HETEROCYCLIC ARCHITECTURE FOR THE SYNTHESIS OF ANTI-HIV PROTEASE AND ANTI-CANCER COMPOUNDS

Abstract

Introduction

Introduction of heterocycle moieties in a bioactive molecule can have important effects on physicochemical and pharmacological properties, because of their chemical stability, their structural rigidity and due to the improving bioavailability and aqueous solubility[1]. Therefore the possibility of H-bond interaction with target backbone atoms can increase their efficacy. The success of this strategy in identifying new biologically active molecules in distinct therapeutic areas has gained a significant growth and different heterocycle are introduced in biological molecules[2]. In particular organic scaffold containing heterocycles have been described as "privileged structures" since they are capable of binding to many receptors with high affinity[3]. Of particular interest is the fact that most know chemicals are based on heteroarene frameworks[4,5]. There are a range of fused [5,6] ring systems that exhibit biological activity. In particular fused [5,6] ring systems, used in the synthetic paths discussed in this work, include indole, benzofuran and benzothiophene.

From several years, in the laboratory where I did my PhD work, research is focused on the introduction of heterocyclic structures into molecules with potential inhibitory activity against the HIV virus. The study concerned the synthesis of compounds designed to block the action of the HIV protease (HIV-Pr), an essential enzyme for the production of mature HIV particles[6]. The object of the study is to introduce heterocycle moieties to the central hydroxyethylaminic *core* that is the structure of a HIV-Pr inhibitors must have to be a good transition-state mimics. Our research is focused on synthesis of analogs of the Darunavir, the last one approved by Food and Drug Administration and the only one active against

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[2] J. Sangshettia, S.K. Pathan, R.Patil, S.A. Ansari, S. Chhajed, R. Arote, D.B. Shind, *Bioorganic & Medicinal Chemistry* **2019**, *27*, 3979.
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[4] C.A. Rouzer, D. Riendeau, J-P Falgueyret, C.K. Lau, M.J. Gresser, *Biochem Pharmacol, 41*, 1365, **1991**.
[5] T.R. Bosin, E.E. Campaigne, *Drug. Res., 11*, 191, **1977**.
[6] A.G. Tomasselli, R.L. Heinrikson, *Biochim. et Biophys. Acta*, *1477*, 189–214, **2000**.

mutated virus[7]. Gosh showed the potency-enhancing effect of fused bicyclic tetrahydrofuran (bis-THF) present in the Darunavir structure that can be involved in an interaction with the backbone atoms [8]. Thus, our design of ligands and templates is focused on introduction of cyclic/heterocyclic structures that could extensively interact by strong hydrogen bonding with the backbone atoms in the protease active site and that could improve bioavailability of PIs. Furtheremore, several studies reported that HIV-inhibitors were endowed with anticancer activity; after the success in treatment of HIV-infected patients affected by Kaposi sarcoma with HIV-1 protease inhibitors, the studies were successfully oriented to other solid tumors[9]. Infact, HIV protease inhibitors seem to be a new class of anticancer drugs with multiple effects[10]. In light on the above, the emergence of drug resistance prompts the scientific community to produce new molecules with lower side effects and higher efficacy, especially if a double biological activity is possible.

First scope of the thesis

Following this line of research and based on past results, this work is focused on the synthesis of novel PIs compounds, bearing hydroxyethylamine *core* with different heteroarenes and sulfonamides, with potentially broad-spectrum activity against drug-resistant variants and anticancer activity. General structure of synthetized compound is reported in figure 1, in which the heterocycle is spaced from the central *core* by different functionality, both carboxyamide function and carbamoyl; the type of heteroaryl group (X = O, NH, S), the sulfonamide moiety and the *core* were modified, with the presence of either H or benzyl as R' substituent.

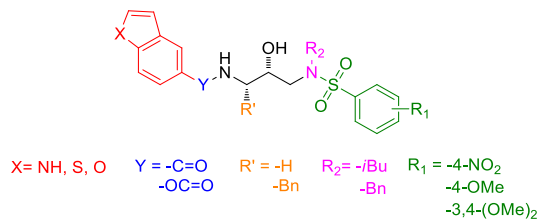
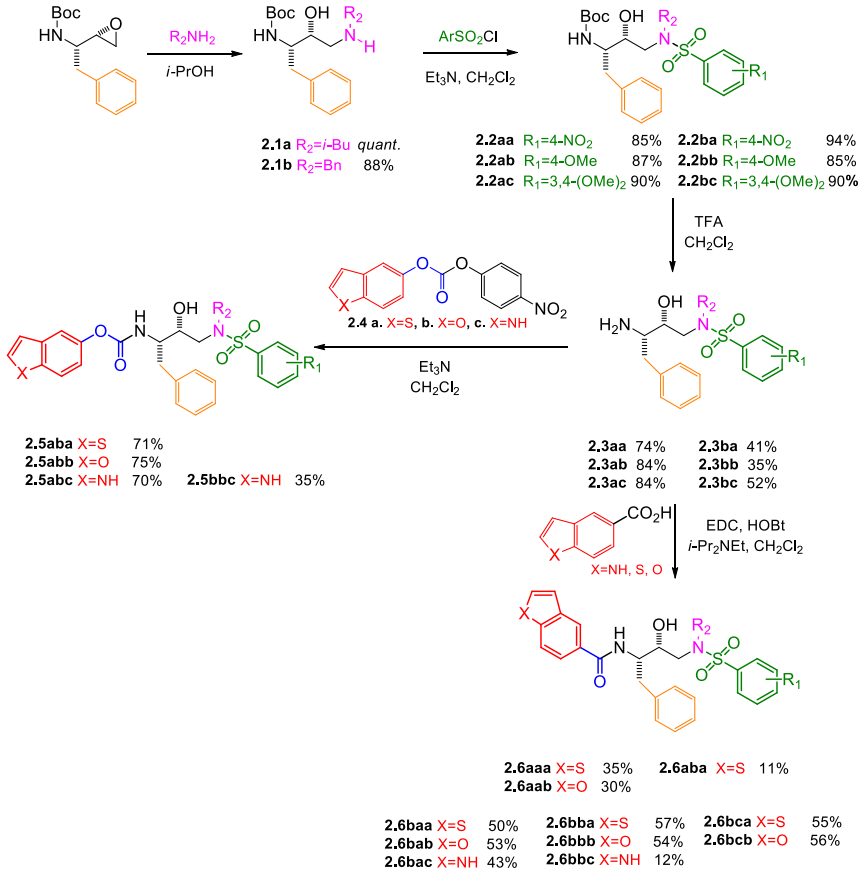


Figure 1

[7] A.K. Gosh, Z.L. Dawson, H. Mitsuya, *Bioorg. Med. Chem.* **2007**, *15*, 7576.
[8] A.K. Ghosh, B.D. Chapsal, I.T. Weber, H. Mitsuya, *Accounts of Chemical Research* **2008**, *41*, No 1, 78.
[9] D. Maksimovic-Ivanic, P. Fagone, J. McCubrey, K. Bendtzen, S. Mijatovic, F. Nicoletti, *Int. J. Cancer*, *140*, 1713, **2017**.
[10] a) MZ Dewan, M. Tomita, H. Katano, *Int J Cancer*, *124*, 622, **2009.** b) S. Gant, J. Carlsson, M. Ikoma, *Antimicrob Agents Chemother*, *55*, 2696, **2011**. c) E. Toschi, C. Sgadari, L. Malavasi L, Int J Cancer, 128, 82, **2011**. d) S. Gantt, A. Cattamanchi, E. Krantz, *J Clin Virol*, *60*, 127, **2014**.

When R' is benzyl group, synthetic path starts from N-Boc protected amino epoxide to obtain a library of compounds with carbamoyl or carboxyamide functionality that spaces heteroarene to the *core* (scheme 1)[11].



Scheme 1

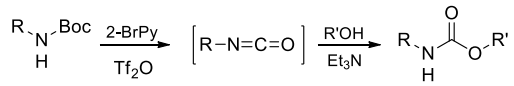
a. R_2=*i*-Bu; **b.** R_2=Bn

a. R_1=4-NO_2; **b.** R_1=4-OMe; **c.** R_1=3,4-$(OMe)_2$

a. X=S; **b.** X=O; **c.** X=NH

[11] a) M. Funicello, L. Chiummiento, F. Tramutola, M.F. Armentano, F. Bisaccia, R. Miglionico, L. Milella, F. Benedetti, F. Berti F., P. Lupattelli, *Bioorganic & Medicinal Chemistry* **2017**, *25*, 4715. b) F. Tramutola, M. F. Armentano, F. Berti, L. Chiummiento, P. Lupattelli, R. D'Orsi, R. Miglionico, L. Milella, F. Bisaccia, M. Funicello, *Bioorganic & Medicinal Chemistry* **2019**, *27*, Issue 9, 1863.

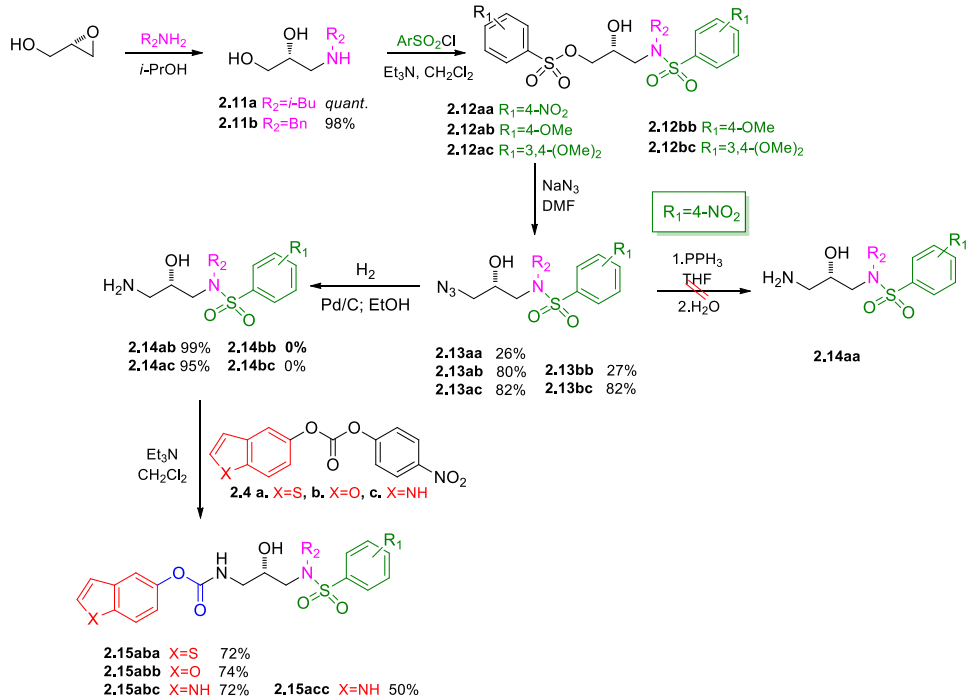
In this work new synthetic paths are studied in order to minimize the steps and to increase the overall yield. In particular one-pot synthesis of carbamates from Boc-protected amines, as reported in scheme 2, was tried, but with poor results.



Scheme 2

Furthermore, diversity-oriented synthesis has been studied, reversing the synthetic steps to change different functionalities according to needs.

With unsubstituted central *core*, synthesis starts from *S*-glycidol as reported in scheme 3.



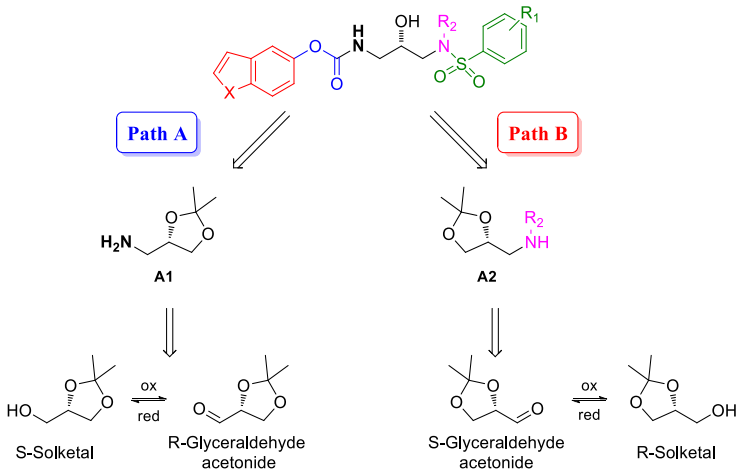
Scheme 3

. R_2=*i*-Bu; **b.** R_2=Bn

a. R_1=4-NO_2; **b.** R_1=4-OMe; **c.** R_1=3,4-$(OMe)_2$

a. X=S; **b.** X=O; **c.** X=NH

Furtheremore, due to the difficulties encountered in this case of using *S*-Glycidol as starting material, in order to prepare a library of compounds, new strategies with different starting material, taken from chiral pool, were investigated depending on which side of the molecule to work on; retrosynthetic paths is reported in scheme 4.



Scheme 4

Synthesized compounds were tested for *in vitro* activity against recombinant protease, using FRET methodology, and for their cellular vitality, using MTT assay.

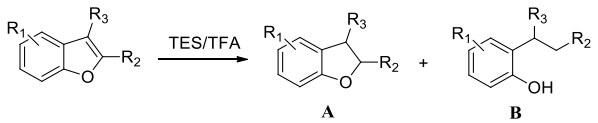
Results by FRET analysis showed that when R_2 is an *i*-Bu, compounds **2.5abb** and **2.5abc**, in which the heteroaryl group is spaced from the *core* by carbamoyl functionality are the most powerful inhibitors, their IC_{50} values being less then 0.6 nM. When R_2 is a benzyl group, instead, longer spaces as carbamoyl moiety between heteroarene and the *core* decreases the inhibition activity, so the carboxyamide functionality is required. In order to explain such results, a series of docking runs were made by Prof. Berti of the University of Trieste on the tested inhibitors, to evaluate the interaction with the enzyme.

Results by MTT assays on hepatocarcinoma cell lines (HepG2), compared to healthy hepatocytes (IHH), showed that compounds with heterocyclic moiety decrease cytotoxic activity as expected; this confirm the probably important activity of the free amine on the left side of the molecule.

Nevertheless the cytotoxic activity of the **2.15abc** derivative, that showed low antiviral activity, is of particular interest.

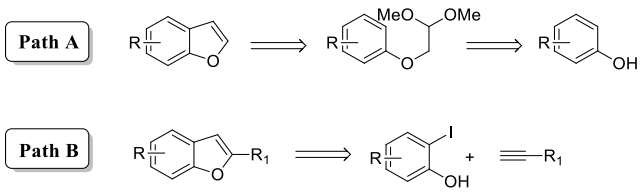
Second scope of the thesis

Another heterocycle that constitutes the core skeleton of a wide number of biologically active compounds is the 2,3-dihydrobenzofuran ring-system[12], deriving by formal hydrogenation of the furan ring fused with a benzene nucleus. There are several approaches to the synthesis of the 2,3-dihydrobenzofuran ring system[13], but general method still lacks. One of these should be the hydrogenation of benzofuran derivatives to the corresponding 2,3-dihydrobenzofurans, but it might be difficult to achieve with respect to other heteroaromatic nuclei[14]. Infact, the catalytic hydrogenation of benzofuran, under all conditions, is accompanied by partial cleavage of the furan ring. In the light of this, another issue is to study the reactivity of substituted benzofuran during reductive reactions, to understand the influence of substituents on the benzene ring or on the furan ring. Among the possible reduction methods, in this work reduction with hydrosilanes was applied (scheme 5).



Scheme 5

Substituted benzofurans ring was obtained by two different strategies because of the different substituents present on benzene ring: **Path A** where benzofuran ring was obtained by preparation and ciclization of aryloxyacetaldehyde acetals[15]; **Path B** where benzofuran ring was obtained by Sonogashira cross-coupling and contemporary cyclization (scheme 6):



Scheme 6

[12] a) M.H. Keylor, B.S. Matsuura, R.J. Stephenson, *Chem. Rev.* **2015**, *115*, 8976. b) K. Chen, M. Pitchakuntla, Y. Jia, *Nat. Prod. Rep*. **2019**, 36, 666.
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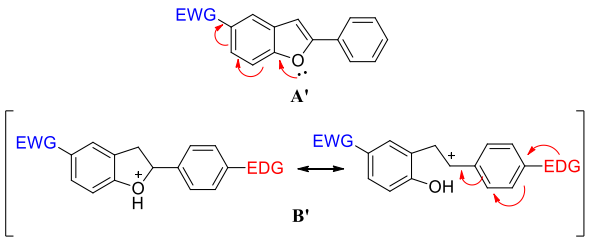
[15] P. Barker, P. Finke, K. Thompson, *Synth. Commun.* **1989**, *19*, 257.

Synthetised benzofurans were used in reduction reaction, according conditions described in scheme 4, to study how the substituents and their position can influence the reactivity in this reaction. A rationalization of reduction results was searched (notation referred to scheme 5):

- if R_2 is an alkylic group reduction occurs and 2,3-dihydrobenzofura, **A**, is obtained, regardless of substituents R_3 and R_1; instead if R_2 is an aryl group, and R_1 and R_3 are H, the product of over reduction, **B**, is obtained, because of the benzyl carbocation stabilization by mesomeric effect;
- for 2,(3),5-substituted benzofurans, the trend is fairly systematic and rationalizable according substituents present, as described in scheme 7:

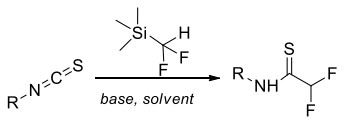


Scheme 7

- for the 2,(3),4,6-substituted benzofurans there is more difficulty acces to protonation and reduction is slower. Thus, identify a general trend becomes complicated and not easily related to the substituents present.

Third scope of the thesis

Last, incorporation of fluorine into bioactive molecules is of special interest to the pharmaceutical industries due to the ability of fluorine to increase metabolic stability and bioavailability. Moreover fluorine increases lipophilicity of hydroxyl or thiol groups and has the capability of H-bonding interactions[16]. Major classes of organofluorine compounds with biological activity include trifluoromethyl- and difluoromethyl groups. In particular, difluoromethyl functional group (CF_2H) has great properties. Therefore, the development of effective and general methodologies for the incorporation of this group via nucleophilic[17], electrophilic[18] and radical pathways[19] has become one of the hotspots in the field of organic chemistry. In this work, in collaboration with the Department of Pharmaceutical Chemistry of University of Wien, CHF_2- moiety was introducted by nucleophilic addition to an electrophilic carbon. Pace group, infact, showed as the nucleophilic addition of functionalized organometallic reagents to isocyanates and isothiocyanates constitutes a versatile, direct, one-pot and high yielding approach to introduce this moiety and represents an attractive tool for preparation of amide-type compounds[20]. Working in this direction, difluoromethyltrimethylsilane was used as organometallic reagent and different isothiocyanates as reactive electrophilic reagents to obtain unprecedented α,α-difluorothiamides, a pivotal class of organic molecules with attractive features (scheme 8).



Scheme 8

This is a new method to introduce this group in order to replace method that needs expensive and toxic elements or fluorinating agent. Optimal conditions for this type of reaction were searched and different isothiocyanates were used to afford a wide range of

[16] T. Zhu, Z. Zhang, J. Tao, K. Zhao, T. Loh, *Organic Letter* **2019**, *21*, 6155.
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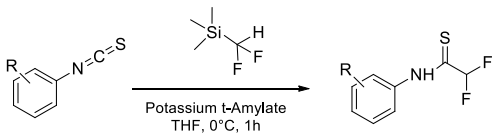
[18] a) C.S. Thomoson, W.R. Dolbier, *J. Org. Chem.* **2013**, *78*, 8904. b) K. Aikawa, K. Maruyama, K. Honda, K. Mikami, *Org. Lett.* **2015**, *17*, 4882.
[19] a) S. Zhang, L. Li, J. Zhang, M. Xue, K. Xu, *Chem. Sci.* **2019**, *10*, 3181. b) P. Xiong, H.-H. Xu, J. Song, H.-C. Xu, *J. Am. Chem. Soc.* **2018**, *140*, 2460. c) P. Dai, X. Yu, P. Teng, W.H. Zhang, C. Deng, *Org. Lett.* **2018**, *20*, 6901.
[20] V. Pace, S. Monticelli, K. de la Vega-Hernandez, L. Castoldi, *Org. Biomol. Chem* **2016**, *14*, 7848.

thioamides in high yields. So a library of scaffolds in medicinal synthesis were obtained (scheme 9)[21]. The nature of the isothiocyanate does not influence the effectiveness of the technique.



Scheme 9

The success of the procedure motivated to evaluate the reactivity of oxo-analogues isocyanates. Then, same study was extendend to Weireb amides. Reactivity of (thio)amides obtained was investigated to afford products with biological activity, but preliminary studies did not afford desired products.

[21] M. Miele, R. D'Orsi, S. Vellaisamy, W. Holzer, V. Pace, *Chem. Commun*. **2019**, *55*, 12960