UNIVERSITY OF LJUBLJANA
Faculty of Chemistry and Chemical Technology

Dr. Slovenko Polanc
Professor of Organic Chemistry

Aškerčeva 5
SI-1000 Ljubljana, Slovenia
Tel: + (386)-1-24-19-236
Fax: + (386)-1-24-19-271
E. mail: slovenko.polanc@fkkt.uni-lj.si

November 11, 2009

Report on the PhD Thesis of Mgr. Juana Monreal Férriz:
'Development of New Potential Antimycobacterial Active Agent Based on the Group of Salicylanilides'

The doctoral thesis of Mgr. Juana Monreal Férriz addresses the development of new potential antimycobacterial active agents originating from salicylanilides that have shown promising antimycobacterial activity against *Mycobacterium tuberculosis* as well as against some atypical strains. The fact that about 2 billion people are presently infected with *Mycobacterium tuberculosis* of which more than a million die every year supports the importance of the topic covered by this work.

The thesis that includes 66 pages is well-written and involves Introduction, Aim of the Thesis, Prodrug Design of Phenolic Drugs, Salicylanilide Modification, Rearrangement of Salisylanilide Derivatives, Conclusions, and References. It is accompanied by an extensive Supplements (79 pages) containing 4 publications, 1 manuscript (currently under revision), and the corresponding Supporting Information (general procedures, characterization data, some of the crucial ^{1}H NMR and ^{13}C NMR spectra, crystallographic data etc.).

The Introduction brings the most basic information concerning tuberculosis as the global problem in terms of its incidence, resistance and co-occurrence with human immunodeficiency virus (HIV). A historical review on the treatment of tuberculosis with the application of the first-line as well as the second-line drugs is also given. Then the overall aim of the thesis is clearly outlined to stress the essential parts of the work, namely the investigation of the most suitable prodrug forms based on salicylanilides.

The chapter 3 describes a prodrug design of the typical phenolic drugs. It shows separately various types of prodrugs: ester prodrugs, their phosphate analogs, carbamates, suphamates and ether prodrugs of phenol. The author has mentioned the same drug (paracetamol, page 12; acetaminophen, page 30) twice and did not use the same number for it what is rather unusual. It is also a pity that the structure of the compound **7** (page 9) and the name of the CA-4 (pages 14 and 15) are incorrect. In spite of the incorrectness mentioned above the chapter is well informative.

The chapter 4 deals with salicylanilide modification. The strategy was oriented towards the synthesis of prodrug forms of the most anti-TB active salicylanilides to

improve their physico-chemical and pharmacokinetic properties that would lead to a new potential antimycobacterial active agents. The results are already published in 3 papers describing the synthesis of the designed esters, their antimycobacterial activity and lipophilicity, the preparation of alkyl carbamates accompanied by the same data as well as their activity against MDR-TB strains. This chapter is the most essential part of the thesis.

Unexpected rearrangement of salicylanilide derivatives is outniled in chapter 5. It takes place after the removal of the Cbz group from the appropriate protected salicylanilides employing HBr in acetic acid following by the treatment with triethylamine. It is interesting to note that mechanism probably involved imidazolidinone intermediate. Namely, it was possible to isolate and characterized such intermediates. The structure of one of them was additionally supported by X-ray analysis.

A conclusion briefly summarizes the main achievements of the thesis. It compares the results with Cbz protected amino acid esters of salicylanilides and their carbamate counterparts. The later seems to be more promising due to the increased antimycobacterial activity and pharmacokinetic properties. Whether this is a simple effect of an increased hydrophobicity and therefore better permeability through the lipophilic mycobacterial cell wall or a direct effect of the carbamate moiety remains to be elucidated.

Finally, the PhD thesis of Mgr. Juana Monreal Férriz is a relevant work which offers a number of new facts about a permanent search for new drugs against tuberculosis and is without any doubt of great interest to the scientific community. The majority of the results have already been published in the prestigious international journals and some of them are currently under revision. In conclusion, let me recommend this thesis for further procedure including a public defense.

My questions:

1. In the introduction (pages 14 and 15) the combretastatin CA-4 is mentioned as an efficient tubulin-binding agent. Is the arrangement around C=C bond in the molecule important for a biological activity of the CA-4 and its derivatives?
2. Alkyl carbamates **7** (page 35, Scheme 13) are formed by treating salicylanilides **1** with the appropriate isocyanates **6**. Which method would you suggest for the preparation of isocyanates when they are not commercially available?
3. ^{13}C NMR of the compound **9a** (page 47) shows two signals (19.4 and 18.6 ppm) for two methyl groups that are attached to the same carbon. Could you explain this fact?

Slovenko Polanc

Opponent: Dr. Slovenko Polanc, Professor of Organic Chemistry