Fragment Based Drug Discovery of New *Leishmania donovani* Nucleoside Hydrolase Inhibitors

Marina A. Alves[1,2,3] (PG)*, Mayara M. Moreira[1] (IC), Charlotte Nirma[1] (PQ), Renata B. Lacerda[2] (PG), Carlos Maurício R. Sant´Anna (PG)[2,4], Lídia M.Lima[2,3](PQ) Luzineide W. Tinoco[1](PQ)

1-Laboratório de Análise e Desenvolvimento de Inibidores Enzimáticos- Instituto de Pesquisa de Produtos Naturais (IPPN/UFRJ);

2-Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio)

3- Programa de Pós-Graduação em Química- Universidade Federal do Rio de Janeiro (UFRJ)

4- Departamento de Química, ICE, UFRRJ;

marinamaral@ufrj.br

Keyword: Leishmaniosis, STD NMR, enzymatic kinetic, nucleoside hydrolase, Leishmania donovani..

Abstract

Using a library of 130 molecular fragments along with the screening by STD NMR was possible to identify new *Leishmania donovani* nucleoside hydrolase inhibitors with activity in µM range.

Introduction

Nucleoside hydrolase (NH) is a strategic target for drug development for the treatment of *leishmaniasis*. NH is part of purines and pyrimidines uptake pathway, which is essential for DNA synthesis of these parasites DNA synthesis of parasites. *Leishmania donovani* nucleoside hydrolase (*Ld*NH) sequence has a high homology with others trypanosomatid and is not found in mammals.[1]

We used STD NMR[2] and molecular fragment based drug discovery techniques to identify new substances for *Ld*NH inhibition. STD can be used as an initial approach to the study of the bioactive products.[3] Unlike the biological tests, STD does require no prior knowledge of protein function or any specific set up the target to its use in pharmaceutical research. We demonstrate in this work that the use of STD along with molecular fragments is an effective and powerful tool for the discovery new ligands to an original target as *Ld*NH

Results and Discussion

Molecular fragment library used here was generated according to the rule of three[4] and structural similarity of fragments with NH substrates. All NMR spectra were acquired on a VNMRSYS-500 (Agilent). ^{1}H NMR spectra were acquired for 130 molecular fragments (120 mM) in DMSO_*d6* stock solution. After performed the assignment for all fragments, they were grouped according to their chemical shifts. Mixtures of 3 to 5 fragments (1 mM) were prepared in 20 mM phosphate buffer at pH 7.4, with 10% of D_2O. STD[5] was used for screening these mixtures of molecular fragments to identify the presence of *Ld*NH ligands. STD spectra of the mixtures of fragments were obtained before protein addition as control. *Ld*NH was cloned, expressed and purified by affinity in Ni-NTA column. For *Ld*NH ligands identification, STD spectra were acquired in 10:1 (fragments: protein) molar ratio.

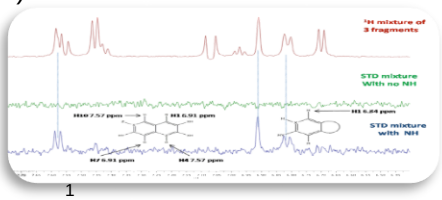

Figure 1: ^{1}H NMR spectra of the mixture of three molecular fragments, STD spectra without protein (control) and STD with *Ld*NH.

Thirteen of 130 molecular fragments tested interact with *Ld*NH. Some of them inhibit the *Ld*NH activity in µM range. The use of molecular modeling strategies would contribute to the development of new compounds from the linking fragments which exhibit good inhibitory capacity. Once synthesized, these novel compounds can be potent and promising inhibitors for planning antileishmanial compounds.

Conclusion

*Ld*NH was successful expressed in high purity and concentration, allowing the screening of 130 compounds library by STD NMR. Among these, thirteen compounds act as ligand of the protein and two molecular fragments are good inhibitor. From these results, it is now possible to use molecular modeling to combine the most active fragments thereof to generate new compounds. In addition, mapping of epitopes groups and analysis by molecular modeling will be used for planning more efficient inhibitors.

Aknowledments

FAPERJ, INCT-INOFAR, CNPQ, UFRJ.

[1] Rennó, M. N. *et al. Eur. J. Med. Chem.* **56,** 301–7 (2012). [2] Meyer, B. & Peters, T. *Angew. Chemie Int. Ed.* **42,** 864–890 (2003). [3] Politi, M., *et al. European J. Org. Chem.* **2005,** 1392–1396 (2005). [4] Congreve, M. *et al. Drug Discov.Today,* **8**, 876 (2003). [5] Meyer & Meyer. *J. Am. Chem. Soc.* **123**, 6108 (2001).