesi di derivati benzotiofenici mediante eterociclizzazione palladio-catalizzata o promossa da specie radicaliche di 1-(2-mercaptoaril)-2-in-1-oli;
4. azione inibitoria dello xanthumolo e di altri composti presenti nel luppolo (*Humulus lupulus* L.) nei confronti del rilascio di mediatori dell'infiammazione quali TNF-α (tumor necrosis factor-α) e MCP-1 (monocyte chemoattractant protein-1).

(1) Sintesi di derivati chinolinici e indolici.

Negli ultimi anni, gli studi condotti per sviluppare sempre più efficienti strategie sintetiche di derivati eterociclici carbonilati a partire da substrati aciclici hanno rivelato come la metodologia che conivolge l'attacco nucleofilico intramolecolare al triplo legame coordinato al palladio [Pd(II)], seguito da alcossicarbonilazione risulti una delle strategie più importanti per la sintesi di composti eterociclici. In quest'ambito, i nostri sforzi si sono rivolti verso la

possibilità di sintetizzare derivati chinolinici e indolici, i quali presentano, come ben noto, svariate proprietà farmacologiche. In particolare, come discusso nel **Capitolo 2**, è stata sviluppata una semplice e selettiva strategia sintetica dei suddetti eterocicli funzionalizzati che prevede reazioni di carbonilazione PdI_2-catalizzate condotte in condizioni ossidative o non-ossidative, a seconda della natura del substrato e delle condizioni di reazione stesse.

(2) Sintesi di derivati chinolici e benzofuranici in liquidi ionici.

L'impiego di liquidi ionici come solventi alternativi per la sintesi di composti eterociclici ha suscitato crescente interesse tra i ricercatori, viste le loro caratteristiche di stabilità, compatibilità ambientale, e la possibilità di essere riciclati. Proprio quest'ultimo aspetto ha spinto il nostro gruppo di ricerca a studiare la reattività di substrati aciclici quali gli 1-(2-aminoaril)-2-in-1-oli e gli 1-(2-allilossifenil)-2-in-1-oli in liquidi ionici, al fine di sintetizzare derivati chinolici e benzofuranici, rispettivamente. Come verrà riportato nel **Capitolo 3**, si è dimostrato che la sintesi di chinoline sostituite può essere condotta in liquidi ionici usando 1-2 mol % di $CuCl_2$ e che il sistema solvente-catalizzatore può essere riutilizzato diverse volte senza perdita apprezzabile di attività catalitica. Nel **Capitolo 4**, inoltre, verrà discusso come anche il processo di catalisi omobimetallica sequenziale che porta alla sintesi di esteri benzofuran-2-acetici a partire da 1-(2-allilossifenil)-2-in-1-oli può essere condotta in liquido ionico, permettendo, anche in questo caso, il conveniente riciclo del sistema catalizzatore-solvente.

(3) Sintesi di derivati benzotiofenici.

I composti aventi nucleo benzotiofenico rivestono particolare importanza in natura in quanto presentano svariate attività biologiche e diverse proprietà nell'ambito delle scienze dei materiali. Dal momento che sono ancora poche in letteratura le strategie sintetiche che coivolgono reazioni di ciclizzazione metallo-

catalizzate per la sintesi di tali derivati, il nostro interesse si è rivolto verso la possibilità di applicare le conoscenze del nostro gruppo di ricerca in materia di eterociclizzazioni metallo-catalizzate alla sintesi di benzotiofeni sostituiti. Nel **Capitolo 5** verrà discussa la sintesi di derivati enil-benzotiofenici ottenuti per eterociclizzazione Pd-catalizzata di 1-(2-mercaptoaril)-2-in-1-oli. Verrà illustrato, inoltre, come a partire dagli stessi substrati e in presenza di un iniziatore radicalico, è possibile ottenere analoghi derivati alcossi-benzotiofenici.

(4) Attività antiinfiammatoria del luppolo.

Il luppolo (*Humulus lupulus* L.) è una pianta erbacea rampicante appartenente alla famiglia delle Cannabaceae. Le sue infiorescenze femminili sono impiegate per la produzione della birra in quanto contribuiscono alla stabilità della schiuma e le confereriscono il sapore e l'aroma caratteristici. Numerosi e dalle svariate proprietà sono i composti estratti da questa pianta; in generale, essi possono essere raggruppati in tre classi: la resina (contenente gli α- e i β-acidi), gli oli essenziali e i polifenoli. Nel **Capitolo 7** verrà dimostrato come alcuni composti presenti nel luppolo presentino interessanti attività antiinfiammatorie. In particolare, verrà riportato come lo xanthumolo possa inibire il rilascio dei mediatori TNF-α (tumor necrosis factor-α) e MCP-1 (monocyte chemoattractant protein-1), entrambi coinvolti nel determinare lo stato di infiammazione nei pazienti obesi.

Contents

Part A

Metal-catalyzed synthesis of heterocycles

CHAPTER 1

General introduction to heterocycles

1.1 General importance of heterocycles

Heterocycles represent an important class of molecules due to their important biological and pharmacological activities, as well as in the industry field. Many molecules, which are active in the cellular metabolism, in fact, present in their structure an heterocyclic moiety. Same example are here reported: precursors of coenzymes and vitamins (such as Thiamine, Riboflavin, Nicotinic Acid, Adenine and Vitamin B12 and E); photosynthetic pigments (Chlorophyll) and oxygen-delivering molecules (Hemoglobin); Purine and pyrimidine bases, amino-acids (Histidine, Tryptophan, Proline) and Hormones (Serotonin, Histamine). Moreover, among natural heterocyclic drugs the most important are purines (Caffeine), alkaloids (nicotine) and antibiotics (such as pennicilline). A large number of heterocyclic synthetic drugs that mimic natural products have been discovered and developed starting from the molecular structure of these biological products. These synthetic drugs belong to very different pharmacological classes: hypnotics (barbiturates), anticonvulsive, antihistamines, antithyroid, fungicides.

Other important practical applications of heterocycles can also be cited; for instance, their use as additives and modifiers in a wide variety of industries including cosmetics, reprography, information storage, plastics, solvents, antioxidants, and vulcanization accelerators. Finally, as an applied science, heterocyclic chemistry is an inexhaustible resource of novel compounds. A vast number of combinations of carbon, hydrogen, and heteroatoms can be designed, providing compounds with the most different physical, chemical, and biological properties. It is, therefore, easy to understand why both the development of new methods and the strategic utilization of known methods for the synthesis of complex heterocyclic compounds continue to drive the field of synthetic organic chemistry.

Organic chemists have focused many efforts in developing new strategies for the synthesis of heterocyclic compounds. Among these strategies, catalytic

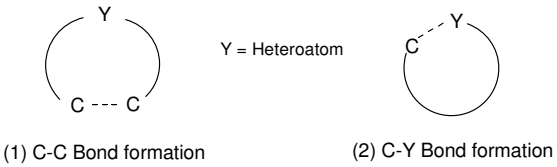
reactions have proved to be one of the most important and powerful approach to the direct synthesis of heterocyclic molecules using both metallic or organic catalysts.

Such protocols are hugely important as they may offer distinct advantages such as improved atom utilization or atom economy by avoidance of common derivation procedures; decreased byproduct formation and, hence, decreased waste resulting from purification procedures required to separate the desired product from impurities; in many cases, reduced energy utilization both in the reaction and purification stages. Further improvements can be provided by the utilization of alternatives more eco-friendly solvents.

1.2. Metal-catalyzed synthesis of heterocycles

Among a variety of new synthetic transformations, transition-metal-catalyzed reactions are some of the most attractive methodologies for the synthesis of heterocyclic compounds, since, throug this approach, complicated molecules can be directly constructed from readily accessible starting materials and under mild conditions. The catalytic construction of heterocyclic skeletons is classified into two major processes, as shown in **Scheme 1**:

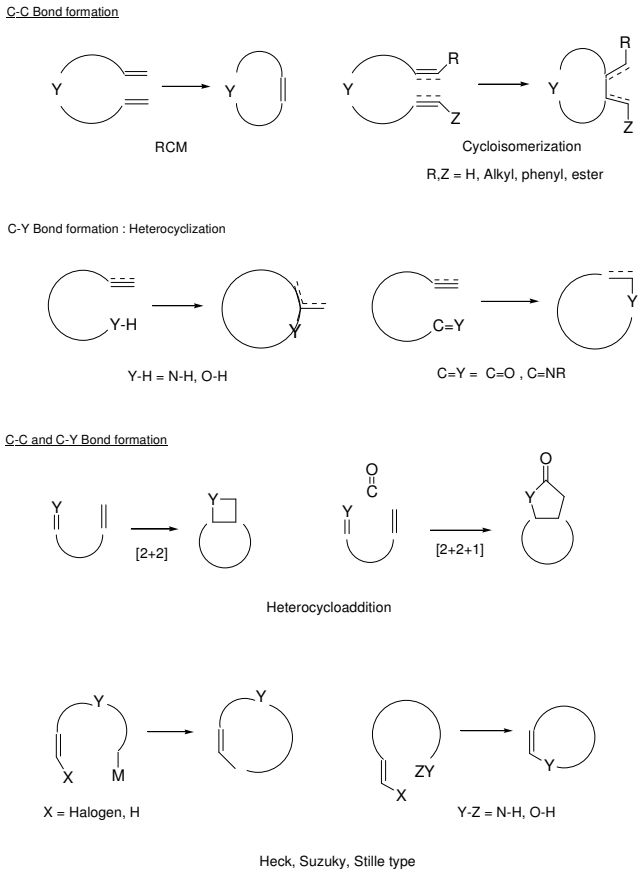
- *(1)* C-C bond formation from the corresponding acyclic precursors and
- *(2)* C-Y bond formation from the corresponding acyclic precursors.



Scheme 1

The synthesis of heterocycles via the olefin metathesis reaction (RCM, ring closing metathesis,) and via the cycloisomerization of dienes, diynes, and enynes belongs to category 1 (**Scheme 2**). The cyclization of alkenes, allenes, and alkynes bearing Y-Z at an appropriate position of the carbon chain is classified under category 2. The two processes, C-C and C-Y bond formation, take place together in the intra- and intermolecular hetero-cycloaddition of alkenes and alkynes bearing a hetero-unsaturated bond at an appropriate position of the carbon chain. Therefore, four-, five- or six-membered heterocycles can be synthesized, depending on the partner of the intra- and intermolecular reaction.

The intramolecular reaction of aryl and vinyl halides via Heck-, Suzuki-, and Stille-type reactions proceeds through the C-C bond formation, while the coupling with a heteroatom proceeds through the C-Y bond formation.



Scheme 2

As can be seen from **Scheme 2**, all the starting materials possess C-C and/or C-heteroatom unsaturated bond(s) in (a) certain position(s) of their structural framework and those functional groups become a reactive site for making a new C-C and/or C-heteroatom bond (C-Y bond). This is a logical outcome, since, the formation of a complex between a transition metal and C-C (or C-Y) unsaturated bond plays an important role in the transition-metal catalyzed reaction and often triggers a key reaction for producing heterocycles.

Compared to the traditional organic transformations leading to heterocycles, the transition-metal catalyzed transformation seems to be not straightforward and not easily understandable in many cases. This is presumably due to the fact that sequential processes are often involved in the catalytic transformation, which makes it difficult to understand the conversion from a starting substrate to a final product.

An important feature in the modern heterocycle synthesis with transition-metal catalysts is that asymmetric catalytic synthesis is becoming very popular and attracting interest of a wide range of organic chemists.

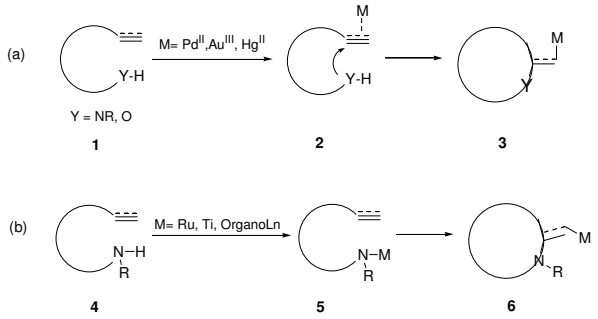
Among all the important methodologies used for the synthesis of heterocyclic compounds I will emphasize the ones that constituted my background and led me to obtain the results show later in this thesis.

1.3 Intramolecular Reaction of Alkenes and Alkynes Bearing N-H, O-H, C=O, and C=N Groups: Heterocyclization

Transition-metal-catalyzed intramolecular reactions of carbon-carbon unsaturated compounds with N-H, O-H, C=O, and C=N groups have been extensively studied and have become a powerful tool for the synthesis of heterocycles.

Alkenes and alkynes have been utilized as a carbon-carbon unsaturated compound, and a wide variety of transition-metal complexes, such as palladium, platinum, gold, copper, titanium, tungsten, and organolanthanides, have been used as a catalyst. In these reactions the heterocyclic compounds are produced via carbon-heteroatom (C-Y) bond formation (see **Scheme 2**).

The transition-metal catalyzed intramolecular addition reaction of Y-H to the C-C unsaturated bonds is classified into two major groups, as illustrated in **Scheme 3**.



Scheme 3

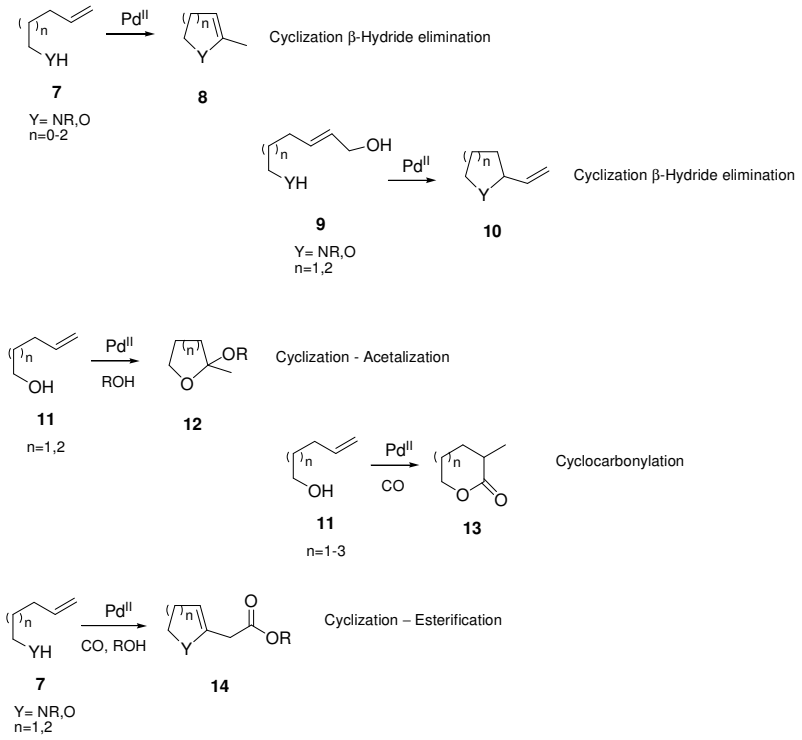
In the presence of a higher valent transition-metal catalyst, such as Pd^{II}, Au^{III}, and Hg^{II}, the reaction of **1** having a Y-H group is initiated by the formation of the π-olefin complex **2** through the coordination of the carbon-carbon unsaturated bond to the transition metal. Subsequent intramolecular nucleophilic attack of the heteroatom to the electron-deficient unsaturated bond produces the new heterocyclic organometallics **3**.

On the other hand, the ruthenium, titanium, and organolanthanide-catalyzed reaction of the amine derivatives **4** starts from the formation of the metal-amido complex **5**, and the following intramolecular aminometalation of the C-C unsaturated bond produces the new heterocyclic organometallics **6**. The organometallic compounds **3** and **6** undergo either β-elimination or the reaction with electrophiles to give the corresponding heterocyclic products.

1.3.1 Alkenes

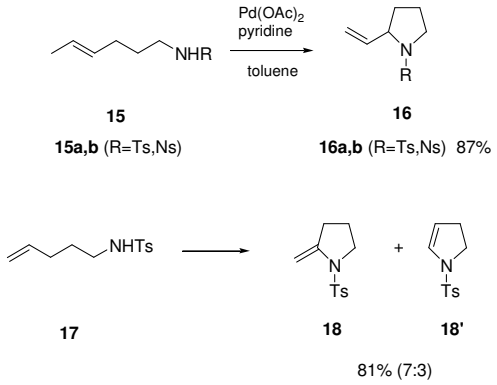
Higher valent palladium(II)-catalyzed reaction of alkenylamines and alkenyl alcohols have been widely investigated, and these reactions are categorized into the five different reaction patterns, as shown in **Scheme 4**. The cyclization of the alkenylamines or alcohols **7** followed by β-hydride elimination gives the cyclic enamines or enols **8**.*(1a-b,2)* The reaction of the substrates **9** having an allyl

alcohol moiety proceeds through the cyclization, and the subsequent β-hydroxy elimination gives the heterocycles **10** bearing a vinyl group .*(1b,6)* The reaction of the alkenols **11** with an external alcohol produces the cyclic acetals **12**.*(1b,7)* The cyclocarbonylation of **11** with carbon monoxide gives the lactones **13**.*(1c,5)* The reaction of the alkenylamines or alkenyl alcohols **7** with carbon monoxide and an alcohol proceeds through the cyclization–esterification to give **14**.*(1d,6)* In these reactions the carbon–carbon double bond of substrates coordinates to the Lewis acidic palladium(II) complex and intramolecular nucleophilic attack of a heteroatom takes place as shown in **Scheme 2**.



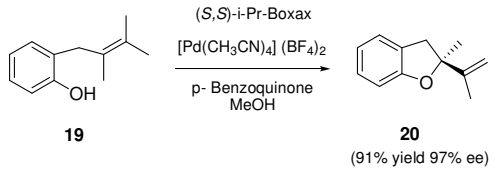
Scheme 4

Fix et al. reported the palladium-catalyzed oxidative cyclization of aminoalkenes.*(2b)* The reaction of the aminoalkenes **15** having a methyl group on the olefin moiety gave the 2-vinylpyrrolidines **16**, while the reaction of the aminoalkene **17** having a terminal olefin gave a mixture of the cyclic enamines **18** and **18'** (**Scheme 5**).



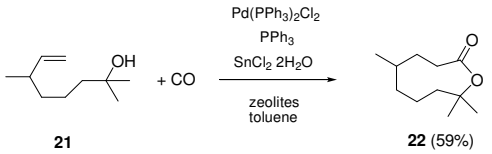
Scheme 5

Uozumi et al. reported the palladium-catalyzed asymmetric heterocyclization. The reaction of the *o*-allylphenol **19** in the presence of $[Pd(CH_3CN)_4](BF_4)_2$ and (*S*,*S*)-*i*-Pr-boxax gave the dihydrobenzofuran **20** in 91% yield with 97% ee (**Scheme 6**).*(2c)*



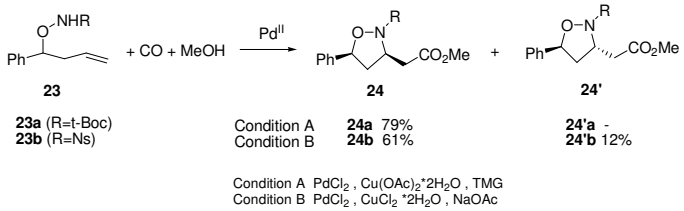
Scheme 6

Lenoble et al. reported the cyclocarbonylation of the alkenyl alcohol **21** with carbon monoxide in the presence of palladium, phosphine, and tin catalysts gave the nine-membered lactone **22** selectively (**Scheme 7**).*(5c)*



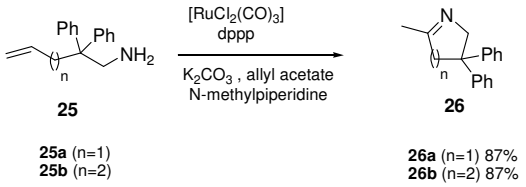
Scheme 7

Bates and Sa-Ei demonstrated that the reaction of *O*-homoallylhydroxyamines **23** with carbon monoxide and methanol in the presence of a palladium catalyst gave the isooxazolidine in good yields.*(6)* The reaction of the carbamate **23a** gave only the cis-isomer **24a** diastereoselectively, while the reaction of the sulfonamide **23b** gave a 5:1 mixture of the cis–trans diastereomers (**Scheme 8**).



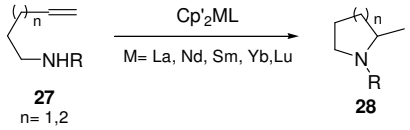
Scheme 8

Mitsudo et al. reported that the ruthenium-catalyzed intramolecular oxidative amination of the aminoalkenes **25** gave the cyclic imines **26** in high yields (Scheme 9).*(7)*



Scheme 9

The organolanthanide-catalyzed intramolecular hydroamination of the aminoalkenes **27** is one of the most useful processes for constructing the nitrogen heterocycles **28**, whose skeletons are often found in naturally occurring alkaloids (**Scheme 10**).*(8-9)*

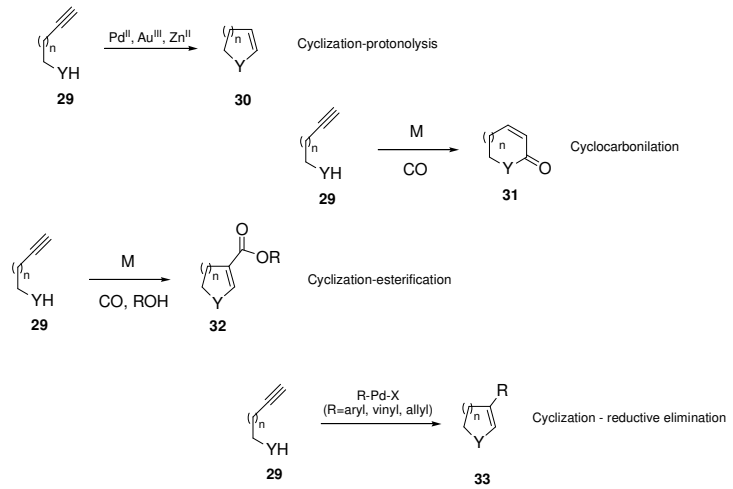


Scheme 10

1.3.2 Alkynes

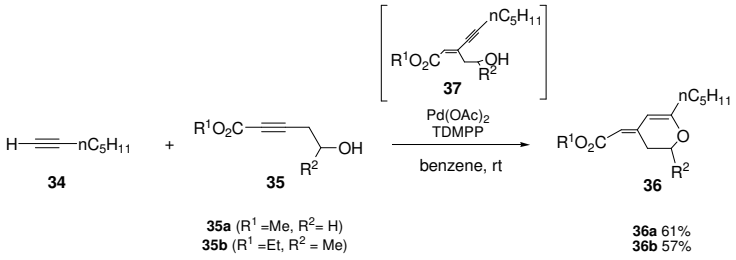
The Lewis acidic metal complexes, such as Pd^{II}, Au^{III}, Zn^{II}, and $W(CO)_6$, promote the intramolecular reaction of an alkyne with an amine, amide, alcohol, and carboxylic acid. These reactions are classified into four types, as shown in **Scheme 11**. The cyclization of **29** and subsequent protonolysis gives the heterocycles **30** having a carbon-carbon double bond.*(1,8,10)* The cyclocarbonylation of **29** occurs under carbon monoxide atmosphere to give the lactones and lactams **31**.*(1,11)* The reaction of the alkynylamines or alkynyl alcohols **29** with carbon monoxide and an alcohol gives the heterocycles **32** having an α-β-unsaturated ester moiety.*(1,12)* In the presence of organopalladium species R-Pd-X, the reaction of **29** proceeds through the cyclization promoted by the Lewis

acidic R-Pd-X, and subsequent reductive elimination of Pd(0) from the resulting cycloalkenylpalladium(II)X complex gives **33**.*(13)*



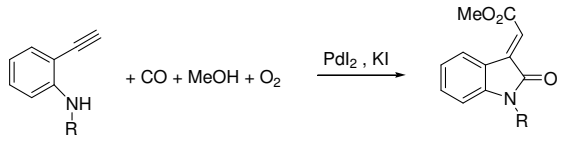
Scheme 11

Trost and Frontier reported that the tandem palladium-catalyzed reaction of 1-heptyne **34** with the alkynols **35** produced the dihydropyrans **36** in good yields (**Scheme 12**).*(10h)* The reaction proceeds through the palladium-catalyzed coupling of 1-heptyne and the alkynols **35**, followed by subsequent palladiumcatalyzed 6-endo-dig cyclization of the resulting enynols **37**.



Scheme 12

Gabriele et al. reported that the palladium-catalyzed reaction of the *o*-ethynylanilines **38** with carbon monoxide, methanol, and oxygen gave the 1,3-dihydroinol-2-one derivatives **39** in good yields (**Scheme 13**).*(11b)* The reaction proceeds through a cyclocarbonylation-esterification (see **Scheme 11**). On the other hand, the reaction of the (*Z*)- (2-en-4-ynyl)amines **40** with carbon monoxide, methanol, and oxygen under CO_2 atmosphere gave the pyrroles **41**, derived from cyclization-esterification, in good yields (**Scheme 14**).*(12c)*



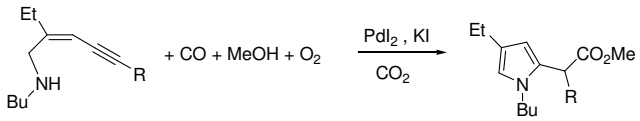
38

38a (R=H)
38b (R=Bn)
38c (R=Bu)

39

39a 53%
39b 50%
39c 45%

Scheme 13



40

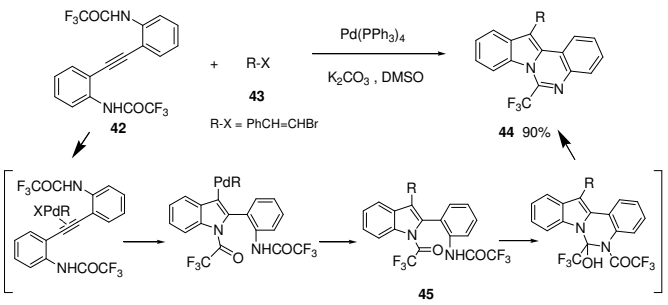
40a (R=Bu)
40b (R=TMS)

41

41a 70%
41b 72% (R=H)

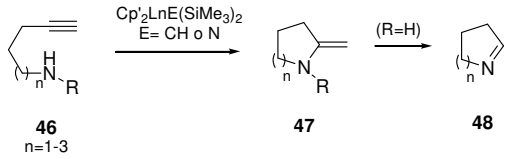
Scheme 14

Arcadi et al. reported that the palladium-catalyzed cyclization of bis(*o*-trifluoroacetamidophenyl)acetylene **42** with aryl and vinyl halides **43** gave the indole[1,2-*c*]quinazolines **44** in high yields (**Scheme 15**).*(13d)* The reaction proceeds through aminopalladation- reductive elimination. The cyclization of the resulting 3-arylinodole derivatives **45** gives the tetracyclic heterocycle **44**.



Scheme 15

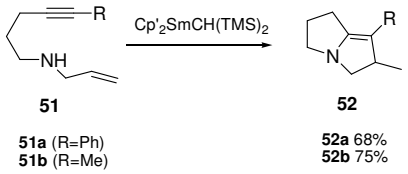
The organolanthanide-catalyzed hydroamination of the aminoalkynes **46** gives the nitrogen-containing heterocycles **47** or **48** (in the case of R = H).*(14)* The reaction of primary amines produces the cyclic imines **48**, while the reaction of secondary amines gives the cyclic enamines **47** (**Scheme 16**). The organolanthanide- catalyzed bicyclization of the aminodiynes, aminoenynes, and aminodienes **49** produces the pyrrolizidine and indolizidine derivatives **50** in a single reaction (**Scheme 17**).*(15)*



Scheme 16

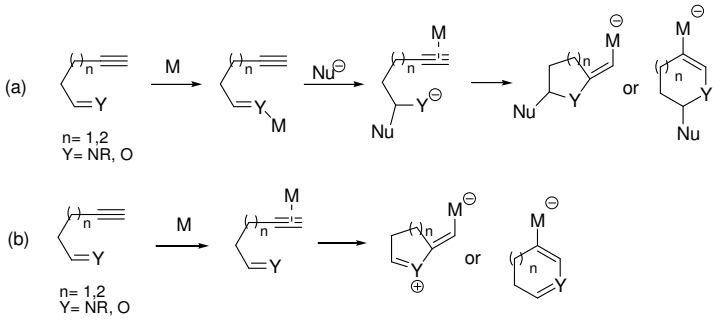
Scheme 17

Li and Marks reported that the reaction of the aminoenynes **51** in the presence of an organolanthanide catalyst gave the pyrrolizines **52** in good yields (**Scheme 18**).*(15)*



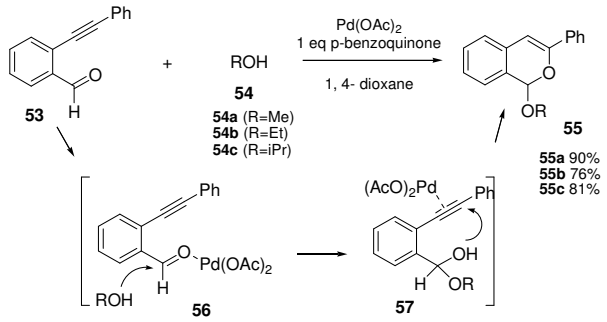
Scheme 18

Heterocyclization of alkynyl aldehydes and imines proceeds through two different mechanistic pathways (**Scheme 19**, routes a and b). A Lewis acidic transition metal is coordinated by a heteroatom of C=Y, the nucleophilic addition of Nu- to the electron-deficient carbon of C=YM takes place first, and then the resulting Y^- attacks an electron-deficient carbon of the alkyne coordinated to M (type a). It should be noted that the M acts simultaneously as a Lewis acid and as a typical transition-metal catalyst, that is to say, as a dual-role catalyst. Alternatively, the triple bond coordinates first to a transition-metal catalyst M, and then the nucleophilic attack of a heteroatom of C=Y takes place (type b).



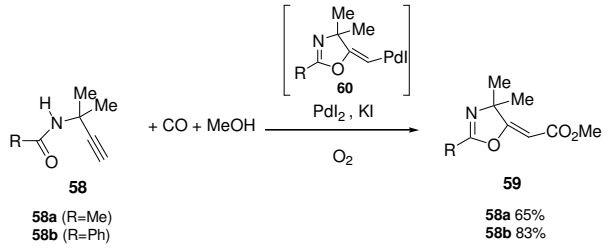
Scheme 19

Yamamoto reported that the palladium-catalyzed reaction of *o*-alkynylbenzaldehyde **53** with the alcohols **54** gave the alkenyl ethers **55** in good to high yields (**Scheme 20**).*(16)* This reaction proceeds through formation of the hemiacetal **57** and subsequent nucleophilic attack of the OH group on the electron-deficient alkyne coordinated by palladium(II). In this reaction, $Pd(OAc)_2$ acted simultaneously as a Lewis acid and as a transition-metal catalyst; the carbonyl group is activated by a Lewis acidic Pd(II) (**56**) to make facile addition of ROH, and the alkynyl moiety is activated by Pd(II), having a typical transition metal characteristic, as shown in **57**, to produce the cyclized alkenyl palladium(II) intermediate that undergoes protonation.



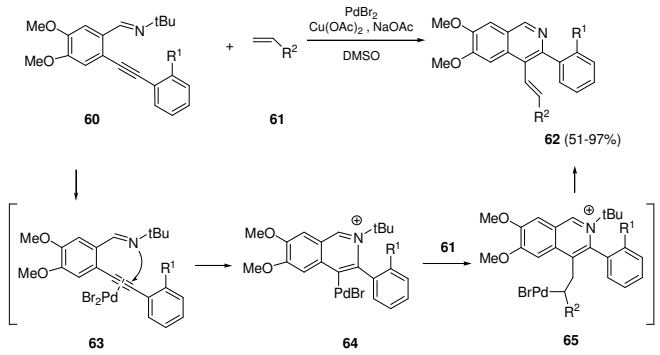
Scheme 20

Bacchi et al. reported the efficient and general synthesis of the 5-(alkoxylcarbonyl)methylene-3-oxazolines **59** by the palladium-catalyzed oxidative carbonylation of the pro-2-ynylamides **58** (**Scheme 21**).*(17)* This reaction is initiated by nucleophilic attack of an oxygen atom of an amide group on an alkyne coordinated by palladium(II), forming the vinylpalladium intermediate **60**. The insertion of CO into the C-Pd bond of **60**, followed by methanolysis of the resulting acylpalladium complex, affords the esters **59** and Pd(0) catalyst. The Pd(0) is oxidized to Pd(II) by molecular oxygen, and thus the catalytic cycle operates well.



Scheme 21

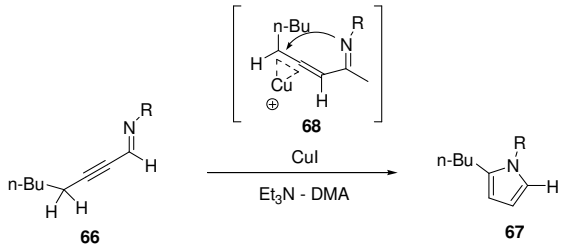
Huang and Larock reported that palladium-catalyzed cyclization/olefination reaction of the *o*-alkynylbenzaldimines **60** with the olefins **61** produced the isoquinolines **62** in good to high yields (**Scheme 22**).*(18)* The reaction proceeds through the nucleophilic attack of the nitrogen atom on electron-deficient alkyne **63**, the formation of the alkenylpalladium intermediate **64**, the insertion of the alkenes **61** into the C-Pd bond **65**, and β-hydride elimination. They reported many examples of this type of reactions.*(19)*



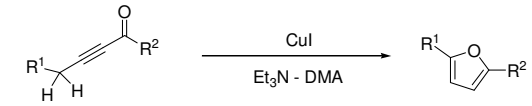
Scheme 22

Kel'in et al. reported that the copper-assisted cycloisomerization of the alkynyl imines **66** gave the pyrroles **67** in high yields (**Scheme 23**).*(20)* Mechanistic studies revealed that this reaction proceeded via the propargyl-allenyl

isomerization of **66** to an allenyl imines and through the nucleophilic attack of the nitrogen atom of imine on the electrondeficient carbon **68**. The cycloisomerization of alkynyl ketones **69** gave 2,5-disubstituted furans **70** (**Scheme 24**).*(21)*



Scheme 23

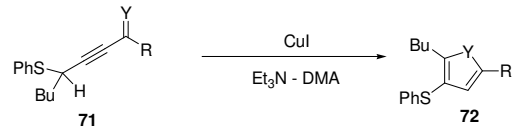


R^1 = H, alkyl, alkoxy
R^2 = alkyl, alkenyl, phenyl

62-94 %

Scheme 24

The 3-thiofurans and pyrroles **72** were synthesized similarly by the copper-catalyzed cycloisomerization of the keto- and iminopropargyl sulfides **71** (**Scheme 25**).*(22)* The reaction proceeds through the copper-catalyzed isomerization of alkyne to the corresponding allenes, followed by the thermal or Cu-mediated 1,2-migration of the thio group.



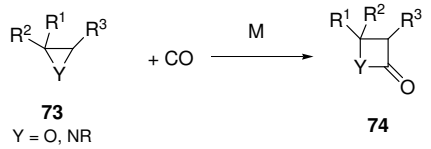
71a (Y= O,R =Ph)
71b (Y= N-Tr, R=H)

72a 91%
72b 85%

Scheme 25

1.4 Synthesis of heterocycles via carbonylation methodologies

The transition-metal catalyzed carbonylation reaction has been extensively investigated, and especially the carbonylative ring expansion reaction of strained heterocycles has been shown to be a useful and efficient procedure to synthesize lactams, lactones, and thiolactones.*(23)* The carbonylation of epoxides and aziridines **73** is a powerful tool to construct the *β*-lactone and *β*-lactam skeletons **74** (**Scheme 26**).*(24)* This type of reactions can be regarded as a hetero-[3 + 1]-cycloaddition.

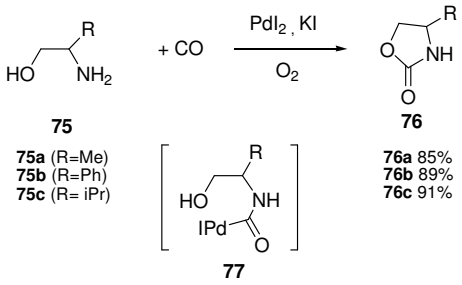


73
Y = O, NR

74

Scheme 26

Gabriele et al. reported that the 2-oxazolidinones **76** can be synthesized by the palladium-catalyzed oxidative carbonylation of the 2-amino-1-alkanols **75** (**Scheme 27**).*(25)* The aminocarbonyl palladium complex **77** is formed as an intermediate, and subsequent ring closure gives **76**.



75

75a (R=Me)
75b (R=Ph)
75c (R= iPr)

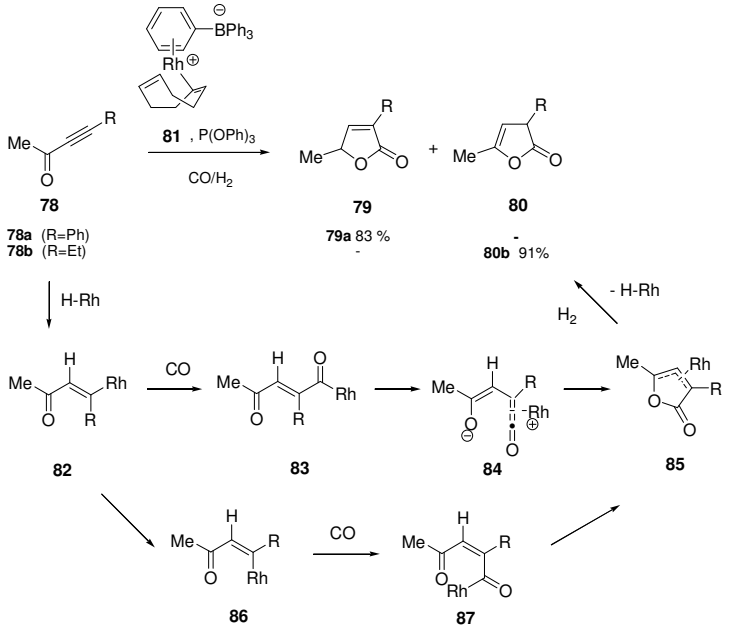
77

76

76a 85%
76b 89%
76c 91%

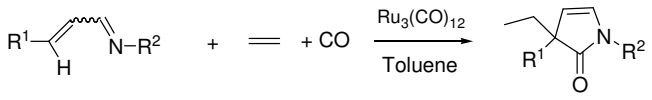
Scheme 27

Van den Hoven et al. reported that the zwitterionic rhodium complex **81**-catalyzed chemo- and regioselective cyclohydrocarbonylation of the α-keto alkynes **78** afforded either the furanone **79** or **80**, depending on the substituent R (**Scheme 28**).*(25)* The reaction of **78a** with R = Ph gave the 2(*3H*)-furanone **79a** in 83% yield, while the reaction of **78b** with R = alkyl afforded 2(*5H*)-furanone **80b** in high yield. The reaction proceeds via hydrorhodation of the triple bond of ynones **78**. The insertion of CO into the C-Rh bond of **82**, rearrangement from **83** to the zwitterionic ketene **84**, and subsequent cyclization of **84** give **85**. Alternatively, the *E-Z* isomerisation of **82** to **86**, CO insertion to the sp2 C-Ru bond of the alkenylruthenium **87**, and intramolecular acylrhodation of the carbonyl moiety of **87** give the same intermediate **85**. The reduction of the ruthenium complex **85** with H_2 gives **79** or **80**.



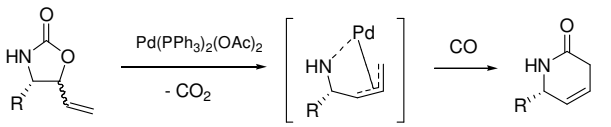
Scheme 28

Imhof et al. and Chatani et al. independently reported that the ruthenium-catalyzed reaction of the α,β-unsaturated imines **88** with alkenes and carbon monoxide gave the β,γ-unsaturated γ-butyrolactams **89** (**Scheme 29**).*(26)*



Scheme 29

Knight et al. reported that the palladium-catalyzed decarboxylative carbonylation of amino acid-derived 5-vinyloxzolidin-2-ones **90** gave the corresponding δ-lactams, 3,6-dihydro-1*H*-pyridin-2-ones **91**, in good to high yields (**Scheme 30**).*(27)* This reaction proceeds through release of carbon dioxide, forming the π-allylpalladium intermediate **92**, followed by insertion of CO.



90

90a (R=iPr)
90b (R=Bn)
90c (R=Ph)

91

92

92a 87%
92b 78%
92c 58%

Scheme 30

1.5 Trends in heterocycles synthesis

The attention for environmental and energetic problems, such as greenhouse effect and other pollution-related issues, and the need of new energy and raw materials sources, have been constantly growing during the last years. It is now widely acknowledged that the solutions for these problems can be provided with innovation in technology, which can lead to a more sustainable progress

without affecting the economical and human development and the level of quality of life.

It is more and more diffuse conviction that scientific research can play a leading role in this field. Chemistry in particular is among the most involved disciplines, and a great effort is being made by many research groups in the world towards a "greener" chemistry. These works try to provide low-waste, low-energy consumption, low-hazard methods as candidates for replacing the traditional industrial processes.

The development of efficient catalytic systems and the use of alternative reaction media are powerful tools to reach the target of a sustainable chemistry in terms of both economy and ecology.

1.5.1 Ionic Liquids

From the above considerations, it comes out clear that a major role on the road towards a more sustainable chemistry can be played by a combination of highly efficient catalysts and green solvents. Amoung the latter, in recent years ionic liquids (ILs) have received a great attention. In fact, the interest of researchers in this field has exponentially grown in the last decade, and they are now recognized as one of the most attractive alternatives to conventional organic solvents. However, although academic research has produced a great number of works in which ILs are used as solvents, cosolvents and/or catalysts, they have not found an application in industry yet, mainly because of their usually very high cost, and secondarily for their, still to be determined, adverse effects and biodegradability.

ILs are commonly defined as salts with a melting point lower than 100°C. ILs that are liquid at ambient temperature are the most widely used because of their easiness of handling. Over their melting points, ILs can be viewed as liquids composed entirely of ions. They are usually non-volatile, easy to recover, and have

a broad (300°C) liquid range. The reason for the relatively low melting point of this salts is the low energy of their crystalline network.

They are usually composed of a bulky and non-symmetric organic cation (generally ammonium or phosphonium) with low charge density and low tendency to intermolecular interactions and an inorganic anion. However, carboxylate-based ionic liquids have been also synthesized in latest years. The most common cations are 1,3-dialkylimidazolium salts, but 1,4-dialkylpyridinium and 1,1-dialkylpyrrolidinium salts are commonly used as well. The most common anions include BF_4^-, PF_6^-, $Al_2Cl_7^-$, RSO_3^-. The chemical, physical and solvent properties of the ILs depend on both the cation and the anion. It is thus possible to design new ionic liquids with the desired properties by opportunely choosing the cation and the anion. Some of the most common anions and cations are reported in **Figure 1**.

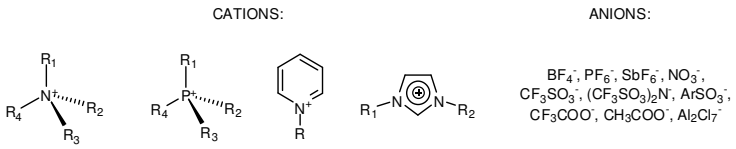
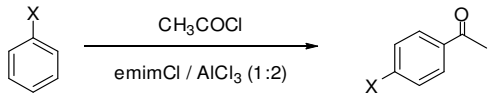


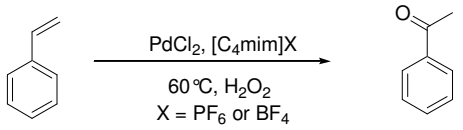
Figure 1

One of the most interesting features of ILs is the opportunity of using them as both solvent and catalyst. Some examples of this kind have been reported by Seddon and coworkers, in the ionic liquid-catalyzed Friedel-Crafts acylation of substituted aromatics *(28)* (**Scheme 31**).



Scheme 31

In other cases the ionic liquid acts as a cocatalyst, for instance in the Pd-catalyzed Wacker oxidation of styrene to acetophenone (**Scheme 32**)*(29)*. It has been reported that in this case the imidazolium cation activates the H_2O_2 which, in turn, reoxidizes palladium(0) to palladium(II) to complete the reaction cycle.



Scheme 32

Obviously there are also several examples in which ILs act simply as a solvent without interacting in the reaction mechanism *(30)*. It is worth noticing that ILs are also a good reaction medium for biocatalyzed transformations, in which they have several advantages over conventional organic solvents, including higher stabilities and enantioselectivity of the reaction system *(31-33)*.

1.6 Aim of the thesis

The aim of this part of the thesis was to develop novel and efficient strategies for the synthesis of heterocyclic compound via metal-catalyzed reactions. Substituted quinolines and indols were found to be easily synthetized by palladium-catalyzed carbonylation of 1-(2-aminoaryl)-2-in-1-ols under oxidative or nonoxidative conditions, depending on the nature of the substrate and on reaction conditions.

A novel synthetic approach to enyl- or alkoxy-benzothiophene derivatives has been developed involving Pd-catalyzed or radical promoted eterocyclization of the corresponding 1-(2-mercaptoaryl)-2-yn-1-ols.

The use of ionic liquids as efficient and convenient reaction media has been studied, showing how important heterocyclic compounds such as quinolines and benzofurans can be easily synthetized. Ionic liquids allow, in fact, both an easy

removal of the product from the reaction mixture and the possibility to recycle the solvent-catalyst system several times without appreciable loss of catalytic activity.

All the synthetic approachs developed show that important heterocyclic class of compounds can be obtained starting from simple substrate easily prepared from commercially available products through a few simple synthetic steps.

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CHAPTER 2

Versatile synthesis of quinoline-3-carboxylic esters and indol-2-acetic esters by palladium-catalyzed carbonylation of 1-(2-aminoaryl)-2-in-1-ols

B. Gabriele, R. Mancuso, G. Salerno, E. Lupinacci, G. Ruffolo, M. Costa

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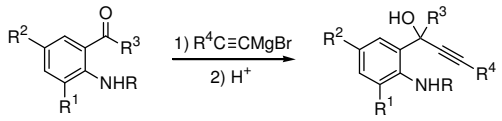
2.1 Introduction

Substituted indoles and quinolines are known to display a wide range of pharmacological activities *(1-3)*.

During the last years, the intramolecular nucleophilic attack to a triple bond coordinated to Pd(II) followed by alkoxycarbonylation has proved to be one of the most important and powerful methodologies for the direct synthesis of carbonylated heterocycles starting from acyclic precursors *(4)*. In this area, our research group have shown that PdI_2 in conjunction with an excess of KI is a very useful and versatile catalyst for achieving several convenient syntheses of carbonylated heterocycles starting from suitably functionalized alkynes, under oxidative as well as nonoxidative conditions *(4-5)*. In this part of the thesis, I have investigated the reactivity of 1-(2-aminoaryl)-2-yn-1-ols under carbonylative conditions in the presence of the PdI_2-KI catalytic system to develop new and selective synthetic approaches to carbonylated nitrogen heterocycles.

2.2 Results and Discussion

1-(2-Aminoaryl)ketones **1**-**5** were reacted with an excess of alkynylmagnesium bromides to give the corresponding (2-aminoaryl)-2-yn-1-ols, according to **Scheme 1**. The crude products **6**-**19** thus obtained could be used as substrates for the subsequent carbonylation reactions without further purification (see the Experimental Section for details).

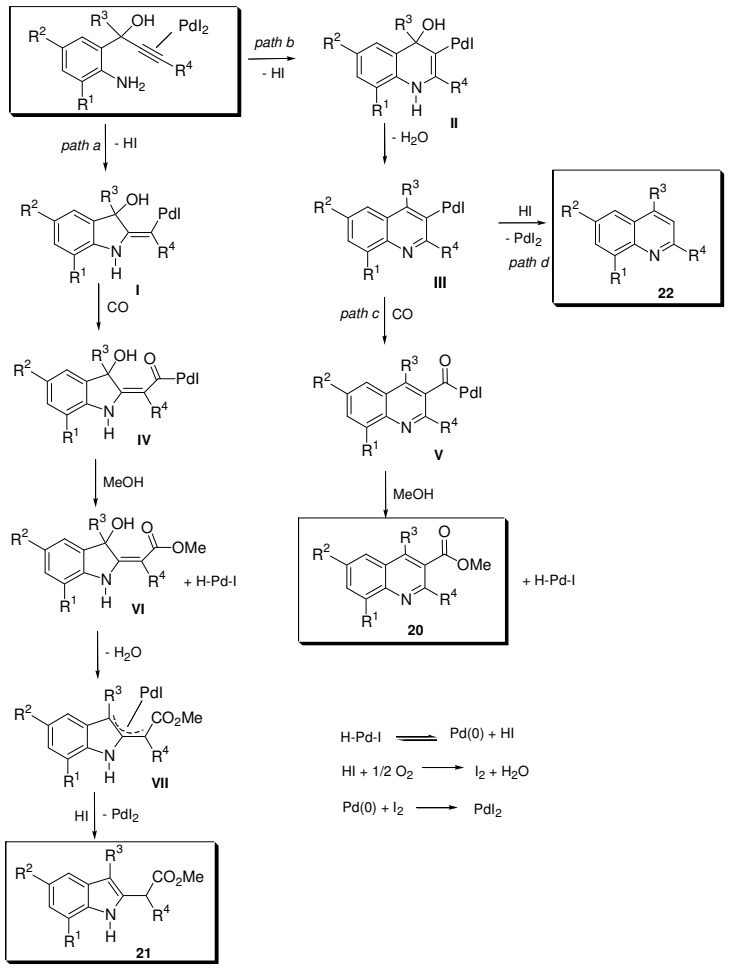


1 R=R^1=R^2=H, R^3=Me
2 R=R^2=H, R^1= OMe, R^3=Me
3 R=R^1=H, R^2=Cl, R^3=Me
4 R=R^1=R^2=H, R^3=Ph
5 R=R^3=Me , R^1=R^2=H

6 R=R^1=R^2=H, R^3=Me, R^4=Bu
7 R=R^2=H, R^1=OMe, R^3=Me, R^4=Bu
8 R=R^1=H, R^2=Cl R^3=Me, R^4=Bu
9 R=R^1=R^2=H, R^3=Me, R^4=Ph
10 R=R^2=H, R^1=OMe, R^3=Me, R^4=Ph
11 R=R^1=H, R^2=Cl, R^3=Me,R^4=Ph
12 R=R^1=R^2=H, R^3=Me, R^4=t-Bu
13 R=R^2=H, R^1=OMe, R^3=Me, R^4=t-Bu
14 R=R^1=H, R^2=Cl, R^3=Me, R^4=*t*-Bu
15 R=R^1=R^2=H, R^3=Ph, R^4=*t*-Bu
16 R=R^1=R^2=H, R^3=Me, R^4=TMS
17 R=R^2=H, R^1=OMe, R^3=Me, R^4=TMS
18 R=R^1=R^2=H, R^3=Ph, R^4=TMS
19 R=R^3=Me, R^1=R^2=H, R^4=tBu

Scheme 1

In principle, different reaction pathways can be followed when 1-(2-aminoaryl)-2-yn-1-ols bearing a primary amino group (R = H) are reacted in the presence of the PdI_2/KI catalytic system under carbonylative conditions (**Scheme 2**, anionic iodide ligands are omitted for clarity).



Scheme 2

The initial intramolecular attack by the amino group to the coordinated triple bond can occur in a 5-*exo-dig* (path a) or a 6-*endo-dig* (path b) cyclization mode, leading to isomeric vinylpalladium intermediates **I** and **II**, respectively, with formal elimination of HI. In contrast to intermediate **I**, however, complex **II** can easily undergo loss of water with simultaneous aromatization to give the 3-quinolinylpalladium species **III**. Under CO pressure, both complexes **I** and **III** can

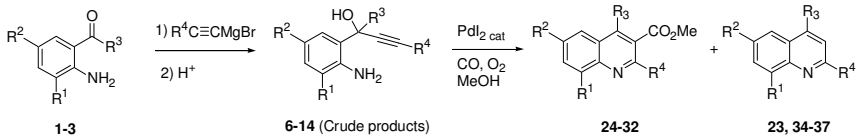
insert carbon monoxide, to give the corresponding acylpalladium intermediates **IV** and **V**, respectively. Eventually, nucleophilic displacement by an external alcohol should afford the corresponding heterocyclic derivatives **VI** and **20**, respectively, with elimination of H-Pd-I [which is known to be in equilibrium with Pd(0)+HI] *(6)*. Because intermediate **VI** still contains an allyl alcoholic function, it can react further with H-Pd-I, according to a known reactivity *(7-8)*, to give the allylpalladium complex **VII**. Protonolysis of the latter by HI would then lead to the indol-2-acetic ester **21** with regeneration of the catalytically active species PdI_2. On the other hand, the process leading to the quinoline-3-carboxylic ester **20** (path c) may become catalytic only in the presence of an external oxidant, such as oxygen, able to reoxidize Pd(0) to PdI_2 *(9)*. However, in the absence of an oxidant, a catalytic cycle is possible also starting with a 6-*endo-dig* cyclization mode, because intermediate **III** may undergo protonolysis by HI to give the noncarbonylated quinoline **22** with simultaneous regeneration of PdI_2 (path d) *(10)*.

On the basis of these mechanistic hypotheses, the reactivity of 1-(2-aminoaryl)-2-yn-1-ols bearing a primary amino group (R = H), such as **6**-**18**, was studied with CO and MeOH in the presence of the PdI_2/KI catalytic system under oxidative (using oxygen as the oxidant) as well as nonoxidative conditions to verify the possibility to find novel approaches to important carbonylated heterocyclic derivatives **5** and **6**, starting from readily available substrates.

The first substrate tested was 2-(2-aminophenyl)oct-3-yn-2-ol **6** (R = R^1 = R^2 = H, R^3 = Me, R^4 = Bu). Crude **6**, obtained by the reaction between commercially available 1-(2-aminophenyl)ethanone **1** and 1-hexynylmagnesium bromide, was already suitable as substrate for the subsequent reactions without further purification. Substrate **6** was initially reacted under oxidative conditions, in MeOH as the solvent (0.22 mmol of substrate per mL of MeOH) at 100 °C and under 20 atm of a 4:1 mixture of CO-air, and in the presence of PdI_2 (2 mol %) and KI as the catalyst (KI: PdI_2 = 10). The substrate conversion was complete after 2 h, and the main reaction product turned out to be 2-butyl-4-methylquinoline **23** (79%

isolated yield, based on starting **6**), whereas the carbonylated quinoline **24** was formed only in traces (**Table 1**, entry 1).

TABLE 1. Reactions of 1-(2-aminoaryl)-2-yn-1-ols **6-14** with CO, O_2 and MeOH in the presence of the PdI_2-KI catalytic system [a]



Entry			R^1	R^2	R^3	R^4		Yield[b] (%)		Yield[b] (%)
1[c,d]	**1**	**6**	H	H	Me	Bu	**24**	traces	**23**	79
2[d]	**1**	**6**	H	H	Me	Bu	**24**	33	**23**	62
3	**1**	**6**	H	H	Me	Bu	**24**	69	**23**	24
4	**2**	**7**	OMe	H	Me	Bu	**25**	65	**33**	28
5	**3**	**8**	H	Cl	Me	Bu	**26**	60		
6	**1**	**9**	H	H	Me	Ph	**27**	45	**34**	30
7	**2**	**10**	OMe	H	Me	Ph	**28**	63	**35**	25
8	**3**	**11**	H	Cl	Me	Ph	**29**	65		
9[e]	**1**	**12**	H	H	Me	t-Bu	**30**	69	**36**	13
10	**2**	**13**	OMe	H	Me	t-Bu	**31**	70	**37**	10
11	**3**	**14**	H	Cl	me	t-Bu	**32**	58		

[a]Crude substrate **6-14** were directly used as substrates without need of further purification. Unless otherwise noted, all reactions were carried out in MeOH as the solvent (0.22 mmol of **1-3** per mL of MeOH) for 2 h in the presence of PdI_2 and KI (**1-3**/PdI_2/KI molar ratio 50:1:10) at 100°C and under 80 atm of a 4:1 mixture of CO-air. Substrate conversion was quantitative in all cases. [b]Isolated yield based on starting **1-3** [c]Substrate concentration was 0.22 mmol/mL di MeOH. [d]The reaction was carried out under 20 atm of a 4:1 mixture CO-air. [e]The reaction also led to the formation of small amounts of 3,3-dimethyl-2-(3-methyl-1*H*-indol-2-yl)butyric acid methyl ester **38** (7%, based on starting **1**).

This result shows that, under the above conditions, **6** selectively undergoes 6-*endo-dig* cyclization (**Scheme 2**, *path b*) followed by dehydration and protonolysis (*path d*) rather than carbonylation (*path c*). Because it is known that protonolysis by HI is slowed down working under less concentrated conditions, the reaction at 0.02 rather then 0.22 mmol of **6** per mL of MeOH was next tried. As expected, the quinoline-3-carboxylic ester **24** was now obtained in appreciable yield (33% isolated), quinoline **23** still being the main reaction product (62% isolated yield, **Table 1**, entry 2). This result did not significantly change working under more diluted conditions.

In order to improve the selectivity toward **24**, the reaction was then carried out under a higher CO pressure, which was expected to favor the carbon monoxide insertion with respect to protonolysis. Indeed, under the same conditions of entry 2, but under 80 atm of a 4:1 mixture of CO-air, **24** was the main reaction product (69% isolated yield), quinoline **23** being formed in only 24% yield (**Table 1**, entry 3). Under the same conditions of entry 3, other 1-(2-aminoaryl)-2-yn-1-ols **7**-**14**, bearing different substituents on the triple bond and on the aromatic ring, were converted into the corresponding quinoline-3-carboxylic esters **25**-**32** in fair to good yields, thus allowing a general synthesis of this class of heterocyclic compounds (**Table 1**, entries 4-11). Minor amounts of noncarbonylated quinolines **33**-**37** were obtained in some cases (entries 4, 6, 7, 9, 10).

The reaction worked well also with substrates bearing a *tert*butyl group on the triple bond, such as **30**-**32**, which led to the corresponding quinolines in 58-70% isolated yields based on starting amino ketones **1**-**3** (**Table** 1, entries 9-11). In the case of **12**, the formation of small amounts (7%) of 3,3-dimethyl-2-(3-methyl-1*H*-indol-2-yl)butyric acid methyl ester **38**, deriving from path a (**Scheme 2**), was observed (**Table 1**,entry 9). Thus, under oxidative conditions, the reaction pathway beginning with a 6-*endo-dig* cyclization (**Scheme 2**, *path b*) is preferentially followed with respect to that beginning with a 5-*exo-dig* cyclization (**Scheme 2**, *path a*), even in the presence of a bulky substituent on the terminal *sp* carbon. This

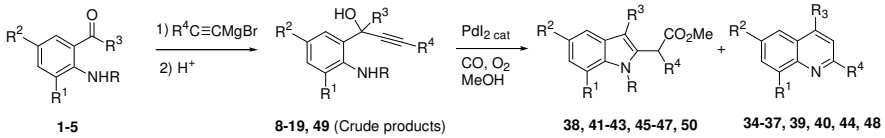
apparently unusual result can be explained as follows. The pathway beginning with a 6-*endo-dig* cyclization is particularly favored by the stabilization ensuing from the subsequent aromatization with formation of the quinoline ring. On the other hand, the pathway beginning with a 5-*exo-dig* cyclization can lead to aromatization only after the reaction between intermediate **VI** and the H-Pd-I species; however, this latter reaction is hindered by the fact that, under oxidative conditions, H-Pd-I is readily reconverted to PdI_2 *(4, 9)*, which may begin a new catalytic cycle leading to **20**.

It was interesting at this point to test the reactivity of 1-(2-aminoaryl)-2-yn-1-ols under nonoxidative conditions. Substrates not bearing a bulky group on the triple bond were found to preferentially undergo 6-*endo-dig* rather than 5-*exo-dig* cyclization. For example, the reaction of 2-(2-aminophenyl)-4-phenylbut-3-yn-2-ol **9** (bearing a phenyl group on the triple bond), carried out under the same conditions of entry 3 (**Table 1**) but under 60 atm of CO and in the absence of oxygen, selectively led to 4-methyl-2-phenylquinoline **34** (59% isolated yield, **Table 2**, entry 12), ensuing from 6-*endo-dig* cyclization (**Scheme 2**, *path b*) followed by aromatization and protonolysis (**Scheme 2**, path d). This result shows that, for a substrate bearing a phenyl on the triple bond, the route leading to indoles **21** (**Scheme 2**, *path a*) it is not competitive with the pathway leading to quinolines **22**. As expected, other substrates bearing a phenyl or a butyl group on the triple bond, such as **10**, **11**, and **8**, behaved similarly, leading to the corresponding noncarbonylated quinolines **35**, **39**, and **40** in 39-49% yields, as shown by the results reported in **Table 2**, entries 13-15.

On the basis of these results and observations, the next logical step was to test the reactivity of substrates bearing a bulky *tert*-butyl or TMS group on the triple bond, under nonoxidative conditions. In this case, in fact, the 5-*exo-dig* pathway leading to indoles **21** was expected to become competitive with the 6-*endo-dig* route leading to quinolines **22** (**Scheme 2**) for steric reasons. Indeed, the reaction of 2-(2-aminophenyl)-5,5-dimethylhex-3-yn-2-ol **12** (R^4 = *tert*-butyl) carried out under the same conditions as those of entries 12-15 (**Table 2**) led to 3,3-

dimethyl-2-(3-methyl-1*H*-indol-2-yl)butyric acid methyl ester **38** in 66% isolated yield [based on starting 1-(2-aminophenyl) ethanone **1**], 2-*tert*-butyl-4-methylquinoline **36** being formed as byproduct (36% isolated yield based on **1a**, **Table 2**, entry 16). The selectivity toward **38** could be improved working under a higher CO pressure: at 90 atm, the yields of **38** and **36** were 75 and 6%, respectively, based on **1** (entry 17, **Table 2**). Under these latter conditions, other substrates bearing a *tert*-butyl group on the triple bond, such as **13**-**15**, led to the corresponding indoles **41**-**43** with good yields and selectivities (entries 18-20, **Table 2**). Minor amounts of noncarbonylated quinolines **37** and **44** were obtained from substrates **13** and **15**, respectively (entries 18 and 20).

TABLE 2. Reactions of 1-(2-aminoaryl)-2-yn-1-ols **8-19, 49** with CO and MeOH in the presence of the PdI_2-KI catalytic system [a]



Entry			R	R^1	R^2	R^3	R^4		Yield[b] (%)		Yield[b] (%)
12[c]	**1**	**9**	H	H	H	Me	Ph			**34**	59
13[c]	**2**	**10**	H	OMe	H	Me	Ph			**35**	54
14[c]	**3**	**11**	H	H	Cl	Me	Ph			**39**	39
15[c]	**3**	**8**	H	H	Cl	Mr	Bu			**40**	49
16[c]	**1**	**12**	H	H	H	Me	t-Bu	**38**	66	**36**	36
17	**1**	**12**	H	H	H	Me	t-Bu	**38**	75	**36**	6
18	**2**	**13**	H	OMe	H	Me	t-Bu	**41**	45	**37**	30
19	**3**	**14**	H	H	Cl	Me	t-Bu	**42**	68		
20	**4**	**15**	H	H	H	Ph	t-Bu	**43**	60	**44**	22
21	**1**	**16**	H	H	H	Me	TMS	**45**[d]	88		
22	**2**	**17**	H	OMe	H	Me	TMS	**46**[d]	42		
23	**4**	**18**	H	H	H	Ph	TMS	**47**[d]	63	**48**[d]	14
24[e]	**5**	**49**	Me	H	H	Me	Bu				
25	**5**	**19**	Me	H	H	Me	t-Bu	**50**	44		

[a]Crude substrate **8-18** were directly used as substrates without need of further purification. Unless otherwise noted, all reactions were carried out in MeOH as the solvent (0.22 mmol of **1-4** per mL of MeOH) for 2 h in the presence of PdI_2 and KI (**1-4**/PdI_2/KI molar ratio 50:1:10) at 100°C and under 90 atm of CO. Substrate conversion was quantitative in all cases. [b]Isolated yield based on starting **1-4**. [c]Reaction was carried out under 60 Atm of CO. [d]R^4=H in the final product. [e]Decomposition of the substrate, with formation of unidentified chromatographically immobile materials, was observed.

As we have already observed in other PdI_2-catalyzed cyclization and oxidative carbonylation reactions *(4, 5, 10)*, in the case of substrates bearing a trimethylsilyl substituent on the triple bond, such as **16**-**18**, the TMS group was lost in the course of the process, thus allowing the synthesis of R-unsubstituted indol-2-acetic esters **45**-**47** (entries 21-23, **Table 2**). 4-Phenylquinoline **48** was obtained as byproduct in the case of the reaction of **18** (entry 23).

The reactivity of 1-(2-alkylaminoaryl)-2-yn-1-ols bearing a secondary rather than a primary amino group was also tested. Clearly, for these substrates, bearing only one hydrogen bonded to nitrogen, paths c and d (**Scheme 2**) could not be followed, thus the possibility to obtain quinoline derivatives **20** or **22** was prevented. The reaction of 2-(2-methylaminophenyl)-oct-3-yn-2-ol **49** (substituted with a butyl group on the triple bond), carried out under nonoxidative conditions, similar to those reported in entry 17 (**Table 2**), led to decomposition of the starting material, with formation of unidentified chromatographically immobile materials (**Table 2**, entry 24). This is conceivable, because, as we have seen, if the substituent on the triple bond is not sterically demanding, the 5-*exo*-*dig* cyclization (path a, **Scheme 2**) is not favored and, as a consequence, the substrate preferentially undergoes decomposition. On the other hand, the reaction of 5,5-dimethyl-2-(2-methylaminophenyl)-hex-3-yn-2-ol **19**, bearing a *tert*-butyl group on the triple bond, did afford the corresponding indol-2-acetic derivative **50**, even though in moderate yield [44% isolated, based on starting 1-(2-methylaminophenyl) ethanone **5**, **Table 2**, entry 25]

2.3 Conclusions

In conclusion, it has been shown that 1-(2-aminoaryl)-2-yn-1-ols **6**-**19** [used as crude products deriving from the Grignard reaction between 1-(2-aminoaryl)ketones **1**-**5** and alkynylmagnesium bromides] may follow different reaction pathways when let to react in the presence of the PdI_2-KI catalytic system under oxidative or nonoxidative carbonylation conditions, depending on the nature

of the substrate and on reaction conditions. In particular, 1-(2-aminoaryl)-2-yn-1-ols, bearing a primary amino group, such as **6**-**14**, selectively undergo 6-*endo-dig* cyclization when allowed to react under oxidative conditions, with selective formation of quinoline-3-carboxylic esters **24**-**32** in fair to good yields [45-70% isolated, based on starting 1-(2-aminoaryl)ketones **1**-**3**]. On the other hand, indol-2-acetic esters **38**, **41**-**43**, **45**-**47**, and **50**, deriving from 5-*exo-dig* cyclization, are obtained in moderate to good yields [42-88%, based on starting 1-(2-aminoaryl)ketones **1**-**5**] under nonoxidative conditions, when the starting material is substituted with a bulky group on the triple bond, as in the case of **12**-**19**. In this latter case, a primary as well as a secondary amino group can be present in the substrate, and R-unsubstituted indol-2-acetic esters are formed from substrates bearing a TMS group on the triple bond, ensuing from loss of the TMS group in the course of the process. The present methodology represents a simple and direct approach to the synthesis of functionalized quinolines and indoles starting from readily available starting materials *(11–14)*.

2.4 Experimental Section

*2.4.1 General Procedure for the Synthesis of Quinoline-3-carboxylic Esters **24-32** (Table 1, entries 3-11).*

To a suspension of Mg turnings (700.0 mg, 28.8 mmol) in anhydrous THF (2.0 mL), maintained under nitrogen and under reflux, was added pure EtBr (0.5 mL) to start the formation of the Grignard reagent. The remaining bromide was added dropwise (ca. 20 min) in THF solution (1.5 mL of EtBr in 15.0 mL of THF; total amount of EtBr added: 2.92 g, 26.8 mmol). The mixture was then allowed to reflux for additional 20 min. After cooling, the solution of EtMgBr thus obtained was transferred under nitrogen to a dropping funnel and was added dropwise to a solution of the 1- alkyne (26.8 mmol) in anhydrous THF (7.0 mL) at 0 °C with stirring. After additional stirring at 0 °C for 15 min, the mixture was allowed to

warm up to room temperature, then it was maintained at 40 °C (R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = *t*-Bu) or 50 °C (R = R^1 = R^2 = H, R^3 = Me, R^4 = Bu; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = Bu; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = Bu; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = Ph; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = Ph; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = Ph; R = R^1 = R^2 = H, R^3 = Me, R^4 = *t*-Bu) for 2 h, and used as such at the same temperature for the next step. 1-(2-Aminoaryl)ketone **1-3** (8.9 mmol) was dissolved under nitrogen in anhydrous THF (7.0 mL) and then added dropwise to the solution of the alkynylmagnesium bromide in THF (prepared as described above) under nitrogen. After stirring at 40 °C for 1.5 h (R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = *t*-Bu), 50 °C for 1 h (R = R^1 = R^2 = H, R^3 = Me, R^4 = Bu; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = Bu; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = Bu) or 50 °C for 2 h (R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = Ph; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = Ph; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = Ph; R = R^1 = R^2 = H, R^3 = Me, R^4 = *t*-Bu), the mixture was cooled to room temperature. Saturated NH4Cl was added with stirring to achieve weakly acidic pH. After additional stirring at room temperature for 15 min. , AcOEt (ca. 20 mL) was added and phases were separated. The aqueous phase was extracted with AcOEt (3 × 30 mL), and the collected organic layers were washed with brine to ca. neutral pH and eventually dried over Na_2SO_4. After filtration, the solvent was evaporated and crude products **6-14** were diluted with MeOH and transferred into a volumetric flask (50 mL). To 7.0 mL of the solution (formally deriving from 1.25 mml of **1-3**) were added 55.5 mL of MeOH (to adjust the substrate concentration to 0.02 mmol / mL of MeOH), and the resulting solution was transferred to an autoclave, previously loaded with PdI_2 (9.0 mg, 2.5 × 10-2 mmol) and KI (41.5 mg, 0.25 mmol). The autoclave was sealed and, while the mixture was stirred, the autoclave was pressurized with CO (64 atm) and air (up to 80 atm). After being stirred at 100 °C for 2 h, the autoclave was cooled, degassed, and opened. The solvent was evaporated, and products **24-32** and **23**, **33-37** were purified by column chromatography on silica gel using 99:1

hexane-acetone as eluent. Non-carbonylated quinolines **23**, **33-37** were eluted first in all cases; in the case of the reaction mixture deriving from **12**, small amounts of 3,3-dimethyl-2-(3-methyl-1*H*-indol-2-yl)butyric acid methyl ester **38** were also isolated (order of elution: **36**, **38**, **30**): **24** [yellow oil, 221.3 mg, 69% based on starting 1-(2- aminophenyl)ethanone **1**]; **23** (yellow oil, 60.3 mg, 24% based on starting **1**); **25** [yellow oil, 233.7 mg, 65% based on starting 1-(2-amino-3-methoxyphenyl)ethanone **2**]; **33** (yellow oil, 79.5 mg, 28% based on starting **2**); **26** [yellow oil, 220.2 mg, 60% based on starting 1-(2-amino-5- chlorophenyl)ethanone **3**]; **27** (yellow solid, mp 58-59 °C, 156.6 mg, 45% based on starting **1**); **34** (yellow oil, 81.8 mg, 30% based on starting **1**); **28** (yellow solid, mp 100-101 °C, 241.2 mg, 63% based on starting **2**); **35** (yellow solid, mp 97-98 °C, 77.3 mg, 25% based on starting **2**); **29** (yellow oil, 254.2 mg, 65% based on starting **3**); **30** (yellow oil, 222.9 mg, 69% based on starting **1**); **38** (yellow solid, mp 115-117 °C, 23.1 mg, 7% based on starting **1**); **36** (yellow oil, 31.5 mg, 13% based on starting **1**); **31** (yellow solid, mp 65-67 °C, 252.3 mg, 70% based on starting **2**); **37** (yellow oil, 30.0 mg, 10% based on starting **2**); **32** (yellow oil, 213.3 mg, 58% based on starting **3**).

*2.4.2 General Procedure for the Synthesis of Indol-2-acetic Esters **38**, **41-43**, **45-47**, **50** (Table 2, entries 17-23, 25).*

To a suspension of Mg turnings (700.0 mg, 28.8 mmol) in anhydrous THF (2.0 mL), maintained under nitrogen and under reflux, was added pure EtBr (0.5 mL) to start the formation of the Grignard reagent. The remaining bromide was added dropwise (ca. 20 min) in THF solution (1.5 mL of EtBr in 15.0 mL of THF; total amount of EtBr added: 2.92 g, 26.8 mmol). The mixture was then allowed to reflux for additional 20 min. After cooling, the solution of EtMgBr thus obtained was transferred under nitrogen to a dropping funnel and was added dropwise to a solution of the 1-alkyne (26.8 mmol) in anhydrous THF (7.0 mL) at 0 °C with stirring. After additional stirring at 0 °C for 15 min, the mixture was allowed to

warm up to room temperature, then it was maintained at 40 °C (R = R^2 =H, R^1 = OMe, R^3 = Me, R^4 = TMS; R = R^3 = Me, R^1 = R^2 = H, R^4 = *t*-Bu; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = *t*-Bu) or 50 °C (R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = TMS; R = R^1 = R^2 = H, R^3 = Ph, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Ph, R^4 = TMS) for 2 h, and used as such at the same temperature for the next step. 1-(2-Aminoaryl)ketone **1-5** (8.9 mmol) was dissolved under nitrogen in anhydrous THF (7.0 mL) and then added dropwise to the solution of the alkynylmagnesium bromide in THF (prepared as described above) under nitrogen. After stirring at 40 °C C for 1.5 h (R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = *t*-Bu; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = TMS), at 40 °C for 2 h (R = R^3 = Me, R^1 = R^2 = H, R^4 = *t*-Bu), 50 °C for 2 h (R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Me, R^4 = TMS; R = R^1 = R^2 = H, R^3 = Ph, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Ph, R^4 = TMS), the mixture was cooled to room temperature. Saturated NH_4Cl was added with stirring to achieve weakly acidic pH. After additional stirring at room temperature for 15 min., AcOEt (ca. 20 mL) was added and phases were separated. The aqueous phase was extracted with AcOEt (3 × 30 mL), and the collected organic layers were washed with brine to ca. neutral pH and eventually dried over Na_2SO_4. After filtration, the solvent was evaporated and crude products **12-19** were diluted with MeOH and transferred into volumetric flask (50 mL). To 7.0 mL of the solution (formally deriving from 1.25 mml of **1-5**) were added 55.5 mL of MeOH (to adjust the substrate concentration to 0.02 mmol / mL of MeOH), and the resulting solution was transferred to an autoclave, previously loaded with PdI_2 (9.0 mg, 2.5×10^{-2} mmol) and KI (41.5 mg, 0.25 mmol). The autoclave was sealed, purged at room temperature several times with CO with stirring (10 atm) and eventually pressurized at 90 atm of CO. After being stirred at 100 °C for 2 h, the autoclave was cooled, degassed, and opened. The solvent was evaporated, and products **38, 41-43, 45-47, 50** and **36**, **37, 44, 48** were purified by column chromatography on silica gel using hexane-acetone from 99:1 to 95:5 (R = R^1 = R^2

= H, R^3 = Me, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Ph, R^4 = *t*-Bu; R = R^1 = R^2 = H, R^3 = Ph, R^4 = TMS; R = R^1 = H, R^2 = Cl, R^3 = Me, R^4 = *t*-Bu) or hexane-AcOEt from 99:1 to 95:5 as eluent (R = R^1 = R^2 = H, R^3 = Me, R^4 = TMS; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = *t*-Bu; R = R^2 = H, R^1 = OMe, R^3 = Me, R^4 = TMS; R = R^3 = Me, R^1 = R^2 = H, R^4 = *t*-Bu). Non-carbonylated quinolines **36**, **37, 44, 48** were eluted first in all cases: **38** (yellow solid, mp 115-117 °C, 244.7 mg, 75% based on starting **1**); **36** (yellow oil, 15.2 mg, 6% based on starting **1**); **41** (colorless solid, mp 83-85 °C, 163.9 mg, 45% based on starting **2**); **37** (yellow oil, 86.8 mg, 30% based on starting **2**); **42** (yellow solid, mp 101-103 °C, 250.6 mg, 68% based on starting **3**); **43** (yellow solid, mp 174-176 °C, 240.9 mg, 60% based on starting **4**); **44** (colorless solid, 77-78 °C, 72.4 mg, 22% based on starting **4**); **45** [yellow solid, mp 78-79 °C (lit.3 77-78 °C), 224.8 mg, 88% based on starting **1**); **46** (yellow oil, 122.3 mg, 42% based on starting **2**); **47** (yellow solid, mp 83-84 °C, 210.1 mg, 63% based on starting **4**); **48** (yellow oil, 36.4 mg, 14% based on starting **4**); **50** (yellow solid, mp 86-88 °C, 149.4 mg, 44% based on starting **5**).

2.5 Characterization data of products

Quinolines **23**, **33**, **34**, **35**, **36**, **37**, **39**, **40**, **44**, and **48** were characterized by comparison with literature data. Complete characterization data for all the other products are given below.

- *2-Butyl-4-methylquinoline-3-carboxylic acid methyl ester (**24**)*. Yield: 221.3 mg, 69% based on starting 1-(2-aminophenyl)ethanone **1a** (Table 1, entry 3). Yellow oil. IR (film): ν = 1731 (s), 1588 (m), 1456 (m), 1435 (m), 1290 (m), 1235 (s), 1161 (w), 1056 (w), 759 (m) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08-8.05 (m, 1 H), 8.01-7.96 (m, 1 H), 7.70 (ddd, *J*= 8.3, 6.9, 1.4, 1 H), 7.53 (ddd, *J* = 8.3, 6.9, 1.4, 1 H), 3.99 (s, 3 H), 2.96-2.88 (m, 2 H), 2.63 (s, 3 H), 1.85-1.72 (m, 2 H), 1.44 (sext, *J* = 7.4, 2 H),

0.95 (t, *J* = 7.4, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.9, 158.3, 147.3, 141.6, 130.0, 129.5, 127.7, 126.3, 125.7, 124.0, 52.4, 37.1, 31.8, 22.9, 15.9, 13.9 ; GC-MS: *m/z* = 257 (6) [M^+], 242 (17), 228 (23), 226 (11), 216 (16), 215 (100), 200 (53), 198 (10), 171 (10), 167 (12), 158 (13), 157 (88), 143 (10), 115 (13); anal. calcd for $C_{16}H_{19}NO_2$ (257.33): C,74.68; H, 7.44; N, 5.44. Found C, 74.76; H, 7.46; N, 5.43

- *2-Butyl-8-methoxy-4-methylquinoline-3-carboxylic acid methyl ester (**25**).* Yield: 233.7 mg, 65% based on starting from **1b** (Table 1, entry 4). Yellow oil. IR (film): ν = 1732 (s), 1567 (w), 1494 (w, 1471 (m), 1436 (m), 1399 (w), 1274 (m), 1257 (m), 1158 (s), 1047 (m), 748 (m) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56 (distorted dd, *J* = 8.4, *J* = 1.2, 1 H), 7.45 (dd, *J*= 8.4, 7.8, 1 H), 7.07 (distorted dd, *J* = 7.8, 1.2, 1 H), 4.07 (s, 3 H), 3.99 (s, 3 H), 3.01-2.94 (m, 2 H), 2.60 (s, 3 H), 1.84-1.71 (m, 2 H), 1.47 (sext, *J* = 7.4, 2 H), 0.94 (t, *J* = 7.4, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.0, 157.2, 155.4, 141.5, 139.3, 128.2, 126.9, 126.2, 115.7, 108.4, 56.3, 52.4, 37.4, 32.1, 22.9, 16.4, 13.9 ; GC-MS: *m/z* = 287 (5) [M^+], 286 (10), 272 (11), 258 (22), 246 (13), 245 (100), 230 (39), 228 (13), 227 (12), 212 (11), 187 (66), 172 (17), 115 (16); anal. calcd for $C_{17}H_{21}NO_3$ (287.35): C, 71.06; H, 7.37; N, 4.87. Found C, 71.17; H, 7.36; N, 4.86.

- *2-Butyl-6-chloro-4-methylquinoline-3-carboxylic acid methyl ester (**26**).* Yield: 220.2 mg, 60% based on starting **1c** (Table 1, entry 5). Yellow oil. IR (film): ν= 1732 (s), 1588 (m), 1487 (m), 1275 (m), 1227 (s), 1095 (m), 832 (w) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.96 (distorted d, *J* = 8.8, 1 H), 7.93 (d, *J* = 2.2, 1 H), 7.62 (dd, *J* = 8.8, 2.2, 1 H), 4.00 (s, 3 H), 2.94-2.85 (m, 2 H), 2.58 (s, 3 H), 1.84-1.71 (m, 2 H), 1.43 (sext, *J* = 7.5, 2 H), 0.95 (t, *J* = 7.4, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.5, 158.6, 145.7,

140.7, 132.1, 131.1, 130.7, 128.4, 126.5, 123.1, 52.5, 37.0, 31.6, 22.8, 15.8, 13.9; GC-MS: *m/z* = 293 (0.2) [$(M+2)^+$], 291 (0.7) [M^+], 276 (12), 262 (21), 251 (34), 250 (16), 249 (100), 236 (14), 234 (45), 232 (11), 193 (25), 192 (11), 191 (73), 167 (12), 154 (13), 115 (10) ; anal. calcd for $C_{16}H_{18}ClNO_2$ (291.77): C, 65.86; H, 6.22; Cl, 12.15; N, 4.80. Found C, 65.98; H, 6.18; Cl, 12.08; N, 4.83

- *4-Methyl-2-phenylquinoline-3-carboxylic acid methyl ester (**27**).* Yield: 156.6 mg, 45% based on starting **1a** (Table 1, entry 6). Yellow solid, mp 58-59 °C. IR (KBr): ν = 1721 (s), 1494 (w), 1436 (w), 1293 (m), 1230 (s), 1114 (m), 1054 (w), 770 (m), 757 (m) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.21-8.15 (m, 1 H), 8.11-8.05 (m, 1 H), 7.81-7.73 (m, 1 H), 7.73-7.65 (m, 2 H), 7.65-7.57 (m, 1 H), 7.51-7.41 (m, 3 H), 3.67 (s, 3 H), 2.75 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.7, 156.1, 147.3, 142.9, 140.5, 130.4, 130.3, 128.8, 128.5, 128.3, 127.8, 127.0, 126.0, 124.1, 52.4, 15.8; GCMS: *m/z* = 277 (24) [M^+], 263 (18), 262 (100), 246 (34), 217 (20), 108 (15); anal. calcd for $C_{18}H_{15}NO_2$ (277.32): C, 77.96; H, 5.45, N, 5.05. Found C, 78.08; H, 5.46; N, 5.06

- *8-Methoxy-4-methyl-2-phenylquinoline-3-carboxylic acid methyl ester (**28**).* Yield: 241.2 mg, 63% based on starting **1b** (Table 1, entry 7). Yellow solid, mp 100-101°C. IR (KBr): ν= 1726 (s), 1560 (w), 1467 (m), 1437 (w), 1398 (w), 1246 (w), 1259 (m), 1160 (s), 1043 (w), 767 (w) (s) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.73-7.67 (m, 2 H), 7.62 (distorted dd, *J* = 8.4, 1.1, 1 H), 7.51 (dd, *J* = 8.4, 7.8, 1 H), 7.45-7.38 (m, 3 H), 7.10 (dd, *J* = 7.8, 1.1, 1 H), 4.05 (s, 3 H), 3.67 (s, 3 H), 2.71 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.9, 155.9, 154.7, 142.6, 140.7, 139.3, 128.6, 128.5, 128.3, 127.8, 127.2, 127.1, 115.7, 108.7, 56.2, 52.4, 16.3; GC-MS: *m/z* =

307 (100) [M^+], 306 (77), 278 (38), 277 (22), 274 (13), 246 (16), 217 (18), 216 (13), 204 (12), 77 (13); anal. calcd for $C_{19}H_{17}NO_3$ (307.34): C, 74.25; H, 5.58; N, 4.56. Found C, 74.18; H, 5.59; N, 4.58

- *6-Chloro-4-methyl-2-phenylquinoline-3-carboxylic acid methyl ester (**29**).* Yield: 254.2 mg, 65% based on starting from **1c** (Table 1, entry 8). Yellow oil. IR (film): ν= 1719 (s), 1291 (m), 1226 (s), 1117 (m), 1090 (w), 833 (m), 716 (w), 698 (w) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13-8.01 (m, 2 H), 7.73-7.64 (m, 3 H), 7.52-7.41 (m, 3 H), 3.68 (s, 3 H), 2.70 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 156.3, 145.7, 142.1, 140.1, 132.9, 131.9, 131.3, 129.0, 128.5, 128.2, 127.9, 126.8, 123.2, 52.5, 15.8; GC-MS: *m/z* = 313 (15) [$(M+2)^+$], 311 (34) [M^+], 298 (32), 297 (16), 296 (100), 282 (17), 281 (19), 280 (44), 253 (13), 217 (28), 216 (28), 114 (14), 77 (13), 75 (11), 73 (33); anal. calcd for $C_{18}H_{14}ClNO_2$ (311.76): C, 69.35; H, 4.53; Cl, 11.37; N, 4.49. Found C, 69.47; H, 4.54; Cl, 11.38, N, 4.50.

- *2-tert-Butyl-4-methylquinoline-3-carboxylic acid methyl ester (**30**).* Yield: 222.9 mg, 69% based on starting **1a** (Table 1, entry 9). Yellow oil. IR (film): ν = 1729 (s), 1634 (m), 1384 (s), 1305 (s), 1196 (w), 1182 (w) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.85-8.80 (m, 1 H), 8.29-8.24 (m, 1 H), 8.07 (ddd, *J* = 8.5, 7.1, 1.3, 1 H), 7.91 (ddd, *J*= 8.5, 7.1, 1.3, 1 H), 4.07 (s, 3 H), 2.86 (s, 3 H), 1.72 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.7, 162.7, 155.1, 137.7, 135.7, 130.5, 128.0, 126.5, 124.7, 122.5, 53.5, 39.8, 29.5, 17.5; GC-MS: *m/z* = 257 (25) [M^+], 243 (18), 242 (100), 226 (13), 224 (12), 215 (34), 210 (20), 200 (16), 182 (11), 167 (21), 157 (21), 143 (37), 115 (19), 90 (11); anal. calcd for $C_{16}H_{19}NO_2$ (257.33): C, 74.68; H, 7.44; N, 5.44. Found C, 74.59; H, 7.42; N, 5.45

- *2-tert-Butyl-8-methoxy-4-methylquinoline-3-carboxylic acid methyl ester (**31**).*
  Yield: 252.3 mg,70% based on starting **1b** (Table 1, entry 10). Yellow solid, mp 65-67°C. IR (KBr): ν= 1726 (s), 1467 (m), 1279 (w), 1260 (m), 1246 (w), 1204 (w), 1081 (s), 1043 (m), 796 (w), 767 (w), 742 (w) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.54 (distorted dd, *J* = 8.4, 1.0, 1 H), 7.43 (dd, *J* = 8.4, 7.8, 1 H), 7.05 (dd, *J* = 7.8, 1.0, 1 H), 4.06 (s, 3 H), 3.96 (s, 3 H), 2.55 (s, 3 H), 1.51 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 161.7, 155.8, 152.0, 141.5, 138.4, 127.1, 126.5, 115.5, 109.0, 56.6, 52.2, 40.2, 30.1, 16.2; GC-MS: *m/z* = 287 (49) [M^+], 286 (37), 273 (18), 272 (100), 258 (13), 257 (13), 256 (16), 254 (21), 245 (18), 212 (17), 198 (13), 187 (16), 173 (33), 158 (12), 115 (11); anal. calcd for $C_{17}H_{21}NO_3$ (287.35): C, 71.06; H, 7.37; N, 4.87. Found C, 71.13; H, 7.36; N, 4.86

- *2-tert-Butyl-6-chloro-4-methylquinoline-3-carboxylic acid methyl ester (**32**).*
  Yield: 213.3 mg, 58% based on starting **1c** (Table 1, entry 11). Yellow oil. IR (film): ν = 1733 (s), 1577 (w), 1482 (m), 1223 (s), 1123 (m), 1092 (m), 1056 (m), 832 (m), 803 (w) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.96 (distorted d, *J* = 8.8, 1 H), 7.93 (d, *J* = 2.2, 1 H), 7.61 (distorted dd, *J*= 8.8, 2.2, 1 H), 3.96 (s, 3 H), 2.52 (s, 3 H), 1.46 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 163.4, 144.8, 140.7, 132.2, 131.8, 130.4, 128.3, 126.0, 122.6, 52.3, 40.0, 30.0, 15.7; GC-MS: *m/z* = 293 (5) [$(M+2)^+$], 291 (25) [M^+], 278 (34), 277 (16), 276 (100), 260 (16), 258 (12), 251 (17), 249 (43), 244 (19), 234 (21), 216 (11), 193 (12), 191 (32), 181 (10), 180 (12), 179 (11), 177 (33), 140 (12), 115 (10), 90 (12); anal. calcd for $C_{16}H_{18}ClNO_2$ (291.77): C, 65.86; H, 6.22; Cl, 12.15; N, 4.80. Found C, 65.95; H, 6.20; Cl, 12.13, N, 4.79.

- *3,3-Dimethyl-2-(3-methyl-1H-indol-2-yl)acetic acid methyl ester (**38**).* Yield: 244.7 mg, 75% based on starting **1a** (Table 2, entry 17). Yellow solid, mp 115-117 °C. IR (KBr): ν = 3401 (s), 1727 (s), 1460 (w), 1340 (w), 1151 (m), 741 (m) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.81 (s, br, 1 H), 7.56-7.48 (m, 1 H), 7.36-7.28 (m, 1 H), 7.21-7.03 (m, 2 H), 3.80 (s, 1 H), 3.69 (s, 3 H), 2.25 (s, 3 H), 1.05 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 135.2, 129.0, 128.4, 121.6, 118.9, 118.4, 110.7, 109.7, 52.3, 51.7, 36.9, 28.0, 9.2 ; GC-MS: *m/z* = 259 (53) [M^+], 203 (45), 202(45), 172 (14), 171 (100), 170 (92), 144 (27), 143 (28), 142 (16), 116 (10), 115 (30); anal. calcd for $C_{16}H_{21}NO_2$ (259.34): C, 74.10; H, 8.16; N, 5.40. Found C, 74.21; H, 8.14; N, 5.38.

- *2-(7-Methoxy-3-methyl-1H-indol-2-yl)-3,3-dimethylbutyric acid methyl ester (**41**).* Yield: 163.9 mg, 45% based on starting **1b** (Table 2, entry 18). Colorless solid, mp 83-85°C. IR (KBr): ν = 3457 (s), 1713 (s), 1582 (w), 1456 (w), 1329 (w), 1259 (m), 1228 (m), 1156 (m), 1047 (w), 723 (w) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.88 (s, br, 1 H), 7.12 (d, *J* = 7.8, 1 H), 7.00 (t, *J* = 7.8, 1 H), 6.60 (d, *J* = 7.8, 1 H), 3.93 (s, 3 H), 3.78 (s, 1 H), 3.69 (s, 3 H), 2.24 (s, 3 H), 1.05 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.7, 146.1, 130.0, 128.9, 126.1, 119.4, 111.4, 110.3, 102.1, 55.3, 52.6, 51.5, 36.8, 28.1, 9.4 ; GC-MS: *m/z* = 289 (50) [M^+], 233 (33), 232 (47), 202 (14), 201 (100), 200 (87), 174 (13); anal. calcd for $C_{17}H_{23}NO_3$ (289.37): C, 70.56; H, 8.01; N, 4.84. Found C, 70.67; H, 8.03; N, 4.82.

- *2-(5-Chloro-3-methyl-1H-indol-2-yl)-3,3-dimethylbutyric acid methyl ester (**42**).* Yield: 250.6 mg, 68% based on starting **1c** (Table 2, entry 19). Yellow solid, mp 101-103 °C. IR (KBr): ν = 3410 (s), 2950 (m), 1715 (s), 1469 (m), 1445 (m), 1348 (w), 1196 (w), 1157 (w), 605 (m) cm1; ^{1}H NMR (300

MHz, $CDCl_3$): δ = 8.87 (s, br, 1 H), 7.49-7.46 (m, 1 H), 7.24 (distorted d, *J* = 8.6, 1 H), 7.10 (distorted dd, *J* = 8.6, 2.2, 1 H), 3.78 (s, 1 H), 3.72 (s, 3 H), 2.21 (s, 3 H), 1.04 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.8, 133.9, 133.5, 130.7, 129.5, 124.6, 121.8, 117.9, 111.7, 52.2, 51.8, 37.0, 28.0, 9.1 ; GC-MS: *m/z* = 295 (6) [$(M+2)^+$], 293 (22) [M^+], 239 (12), 238 (15), 237 (34), 236 (30), 207 (34), 206 (25), 205 (100), 204 (44), 178 (17), 177 (15), 141 (10), 140 (12); anal. calcd for $C_{16}H_{20}ClNO_2$ (293.79): C, 65.41; H, 6.86; Cl, 12.07; N, 4.77. Found C, 65.49; H, 6.88; Cl, 12.09; N, 4.76.

- *Dimethyl-2(3-phenyl-1H-indol-2-yl)butyric acid methyl ester (**43**).* Yield: 240.9 mg, 60% based on starting **1d** (Table 2, entry 20). Yellow solid, mp 174-176 °C. IR (KBr): ν = 3406 (s), 1713 (s), 1435 (w), 1350 (w) 1256 (w), 1196 (m), 1155 (m), 738 (m), 705 (w) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.06 (s, br, 1 H), 7.61-7.55 (m, 1 H), 7.51-7.37 (m, 5 H), 7.37-7.28 (m, 1 H), 7.20 (ddd, *J* = 8.2, 7.1, 1.2, 1 H), 7.09 (ddd, *J* = 7.9, 7.1, 1.2, 1 H), 4.09 (s, 1 H), 3.75 (s, 3 H), 0.88 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 135.2, 135.1, 130.2, 129.4, 128.7, 127.4, 126.3, 122.1, 119.8, 119.1, 117.6, 110.9, 51.92, 51.85, 36.7, 27.9; GC-MS: *m/z* = 321 (36) [M^+], 266 (10), 265 (49), 264 (23), 234 (14), 233 (77), 206 (18), 205 (29), 204 (100), 203 (11); anal. calcd for $C_{21}H_{23}NO_2$ (321.41): C, 78.47; H, 7.21; N, 4.36. Found C, 78.52; H, 7.20; N, 4.35.

- *(3-Methyl-1H-indol-2-yl)acetic acid methyl ester (**45**).* Yield: 224.8 mg, 88% based on starting **1a** (Table 2, entry 21). Yellow solid, mp 78-79°C (lit.3 77-78 °C). IR (KBr): ν = 3359 (s), 1720 (s), 1460 (w), 1435 (w), 1307 (m), 1240 (m), 1163 (w), 1006 (w), 746 (m) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.40 (s, br, 1 H), 7.51-7.45 (m, 1 H), 7.24-7.19 (m, 1 H), 7.15-7.05 (m, 2 H), 3.69 (s, 2 H), 3.67 (s, 3 H), 2.21 (s, 3 H); ^{13}C NMR (75

MHz, $CDCl_3$): δ = 171.1, 135.8, 129.0, 126.3, 121.8, 119.2, 118.5, 110.7, 109.0, 52.1, 31.7, 8.3 ; GC-MS: *m/z* = 203 (45) [M^+], 145 (11), 144 (100), 143 (23), 115 (9); anal. calcd for $C_{12}H_{13}NO_2$ (203.24): C, 70.92; H, 6.45; N, 6.89. Found C, 71.15; H, 6.47; N, 6.87

- *(7-Methoxy-3-methyl-1H-indol-2-yl)acetic acid methyl ester (**46**).* Yield: 122.3 mg, 42% based on starting **1b** (Table 2, entry 22). Yellow oil. IR (film): ν = 3373 (s), 1734 (s), 1575 (w), 1463 (m), 1335 (w), 1259 (s), 1171 (m), 1047 (m), 1002 (m), 773 (w), 716 (m) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.57 (s, br, 1 H), 7.11 (distorted d, *J* = 7.7, 1 H), 7.00 (t, *J* = 7.7, 1 H), 6.60 (d, *J* = 7.7, 1 H), 3.92 (s, 3 H), 3.75 (s, 2 H), 3.70 (s, 3 H), 2.23 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 145.8, 130.1, 126.0, 125.8, 119.6, 111.3, 109.5, 101.9, 55.3, 52.2, 31.8, 8.7 ; GC-MS: *m/z* = 233 (60) [M^+], 175 (12), 174 (100), 172 (11), 160 (10), 159 (29), 158 (10), 144 (10), 131 (19), 130 (16), 103 (10); anal. calcd for $C_{13}H_{15}NO_3$ (233.26): C, 66.94; H, 6.48; N, 6.00. Found C, 67.06; H, 6.47; N, 6.01.

- *(3-Phenyl-1H-indol-2-yl)acetic acid methyl ester (**47**).* Yield: 210.1 mg, 63% based on starting **1d** (Table 2, entry 23). Yellow solid, mp 83-84 °C. IR (KBr): ν = 3356 (s), 3336 (m), 1724 (s), 1431 (w), 1256 (m), 1140 (m), 1011 (w), 748 (m), 695 (m) cm-1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.84 (s, br, 1 H), 7.65 (d, br, *J* = 7.7, 1 H), 7.52-7.41 (m, 4 H), 7.35 (distorted d, *J* = 8.1, 1 H), 7.34-7.28 (m, 1 H), 7.20 (td, *J* = 7.7, 1.0, 1 H), 7.11 (td, *J* = 8.1, 1.0, 1 H), 3.88 (s, 2 H), 3.73 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.2, 136.0, 134.7, 129.8, 128.7, 127.6, 126.8, 126.4, 122.4, 120.2, 119.4, 116.6, 110.9, 52.3, 32.1; GC-MS: *m/z* = 265 (90) [M^+], 207 (18), 206 (100), 205 (34), 204 (44), 179 (32), 178 (24); anal. calcd for $C_{17}H_{15}NO_2$ (265.31): C, 76.96; H, 5.70; N, 5.28. Found C, 77.12; H, 5.68; N, 5.29.

- *2-(1,3-Dimethyl-1H-indol-2-yl)-3,3-dimethylbutyric acid methyl ester (**50**).* Yield: 149.4 mg, 44% based on starting **1e** (Table 2, entry 25). Yellow solid, mp 86-88°C. IR (KBr): ν = 3457 (m, br), 1741 (s), 1471 (m), 1331 (w), 1202 (w), 1144 (s), 1040 (w), 1021 (w), 743 (m) cm1; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57-7.51 (m, 1 H), 7.30-7.18 (m, 2 H), 7.15-7.07 (m, 1 H), 3.75 (s, 1 H), 3.71 (s, 3 H), 3.60 (s, 3 H), 2.31 (s, 3 H), 1.13 (s, 9 H); GC-MS: *m/z* = 273 (99) [M^+], 218 (10), 217 (66), 216 (99), 214 (180), 186 (30), 185 (99), 184 (100), 182 (11), 169 (12), 168 (15), 167 (11), 158 (65), 157 (26), 156 (26), 154 (10), 144 (16), 128 (10), 115 (13); anal. calcd for $C_{17}H_{23}NO_2$ (273.37): C, 74.69; H, 8.48; N, 5.12. Found C, 74.61; H, 8.49; N, 5.13

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CHAPTER 3

Recyclable catalytic synthesis of substituted quinolines: copper-catalyzed heterocyclization of 1-(2-aminoaryl)-2-yn-1-ols in ionic liquids

Bartolo Gabriele, Raffaella Mancuso, Elvira Lupinacci, Rosella Spina, Giuseppe Salerno, Lucia Veltri, and Angela Dibenedetto

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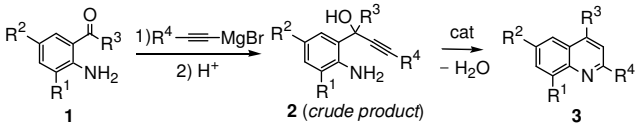
3.1 Introduction

The application of ionic liquid (ILs) as alternative reaction media has attracted increasing attention. ILs have been successfully used in a variety of catalytic reactions as environmentally benign solvents and catalysts. Ionic liquids present several useful characteristics: they are stable, non-flammable, non-volatile, recyclable, and in several cases may even promote organic reactions.*(1,2)* Another attractive facet of these solvents is related to the possibility to easily separate the products from the reaction mixture (by simple extraction procedures) and, in catalytic reactions, to recycle the solvent-catalytic system several times.*(1,2)*

Our research group has very recently reported that 1-(2-aminoaryl)-2-yn-1-ols **2**, easily obtained by the Grignard reaction between the appropriate alkynylmagnesium bromide and 2-aminoaryl ketones **1**, can undergo a selective Cu- or Pd-catalyzed 6-*endo*-dig dehydrative heterocyclization to give substituted quinolines **3** in good yields (**Scheme 1**).*(3,4)* The crude substrates **2** could be used without further purification for the subsequent step. Heterocyclizations were typically carried out in MeOH or 1,2-dimethoxyethane (DME) as the solvent at 100 °C in the presence of $CuCl_2$ (2 mol%) or PdX_2 (2 mol %) in conjunction with an excess of KX (X = Cl, I) as the catalyst.



Scheme 1. Heterocyclodehydration of 1-(2-aminoaryl)-2-yn-1-ols **2** (crude products obtained by alkynylation of 2-aminoaryl ketones **1**) leading to quinolines **3**.

In this thesis, it has been found that substituted quinolines **3** can be synthetized in ionic liquids as the reaction media, using 1-2 mol % of $CuCl_2$, and

that the solvent-catalyst system can be conveniently recycled several times without appreciable loss of catalytic activity.

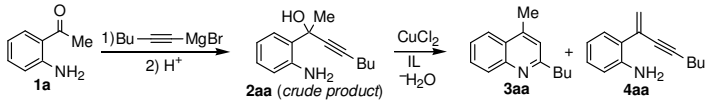
3.2 Results and discussion

As first step, the reactivity of the 2-(2-aminophenyl)-oct-3-yn-2-ol (**2a**) (R^1 = R^2 = H, R^3 = Me, R^4 = Bu) in the ionic liquid $BmimBF_4$ (1-butyl-3-methylimidazolium tetrafluoroborate) was examined. The reaction of **2aa** was carried out in the presence of $CuCl_2$ (2 mol %) at 100 °C for 15 h. The result was very promising since the corresponding quinoline **3aa** was obtained in 71% yield based on starting 2-aminoacetophenone **1a** (**Table 1**, entry 1, run 1) *(5)*, showing that heterocyclodehydration reaction could indeed occur in an ionic liquid as the solvent. The possibility to recycle the solvent-catalyst was then verified. In order to separate the product, the reaction crude was extracted several times with Et_2O, while freshly prepared **2aa** was added to the ionic liquid phase, and the resulting mixture let to react again under the abovementioned conditions (see the Experimental Section for details) As can be seen from the results shown in **Table 1**, entry 1, runs 2-7, no significant decrease of activity was observed, even after the sixth recycle.

When the reaction was carried out with 1 mol % of catalyst for 15 h, a mixture of **3aa** (66% yield) and the enynic derivative **4aa** (7%), deriving from dehydration of the alcoholic function of the substrate, was obtained (**Table 1**, entry 2, run 1). Similar results were observed after 4 recycles (**Table 1**, entry 2, runs 2-5). We have verified that **4aa** can be a possible intermediate in the formation of **3aa**: in fact, when pure **4aa** was let to react under the same reaction conditions as those reported in **Table 1** (entry 2), **3aa** was obtained in 95% isolated yield. In agreement with this result, when the reaction of Entry 2 was carried out for 24 h rather than 15 h, no formation of **4aa** was observed, and the yield of **3aa** increased to 76% (**Table 1**, entry 3, run 1). The yield obtained under these conditions is similar to that

observed in MeOH as the solvent (80%), which, however, was obtained using 2 mol % of $CuCl_2$.

Table 1: Reactions of 2-(2-aminophenyl)oct-3-yn-2-ol (**2aa)** in $BmimBF_4$ [a] .

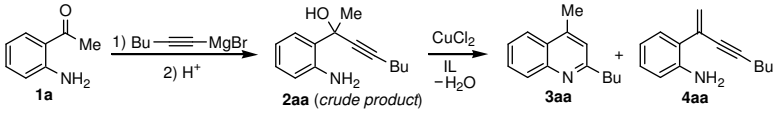


Entry	Solvent	Mol % of $CuCl_2$[b]	Time [h]	Run	Yield of 3aa [%][c]	Yield of 4aa [%][c]
1	$BmimBF_4$	2	15	1	71	
				2	74	
				3	70	
				4	69	
				5	70	
				6	71	
				7	70	
2	$BmimBF_4$	1	15	1	66	7
				2	65	5
				3	63	5
				4	64	5
				5	66	4
3	$BmimBF_4$	1	24	1	76	
				2	70	
				3	71	
				4	69	
				5	68	
				6	72	
				7	68	

[a]All reactions were carried out at 100 °C in $BmimBF_4$ as the solvent (0.22 mmol of starting 2-aminoacetophenone **1a** per mL of solvent, 1 mmol scale based on **1a**). Conversion of **2aa** was quantitative in all cases. [b]Mol % of $CuCl_2$ with respect to starting **1a**. [c]Isolated yield based on starting **1a**.

The effect of the nature of the ionic liquid on the reactivity was then tested. **Table 2** shows the results achieved using $BmimNTf_2$, BmimOTf, BmimCl, and $BmimPF_6$. As can be seen, among the LIs tested, $BmimBF_4$ led to the most satisfactory results. In particular, when the reaction was carried out in $BmimNTf_2$ and BmimOTf, quinoline **3aa** was obtained in consistently lower yields of with respect to that achieved with $BmimBF_4$ (compare **Table 2**, entries 1 and 2 with **Table 1**, entry 3). In the case of BmimCl, the main product **3aa** was obtained in a mixture with **4aa** (**Table 2**, entry 3). With $BmimPF_6$, the yields in the first two runs (**Table 2**, entry 4, runs 1-2) were higher with respect to those observed with $BmimBF_4$ (**Table 1**, entry 3, runs 1-2). However, starting from the third recycle, the reaction led to a mixture of **3aa** and **4aa** (**Table 2**, entry 4, runs 3-7).

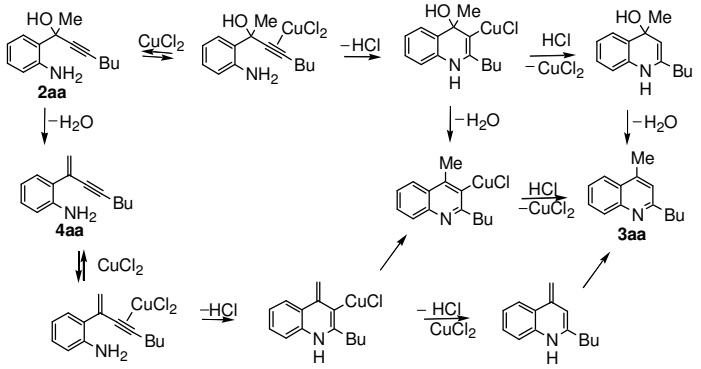
Table 2: Synthesis of 2-butyl-4-methyl-quinoline **3aa** by $CuCl_2$-catalyzed heterocyclization of 2-(2-amino-phenyl)-oct-3-yn-2-ol **2aa** in ionic liquids [a]



Entry	Solvent	Mol % of $CuCl_2$	Time [h]	Run	Yield of 3aa [%][b]	Yield of 4aa [%][b]
1	$BmimNTf_2$	1	24	1	76	
					70	
					71	
					69	
					68	
					72	
					68	
2	BmimOTf	1	24	1	51	
					48	
					54	
					53	
					58	
					56	
					55	
3	BmimCl	1	24	1	48	17
				2	40	16
				3	38	15
				4	42	18
				5	43	20
4	$BmimPF_6$	1	24	1	82	
				2	76	
				3	67	6
				4	59	10
				5	60	11
				6	56	11
				7	48	18

[a] All reactions were carried out in the presence of 1 Mol % of $CuCl_2$ with respect to starting **1a** at 100 °C for 24 h in the given ionic liquid as the solvent (0.22 mmol of starting 2-aminoacetophenone **1a** per mL of solvent, 1 mmol scale based on **1a**). Conversion of **2aa** was quantitative in all cases. [b] Isolated yield based on starting **1a.**

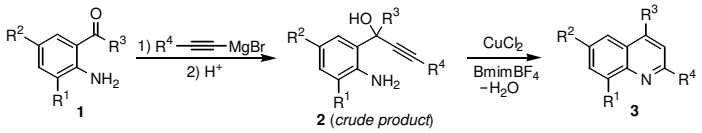
A plausible reaction mechanism for the formation of **3aa** from **2aa** or **4aa** is shown in **Scheme 2**. The key steps of the mechanism involve the 6-*endo*-dig nucleophilic attack of the amino group to the triple bond of **2aa** or **4aa** coordinated to $CuCl_2$, followed by protonolysis and aromatization or vice versa.



Scheme 2. Proposed reaction mechanism for the conversion of 2-(2-aminophenyl)oct-3-yn-2-ol **2aa** and 2-(1-methylenehept-2-ynyl)aniline **4aa** into 2-butyl-4-methylquinoline **3aa**.

The next experiments, aimed at generalizing the process to the use of variously substituted 1- (2-aminoaryl)-2-yn-1-ols, were therefore carried out using $BmimBF_4$ as the solvent, under the same conditions as those of entry 3, **Table 1**. The results obtained with substrates bearing different substituents on the triple bond, at the benzylic position, and on the phenyl ring are shown in **Table 3**.

Table 3: Synthesis of quinolines **3** by heterocyclization $CuCl_2$-catalyzed of 1-(2-aminoaryl)-2-yn-1-ols **2** in $BmimBF_4$ [a]



Entry	1	R^1	R^2	R^3	R^4	2	Mol % $CuCl_2$[b]	3	Run	Yield of 3 [%][c]
1	**1b**	OMe	H	Me	Bu	**2ba**	1	**3ba**	1	46[d]
2	**1b**	OMe	H	Me	Bu	**2ba**	2	**3ba**	1	73
									2	66
									3	68
									4	66
									5	65
									6	66
									7	66
3	**1c**	H	Cl	Me	Bu	**2ca**	1	**3ca**	1	71
									2	69
									3	66
									4	65
							1		5	66
									6	65
									7	65
4[e]	**1d**	H	H	Ph	Bu	**2da**	1	**3da**	1	81
									2	81
									3	75
									4	83
									5	79
									6	76
									7	75

5	**1a**	H	H	Me	tBu	**2ab**	1	**3ab**	1	70
									2	68
									3	62
									4	60
									5	63
									6	61
									7	61
6	**1a**	H	H	Me	TMS	**2ac**	1	**3ac**[f]	1	57[g]
7	**1a**	H	H	Me	TMS	**2ac**	2	**3ac**[f]	1	61
									2	59
									3	60
									24	58
									35	60
									46	61
									7	61
8	**1a**	H	H	Ph	Ph	**2dd**	2	**3dd**	1	60
									2	58
									3	57
									4	55
									5	51
									6	50
									7	52

[a] Unless otherwise noted, all reactions were carried out at 100 °C for 24 h in BmimBF4 as the solvent (0.22 mmol of starting 2-aminoarylchenone **1** per mL of solvent, 1 mmol scale based on **1**). Conversion of **2** was quantitative in all cases. [b] Mol % of $CuCl_2$ with respect to starting 1a. [c] Isolated yield based on starting **1**. [d] The reaction also led to the formation of 2-methoxy-6-(1-methylenehept-2-ynyl)aniline **4ba** in 9% isolated yield. [e] Reaction time was 15 h. [f] R^4 = H in the final quinoline **3ac**. [g] The reaction also led to the formation of 2-(1-methylene-3-trimethylsilanylprop-2-ynyl)aniline **4ac** in 18% isolated yield.

As can be seen, the corresponding quinolines were consistently obtained in good yields, and in all cases the ionic liquid containing the catalyst could be recycled and reused up to 6 times without appreciable loss of activity. In some cases, the reaction led to better results working with 2 mol % of $CuCl_2$ rather than 1 mol % (entries 2, 7-8). In the case of 2-(2-aminophenyl)-4- trimethylsylanylbut-3-yn-2-ol **2ac** (R^1 = R^2 = H, R^3 = Me, R^4 = TMS), as we already observed in the process carried out in DME, *(3)* the TMS group was lost in the course of the process (entries 6-7).

3.3 Conclusion

In conclusion, in this part of the thesis I have shown that an ionic liquid, such as $BmimBF_4$ can be efficiently used as the reaction medium for the $CuCl_2$-catalyzed heterocyclodehydration of 1-(2-aminoaryl)-2-yn-1-ols. The yields obtained in $BmimBF_4$ are similar to those obtained under the "classical" conditions (in MeOH or DME as the solvent); *(3)* however, the use of the ionic liquid has allowed to recycle the solvent-catalytic system several times, without significant loss of activity. The present recyclable catalytic method for synthesis of substituted quinolines thus represents a simple and convenient approach for the production of a very interesting class of heterocyclic compounds, whose importance in various fields of Science is well known. *(6-9)*

3.4 Experimental Section

Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C on a Bruker DPX Avance 300 spectrometer in $CDCl_3$ solutions at 300 MHz and 75 MHz, respectively, with Me_4Si as internal standard. Chemical shifts (δ) and coupling constants (J) are given in ppm and in Hz, respectively. IR spectra were taken with a Jasco FT-IR 4200 spectrometer. Mass spectra were obtained using a Shimadzu QP-2010 GCMS apparatus at 70 eV ionization voltage. Microanalyses were carried out

with a Carlo Erba Elemental Analyzer Mod. 1106. All reactions were analyzed by TLC on silica gel 60 F254 and by GLC using a Shimadzu GC-2010 gas chromatograph and capillary columns with polymethylsilicone + 5% phenylsilicone as the stationary phase (HP-5). Column chromatography was performed on silica gel 60 (Merck, 70-230 mesh).

3.4.1 Preparation of Substrates and Ionic Liquids.

2-Aminoacetophenone **1a** and 2-aminobenzophenone **1d** were commercially available (Aldrich, Fluka) and were used as received. 2-Amino-3-methoxyacetophenone **1b** and 2-amino-5- chloroacetophenone **1c** were prepared as we already reported. *(3)* Ionic liquids BmimNTf$_2$ *(10)* BmimOTf *(11)* were prepared according to literature procedures. All the other ionic liquids were prepared as described below.

3.4.2 Preparation of BmimCl.

A mixture of 1-methylimidazole (40 mL, 41.2 g, 502 mmol) and toluene (50 mL) maintained at 0 °C under nitrogen was stirred for 10 min. 1-Chlorobutane (58 mL, 51.4 g, 555 mmol) was quickly added at 0 °C and the resulting mixture was vigorously stirred for 15 min. at the same temperature. The solution was allowed to warm up to room temperature and then heated at 110 °C for 24 h with stirring. After cooling to room temperature, the mixture was refrigerated (-20 °C) and allowed to stand for 24 h. After this time, two phases separated; toluene was removed by decantation, while the residue was taken up with MeCN. The solvent was removed under vacuum and MeCN (ca. 30 mL) and THF (ca. 30 mL) were added. The resulting mixture was cooled with the aid of an ice-water bath, to give, on standing, BmimCl as a whitish solid. The mixture was then cooled at -20 °C overnight. After decantation and removal of the solvent, the residue was washed with cold THF and eventually dried in vacuo to give pure BmimCl as a whitish solid, which was stored at –20 °C under nitrogen (77.6 g, 89%).

3.4.3 Preparation of $BmimBF_4$.

$NaBF_4$ (5.7 g, 51.9 mmol) was added to 9.0 g (51.8 mmol) of BmimCl maintained at 80 °C under vigorous stirring. The mixture was allowed to stir at 80 °C for 8 h and then at room temperature for 15 h. CH_2Cl_2 (ca. 30 mL) was added with stirring, and the solution was cooled to –20 °C and allowed to stand at this temperature overnight. The precipitate (NaCl) was removed by filtration, and the solvent was removed under vacuum to give pure $BmimBF_4$, which was stored under nitrogen at room temperature (9.3 g, 80%).

3.4.4 Preparation of $BmimPF_6$.

KPF_6 (9.5 g, 51.6 mmol) was added to 9.0 g (51.8 mmol) of BmimCl maintained at 80 °C under vigorous stirring. The mixture was allowed to stir at 80 °C for 8 h and then at room temperature for 15 h. CH_2Cl_2 (ca. 30 mL) was added with stirring, and the solution was cooled to –20 °C and allowed to stand at this temperature overnight. The precipitate (KCl) was removed by filtration, and the solvent was removed under vacuum to give pure $BmimPF_6$, which was stored under nitrogen at room temperature (11.8 g, 81%).

*3.4.5 General Procedure for the Synthesis of Quinolines **3** in Ionic Liquids (Tables 1-3).*

To a suspension of Mg turnings (700.0 mg, 28.8 mmol) in anhydrous THF (2.0 mL), maintained under nitrogen and under reflux, was added pure EtBr (0.5 mL) to start the formation of the Grignard reagent. The remaining bromide was added dropwise (ca. 20 min) in THF solution (1.5 mL of EtBr in 15.0 mL of THF; total amount of EtBr added: 2.92 g, 26.8 mmol). The mixture was then allowed to reflux for additional 20 min. After cooling, the solution of EtMgBr thus obtained was transferred under nitrogen to a dropping funnel and was added dropwise to a solution of the 1-alkyne (26.8 mmol) in anhydrous THF (7.0 mL) at 0 °C with stirring. After additional stirring at 0 °C for 15 min, the mixture was allowed to warm up to room temperature, maintained at 50 °C for 2 h, and then used as such

for the next step. 2-Amino ketone **1** (8.9 mmol) was dissolved under nitrogen in anhydrous THF (7.0 mL) and then added dropwise to the solution of the alkynylmagnesium bromide in THF (prepared as described above) at 50 °C under nitrogen. After stirring at 50 °C for 1 h ($R^1 = R^2 = H$, $R^3 = Me$, $R^4 = Bu$; $R^1 = OMe$, $R^2 = H$, $R^3 = Me$, $R^4 = Bu$; $R^1 = H$, $R^2 = Cl$, $R^3 = Me$, $R^4 = Bu$), 2 h ($R^1 = R^2 = H$, $R^3 = Me$, $R^4 = t\text{-}Bu$; $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = TMS$) or 3 h ($R^1 = R^2 = H$, $R^3 = Ph$, $R^4 = Bu$; $R^1 = R^2 = H$, $R^3 = R^4 = Ph$), the mixture was cooled to room temperature. Saturated NH_4Cl was added with stirring to achieve a weakly acidic pH. After additional stirring at room temperature for 15 min, AcOEt (ca. 20 mL) was added and phases were separated. The aqueous phase was extracted with AcOEt (3 x 30 mL), and the collected organic layers were washed with brine to neutral pH and eventually dried over Na_2SO_4. After filtration, the solvent was evaporated and crude products **2** were diluted with Et_2O and transferred into a volumetric flask (50 mL). 6.2 mL of the solution (formally deriving from 1.10 mmol of **1**) were transferred under nitrogen to a Schlenk flask containing the ionic liquid (5.0 mL) and $CuCl_2$ (1.5 mg, 1.1 x 10^{-2} mmol, **Tables 1**, entries 2-3; **Tables 2**, entries 1-4, or 3.0 mg, 2.2 x 10^{-2} mmol, **Tables 1**, entry 1; **Table 3**, entries 2, 7-8). Et_2O was removed under vacuum, and the resulting mixture was heated at 100 °C for 15 h (**Table 2**, entries 1-2; **Table 3**, entry 4) or 24 h (**Table 1**, entry 3; **Table 2**, entries 1-4; **Table 3**, entries 1-3, 5-8). After cooling, the product was extracted with Et_2O (6 x 4 mL), and the residue (still containing the catalyst dissolved in the ionic liquid) was used as such for the next recycle (see below). The collected ethereal phases were concentrated and the product purified by column chromatography (SiO_2, hexane-AcOEt from 99:1 to 95:5) to give pure quinolines **3**. In the case of the reactions also leading to the formation of enynes **4** (**Table 1**, entry 2; **Table 2**, entries 3-4; **Table 3**, entries 1-6), the order of elution was **4**, **3**. The isolated yields obtained in each experiment are reported in **Tables 1-3**.

3.4.6 Recycling Procedure (Tables 1-3).

To the residue obtained as described above, still containing the catalyst dissolved in the ionic liquid, were added 6.2 mL of the ethereal solution containing crude **2**. Et_2O was removed under vacuum, and then the same procedure described above was followed.

*3.4.7 Conversion of 2-(1-Methylenehept-2-ynyl)aniline **4aa** into 2-Butyl-4-methylquinoline **3aa**.*

A solution of pure **4aa** (297.0 mg, 1.49 mmol) in Et_2O (3.0 mL) was transferred under nitrogen to a Schlenk flask containing $BmimBF_4$ (6.8 mL) and $CuCl_2$ (2.0 mg, 1.5 x 10^{-2} mmol). Et_2O was removed under vacuum, and the resulting mixture was heated at 100 °C for 15 h. After cooling, the product was extracted with Et_2O (6 x 4 mL). The collected ethereal phases were concentrated, and the residue was purified by column chromatography (SiO_2, 95:5 hexane-AcOEt) to give 281.7 mg of quinoline **3aa** (95%).

3.5 Characterization of Products

All quinolines **3** were characterized by comparison with literature data.*(3)* Enynic derivatives **4** were fully characterized by elemental analysis, MS spectrometry, and IR, ^1H NMR, and ^{13}C NMR spectroscopies, as reported below.

- *2-(1-Methylenehept-2-ynyl)aniline (**4aa**).*Pale yellow oil. IR (film): ν = 3462 (m, br), 3375 (m, br), 2957 (m), 2931 (m), 2871 (w), 2220 (w), 1618 (s), 1494 (m), 1455 (m), 1308 (m), 904 (m), 748 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (1 H, dd, J=7.8 Hz, 1.6 Hz, H-3), 7.12-7.05 (1 H, m, H-5), 6.72 (1 H, td, J=7.5 Hz, 1.2 Hz, H-4), 6.65 (1 H, dd, J=7.7 Hz, 1.2 Hz, H-6), 5.67 (1 H, d, J=2.0 Hz, =CHH), 5.56 (1 H, d, J=2.0 Hz, =CHH), 4.15 (1 H, s, br, NH_2), 2.34 (2 H, t, J=7.1 Hz, ≡CCH_2), 1.60-1.34 (4 H, m,

$CH_2CH_2CH_3$), 0.91 (3 H, t, J=7.3 Hz, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.7, 129.7, 129.4, 128.9, 125.2, 124.3, 118.3, 116.0, 92.1, 79.9, 30.7, 22.1, 19.1, 13.6. MS (70 eV, EI): m/z (%): 199 (64) [M^+], 184 (9), 170 (35), 157 (100), 156 (85), 155 (27), 154 (40), 144 (16), 130 (38), 129 (43), 128 (44), 127 (20), 115 (20), 89 (13), 77 (28). Anal. Calcd for $C_{14}H_{17}N$ (199.29): C 84.37, H 8.60, N 7.03. Found: C 84.45, H 8.56, N 7.01.

- *2-Methoxy-6-(1-methylenehept-2-ynyl)aniline (**4ba**)*. Pale yellow oil. IR (film): ν = 3471 (m, br), 3378 (m, br), 2957 (s), 2932 (s), 2864 (w), 2231 (w), 1614 (m), 1562 (m), 1475 (s), 1287 (m), 1211 (m), 1048 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.84 (1 H, distorted dd, J=7.3 Hz, 1.6 Hz, H-3), 6.78-6.65 (1 H, m, H-4+H-5), 5.68 (1 H, distorted d, J=2.0 Hz, =CHH), 5.59 (1 H, distorted d, J=2.0 Hz, =CHH), 4.36 (2 H, s, br, NH_2), 3.85 (3 H, s, OMe), 2.35 (2 H, t, J=7.1 Hz, $\equiv CCH_2$), 1.60-1.35 (4 H, m, $CH_2CH_2CH_3$), 0.91 (3 H, t, J=7.3 Hz, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.1, 133.9, 129.5, 124.9, 124.2, 121.4, 117.2, 109.6, 92.0, 79.9, 55.7, 30.7, 22.1, 19.1, 13.6. MS (70 eV, EI): m/z (%): 229 (100) [M^+], 214 (14), 200 (19), 187 (51), 186 (40), 172 (54), 171 (27), 170 (27), 154 (25), 144 (17), 127 (15), 115 (22). Anal. Calcd for $C_{15}H_{19}NO$ (229.32): C 78.56, H 8.35, N 6.11. Found: C 78.65, H 8.33, N 6.10.

- *2-(1-Methylene-3-trimethylsilanylprop-2-ynyl)aniline (**4ac**).* Pale yellow oil. IR (film): ν = 3466 (m, br), 3378 (m, br), 2960 (m), 2144 (m), 1620 (s), 1495 (m), 1455 (m), 1250 (s), 957 (m), 842 (vs), 759 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.16 (1 H, distorted ddd, J=7.5 Hz, 1.6 Hz, 0.3 Hz, H-3), 7.09 (1 H, distorted ddd, J=8.0 Hz, 7.5 Hz, 1.6 Hz, H-5), 6.72 (1 H, td, J=7.5 Hz, 1.1 Hz, H-4), 6.64 (1 H, distorted ddd, J=8.0 Hz, 1.1 Hz, 0.3 Hz, H- 6), 5.79 (1 H, d, J=1.8 Hz, =CHH), 5.67 (1 H, d, J=1.8 Hz, =CHH), 4.17

(2 H, s, br, NH_2), 0.20 (9 H, s, TMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ= 144.2, 129.8, 129.7, 129.5, 126.8, 124.4, 118.7, 116.4, 104.3, 96.3, 0.2. MS (70 eV, EI): m/z (%): 215 (100) [M^+], 200 (97), 198 (33), 184 (44), 174 (16), 170 (15), 160 (56), 100 (14). Anal. Calcd for $C_{13}H_{17}NSi$ (215.37): C 72.50, H 7.96, N 6.50. Found: C 72.64, H 7.99, N 4.47.

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CHAPTER 4

Tandem catalysis in ionic liquids: a recyclable catalytic synthesis of benzofuran derivatives

Bartolo Gabriele, Raffaella Mancuso, Elvira Lupinacci, Giuseppe Salerno, Lucia Veltri,

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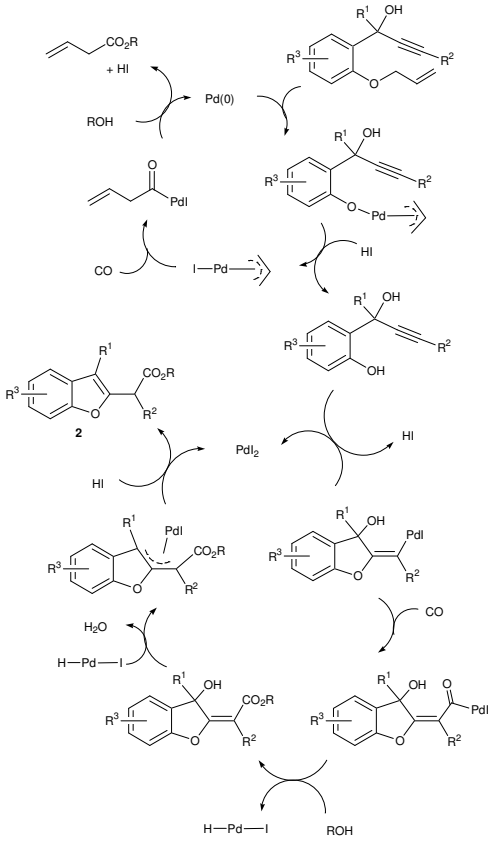
4.1 Introduction

Cascade reactions are powerful synthetic tools for constructing functionalized molecules in one step starting from simple and readily available building blocks *(1)* In this view, 'sequential' or 'tandem' catalysis, in which the product of a first catalytic cycle becomes the substrate of a second catalytic cycle and so on, has recently acquired a growing importance in synthesis (**Scheme 1**) *(2)*.

Scheme 1

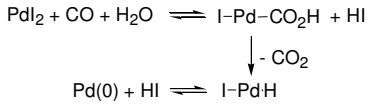
Some years ago, our research group disclosed a novel approach to benzofuran-2-acetic esters **2** starting from 1-(2-allyloxyphenyl)-2-yn-1-ols **1** through the sequential combination between two catalytic cycles (**Scheme 2**) *(3)*



Scheme 2

The first cycle corresponded to the deprotection of the phenolic oxygen of **1**, while the second process corresponded to a carbonylative heterocyclization process eventually leading to the final benzofuran derivative in high yields and selectivities. For this particular type of tandem catalysis we coined the term 'sequential homobimetallic catalysis', since the two cycles were catalyzed by the same metal, but in two different oxidation states: in particular, the first cycle was catalyzed by a Ph_3P-stabilized Pd(0) species, while the second cycle was catalyzed by a PdI_2-based species (**Scheme 2**).

Reactions were typically carried out in MeOH as the solvent at 100 °C and under 30-90 atm of CO, in the presence of catalytic amounts of PdI_2 in conjunction with KI, PPh_3, and H_2O. Under these conditions, PdI_2, besides being the catalyst for the second catalytic cycle, also acted as a precursor for the formation in situ of the Pd(0) species promoting the first catalytic cycle, according to **Scheme 3**.

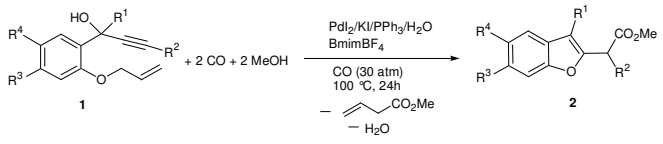


Scheme 3

In this part of the thesis, I report the results obtained when the tandem catalytic process was carried out in ionic liquids (ILs) as the reaction media, using MeOH as external nucleophile. Ionic liquids are a well established class of non-conventional solvents, characterized by low flammability, low volatility, and low toxicity *(4,5)*. A very attractive feature of ILs is the possibility to recycle them and to easily recover the product (by simple extraction procedures). Moreover, in the case of catalytic reactions, it may also be possible to recycle the catalyst-solvent system several times, which makes the use of ILs particularly convenient *(4,5)*.

4.2 Results and discussion

We have found that the catalytic transformation of 1-(2-allyloxyphenyl)-2-yn-1-ols **1** into benzofurans **2** can be expediently accomplished in an ionic liquid, such as $BmimBF_4$, as the reaction medium (**Eq.1**), and that the solvent-catalytic system can be recycled several times without appreciable loss of catalytic activity.

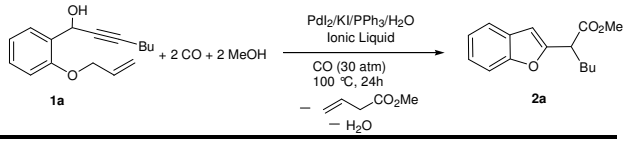


(1)

The possibility to employ an ionic liquid as the solvent for this reaction and to recycle the catalyst thus makes the process both more practical and attractive. In fact, the product can be easily separated from the palladium catalyst, by a simple extraction procedure. Even more importantly, particularly for a rather expensive metal, such as palladium, the catalyst recyclability allows for a significant increase of the ratio between the total amount of product obtained and the amount of catalyst employed with respect with the reaction carried out in conventional solvents.

The results obtained from the reaction of 1-(2-allyloxyphenyl) hept-2-yn-1-ol **1a** ($R^1 = R^3 = R^4 = H$, R^2 =Bu) (270 mg, 1 equiv) carried out in different ionic liquids as the solvent (3.75 mL) at 100 °C under 30 atm of CO for 24 h, in the presence of PdI_2 (4.0 mg, 1 mol %), KI (183 mg, 1 equiv), PPh_3 (11.5 mg, 4 mol %), H_2O (40 mL, 2 equiv), and MeOH (1.25 mL, 28 equiv) are shown in **Table 1.**

Table 1. Reactions of 1-(2-allyloxyphenyl)hept-2-yn-1-ol **1a** with CO in the presence of PdI_2/KI/PPh_3/H_2O/MeOH in different ionic liquids (ILs)[a]



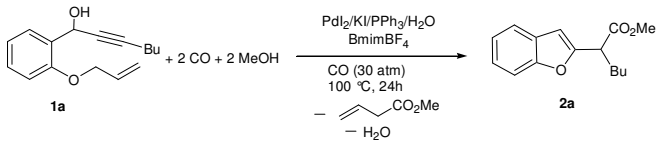
Entry	Solvent	Conversion of 1a (%)[b]	Yield of 2a [%][c]
1	BmimCl	100	20
2	$BmimBF_4$	100	84
3	$BmimNTf_2$	99	65
4	$BmimPF_6$	0	–

[a]All reactions were carried out at 100 °C under 30 atm of CO for 24 h in the given ionic liquid as the solvent (3.75 mL), in the presence of PdI_2 (4.0 mg, 1 mol %), KI (183 mg, 1 equiv), PPh_3 (11.5 mg, 4 mol %), H_2O (40 mL, 2 equiv), **1a** (270.0 mg, 1 equiv), and MeOH (1.25 mL, 28 equiv). Conversion of **1a** was quantitative in all cases.[b]Based on starting **1a**, by GLC analysis of the ethereal extract. See the Experimental section for details.[c]Isolated yield based on starting **1a**.

As can be seen from the Table, the sequential catalytic process leading to the corresponding benzofuran derivative (2-benzofuran-2-ylhexanoic acid methyl ester, **2a**) took place in BmimCl, $BmimBF_4$, and $BmimNTf_2$ (**Table 1**, entries 1-3), even though with appreciable differences in the product yield,while it did not work in $BmimPF_6$ (**Table 1**, entry 4). The best yield of **2a** (84%) was obtained in $BmimBF_4$ (**Table 1**, entry 2), which was accordingly chosen as the reference solvent for the next experiments.

The recyclability of the catalyst-solvent system was then assessed. The reaction crude was extracted several times with Et_2O, in order to separate the product, while a freshly prepared solution of **1a** (270 mg, 1 equiv) and H_2O (40 mL, 2 equiv) in MeOH (1.25 mL) was added to the ionic liquid phase. The resulting mixture was then allowed to react again under the usual conditions. The results obtained after six recycles, shown in **Table 2** (entry 1), show that the catalytic activity of the ionic liquid phase slightly decreased after the first recycle, and then remained practically unchanged even after the sixth recycle. The decrease in yield of **2a** after the first run may be due to a partial deactivation of the catalytic system, which, however, after the second recycle, tended to maintain its activity quite constant. We refrain to propose a straightforward explanation for this behavior, which would require additional investigations. It is worth noting that it was not necessary to add PPh_3 after each recycle. In fact, when fresh PPh_3 was added, the results turned out to be less satisfactory, and lower yields of the product were obtained after the recycling procedure (**Table 2**, entries 2 and 3).

Table 2. Recyclable catalytic synthesis of 2-benzofuran-2-ylhexanoic acid methyl ester **2a** by Pd-catalyzed carbonylative heterocyclization of 1-(2-allyloxyphenyl)hept-2-yn-1-ol **1a** in $BmimBF_4$[a]

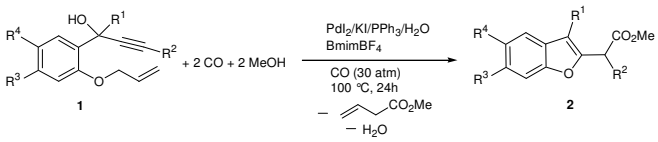


Entry	Mol % PPh_3[b]	Run	Yield of 2aa [%][c]
1	4	1	84
		2	68
		3	69
		4	68
		5	66
		6	66
		7	67
2	4	1	84
	1	2	63
	1	3	63
	1	4	62
	1	5	60
	1	6	60
	1	7	60
3	4	1	83
	4	2	63
	4	3	60
	4	4	60
	4	5	61
	4	6	62
	4	7	60

[a] All reactions were carried out at 100 °C under 30 atm of CO for 24 h in $BmimBF_4$ as the solvent, (3.75 mL) in the presence of PdI_2 (4.0 mg, 1 mol %), KI (183 mg, 1 equiv), PPh_3 (see footnote b), H_2O (40 mL, 2 equiv), **1a** (270.0 mg, 1 equiv) and MeOH (1.25 mL, 28 equiv). Conversion of **1a** was quantitative in all cases. [b] Mol % of PPh3 added in each run to the ionic liquid phase. [c] Run 1 corresponds to the first experiment, the next runs to recycles. See text for details.[d] Isolated yield based on starting **1a**.

Having established the feasibility of the sequential homobimetallic catalytic process leading to **2a** in an ionic liquid, and the possibility to recycle to catalyst-solvent system, we then tested the reactivity of differently substituted substrates **1b-i** in order to verify the general applicability of the recyclable synthetic procedure. The results obtained using substrates bearing a π-donating or electron-withdrawing group on the ring, an aryl or sterically demanding substituent on the triple bond, or an additional substituent a to the hydroxyl group are shown in **Table 3**.

Table 3. Recyclable catalytic synthesis of benzofuran-2-acetic esters **2** by Pd-catalyzed carbonylative heterocyclization of 1-(2-allyloxyphenyl)-2-yn-1-ols **1** in $BmimBF_4$[a]



Entry	1	R^1	R^2	R^3	R^4	2	Run[b]	Yield of 3 [%][c]
1	**1b**	Me	Bu	H	H	**2b**	1	84
							2	85
							3	85
							4	80
							5	86
							6	85
							7	88
2	**1c**	Ph	Bu	H	H	**2c**	1	84
							2	82
							3	82
							4	79
							5	83
							6	81
							7	82
3	**1d**	H	Ph	H	H	**2d**	1	66
							2	63
							3	64
							4	65
							5	66
							6	65
							7	65
4	**1e**	H	*t*-Bu	H	H	**2e**	1	76
							2	77
							3	80
							4	79
							5	73
							6	74
							7	73

5	**1f**	H	Bu	OMe	H	**2f**	1	80
							2	78
							3	81
							4	82
							5	79
							6	77
							7	80
6	**1g**	H	Bu	H	OMe	**2g**	1	76
							2	75
							3	79
							4	78
							5	70
							6	70
							7	72
7	**1h**	H	Bu	H	Cl	**2f**	1	85
							2	81
							3	78
							24	72
							35	77
							46	78
							7	81
8	**1i**	H	Ph	H	Cl	**2i**	1	80
							2	75
							3	76
							4	77
							5	74
							6	78
							7	79

[a] All reactions were carried out at 100 °C under 30 atm of CO for 24 h in $BmimBF_4$ as the solvent (3.75 mL), in the presence of PdI_2 (4.0 mg, 1 mol %), KI (183 mg, 1 equiv), PPh_3 (11.5 mg, 4 mol %), H2O (40 mL, 2 equiv), **1** (1 equiv), and MeOH (1.25 mL, 28 equiv). Conversion of **1** was quantitative in all cases. [b] Run1corresponds to thefirst experiment, thenext runstorecycles. See text for details. [c] Isolated yield based on starting **1**.

As can be seen from the Table, the reaction worked nicely in all cases, thus allowing a general recyclable synthesis of benzofuran-2-acetic esters from readily available substrates.

4.3 Conclusion

In conclusion, it has been shown that the sequential homobimetallic catalytic process, consisting of Pd(0)-catalyzed deallylation followed by Pd(II)-catalyzed carbonylative heterocyclization, leading to benzofuran-2-acetic esters **2** from 1-(2-allyloxyphenyl)-2-yn-1-ols **1**, can be conveniently carried out in an ionic liquid, such as $BmimBF_4$, as the solvent. The use of $BmimBF_4$ allows both an easy removal of the product from the reaction mixture and the possibility to recycle the solvent-catalyst system several times without appreciable loss of catalytic activity. Benzofuran-2-acetic ester derivatives **2** are an important class of benzofuran derivatives *(3b,6-8)*, which are known to display interesting biological activities *(9)*. The present recyclable catalytic method represents an attractive, practical, and convenient approach for their production, starting from readily available starting materials.

4.4 Experimental section

Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded at 25 °C on a Bruker DPX Avance 300 spectrometer in $CDCl_3$ solutions at 300 MHz and 75 MHz, respectively, with Me_4Si as internal standard. IR spectra were taken with a Jasco FT-IR 4200 spectrometer. Mass spectra were obtained using a Shimadzu QP-2010 GC-MS apparatus at 70 eV ionization voltage. Microanalyses were carried out with a Carlo Erba Elemental Analyzer Mod. 1106. All reactions were analyzed by TLC on silica gel 60 F_{254} and by GLC using a Shimadzu GC-2010 gas chromatograph and capillary columns with polymethylsilicone +5% phenylsilicone as the stationary phase (HP-5). Column chromatography was performed on silica gel 60 (Merck, 70-230 mesh).

4.4.1 Preparation of substrates and ionic liquids.

Substrates **1a-i** were prepared as we already described *(3)*. Ionic liquids BmimCl and $BmimBF_4$ were prepared as we already reported *(10)*. Ionic liquids

$BmimNTf_2$ *(11)* and BmimOTf *(12)* were prepared according to literature procedures.

*4.4.2 General procedure for the synthesis of benzofuran-2-acetic esters **2** in ionic liquids (Tables 1-3).*

A 35 mL stainless steel autoclave was charged with PdI_2 (4.0 mg, 1.1 x 10^{-2} mmol), KI (183 mg, 1.1 mmol), PPh_3 (11.5 mg, 4.4 x 10^{-2} mmol), and a solution of **1** (1.1 mmol) in anhydrous MeOH (1.25 mL, 30.8 mmol). The ionic liquid (3.75 mL) and H_2O (40 mL, 2.2 mmol) were then added, and the autoclave was sealed, purged at room temperature several times with CO with stirring (10 atm) and eventually pressurized at 30 atm. After stirring at 100 °C for 24 h, the autoclave was cooled and degassed. The mixture was then extracted with Et_2O (6 x 4 mL), and the residue (still containing the catalyst dissolved in the ionic liquid) was used as such for the next recycle (see below). The collected ethereal phases were concentrated and the product purified by column chromatography on silica gel to give pure benzofuran-2-acetic esters **2** (eluent: 1:1 hexane-CH_2Cl_2 for **2a**; 9:1 hexane-AcOEt for **2b**; 95:5 hexane-AcOEt for **2c**; 8:2 hexane-acetone for **2d** and **2i**; 8:2 hexane-AcOEt for **2e**, **2f**, and **2g**; 7:3 hexane-AcOEt for **2h**), whose characterization data agreed with those we already reported *(3)*. The isolated yields obtained in each experiment are given in **Tables 1-3**.

4.4.3 Recycling procedure (Tables 2 and 3).

After removal of Et_2O under vacuum, the residue obtained as described above, still containing the catalyst dissolved in the ionic liquid,was transferred into the autoclave. A solution of **1** (1.1 mmol) in anhydrous MeOH (1.25 mL, 30.8 mmol) and H_2O (40 mL, 2.2 mmol) was added, and then the same procedure described above was followed.

4.5 Characterization of products

Complete characterization data for all the products are given below.

- *Benzofuran-2-ylhexanoic acid methyl ester (**2a**)*. Colorless oil. IR (film): 1743 (s), 1600 (w), 1585 (w), 1454 (m), 1252 (m), 1160 (m), 751 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.53-7.49 (1H, m, H-4 or H-7), 7.47-7.42 (1H, m, H-7 or H-4), 7.27-7.15 (2H, m, H-5þH- 6), 6.59-6.58 (1H, m, H-3), 3.82 (1H, t, J = 7.3 Hz, $CHCH_2$), 3.72 (3H, s, CO_2Me), 2.18-1.93 (2H, m, $CHCH_2$), 1.43-1.24 (4H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, J = 6.8 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 155.3, 154.8, 128.4, 123.9, 122.7, 120.7, 111.1, 103.8, 52.3, 45.7, 30.6, 29.5, 22.4, 13.8. GC-MS (EI, 70 eV): m/z (%): 246 (33) [M^+], 190 (13), 189 (8), 187 (36), 145 (7), 144 (10), 132 (11), 131 (100), 115 (12). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.41; H, 7.35.

- *(3-Methylbenzofuran-2-yl)hexanoic acid methyl ester (**2b**)*. Pale yellow oil. IR (film): 1743 (s),1643 (w),1613 (w),1589 (w),1455 (m), 1255 (m), 1246 (m), 1185 (m), 1167 (m), 747 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.47-7.40 (2H, m, H-4 + H-7), 7.27-7.17 (2H, m, H-5 + H-6), 3.84 (1H, dd, J = 9.1, 6.6 Hz, $CHCH_2$), 3.68 (3H, s, CO_2Me), 2.21 (3H, s, $=CCH_3$), 2.23-1.96 (2H, m, $CHCH_2$) 1.41-1.17 (4H, m, $CH_2CH_2CH_3$), 0.87 (3H, t, J = 7.1 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 154.1, 149.6, 130.0, 123.8, 122.2, 119.0, 112.2, 111.1, 52.2, 43.6, 29.7, 29.5, 22.4, 13.9, 7.9. GC-MS (EI, 70 eV): m/z (%): 260 (23) [M^+], 203 (5), 202 (8), 201 (53), 171 (10), 158 (5), 157 (5), 146 (11), 145 (100), 131 (5), 128 (5), 115 (9). Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found C, 73.76; H, 7.75.

- *(3-Phenylbenzofuran-2-yl)hexanoic acid methyl ester (**2c**)*. Pale yellow oil. IR (film): 1745 (s),1611 (w),1496 (w),1454 (m),1256 (m), 1215 (m), 1190 (m), 1167 (m), 1013 (w), 968 (w), 749 (m), 702 (m) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59-7.36 (7H, m, Ph + H-4 + H-7), 7.34-7.20 (2H, m, H-5 + H-6), 3.97 (1H, dd, J = 9.0, 6.8 Hz, $CHCH_2$), 3.72 (3H, s, CO_2Me), 2.20-1.98 (2H, m, $CHCH_2$), 1.28-1.08 (4H, m, $CH_2CH_2CH_3$), 0.79 (3H, t, J = 6.8 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 154.3, 150.3, 132.0, 129.3, 128.9, 128.6, 127.6, 124.3, 122.8, 119.9, 119.6, 111.4, 52.3, 43.7, 30.0, 29.4, 22.3,13.7. GC-MS (EI, 70 eV): m/z (%): 322 (70) $[M^+]$, 264 (26), 263 (99), 219 (11), 208 (20), 207 (100), 205 (35), 179 (35), 178 (25), 165 (8). Anal. Calcd for $C_{21}H_{22}O_3$: C, 78.23; H, 6.88. Found C, 78.45; H, 6.86.

- *Benzofuran-2-ylphenylacetic acid methyl ester (**2d**)*. Yellow oil. IR (film): 1739 (s), 1600 (w), 1584 (w), 1453 (m), 1253 (m), 1199 (m), 1156 (s), 1010 (m), 751 (s), 723 (m), 699 (m) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50e7.26 (7H, m, Ph + H-4 + H-7), 7.25-7.12 (2H, m, H-5 + H-6), 6.57 (1H, t, J = 1.0 Hz, H-3), 5.14 (1H, br s, CHPh), 3.74 (3H, s, CO_2Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 155.0, 154.6, 128.8, 128.7, 128.2, 128.0, 124.1, 123.6, 122.7, 120.9, 111.1, 105.2, 52.6, 51.7. GC-MS (EI, 70 eV): m/z (%): 266 (20) $[M^+]$, 208 (17), 207 (100), 179 (8), 178 (31), 176 (6), 152 (6), 89 (5). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found C, 76.81; H, 5.29.

- *2-Benzofuran-2-yl-3,3-dimethylbutanoic acid methyl ester (**2e**)*. Pale yellow solid, mp 60-61 °C. IR (KBr): 1733 (s), 1577 (w), 1474 (w), 1456 (m), 1435 (m), 1371 (m), 1321 (m), 1243 (m), 1205 (m), 1150 (s), 1043 (m), 1007 (m), 827 (m), 755 (m), 747 (m) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54-7.49 (1H, m, H-4 or H-7), 7.46-7.41 (1H, m, H-7 or H-4), 7.25-7.14 (2H, m, H-5

+ H-6), 6.74 (1H, dd, J = 1.0, 0.3 Hz, H-3), 3.73 (1H, d, J = 0.3 Hz, $CHCMe_3$), 3.69 (3H, s, CO_2Me),1.08 (9H, s, CMe_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 154.5, 153.9, 128.5, 123.7, 122.7, 120.7, 111.0, 105.5, 55.9, 51.7, 35.1, 28.0. GC-MS (EI, 70 eV): m/z (%): 246 (11) [M^+], 191 (12), 190 (100), 187 (8), 175 (22), 158 (35), 131 (27), 130 (9), 102 (8), 57 (30). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found C, 73.02; H, 7.38.

- *2-(6-Methoxybenzofuran-2-yl)hexanoic acid methyl ester (**2f**).* Yellow oil. IR (film): 1741 (s),1624 (m),1587 (w),1492 (m),1438 (m), 1293 (m), 1274 (m), 1195 (m), 1149 (m), 1107 (m), 1027 (m), 961 (w), 823 (m) cm^{-1}. ^{1}HNMR (300 MHz, $CDCl_3$): δ 7.35 (1H, d, J = 8.6 Hz, H-4), 6.99 (1H, d, J = 2.2 Hz, H-7), 6.83 (1H, dd, J = 8.6, 2.2 Hz, H-5), 6.50 (1H, s, H-3), 3.83-3.75 (1H, m, CHBu), 3.81 (3H, s, $ArOCH_3$), 3.71 (3H, s, CO_2Me), 2.15-1.90 (2H, m, $CHCH_2$), 1.43-1.25 (4H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, J = 6.9 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 157.8, 155.7, 154.3, 121.7, 120.8, 111.7, 103.5, 96.0, 55.7, 52.2, 45.7, 30.5, 29.5, 22.4, 13.9. GC-MS (EI, 70 eV): m/z (%): 276 (54) [M^+], 219 (33), 218 (15), 217 (95),174 (8),162 (12),161 (100),159 (16). Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found C, 69.45; H, 7.30.

- *2-(5-Methoxybenzofuran-2-yl)hexanoic acid methyl ester (**2g**)*. Yellow oil. IR (film): 1741 (s), 1615 (w), 1602 (w), 1477 (m), 1447 (m), 1435 (m), 1205 (m), 1167 (m), 1031 (w) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (1H, dt, J = 8.8, 0.7 Hz, H-7), 6.98-6.96 (1H, m, H-4), 6.84 (1H, dd, J = 8.8, 2.7 Hz, H-6), 6.52 (1H, t, J = 0.7 Hz, H-3), 3.82-3.76 (1H, m, CHBu), 3.80 (3H, s, $ArOCH_3$), 3.71 (3H, s, CO_2Me), 2.16-1.91 (2H, m, $CHCH_2$), 1.43-1.24 (4H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, J = 6.9 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 156.1, 156.0, 149.8, 129.0, 112.5, 111.5,

103.9, 103.4, 55.9, 52.3, 45.8, 30.6, 29.5, 22.4, 13.9. GC-MS (EI, 70 eV): m/z (%): 276 (33) [M^+], 220 (12), 219 (10), 218 (7), 217 (43), 216 (6), 191 (6), 162 (12), 161 (100). Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found C, 69.69; H, 7.29.

- *2-(5-Chlorobenzofuran-2-yl)hexanoic acid methyl ester (**2h**)*. Yellow oil. IR (film): 1742 (s),1594 (w),1447 (m),1259 (m),1159 (m), 1061 (w), 801 (m), 696 (w) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.46 (1H, dt, J = 2.0, 0.7 Hz, H-4), 7.34 (1H, dt, J = 8.6, 0.7 Hz, H-7), 7.18 (1H, dd, J = 8.6, 2.0 Hz, H-6), 6.54 (1H, t, J = 0.7 Hz, H-3), 3.81 (1H, t, J = 7.6 Hz, CHBu), 3.72 (3H, s, CO_2Me), 2.17-1.91 (2H, m, $CHCH_2$), 1.43-1.24 (4H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.8, 157.0, 153.1, 129.8, 128.3, 124.1, 120.3, 112.1, 103.5, 52.4, 45.7, 30.5, 29.5, 22.4, 13.8. GC-MS (EI, 70 eV): m/z (%): 282 (7) [$(M^{+2})^+$], 280 (22) [M^+], 224 (17), 223 (15), 221 (34), 167 (33), 166 (11), 165 (100), 115 (12). Anal. Calcd for $C_{15}H_{17}ClO_3$: C, 64.17; H, 6.10; Cl, 12.63. Found C, 64.26; H, 6.08; Cl, 12.61.

- *(5-Chlorobenzofuran-2-yl)phenylacetic acid methyl ester (**2i**)*. Yellow oil. IR (film): 1743 (s), 1593 (m), 1446 (m), 1259 (m), 1199 (m),1153 (s),1060 (w),1010 (m), 801 (m), 723 (m), 695 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.45-7.26 (7H, m, Ph + H-4 + H-7), 7.16 (1H, dd, J = 8.8, 2.2 Hz, H-6), 6.52 (1H, t, J = 1.0 Hz, H-3), 5.11 (1H, br s, CHPh), 3.74 (3H, s, CO_2Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3,156.2, 153.3, 135.1, 129.6, 128.9, 128.6, 128.3, 128.2, 124.3, 120.5, 112.1, 104.9, 52.7, 51.6. GC-MS (EI, 70 eV): m/z (%): 302 (9) [$(M^{+2})^+$], 300 (26) [M^+], 243 (43), 242 (21), 241(100), 206(10), 205(13), 178(38), 176(14). Anal. Calcd for $C_{17}H_{13}ClO_3$: C, 67.89; H, 4.36, Cl, 11.79. Found C, 67.95; H, 4.35; Cl, 11.77.

4.6 References and notes

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CHAPTER 5

Versatile synthesis of benzothiophene derivatives by Pd-catalyzed or radical-promoted heterocyclization of 1-(2-mercaptoaryl)-2-yn-1-ols.

Bartolo Gabriele, Raffaella Mancuso, Elvira Lupinacci, Giuseppe Salerno

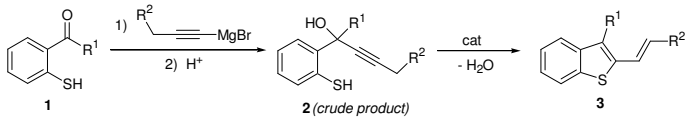
Results to be published

5.1 Introduction

Multi-substituted benzothiophenes are of considerable importance, as they exhibit various biological activities*(1)* and also provide useful properties in materials science.*(2)* A number of methods to synthesise this class of compounds have been reported in recent years, most of which involve the cyclisation of benzenethiol derivatives.*(3)* However, facile and versatile methods to access multi-substituted benzothiophenes are still limited. Furthermore, catalytic cyclisation approaches using transition metals for the construction of the benzothiophene skeleton, which would provide a more efficient and practical route, are extremely rare in the literature, presumably due to of sulfur's long-standing reputation as a catalyst poison.*(4–6)* Only a few reports of this nature have appeared,*(7)* in which Au*(7a)* or Pd*(7b)* catalysts have been employed to effect C–S bond formation.

In connection with our studies on the synthesis of heterocyclic compounds via transition metal-catalysed reactions, our research group has recently reported several examples of copper- or palladium-catalyzed cycloisomerization reactions leading to heterocyclic derivatives starting from suitably functionalized alkyne derivatives.*(8)* In particular, our research group has reported a general methodology for the regioselective synthesis of substituted furans*,(9)* thiophenes,*(10)* and pyrroles*(11)* starting from (*Z*)-2-en-4-yn-1-ols, (*Z*)-2-en-4-yne-1-thiols, and (*Z*)-(2-en-4-ynyl)amines, respectively, through 5-*exo-dig* heteroannulation-aromatization promoted by PdX_2 in conjunction with KX (X = Cl, I) or by $CuCl_2$ as the catalytic system. We have also reported a divergent synthesis of (*Z*)-1-alkylidene-1,3-dihydroisobenzofurans and 1*H*-isochromenes by PdI_2/KI-catalyzed 5-*exo-dig* or 6-*endo-dig*, respectively, cycloisomerization of 2-alkynylbenzyl alcohols.*(12).*

In this context, we became interested in whether this metal-catalyzed cycloisomerization process could be extended to C–S bond formation to prepare sulfur-based heterocycles, such as benzothiophenes (**Scheme 1**).



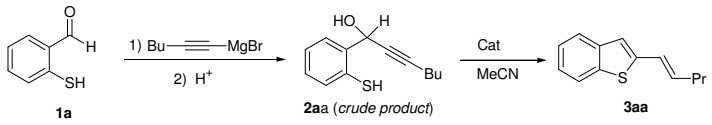
Scheme 1

In this part of the thesis, I report on the versatile synthesis of benzothiophene derivatives by Pd-catalyzed or radical promoted eterocyclization of 1-(2-mercaptoaryl)-2-yn-1-ols.

5.2 Results and discussion.

Initial studies to determine the optimal reaction conditions were performed using 1-(2-mercapto-phenyl)-hept-2-yn-1-ol **2aa** as a substrate (**Table 1**).

Table 1: Synthesis of 2-pent-1-enyl-benzothiophene **3aa** by metal-catalyzed heterocyclization of 1-(2-Mercapto-phenyl)-hept-2-yn-1-ol **2aa**[a].



1a **2a**a (*crude product*) **3aa**

Entry	Catalyst	Yield of 3aa(%)[b]
1	PdI_2 + 10 KI	54
2	$PdCl_2$ + 10 KCl	25
3	$Pd(NO_2)$ x $2H_2O$	13
4	$ZnCl_2$	3
5	$CuCl_2$	10
6	CuI	traces
7	AuCl	11
8	$AuCl_3$	24
9[b]	/	/

[a] All reactions were carried out for 6 h at 100 °C in MeCN as the solvent (0.22 mmol of starting 2-mercaptobenzaldheyde **1a** per mL of solvent, 1 mmol scale based on **1a**). Conversion of **2aa** was quantitative in all cases. [b] Isolated yield based on starting **1a**. [b] Reaction was carried out without any catalytic system.

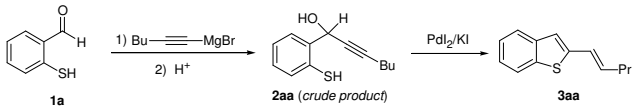
Despite extensive screening of a range of catalytic systems, the desired 2-pent-1-enyl-benzothiophene **3aa** was obtained in a satisfactory yield (54% yield based on starting **1a**) only when the PdI_2-KI catalytic system was employed. In particular, the reaction of **2aa** using 2 mol% of Cu-based catalylists in MeCN at 100 °C resulted in the formation of **3aa** in only traces (**Table 1**, entry 5-6), while the use of AuCl and $AuCl_3$ as catalysts delivered **3aa** in 11 and 24% yield, respectively (**Table 1**, entry 7-8). From subsequent examinations of various

palladium sources, PdI_2 and in minor amount $PdCl_2$ proved to be the best catalysts (**Table 1**, entries 1-2). None of the desired product, **3aa**, was obtained in the absence of any catalyst (**Table 1**, entry 9). Therefore, the effective catalyst choosen for the subsequent reactions was the PdI_2 catalyst.

One drawback of this synthetic approach was related to the instability of **2aa** during the purification procedures, which in several cases caused its partial decomposition after column chromatography.*(13)* However, it was found that the cyclization reaction worked nicely even on the crude product, which also facilitated the synthetic protocol (see Experimental Section for details). Thus, when crude **2aa** was let to react at 100 °C in MeCN as the solvent in the presence of PdI_2 and KI (**2aa**/KI/PdI_2 molar ratio = 50:10:1), benzothiophene **3aa** was obtained in 54% isolated yield based on starting 2-mercaptobenzaldehyde **1aa** (**Table 1**, entry 1)

Using PdI_2 as the catalyst, we next tested the reactivity of **2aa** in different solvents. The results, shown in **Table 2**, clearly indicate MeCN as the solvent of choice for the reaction.

Table 2: Synthesis of 2-pent-1-enyl-benzothiophene **3aa** by PdI_2-catalyzed heterocyclization of 1-(2-Mercapto-phenyl)-hept-2-yn-1-ol **2aa**[a]



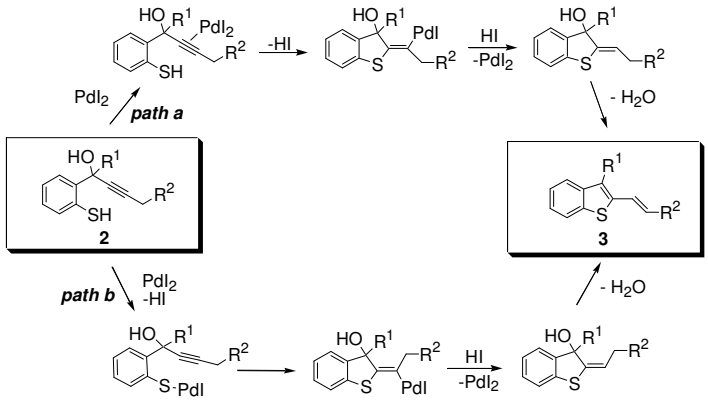
Run	Solvent	Yield[b] 3aa (%)
1	MeCN	54
2	DME	45
3	DMA	45
4	Dioxane	36
5[c]	MeOH	/

[a] All reactions were carried out for 6 h at 100 °C in different solvents (0.22 mmol of starting 2-mercaptobenzaldheyde **1a** per mL of solvent, 1 mmol scale based on **1a**) in the presence of PdI_2 and KI (**2aa**/KI/PdI_2 molar ratio = 50:10:1). Conversion of **2aa** was quantitative in all cases. [b] Isolated yield based on starting **1a**. [c] The reaction let to the formation of 2-(1-methoxy-pentyl)-benzothiophene **4aa** in 52 % yield.

Interestingly, when the reaction was carried out in MeOH as the solvent, the desired product, **3aa**, was not obtained, and the formation of 2-(1-methoxy-pentyl)-benzothiophene **4aa** in 52 % yield (based on starting **1a**) was observed. The latter result let us to hypotize that the formation of the methoxy-substituted benzothiophene could occur via a different reaction mechanism which does not involve the PdI_2-catalyst. Thus, a reaction without catalyst was conducted in MeOH as the solvent, leading to the desired product 2-(1-methoxy-pentyl)-benzothiophene **4aa** in 60 % yield. A radical-promoted eterocyclization mechanism was then hypotized, therefore a reaction with the radical iniziator AIBN (azabisisoisobutyronitrile, 20% with respect to substrate weight, see experimental

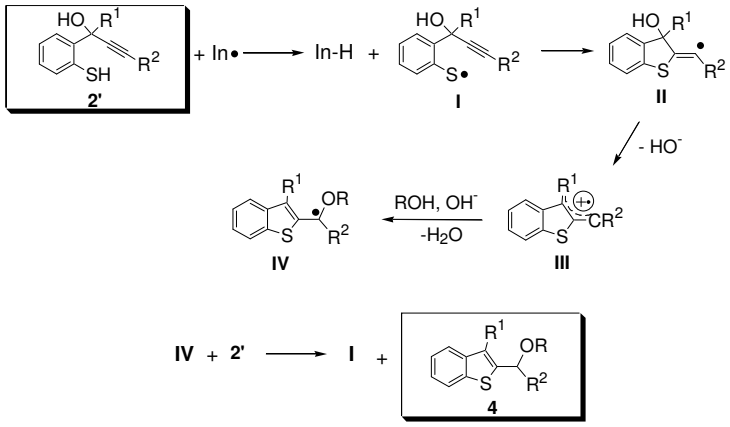
section for details) was carried out. The reaction of 1-(2-mercapto-phenyl)-hept-2-yn-1-ol **2aa** thus afforded the corresponding benzothiophene **4aa** in 62 % isolated yield (based on starting **1a**), confirming the validity of our hypothesis. Moreover, when substrate **2aa** was let to react in the presence of the radical inhibitor TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) no conversion of the substrate was observed, which confirmed a radical mechanism.

The two plausible reaction mechanisms for the formation of benzothiophenes **3aa** and **4aa** from substrate **2aa** are shown in **Scheme 2** and **Scheme 3**, respectively.



Scheme 2

When the reaction is carried out with PdI_2 as the catalyst and MeCN as the solvent, two different reaction pathways can occur (**Scheme 2**). In *path a*, intramolecular nucleophilic attack of the –SH group to the triple bond coordinated to the metal center is followed by protonolysis and dehydration. In *path b* the formation of a palladium-sulfide intermediate is followed by triple bond insertion, protonolysis and dehydration.



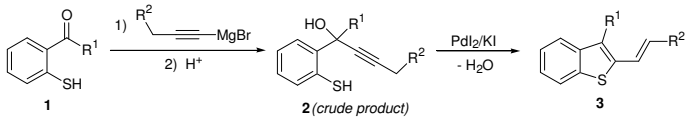
Scheme 3

On the other hand, when the reaction is carried out without catalyst, in MeOH as the solvent and AIBN as the radical iniziator, formation of the thyil radical **I** occurs, followed by 5-*exo-dig* cyclization to give intermediate **II** (**Scheme 3**). The elimination of HO^- from **II** leads to the radical cation species **III**, whose reaction with ROH gives a benzylic-type radical **IV**. The radical chain then propagates through the reaction between **IV** and **2'**, with formation of product **4** and regeneration of the thyil radical **I** (**Scheme 3**).

Clearly, while in radical-promoted reactions the substrate can bear any kind of substituent on the triple bond (R^2), in metal-catalyzed reactions, in order to obtain the enyl-benzothiophenic products, 1-(2-mercaptoaryl)-2-yn-1-ols must present a methylenic spacing-group between the triple bond and the R^2-substituent (compare substrate **2'** in **Scheme 3** with substrate **2** in **Scheme 2**).

The generality of the two processes was then verified in both cases by varying the nature of substituents R^1 (at the benzylic position), and R^2 (on the triple bond). The results are shown in **Table 3** and **Table 4**.

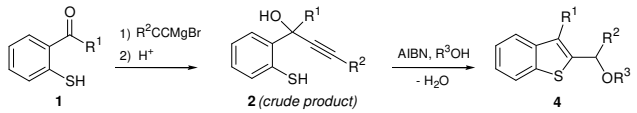
Table 3: Synthesis of benzothiophenes **3** by heterocyclization PdI_2/KI-catalyzed of 1-(2-mercaptoaryl)-2-yn-1-ols **2**[a]



Entry	1	R^1	R^2	2	t(h)	3	Yield of 4 (%)[b]
1[c,d]	**1a**	H	Pr	**2aa**	6	**3aa**	63
2[d]	**1b**	Me	Pr	**2ba**	5	**3ba**	72
3	**1c**	Ph	Pr	**2ca**	15	**3ca**	82
4	**1a**	H	CH_2Ph	**2ad**	15	**3ad**	52
5	**1b**	Me	CH_2Ph	**2bd**	15	**3bd**	68
6	**1c**	Ph	CH_2Ph	**2cd**	15	**3cd**	53
7	**1a**	H	Ph	**2ae**	5	**3ae**	72
8	**1c**	Ph	Ph	**2ce**	15	**3ce**	73

[a] Unless otherwise noted, all reactions were carried out at 80 °C in MeCN as the solvent (0.02 mmol of starting 2-mercaptoaryl ketones **1** per mL of solvent, 1 mmol scale based on **1**) in the presence of PdI_2 and KI (**2**/KI/PdI_2 molar ratio = 50:10:1) Conversion of **2** was quantitative in all cases. [b] Isolated yield based on starting **1**. [c] **2**/KI/PdI_2 molar ratio = 50:10:2. [d] Substrate concentration was 0.22 mmol/mL of MeCN

Table 4: Synthesis of benzothiophenes **4** by radical-promoted heterocyclization of 1-(2-mercaptoaryl)-2-yn-1-ols **2**[a]



Entry	1	R^1	R^2	R^3	2	t(h)	4	Yield of 4 (%)[b]
1[c]	**1a**	H	Bu	Me	**2aa**	8	**4aa**	70
2[c]	**1a**	H	*t*-Bu	Me	**2ab**	8	**4ab**	48
3[c]	**1a**	H	Ph	Me	**2ac**	15	**4ac**	60
4	**1b**	Me	Bu	Me	**2ba**	15	**4ba**	99
5	**1b**	Me	*t*-Bu	Me	**2bb**	15	**4bb**	55
6[c]	**1b**	Me	Ph	Me	**2bc**	8	**4bc**	58
7	**1c**	Ph	Bu	Me	**2ca**	8	**4ca**	46
8	**1c**	Ph	*t*-Bu	Me	**2cb**	8	**4cb**	59
9	**1c**	Ph	Ph	Me	**2cc**	15	**4cc**	80
10	**1a**	H	$(CH_2)_2Ph$	Me	**2ad**	15	**4ad**	51
11[c]	**1b**	Me	$(CH_2)_2Ph$	Me	**2bd**	15	**4bd**	65
12	**1c**	Ph	$(CH_2)_2Ph$	Me	**2cd**	15	**4cd**	73
13	**1a**	H	CH_2Ph	Me	**2ae**	8	**4ae**	49
14[c]	**1c**	Ph	CH_2Ph	Me	**2ce**	15	**4ce**	43
15[c]	**1a**	H	Bu	Et	**2aa**	15	**4aa'**	63
16[c]	**1a**	H	*t*-Bu	Et	**2ab**	8	**4ab'**	32
17[c]	**1a**	H	Ph	Et	**2ac**	15	**4ac'**	51
18[c]	**1b**	Me	Bu	Et	**2ba**	15	**4ba'**	95
19[c]	**1b**	Me	*t*-Bu	Et	**2bb**	15	**4bb'**	54
20[c]	**1b**	Me	Ph	Et	**2bc**	15	**4bc'**	47
21	**1c**	Ph	Bu	Et	**2ca**	8	**4ca'**	52
22[c]	**1a**	H	Bu	*i*-Pr	**2aa**	15	**4aa''**	55
23[c]	**1a**	H	*t*-Bu	*i*-Pr	**2ab**	15	**4ab''**	60
24[c]	**1a**	H	Ph	*i*-Pr	**2ac**	8	**4ac''**	35

[a]Unless otherwise noted, all reactions were carried out at 100 °C in R^3OH as the solvent (0.02 mmol of starting 2-mercaptoaryl ketones **1** per mL of solvent, 1 mmol scale based on **1**) in the presence of AIBN (20% respect to the weight of substrate **2**). Conversion of **2** was quantitative in all cases. [b]Isolated yield based on starting **1**. [c] The reaction was carried out at 80 °C.

As can be seen from **Table 4**, the radical-promoted reaction works nicely also when EtOH (entries 15-21) and *i*-PrOH (entries 22-24) were employed as solvents, leading to the corresponding alcohoxy-derivatives in fair to good yield.

5.3 Conclusion

In conclusion, in this part of the thesis, I have reported a novel and practical synthesis of benzothiophene derivatives through a two-step procedure involving Grignard addition of alkynylmagnesium bromides to 2-mercaptoarylketones followed by Pd-catalyzed or radical promoted eterocyclization of the corresponding 1-(2-mercaptoaryl)-2-yn-1-ols. The latter intermediates could be used without further purification for the subsequent cyclization step, thus further facilitating the synthetic procedure. The generality of the process has been verified by varying the nature of substituents on the aromatic ring as well as at the benzylic position and on the triple bond.

5.4 Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in $CDCl_3$ solutions at 300 or 500 MHz and 75 or 126 MHz, respectively, with Me_4Si as internal standard. Chemical shifts (δ) and coupling constants (*J*) are given in ppm and in Hz, respectively. IR spectra were taken with an FT-IR spectrometer. Mass spectra were obtained using a GC-MS apparatus at 70 eV ionization voltage. Microanalyses were carried out at our analytical laboratory. All reactions were analyzed by TLC on silica gel 60 F_{254} and by GLC using a gas chromatograph and capillary columns with polymethylsilicone + 5% phenylsilicone as the stationary phase. Column chromatography was performed on silica gel 60 (70-230 mesh). Evaporation refers to the removal of solvent under reduced pressure.

*5.4.1 Preparation of 2-mercaptobenzaldehyde (**1a**)*

2-Mercaptobenzaldehyde was prepared by the general method of Toste.*(14)* To a solution of thiophenol (3.0 g, 2.8 ml, 27.2 mmol), N,N,N',N'-tetramethylethylendiamine (9 ml, 60.0 mmol) in hexane (75 mL) was carefully added buthyllithium (59.9 mmol, 48 mL of 1.6 M solution in hexane) at 0 °C over 20 min. The reaction mixture was stirred overnight at room temperature. Then, anhydrous DMF was added (4.88 g, 5.2 ml, 67 mmol) and the mixture was allowed to react for additional 16 h. The reaction mixture was slowly quenched with HCl 1 M solution. Et_2O (ca. 20 mL) was added and phases were separated. The aqueous phase was extracted with Et_2O (3 x 50 mL) and the collected organic layers were washed with water till neutral pH and eventually with brine. The solution was then dried on Na_2SO_4 and after filtration, the solvent was evaporated to give 2-mercaptobenzaldehyde (yellow oil, 3.16 g, 22.9 mmol, 85 % yield). Crude product 1a was diluted with Et_2O and transferred into a volumetric flask (250 mL).

*5.4.2 Preparation of 2-mercaptoacetophenone (**1b**).*

2-Mercaptoacetophenone was prepared by the general method of Topolsky.*(15)* To a solution of thiosalicylic acid (3.08 g, 20 mmol) in anhydrous THF (100 mL) was carefully added methyllithium (80 mmol, 51 mL of 1.6 M solution in ether) at 0°C. The reaction mixture was stirred overnight at room temperature and it was slowly quenched with water and then saturated NH_4Cl solution. The organic phase was separated and subsequently washed with 5% $NaHC0_3$ and brine. It was then dried on Na_2SO_4 and after filtration, the solvent was evaporated to give 2-mercaptoacetophenone (yellow oil, 2.85 g, 18.8 mmol, 94 % yield). Crude product 1b was diluted with Et_2O and transferred into a volumetric flask (250 mL).

*5.4.3 Preparation of 2-mercaptobenzophenone (**1c**).*

2-Mercaptobenzophenone was prepared by the general method of Schellenberg.*(16)* 2-Aminobenzophenone (2 g, 10.15 mmol), 2,2 ml of concentrated hydrochloric acid, and 20 ml of water were stirred at -2 °C, while a solution of 730 mg of sodium nitrite in 2 ml of water was added over 15 min; the mixture was stirred for an additional 20 min. The excess nitrite was destroyed with 50 mg of urea in 3 ml of water. The suspension of yellow diazonium salt was added over 0.5 hr to a stirred solution at room temperature of 8 g of potassiumbethyl xanthate in 10 ml of water. The temperature of the mixture rose to 40 °C during mixing; it was heated to 80 °C for 0.5 hr, cooled to O °C, and extracted with ether. After the ether solution had been washed and the ether removed by rotary evaporation, the aryl ethyl xanthate was saponified by refluxing with 20 ml of ethanol and 2 g of potassium hydroxide under nitrogen for 16 hr. The mixture was acidified and the mercaptan was extracted with ether, washed with acid and water, and extracted into 1 N sodium hydroxide. The alkaline extract was acidified and re-extracted with ether, which was washed and then dried with sodium sulfate; the ether was removed. The oil prepared from 2 g of 2-aminobenzophenone was dissolved in acetone and purified by column chromatography on silica gel to give 2-mercaptobenzophenone (yellow solid, 1.02 g, 4.77 mmol, 47 % yield).

*5.4.4 Preparation of 1-(2-mercaptoaryl)-2-yn-1-ols (**2**).*

To a suspension of Mg turnings (500.0 mg, 20.5 mmol) in anhydrous THF (2.0 mL), maintained under nitrogen and under reflux, was added pure EtBr (0.5 mL) to start the formation of the Grignard reagent. The remaining bromide was added dropwise (ca. 20 min) in THF solution (1.0 mL of EtBr in 15.0 mL of THF; total amount of EtBr added: 2.15 g, 19.8 mmol). The mixture was then allowed to reflux for additional 20 min. After cooling, the solution of EtMgBr thus obtained was transferred under nitrogen to a dropping funnel and was added dropwise to a

solution of the 1-alkyne (19.8 mmol) in anhydrous THF (7.0 mL) at 0 °C with stirring. After additional stirring at 0 °C for 15 min, the mixture was allowed to warm up to room temperature, maintained at 50 °C for 2 h, and then used as such for the next step.

A solution of 2-mercapto-benzaldehyde **1a** or 2-mercapto-aryl ketones **1b-c** (7.92 mmol) in anhydrous THF (22.0 mL) was then added dropwise to a solution of the alkynylmagnesium bromide (prepared as described above) at 50 °C under nitrogen. After stirring at 50 °C for 2 h, the mixture was cooled to room temperature and saturated NH_4Cl was added with stirring.

After additional stirring at room temperature for 15 min., Et_2O (ca. 20 mL) was added and phases were separated. The aqueous phase was extracted with Et_2O (3 x 50 mL) and the collected organic layers were washed with brine and eventually dried over Na2SO4. After filtration, the solvent was evaporated and crude products 2 were diluted with Et_2O and transferred into a volumetric flask (50 mL).

*5.4.5 Preparation of 2-pent-1-enyl-benzothiophene (**3aa**) in different catalytic systems.*

Reactions were carried out at 100 °C in MeCN as the solvent (0.22 mmol of starting 2-mercaptobenzaldehyde **1a** per mL of solvent, 1 mmol scale based on **1a**). The catalytic system [PdI_2 (0.02 mmol) in conjunction with KI (0.2 mmol); or $PdCl_2$ (0.02 mmol) in conjunction with KCl (0.2 mmol); or $Pd(NO_2)$ x $2H_2O$ (0.02 mmol), or $ZnCl_2$ (0.02 mmol), or $CuCl_2$ (0.02 mmol), or CuI (0.02 mmol), or AuCl (0.02 mmol), or $AuCl_3$ (0.02 mmol)], was added to a solution of crude **2a** (1.0 mmol, 220 mg) in anhydrous CH_3CN (4.5 mL) in a Schlenk flask. The resulting mixture was stirred at 100°C and for 6 h. Solvent was evaporated and the crude product purified by column chromatography on silica gel (**4aa**: yellow solid, mp 56.9-58.9 °C, 95:5 hexane-Et_2O). The yields obtained in each experiment are reported in **Table 1**.

The catalyst-free reaction was carried out as follows: a solution of crude **2a** (1.0 mmol, 220 mg) in anhydrous MeCN (4.5 mL) was allowed to react without any

catalytic system in a Schlenk flask at 100°C and for 6 h (run 9, Table 3). After removing the solvent, the resulting crude product was diluted with Et_2O, transferred into a volumetric flask (10 mL) and quantified by HPLC using tosylhydrazide as standard. A Shimadzu LC-20AB model liquid chromatograph equipped with a SIL-20A HT autosampler and a SPD-20A UV-detector was used. The analytical column (SUPELCO Discovery C18) was packed with C-18 particles (5 μm). The mobile phase consisted of solvent **A**: water and solvent **B**: acetonitrile. The gradient employed was: 0–5 min: isocratic 25% **B** in **A**; 5–20 min: linear gradient from 25% **B** in **A** to 60% **B** in **A**; 20–35 min: linear gradient from 60% **B** in **A** to 88% **B** in **A**; 35–37 min: isocratic 88% **B** in **A**; 37–38 min: linear gradient from 88% **B** in **A** to 25% **B** in **A**; 38–43 min: isocratic 25% **B** in **A**. The flow-rate was 1 mL/min; UV-detection was set at 280 nm.

*5.4.6 Preparation of benzothiophenes (**3**).*

PdI_2 (0.01 mmol) in conjunction with KI (0.1 mmol) was added to a solution of crude **2** (0.5 mmol) in anhydrous MeCN (25 mL) in a Schlenk flask. The resulting mixture was stirred at the temperature and for the time indicated in Table 2. Solvent was evaporated and the crude products purified by column chromatography on silica gel: **3aa** (yellow solid, 62.6 mg, 0.31 mmol, mp 56.9-58.9 °C, 95:5 hexane-Et_2O); **3ba** (yellow solid, 77.7 mg, 0.36 mmol, mp 63.2-64.6 °C, 95:5 hexane- Et_2O); **3ca** (white solid, 113.9 mg, 0.41 mmol, mp 60.8-63.0 °C, 95:5 hexane- Et_2O); **3ad** (yellow solid, 65.0 mg, 0.26 mmol, mp 58.8-60.0 °C, 95:5 hexane- Et_2O); **3bd** (white solid, 89.7 mg, 0.34 mmol, mp 61.4-62.8 °C, 95:5 hexane- Et_2O); **3cd** (yellow solid, 84.7 mg, 0.26 mmol, mp 58.8-62.0 °C, 95:5 hexane- Et_2O); **3ae** (yellow solid, 84.9 mg, 0.36 mmol, mp 122.3-124.8 °C, 95:5 hexane- Et_2O); **3ce** (yellow solid, 112.3 mg, 0.36 mmol, mp 56.8-59.9 °C, 95:5 hexane- Et_2O). The yields obtained in each experiment are reported in **Table 3**.

*5.4.7 Preparation of benzothiophenes (**4**).*

AIBN (20% respect to the substrate weight) was added to a solution of crude 2 (0.5 mmol) in anhydrous MeOH (or EtOH or *i*-PrOH) (25 mL) in a Schlenk flask. The resulting mixture was stirred at the temperature and for the time indicated in **Table 4**. Solvent was evaporated and the crude products purified by column chromatography on silica gel: **4aa** (yellow oil, 81.9 mg, 0.35 mmol, 95:5 hexane-AcOEt); **4ab** (yellow solid, 56.1 mg, 0.24 mmol, mp 96.4-97.5 °C, 99:1 hexane-AcOEt); **4ac** (yellow solid, 76.2 mg, 0.30 mmol, mp 60.3-61.2 °C, 9:1 hexane-AcOEt); **4ba** (yellow oil, 121.5 mg, 0.49 mmol, 9:1 hexane-AcOEt); **4bb** (white solid, 66.9 mg, 0.27 mmol, mp 56.1-58.2 °C, 9:1 hexane-AcOEt); **4bc** (yellow oil, 77.7 mg, 0.29 mmol, 9:1 hexane-AcOEt); **4ca** (yellow oil, 77.7 mg, 0.23 mmol, 95:5 hexane-AcOEt); **4cb** (white solid, 89.9 mg, 0.29 mmol, mp 99.1-99.5 °C, 95:5 hexane-AcOEt); **4cc** (yellow solid, 132.0 mg, 0.40 mmol, mp 106.2-107.1 °C, 95:5 hexane-AcOEt); **4ad** (yellow oil, 70.5 mg, 0.25 mmol, 99:1 hexane-AcOEt); **4bd** (yellow oil, 94.7 mg, 0.32 mmol, 99:1 hexane-AcOEt); **4cd** (yellow solid, 128.8 mg, 0.36 mmol, 95:5 hexane-AcOEt); **4ae** (yellow solid, 64.3 mg, 0.24 mmol, mp 61.5-63.0 °C, 95:5 hexane-AcOEt); **4ce** (yellow solid, 72.2 mg, 0.21 mmol, mp 70.3-71.0 °C, 95:5 hexane-AcOEt); **4aa'** (yellow oil, 76.8 mg, 0.31 mmol, 95:5 hexane-AcOEt); **4ab'** (white solid, 39.7 mg, 0.16 mmol, mp 27.0-27.5 °C, 95:5 hexane-AcOEt); **4ac'** (yellow solid, 67.0 mg, 0.25 mmol, mp 34.0-34.9 °C, 95:5 hexane-AcOEt); **4ba'** (colourless oil, 123.1 mg, 0.47 mmol, 9:1 hexane-AcOEt); **4bb'** (white solid, 70.7 mg, 0.27 mmol, mp 49.3-51.2 °C, 95:5 hexane-AcOEt); **4bc'** (yellow oil, 64.8 mg, 0.23 mmol, 95:5 hexane-AcOEt); **4ca'** (colourless oil, 84.2 mg, 0.26 mmol, 99:1 hexane-AcOEt); **4aa''** (yellow oil, 70.7 mg, 0.27 mmol, 95:5 hexane-AcOEt); **4ab''** (yellow solid, 78.6 mg, 0.30 mmol, mp 83.2-83.8 °C, 95:5 hexane-AcOEt); **4ac''** (yellow solid, 47.9 mg, 0.17 mmol, mp 29.0-29.3 °C, 95:5 hexane-AcOEt); The yields obtained in each experiment are reported in **Table 4**.

5.5 Characterization of products

Complete characterization data for all the other products are given below.

- *2-Pent-1-enyl-benzo[b]thiophene (**3aa**).* Yellow solid, mp. 56.9-58.9 °C. IR (KBr): ν = 3057 (m), 2960 (s), 2870 (m), 1677 (w), 1457 (s), 1436 (s), 1378 (w), 1275 (s), 1225 (w), 1067 (w), 953 (s), 839 (m), 744 (s), 725 (s), 565 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.75-7.68 (m, 1 H aromatic), 7.65-7.58 (m, 1 H aromatic), 7.28-7.22 (m, 2 H aromatic), 7.02 (s, 1 H, H-3), 6.63-6.54 (m, 1 H, C*H*=CH), 6.15 (dt, *J* = 15.3, 7.2, 1H, CH=C*H*), 2.25-2.12 (m, 2H, $CH_2CH_2CH_3$), 1.50 (q, *J* = 7.3, 2H, $CH_2CH_2CH_3$), 0.95 (t, 7.3, 3H, CH_3); ^{13}C NMR (75 MHz): δ = 169.2, 150.8, 145.1, 144.1, 130.2, 123.5, 113.2, 106.1, 105.5, 55.9, 52.4, 34.3; GC-MS: m/z = 204 (3) [$(M+2)^+$], 203 (10), 202 (62) [M^+], 184 (4), 174 (14), 173 (100), 172 (13), 171 (19), 160 (22), 159 (23), 147 (9), 134 (15), 129 (32), 128 (14), 115 (14), 45 (35).; anal. calcd for $C_{13}H_{14}S$ (202.08): C, 77.18; H, 6.97; S, 15.85; found C, 77.19 H, 6.99; S, 15.82.

- *3-Methyl-2-pent-1-enyl-benzo[b]thiophene (**3ba**).* Yellow solid, mp. 63.2-64.6 °C. IR (KBr): ν = 3060 (w), 2957 (m), 2870 (m), 1460 (m), 1435 (m), 1379 (w), 1198 (w), 1017 (w), 950 (s), 752 (s), 744 (s), 727 (s) cm^{-1}; ^1H NMR (300 MHz): δ = 7.75-7.64 (m, 1 H aromatic), 7.63-7.51 (m, 1 H aromatic), 7.35-7.16 (m, 2 H aromatic), 6.75-6.62 (m, 1 H, C*H*=CH), 6.12 (dt, *J* = 15.3, 7.1, 1H, CH=C*H*), 2.39 (s, 3H, CH_3), 2.27-2.15 (m, 2H, $CH_2CH_2CH_3$), 1.50 (q, *J*=7.3, 2H, $CH_2CH_2CH_3$), 0.95 (t, *J*=7.3, 3H, $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ = 141.3, 137.7, 136.8, 133.3, 127.5, 124.4, 123.9, 122.1, 122.0, 121.4, 35.4, 22.5, 13.7, 11.6; GC-MS: *m/z* = 218 (5) [$(M+2)^+$], 217 (12), 216 (82) [M^+], 187 (100), 174 (19), 173 (18), 172

(48), 171 (37), 154 (11), 147 (24), 128 (16), 115 (19); anal. calcd for $C_{14}H_{16}S$ (216.10): C, 77.72; H, 7.45; S, 14.82; found C, 77.76; H, 7.47; S, 14.84.

- *2-Pent-1-enyl-3-phenyl-benzo[b]thiophene (**3ca**)*. Yellow solid, mp. 60.8-63.1 °C. IR (KBr): ν = 3058 (w), 3024 (w), 2958 (s), 2928 (s), 2870 (m), 1487 (w), 1457 (m), 1434 (s), 1205 (m), 1157 (w), 1071 (w), 956 (s), 767 (s), 753 (m), 732 (s), 717 (m), 701 (s) cm^{-1}; ^1H NMR (300 MHz): δ = 7.79-7.71 (m, 1H aromatic), 7.52-7.44 (m, 3H aromatic), 7.44-7.35 (m, 3H, aromatic), 7.30-7.20 (m, 2 H aromatic), 6.53 (dt, *J*=15.5, 1.4, 1H, CH=C*H*), 6.20 (dt, *J*=15.5, 7.0, 1H, CH=C*H*), 2.20-2.07 (m, 2H, $CH_2CH_2CH_3$), 1.44 (q, *J*=7.3, 2H, $CH_2CH_2CH_3$), 0.91 (t, *J*=7.3, 3H, $CH_2CH_2CH_3$);^{13}C NMR (75 MHz): δ = 140.6, 138.8, 137.7, 135.0, 134.3, 130.4, 128.5, 127.5, 124.6, 124.3, 122.9, 122.8, 122.0, 121.5, 35.2, 22.4, 13.7; GC-MS: *m/z* = 280 (5) [$(M+2)^+$], 279 (17), 278 (75) [M^+], 250 (12), 249 (60), 247 (18), 236 (26), 235 (100), 234 (86), 221 (33), 216 (16), 215 (20), 202 (12), 171 (10), 115 (10); anal. calcd for $C_{19}H_{18}S$ (278.11): C, 81.97; H, 6.52; S, 11.52; found C, 81.99; H, 6.55; S, 11.58.

- *2-(3-Phenyl-propenyl)-benzo[b]thiophene (**3ad**)*. Yellow solid, mp. 58.8-60.0 °C. IR (KBr): ν = 3059 (w), 2947 (m), 2877 (m), 1450 (m, br), 1385 (m), 1215 (w), 1026 (w), 949 (m), 833 (w), 749 (s), 698 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.80-7.59 (m, 2 H aromatic), 7.45-7.15 (m, 7 H aromatic), 7.06 (s, 1 H, H-3), 6.70-6.56 (m, 1H, C*H*=CH), 6.38-6.22 (m, 1H, CH=C*H*), 3.55 (d, *J*=7.3, 2 H, CH_2Ph); ^{13}C NMR (75 MHz): δ =142.7, 140.2, 139.5, 138.7, 132.0, 128.8, 128.6, 126.4, 125.0, 124.4, 124.3, 123.2, 122.1, 121.8, 39.2 ; GC-MS: m/z = 252 (10) [$(M+2)^+$], 251 (24), 250 (100) [M^+], 249 (51), 235 (32), 215 (18), 202 (12), 173 (21), 171 (15), 147 (22),

134 (13), 115 (34), 91 (10), 98 (11); anal. calcd for C17H14S (270.08): C, 81.56; H, 5.64; S, 12.81 ; found C, 81.56; H, 5.67; S, 12.82.

- *3-Methyl-2-(3-Phenyl-propenyl)-benzo[b]thiophene (**3bd**).* White solid, mp. 61.4-62.8 °C. IR (KBr): ν = 3429 (w), 3060 (w), 3025 (w), 2918 (m), 2855 (w), 1601 (w), 1495 (m), 1437 (m), 1418 (w), 1212 (w), 1075 (w), 1029 (w), 957 (m), 933 (w), 752 (m), 741 (s), 727 (m), 700 (m), 670 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.72-7.65 (m, 1H aromatic), 7.60-7.54 (m, 1H aromatic), 7.38-7.11 (m, 7H, aromatic), 6.76 (dt, *J* =15.4, 1.4, 1H, CH=C*H*), 6.25 (dt, *J*=15.4, 7.0, 1H, CH=C*H*), 3.56 (d, *J* = 6.9, 2H, CH_2), 2.35 (s, 3H, CH_3);^{13}C NMR (75 MHz): δ = 141.1, 139.7, 137.8, 136.2, 131.3, 128.7, 128.5, 128.3, 126.3, 124.6, 124.0, 123.2, 122.1, 121.6, 39.5, 11.7; GC-MS: m/z = 266 (6) [$(M+2)^+$], 265 (20), 264 (100) [M^+], 250 (13), 249 (64), 247 (10), 234 (11), 216 (15), 215 (10), 173 (23), 171 (22), 161 (16), 160 (13), 147 (15), 130 (10), 128 (16), 116 (19), 115 (95), 91 (30), 77 (13); anal. calcd for $C_{18}H_{16}S$ (264.10): C, 81.77; H, 6.10; S, 12.13; found C, 81.79; H, 6.12; S, 12.12.

- *3-Phenyl-2-(3-Phenyl-propenyl)-benzo[b]thiophene (**3cd**).* Yellow solid, mp. 58.8-62.0 °C. IR (KBr): ν =3170 (w), 3059 (m), 3024 (m), 1597 (w), 1489 (m), 1435 (m), 1385 (w), 1207 (m), 1153 (w), 1068 (m), 1022 (w), 949 (s), 748 (s), 694 (s) cm^{-1}; ^1H NMR (300 MHz): δ = 7.80-7.76 (m, 1H aromatic), 7.61-7.54 (m, 6H aromatic), 7.35-7.08 (m, 7H, aromatic), 6.70-6.58 (m, 1H, CH=C*H*), 6.30 (dt, *J*=15.5, 7.2, 1H, CH=C*H*), 3.48 (d, *J*=7.3, 2H, CH_2); ^{13}C NMR (75 MHz): δ = 140.4, 139.7, 138.2, 137.8, 134.9, 134.4, 132.2, 130.4, 128.5, 127.6, 126.3, 124.8, 124.3, 124.1, 122.9, 122.1, 39.4; GC-MS: m/z = 328 (2) [$(M+2)^+$], 327 (14), 326 (37) [M^+], 236 (23), 235

(100), 234 (89), 222 (10), 221 (28), 115 (19), 91 (21); anal. calcd for $C_{23}H_{18}S$ (326.11): C, 84.62; H, 5.56; S, 9.82; found C, 84.66; H, 5.57; S, 9.84.

- *2-Styryl-benzo[b]thiophene (**3ae**)*. Yellow solid, mp. 122.3-124.8 °C. *(17)* IR (KBr): ν = 2921 (w), 2850 (w), 1447 (w), 1384 (s), 1225 (w), 1143 (w), 1074 (w), 948 (m), 818 (m), 740 (s), 691 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.82-7.74 (m, 1H aromatic), 7.72-7.67 (m, 1H aromatic), 7.57-7.46 (m, 2H, aromatic), 7.42-7.22 (m, 7H, 4H aromatic + H_3 + 2CH=CH), 7.04-6.95 (m, 1H aromatic); ^{13}C NMR (75 MHz): δ = 142.9, 140.2, 136.7, 130.9, 128.8, 128.3, 128.0, 126.6, 124.8, 124.5, 123.4, 123.3, 122.3, 122.2; m/z = 238 (5) [$(M+2)^+$], 237 (20), 236 (100) [M^+], 235 (87), 234 (55), 221 (18), 202 (21), 189 (6), 134 (5), 117 (14), 104 (8), 89 (5), 77 (7), 63 (7), 51 (5); anal. calcd for $C_{16}H_{12}S$ (236.07): C, 81.31; H, 5.12; S, 13.57; found C, 81.33; H, 5.13; S, 13.59.

- *3-Phenyl-2-Styryl-benzo[b]thiophene (**3ce**)*. Yellow solid, mp. 56.8-59.2 °C. IR (KBr): ν = 3058 (m), 3028 (m), 2923 (m), 2850 (w), 1599 (m), 1494 (m), 1432 (m), 1318 (w), 1221 (w), 1072 (w), 951 (m), 769 (s), 692 (m), cm^{-1}; ^1H NMR (300 MHz): δ = 7.82-7.75 (m, 1 H aromatic), 7.58-6.94 (m, 13 H aromatic, 2H C*H*=C*H*); ^{13}C NMR (75 MHz): δ = 140.5, 138.5, 138.0, 136.8, 135.9, 134.8, 131.1, 130.4, 128.6, 127.9, 127.7, 126.6, 125.1, 124.5, 123.0, 122.1, 121.4, 29.7; GC-MS: m/z = 314 (7) [$(M+2)^+$], 313 (24), 312 (100) [M^+], 311 (40), 297 (17), 235 (37), 234 (46), 221 (10), 202 (11), 78 (28), 77 (18); anal. calcd for $C_{22}H_{16}S$ (312.10): C, 84.57; H, 5.16; S, 10.26; found C, 84.56; H, 5.17; S, 10.26.

- *2-(1-Methoxy-pentyl)-benzo[b]thiophene (**4aa**)*. Yellow oil. IR (film): ν= 2956 (s), 2931 (s), 2859 (m), 2821 (m), 1458 (s), 1436 (m), 1351 (w), 1251

(w), 1193 (w), 1126 (m), 1091 (s), 829 (w), 746 (m), 727 (m), 603 (w) cm-[1]; [1]H NMR (300 MHz): δ = 7.86-7.78 (m, 1H aromatic), 7.74 -7.68 (m, 1H aromatic), 7.39-7.25 (m, 2H, aromatic), 6.18 (s, 1H, C*H*=CS), 4.41 (t, *J*=6.7, 1H, C*H*OCH_3), 3.30 (s, 3H, OCH_3), 2.06-1.88 (m, 1H, C*H*H$CH_2CH_2CH_3$), 1.86-1.69 (m, 1H, CH*H*$CH_2CH_2CH_3$), 1.49-1.17 (m, 4H, $CH_2CH_2CH_3$), 0.88 (t, *J*=6.9, 3H, CH_3); [13]C NMR (75 MHz): δ = 147.5, 139.7, 129.1, 124.1, 123.3, 122.6, 121.8, 80.2, 56.7, 37.7, 29.7, 27.9, 22.5, 14.0; GC-MS: m/z = 236 (1) [$(M+2)^+$], 235 (2), 234 (14) [M^+], 178 (13), 177 (100), 162 (12), 161 (16), 147 (12), 134 (11) ; anal. calcd for C14H18OS (234.11): C, 71.75; H, 7.74; S, 13.68; found C, 71.78; H, 7.79; S, 13.69.

- *2-(1-Methoxy-2,2-dimethyl-propyl)-benzo[b]thiophene (**4ab**).* Yellow solid, mp. 96.4-97.5°C. IR (KBr): ν = 3447 (w), 2960 (m), 2925 (m), 2867 (w), 1460 (m), 1384 (s), 1187 (w), 1127 (m), 1087 (s), 966 (w), 836 (s), 759 (s), 728 (s), 676 (w) cm^{-1}; [1]H NMR (300 MHz): δ = 7.86-7.78 (m, 1H aromatic), 7.76-7.68 (m, 1H aromatic), 7.40-7.23 (m, 2H, aromatic), 7.14 (s, 1H, H-3), 4.07 (s, 1H, C*H*OCH_3), 3.30 (s, 3H, OCH_3), 1.01 (s, 9H, $C(CH_3)_3$); [13]C NMR (75 MHz): δ = 144.9, 139.8, 139.3, 124.0, 123.8, 123.2, 123.1, 122.2, 88.9, 57.9, 35.7, 29.7, 26.3; GC-MS: m/z = 236 (0.4) [$(M+2)^+$], 235 (1), 234 (9) [M^+], 178 (12), 177 (100), 162 (12), 161 (17), 134 (10), 89 (5); anal. calcd for $C_{14}H_{18}OS$ (234.11): C, 71.75; H, 7.74; S, 13.68; found C, 71.76; H, 7.77; S, 13.68.

- *2-(Methoxy-phenyl-methyl)-benzo[b]thiophene (**4ac**)*. Yellow solid, mp. 60.3-61.2 °C. IR (KBr): ν = 3003 (w), 2930 (m), 2830 (m), 1493 (m), 1451 (m), 1384 (w), 1307 (w), 1271 (w), 1195 (m), 1090 (s), 1157 (w), 1026 (w), 964 (w), 833 (s), 782 (s), 715 (s), 660 (m), 640 (m) cm^{-1}; [1]H NMR (300 MHz): δ = 7.84-7.73 (m, 1H aromatic), 7.71-7.62 (m, 1H aromatic),

7.53-7.20 (m, 7H, aromatic), 7.07 (s, 1H, H-3), 5.52 (s, 1H, C*H*OCH_3), 3.43 (s, 3H, OCH_3); ^{13}C NMR (75 MHz): δ = 147.1, 140.8, 140.1, 139.4, 128.5, 128.1, 126.9, 124.1, 123.5, 122.4, 121.8, 81.9, 57.1, 29.7; GC-MS: m/z = 256 (4) [$(M+2)^+$], 255 (9), 254 (53) [M^+], 224 (20), 223 (100), 222 (21), 221 (32), 178 (17), 177 (19), 161 (17), 111 (15), 105 (43), 89 (21); anal. calcd for $C_{16}H_{14}OS$ (254.08): C, 75.55; H, 5.55; S, 12.61; found C, 75.56; H, 5.57; S, 12.68.

- *2-(1-Methoxy-pentyl)-3-methyl-benzo[b]thiophene (**4ba**)*. Yellow oil. IR (KBr): ν = 3447 (w), 2955 (s), 2931 (s), 2859 (m), 2818 (m), 1460 (m), 1437 (m), 1380 (w), 1339 (w), 1192 (w), 1130 (w), 1177 (w), 1094 (s), 754 (s), 729 (s), 713 (w) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.80 (dt distorted, *J*=7.7, 0.8, 1H, aromatic), 7.65 (dt distorted, *J*=7.7, 0.8, 1H, aromatic), 7.38 (td , *J*=7.3, 1.2, 1H, aromatic), 7.31 (td, *J*=7.3, 1.6, 1H, aromatic), 4.60 (t, *J*=6.9, 1H, C*H*OCH_3), 3.28 (s, 3H, OCH_3), 2.38 (s, 3H, CCH_3), 2.07-1.89 (m, 1H, C*H*$HCH_2CH_2CH_3$), 1.85-1.68 (m, 1H, CH*H*$CH_2CH_2CH_3$), 1.48-1.16 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.88 (t, *J*=6.9, 3H, $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ = 141.3, 140.6, 138.9, 128.7, 124.2, 123.8, 122.6, 121.5, 76.6, 56.6, 37.7, 29.7, 27.9, 22.6, 14.0, 11.9; GC-MS: m/z = 250 (1) [$(M+2)^+$], 249 (2), 248 (12) [M^+], 192 (14), 191 (100), 176 (14), 175 (13), 147 (16), 128 (5), 115 (5); anal. calcd for $C_{15}H_{20}OS$ (248.12): C, 72.53; H, 8.12; S, 12.91; found C, 72.56; H, 8.13; S, 12.90.

- *2-(1-Methoxy-2,2-dimethyl-propyl)-3-methyl-benzo[b]thiophene (**4bb**).* White solid, mp. 56.1-58.2 °C. IR (KBr): ν = 3446 (w), 2953 (s), 2865 (m), 2818 (m), 1480 (m), 1460 (s), 1385 (s), 1362 (s), 1264 (m), 1169 (s), 1156 (m), 1092 (s), 1015 (w), 960 (s), 912 (m), 755 (s), 739 (s), 705 (w), 624 (w) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.83-7.76 (m, 1H, aromatic), 7.70-7.63 (m, 1H,

aromatic), 7.40-7.26 (m, 2H, aromatic), 4.28 (s, 1H, C*H*OCH_3), 3.24 (s, 3H, OCH_3), 2.37 (s, 3H, CCH_3), 1.03 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz): δ = 140.3, 139.1, 139.0, 130.0, 124.1, 123.5, 122.2, 121.3, 86.1, 65.8, 57.6, 36.9, 26.5, 15.3, 12.8; GC-MS: m/z = 250 (1) [$(M+2)^+$], 249 (2), 248 (9) [M^+], 192 (17), 191 (100), 176 (19), 175 (17), 147 (17); anal. calcd for $C_{15}H_{20}OS$ (248.12): C, 72.53; H, 8.12; S, 12.91; found C, 72.56; H, 8.10; S, 12.91.

- *2-(Methoxy-phenyl-methyl)-3-methyl-benzo[b]thiophene (**4bc**).* Yellow oil. IR (film): ν = 3060 (m), 2985 (w), 2929 (m), 2820 (m), 1602 (w), 1493 (m), 1460 (s), 1436 (s), 13.80 (w), 1312 (m), 1171 (m), 1094 (s), 1070 (s), 1029 (m), 960 (m), 915 (w), 732 (s), 730 (s), 700 (s), 648 (w) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.80-7.74 (m, 1H, aromatic), 7.68-7.62 (m, 1H, aromatic), 7.48-7.41 (m, 2H, aromatic), 7.37-7.24 (m, 5H, aromatic), 5.70 (s, 1H, C*H*OCH_3), 3.45 (s, 3H, OCH_3), 2.41 (s, 3H, CCH_3); ^{13}C NMR (75 MHz): δ = 140.9, 140.5, 140.3, 139.1, 128.5, 127.8, 126.7, 124.2, 123.8, 122.5, 121.6, 79.7, 57.1, 12.0; GC-MS: m/z = 270 (3) [$(M+2)^+$], 269 (10), 268 (52) [M^+], 253 (16), 238 (18), 237 (100), 236 (12), 222 (12), 221 (18), 191 (21), 175 (11), 147 (15), 105 (16), 91 (10); anal. calcd for $C_{17}H_{16}OS$ (268.09): C, 76.08; H, 6.01; S, 11.95; found C, 76.06; H, 6.00; S, 11.95.

- *2-(1-Methoxy-pentyl)-3-phenyl-benzo[b]thiophene (**4ca**)*. Yellow oil. IR (film): ν = 3058 (m), 2984 (w), 2857 (m), 2818 (m), 1603 (w), 1489 (m), 1456 (s), 1435 (s), 1350 (w), 1335 (w), 1251 (w), 1194 (m), 1124 (w), 1089 (m), 1030 (w) 949 (w), 768 (s), 733 (s), 701 (s), 649 (w), 603 (m) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.89-782 (m, 1H, aromatic), 7.56-7.40 (m, 4H, aromatic), 7.39-7.24 (m, 4H, aromatic), 4.45 (t, *J*=6.8, 1H, C*H*OCH_3), 3.21 (s, 3H, OCH_3), 2.03-1.87 (m, 1H, C*H*H$CH_2CH_2CH_3$), 1.85-1.70 (m, 1H,

CH*H*$CH_2CH_2CH_3$), 1.43-1.13 (m, 4H, CH_2C$H_2$$CH_3$), 0.82 (t, *J*=7.0, 3H, $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ = 143.7, 140.1, 138.8, 136.0, 135.1, 130.0, 128.6, 127.7, 124.5, 124.1, 123.0, 122.5, 77.5, 56.5, 38.1, 27.9, 22.5, 13.9; GC-MS: m/z = 312 (1) [$(M+2)^+$], 311 (3), 310 (14) [M^+], 254 (18), 253 (100), 237 (16), 221 (33), 165 (11); anal. calcd for $C_{20}H_{22}OS$ (310.14): C, 77.38; H, 7.14; S, 10.33; found C, 77.39; H, 7.16; S, 10.37.

- *2-(1-Methoxy-2,2-dimethyl-propyl)-3-phenyl-benzo[b]thiophene (**4cb**).* White solid, mp. 99.1-99.5 °C. IR (KBr): ν = 3055 (m), 2983 (s), 2919 (m), 2868 (m), 2817 (m), 1600 (w), 1477 (m), 1459 (s), 1364 (m), 1311 (w), 1180 (w), 1134 (w), 1068 (s), 963 (m), 755 (s), 735 (s), 700 (s), 654 (m) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.85 (d, *J*=7.3, 1H, aromatic), 7.54-7.41 (m, 3H, aromatic), 7.40-7.22 (m, 5H, aromatic), 4.22 (s, 1H, C*H*OCH_3), 3.30 (s, 3H, OCH_3), 0.90 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75 MHz): δ = 140.8, 140.1, 139.1, 137.6, 135.7, 128.6, 127.5, 124.4, 123.9, 122.9, 122.1, 85.6, 57.3, 36.4, 26.6; GC-MS: m/z = 312 (0.3) [$(M+2)^+$], 311 (0.9), 310 (4) [M^+], 254 (18), 253 (100), 237 (15), 221 (30), 165 (9); anal. calcd for $C_{20}H_{22}OS$ (310.14): C, 77.38; H, 7.14; S, 10.33; found C, 76.36; H, 7.17; S, 10.34.

- *2-(Methoxy-phenyl-methyl)-3-phenyl-benzo[b]thiophene (**4cc**).* Yellow solid, mp. 106.2-107.1 °C. IR (KBr): ν= 3055 (m), 2994 (m), 2925 (m), 2815 (m), 1494 (m), 1440 (s), 1384 (m), 1317 (m), 1185 (m), 1133 (s), 1077 (s), 1028 (m), 970 (m), 857 (w), 771 (s), 751 (s), 700 (s), 667 (m), 630 (w) cm-1; 1H NMR (300 MHz): δ = 7.83-7.74 (m, 1H aromatic), 7.55-7.12 (m, 14H aromatic), 5.54 (s, 1H, CHOCH3), 3.35 (s, 3H, OCH3); 13C NMR (75 MHz): δ = 142.5, 141.2, 140.0, 139.3, 135.8, 134.9, 130.1, 128.7, 128.4, 127.8, 127.7, 126.6, 124.6, 124.1, 123.1, 122.4, 79.4, 56.8; GC-MS: m/z = 332 (4) [$(M+2)^+$], 331 (16), 330 (61) [M^+], 300 (18), 299 (70), 298 (27),

297 (13), 253 (15), 237 (11), 222 (17), 221 (100), 165 (15), 105 (16); anal. calcd for C22H18OS (330.11): C, 79.96; H, 5.49; S, 9.70; found C, 79.99; H, 5.47; S, 9.75.

- *2-(1-Methoxy-3-phenyl-propyl)-benzo[b]thiophene (**4ad**)* Yellow oil. IR (film): ν = 3062 (w), 3016 (w), 2934 (m), 2860 (m), 2820 (w), 1605 (m), 1486 (w), 1458 (w), 1437 (m), 1330 (m), 1188 (w), 966 (w), 794 (m), 780 (s), 655 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.88-7.76 (m, 2H aromatic), 7.48-6.90 (m, 8H aromatic), 4.14 (s, 1H, C*H*OCH_3), 3.28 (s, 3H, OCH_3), 2.51 (s, 2H, CH_2CH_2Ph), 2.01 (s, 2H, CH_2CH_2Ph); ^{13}C NMR (75 MHz): δ = 146.1, 139.0, 138.8, 137.1, 128.6, 128.5, 128.3, 128.2, 125.8, 124.4, 124.3, 123.8, 122.6, 120.7, 81.9, 40.3, 51.4, 29.5; GC-MS: *m/z* = 284 (1) [$(M+2)^+$], 283 (4), 282 (15) [M^+], 178 (25), 177 (100), 162 (12), 161 (15), 134 (10), 91 (16); anal. calcd for $C_{18}H_{18}OS$ (282.11): C, 76.56; H, 6.42; S, 11.35; found C, 76.56; H, 6.47; S, 11.38

- *2-(1-Methoxy-3-phenyl-propyl)-3-methyl-benzo[b]thiophene (**4bd**).* Yellow oil. IR (film): ν = 3061 (w), 3026 (w), 2924 (m), 2859 (w), 2818 (w), 1603 (w), 1496 (w), 1455 (m), 1437 (m), 1340 (w), 1178 (w), 942 (w), 1102 (s), 754 (s), 700 (m), 634 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.85-7.77 (m, 1 H aromatic), 7.70-7.60 (m, 1 H aromatic), 7.41-7.12 (m, 7 H, aromatic), 4.58 (dd, *J*=7.3, 6.1, 1 H, CHOCH_3), 3.27 (s, 3 H, OMe), 2.82-2.61 (m, 2 H, CH_2CH_2Ph), 2.42-2.20 (m, 1 H, CH_2C*H*HPh), 2.29 (s, 3H, CH_3), 2.17-1.98 (m, 1H, CH_2CH*H*Ph); ^{13}C NMR (75 MHz): δ =141.5, 140.7, 140.6, 138.9, 128.9, 128.5, 128.4, 125.9, 124.3, 123.8, 122.6, 121.5, 77.4, 56.6, 39.2, 31.9, 11.8; GC-MS: *m/z* =298 (1) [$(M+2)^+$], 296 (14) [M^+], 192 (14), 191 (100), 176 (12), 175 (11), 147 (14), 91 (28); anal. calcd for $C_{19}H_{20}OS$ (296.12): C, 76.98; H, 6.80; S, 10.82; found C, 76.96; H, 6.82; S, 10.83.

- *2-(1-Methoxy-3-phenyl-propyl)-3-phenyl-benzo[b]thiophene (**4cd**).* Yellow solid, mp. 61.5-63.0°C. IR (KBr): ν = 3062 (w), 2931 (m), 2858 (w), 1493 (w), 1442 (m), 1385 (w), 1261 (w), 1074 (s), 1026 (w), 941 (w), 748 (s), 690 (s) cm^{-1}; δ = 7.93-7.80 (m, 1 H aromatic), 7.56-7.04 (m, 13 H aromatic), 4.53-4.42 (m, 1 H, CHOC*H*$_3$), 3.21 (s, 3 H, OMe), 2.84-2.53 (m, 2 H, C*H*$_2$CH$_2$Ph), 2.38-2.18 (m, 1 H, CH$_2$C*H*HPh), 2.14-1.96 (m, 1H, CH$_2$CH*H*Ph); ^{13}C NMR (75 MHz): δ =153.7, 143.3, 141.2, 140.1, 138.9, 136.0, 134.8, 129.9, 128.6, 128.4, 127.6, 125.8, 124.6, 124.1, 123.0, 122.5, 56.6, 39.8, 31.9, 29.7; GC-MS: *m/z* = 360 (1) [(M+2)$^+$], 359 (5), 358 (17) [M$^+$], 253 (100), 237 (17), 234 (10), 221 (37), 165 (12), 91 (23); anal. calcd for $C_{24}H_{22}OS$ (358.14): C, 80.41; H, 6.19; S, 8.94; found C, 80.43; H, 6.21; S, 8.96.

- *2-(1-Methoxy-2-phenyl-ethyl)-benzo[b]thiophene (**4ae**).* Yellow solid, mp. 63.5-64.4 °C. IR (KBr): ν = 3052 (w), 2927 (m), 2852 (w), 1495 (w), 1456 (m), 1384 (s), 1316 (w), 1190 (w), 1133 (w), 1102 (s), 1079 (w), 1026 (w), 871 (w), 842 (m), 756 (s), 727 (m), 705 (m), 669 (w), 603 (m) cm^{-1}; ^{1}H NMR (300 MHz): δ = 7.98-7.73 (m, 1H aromatic), 7.71-7.62 (m, 1H aromatic), 7.41-7.17 (m, 7H, aromatic), 7.07 (s, 1H, H-3), 4.64 (s, 1H, C*H*OCH$_3$), 3.30 (s, 3H, OCH$_3$), 3.01 (s, 2H, C*H*$_2$Ph); ^{13}C NMR (75 MHz): δ = 146.4, 139.7, 139.3, 137.8, 129.4, 128.2, 126.4, 124.2, 123.4, 122.6, 122.2, 122.1, 81.3, 56.9, 44.6; GC-MS: *m/z* = 270 (0.2) [(M+2)$^+$], 269 (0.8), 268 (4) [M$^+$], 178 (12), 177 (100), 162 (12), 161 (14), 134 (9); anal. calcd for $C_{17}H_{16}OS$ (268.09): C, 76.08; H, 6.01; S, 11.95; found C, 76.06; H, 6.03; S, 11.98.

- *2-(1-Methoxy-2-phenyl-ethyl)-3-phenyl-benzo[b]thiophene (**4ce**).* Yellow solid, mp. 70.3-71.0 °C. IR (KBr): ν = 3435 (m), 2921 (w), 2820 (w), 1440

(w), 1304 (s), 1333 (w), 1195 (w), 1092 (m), 974 (w), 770 (m), 757 (m), 698 (m), 598 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.95-7.82 (m, 1 H aromatic), 7.49-7.22 (m, 7 H aromatic), 7.21-7.12 (m, 3 H, aromatic), 7.10-6.94 (m, 3 H, aromatic), 4.60 (dd, *J*=7.3, 6.5, 1 H, CHOCH_3), 3.18 (s, 3 H, OMe), 1.57 (s, H, C*H*HPh), 1.26 (s, H, CH*H*Ph); ^{13}C NMR (75 MHz): δ =142.2, 140.1, 138.9, 137.5, 136.8, 134.6, 129.8, 129.5, 128.4, 128.2, 127.5, 126.4, 124.6, 124.1, 123.1, 122.6, 79.0, 56.5, 44.7; GC-MS: *m/z* = 346 (0.1) [$(M+2)^+$], 345 (0.5), 344 (2) [M^+], 254 (18), 253 (100), 237 (21), 221 (37), 165 (14), 91 (30); anal. calcd for $C_{17}H_{16}OS$ (344.12): C, 80.19; H, 5.85; S, 9.31; found C, 80.20; H, 5.87; S, 9.33.

- *2-(1-Ethoxy-pentyl)-benzo[b]thiophene (**4aa'**).* Yellow oil. IR (film): ν = 3446 (w), 2955 (m), 2936 (m), 2859 (m), 1457 (m), 1398 (w), 1318 (m), 1241 (w), 1159 (w), 1105 (m), 1087 (m), 938 (w), 872 (w), 836 (s), 750 (s), 727 (s), 670 (w), 605 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.84-7.77 (m, 1 H aromatic), 7.74-7.68 (m, 1 H aromatic), 7.38-7.24 (m, 2 H aromatic), 7.16 (s, 1H, H-3), 4.52 (t, *J*=6.9, 1H, C*H*OCH_2CH_3), 3.60-3.35 (m, 2H, OCH_2CH_3) 2.01-1.88 (m, 1H, C*H*H$CH_2CH_2CH_3$), 1.84-1.71 (m, 1H, CH*H*$CH_2CH_2CH_3$), 1.40-1.15 (m, 4H, CH_2CH_2CH_3), 1.20 (t, *J*=7.1, 3H, OCH_2CH_3), 0.88 (t, *J*=7.1, 3H, $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ = 148.5, 139.6, 139.4, 124.1, 124.0, 123.2, 122.6, 121.3, 64.3, 37.9, 29.7, 27.9, 22.5, 15.3, 14.0; GC-MS: m/z = 250 (1) [$(M+2)^+$], 249 (3), 248 (20) [M^+], 192 (15), 191 (100), 163 (75), 161 (15), 147 (23), 135 (42), 134 (13), 115 (10), 91 (23); anal. calcd for $C_{15}H_{20}OS$ (248.12): C, 72.53; H, 8.12; S, 12.91; found C, 72.56; H, 8.17; S, 12.90.

- *2-(1-Ethoxy-2,2-dimethyl-propyl)-benzo[b]thiophene (**4ab'**)*. White solid, mp. 27.0-27.5 °C. IR (KBr): ν = 3420 (w), 2963 (m), 2866 (m), 1458 (m),

1388 (m), 1365 (m), 1314 (w), 1205 (w), 1119 (w), 1090 (s), 1014 (w), 939 (w), 892 (w), 837 (s), 752 (s), 728 (s), 683 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.84-7.76 (m, 1 H aromatic), 6.74-6.78 (m, 1 H aromatic), 7.30 (qd, *J*=7.3, 1.6, 2H aromatic), 7.12 (s, 1 H, H-3), 4.17 (s, 1H, C*H*OCH_2CH_3), 3.60-3.48 (m, 1H, OC*H*HCH_3), 3.40-3.28 (m, 1H, OCH*H*CH_3), 1.17 (t, *J*=7.1, 3H, OCH_2CH_3), 1.00 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz): δ = 146.0, 139.7, 139.4, 123.9, 123.7, 123.0, 122.6, 122.2, 86.4, 65.2, 35.6, 29.7, 26.3, 15.1; GC-MS: m/z = 250 (1) [$(M+2)^+$], 249 (2), 248 (14) [M^+], 192 (13), 191 (100), 163 (82), 162 (10), 161 (21), 135 (40), 134 (17), 91 (27), 89 (10); anal. calcd for $C_{15}H_{20}OS$ (248.12): C, 72.53; H, 8.12; S, 12.91; found C, 73.56; H, 8.13; S, 12.90.

- *2-(Ethoxy-phenyl-methyl)-benzo[b]thiophene (**4ac'**)*. Yellow solid, mp. 34.0-34.9 °C. IR (KBr): ν = 3443 (w), 2971 (m), 2890 (m), 2866 (m), 1492 (w), 1457 (s), 1433 (s), 1436 (m), 1384 (m), 1312 (w), 1216 (w), 1139 (m), 1083 (s), 1072 (s), 1009 (w), 851 (m), 756 (s), 728 (m), 701 (s), 640 (m) cm^{-1}; δ = 7.79-7.72 (m, 1 H aromatic), 7.69-7.62 (m, 1 H aromatic), 7.50-7.43 (m, 2 H aromatic), 7.40-7.21 (m, 5 H aromatic), 7.08 (s, 1H, H-3), 5.64 (s, 1H, C*H*OCH_2CH_3), 3.68-3.54 (m, 2H, OCH_2CH_3), 1.29 (t, *J*=7.0, 3H, OCH_2CH_3); ^{13}C NMR (75 MHz): δ = 147.7, 141.3, 140.0, 139.4, 128.5, 128.0, 126.9, 124.1, 124.0, 123.5, 122.4, 121.6, 80.0, 64.9, 15.3; GC-MS: *m/z* = 270 (3) [$(M+2)^+$], 269 (11), 268 (56) [M^+], 224(32), 223 (100), 222 (20), 221 (30), 179 (11), 178 (21), 163 (11), 161 (12), 135 (10), 111 (12), 105 (53), 105 (53), 91 (10), 89 (21); anal. calcd for $C_{17}H_{16}OS$ (268.09): C, 76.08; H, 6.01; S, 11.95; found C, 76.06; H, 6.00; S, 11.98.

- *2-(1-Ethoxy-pentyl)-3-methyl-benzo[b]thiophene (**4ba'**)*. Colorless oil. IR (film): ν = 3061 (w), 2957 (m), 2931 (s), 2861 (s), 1460 (m), 1438 (m),

1380 (w), 1331 (w), 1180 (w), 1104 (m), 1089 (m), 1002 (w), 756 (s), 729 (s) cm^{-1}; ^1H NMR (300 MHz): δ = 7.78 (d, *J*=8.1, 1H aromatic), 7.64 (d, *J*=8.1, 1H aromatic), 7.42-7.19 (m, 2 H aromatic), 4.70 (t, *J*=6.6, 1H, C*H*OCH_2CH_3), 3.60-3.30 (m, 2H, C*H*$_2CH_2CH_2CH_3$), 2.36 (s, 3H, Me), 2.10-1.92 (m, 1H, $CH_2CHHCH_2CH_3$), 1.83-1.68 (m, 1H, $CH_2CHHCH_2CH_3$), 1.45-1.25 (m, 4H, $CH_2CH_2CH_2CH_3$ + OCH_2CH_3), 1.19 (t, *J*=6.6, 3H, OCH_2CH_3), 0.88 (t, *J*=7.3, 3H, $CH_2CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ =142.3, 140.7, 138.8, 128.1, 124.1, 123.7, 122.5, 121.4, 76.0, 64.2, 37.9, 27.9, 22.6, 15.3, 14.0, 11.9; GC-MS: *m/z* = 264 (2) [$(M+2)^+$], 263 (8), 262 (39) [M^+], 206 (26), 205 (100), 178 (11), 177 (85), 175 (10), 161 (20), 149 (42), 147 (17), 134 (20); anal. calcd for $C_{16}H_{22}OS$ (262.14): C, 73.23; H, 6.10; S, 12.22; found C, 73.25; H, 6.17; S, 12.21.

- *2-(1-Ethoxy-2,2-dimethyl-propyl)-3-methyl-benzo[b]thiophene (**4bb'**)*. Yellow solid, mp. 49.3-51.2 °C. IR (KBr): ν = 2964 (m), 2931 (m), 2863 (m), 2834 (m), 1461 (m), 1384 (m), 1316 (m), 1263 (w), 1160 (m), 1072 (s), 996 (m), 916 (w), 763 (s), 736 (m), 715 (m), 634 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.78 (d, *J*=7.3, 1 H aromatic), 7.69 (d, *J*=7.3, 1 H aromatic), 7.40-7.25 (m, 2 H aromatic), 4.37 (s, 1H, C*H*OCH_2CH_3), 3.52-3.38 (m, 1H, OC*H*HCH_3), 3.35-3.20 (m, 1H, OCH*H*CH_3), 1.15 (t, *J*=7.0, 3H, OCH_2CH_3), 1.02 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz): δ = 140.37, 140.2, 139.1, 129.4, 123.9, 123.5, 122.1, 121.3, 83.7, 64.9, 36.9, 26.5, 15.1, 12.8; GC-MS: *m/z* = 264 (0.5) [$(M+2)^+$], 263 (2), 262 (8) [M^+], 206 (14), 205 (100), 177 (61), 149 (31), 147 (12), 134 (16); anal. calcd for $C_{16}H_{22}OS$ (262.14): C, 73.23; H, 8.45; S, 12.22; found C, 7.24; H, 8.47; S, 12.25.

- *2-(Ethoxy-phenyl-methyl)-3-methyl-benzo[b]thiophene (**4bc'**)*. Yellow oil. IR (KBr): ν = 3061 (m), 2974 (m), 2917 (s), 2849 (m), 1493 (m), 1436

(m), 1381 (w), 1305 (w), 1260 (w), 1170 (m), 1155 (m), 1070 (s), 1066 (m), 898 (w), 733 (s), 729 (s), 700 (s) cm^{-1}; ^1H NMR (300 MHz): δ = 7.74 (d, *J*=7.3, 1 H aromatic), 7.63 (d, *J*=7.3, 1 H aromatic), 7.44 (d distorted, *J*=7.3, 1 H aromatic), 7.38-7.19 (m, 2 H aromatic), 5.80 (s, 1H, C*H*OCH_2CH_3), 3.71-3.51 (m, 2H, OCH_2CH_3), 2.39 (s, 3H, CH_3), 1.33-1.21 (m, 3H, OCH_2CH_3); ^{13}C NMR (75 MHz): δ = 141.3, 141.1, 140.6, 139.1, 128.4, 127.7, 126.7, 124.1, 123.8, 122.4, 121.5, 77.8, 64.8, 29.7, 15.2, 12.0; GC-MS: *m/z* = 284 (4) [$(M+2)^+$], 283 (13), 282 (57) [M^+], 267 (11), 239 (11), 238 (24), 237 (100), 236 (17), 235 (14), 222 (12), 221 (18), 205 (11), 105 (24); anal. calcd for $C_{18}H_{18}OS$ (282.11): C, 76.56; H, 6.42; S, 11.35; found C, 76.56; H, 6.47; S, 11.38.

- *2-(1-Ethoxy-pentyl)-3-phenyl-benzo[b]thiophene (**4ca'**).* Colorless oil. IR (film): ν = 2935 (s), 2856 (s), 1457 (m), 1377 (m), 1193 (w), 1085 (m), 968 (w), 768 (m), 734 (m), 701 (m) cm^{-1}; δ = 7.89-7.80 (m, 1 H aromatic), 7.55-7.22 (m, 8 H aromatic), 4.56 (t, *J*=6.7, 1H, C*H*OCH_2CH_3), 3.61-3.42 (m, 1H, OC*H*HCH_3), 3.35-3.18 (m, 1H, OCH*H*CH_3), 2.11-1.87 (m, 1H, C*H*$HCH_2CH_2CH_3$), 1.86-1.70 (m, 1H, CH*H*$CH_2CH_2CH_3$), 1.44-1.18 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.12 (t, *J*=7.3, 3H, CH_2CH_3), 0.82 (t, *J*=7.3, 3H, $CH_2CH_2CH_2CH_3$); ^{13}C NMR (75 MHz): δ = 144.8, 140.2, 138.8, 135.4, 135.2, 130.0, 128.6, 127.6, 124.4, 124.0, 122.9, 122.5, 75.6, 64.0, 38.3, 27.9, 22.4, 15.2, 13.9; GC-MS: *m/z* = 326 (1) [$(M+2)^+$], 325 (3), 324 (14) [M^+], 268 (20), 267 (111), 239 (40), 221 (16), 211 (16), 178 (14), 165 (11); anal. calcd for $C_{21}H_{24}OS$ (324.15): C, 77.73; H, 7.46; S, 9.88; found C, 77.75; H, 7.48; S, 9.68.

- *2-(1-isopropoxy-pentyl)-benzo[b]thiophene (**4aa''**).* Yellow oil. IR (film): ν = 3433 (w), 2957 (s), 2925 (s), 2354 (s), 1603 (m), 1458 (s), 1438 (w),

1378 (m), 1315 (w), 1245 (m), 1128 (w), 1024 (w), 822 (w), 746 (s), 727 (m), 699 (m) cm^{-1}; ^1H NMR (300 MHz): δ = 7.84-7.76 (m, 2H aromatic), 7.32 (d, *J*=7.35, 2H, aromatic), 6.91 (s, 1H, H-3), 4.18 (s, 1H, C*H*OCH$(CH_3)_2$, 3.24 (s, 1H, C*H*$(CH_3)_2$), 1.72 (d, *J*=6.4, 2H, C*H*$_2(CH_2)_2CH_3$), 1.35-1.28 (m 4H $CH_2(CH_2)_2CH_3$) 1.16 (s, 6H, CH(C*H*$_3)_2$) 0.99 (s, 3H, CC*H*$_3$); ^{13}C NMR (75 MHz): δ = 146.1, 139.0, 137.2, 124.4, 124.3, 123.8, 122.6, 120.7, 77.5, 65.8, 38.5, 26.4, 23.4 23.3 14.3; GC-MS: *m/z* =264 (1) [$(M+2)^+$], 263 (2), 262 (10) [M^+], 205 (23), 164 (11), 163 (100), 147 (14), 135 (20); anal. calcd for $C_{16}H_{22}OS$ (262.14): C, 73.23; H, 8.45; S,12.22; found C, 73.26; H, 8.47; S, 12.28.

- *2-(1-isopropoxy-2,2-dimethyl-propyl)-benzo[b]thiophene (**4ab''**).* Yellow solid, mp. 83.2-83.8 °C. IR (KBr): ν = 3436 (w), 2970 (s), 2929 (m), 2866 (m), 1462 (m), 1304 (s), 1315 (w), 1185 (w), 1125 (s), 1060 (s), 1031 (w), 937 (w), 868 (w), 834 (s), 751 (s), 728 (m), 719 (w), 684 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.84 (d, *J*=7.3, 1H aromatic), 7.74-7.66 (m, 1H aromatic), 7.30 (qd, *J*=7.3, 1.5, 2H, aromatic), 7.11 (s, 1H, H-3), 4.28 (s, 1H, C*H*OCH$(CH_3)_2$, 3.54 (q, *J*=6.4, 1H, C*H*$(CH_3)_2$), 1.12 (dd, *J*=6.4, 6H, C*H*$(CH_3)_2$), 0.99 (s, 9H, C$(CH_3)_3$); ^{13}C NMR (75 MHz): δ =149.9, 139.7, 139.4, 123.9, 123.6, 123.0, 122.4, 122.1, 83.5, 69.6, 35.5, 26.4, 23.3, 20.8; GC-MS: m/z =264 (1) [$(M+2)^+$], 263 (1), 262 (5) [M^+], 205 (26), 164 (12), 163 (100), 161 (12), 135 (22), 91 (11); anal. calcd for $C_{16}H_{22}OS$ (262.14): C, 73.23; H, 6.10; S, 12.22; found C, 73.56; H, 6.47; S, 12.68.

- *2-(1-isopropoxy-phenyl-methyl)-benzo[b]thiophene (**4ac''**).* Yellow solid, mp. 29.0-29.3°C. IR (KBr): ν = 3448 (w), 3059 (w), 2968 (m), 2925 (m), 1306 (w), 1453 (m), 1437 (w), 1373 (w), 1117 (w), 1049 (m), 938 (w), 818 (m), 747 (s), 727 (s), 700 (s), 630 (w) cm^{-1}; ^1H NMR (300 MHz): δ = 7.82

(d, *J*=7.4, 2H aromatic), 7.34-7.18 (m, 7H aromatic), 6.91 (s, 1H, H-3), 5.48 (s, 1H, C*H*OCH$(CH_3)_2$, 3.24 (q, *J*=6.7, 1H, C*H*$(CH_3)_2$), 1.16 (dd, *J*=6.7, 6H, C*H*$(CH_3)_2$); ^{13}C NMR (75 MHz): δ =146.9, 139.7, 139.4, 137.2, 128.4, 128.3, 127.7, 127.6, 127.5, 124.4, 124.3, 123.9, 122.4, 121.6, 80.5, 65.4, 23.3; GC-MS: *m/z* =284 (3) [$(M+2)^+$], 283 (7) , 282 (53) [M^+], 225 (14), 224 (45), 223 (99), 222 (23), 221 (40), 211 (21), 189 (11), 179 (11), 178 (27), 163 (21), 161 (15), 135 (34), 134 (12), 111 (20), 105 (100), 91 (16), 89 (24); anal. calcd for $C_{18}H_{18}OS$ (282.11): C, 76.56; H, 6.42; S, 11.35; found C, 76.58; H, 6.47; S, 11.68.

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Part B

Anti-inflammatory activity of hop (*Humulus lupulus* L.)

CHAPTER 6

General introduction to hop

6.1 Introduction.

Hop is a member of the plant family Cannabaceae, which is known since ancient times an has been used for several purposes, e.g. as ornamental plant, for bread-making, for stuffing of pillows, and as a salad *(1)*. It is still unclear when precisely hop was introduced in beer brewing. Today, cultivated hop is used for commercial purposes since its female flowers are the source of beer bitter substances, giving to this drink the characteristic flavour. Moreover, hop is also used for culinary, medicinal and cosmetic purposes.

6.2 Chemical properties of hop extract.

Fresh hop cones contain about 80% moisture which is reduced to 8-12% by drying before storage. There are hundreds of components in hop cones, but the interesting compounds can be grouped in three classes: resin (containing α- and β-acids), hop oil and polyphenols. These three classes are important as biomedical markers to differenciate hop varieties *(2)*. The major components are shown in **Table 1**.

Table 1. The chemical composition of dried hop cones.

Components	Amount (%, w/w)
α-Acids	2-18
β-Acids	1-10
Essential oil	0.5-3.0
Polyphenols	2-5
Oil and fatty acids	Up to 25
Protein	15
Cellulose	40-50
Chlorophyll	2
Ash-salts	10
Water	8-12

The dried hop cones contain polyphenols, mainly phenolic acids, prenylated chalcones, flavonoids, catechins and proanthocyanidins *(3)*. Only 20-30% of beer polyphenols are derived from hop, whereas 70-80% originate from malt *(4)*. Polyphenols are natural antioxidants which can protect beer against oxidation. Polyphenols also contribute to the colour of the beer *(5)* and to haze formation. However, polyphenols may cause an upleasant astringency to beer *(6)*.

Phenolic acids can be precursors to specific beer flavours. Ferulic acid for example is a precursor of wheat aroma. The main prenyflavonoid of hop cones is xanthohumol. It is a simple prenylated chalcone only present in hop. It posseses an anticancer activity *(7)*. Other polyphenols, namely tannis, play a role in clarifying beer by precipitating proteins during boiling.

Hop oil contributes to the aroma of beer. There are more than 300 compounds in hop oil. It can be divided into a non-polar (hydrocarbon) and a polar (oxygenated and sluphur-containing) fraction. Around 40-80% of hop oil is of hydrocarbon-nature and consists manly of the monoterpene myrcene and the sesquiterpenes caryophyllene, humulene, and farnesene. Alcohols are also present in hop oil. Linalool is the major terpene alcohol found in hop; it is present up to 1% of total oil. Hop oils contain organic sulphur compounds which have a negative effect on beer flavour. Although sulphur compound are present in very low quantites in hop, some have flavour thresholds of a few parts per billion or even lower *(8)*.

While essential oils are responsible for the aroma, the resinous compounds, especially α-acids are responsible for the bitter taste of beer. Hop resin is species-specific compounds. There are two types of hop resins: α- and β-acids. Total resin is defined as the portion of a diethyl ether axtract of hop that is soluble in cold methanol and consists of hard resin and soft resin. It is different from hop wax, a mixture of long chain alcohols, acids, esters, and hydrocarbons, which are poorly soluble in cold methanol. Hard resin is defined as that is insoluble in hexane , while soft resin is soluble in hexane. Hard resin consist of α- and β-acids-oxidation

products, xanthohumol, iso-xanthohumol, and some flavones. Soft resin is very important in brewing because it consists of deoxyhumulones, α-acids (humulone, cohumulone, adhumulone, prehumulone, and posthumulone) and β-acids (lupulone, colupulone, adlupulone, prelupulone and postlupulone), known as bitter acids. Because soft resin is found in lupulin glands, it is also called lupulinic resin. For the brewery the most important compounds of hop are the α-acids. These are weak acids (pKa values of humulone = 5.1) and have a very poor solubility in aqueous beer medium (pH between 5 and 5.2 in pilsener beer). During wort boiling they are isomerized to the bitter-tasting iso-α-acids which are much more soluble (pKa values of trans-isohumulone = 3.1) *(9)*.

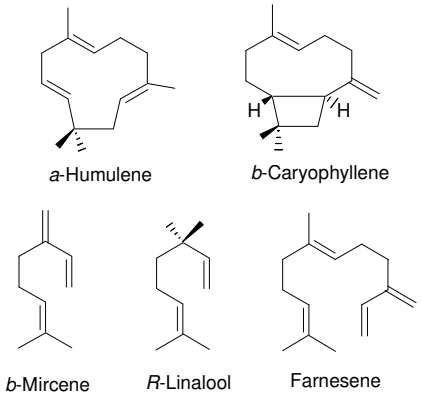


Figure 1. Chemical structures of major hop oil compounds.

Compared to iso-α-acids, β-acids are much less bitter. Thus, these compound do not play a role in the quality of beer. β-Acids tends to precipitate in wort and beer. The have pKa values around 6. they comprise five compounds, namely lupulone, colupulone, adlupulone, prelupulone and postlupulone *(4, 9b)*.

Soft resin and hop oil are not stable products. They easly degrade at room temperature. Oxidation of humulone produces the oxidation products humulinone

(γ-acids), tricyclodehydroisohumulone, and hydroxyl humulinic acids. None of these oxidation product is very bitter. Tricyclodehydroisohumulone, is about 70% as bitter as iso-α-acids. The oxidation of β-acids produces δ-acids (hulupones) *(9b, 10)*.

6.3 Bitter acids.

The hop bitter acids are classified as either ‘‘α-acids’’ or ‘‘β-acids’’ which are, respectively, di- or tri-prenylated phloroglucinol derivatives. In addition, they each contain a 3-, 4-, 5-, or 6-carbon oxo-alkyl side chain (**Figure 2**).

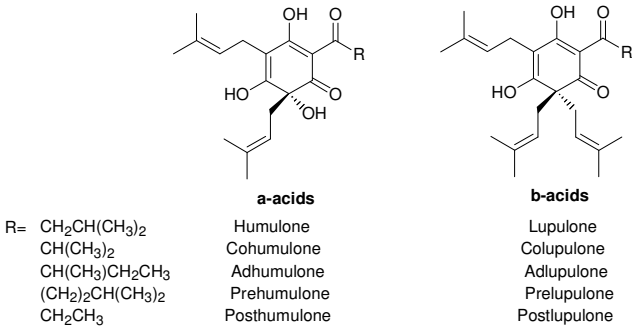


Figure 2. Chemical structure of α- and β-acids.

The α-acids were originally distinguished by the fact that they are precipitated from a crude extract of hops by the addition of lead acetate. The β-acids, by definition, would remain in solution. The α-acids, particularly humulone (35–70% of total α-acids), cohumulone (20–65%), and adhumulone (10–15%) are regarded as the most important constituents in determining the quality of hops *(10)*.

The α-acids occur in beers in concentrations up to 4 mg/ml and contribute to foam stability as well as imparting antibacterial properties. While regarded as

the principal ‘‘bitter acids’’ from hops, they perhaps paradoxically do not have a bitter taste, even at concentrations of 100 mg/ml *(10).*

The hop α-acids isomerize to the corresponding ‘‘iso-α-acid’’ under a variety of reaction conditions (**Figure 3**).

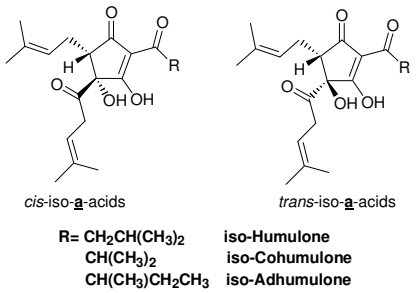
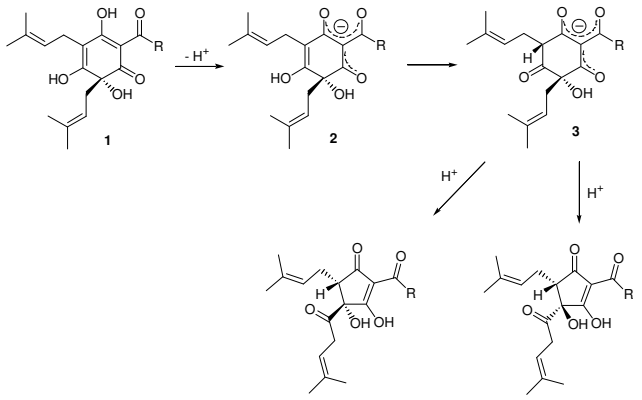


Figure 3. Chemical structure of iso-α-acids.

The isomerisation of α-acids to iso-α-acids occurs during wort boiling by an acyloin-type ring contraction. Each α-acid converts to a pair of *trans-/cis*-iso-α-acids. These two epimers are different depending on the position of the tertiary alcohol function at C-4 and the prenyl side chain at C-5. Thus three major α-acids convert to six major α-acids (*trans-/cis*-isohumulone, *trans-/cis*-isocohumulone, *trans-/cis*-isoadhumulone) which are present in beer. The ratio of the iso-α-acids depends on the reaction conditions. In the wort medium it is normally 75:25 (*cis-/trans*-isomers). A major problem is the poor solubility of the α-acids in the wort medium which limit their conversion. Iso-α-Acids (pKa values around 5.5) are not well soluble in the wort medium which has a pH between 5.0 and 5.2. On the other hand, iso-α-acids (pKa values around 3.0) are much more soluble in wort medium. A divalent cation, such as magnesium or calcium, strongly catalysed the isomerisation reaction, producing a 1:1 of *cis-/trans*-isomers composition *(4, 9a).* The isomerisation of the α-acids can also be performed by irradiation with UV light

in the wavelength region of 365-366 nm. However, α-acids are converted exclusively to the *trans*-isomers by this method.

Ionisation of the acidic function of humulone **1** deconjugates the double bond system in **2**, so that ketonisation can occur yielding **3**, an intermediate with two isoprenyl groups in trans-position (**Scheme 1**). The ring contraction gives a mixture of *trans*- and *cis*-isohumulones. The relative amount of the two diastereoisomers formed remains relatively constant (25% *trans*-form and 75% *cis*-form) over a wide range of pH when only monovalent cations are used. With bivalent or multivalent cations derived from e.g. magnesium, lead or iron other composition or ever equilibrium situations can occur *(9b)*.



Scheme 1. Ionisation of humulone to *trans*- and *cis*-isohumulone

The concentration of iso-α-acids in beer is quite low (15-18 ppm). However the quality of beer is much influenced by these compounds, since 80% of the bitter taste derives from iso-α-acids. The bitter taste threshold value of the iso-α-acids in water is around 6 mg/liter *(9a)*.

Individual stereoisomers have different characteristics both in bitter taste and stability. Generally, the *trans*-isomers are less bitter than the *cis*-counterparts *(11)*. The *trans*-isomers were also reported to be more prone to oxidation than their

cis-counterparts *(12)*. The quality of beer therefore may be improved by using only the *cis*-isomers mixture.

Iso-α-acids are very unstable compounds and their degradation compounds are tought to be partially responsible for the off-flavour characteristic of ageing beer including stale and cardboard flavours which are connected with their oxidative degradation. The compounds that are responsible for these off-flavours are usaturated aldehydes, such as *trans*-nonen-2-al, formed by the oxidative degradation of isohumulones *(13)*. Other compounds are the vicinal diketones which are formed from oxidative decarboxylation of 2-acetohydroxycarboxylic acids. The taste threshold values for these compounds are very low ($<10^{-2}$ mg/l), and and even as low as to 5×10^{-4} mg/l for *trans*-2-nonenal. In higher concentration it will give beer a very unpleasant resinous taste. Beer is no longer drinkable if the concentration of these compounds is about 1 mg/l *(10)*.

Furthermore, iso-α-acids are sensitive to light, and their degradation products (3-methyl-2-butene-1-thiol and dehydrohumulinic acid) are responsible for the light struck flavour of beer. In order to reduce this, beer is usually bottled in dark-coloured glass. Alternatively, light stable reduced-iso-α-acids are used.

Iso-α-acids exibit other interesting features: they stabilize the beer foam and inhibit the growth of gram-positive bacteria. However, lactic acid bacteria in beer are resistant to iso-α-acids. As isocohumulone is less foam-active compared to the other iso-α-acids, the cohumulone content of different hop varieties should be taken into consideration *(14)*.

6.4 Prenylated flavonoids

A mixture of prenylated, geranylated, oxidized, and/or cyclized chalcones, 30 of which have been isolated to date, is secreted along with bitter acids and essential oils by lupulin glands of the female hop inflorescences (hop cones). *(15-16)*. Since lupulin glands lack the enzyme necessary for the conversion of

chalcones to flavanones, they produce (exclusively) flavonoids of the chalcone type with XN as the most abundant (82–89%) of the total amount of prenylated flavonoids in European hop varieties, present at a concentration of ca. 0.01–0.5% *(16-17)*.

A majority of the known flavonoids from hops can be considered as derivatives of the compound 20,4,40,60-tetrahydroxy-30-prenylchalcone, commonly known as DMX. With the exception of two geranylated compounds, which have only been detected by MS *(16)*, all known constituents of hops from this series are prenylated at either the 6- or 8-position, or both. There are three known series of compounds present in hops based on this nucleus:

- the XH series (5-methoxy),
- the XG series (7-methoxy),
- the DMX series.

During the brewing process, XN is converted into the corresponding isomeric prenylflavanone isoxanthohumol, which represents the major prenylflavonoid in beer; the reaction reportedly taking place more rapidly with increasing pH *(3, 16)*. The corresponding isomerization is possible in any chalcone containing a free 20- or 60-hydroxyl group, and in cases where there is a hydroxyl at both 20 and 60, two isomers are produced. Chemical structures of these compounds are given in **Figure 4**.

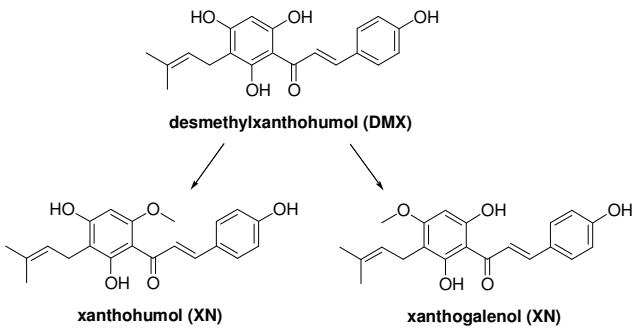


Figure 4. Chemical structure of prenylated chalcones

6.5 Pharmacognosy of hop

Numerous studies have been published citing hops to be active in specific bioassays. Hop extracts and/or compounds have been reported to be ‘‘active’’ in the following assays: various antioxidant and/or chemoprevention *(18-23)*, antimicrobial, particularly against Gram positive bacteria *(24)* and cytotoxicity *(25)*. Many constituents have been shown to possess, in addition to the estrogenic properties, other types of biological activity such as potential cancer chemopreventive activity *(18, 26)* and suppression of COX-2 gene transcription *(27)*. The major chalcone XH, as well as the flavanones IX, and 6PN and 8PN show various forms of antioxidant activity in vitro in the micromolar range, and are weakly cytotoxic to certain cancer cell lines *(15, 18, 26)*.

In particular, besides their important antibiotic properties, α- and iso-α-acids possess many biological activities relevant for cancer prevention. Humulones and lupulones have radical-scavenging and lipid peroxidation inhibitory activity *(23)*. These effects might contribute to the potent inhibition of bone resorption. Humulone demonstrated significant anti-inflammatory activity by suppression of Cox-2 gene transcription in murine osteoblastic MC3T3-E1 cells *(27)*. Moreover, humulone treatment at low concentrations resulted in induction of cell

differentiation in human HL-60 promyelocytic leukaemia cells and several other leukaemia cells lines.

In addition to the inhibition of cell proliferation by induction of differentiation, humulone was also found to inhibit angiogenesis.*(28)*.

Two recent reports suggest that iso-α-acids might have beneficial effects for the treatment of diabetic symptoms by inhibition of aldose reductase, activation of peroxisome proliferator-activated receptor (PPAR) α and γ and reduction of insulin resistance *(29)*. These reports are of interest in view of the potential role of PPAR α and ligands in cancer prevention, but also as a causal factor in colon carcinogenesis *(30)*

As regards prenylflavonoids from hops, cancer-related studies have mainly focused on the in vitro activities of xanthohumol as a cancer chemopreventive agent. Cancer chemoprevention is targeted at the initiation, promotion, and progression stages of carcinogenesis and requires long-term exposure to non-toxic nutrients, food supplements or pharmacological agents with the aim to prevent the development of malignancies.

Xanthohumol, isoxanthohumol, 8-prenylnaringenin, and nine other prenylflavonoids from hops were shown to strongly inhibit the cDNA-expressed human cytochrome P450 enzymes, Cyp1A1, Cyp1B1, and Cyp1A2, These cytochrome P450s form a group of enzymes that mediate the metabolic activation of many chemical carcinogens, and the inhibitory effects of hop prenylflavonoids on cytochrome P450s may offer an explanation for the reported inhibitory effects of beer on mutagenesis and DNA adduct formation induced by carcinogens *(31)*.

Uncontrolled proliferation of tumor cells has been associated with inflammation and increased production of hormone-like mediators such as prostaglandins. Food constituents that can interfere with proliferation mechanisms are of great interest as cancer chemopreventive agents due to their long-term exposure. Antiproliferative and cytotoxic effects of xanthohumol and five other prenylated hop flavonoids were tested in breast cancer (MCF-7), colon cancer (HT-

29), and ovarian cancer (A-2780) cells in vitro *(25b)*. Xanthohumol inhibited the proliferation of these cell lines in a dose-dependent manner.

Gerhauser et al. *(7a)* showed that xanthohumol can be an effective anti-inflammatory agent by inhibition of endogenous prostaglandin synthesis through inhibition of cyclooxygenase (constitutive COX-1 and inducible COX-2) enzymes. Prostaglandins are also known to initiate formation of new blood vessels (angiogenesis), an important event in tumor growth.

Zhao et al. *(32)* have demonstrated that xanthohumol inhibits the production of nitric oxide (NO) by suppressing the expression of inducible nitric oxide synthase (iNOS). This finding may be relevant to angiogenesis because excessive and prolonged NO generation promotes the production of vascular endothelial growth factor (VEGF), a known inducer of angiogenesis.

Only a few studies have reported on the antioxidant effects of prenylated flavonoids, probably because of their low dietary intake compared to flavonols and anthocyanins. In a study conducted by Miranda et al. *(33)*, prenylated chalcones from hops protected low density lipoprotein (LDL) from oxidation in vitro. Of the 12 tested prenylated chalcones, xanthohumol and desmethylxanthohumol were the most effective antioxidants. Scavenging of reactive oxygen species by xanthohumol and isoxanthohumol was studied by Gerhauser et al. *(7a)*, who found that xanthohumol was a potent scavenger of superoxide anion radicals.

Regarding the estrogenic effects of hops, they have been recognized for decades. The active estrogenic principle remained unclear, however, several bioassay-guided fractionation of hop extracts led to the isolation 8-prenylnaringenin as a potent phytoestrogen *(15)*. Although 8-prenylnaringenin is a much weaker estrogen than 17b-estradiol (<1%), it is considered the most potent phytoestrogen known to date. Weak estrogenic activities were observed for 6-prenylnaringenin, 8-geranylnaringenin, and 6,8-diprenylnaringenin, while other hop prenylflavonoids, including xanthohumol and xanthogalenol showed very weak or no estrogenic activities *(34)*.

6.6 Aim of the thesis.

This thesis project was carried on in the Netherland in the framework of a collaboration between the Departments of Human Nutrition and Food Chemistry of Wageningen University and the University of Calabria. The aim of the project was the study of the chemical composition of a hop crude extract (HCE) and its investigation by ultrahigh-performance liquid chromatography (UHPLC). The HCE and various fractions obtained by preparative HPLC were tested for their ability to inhibit production of two pro-inflammatory cytokines, monocyte chemoattractant protein-1 (MCP-1, CCL2) and tumor necrosis factor-α (TNF-α), which play crucial roles in the complications of obesity. The hop chalcone xanthohumol was found to be the most potent inhibitor of both cytokines in LPS-activated RAW 264.7 mouse macrophages and U937 human monocytes. Moreover, other constituents, namely, iso-α-acids, in combination with the β-acid hulupone, showed a moderate but selective inhibitory activity only on MCP-1 release.

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CHAPTER 7

Xanthohumol from hop (*Humulus lupulus* L.) is an efficient inhibitor of monocyte chemoattractant protein-1 and tumor necrosis factor-α release in LPS-stimulated RAW 264.7 mouse macrophages and U937 human monocytes

Elvira Lupinacci, Jocelijn Meijerink, Jean-Paul Vincken, Bartolo Gabriele, Harry Gruppen, and Renger F. Witkamp

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7.1 Introduction.

The increased incidence of obesity and its pathological consequences including atherosclerosis, hypertension, and insulin resistance, has reached epidemic dimensions *(1)*. It is now generally accepted that important factors for the development of these disorders includes an excessive growth of abdominal fat, deposition of lipids into nonadipose tissues such as liver and muscles, and a state of chronic inflammation. In fact, adipose tissue is not just a site of energy storage but also behaves as a dynamic endocrine organ *(2)*, producing a large number of both adipokines, such as leptin, adiponectin, and cytokines including IL-6, tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1). Some of these molecules affect energy metabolism and insulin sensitivity in other tissues such as muscle and liver *(3)*.

During obesity, lipid storage in adipocytes is increased, which triggers the release of adipokines *(4)*. Recent studies have shown that among the adipokines up-regulated in lipid-loaded adipocytes, MCP-1 (CCL2) plays a crucial role in inducing macrophage infiltration into adipose tissues, leading to the amplification of the adipose tissue inflammatory response (5). MCP-1 belongs to the subfamily of the CC chemokines, which are involved in the recruitment of monocytes and T lymphocytes at sites of infection and injury *(6)*. Its expression in adipose tissue has been found to correlate positively with the degree of obesity *(7)*. Directed among others by the chemoattractant signal of MCP-1, infiltrating macrophages secrete large amounts of TNF-α, which, in turn, stimulate the further release of MCP-1. This results in a chronic inflammatory state due to the persistence of a vicious cycle of macrophage infiltration and pro-inflammatory cytokine production *(8)*. Macrophage-secreted TNF-R increases lipolysis and decreases triglyceride synthesis in adipocytes. This contributes to an excess of circulating free fatty acids (FFA), one of the molecules that triggers insulin resistance *(3)*. Therefore, an increased level of TNF-α and MCP-1 in adipose tissue is closely associated with

obesity-related complications such as insulin resistance, thus providing useful therapeutic targets for modulating visceral obesity-related pathologies *(9)*.

An attractive way of reducing the chronic inflammatory process in obesity consists of the use of natural products, in particular, compounds present in foods that have a long tradition of use. Among the relevant candidates there is the hop plant (*Humulus lupulus* L.; Cannabaceae), which is known in traditional medicine for its bacteriostatic action *(10)* and is used worldwide as an essential flavoring ingredient in beer. Hop displays a wide range of biological activities, including bacteriostatic and antidiabetic activity *(11)*. Hundreds of compounds have been identified in hop, including phenolic compounds such as xanthohumol, hop oils, and bitter acids. The bitter acids consist of two classes: the α-acids (humulones) and the β-acids (lupulones). Representatives of both are present in complex mixtures in hop strobiles and differ from each other in degree of prenylation. Some hop compounds have been reported to exhibit immunomodulatory activity. For example, xanthohumol inhibits the production of both the pro-inflammatory cytokine IL-1β *(12)* and nitric oxide *(13)*. In addition, it has been shown to possess anticancer activity *(14)*. The bitter acids seem to inhibit bone resorption *(15)* and COX-2 expression *(16)* and reduce insulin resistance through the activation of PPAR-α and -γ *(17)*.

In this part of the thesis, I will show the study of the chemical composition of the crude extract from hop (HCE) by ultrahigh-performance liquid chromatography (UHPLC) and the investigation of the anti-inflammatory activity of HCE and of the fractions obtained from HCE by preparative HPLC. This was done by testing their inhibitory activity on the production of both MCP-1 and TNF-α in lipopolysaccharide (LPS)-stimulated mouse macrophages RAW 264.7 and U937 human monocytes.

7.2 Matherials and Methods

7.2.1 Chemical Reagents.

HPLC grade MeCN (for UHPLC analysis and preparative HPLC), xanthohumol, PMA, and LPS were purchased from Sigma-Aldrich (Schnelldorf, Germany). Water for the mobile phase was purified with a Milli-Q system (Millipore, Bedford, MA). Acetic acid, reagent grade ethyl acetate, methanol, ethanol, and hexane were obtained from Merck (Darmstadt, Germany). Hop pellets SAAZ (variety Hallertauer; 3.6% α-acids) were obtained from BREWFERM (Beverlo, Belgium). Cell culture media, fetal bovine serum (FBS), penicillin, and streptomycin were from Lonza (Verviers SPRL, Belgium).

7.2.2 Extraction Procedure.

Hop pellets (25.04 g) were extracted by sonication for 1 h with 100 mL of ethyl acetate. Extractions were repeated three times, and the dark green extracts were combined and subsequently filtered through a Buchner funnel. The solvent was evaporated under vacuum. To remove volatile components and oils from the extract, a volume of 200 mL of methanol was added and the mixture was incubated for 30 min at 90 °C. Separation of oils and methanol was achieved by storing the mixture at -20 °C overnight. The methanol fraction was then centrifuged (30 min, 16000g, 5 °C). Liquid-liquid extraction with hexane was carried out to remove the remaining oil residue from the methanol fraction. Next, the methanol fraction was evaporated under vacuum, and the total yield was calculated (2.5 g; 10%). A stock solution of 100 mg/mL was prepared in methanol and stored at -20 °C in the dark. This solution is denoted hop crude extract (HCE).

7.2.3 Fractionation of the Hop Crude Extract.

The HCE was subjected to preparative HPLC, using a Waters 2690 system equipped with a 2767 automatic sample injector and a 2525 binary gradient module

(Waters Inc., Etten-Leur, The Netherlands). All separations were performed with an XTerra RP18 column (5 μm, 19 x 150 mm, i.d., Waters Inc.), eluted at 17mL/min with (A)MQwater + 0.1% (v/v) acetic acid and (B) acetonitrile + 0.1% (v/v) acetic acid for 85 min. The gradient was as follows: 0-10 min, 10% B isocratic; 10-40 min, 10-55% B; 40-70 min, 55-70 % B; 70-75 min, 70-100% B; 75-80 min, 100-10% B; 80-85 min, 10% B isocratic. LC-UV traces were recorded in-line with a 2996 PDA detector and an UV fraction manager with detection at 205 and 280 nm (Waters Inc.). The HCE was filtered through a 0.45 μm syringe filter and injected (1 mL) onto the column.

Fractions (18 mL each) were obtained by collecting the effluent during 1 min intervals. On the basis of the UV 280 nm signal, appropriate fractions were combined in 21 pools, and the organic solvent was removed by evaporation. Subsequently, the pools were freeze-dried and redissolved in methanol before use. Each pool was analyzed by UHPLC and assayed for the inhibition of MCP-1 and TNF-α release.

7.2.4 Analysis of Hop Compounds by UHPLC-MS.

The analyses were performed using a Thermo Accela UHPLC system (San Jose, CA) equipped with pump, autosampler, and PDA detector (range 205-400 nm) and a MS detector (LTQ, Thermo Fisher Scientific, Bellefonte, PA). Separation of hop compounds was carried out with a Hypersil GOLD column (1.9 μm, 2.1 x 50 mm i.d.) (Thermo Fisher Scientific) using (A) MQwater + 0.1% (v/v) acetic acid and (B) acetonitrile + 0.1% (v/v) acetic acid as mobile phases. The optimized elution program was as follows: 0-4 min,9-30% B; 4-15 min, 30-50% B; 15-18 min, 50% B isocratic; 18-21 min, 50-70% B; 21-22 min, 70-100% B; 22-25 min, 100% B isocratic; 25-27 min, 100-10% B; 27-29 min, 10% B isocratic. The column was run at 30 °C at a flow rate of 0.3mL/min. UV detection was set at 280 nm.

MS experiments were performed on a linear quadrupole ion trap mass spectrometer (LTQ, Thermo Fisher Scientific) equipped with an electrospray

ionization source interface (negative ion mode). The operating parameters were as follows: the heated capillary temperature was set at 250 °C, vaporizer temperature at 250 °C, auxiliary gas at 2 arbitrary units, and sheath gas at 40 psi. For MS/MS analysis, helium (He) was used as target gas in the collision. The collision energy was set at 35 V.

7.2.5 Cell Culture.

The RAW 264.7 macrophage cell line, obtained from American Type Culture Collection (Teddington, U.K.), was cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS), 100U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a 5% (v/v) CO_2 humidified air atmosphere. RAW2 64.7 cells were seeded in a 96-well culture plate at a density of 25 x 10^3 per well and incubated overnight. After removal of the supernatant, adherent cells were treated with 100 ng/mL LPS in combination with HCE, the different hop pools, or pure xanthohumol. Hop samples were dissolved in methanol, whereas pure xanthohumol was dissolved in ethanol (final concentration of both solvents never exceeded 0.1% v/v). Following 4 h of incubation, the medium was collected for enzyme-linked immunosorbent assay (ELISA).

Human myelomonocytic leukemia U937 cells were obtained from the American Type Culture Collection and cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2. For differentiation, U937 cells were seeded in a 96-well plate at a density of 25 x 10^3 per well and incubated overnight with 10 ng/mL PMA. After the supernatant was removed, fresh medium was added, and cells were incubated for 2 days. Differentiated U937 cells were then treated with 1 ng/mL LPS with different concentrations of xanthohumol. Following 4 h of incubation, the medium was used for ELISA.

7.2.6 Measurement of MCP-1 and TNF-α Production.

MCP-1 and TNF-α concentrations were determined by ELISA. ELISA was performed according to the manufacturer's protocol using R&D Systems kits (Abingdon, U.K.) for mouse and human MCP-1 and human TNF-α, whereas an Invitrogen kit (Breda, The Netherlands) was used for mouse TNF-α. Controls contained medium with equivalent amounts of solvent as compared to the treatments. These were incubated both with and without LPS. The concentrations of TNF-α and MCP-1 were quantified from a standard curve. Data were expressed as percentage of the positive LPS-treated control (set at 100%).

7.2.7 Viability and Cytotoxicity Assays.

The determination of cellular viability was carried out using an XTT Cell Proliferation Kit II (Roche Applied Science,Almere, The Netherlands). Briefly, RAW 264.7 cells were incubated for 24 h with the samples and 100 ng/mL of LPS. After incubation, the supernatant was carefully removed, and 100 μL of fresh medium, together with sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium] bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT) (final concentration = 0.45 mM) and N-methyldibenzopyrazine methyl sulphate (1.25 mM), was added. After 4 h of incubation at 37 _C, the amount of formazan accumulated in the growth medium was measured at 450 nm using an ELISA plate reader (Multiskan Ascent, Thermo Labsystem, Breda, TheNetherlands). The conditions were considered to be toxic if the cell's ability to metabolize XTT to formazan was lowered by >20% in comparison to that of the untreated control.

Cytotoxicity of the samples was evaluated through an LDH Cytotoxicity Detection Kit (Roche Applied Science). RAW 264.7 macrophages were treated for 24 h with the samples in the presence of 100 ng/mL of LPS. After the supernatant had been carefully removed, a mixture of the catalyst (diaphorase/NAD+ mixture, 250 μL) and the dye solution (iodotetrazolium chloride and sodium lactate, 11.25 mL) was added to adherent cells (100 μL/well). After 30 min of incubation at 25

°C, the absorbance was measured at 492 nm using an ELISA plate reader (Multiskan Ascent).

7.2.8 Statistical Analysis.

Each experiment was performed independently at least three times in duplicate or triplicate. Data are expressed as means (SEM) of the normalized values. Statistical differences between the treatments and the control were evaluated by two-way ANOVA followed by post hoc Bonferroni's test. A value of $p<0.05$ or 0.01 was accepted as statistically significant.

7.3 Results and discussion

7.3.1 Identification of Hop Compounds in Hop Crude Extract.

HCE was analyzed by UHPLC. A representative chromatogram is shown in **Figure 1**.

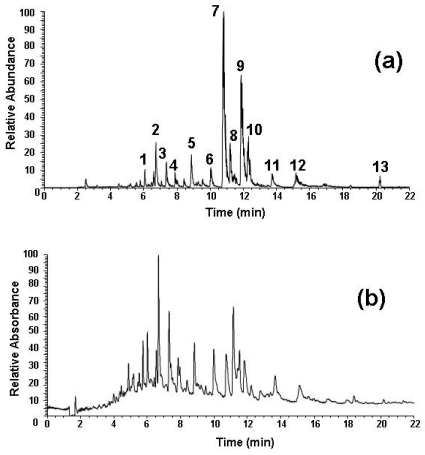

Figure 1 UPLC chromatograms of HCE: **a)** base peak intensity chromatogram and **b)** UV profile at 280 nm. Peak numbers are referred to **Table 1**.

Eleven main compounds (Table 1) were detected in the HCE and identified on the basis of their molecular ions, characteristic fragmentation patterns, UV spectroscopic characteristics, and literature data (Figure 2) *(18-21)*.

Table 1. Specific structure information of compounds identified in HCE by UPLC-ESI-MS/MS.

N°	R_t (min)	UV λ_{max} (nm)	Identification	Molecular formula	$[M-H]^-$	Fragmentation
1	6.07	213-257	ox cohumulinone	$C_{20}H_{28}O_7$	379.1	335.0-249.0-247.0-223.0-361.1-223.0-237.0-205.1
2	6.73	217	ox humulinone/ adhumulinone	$C_{21}H_{30}O_7$	393.2	349.0-263.1-375.1-223.0-251.0-165.0
3	7.37	215-275	ox humulinone/ adhumulinone	$C_{21}H_{30}O_8$	409.1	263.0-251.1-392.1-295.9-365.0-348.2-323.0-377.1-279.0
4	7.89	214-257	cohumulinone	$C_{20}H_{28}O_6$	363.2	261.0-192.0-251.1-233.0-345.2-319.0
5	8.87	219-271	humulinone/ adhumulinone	$C_{21}H_{30}O_6$	377.2	263.0-281.0-359.2-223.0-207.0-308.0
6	10.04	217-257	cohumulinone	$C_{20}H_{28}O_6$	363.2	261.0-192.0-251.1-233.0-345.2-319.0
7	10.81	220-257	cohulupone	$C_{19}H_{26}O_4$	317.3	248.0-180.0-220.1-233.1-205.1-152.0
8	11.21	218-257	humulinone/ adhumulinone	$C_{21}H_{30}O_6$	377.2	263.0-281.0-359.2-223.0-207.0-308.0
9	11.88	219-257	hulupone/ adhulupone	$C_{20}H_{28}O_4$	331.2	262.0-194.1-219.0-247.0-166.0-234.1
10	12.32	219-257	hulupone/ adhulupone	$C_{20}H_{28}O_4$	331.2	262.0-194.1-219.0-247.0-166.0-234.1
11	13.74	221	iso-cohumulone	$C_{20}H_{28}O_5$	347.2	251.1-329.0-235.0-278.0-182.2-207.0-303.0
12	15.18	221	iso-humulone/ adhumulone	$C_{21}H_{30}O_5$	361.2	265.1-343.0-317.0-235.1-292.0-165.0
13	20.23	222	xanthohumol	$C_{21}H_{22}O_5$	353.1	233.0-119.2-247.0-251.0-189.1-218.0-145.1-165.0

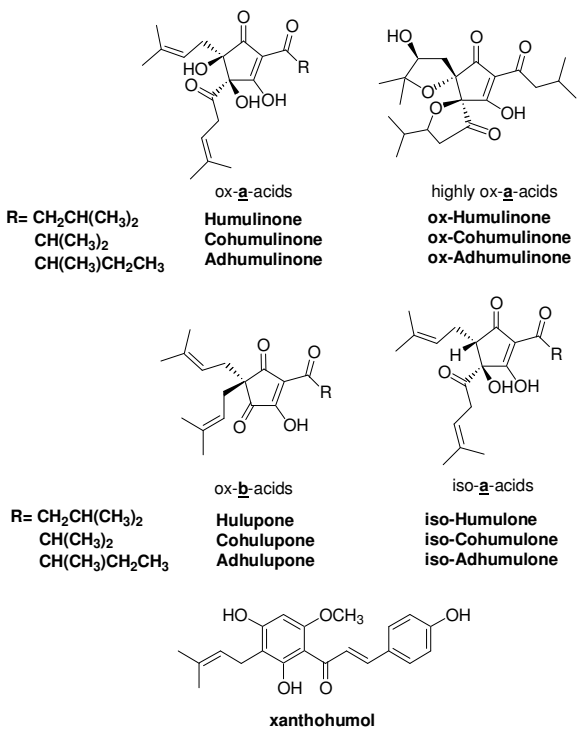

Figure 2: Molecular formulas of identified compounds.

The amounts of oxidized compounds detected in the mixture were relatively high, as might be expected under the extraction conditions employed *(18)*. Peaks **1** and **2**, with molecular ions ($[M–H]^-$) of *m/z* 379.1 and 393.2, respectively, corresponded to oxidized cohumulinone and oxidized humulinone/adhumulinone (overlap), compounds derived from cohumulone and humulone/adhumulone respectively, in which two oxygen atoms have been incorporated *(18)* (**Figure 1a, Table 1**). In a similar way, peak **3**, with an *m/z* ratio of 409.1, was thought to be a triple oxidized product of humulinone/adhumulinone (**Figure 1a, Table 1**) *(18)*. Peaks **4** and **6**, both having a molecular ion of *m/z* 363.2 were assigned as cohumulinone, the product of oxidation of the α-acids

cohumulone, while peak **5** with an *m/z* ratio of 377.2 was identified as humulinone/adhumulinone (overlap), a compound formed after oxidation of the α-acid humulone/adhumulone (**Figure 1a, Table 1**). Peak **7**, with *m/z* 317.3, corresponded to cohulupone, the oxidized derivative of the β-acid colupulone (**Figure 1a, Table 1**). Peak **9** and **10** contained the molecular ion of *m/z* 331.2, which was assigned as hulupone/adhulupone (overlap), the oxidation product of the β-acid lupulone/adlupulone (**Figure 1a, Table 1**). Interestingly, two iso-α-acids in HCE, corresponding to iso-cohumulone (peak **11**) and iso-humulone/iso-adhumulone (overlap) (peak **12**) were also found (**Figure 1a, Table 1**). Iso-α-acids are usually formed from α-acids during the brewing process of beer. Possibly, the high temperature employed during the extraction procedure of the hop material has caused isomerization of α-acids into the corresponding iso-α-acids. The α- and iso-α-acids have the same molecular mass, but they can be discriminated by their MS/MS fragmentation pattern. It is known from literature *(22, 18)* that the main product ion observed for α-acids corresponds to the loss of a C_5H_9 side chain (69 amu), whereas for iso-α-acids mainly a loss of a C_6H_8O side chain (96 amu) is observed. Peak **13** showed a molecular ion (*m/z* 353.1) corresponding to the chalcone xanthohumol (**Figure 1a, Table 1**).

7.3.2 Effect of hop crude extract on MCP-1 and TNF-α release in LPS-stimulated RAW 264.7 mouse macrophages.

The next part of the work consisted in the investigation of the effect of HCE on RAW 264.7 cells stimulated with 100 ng/mL of LPS. Macrophages were treated with four different concentrations of HCE, i.e. 100, 10, 1, and 0.1 µg/mL. After 4 h of incubation, the concentrations of MCP-1 and TNF-α were measured by ELISA. As shown in **Figure 3a**, HCE appeared to be an effective inhibitor of MCP-1 release. A dose of 0.1 µg/mL of the extract caused a significant inhibition by 36% compared to control values. Higher doses from 10 to 100 µg/mL produced more pronounced effects and in a dose dependent manner. The inhibitory effect of

the HCE on the production of TNF-α was less pronounced, as only the highest dose (100 µg/mL) elicited a 70% decrease (**Figure 3b**). Consequently, no dose-dependency was observed for TNF-α within the tested range.

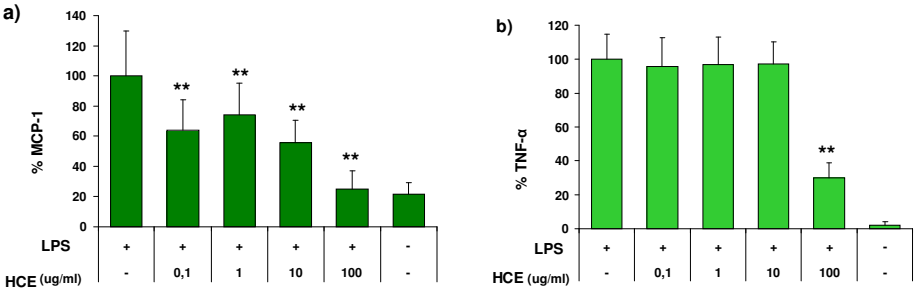

Figure 3: Inhibitory effect of HCE on the production of pro-inflammatory cytokines in LPS-activated RAW 264.7 mouse macrophages. **a)** MCP-1 protein level of RAW 264.7 cells treated with HCE (0.1, 1, 10 and 100 µg/mL) in the presence of LPS (100 ng/mL) for 4 h. Data represent mean ± S.E.M. of five independent observations performed in duplicate. **b)** TNF-α protein level of RAW 264.7 cells incubated with different concentration of HCE (0.1, 1, 10 and 100 µg/mL) and LPS (100 ng/mL) for 4 h. Data are expressed as mean ± S.E.M. of five independent experiments performed in duplicate. **p<0.01, significant compared to the LPS-activated group.

The viability test showed that the viability of the RAW 264.7 cells was not affected by HCE (**Table 2**). A cytotoxicity assay was also performed and confirmed the results obtained with the XTT assay.

Table 2. Effect of various samples on cell viability.

Sample	Cell Viability[a]
medium	100 ± 8.5
medium + LPS	110.1 ± 1.4
medium + 0.1% ethanol	111.5 8.6
medium + 0.1% ethanol + LPS	97.6 ± 8.4
medium + 0.1% methanol	92.0 ± 3.9
medium + 0.1% methanol + LPS	109.0 ± 10.1
HCE	96.2 ± 2.4
HCE + LPS	91.5 ± 4.0
pool 1 + LPS	109.0 ± 8.1
pool 2 + LPS	101.1 ± 12.1
pool 3 + LPS	103.8 ± 9.8
pool 4 + LPS	107.1 ± 7.2
pool 5 + LPS	105.5 ± 7.1
pool 6 + LPS	96.6 ± 8.3
pool 7 + LPS	97.5 ± 6.7
pool 8 + LPS	100.2 ± 3.3
pool 9 + LPS	105.6 ± 6.1
pool 10 + LPS	92.5 ± 9.6
pool 11 + LPS	96.2 ± 8.3
pool 12 + LPS	101.2 ± 7.7
pool 13 + LPS	105.0 ± 4.4
pool 14 + LPS	97.1 ± 9.6
pool 15 + LPS	85.1 ± 4.7
pool 16 + LPS	95.0 ± 3.2
pool 17 + LPS	107.4 ± 5.4
pool 18 + LPS	96.5 ± 8.4
pool 19 + LPS	106.0 ± 10.5
pool 20 + LPS	105.2 ± 7.5
pool 21 + LPS	108.9 ± 5.0
XN (5 µg/mL)	105.5 ± 10.2
XN (5 µg/mL) + LPS	89.3 ± 8.2

[a]Data represent mean (SEM of three independent experiments performed in triplicates). Samples were tested at a concentration of 100 µg/mL.

Thus, these experiments demonstrate that HCE is able to inhibit the production of MCP-1 and TNF-α in activated RAW 264.7 macrophages. As mentioned before, these cytokines have been found to play a pivotal rule in the obesity-related inflammatory process and the development of insulin resistance *(29, 30)*. In mice, it has recently become evident that MCP-1 contributes to infiltration of macrophages into adipose tissue *(5)*. In addition, Kanda *et al. (31)* reported that MCP-1 is involved in the development of insulin resistance in mouse models, and might be the link between adipose tissue inflammation and insulin resistance. Although the anti-inflammatory properties of hop have been described before, the majority of data refer to inhibition of cyclo-oxygenase (COX) *(14, 16)*.

7.3.3 Effect of hop fractions on MCP-1 and TNF-α release in LPS-stimulated RAW 264.7 mouse macrophages.

In order to identify which classes of compounds were responsible for the biological activity, HCE was fractionated by preparative-HPLC. Twenty-one fractions were collected and re-analyzed by UPLC-ESI-MS/MS. The various compounds present in HCE were grouped in five different classes: iso-α-acids, oxidized-α-acids, highly oxidized-α-acids, oxidized-β-acids and chalcones. Since no standards for the quantification of oxidized compounds from hop were commercially available, the amount of compounds present in each fraction was estimated on the basis of the peak areas detected by UV spectroscopy, assuming equal response factors. As shown in **Table 3**, fractions 1 and 2 mainly contained highly oxidized-α-acids, while fractions 3, 4, and 6 consisted mainly of oxidized-α-acids. Oxidized-β-acids were mainly detected in fractions 5,7 and 8, while xanthohumol was found in fractions 14-16. On the other hand, iso-α-acids were predominantly present in fractions 9-13, 17-19 and 21.

Tab. 3. Inhibitory effect of hop pools on the secretion of MCP-1 and TNF-α by LPS-stimulated RAW 264.7 macrophages.

	Main classes of compounds					MCP-1[f,h]	TNF-α[g,h]
pool	iso-α[a]	high ox-α[b]	ox-α[c]	ox-β[d]	c[e]	%	%
1	-	+++	+	-	-	75 ± 42	115 ± 31
2	-	+++	+	-	-	98 ± 42	115 ± 34
3	-	++	+++	-	-	108 ± 37	118 ± 32
4	-	+	+++	-	-	88 ± 30	112 ± 33
5	-	+	+	+++	-	97 ± 36	110 ± 28
6	-	+	+++	+	-	93 ± 29	100 ± 28
7	-	+	+	+++	-	81 ± 28 **	108 ± 28
8	+	+	-	+++	-	56 ± 14 **	96 ± 23
9	+++	+	-	++	-	79 ± 26 **	115 ± 34
10	+++	-	-	+	-	65 ± 26 **	110 ± 34
11	+++	-	+	-	-	82 ± 27 **	114 ± 33
12	+++	-	-	+	-	86 ± 30	107 ± 28
13	+++	+	-	+	-	76 ± 26 **	110 ± 30
14	+	-	-	+	++	74 ± 22 **	101 ± 29
15	+	-	-	-	++	35 ± 9 **	77 ± 23 **
16	++	-	-	+	++	36 ± 8 **	67 ± 19 **
17	+++	-	-	+	-	86 ± 26 *	112 ± 32
18	+++	-	+	+	-	95 ± 30	117 ± 33
19	+++	-	+	++	-	100 ± 32	118 ± 35
20	++	+	++	++	-	86 ± 26	117 ± 35
21	+++	+	+	+	-	90 ± 29	108 ± 33

[a]iso-α: iso-α-acids. [b]high ox-α: highly oxidized α-acids. [c]ox-α: oxidized α-acids. [d]ox-β: oxidized β-acids. [e]c: chalcones. [f]MCP-1 (%): MCP-1 production compared to LPS-treated control group (latter was set at 100%). [g]TNF-α (%): TNF-α production compared to LPS-treated control group (latter was set at 100%);-: peak area < 5%;+, 5% < peak area < 25%;++: 25% < peak area < 50%;+++: peak area>50%. [h]MCP-1 and TNF-α protein release from RAW 264.7 cells was determined after 4 h upon treatment of the cells with hop pools (100 µg/mL) in the presence of LPS (100 ng/mL). Data are expressed as mean (SEM of four independent experiments performed in duplicates. *, $p < 0.05$, and **, $p < 0.01$, significant compared to the LPS-activated group. MCP-1 and TNF-α protein levels for negative control were 22 ± 8 and 1 ± 0.2, respectively.

The bioactivity of the fractions was assessed by treating LPS-stimulated RAW 264.7 mouse macrophages for 4 h. The production of TNF-α in LPS-stimulated RAW 264.7 cells was not affected by the majority of the fractions tested (**Table 3**), except for the ones containing xanthohumol (fractions 14-16) which showed a moderate decrease of TNF-α secretion.

The inhibitory effect on MCP-1 release was more complex. Also here, the xanthohumol-containing fractions 14-16 showed the strongest effect, with fraction 15 being the most potent. Only some of the iso-α-acid-containing fractions (9-11, and 13) showed a moderate inhibition of MCP-1 release. In these fractions, the iso-α-acids humulone/adhumulone were mainly present. For oxidized β-acid-containing fractions, particularly fraction 8, and to a lesser extent fraction 7, reduced the MCP-1 level. Hulupone/adhulupone were the most abundant oxidized β-acids contained in these fractions. Both the oxidized and the highly oxidized α-acids appeared to be unable to inhibit MCP-1 release.

A viability assay was performed which showed that none of the tested fractions affected the viability of the RAW 264.7 cells (**Table 2**). A cytotoxicity test was also carried out and displayed comparable results.

Considering the chemical structures and the inhibitory activities of detected compounds, it was possible to identify some structural features which may affect the activity. Compounds derived from oxidation of α-acids are characterized by an hydroxyl group in position 5 of the five-member ring (**Figure 2**). Oxidized β-acids and iso-α-acids, on the other hand, have in the same position a prenyl group and an hydrogen atom, respectively (**Figure 2**). Oxidized α-acids did not show inhibitory effect indicating that oxidation in position 5 of the ring may reduce the inhibitory activity on both TNF-α and MCP-1 production. When considering the structure of highly oxidized-α-acids (**Figure 2**), it was found that the presence of a cyclic side chain in the molecule may inhibit the anti-inflammatory activity. Oxidized β-acids exhibited weaker inhibitory effect on MCP-1 release than iso-α-acids. They have a ketone moiety in position 3 of their ring (**Figure 2**), suggesting

that this group may not be important for inhibitory activity. Interestingly, fractions mostly containing the iso-α-acids iso-humulone/iso-adhumulone together with the oxidized β-acids hulupone (fractions 9, 10 and 13) showed a moderate inhibition of MCP-1 production. These molecules are characterized by the same side-chain (**Figure 2**), meaning that this substituent may be necessary for improving the inhibitory activity. However further investigations are needed to evaluate these hypothesis.

7.3.4 Effect of xanthohumol on MCP-1 and TNF-α release in LPS-stimulated RAW 264.7 mouse macrophages.

To assess whether the inhibition of MCP-1 and TNF-α secretion observed for fractions 14 to 16 was actually due to the presence of xanthohumol, the pure chalcone was tested. RAW 264.7 macrophages were stimulated with four different concentrations of xanthohumol (5, 2.5, 1 and 0.1 μg/mL corresponding to 14, 7, 2 and 0.2 μM of the compound) in the presence or absence of LPS. As displayed in **Figure 4a**, after 4 h of incubation the release of MCP-1 by RAW 264.7 cells was strongly and significantly inhibited by xanthohumol in a dose-dependent manner. In particular, a dose of 2.5 μg/mL significantly evoked an inhibition of the MCP-1 production of 46% when compared to control. The inhibitory effect of xanthohumol on the LPS-induced TNF-α release by RAW 264.7 macrophages was moderate (**Figure 4b**) confirming the results shown in **Table 3**. The viability assay carried out revealed that the highest concentration of xanthohumol (5 μg/mL) did not affect the viability of the RAW 264.7 cells (**Table 2**). A cytotoxicity test was also performed and confirmed results obtained with the XTT assay.

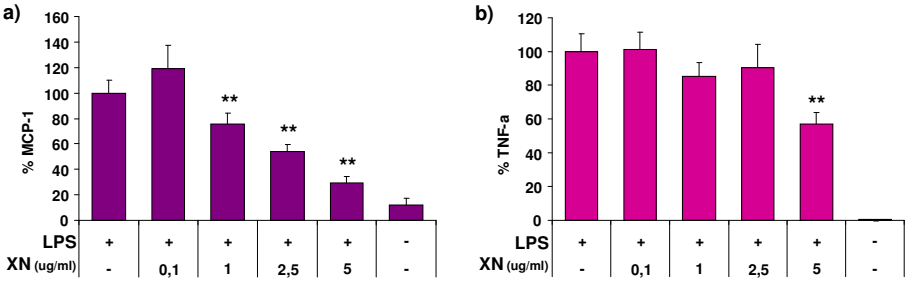

Figure 4: Inhibitory effect of xanthohumol (XN) on the production of pro-inflammatory cytokines in LPS-activated RAW 264.7 mouse macrophages. **a)** MCP-1 protein level of RAW 264.7 cells treated with XN (0.1, 1, 2.5 and 5 μg/mL) in the presence of LPS (100 ng/mL) for 4 h. Data represent mean ± S.E.M. of three independent observations performed in triplicate. **b)** TNF-α protein level of RAW 264.7 cells incubated with XN (0.1, 1, 2.5 and 5 μg/mL) and LPS (100 ng/mL) for 4 h. Data are expressed as mean ± S.E.M. of three independent experiments performed in triplicate. **p<0.01, significant compared to the LPS-activated group.

Thus, these data show that MCP-1 protein levels can be effectively decreased in mouse macrophages by xanthohumol. In particular, the effect on MCP-1 shows dose-dependent trend, with a maximum inhibitory effect observed at 5 μg/mL. This was the first time that the inhibitory activity of xanthohumol on the MCP-1 release by LPS-activated mouse macrophages was reported. Xanthohumol is the most abundant prenylated chalcone in hops and beer *(23)*, and has recently gained considerable interest due to the discovery of many biological activities. While its anti-cancer effects have been widely reported on various cancer cell lines *(24, 25, 14, 26)*, less attention has been paid on its anti-inflammatory activity *(12, 13, 14)*. Interestingly, recent studies have demonstrated that xanthohumol inhibits lipid secretion from HepG2 cells *(27)* and improves lipid and glucose metabolism in obesity and type 2 diabetes mice models *(28)*.

7.3.5 Effect of xanthohumol on MCP-1 and TNF-α release in LPS-stimulated U937 human monocytes.

To confirm that xanthohumol-induced TNF-α and MCP-1 inhibition is not restricted to mice macrophages, xanthohumol was also tested in human monocytic U937 cells. At a dose of 2.5 µg/mL, xanthohumol inhibited the secretion of MCP-1 in LPS-treated U937 cells, in a dose-dependent manner. After 4 h of incubation, xanthohumol evoked a significant inhibition of 42% (**Figure 5a**). As shown in **Figure 5b**, stimulation of differentiated U937 cells with 1 ng/mL LPS and various concentrations of xanthohumol also resulted in a decrease of TNF-α secretion. A dose of 2.5 µg/mL significantly elicited an inhibition of the TNF-α production of 34%.

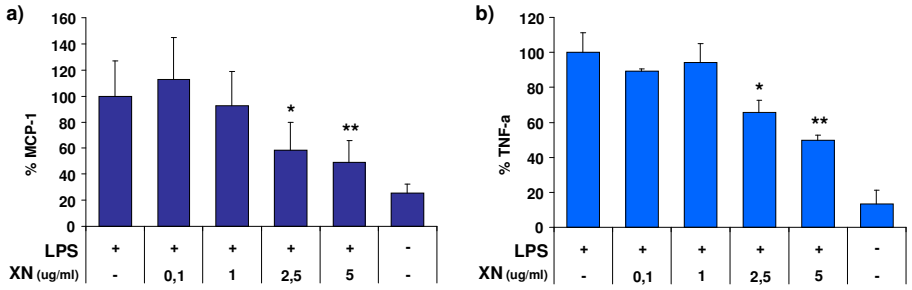

Figure 5: Inhibitory effect of xanthohumol (XN) on the production of pro-inflammatory cytokines in LPS-activated U937 human monocytes. **a)** MCP-1 protein level of differentiated U937 cells treated with 1 ng/mL LPS and XN (0.1, 1, 2.5 and 5 µg/mL) for 4 h. Data represent mean ± S.E.M. of three independent experiments performed in triplicate. **b)** TNF-α protein level of differentiated U937 cells incubated with various concentration of XN (0.1, 1, 2.5 and 5 µg/mL) and LPS (1 ng/mL) for 4 h. Data are expressed as mean ± S.E.M. of three independent observations performed in triplicate. *$p<0.05$ and **$p<0.01$, significant compared to the LPS-activated group.

These results demonstrated for the first time that xanthohumol produces effects in human monocytes that are associated with suppression of inflammation. Xanthohumol reduced MCP-1 secretion in differentiated U937 human monocytes

at levels similar to RAW 264.7 mouse macrophages. MCP-1 is considered to play a unique role among the chemokines in adipose tissue of obese persons *(30)*. Plasma MCP-1 levels correlated positively with BMI since weight reduction in morbid obese subjects was found to be associated with a decrement in circulating MCP-1 levels *(7)*. Moreover, MCP-1 secretion was associated with markers of the metabolic syndrome, including insulin resistance, obesity and increased serum triglyceride concentration *(32)*.

Interestingly, it was also found that the HPLC pools mainly containing iso-α-acids produce a moderate but significant decrease of MCP-1, but not of TNF-α production. Recent studies have demonstrated that iso-α-acids can prevent diet-induced obesity and insulin resistance in mice, modulating lipid metabolism and inhibiting intestinal lipid absorption *(33)*. Moreover, a preliminary clinical study indicated that iso-α-acids can activate both PPAR-α and -γ and improve insulin sensitivity in type 2 diabetic patients *(17)*. In addition, Toyoda *et al. (34)* showed that PPAR-α ligands can reduce MCP-1 and TNF-α expression and secretion, providing evidence for the anti-inflammatory effect of PPAR-α ligands. In agreement with these observations, our data suggest that iso-α-acids might also have an immunomodulatory effect on MCP-1.

7.4 Conclusions.

In conclusion, with this study xanthohumol has been identified as a bioactive compound present in hop with potent anti-inflammatory properties in vitro. Future studies should address the issue of bioavailability in humans of the different hop components in general and that of xanthohumol in particular. Although in vivo activity of xanthohumol was reported in mice *(31)*, other reports suggested low oral bioavailability of the pure compound, at least in rat *(36)*. Whether active plasma concentrations will be achievable in humans will also depend on matrix and possible coabsorption effects or the development of a suitable formulation. The effects found on MCP-1 and TNF-α secretion by

activated macrophages at least support further studies with hop in overweight and obesity or in other inflammatory conditions.

7.5 References

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List of publications

- Gabriele, B., Mancuso, R., Salerno, G., Lupinacci, E., Ruffolo, G., Costa, M., "Versatile synthesis of Quinoline-3-Carboxylic Esters and Indol-2-Acetic Esters by Palladium-Catalyzed Carbonylation of 1-(2-Aminoaryl)-2-Yn-1-Ols", *J. Org. Chem.* **2008**, *73*, 4971-4977
- Gabriele, B., Mancuso, R., Lupinacci, E., Spina R., Salerno, G., Veltri, L., Dibenedetto, A., "Copper-Catalyzed Heterocyclodehydration of 1-(2-Aminoaryl)-2-Yn-1-Ols in Ionic Liquids: a "Green" Approach to Substituted Quinolines" *Tetrahedron*, **2009**, *65*,8507–8512
- Lupinacci, E., Meijerink, J., Vincken, J-P., Gabriele, B., Gruppen, H., Witkamp, R., "Xanthohumol from Hop (Humulus lupulus L.) Is an Efficient Inhibitor of Monocyte Chemoattractant Protein-1 and Tumor Necrosis Factor-α in LPS-Stimulated RAW 264.7 Mouse Macrophages and U937 Human Monocytes" *J. Agric. Food Chem.*, **2009**, *57*, (16), 7274-7271.
- Lupinacci, E., Meijerink, J., Vincken, J-P., Poland, M., Gabriele, B., Witkamp, R., "Inhibition of proinflammatory mediators production in LPS-activated macrophages by hops (Humulus lupulus L.)" *Naunyn-Schmiedebergs Archives Of Pharmacology* ,**2009**, *380* (3), 261-261
- Gabriele, B., Mancuso, R., Lupinacci, E., Salerno, G., Veltri, L., "Tandem catalysis in ionic liquids: a recyclable catalytic synthesis of benzofuran derivatives" *Tetrahedron*, **2010**, *66*, 6156–6161

Education: overview of completed training activities

Meetings

- "*XXXI Convegno Nazionale della Divisione di Chimica Organica della Società Chimica Italiana*", 10-14 Settembre 2007, Università della Calabria - Rende (Cs)
- "*VII Sigma Aldrich Young Chemists Symposium*", 22-24 Ottobre 2007, Riccione
- "*Convegno congiunto SCI delle sezioni Calabria e Sicilia*", 3-4 Dicembre 2007, Messina
- *"FIGON Dutch medicine days"*, 6-8 Ottobre 2008, Lunteren, Olanda
- *"Convegno congiunto SCI delle sezioni Calabria e Sicilia"*, 1-3 Dicembre 2008, Università della Calabria, Rende (CS)
- "*XXIII Scuola di Spettrometria di Massa per Dottorandi di Ricerca 2009*", 8-13 Marzo 2009, Certosa di Pontignano, Siena
- "*First International symposium on microwave assisted organic and peptide synthesis*", (MAOPS 2009), 4-5 Giugno 2009, La Grande Motte, Francia
- "*7th International School of Organometallic Chemistry*" (ISOC09), 5-9 settembre 2009, Camerino (Macerata)

Courses

- Prof. Casciaro, "Numerical post-buckling analysis" 25-01-2007
- Dr. K. Hopmann "Preliminary study on the working mechanism of PAL enzyme" 07-03-2007
- Dr. F. Lucas "Theoretical calculations on the enzyme pyruvate formate lyase" 08-03-2007

- Prof. Ragno “I farmaci di automedicazione, vantaggi e limiti” 21-03-2007
- Dott.ssa Ioele “Sistemi di stabilizzazione di farmaci fotosensibili” 21-03-2007
- Dott.ssa Maiuolo “L’uso di Lantanidi nella catalisi in chimica organica” 29-03-2007
- WORKSHOP “Drug delivery systems” 03-04-2007
- Dott.ssa. Trombino “Attività antiossidante di composti naturali e loro veicolazione per applicazioni in campo farmaceutico” 03-04-2007
- Dott.ssa. Maiuolo “Reazioni pericicliche: cicloaddizioni, sigmatropiche e elettrocicliche” 11-04-2007
- Prof Garofalo “Enzimi (recettori catalitici): aspetti chimico-farmaceutici (Parte I)” 11-04-2007
- Dott.ssa Trombino “Direzionamento di famaci al Sistema Nervoso Centrale” 26-04-2007
- Dott.ssa Iemma “Polimeri per applicazioni farmaceutiche (Parte I)” 02-05-2007
- Prof. Bortolini “Epossidazione di Substrati Organici (Parte I)” 03-05-2007
- Prof. Bortolini “Epossidazione di Substrati Organici (Parte II)” 10-05-2007
- WORKSHOP "Le Piu’ Recenti Innovazioni Della Spettrometria Di Massa Nel Settore Agro-Alimentare, Ambientale e Farmaceutico" 16-05-2007
- Prof. Menichini “Aspetti fitochimici nella ricerca sulle piante medicinali” 17-05-2007
- Prof Garofalo “Enzimi (recettori catalitici): aspetti chimico-farmaceutici (Parte II)” 24-05-2007
- Prof. Serge Carreau “Roles of estrogens in spermatogenesis” 21-05-2007
- Prof. Salerno “Sintesi di derivati carbo- ed eterociclici mediante catalisi organometallica (Parte I)” 30-05-2007

- Prof. Salerno “Sintesi di derivati carbo- ed eterociclici mediante catalisi organometallica (Parte II)” 31-05-2007
- Prof. Menichini “Valutazione dell'attività biologica di principi attivi di origine naturale” 31-05-2007
- Dott.ssa Muzzalupo “Strategie utilizzate per superare le problematiche inerenti alla veicolazione di principi attivi (Parte I)” 04-06-2007
- Prof. Gabriele “Sintesi di eterocicli PdI_2-catalizzata” 06-06-2007
- WORKSHOP Prof Himo “Enzymatic Reaction Mechanisms: Oxidation and Reduction” 14-06-2007
- WORKSHOP “Qualità e salubrità degli alimenti. Il contributo della spettrometria di massa” 15-06-2007
- Prof. Nouri Neamati, “Design and discovery of novel small-molecules drugs with anticancer and antiviral activities” 20-06-2007
- Prof. Takashi Oshima, “Mechanism of action of a novel anticancer agent” 20-06-2007
- Prof. Porto “Controllo dei Parametri necessari per la validazione dei metodi di analisi (Parte I)” 20-06-2007
- WORKSHOP “Human and vegetable proteomics” 25-06-2007
- Prof. Porto “Controllo dei Parametri necessari per la validazione dei metodi di analisi (Parte II)” 27-06-2007
- Dott.ssa Fazio “Le ciclodestrine nella chimica degli alimenti” 28-06-2007
- Prof De Nino “Le reazioni multicomponente (MCRs)” 05-07-2007
- Prof De Nino “L’evoluzione dei modelli per la reazione di addizione al Carbonile” 26-07-2007
- Prof. Picci “Nuove strategie di rilascio controllato di farmaci (Parte I)” 11-10-2007
- Dr. Tagarelli “Mass Spectrometry Basics” 17-10-2007
- Dott.ssa Ioele “Farmaci utilizzati nella terapia pediatrica” 22-10-2007

- Dott.ssa Leggio “Peptidomimetici (Parte I)” 24-10-2007
- Prof Liguori “Chimica degli steroidi” 25-10-2007
- Prof Liguori “Sintesi di amminoacidi e peptici modificati” 25-10-2007
- Dott.ssa Napoli “Mass Spectrometry Advanced” 26-10-2007
- Dott.ssa Di Gioia “Tecniche di sintesi su supporto solido” 29-10-2007
- Prof. Picci “Nuove strategie di rilascio controllato di farmaci (Parte II)” 31-10-2007
- Prof. Siciliano “Analisi NMR di biomolecole: la terza dimensione” 05-11-2007
- Prof. Siciliano “L’analisi strutturale NMR nella progettazione e preparazione di analoghi di biomolecole aventi potenzialità farmaceutiche” 06-11-2007
- Dott.ssa Aiello “Drug-like properties and the causes of poor solubility and poor permeability (Parte I)” 09-11-2007
- Dott.ssa Aiello “Drug-like properties and the causes of poor solubility and poor permeability (Parte II)” 16-11-2007
- Prof. R. C. Larock “Adventures in bioplastics, iodine, palladium and aryne Chemistry” 20-11-2007
- Prof. Ragno “Elaborazione statistica dei dati analitici. Approccio chemiometrico” 29-11-2007
- Prof. Zanotti “Reactions of bridging C3 ligands in diiron complexes: unconventional routes to new functionalized organic frames” 19-01-2008
- Prof B. Silvi and M. Curie "Electronic reaction mechanisms from molecular geometry" 20-01-2008
- Prof. I. Andreu “Photosensitisation of lipids by diaryl-ketones in model dyads” 23-01-2008
- Prof. L. Coitino “Targeting DNA by metallic complexes useful in therapy and early of human diseases with in silico approaches” 30-01-2008

- Prof Ragno “Sistemi farmaceutici anticoncezionali” 07-02-2008
- Prof. A. Caruso “Effetti dell’oleuropeina su colture primarie di endoteli umani: Attività antinfiammatoria e antivirale” 13-02-2008
- Dott.ssa Trombino “Principali tecniche per la clonazione animale” 19-02-2008
- Dr. C. M. Athanassopoulos, “Total syntheses of medicinally interesting polyamine analogues” 29-02-2008
- Workshop “Analisi degli alimenti e delle acque mediante LC/MS/MS" 02-04-2009
- Prof. R. Wigmans “Dual readout calorimetry” 19-05-2009