Synthesis of new aminopyrazole analogues with promising anti-leishmaniosis activity: A University-DND*i* Open Synthesis Project

Carlotta Cecchini[1], Hesham M. Ismail[1], Nathalie Deschamps[1], Léonie Pellissier[1], Alice Mainetti[1], Adriano Rutz[1], Students from BUSP2[2], Elisabeth Rivara-Minten[1], Emmanuel Varesio[1], Benjamin Perry[3], Sébastien Tardy[1], Leonardo Scapozza[1]

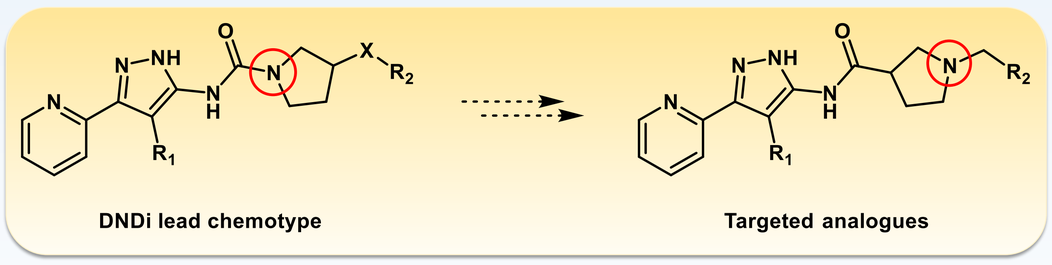
[1] School of Pharmaceutical Sciences, University of Geneva, Rue Michel-Servet 1, 1206 Geneva

[2] Students from the second year of the bachelor in Pharmaceutical Sciences performing pharmaceutical chemistry practical exercise, University of Geneva, Rue Michel-Servet 1, 1206 Geneva

[3] Drugs for Neglected Diseases initiative (DNDi), 15 Chemin Louis-Dunant, 1202 Geneva

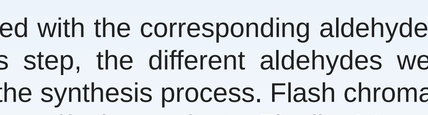
Introduction: Leishmaniasis is a neglected tropical disease, an illness that kills up to 30,000 people yearly. Existing drugs have serious drawbacks in terms of safety, resistance, stability, difficulty of administration and cost. Thus, there is a need for new treatments. The aminopyrazole class of compounds originally from Pfizer has shown promising early profiles for the treatment of both visceral and cutaneous leishmaniasis[1-3]. In the frame of an Open Synthesis Network (OSN), Drugs for Neglected Diseases initiative (DND*i*) medicinal chemists, in collaboration with researchers and bachelor students of the University of Geneva, designed a number of aminopyrazole analogues to test different hypothesis for early stage discovery towards new treatments for leishmaniasis.

Aim: Synthetising a series of aminopyrazole analogues with putative anti-leishmaniasis activity during the pharmaceutical chemistry practical course to be tested on a series of relevant anti-leishmaiasis assays.

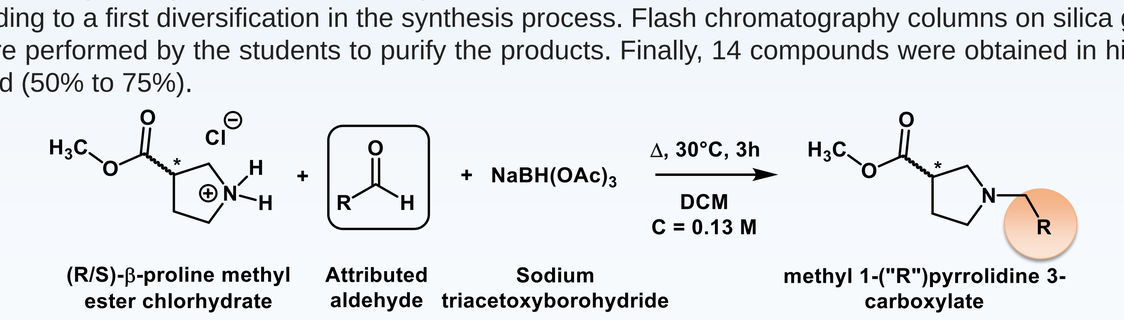


Hit optimization process

Methods: In order to explore the aminopyrazole chemotypes, we set up a 5-steps synthesis starting from (R/S)-β-proline, 14 different aldehydes and 3 different aromatic cores to obtain 42 different products. The two key reactions are reductive amination, during which a first structural diversification occurs and the last coupling by amidation that leads to the final expected compounds.



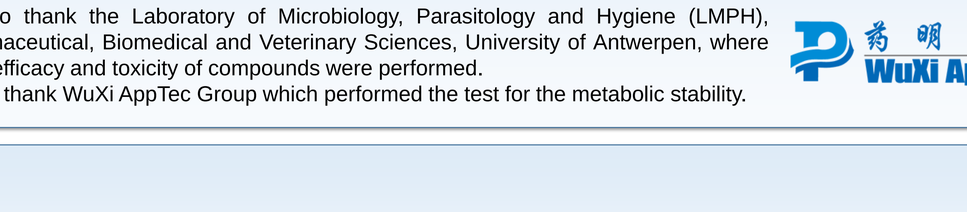
X = C-H, C-Cyclopropyl, or C-Cl



General scheme of expected compounds

Step 1: (R/S)-β-proline protection

(R/S)-β-proline protection through esterification. This first step of synthesis worked in quantitative yield, leading to the corresponding product as an hydrochloride salt.

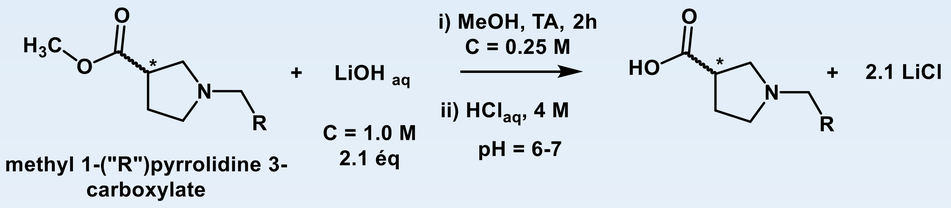


Step 2: reductive amination

The ester chlorhydrate was coupled with the corresponding aldehyde by reductive amination, giving the corresponding amine. In this step, the different aldehydes were attributed to the students, leading to a first diversification in the synthesis process. Flash chromatography columns on silica gel were performed by the students to purify the products. Finally, 14 compounds were obtained in high yield (50% to 75%).

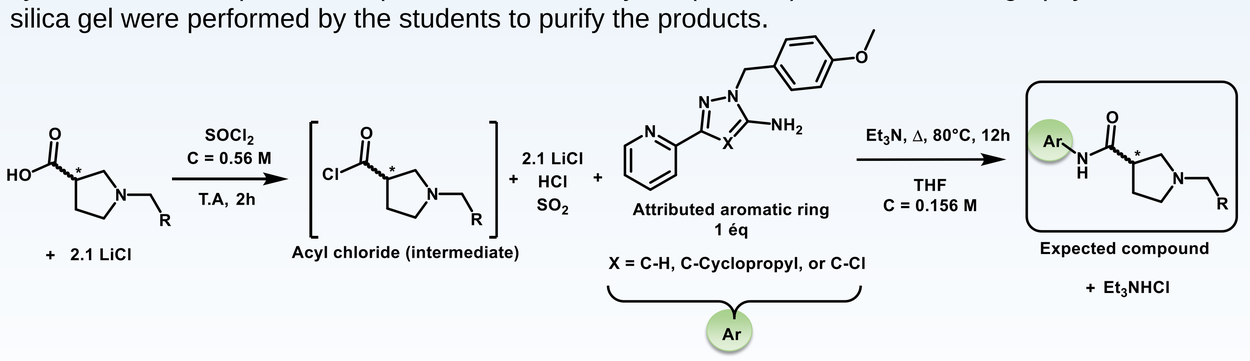
Step 3: Saponification

The different esters were cleaved back into carboxylic acids under basic conditions ($LiOH_{aq}$).
The reaction led to the expected compounds and the yields were quantitative. In the work-up, HCl_{aq} was used to neutralize the reaction mixture leading to the formation of a significant amount of LiCl salt. Being the expected compounds highly soluble in water, it was difficult to extract them with an organic phase, so they were evaporated in vacuum sequentially (H_2O/MeOH medium).



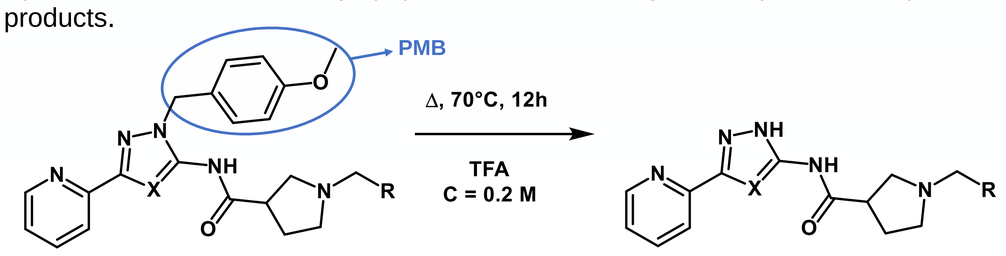
Step 4: amidation coupling via acyl chloride intermediate

The amidation of carboxylic acids *via* acyl chlorides involves first the conversion of the acid into the acyl chloride, followed by the coupling with the amine to give the corresponding amide. Students performed this reaction using tetrahydrofuran (THF) and triethylamine (Et_3N), leading to successful synthesis of the expected compounds in sufficient yield (32-36%). Flash chromatography columns on silica gel were performed by the students to purify the products.

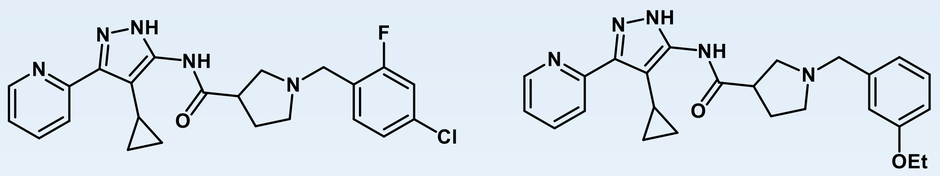


Step 5: p-methoxybenzyl (PMB) deprotection

p-Methoxybenzyl (PMB) deprotection worked quantitatively using triflouroacetic acid media under reflux (70%) for 12h. Flash chromatography columns on silica gel were performed by the students to purify the products.



Results and discussion: This multi-steps synthesis led to 14 new compounds that have been fully characterized by HRMS, ^{1}H-NMR, ^{13}C-NMR and HPLC. Despite difficulties occurring in the amidation coupling, a new valid protocol was found to obtain the expected compounds in a sufficient yield (32-36%) and purities > 95%. All final compounds have been tested *in vitro* for both efficacy and toxicity. Two of them showed high potency against *Leishmania infantum* (IC_{50} 0.3 and 1.5 µM) and a selectivity index (CC_{50}/IC_{50}) ranging from 27 to 213.



GA17 deprotected

IC_{50} = 1.48 µM

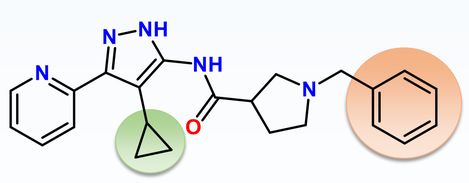
GB21b deprotected

IC_{50} = 0.32 µM

In vitro ADME studies for this 2 compounds were then performed by WuXi AppTec Co. Ltd. (Shangai) to set the metabolic stability in Human Liver Microsome. As shown in the following table, both compounds are very rapidly metabolized, with the hepatic extraction ratio (EH ratio) nearing 0.9. Even if the values on metabolic stability are not yet satisfactory, we still don't know if the metabolites are compounds resulting from hydrolysis of the bond and thus degraded or there is an oxidation and the resulting metabolites are actives. Further analysis on the metabolic profile will answer these questions and will help the design of compounds with improved stability.

Sample Name	Human liver microsome							
	R^2	$T_{1/2}$ (min)	$CL_{int(mic)}$ (µL/min/mg)	$CL_{int(liver)}$ (mL/min/kg)	CL_H (mL/min/kg)	EH	Remaining (T=60min)	Remaining (NCF=60min)
GA17	0,96	7,80	222,25	200,03	18,18	0,91	0,75%	91,06%
GB21b	0,99	8,98	193,00	173,70	17,93	0,90	1,22%	93,82%

Based on the results of the 14 compounds series, some structure-activity relationships (SAR) can be hypothetized for the synthesis of new analogues.



The phenyl-moiety is essential for improving the activity. Its substitution with electron withdrawing groups is significantly improving the activity on *Leishmania infantum*

Cyclopropyl substitution is significantly improving the activity on *Leishmania infantum*

Conclusions and perspectives: The R&D character of the project and its open source nature allowed to deepen the learning of the student during laboratory work and their scientific rigor of work which includes the preparation and the follow-up plans of experiment. The collaborative spirit of the students has been instrumental for the success of the synthesis project. The promising anti-leishmaniosis activity clearly indicates an SAR and the possibility of further exploring the current chemotype to improve compounds efficacy, selectivity and most of all stability.

Acknowledgments:

The authors of this poster would like to thank DND*i*, for the chance of getting this project and for providing the aromatic amine compounds.
The authors also thank the Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerpen, where the tests for the efficacy and toxicity of compounds were performed.
The authors also thank WuXi AppTec Group which performed the test for the metabolic stability.

References:

[1] Oehler RS et al. MRes Drug Discovery & Development, 2017
[2] Fernández-Llaneza D et al. MRes Drug Discovery & Development, 2017
[3] Mowbray CE et al. J Med Chem 2015; 58 (24): 9615-9624

Poster presented at «Young Researcher Fellow Meeting 2019»