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Syntheses and transformation of alicyclic β-amino acid derivatives utilizing conventional and green chemistry methods

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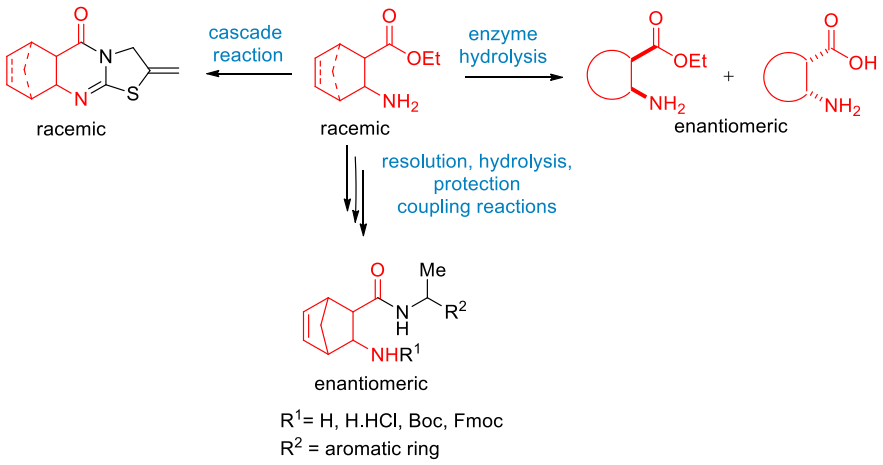
1. INTRODUCTION AND AIMS

The synthesis of natural compounds means a huge challenge for scientists. Numerous diverse natural products have the oxindole or dihydroquinazoline core and these compounds own a lot of scientific attention thanks to their wide range of biological activities, such as antioxidative, anti-HIV and neuroprotection activity. Recently, there has been a lot of interest in developing more sustainable synthesis pathways for pharmacologically relevant derivatives. Because of the importance of a sustainable future in today's world, green chemistry is becoming desirable and more and more attractive. There is still a need for new, environmentally benign and effective catalytic systems for organic compounds in pharmaceutical chemistry. Dihydroquinazolinone derivatives, which have attracted the interests of researchers, recently, have important biological activities like antitumor and anticancer effects. Synthesis of different kind of dihydroquinazolinone compounds and finding greener synthetic procedures to obtain these kinds of derivatives requires on-going development.

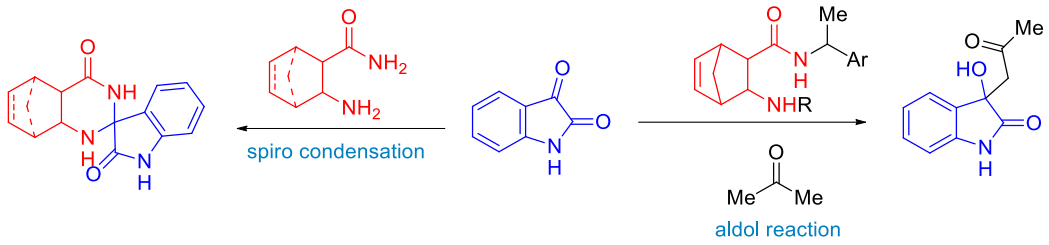
In view of the growing importance of β-amino acid derivatives, the first aim of my PhD work was to develop a simple route for the synthesis of 2-methylenethiazolo[2,3-*b*]quinazolinone derivatives. [I] Another goal was developing efficient strategies for the enzymatic resolution of 5–8-membered alicyclic amino esters through hydrolysis in green organic media, under solvent-free conditions and using ball milling. [II] We achieved the syntheses of enantiomeric form of 2-aminocycloalkane-carboxylic acid and ester derivatives starting from the appropriate racemic esters (Scheme 1).



Scheme 1

Additional aim was examining greener synthesis procedures and organocatalytic applicability of the alicyclic β-amino acid derivatives. [III] Also testing the catalytic applicability of substituted enantiomeric alicyclic β-amino amides in asymmetric aldol reaction of isatin and acetone was amongst our intentions. [III]

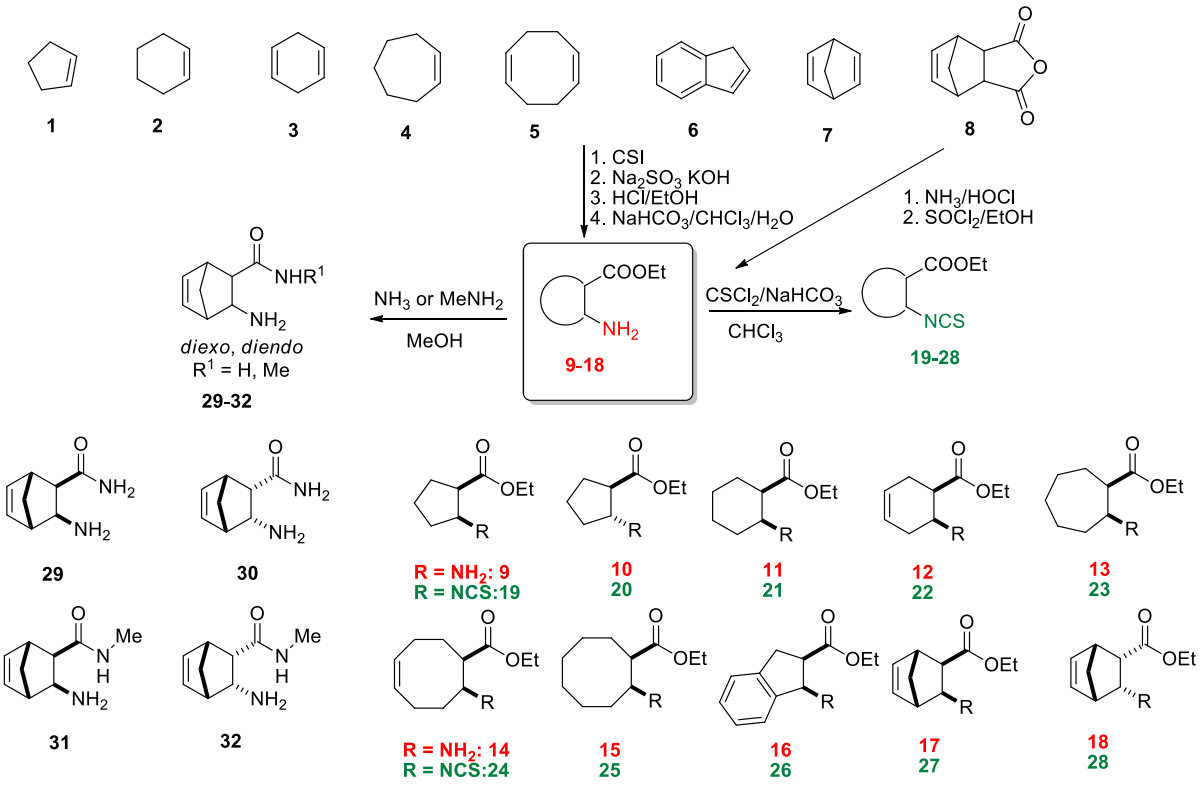
By spiro-condensation reactions of isatin and alicyclic β-amino amides obtaining racemic tetrahidro-1*H*-spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives were targeted (Scheme 2). [IV]



Scheme 2

2. RESULTS AND DISCUSSIONS

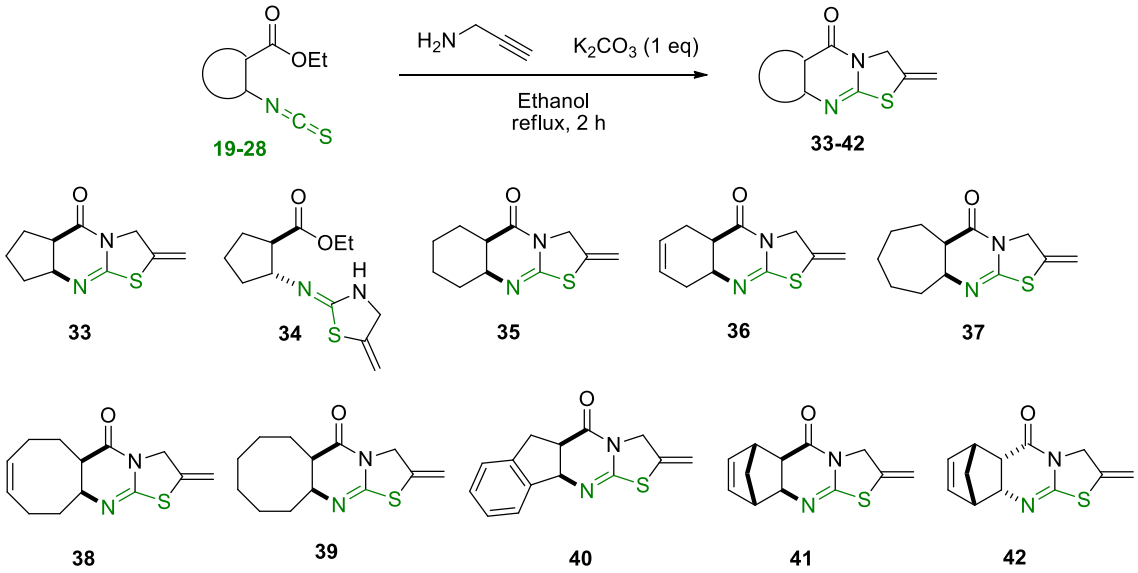
In order to achieve our goals, the first steps were to synthesise the starting compounds. The reaction of chlorosulfonyl isocyanate (CSI) with different cycloalkenes is a well-known route for the synthesis of racemic cycloalkane-fused β-lactams. The synthesis routes were accomplished according to methods already known starting from cyclopentene (**1**), cyclohexene (**2**), 1,4-cyclohexadiene (**3**), cycloheptene (**4**), 1,5-cyclooctadiene (**5**), indene (**6**) and norbornadiene (**7**). Treatment of the β-lactams with ethanolic HCl led to racemic alicyclic amino esters **9**, **11**–**14**, **16** and **17**. Isomerisation of *cis* amino ester **9**, induced by sodium ethoxide, resulted in the formation of *trans* amino ester **10**. In order to get saturated **15**, ethyl *cis*-2-aminocyclooct-5-ene carboxylate **14** was reduced catalytically under H_2. Compound *diendo*-β-amino ester **18** was prepared by hypochlorite-mediated Hofmann degradation of the carboxamide obtained by ammonolysis of anhydride **8**, followed by esterification in the presence of ethanol and then thionyl chloride gave the required product. Ethyl *cis*- and *trans*-, *diexo*- and *diendo*-2-isothiocyanato-1-cyclalkanecarboxylates **19**–**28** were prepared by conventional methods, with the reactions of the corresponding alicyclic ethyl 2-amino-1-carboxylates **9**–**18** and thiophosgene. The *diexo*- and *diendo*-2-aminonorbornene esters **17** and **18** were treated with ammonia to furnish the *diexo*- and *diendo*-2-aminonorbornene carboxamides **29** and **30**. The *diexo*- and *diendo*-*N*-methylcarboxamides **31** and **32** were obtained in the reaction of ethyl ester **17** and **18** with methanolic methylamine (Scheme 3).



Scheme 3

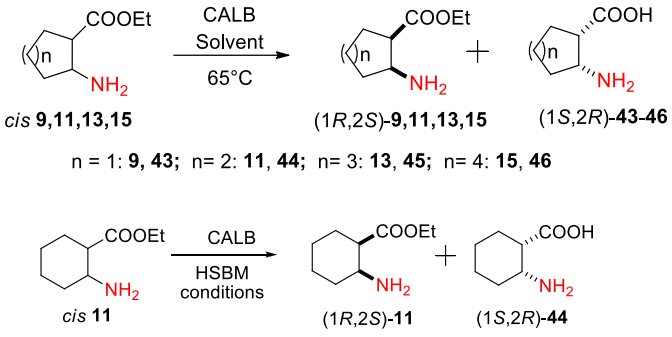
The isothiocyanates **19**–**28** underwent a base-promoted cascade reaction with propargyl amine, resulting in 2-methylene-substituted thiazolo[2,3-*b*]quinazolinones and alicyclic thiazolo[3,2-*a*]pyrimidinones **33**–**42**

(Scheme 4). Note that *trans* isomer **20** failed to cyclise, but gave methylenethiazolidin-2-ylidene intermediate **34**. The cascade reaction proceeds by way of a favoured 5-*exo*-*dig* process during the second ring closure, as confirmed by full NMR spectroscopic assignments. The biological activity of compounds **33**–**42** was tested and modest activities were detected mainly against breast cancer cells. (5a*S**,10b*S**)-2-Methylene-2,3,5a,6-tetrahydroindeno[1,2-*d*]-thiazolo[3,2-*a*]pyrimidin-5(10b*H*)-one (**40**) showed an outstanding biological feature, which may certainly be helpful in developing anticancer drugs. [I]



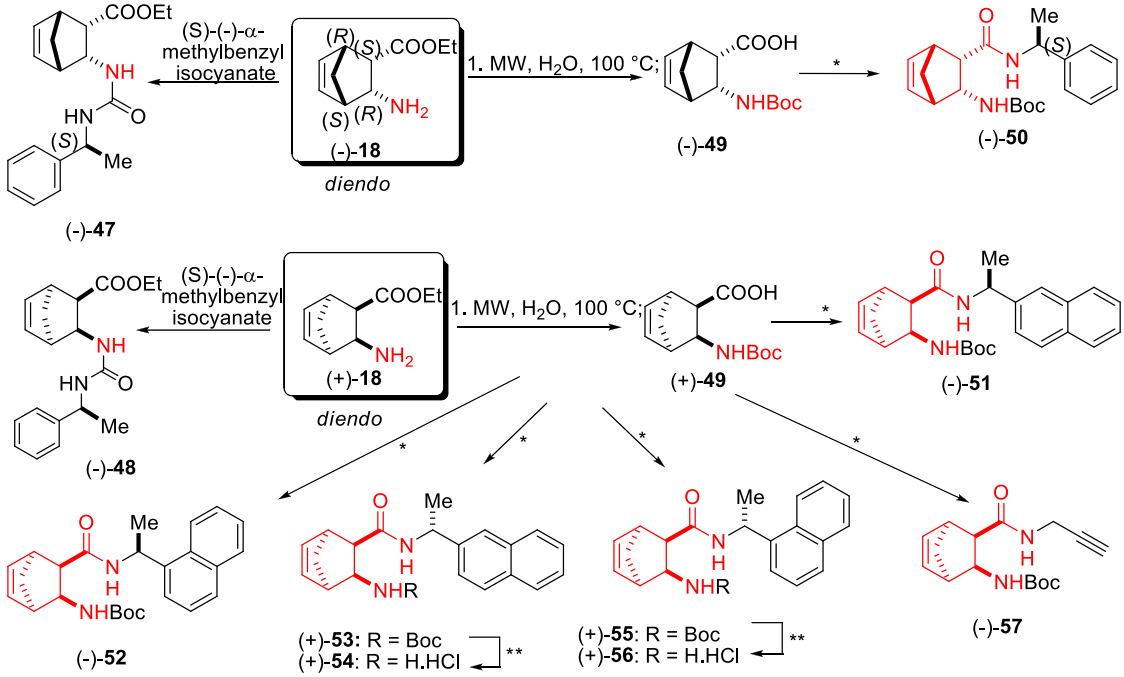
Scheme 4

Furthermore, cyclopentane, cyclohexane, cycloheptane and cyclooctane skeletons bearing β-amino esters **9**, **11**, **13** and **15** were successfully synthesised according to literature procedures. These compounds were starting materials in enzymatic hydrolysis with the use of CALB enzyme to obtain unreacted (1*R*,2*S*)-**9**, **-11**, **-13** and **-15** β-amino ester enantiomers and enantiomeric β-amino acids (1*S*,2*R*)-**43**–**46** as a part of greener approaches (Scheme 5). In addition, these enzymatic reactions were carried out under HSBM conditions to make them more environmentally benign. HSBM proved to be a beneficial method in the case of enzymatic hydrolysis. What is more, green solvents like propylene carbonate, 2-Me-THF, 2M2B and *t*BuOMe were tested in enzymatic hydrolysis. Of these, *t*BuOMe turned out to be the best working solvent in CALB-assisted enzymatic hydrolysis reactions. Preparative-scale reactions were also done under HSBM conditions. [II]

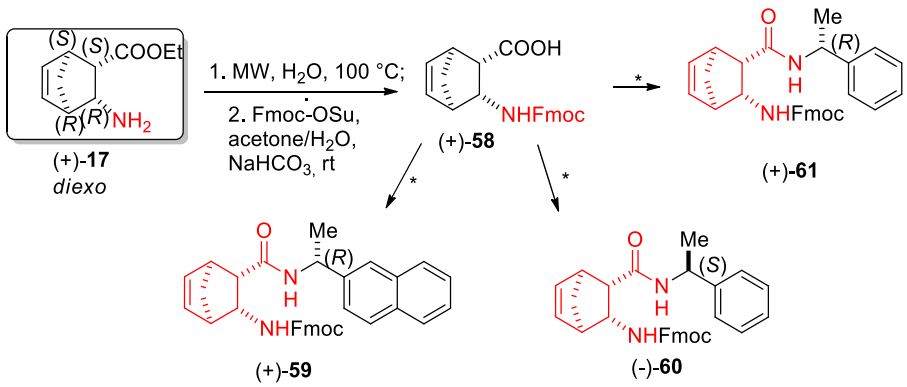


Scheme 5

Starting from enantiomeric ethyl 3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylates (+)-**17**, (–)-**18** and (+)-**18**, a large number of organocatalyst **47**–**61** were synthesised, characterised and tested in the aldol reaction between isatin (**62**) and acetone (**63**) as a model reaction. Our novel compounds can be divided into the following three different groups: β-carbamido esters (–)-**47**, (–)-**48**, β-amino amide hydrochloride salts (+)-**54**, (+)-**56** and *N*-protected β-amino amides (–)-**50**–(–)-**57** and (+)-**59**–(+)-**61** (Scheme 6, Scheme 7).



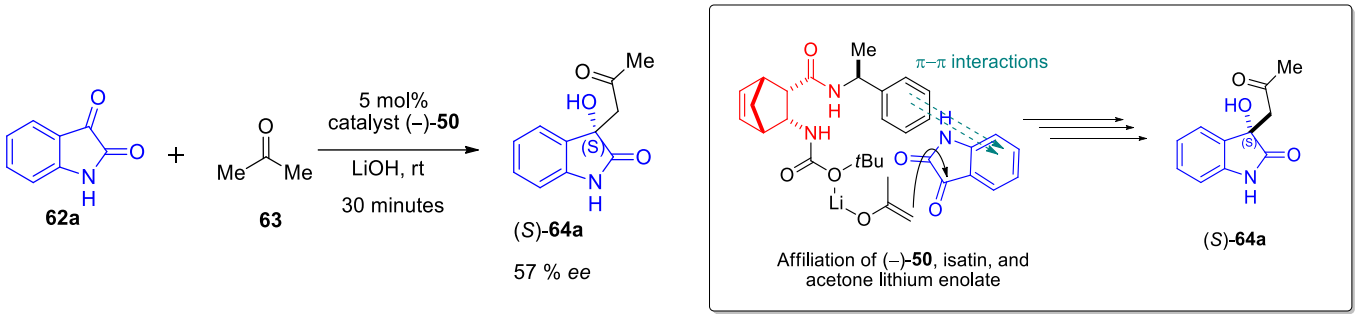
Scheme 6



Scheme 7

The applicability of these compounds as organocatalysts were examined in the asymmetric aldol reaction as a chosen model reaction. The model reaction was optimised by screening the additives and solvents used, catalyst loadings and temperatures to enhance the enantioselectivity of the reaction. The optimised conditions were the following: utilising a very small amount of LiOH as additive, acetone as solvent and reagent, 5 mol%

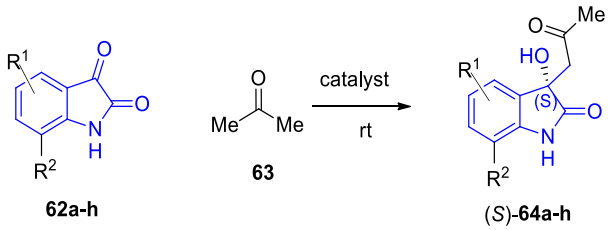
catalyst loading and room temperature. These are the safest and most green conditions. Note that we could remarkably shorten the reaction time to 30 minutes and reduced the used amount of catalyst down to 5 mol% in the case of product (*S*)-3-hydroxy-3-(2-oxopropyl)indolin-2-one (**64a**) (Scheme 8).



Scheme 8

To enhance the better understanding of possible reaction mechanisms in this field, we proposed to use our different groups of catalysts. In a nutshell, the reaction of LiOH with acetone generates a lithium enolate. In the presence of OH^-, the enolate of acetone is generated, which is rather reactive. Hence, OH^- is needed for accelerating the reaction. On the other hand, enantioselectivity specifically requires Li^+, because its unique coordinative ability is crucial for the orientation of the enolate (Scheme 8).

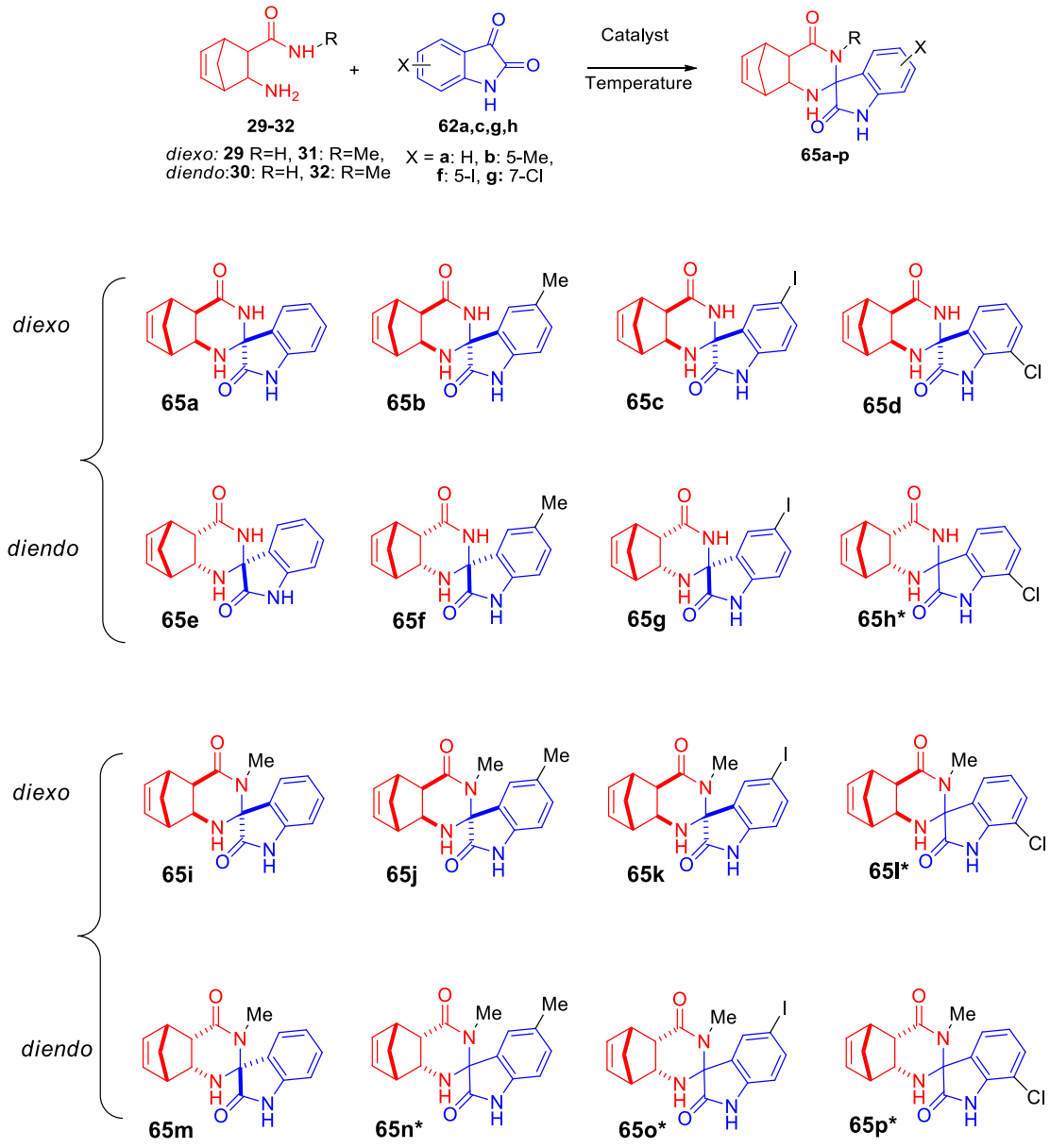
Substrate screening was also accomplished in the aldol reaction of isatin and acetone with numerous substituted isatins, using, for example 5-methylisatin (**62b**), 5-fluoroisatin (**62c**), 5-bromoisatin (**62d**), 5-nitroisatin (**62e**), 5-iodoisatin (**62f**), 7-chloroisatin (**62g**) and 4,7-dichloroisatin (**62h**) (Scheme 9). In harmony with literature data applying substituted isatins, in most cases better results could be achieved up to excellent 99% in *ee* compared to isatin **62a** itself. Moreover, we put an effort to make the model reaction more sustainable through performing the aldol reaction in HSBM. Neither too high nor too low frequencies were favourable in the model reaction and the use of 15 or 20 Hz proved to be a good solution. Moderate results were obtained. Note, however, that investigating the possible effect of the used number of balls, presented an astonishingly good result of 96% *ee* under ball milling condition. [III]



Scheme 9

Moreover, in order to develop useful derivatives with antitumor and antiallergic features, novel spiro-quinazolinone compounds were designed and synthesised in the following way: *diexo*- and *diendo*-aminobicyclocarboxamides **29**–**32** were transformed through condensation reactions with isatins

(Scheme 10). For instance, isatin (**62a**), 5-methylisatin (**62b**), 5-iodoisatin (**62f**) and 7-chloroisatin (**62g**) were applied to obtain novel spiro-quinazolinone derivatives **65a**–**p**. Catalysts, solvents and temperatures were screened in order to optimize the reaction. In accordance with our goals, investigations were done under CF, MW and HSBM conditions in order to make the condensation reaction of β-amino amides and isatins more environmentally benign. Utilising MW irradiation afforded the products with excellent results in yields up to 85%. Moreover, with HSBM usage it had become possible to reduce the amount of used solvent and the product was reached in good yields.



Scheme 10

In silico docking studies were done with spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives **65a**–**p** in order to facilitate the design of potential biologically useful molecules and promote drug developing. Our innovative compounds showed to be good ligands fitting to SARS-CoV-2 main protease (PDB: 6LU7) and human mast cell tryptase (PDB: 2ZA5). The most promising ligand was compound **65d**, affording

excellent results in both cases of macromolecules (Figure 1, Figure 2). In addition, an Absorption, Distribution, Metabolism and Toxicity (ADMET) prediction was also made to forecast, whether our novel compounds are capable of producing orally active drugs or they can penetrate the skin. The importance of the docking and ADMET studies regarding the results of the ADMET prediction, that spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives **65a**–**p** theoretically were shown to be feasible for drug development.

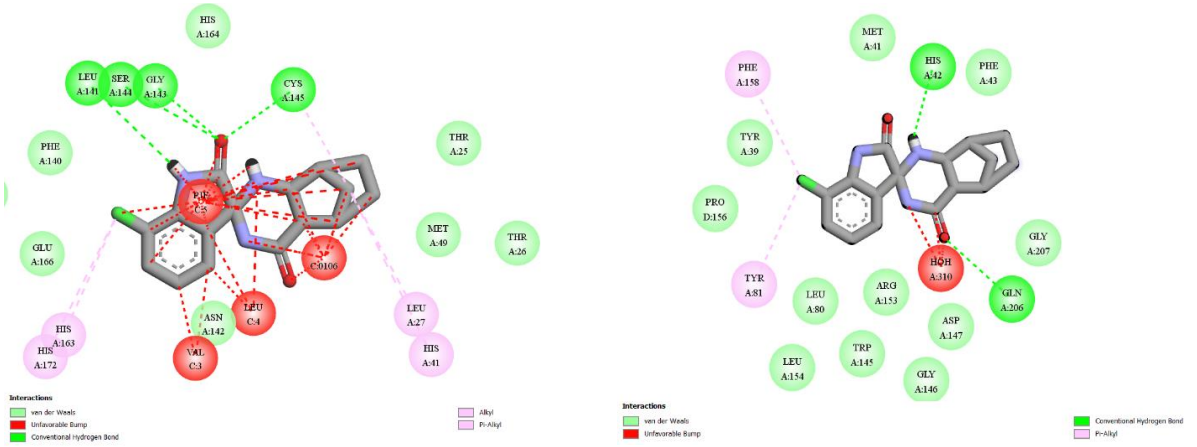


Figure 1: Left:2D interaction map between PDB: 6LU7 and **65d**. Right: 2D interaction map between PDB: 2ZA5 and **65d.**

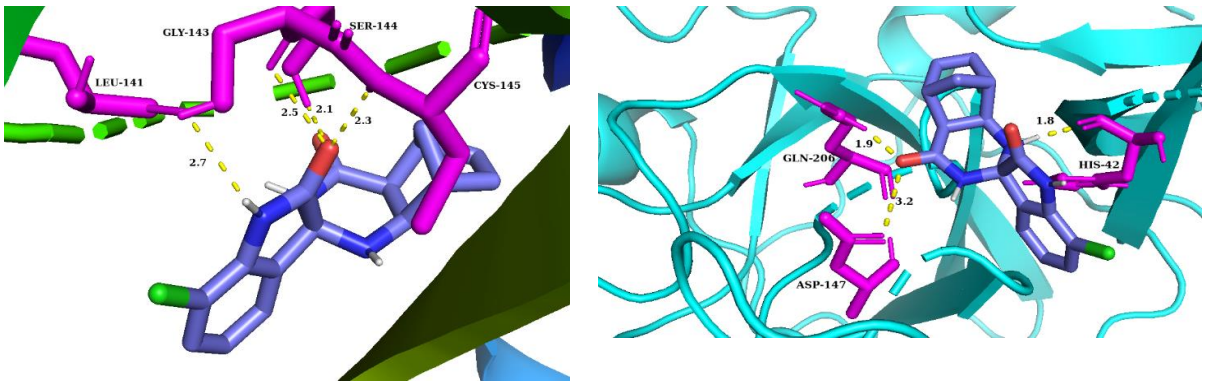


Figure 2 Left: Molecular interactions and binding pose of compound **65d** at the interface of human tryptase with SARS-CoV-2 main protease (PDB: 6LU7), H bonds between the macromolecule and compound **65d** shown as yellow dashes and distances in Å units; Right: Molecular interactions and binding pose of compound **65d** at the interface of human tryptase with potent non-peptide inhibitor (PDB: 2ZA5), H-bonds between the macromolecule and compound **65d** shown as yellow dashes and distances in Å units.

3. PUBLICATIONS

Papers related to the thesis

I. Mohamed El Haimer, **Tünde Faragó**, Zsuzsanna Schelz, István Zupkó, Márta Palkó
Synthesis of alicyclic 2-methylenethiazolo[2,3-*b*]quinazolinone derivatives via base-promoted cascade reaction
Synthesis, **2022**, *54*, 3809–3816. (DOI: 10.1055/s-0040-1720028) **IF: 2.6**

II. Sayeh Shahmohammadi, **Tünde Faragó**, Márta Palkó, Enikő Forró
Green strategies for the preparation of enantiomeric 5–8-membered carbocyclic β-amino acid derivatives through CALB-catalyzed hydrolysis
Molecules, **2022**, *27*, 2600. (DOI: 10.3390/molecules27082600) **IF: 4.6**

III. **Tünde Faragó**, Attila M. Remete, István Szatmári, Rita Ambrus, Márta Palkó
The synthesis of pharmacologically important oxindoles via the asymmetric aldol reaction of isatin and the investigation of the organocatalytic activity of new alicyclic β-amino acid derivatives
RSC Advances, **2023**, *13*, 19356–19365. (DOI: 10.1039/D3RA03528J) **IF: 3.9**

IV. **Tünde Faragó**, Rebeka Mészáros, Edit Wéber, Márta Palkó
Synthesis and docking studies of novel spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives
Molecules, **2024**, *29*, 5112. (DOI: 10.3390/ molecules29215112) **IF: 4.2**

Other papers which not related to the thesis

V. Attila Gácsi, Bence Kutus, Zita Csendes, **Tünde Faragó,** Gábor Peintler, István Pálinkó, Pál Sipos
Calcium L-tartrate complex formation in neutral and in hyperalkaline aqueous solutions
Dalton Transactions, **2016**, *45*, 17296–17303. (DOI: 10.1039/c6dt03463b) **IF: 4.6**

4. CONFERENCE LECTURES

I. **Faragó Tünde**, Palkó Márta:
Aliciklusos β-aminosav származékok organokatalitikus alkalmazhatóságának vizsgálata: farmakológiailag jelentős oxindolok szintézise izatin aszimmetrikus aldol reakciójával
Szegedi Ifjú Kémikusok Támogatásáért Alapítvány ZOOM konferencia keretében szervezett előadóülése
Szeged, May 25, 2021. (oral presentation).

II. **Tünde Faragó**, Márta Palkó, István Szatmári:
Organocatalytic activity of novel alicyclic β-amino amides in asymmetric aldol reaction
2022 #RSC Poster Twitter Conference
March 1, 2022. (online, poster presentation)

III. Shahmohammadi Sayeh, **Faragó Tünde**, Palkó Márta, Forró Enikő:
Green enzymatic strategies for the preparation of enantiomeric carbocyclic β-amino acid derivatives
4th International Green Catalysis Symposium, GreenCat
Rennes/France, April 19–22, 2022. P6 (poster presentation)

IV. **Faragó Tünde**, Palkó Márta, Szatmári István:
Változatosan szubsztituált enantiomertiszta aliciklusos β-aminosav-származékok szintézise és organokatalitikus alkalmazhatóságának vizsgálata izatin aszimmetrikus aldol reakciójával
MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése,
Balatonszemes, May 23–25, 2022. (oral presentation)

V. Márta Palkó, **Tünde Faragó**, István Szatmári:
Synthesis of glycosyl triazolyl methanopyrroloquinazoline and methanoisoindoloquinazoline derivatives via domino- and click reactions
22nd Tetrahedron Symposium
Lisbon/Portugal, June 28–July 1, 2022. Abstract reference number: 188, P2.27 (poster presentation)

VI. **Tünde Faragó**, Márta Palkó, István Szatmári:
Novel alicyclic β-amino amide organocatalysts for the synthesis of pharmaceutically relevant oxindoles
22ndTetrahedron Symposium
Lisbon/Portugal, June 28–July 1, 2022. Abstract reference number: 198, P2.36 (poster presentation)

VII. **Faragó Tünde**:
Aliciklusos β-aminosav származékok előállítása és organokatalitikus alkalmazhatóságának vizsgálata: farmakológiailag jelentős oxindolok szintézise izatin aszimmetrikus aldol reakciójával
MTA Heterociklusos és Elemorganikus Kémiai Munkabizottságának a Patonay Tamás-díj átadásával egybekötött nyílt ülése
Budapest, November 25, 2022. (oral presentation)

VIII. **Tünde Faragó**, Márta Palkó:
Synthesis of novel spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives starting from alicyclic β-amino amides
23ndTetrahedron Symposium
Gothenburg/Sweden, June 27–30, 2023. Abstract reference number: TETR2023_0366, P3.001 (poster presentation)

IX. **Tünde Faragó**, Márta Palkó:
Synthesis and docking studies of novel spiro[5,8-methanoquinazoline-2,3'-indoline]-2',4-dione derivatives
#RSC Poster Conference 2024 Linkedin
March 5, 2024. (online, poster presentation)