ÚOCHB AV ČR
ÚSTAV ORGANICKÉ CHEMIE A BIOCHEMIE
AKADEMIE VĚD ČESKÉ REPUBLIKY
INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

Review of the Dissertation Thesis of Luís André Vale Silva:
Antifungal Drug Discovery: Focus on Incrustoporin Derivatives

The dissertation thesis of Luís André Vale Silva addresses an increasingly important topic of antifungal therapy, development and *in vitro* testing of novel compounds possessing an antifungal activity. A significant part of the work is devoted to the efforts to understand the mechanism of action of the most promising compounds.
The whole thesis (114 pages) is written in a concise style, and structured in a standard way (Introduction, Theoretical Part, Materials and Methods, Results, Discussion, Conclusions, Literature References).
The Theoretical Part is comprehensive and well arranged. It describes the history and the current state of the antifungal drug development in a very informative way. It could serve as a tutorial for newcomers to the field, as well as an expert-level synopsis of structures and mechanisms of currently available antifungal drugs and susceptibility testing methods.
The methodological part is well written, all experimental procedures are clearly and explicitly described.
Most of the results presented in this thesis are related to the testing of potential antifungals synthesized in the laboratory of Assoc.Prof. M. Pour. Minimum inhibitory concentration was determined for all of the compounds tested, and in the case of incrustoporine derivatives the study was extended to the flow cytometry analysis, in order to find a clue to the investigation of mechanism of antifungal action. It is obvious that enormous amount of work has been done. More than 130 compounds were tested in a routine type of antifungal activity screening using 13 fungal strains. Another 190 fungal strains were used in some parts of the work where more extensive approach was needed. Several sets of valuable data were obtained this way and promising structures of potential antifungals were identified. Some of the compounds were active against *Candida glabrata* and *Candida krusei*. This result is of a particular interest, because these pathogens are often resistant to the drugs currently available on the market.
It is therefore a pity that some of the figures are not arranged clearly enough (Fig. 17, page 60; Fig. 22, page 65). Moreover, the data are summarized in tables and figures, but the explanations concerning the rationale and interpretation of the results are rather scanty. The interpretation of the results has been shifted to the Discussion, making the text less comprehensive and less handy to read.
The Results section is organized according to the chemical groups of the tested drugs. However, the chapter 4.1 describing the incrustoporine derivatives might have been further substructured according to the type of analysis. This would have helped the reader to better understand logical relationships among the individual experiments.
Incrustoporin is a relatively novel leading structure and its analogs have promising antibiotic and antifungal properties. Thus, the attention paid to these compounds is fully justified. Luís André Vale Silva used flow cytometry methods and different fluorochromes to investigate

mode of action of these compounds, showing that the antifungal effect is due to the impairment of the plasma membrane integrity. Although the results are well-documented and not over-interpreted, it would be highly advisable to add more experiments and a wider range of methods to elucidate this phenomenon.
In conclusion: the work presented by Luís André Vale Silva is highly relevant and reveals new facts about novel classes of antifungals. Moreover, the results have been published in several peer-reviewed journals. Therefore, I recommend his dissertation thesis to be accepted for defence and further procedures.

I have following questions:

1. Is the mechanism of action of the incrustoporine derivatives subject to a further investigation?
2. Did Luís André Vale Silva participate in the design of antifungal compounds, or just in the antifungal susceptibility testing?

Opponent: RNDr. Olga Hrušková-Heidingsfeldová, CSc.

Prague, 30th May 2006.

.