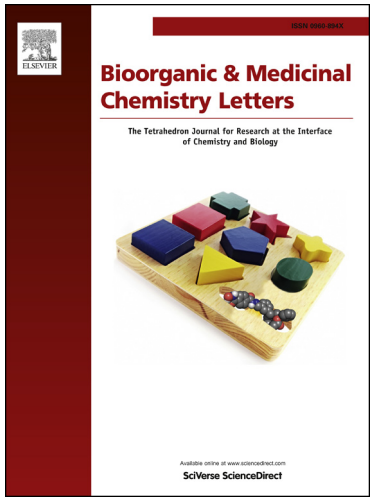
Accepted Manuscript



Synthesis and activity of novel tetrazole compounds and their pyrazole-4-carbonitrile precursors against Leishmania spp

Jéssica V. Faria, Maurício S. dos Santos, Alice M.R. Bernardino, Klaus M. Becker, Gérzia M.C. Machado, Raquel F. Rodrigues, Marilene M. Canto-Cavalheiro, Leonor L. Leon

PII: S0960-894X(13)01136-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.09.062
Reference: BMCL 20914

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 23 August 2013
Revised Date: 20 September 2013
Accepted Date: 23 September 2013

Please cite this article as: Faria, J.V., dos Santos, M.S., Bernardino, A.M.R., Becker, K.M., Machado, G.M.C., Rodrigues, R.F., Canto-Cavalheiro, M.M., Leon, L.L., Synthesis and activity of novel tetrazole compounds and their pyrazole-4-carbonitrile precursors against Leishmania spp, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.09.062

Synthesis and activity of novel tetrazole compounds and their pyrazole-4-carbonitrile precursors against *Leishmania spp.*

Jéssica V. Faria[1], Maurício S. dos Santos[2], Alice M.R.Bernardino[1], Klaus M. Becker[3], Gérzia M. C. Machado[3], Raquel F. Rodrigues[3], Marilene M. Canto-Cavalheiro[3], Leonor L. Leon[3].

[1]Departamento de Química Orgânica, Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, 24020-150, Niterói, RJ, Brazil

[2]Instituto de Física e Química, Centro de Estudos e Inovação em Materiais Biofuncionais Avançados, Universidade Federal de Itajubá, 37500-903, Itajubá, MG, Brazil

[3]Laboratório de Bioquímica de Tripanosomatídeos, IOC, Fundação Oswaldo Cruz, 21040-900, Rio de Janeiro, RJ, Brazil

**Corresponding author. Tel.: +55 35 3629-1628; Fax: +55 35 3629-1140*

E-mail address: mauriciounifei@yahoo.com.br

ABSTRACT

A new series of 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1H-tetrazole derivatives (**4a-m**) and their precursor 1-aryl-3-methyl-1*H*-pyrazole-4-carbonitriles (**3a-m**) were synthesized and evaluated as antileishmanials against *Leishmania braziliensis* and *Leishmania amazonensis* promastigotes *in vitro*. In parallel, the cytotoxicity of these compounds was evaluated on the RAW 264.7 cell line. The results showed that among the assayed compounds the substituted 3-chlorophenyl (**4a**) (IC_{50}/24h =15±0.14 µM) and 3,4-dichlorophenyl tetrazoles (**4d**) (IC_{50}/24h=26±0.09µM) were the most potent against *L. braziliensis* promastigotes, as compared the reference drug pentamidine, which presented IC_{50}=13±0.04µM. In addition, **4a** and **4d** derivatives were less cytotoxic than pentamidine. However, these tetrazole derivatives (**4**) and pyrazole-4-carbonitriles precursors (**3**) differ against each of the tested species and were more effective against *L.braziliensis* than on *L. amazonensis.*

Keywords: Antileishmanial activity, *Leishmania braziliensis, Leishmania amazonensis*, Tetrazole

The parasitic protozoans of the genus *Leishmania* are the causative agents of several forms of cutaneous, mucocutaneous, and visceral leishmaniasis. It is transmitted to human beings and other a mammals by the bite of an infected female Phlebotomine sandfly.[1] Leishmania has two evolutive forms: an extracellular flagellated promastigotes within the Phlebotomine vector and the intracellular amastigotes within mononuclear phagocytes in mammalian host. According to WHO estimations, leishmaniasis affect almost 12 million people in 88 countries on five continents (Africa, Asia, Europe, North and South America), representing

a worldwide public health problem. It is estimated that 1.6 million new cases each year, of which an estimated 400 000 are visceral and 1.2 million are cutaneous.[2,3] Cutaneous leishmaniasis is mainly caused by *Leishmania major* in the Old World, and by *L. braziliensis* or *L. amazonensis* in the Americas, in particular in Brazil.[4] *L. braziliensis* cause a disease which is characterized by its chronicity and the possibility to metastasize leading to the mucocutaneous clinical form. On the other hand, *L. amazonensis* is found in different regions and it has been described associated with various clinical forms such as: cutaneous, mucosa, diffuse cutaneous and visceral leishmaniasis, therefore it is considered a specie with an epidemiological significance.[5,6]

Despite the high worldwide prevalence, few advances were made in the treatment of this disease. There are no vaccines for leishmaniasis and chemotherapy is the main control strategy. For more than fifty years, pentavalent antimonials have been the first-line drugs in the treatment for all types of leishmaniasis, however these drugs require long-term and painful treatment which cause serious side effects and the rate of treatment failure of cutaneous leishmaniasis (CL) is increasing due to emerging of drug resistance. In the case of relapse, patients need treatment with second-line medicines, such as pentamidine and amphotericin B which have demonstrated nephrotoxicity. The recent and first oral drug miltefosine is really a progress, but drug resistance is at risk.[7,8] In addition, several reports regarding natural and synthetic new antileishmanial compounds have been described,[9] including some by our research group.[10] Among the several other compounds at various stages of development there are the azoles derivatives, which have been revealed as potential new drugs against *L. amazonensis* infection.[11,12] We have also demonstrated the leishmanicidal activity of the 5-amino-1-(3,5-dichlorophenyl)-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles i*n vivo* against *L.amazonensis* and exhibited significant inhibition in comparison to untreated control.[13] Based on these promising results obtained by our research group with azoles compounds, we planned to synthesize pyrazole-tetrazole hybrids, keeping the pyrazole ring in the structure of the target molecules. Thus, a new class of the 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1*H*-tetrazole derivatives (**4a-m**) and their respective precursors (**3a-m)** were synthesized and evaluated against *L. braziliensis* and *L. amazonensis***.**

The synthesis of the 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)1*H*-tetrazoles (**4a-m**) is summarized in Scheme 1. Arylhydrazine hydrochlorides (**1a-m**) were reacted with 1-ethoxyethylidenemalononitrile and sodium acetate in ethanol, under reflux to form 5-amino-1-aryl-3-methyl-1*H*-pyrazole-4-carbonitriles (**2a-m**). Then, the compounds (**2a-m**) were converted to 1-aryl-3-methyl-1*H*-pyrazole-4-carbonitriles (**3a-m**) by the aprotic deamination using *t*-butyl nitrite and tetrahydrofuran (THF), under reflux.[11,14] Ultimately, the target 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1*H*-tetrazoles (**4a-m**) were obtained by the reaction of 1-aryl-3-methyl-1*H*-pyrazole-4-carbonitriles (**3a-m**) with sodium azide, ammonium chloride and dimethylformamide (DMF) at 130°C.[14]

The *in vitro* biological activities of tetrazole derivatives and their pyrazole-4-carbonitriles precursors were assayed against *L. amazonensis* (MHOM/BR/77/LTB0016 strain) and *L. braziliensis* (MCAN/BR/98/R619 strain), using pentamidine isethionate as reference drug. Table 1 displays IC_{50} (inhibitory concentration-the concentration of a compound to inhibit the growth of 50% of parasites), CC_{50} (cytotoxic concentration-the concentration of a compound which cause a 50% reduction in cell viability) and SI (selective index between CC_{50}/IC_{50} in *L. braziliensis*) values of synthesized 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1*H*-tetrazoles (**4a-m**) and pyrazole-4-carbonitriles (**3a-m**) against *Leishmania* promastigotes and macrophage cell line (RAW 264.7). Interestingly, the activity of the tetrazoles and 4-carbonitriles differs for each of the tested species and were more effective against *L. braziliensis* than on *L. amazonensis*. The substituted 3-chlorophenyl tetrazole **4a** (IC_{50}=15±0.14 µM) and 3,4-dichlorophenyl (IC_{50}=26±0.09µM) **4d** were found to be the most potent in inhibiting promastigotes forms of *L. braziliensis*. Furthermore, the CC_{50} (cell line RAW 264.7) and SI for *L. braziliensis* indicate that these two compounds exhibited low levels of the cytotoxicity, with a CC_{50} of 151.20 and 244.0 µM, respectively. On the other hand, the reference drug pentamidine presented IC_{50}=13±0,04 µM with SI of 1.96 for *L. braziliensis*. The overall activity profile of the tested compounds demonstrated that the biological activities were highly influenced by the tetrazole ring. This can be clearly seen through the corresponding tetrazole **4a** (IC_{50} of 15.0 and 123.0 µM) and the precursor **4b** (IC_{50} of 147.0 and >1000 µM) for *L. braziliensis* and *L. amazonensis,* respectively. Additionally, in our previous structure activity studies we found that there is no significant difference in the antileishmanial effect related to tetrazole and imidazole rings.[13] However, the antipromastigote activity was also noticeably influenced by the type of substituent attached on the phenyl nucleus. Compounds **4a, 4c** and **4d** consisting a 3-chloro substituted group showed the lowest IC_{50} and low toxicity (table 1). Moreover, the replacement of 3-chloro group with 3-fluoro (**4h**) or 3-bromo (**4l**) resulted in decreased of antileishmanial activity, while that non-halogen derivative with a 4-OMe (**4m**) displayed a negligible *in vitro* activity against both *Leishmania* species.

In conclusion, this study indicates that the pyrazole-tetrazole are a new structural class of azoles with antileishmanial activity. Among all the tested compounds it was found that 3-chlorophenyl tetrazole derivative **(4a)** should be further investigated for the development of a highly selective antileishmanial compound emphasizing that this compound was more effective against *L. braziliensis*. Here it has also been reported the positive effect of introducing tetrazole group, leading to increased activity of *Leishmania* spp.

Acknowledgements

We thank the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Programa

Estratégico de Apoio à Pesquisa em Saúde (PAPES), Coordenação de Aperfeiçoamento de Pessoal Docente (CAPES), Universidade Federal Fluminense (PROPP/UFF), the Instituto Oswaldo Cruz/Fundação Oswaldo Cruz(IOC/Fiocruz), Rede Mineira de Química (RMQ) and Centro de Estudos e Inovação em Materiais Biofuncionais Avançados (UNIFEI) for fellowships and financial support.

Supplementary data

Supplementary data associated with this article can be found in the online version.

References

1. Sharma, U.; Singh, S. J. *Vector Borne Dis.* **2008**, *45*, 255.

2. Alvar, J.; Vélez, I. D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J.; den Boer, M. *PLoS One* **2012**, *7*, e35671.

3. Crompton, D. W. T., Peters, P. *Working to Overcome the Global Impact of Neglected Tropical Diseases: First WHO Report on Neglected Tropical Diseases*; WHO Press, 2010, pp 91-96.

4.Azeredo-Coutinho, R. B.; Conceição-Silva, F.; Schubach, A.; Cupolillo, E.; Quintella. L. P.; Madeira, M. F.; Pacheco, R. S.; Valete-Rosalino, C. M.; Mendonça, S. C. *Trans. R. Soc. Trop. Med. Hyg.* **2007**,*101,* 735.

5. Barral, A.; Pedral-Sampaio, D.; Grimaldi Jr, G.; Momen, H.; McMahon-Pratt, D.; Jesus, A.R.; Almeida, R.; Badaro, R.; Barral-Netto, M.; Carvalho, E.M.; Johnson, W.D. *Am. J. Trop. Med. Hyg.* **1991**,*44*, 536.

6. Leon, L. L.; Machado, G. M.; Paes, L. E.; Grimaldi Jr, G. *Trans. R. Soc. Trop. Med. Hyg.* **1990**, *84*, 678.

7. Croft, S. L.; Olliaro, P. *Clin. Microbiol. Infect.* **2011**, *17*, 1478.

8.Richard, J. V.; Werbovetz, K. A. *Curr.Opin.Chem. Biol*. **2010**,*14*, 447.

9.Kobets, T.; Grekov, I.; Lipoldova, M. *Curr .Med. Chem*. **2012** ,*19*,1443.

10. Marra, R. K. F.; Bernardino, A. M. R.; Proux, T. A.; Charret, K. S.; Lira, M. L. F.; Castro, H. C.; Souza, A. M. T.; Oliveira, C. D.; Borges, J. C.; Rodrigues, C. R.; Canto-Cavalheiro, M. M.; Leon, L. L.; Amaral, V. F. *Molecules* **2012**, *17*, 12961.

11. Santos, M. S.; Gomes, A. O.; Bernardino, A. M. R.; Souza, M. C.; Khan, M. A.; Brito, M. A.; Castro, H. C.; Abreu, P. A.; Rodrigues, C. R.; Léo, R. M. M.; Leon, L. L.; Canto-Cavalheiro, M. M. *J. Braz. Chem. Soc.* **2011**, *22*, 352.

12. Charret, K. S.; Rodrigues, R. F.; Bernardino, A. M. R.; Gomes, A. O.; Carvalho, A. V.; Canto- Cavalheiro, M. M.; Leon, L. L.; Amaral, V. F. *Am. J. Trop. Med. Hyg.* **2009**, *80*, 568.

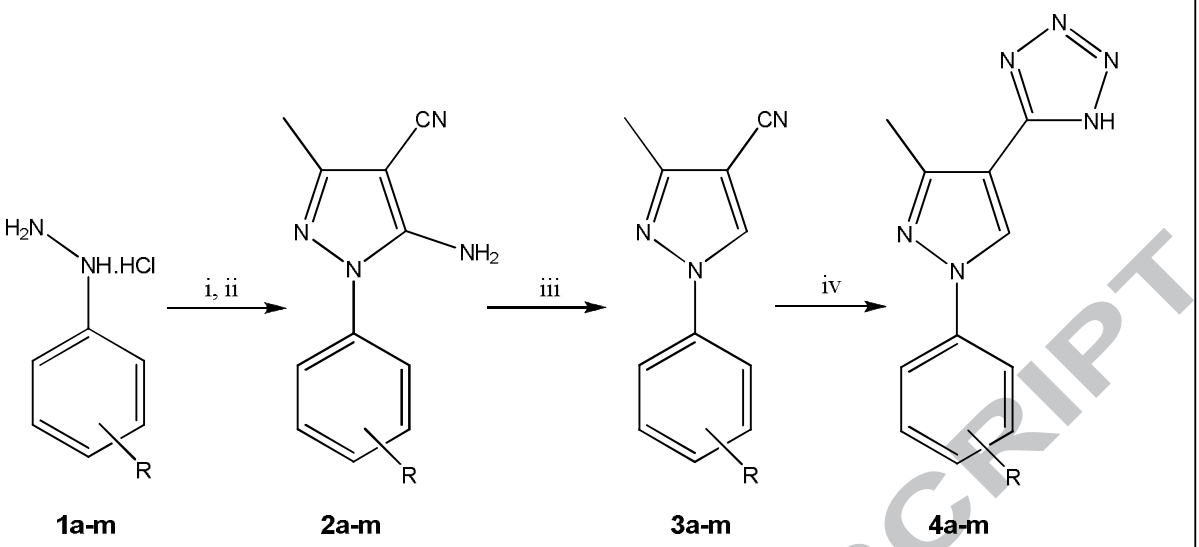
13. Santos, M. S.; Oliveira, M. L. V.; Bernardino, A. M. R.; Léo, R. M.; Amaral, V. F.; Carvalho, F. T.; Leon, L. L.; Canto-Cavalheiro, M. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7451.

14. Santos, M. S.; Bernardino, A. M. R.; Pinheiro, L. C. S.; Canto-Cavalheiro, M. M.; Leon, L. L. *J. Heterocyclic Chem.* **2012**, *49*, 1425.

Table 1. IC_{50} [1#] (μM) values of 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1*H*-tetrazoles (**4a-m**) and pyrazole-4-carbonitriles precursors (**3a-m**) on promastigotes of *Leishmania spp.*, cytotoxicity (CC_{50}) in macrophage cell line (RAW 264.7) and selectivity index (SI).

Compounds	*L. braziliensis* (IC_{50} μM)	*L. amazonensis* (IC_{50} μM)	Cytotoxicity (CC_{50} μM)	SI (CC_{50}/ IC_{50} *L. braziliensis*)
3a(3-Cl)	147.0±0.10	>900	404.25±0.77	2.75
3b(2,4-diCl)	119.0±0.26	>900	264.04±0.93	2.21
3c(3,5-diCl)	180.0±0.12	277.78±0.38	440.0±0.09	2.4
3d (3,4-diCl)	ND	ND	ND	-
3e(4-Cl)	658.99±0.30	221.20±0.29	ND	-
3f(2-F)	>900	>900	ND	-
3g(4-F)	378.11±0.41	>900	ND	-
3h(3-F)	144.28±0.61	417.91±0.33	363.42±0.98	2.52
3i(2-Br)	706.11±0.21	137.40±0.38	ND	-
3j(4-Br)	137.40±2.99	206.11±0.24	219.53±0.48	1.5
3l(3-Br)	694.66±0.17	274.81±0.14	ND	-
3m(4-OMe)	361.50±3.20	>900	ND	-
4a(3-Cl)	15.38±0.14	123.08±0.44	151.2±0.06	9.83
4b(2,4-diCl)	152.54±0.13	105.08±0.18	ND	-
4c(3,5-diCl)	85.41±0.12	160.14±0.22	272±0,05	3.18
4d(3,4-diCl)	26.69±0.09	266.90±0.35	244±0,08	9.14
4e(4-Cl)	111.54±0.49	246.15±0.23	>1000	9.0
4f(2-F)	225.41±0.27	401.64±0.10	ND	-
4g(4-F)	459.02±0.18	512.30±2.21	ND	-
4h(3-F)	77.87±0.21	270.49±0.15	284.0±0.06	3.6
4i(2-Br)	111.48±0.12	144.26±0.13	90.34±2.81	0.81
4j(4-Br)	68.75±0.38	295.08±0.09	295.0±0.06	4.29
4l(3-Br)	109.84±0.37	134.43±0.12	>1000	9.1
4m(4-OMe)	660.16±0.22	660.16±0.32	ND	-
Pentamidine	13.0±0.04	3.38±0.02	25.5±0.24	1.96

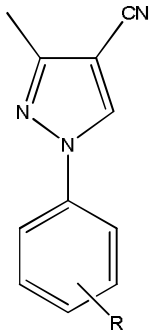
[1#] IC_{50} values were obtained from the drug concentration-response curve, and the results were expressed as the mean±/standard deviation determined from three independent experiments.
ND: not done.



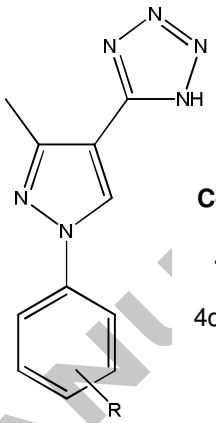
a: 3-Cl; **b**: R=2,4-diCl; **c**: R=3,5-diCl; **d**: R=3,4-diCl; **e**: R=4-Cl; **f**: R=2-F; **g**: R=4-F; **h**: R=3-F; **i**: R=2-Br; **j**: R=4-Br; **l**: R=3-Br; **m**: R=4-OCH_3.

Scheme 1. Reagents and conditions: i) sodium acetate, ethanol, 0,5h reflux; ii) 1-ethoxyethylidenemalononitrile, ethanol, 1h, reflux; iii) *t*-butyl nitrite, THF, 2h, reflux; iv) sodium azide, ammonium chloride, DMF at 130°C, 14h.

A series of 5-(1-aryl-3-methyl-1*H*-pyrazol-4-yl)-1H-tetrazole derivatives (**4a-m**) and their 1-aryl-3-methyl-1*H*-pyrazole-4-carbonitriles (**3a-m**) precursors were synthesized and evaluated for their antileishmanial activity.



3a-m



4a-m

Compound	IC_{50} (μM)
4a (3-Cl)	15±0.14
4d (3,4-diCl)	26±0.09