Stereoselective syntheses and application of (–)-isopulegol and (+)-neoisopulegol-based bi-, tri- and tetrafunctionalised monoterpenes

Ph.D. Thesis

Fatima Zahra Bamou

Supervisors

Prof. Dr. Zsolt Szakonyi

Dr. Tam Minh Le

Institute of Pharmaceutical Chemistry

University of Szeged

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Doctoral School of Pharmaceutical Sciences

Educational Program: Pharmaceutical Chemistry and Drug Research

Programme director: Prof. Dr. István Szatmári

Institute: Institute of Pharmaceutical Chemistry

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Complex exam committee:

Head: Prof. Dr. Loránd Kiss

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Prof. Dr. János Wölfling

Reviewer committee:

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Reviewers: Dr. Mihály Herczeg

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Introduction and aims

In the past decade, chiral synthons have received much attention in organic synthesis due to their application as starting materials in asymmetric transformations or as auxiliaries and chiral catalysts in enantioselective syntheses. Among them, 1,2-aminoalcohols, aminodiols, and aminotriols are of increasing importance, which are not only known to be used as building blocks, but also possess significant biological activities. A large majority of these compounds are derived from commercially available monoterpenes, such as (–)-isopulegol, *α*- or *β*-pinene.

There are several different substances with the 1,2-aminoalcohol structure that have physiologically and pharmaceutically relevant characteristics. For instance, substances with a backbone constituted of hydroxyethylamine may block aspartic protease enzymes and are frequently employed as anti-HIV, antimalarial, and anti-leishmanial agents. A wide variety of *β*-adrenergic blockers, including those used to treat high blood pressure, angina pectoris, cardiac arrhythmias, and other problems of the sympathetic nervous system, include the 1,2-aminoalcohol moiety.
In asymmetric synthesis, it has also been shown that 1,2-aminoalcohols are effective chiral auxiliaries and chiral catalysts.

Chiral aminodiols and aminotriols derived from available natural monoterpenes are known as good starting materials and excellent chiral catalysts in asymmetric addition reactions, including Grignard addition, intramolecular [2 + 2] photocycloaddition, and intramolecular radical cyclisation. They are also used as building blocks for the synthesis of heterocyclic ring systems, mainly 1,3-oxazines and 1,3-thiazines, which have a wide range of biological properties, such as antiproliferative, analgesic, anticonvulsant, anti-inflammatory, antibiotic, antimicrobial, antimalarial, antihypertensive, and anticancer properties. For example, Aristeromycin analogues are widely used as antiviral agents against hepatitis B and C, human immunodeficiency, influenza, herpes simplex, and other viruses. Moreover, (2*R*,3*R*,7*Z*)-2-aminotetradec-7-ene-1,3-diol are known as potent antimicrobial metabolites. In addition, sphingolipid analogues have diverse biological roles, including the treatment of inflammation, cancer and Alzheimer's disease.

O-Benzyl azole derivatives have been widely employed as pharmacophores and synthons in organic chemistry and drug discovery and have played key roles in the history of heterocyclic

chemistry. As a result of their high therapeutic index, superior absorption, and acceptable safety profile, azoles such as imidazole and triazole have become the most researched class of antifungal drugs. It is noted to mention that the *O*-benzyl substituent is crucial to the enhanced antibacterial action of these compounds.

On the other hand, pyrimidines are determined to be a prominent group in heterocyclic chemistry due to their considerable biological activity. According to their significant chemopreventive and chemotherapeutic properties, 2,4-diaminopyrimidines have gained substantial attention among the enormous number of structurally varied pyrimidine derivatives now available. In the domain of drug research and development, 2,4-diaminopyrimidine derivatives with antiviral, antiparasitic, antibacterial, and anti-inflammatory properties have attracted substantial attention in recent years.

The aim of this Ph.D. work was to synthesise (–)-isopulegol and (+)-neoisopulegol based bi-, tri- and tetrafunctionalised building blocks such as 1,2-aminoalcohols, aminodiols, and aminotriols, whereas the amino function can be primary, secondary, or tertiary, a part of azole or pyrimidine ring system, starting from commercially available (–)-isopulegol.

Furthermore, we planned to study the ring closure properties of the prepared 1,2-aminoalcohols, as well as the synthesis of 1,3-oxazines, 1,3-oxazoles, 1,3-thiazines and 1,3-thiazoles. In addition, to expand our work we prepared a new series of 2,4-diaminopyrimidine derivatives.
The resulting 1,2-aminoalcohols and their ring-closed derivatives were planned to apply as chiral catalysts in the enantioselective addition of Et_2Zn to aldehydes.

On the other hand, according to the literature background, we planned to study the antimicrobial activities of the prepared chiral synthon 1,2-aminoalcohols, aminodiol and aminotriol chiral synthons against two yeast species as well as against two Gram-positive and two Gram-negative bacteria. The antiproliferative activities, in turn, were determined in the case of 1,3-oxazine, 1,3-oxazole, 1,3-thiazine and 2,4-diaminopyrimidine derivatives using *in vitro* assay against different human cancer cell lines.

Methods

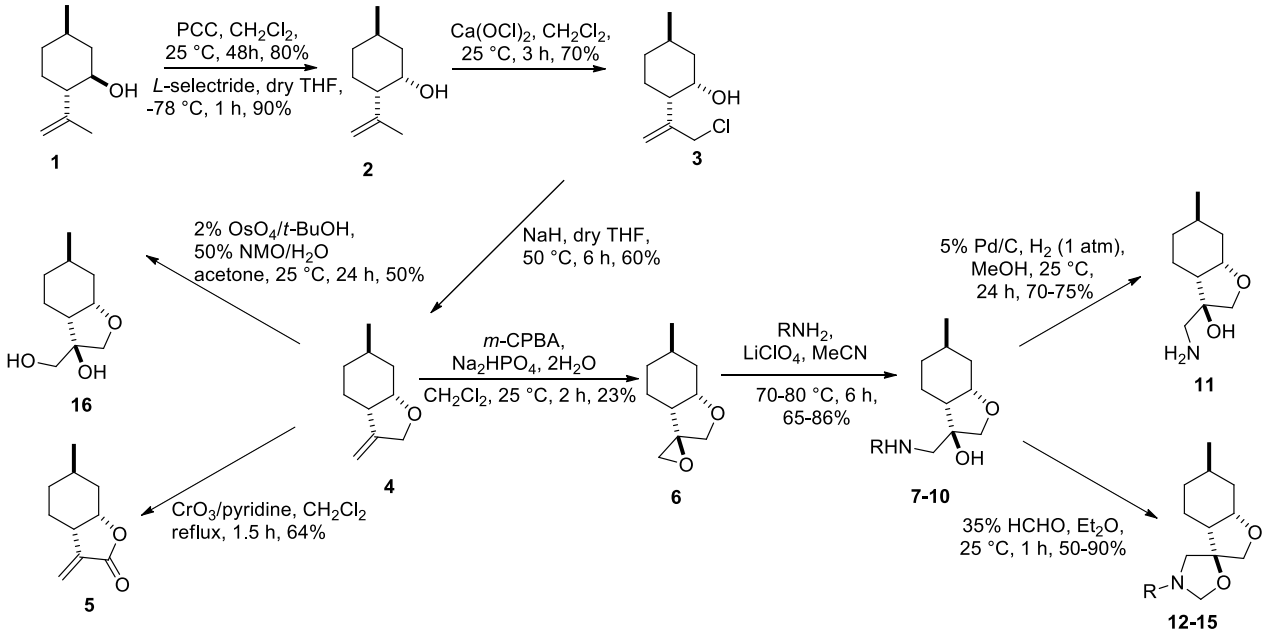
Reactions were performed on a mmol scale, and products were purified by column chromatography on silica gel or by crystallisation. All new compounds were characterised by their mass spectrometry, melting point measurement, 1D- and 2D-NMR, elemental analysis,

and optical rotation. The enantiomeric excess of 1-phenyl-1-propanols was checked by chiral GC. The antiproliferative properties were determined by Prof. Dr. István Zupkó *et al*.. Calculations were performed by means of the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA). Antimicrobial activities were measured by Dr. András Szekeres and co-workers. Docking study and *in-Silico* ADMET prediction were accomplished by Accelrys discovery studio 2.5 software.

Results and discussions

1. Synthesis of (+)-neoisopulegol-based 1,2-aminoalcohols

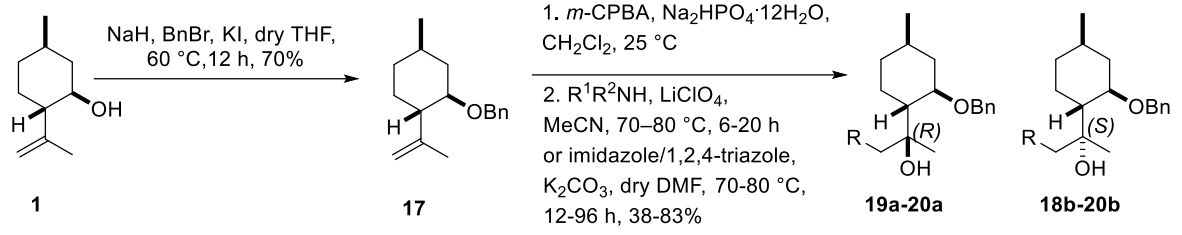
The key intermediate **4** was synthesised starting from commercially available (–)-isopulegol **1,** which was transformed into (+)-neoisopulegol **2**, followed by allylic chlorination and cyclisation. The resulting **4** was treated with Collins reagent (CrO_3/pyridine) resulting in compound **5**, whereas using *m*-CPBA and Na_2HPO_4, $12H_2O$ afforded the oxirane **6**, which gave secondary aminoalcohols **7**–**10** by aminolysis using different primary amines. Latter underwent catalytic hydrogenations to produce **11** and on the other hand, cyclisation of compounds **7**–**10** with formaldehyde resulted in the formation of spiro-oxazolidines **12**–**15**. Compound **4** was subjected to *syn*-selective dihydroxylation to form **16** (Scheme 1).



Scheme 1

2. Synthesis of (–)-isopulegol and (+)-neoisopulegol-based *O*-benzyl derivatives

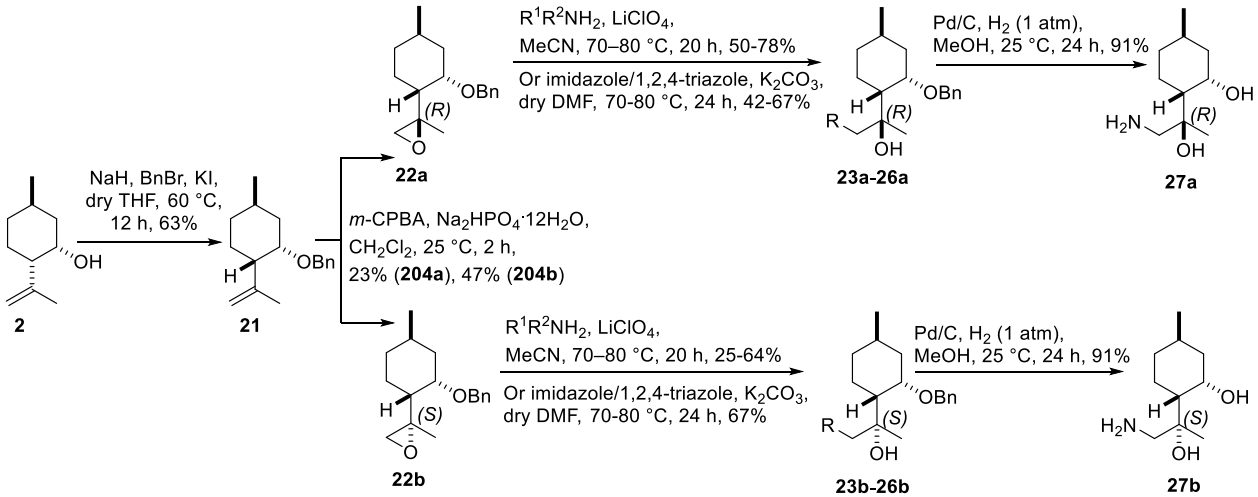
O-benzylation of (–)-isopulegol **1**, followed by epoxidation of **17** with *m*-CPBA and aminolysis with dibenzylamine in the presence of $LiClO_4$ as a catalyst, and imidazole/1,2,4-triazole with K_2CO_3 in dry DMF resulted in compounds **18b**, **19a**–**b** and **20a**–**b** (Scheme 2).



18b: R = $N(CH_2Ph)_2$, **19a-b:** R = imidazole, **20a-b:** R = 1,2,4-triazole

Scheme 2

(+)-Neoisopulegol **2** was subjected to the *O*-benzylation followed by epoxidation of the resulting **21**, produced a 1:2 mixture of epoxides **22a** and **22b** in good yields, which were easily separated using column chromatography. Ring opening of the oxiranes using different nucleophiles (including benzylamine, dibenzylamine, imidazole and 1,2,4-triazole) afforded **23a**–**26a and 23b**–**26b**. Debenzylation of **23a** and **23b** resulted in the formation of **27a** and **27b** in excellent yields (Scheme 3).

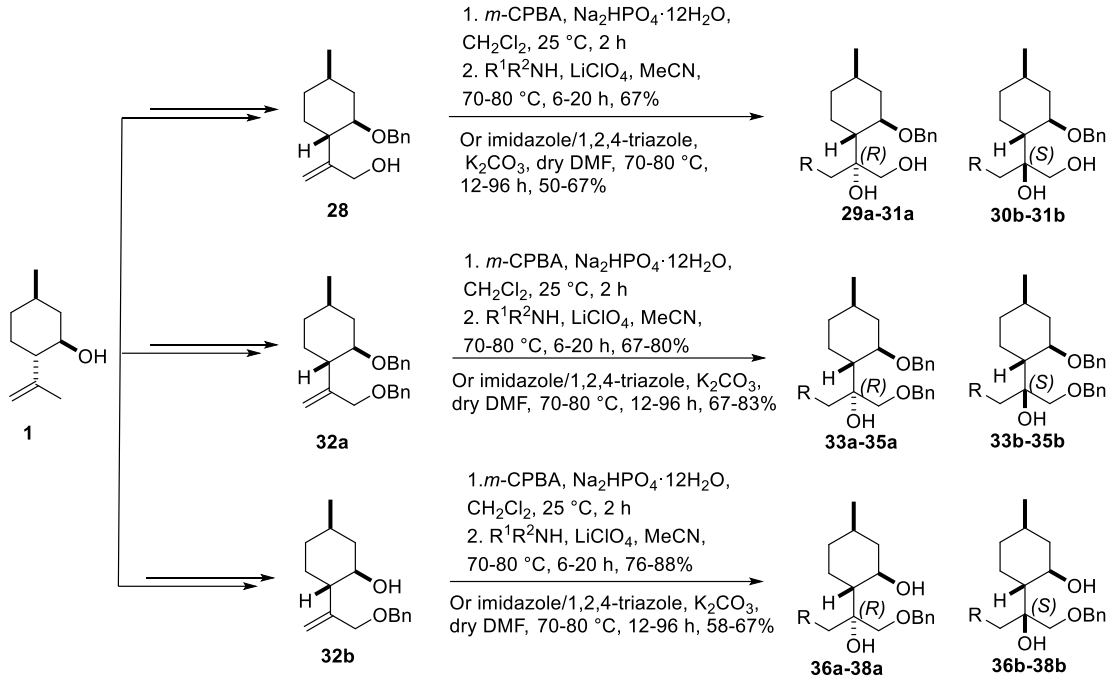


23a-b: R = $NHCH_2Ph$, **24a-b**: R = $N(CH_2Ph)_2$, **25a-b**: R = imidazole, **26a-b**: 1,2,4-triazole

Scheme 3

Furthermore, (–)-isopulegol-based *O*-benzyl aminotriol derivatives were also synthesised, applying the same above process starting from the prepared compounds **28**, **32a**, **32b** and

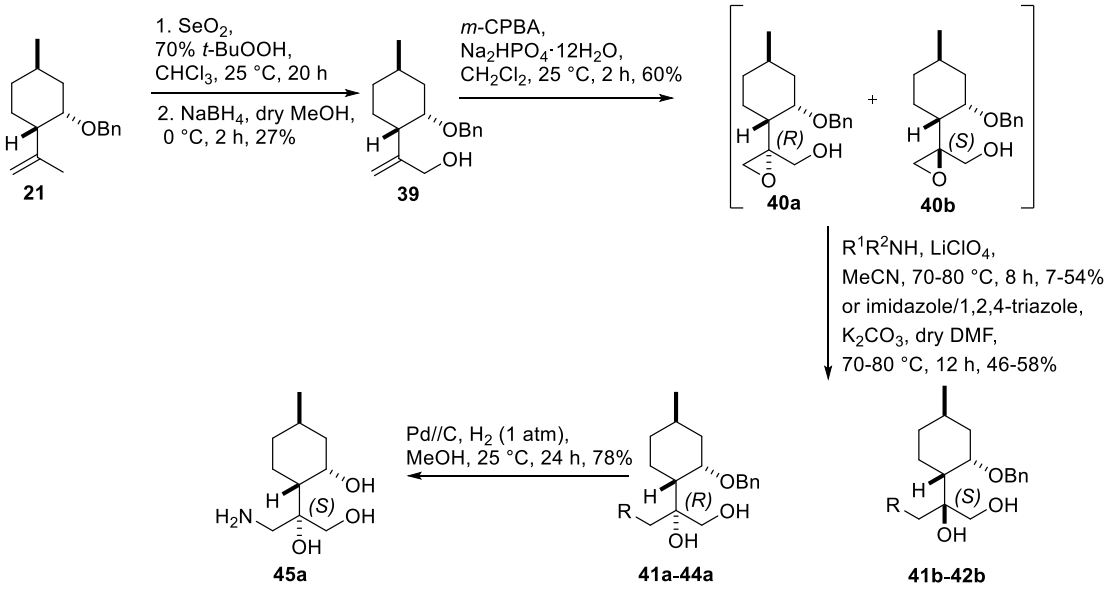
using dibenzylamine, imidazole and 1,2,4-triazole as nucleophiles for the ring-opening epoxides (Scheme 4).



29a, **33**, **36**: R = $N(CH_2Ph)_2$; **30**, **34**, **37**: R = imidazole; **31**, **35**, **38**: R = 1,2,4-triazole

Scheme 4

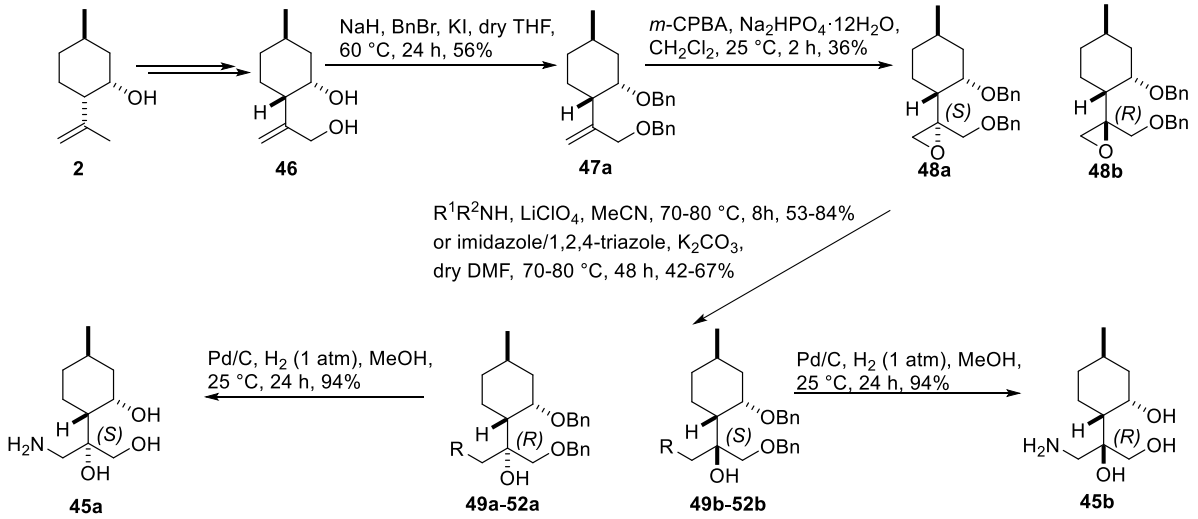
The (+)-neoisopulegol *O*-benzyl aminotriols were prepared starting from allylic oxidation of **39** followed by epoxidation, which produced a 4:1 mixture of epoxides **40a** and **40b.** Ring opening of the mixture resulted in compounds **41**–**44**. The separation of *N*-benzyl derivatives was successfully achieved, whereas, for the azole derivatives, only the major compounds were generated. Hydrogenation of **41a** over 5% Pd/C in MeOH furnished primary aminotriol **45a** in 78% yield (Scheme 5).



41a-b: R = $NHCH_2Ph$, **42a-b**: R = $N(CH_2Ph)_2$, **43a**: R = imidazole, **44a**: R = 1,2,4-triazole

Scheme 5

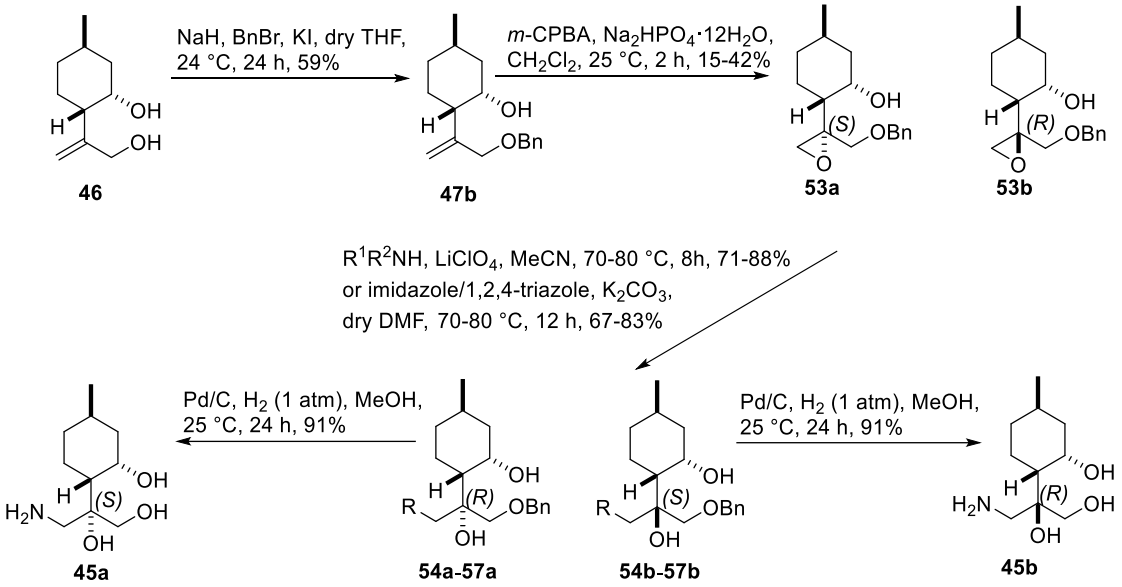
In addition, diol **46** obtained from oxidation of (+)-neoisopulegol **2** was subjected to di-*O*-benzylation instead, using NaH/BnBr/KI system under reflux in dry THF resulting in **47a**. A 1:1 mixture of epoxides **48a** and **48b** was formed by epoxidation of **47a** with *m*-CPBA system, followed by purification and ring-opening with multiple nucleophiles giving a family of di-*O*-benzyl derivatives **49a**–**52a** and **49b**–**52b**, respectively. Hydrogenation of the resulting compounds of **49a** and **49b** over Pd/C resulted in the formation of primary aminotriols **45a** and **45b** in excellent yields (Scheme 6).



49a-b: R = $NHCH_2Ph$, **50a-b**: R = $N(CH_2Ph)_2$, **51a-b**: R = imidazole, **52a-b**: 1,2,4-triazole

Scheme 6

In the other hand, diol **46** was subjected to mono-*O*-benzylation to form compound **47b**, followed by epoxidation using *m*-CPBA produced a mixture of epoxides **53a** and **53b** with 3:1 ratio, followed by separation using column chromatography. Aminolysis of the individuals with different nucleophiles resulted in the formation of novel *O*-benzyl derivatives **54a**–**57a** and **54b**–**57b**, respectively. Catalytic hydrogenation of **54a** and **54b** with Pd/C yielded primary aminotriols **45a** and **45b** (Scheme 7).

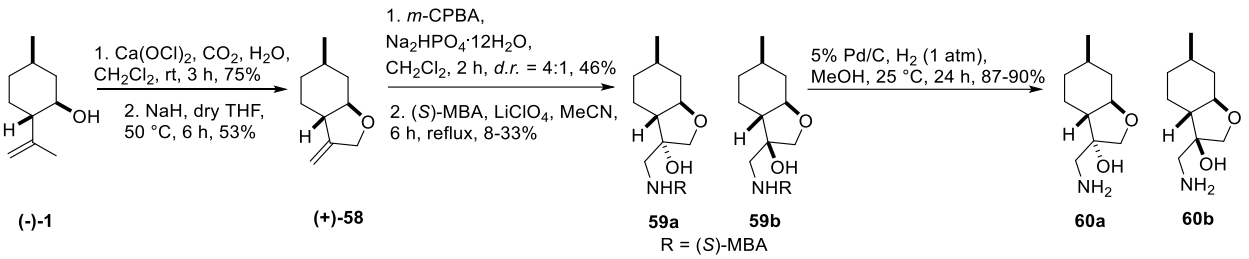


54a-b: R = $NHCH_2Ph$, **55a-b**: R = $N(CH_2Ph)_2$, **56a-b**: imidazole, **57a-b**: 1,2,4-triazole

Scheme 7

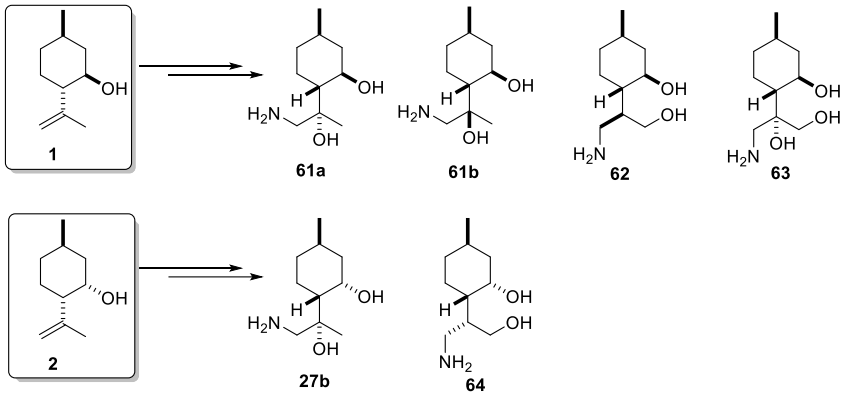
3. Synthesis of 1,3-oxazines, oxazolidines, 1,3-thiazines and thiazolidines

We prepared the starting materials aminoalcohols **60a**–**b** starting from **(**–**)-1** *via* allylic chlorination, followed by cyclisation to produce *exo*-methylene tetrahydrofuran **(**+**)-58**. The epoxidation of **(**+**)-58** produced a mixture of epoxides, followed by ring-opening reaction using (*S*)-methylbenzylamine to obtain secondary aminoalcohols **59a**–**b,** which were separated using column chromatography, and the individual compounds were subjected to catalytic hydrogenation over Pd/C to give primary aminoalcohols **60a–b** (Scheme 8).



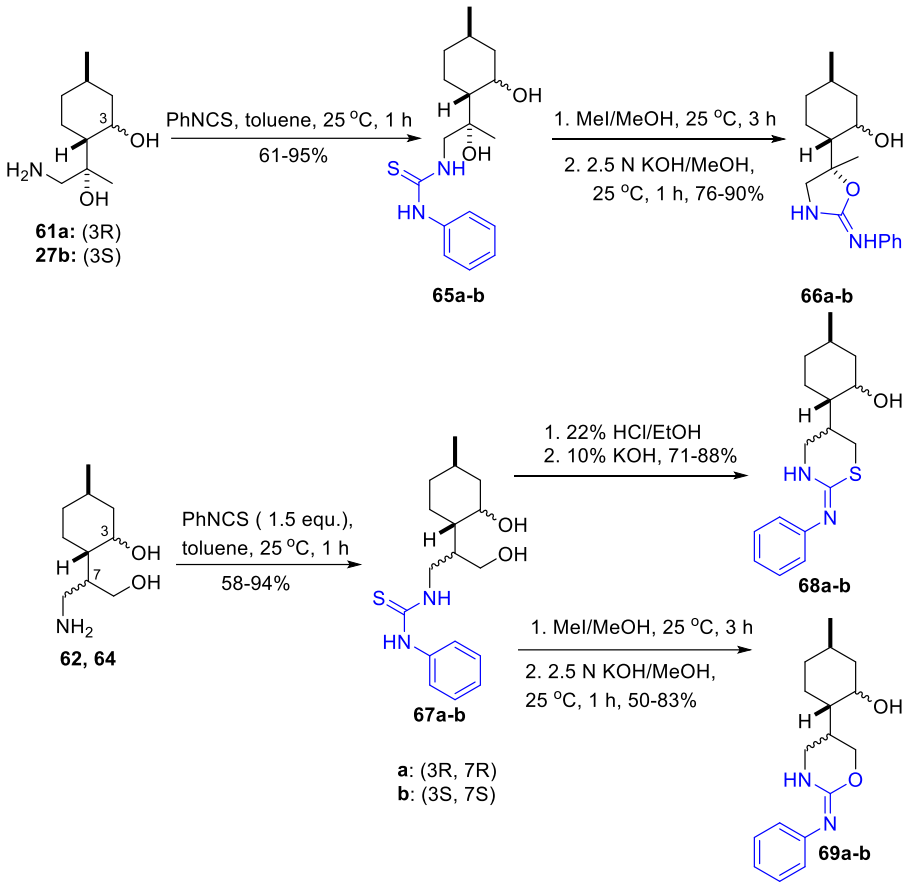
Scheme 8

Furthermore, other starting materials including (–)-isopulegol and (+)-neoisopulegol-based aminodiols **61a**–**b**, **62**–**63** and aminotriols **27b** and **64** were also prepared using literature methods (Scheme 9).



Scheme 9

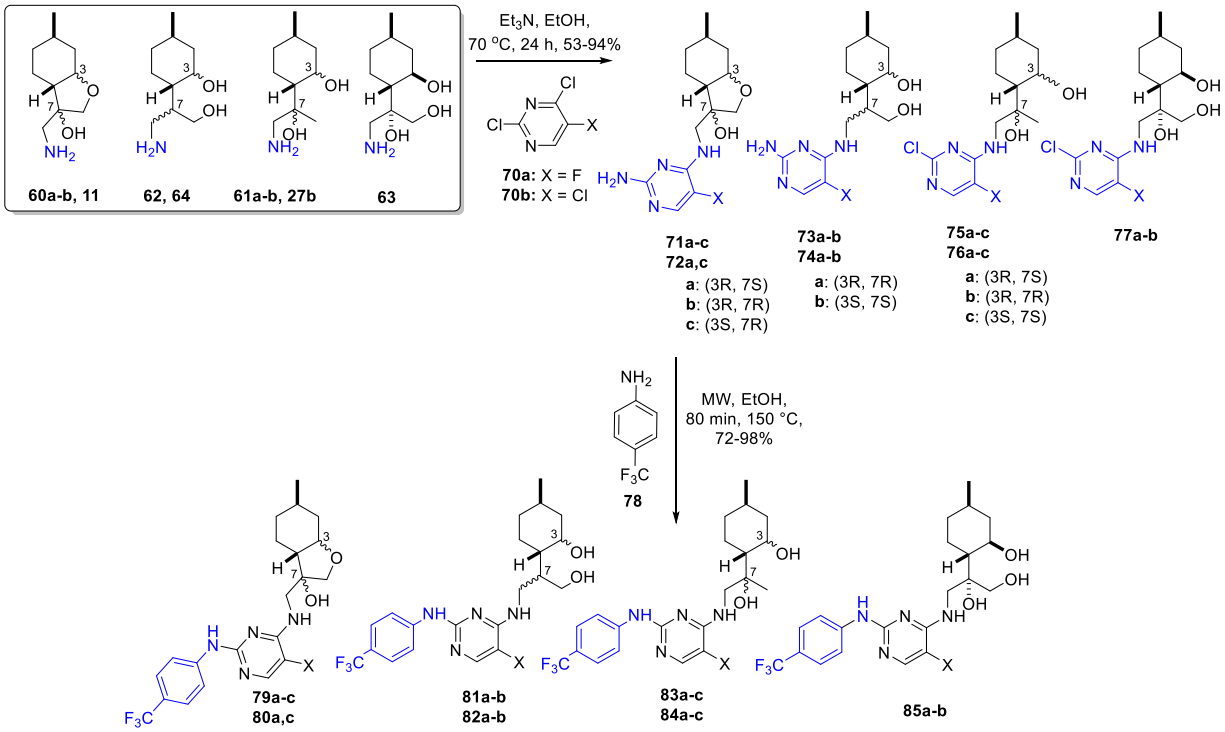
The prepared aminodiols were used for the synthesis of 1,3-oxazolidines **66a**–**b**, 1,3-thiazines **68a**–**b**, and 1,3-oxazines **69a**–**b**, through their reaction with PhNCS in toluene which gave thioureas **65a**–**b** and **67a**–**b**. Treatment of those intermediates with MeI and under alkaline conditions provided 1,3-oxazolidines **66a**–**b** and 1,3-oxazines **69a**–**b**. On the other hand, thiazines **68a**–**b** were formed by acid catalysed cyclisation with 22% HCl in EtOH, whereas in the case of **65a**–**b**, the thiazolidines were not obtained due to the steric hindrance.



Scheme 10

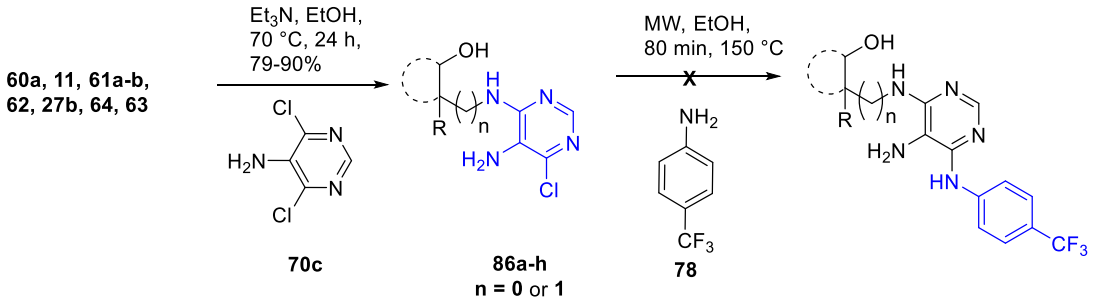
4. Synthesis of 2,4-diaminopyrimidine derivatives

Primary aminodiols **27b**, **61a**–**b**, **62** and **64**, and aminotriol **63**, as well as aminoalcohols **11** and **60a**–**b**, were applied as starting materials for the preparation of 2,4-diaminopyrimidine derivatives through the addition of 2,4-dichloro-5-fluoropyrimidine **70a** and 2,4,5-trichloropyrimidine **70b** which provided the intermediates **71**–**77**. Microwave-assisted SNAr coupling reaction of the resulted intermediates with 4-aminobenzotrifluoride **78** was applied to form the 2,4-diaminopyrimidines **79**–**85**.



Scheme 11

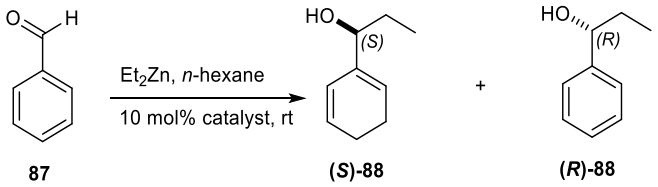
Following the same above conditions, for the synthesis of **86a–h** by addition of 5-amino-4,6-dichloropyrimidine **70c** to **60a**, **11**, **61a–b**, **62**, **27**, **64** and **63** respectively. Unfortunately, due to the steric effect of the amino group at the *ortho* position, the addition of 4-aminobenzotrifluoride was not successful.



Scheme 12

5. Catalytic addition of diethylzinc to benzaldehyde

The aminoalchols **7**–**15** were evaluated as chiral catalysts for the catalytic addition of diethylzinc to benzaldehyde. The results showed low to moderate enantiomeric excess (*ee*%) values with (*S*)-selectivity, the highest catalytic performance was noticed in the case of ligand **8** with a 40% *ee* value and (*S*)-selectivity (Scheme 13).



Scheme 13

6. Antimicrobial effects, antiproliferative activity

In vitro antimicrobial activity of the synthesised 1,2-aminoalcohol derivatives **7**–**15** and diol **16** was evaluated against two yeasts as well as two Gram-positive and two Gram-negative bacteria, the results clearly show that *N*-substituted aminoalcohols have a moderate antibacterial effect on multiple bacterial strains, while a significant antifungal action was noticed in the case of the diol **16**. the *O*-benzyl substituent on the cyclohexyl ring in aminodiol and aminotriol derivatives is essential to have an antimicrobial effect.

The *in vitro* antiproliferative activities of the prepared 2,4-diaminopyrimidine derivatives were tested on different human cancer cell lines (HeLa, SiHa, A2780, and MDA-MB-231), and the results showed that the introduction of the N^2-aryl function into the 2,4-diaminopyrimidine sketelon has an important impact on the high potency.

7. Docking studies

Docking studies were employed to examine the interactions between the ligand **83b** and Aurora A kinase PDB (Code: 4DEEhe results revealed that compound **83b** has several interactions with Aurora A which leads to a high *in vitro* potency.

PUBLICATION LIST

Papers related to the thesis

I. **Fatima Zahra Bamou**, Tam Minh Le, Bettina Volford, András Szekeres, Zsolt Szakonyi
Synthesis and application of 1,2-aminoalcohols with neoisopulegol-based octahydrobenzofuran core
Molecules **2020**, *25*, 21. DOI: 10.3390/molecules25010021

IF: 4.412

II. Tam Minh Le, Thu Huynh, **Fatima Zahra Bamou**, András Szekeres, Ferenc Fülöp, Zsolt Szakonyi
Novel (+)-neoisopulegol-based *O*-benzyl derivatives as antimicrobial agents
Int. J. Mol. Sci, **2021**, *22*, 5626. DOI: 10.3390/ijms22115626

IF: 6.208

III. **Fatima Zahra Bamou**, Tam Minh Le, Bizhar Ahmed Tayeb, Seyyed Ashkan Senobar Tahaei, Renáta Minorics, István Zupkó, Zsolt Szakonyi
Antiproliferative activity of (–)-isopulegol-based 1,3-oxazine, 1,3-thiazine and 2,4–diaminopyrimidine derivatives
ChemistryOpen **2022**, accepted. DOI: 10.1002/open.202200169

IF: 2.630

Papers not related to the thesis

IV. Abdoullah Bimoussa, Ali Oubella, **Fatima Zahra Bamou**, Zein Alabdeen Khdar, Mourad Fawzi, Yassine Laamari, My Youssef Ait Itto, Hamid Morjani, Aziz Auhmani
New 1,3,4-thiadiazoles derivatives: synthesis, antiproliferative activity, molecular docking and molecular dynamics
Future Med. Chem, **2022**, *14*, 881–897. DOI: 10.4155/fmc-2022-0016

IF: 4.767

V. Mourad Fawzia, Ali Oubella, Abdoullah Bimoussa, **Fatima Zahra Bamou**, Zein Alabdeen Khdar, Aziz Auhmani, Abdelkhalek Riahi, Anthony Robert, Hamid Morjani, My Youssef AitIttoa
Design, synthesis, evaluation of new 3-acetylisoxazolines and their hybrid analogous as anticancer agents: *In vitro and in silico* analysis
Comput. Biol. Chem, **2022**, *98*, 107666. DOI: 10.1016/j.compbiolchem.2022.107666

IF: 3.737

Scientific lectures

I. Tam Minh Le, **Fatima Zahra Bamou**, Tamás Szilasi, Ferenc Fülöp, Zsolt Szakonyi
Synthesis and transformation of isopulegol-based bi-, tri- and tetrafunctional chiral ligands
MTA Alkaloid-és Flavonoidkémiai Munkabizottság ülése Mátrafüred, April 11-12, 2019

II. Zsolt Szakonyi, Tam Minh Le, **Fatima Zahra Bamou**, Tamás Szilasi
Izopulegol alapú bi-, tri és tetra funkciós kismolekulák sztereoszelektív előállítása és viszgálata Gyógyszerkémai és Gyógyszertechnológiai
Szimpózium ´19, Kecskemét, September 5-6, 2019

III. **Fatima Zahra Bamou**
Synthesis and application of neoisopulegol-based 1,2-aminoalcohols with octahydrobenzofuran core
XLII. KÉMIAI ELŐADÓI NAPOK, October 28-30, 2019

IV. **Fatima Zahra Bamou,** Tam Minh Le, Bettina Volford, András Szekeres, Zsolt Szakonyi
Synthesis, transformation, and application of isopulegol and neoisopulegol-based bi- and tridentatechiral ligands
The 24th International Electronic Conference on Synthetic Organic Chemistry, 15 Nov–15 December, 2020

V. Tam Minh Le, Mounir Raji, **Fatima Zahra Bamou**, Thu Huynh, Szekeres András, Szakonyi Zsolt
Stereoselective synthesis and antimicrobial evaluation of monoterpene-based bi-, tri and tetrafunctionalized chiral synthons
Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium ’21, September 20-21, 2021

VI. Tam Minh Le, Thu Huynh, **Fatima Zahra Bamou**, András Szekeres, Ferenc Fülöp, Zsolt Szakonyi
Novel (–)-isopulegol-based *O*-benzyl derivatives as antimicrobial agents.

56th International Conference on Medicinal Chemistry (RICT 2021), Interfacing Chemical Biology and Drug Discovery; July, 7-9, 2021

VII. **Fatima Zahra Bamou**, Tam Minh Le, Bizhar Ahmed Tayeb, Seyyed Ashkan Senobar Tahaei, Renáta Minorics, and Zsolt Szakonyi

Antiproliferative activity of (–)-isopulegol-based 1,3-oxazine, 1,3-thiazine, and 2,4-diaminopyrimidine derivatives

17th Belgian Organic Synthesis Symposium, Namur, Belgium, July 3-8, 2022