Docking Study of *L. amazonensis* Trypanothione Reductase Inhibition by *N,N'*-Diphenyl-benzamidines: TST, NADPH and FAD Binding Sites

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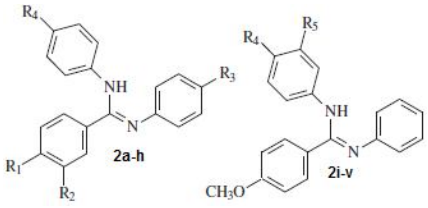
Keywords: *N,N'-Diphenyl-benzamidines,* Leishmania amazonensis, *Docking Method, Trypanothione Reductase.*

Introduction

Aromatic amidines have been shown to present good activity against some infectious diseases, but only one of these compounds, pentamidine (**1**), has achieved clinical use. New amidines synthesized by Echevarria *et al*. (Scheme 1) were active against *Leishmania amazonensis*[1], a parasite associated to leishmaniasis, one of the most serious diseases caused by protozoans worldwide.

Scheme 1. *N,N'*-Diphenyl-benzamidines (NDBs)[1].

	R_1	R_2	R_3	R_4		R_4	R_5
2a	OCH_3	H	H	H	**2i**	Cl	H
2b	H	OCH_3	H	H	**2j**	H	Cl
2c	H	H	OCH_3	H	**2k**	F	H
2d	H	OCH_3	OCH_3	H	**2l**	H	F
2e	OCH_3	H	OCH_3	H	**2m**	Br	H
2f	H	H	OCH_3	OCH_3	**2n**	H	OCH_3
2g	OCH_3	H	OCH_3	OCH_3	**2o**	NO_2	H
2h	OCH_2O	OCH_2O	OCH_3	H	**2p**	H	NO_2
					2q	OH	H
					2r	H	OH
					2s	CH_3	H
					2t	H	CH_3
					2u	SO_2NH_2	H
					2v	Cl	NO_2



The mechanism of action associated to the activity remains unknown. To help elucidate it, a possible target, trypanothione reductase (TR), was studied in this work. The knowledge of which binding site – trypanothione (TST), FAD or NADPH – these NDBs are most probably bonded at, would help the development of more active compounds.

Results and Discussion

A homology model was created for *L. amazonensis* TR with the Swiss-Model server, using as a template the *L. infantum* TR crystallographic structure (PDB code 2KJ6[2]). The NDBs were constructed and energy-minimized with PM3 method, through Spartan'08 software (Wavefunction Inc.). The docking study was carried out with GOLD 5.2 (CCDC Software Ltd.) into the TST, FAD and NADPH binding sites. All the four scoring functions available were tested through the redocking method and the ChemPLP function presented the best result. In table 1, values refer to poses with the best fitness scores obtained in each binding site with this scoring function. The NDBs with the lower IC_{50} values and with the higher scores are highlighted in table 1 and they are the same only in the case of the FAD binding site.

Table 1. IC_{50} (μM) against *L. amazonensis* promastigotes[1] and docking scores (ChemPLP) of NDBs into TRP, NADPH and FAD binding sites.

	IC_{50}	TRP	NAD	FAD		IC_{50}	TRP	NAD	FAD
2a	1.85	65.4	57.6	73.1	**2m**	***1.34***	65.8	57.1	***80.7***
2b	2.56	66.4	62.8	80.6	**2n**	1.68	66.5	63.2	79.9
2c	2.80	***68.3***	62.3	70.4	**2o**	***1.22***	65.6	62.6	***80.9***
2d	2.57	63.7	63.6	77.7	**2p**	2.59	66.6	***66.0***	75.5
2e	2.80	60.4	***64.5***	66.5	**2q**	2.51	63.6	59.9	69.5
2f	2.24	***71.3***	56.2	72.2	**2r**	2.65	64.8	61.2	76.6
2g	>3.0	63.8	61.3	73.3	**2s**	2.42	65.2	62.9	67.9
2h	1.60	63.9	63.8	67.4	**2t**	2.42	66.5	***64.7***	80.1
2i	1.90	66.9	59.6	79.3	**2u**	1.79	***69.4***	60.9	76.8
2j	***1.11***	65.5	59.8	***82.8***	**2v**	***1.10***	***67.0***	***63.9***	***84.4***
2k	1.43	66.4	59.2	80.6	**1**	1.05	70.0	72.7	78.7
2l	1.62	65.8	62.4	79.9					

Conclusions

The ChemPLP score values suggest that NDBs are better ligands to the TR FAD binding site, suggesting allosteric inhibition. This is possibly a consequence of aromatic nature of the residues in the FAD binding site, which results in more favorable interaction energies with NDBs.

The activity could be improved by removal of the protonation site, improving factors such as absorption. The NDBs interactions are suggestive that hydrophobicity could play an important role for the improvement of the antileishmanial activity.

Acknowledgements

CAPES, CNPq, INCT-INOFAR, FAPERJ

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