Bioorganic & Medicinal Chemistry Letters 20 (2010) 291–293

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of substituted aryloxy alkyl and aryloxy aryl alkyl imidazoles as antileishmanial agents ☆

Kalpana Bhandari [a,*], Nagarapu Srinivas [a], Vijay K. Marrapu [a], Aditya Verma [b], Saumya Srivastava [b], Suman Gupta [b]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute, CSIR, Lucknow 226 001, India
[b] Division of Parasitology, Central Drug Research Institute, CSIR, Lucknow 226 001, India

ARTICLE INFO

Article history:
Received 20 July 2009
Revised 24 September 2009
Accepted 27 October 2009
Available online 30 October 2009

Keywords:
Leishmania
Azoles
Imidazoles
Aryloxy cyclohexane

ABSTRACT

A series of aryloxy alkyl/aryl alkyl imidazoles were synthesized and evaluated in vitro as antileishmanials against *Leishmania donovani*. All the 19 compounds exhibited 94–100% inhibition at 10 μg/mL against promastigotes and 12 compounds exhibited high inhibition with an IC_{50} in the range of 0.47–4.85 μg/mL against amastigotes. Promising compounds were tested further in vivo. Among all, compounds **4** and **23** with 4-CF_3 aryloxy moiety exhibited medium in vivo inhibition of 58–60%, thus providing new structural lead for antileishmanials.

Leishmaniasis is a complex disease, with visceral and cutaneous manifestations, and is caused by over 17 different species of the protozoan parasite genus Leishmania.[1] It affects more than 12 million people worldwide.[2] The clinical manifestations may range from single cutaneous lesions to fatal visceral leishmaniasis.[3] Conventional chemotherapy relies on injected pentavalent antimonials that are considerably toxic and prone to induce resistance.[4] Amphotericin B[5] and the oral anticancer drug miltefosine[6] are considered at the moment to be the best second-line therapeutic solutions. Nevertheless, they do not represent a safe treatment in all clinical cases and necessitate the research of new antileishmanial molecules. There are several other potential drugs at various stages of development. These include the azoles that were initially developed as antifungal drugs.[7] Leishmania resemble fungi in synthesizing 24-substituted sterols such as ergosterol, whereas mammals have just cholesterol. Azoles, such as ketoconazole, inhibit 14a-demethylase, a key enzyme in this sterol biosynthesis pathway.[8] Ketoconazole, miconazole, itraconazole and fluconazole have undergone several trials for CL and VL with equivocal results.[3,9]

More recently, we have demonstrated the high leishmanicidal activity of novel aryloxy benzocycloalkyl azoles, aryloxy cyclohexane-based bis (**I**) and mono-imidazoles (**II**)[10] (Fig. 1) which makes these compounds promising hits for the development of an effective therapeutic agent.

Based on the above report and our continuation of studies on chemotherapy of Leishmania, we have planned to synthesize an expanded series of aryloxy alkyl/aryl alkyl azoles (**IV**) and investigated their biological effects against the Leishmania parasites. Thus, we planned the synthesis of derivatives **4**–**14**, **18**–**25** which are related acyclic analogues (Fig. 1). These compounds represent new structure scaffolds significantly different from those of the

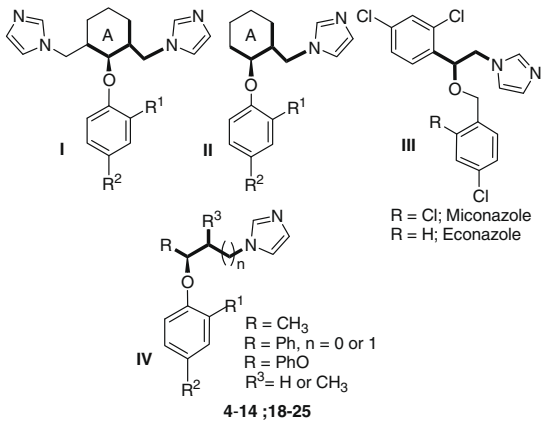
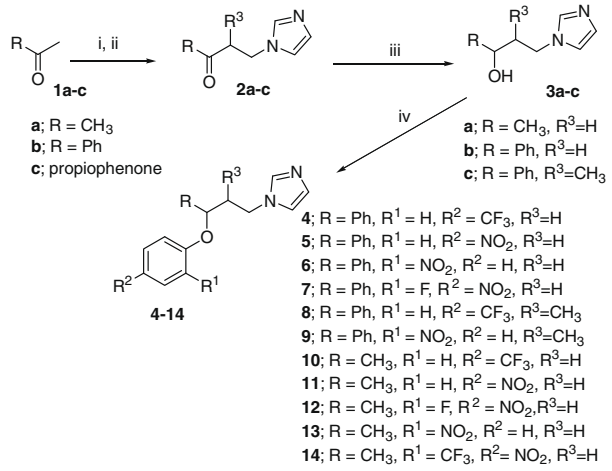


Figure 1. Representative antileishmanial and antimicrobial agents.

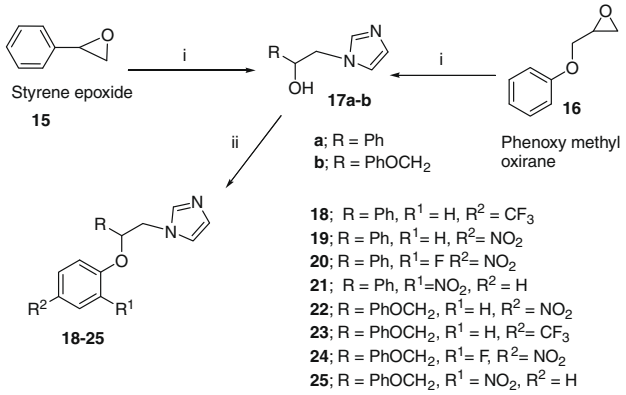
☆ CDRI communication number 7813.

* Corresponding author. Tel.: +91 522 2612411 18; fax: +91 522 2623405.
E-mail addresses: kalpana_bhandari@cdri.res.in, bhandarikalpana@rediffmail.com (K. Bhandari).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.117



Scheme 1. Reagents and conditions: (i) pyrrolidine, $(HCHO)_n$, L-proline/DMSO, 6–8 h; (ii) corresponding Mannich salt, imidazole/ethanol:H_2O (3:2), 5 h; (iii) $NaBH_4$/MeOH, 2 h; (iv) K(*t*-OBu), DMSO, substituted aryl halides, 2–3 h.



Scheme 2. Reagents and conditions: (i) imidazole/abs ethanol, reflux, 5 h; (ii) K(*t*-OBu), DMSO, substituted aryl halides, 2–3 h.

existing classes of azoles with good antileishmanial activity and here we described the details of our investigations.

The synthesis of the aryloxy alkyl/aryl alkyl imidazoles is summarized in Scheme 1. Ketone (acetone, acetophenone or propiophenone) **1a**–**1c** were reacted with pyrrolidine and paraformaldehyde under asymmetric Mannich conditions (to avoid mixture of products) in the presence of L-proline to give the corresponding Mannich products.[11] Subsequent replacement of the pyrrolidine with imidazole (amine exchange reaction to give **2a** and **2b**; **2c** was synthesized by our recently reported procedure),[12] followed by sodium borohydride reduction gave the hydroxyl intermediates **3a–3c** [**3c** with cis stereoselectivity (8.5:1.5)]. Condensation of the hydroxyl intermediates **3a, 3b** and *cis* **3c** isomer (major product) with substituted aryl halides furnished the required ethers **4–14** (**8** and **9** were obtained as *cis* diastereomers).

Formation of final compounds aryloxy aryl alkyl imidazoles (**18–21**) and diaryloxy alkyl imidazoles (**22–25**), followed the sequence shown in Scheme 2. Regioselective ring opening of styrene epoxide (**15**) or phenoxy methyl oxirane (**16**)[13] with imidazole gave the corresponding alcohols **17a and 17b**, SNAr substitution with an aryl fluoride generated the targeted aryloxy ethers **18–25**.

The compounds selected for study were evaluated in vitro against transgenic *Leishmania donovani* promastigotes at 10 μg/mL concentrations.[10,14] All the compounds showed 94–100% inhibition against promastigotes (Table 1). The compounds were further screened for antiamastigote (against intracellular Luc amastigotes) and cytotoxicity responses using mouse macrophage cell line (J-774-A-1)[14] and taking sodium stibogluconate, pentamidine and Amphotericin B as controls. The cell viability was determined using the MTT assay.[15] CC_{50} values were estimated as described by Huber and Koella.[16] IC_{50} of antileishmanial activity was calculated by nonlinear regression analysis of the concentration–response curve using the four-parameter Hill equation. Any synthesized analogues with in vitro IC_{50} value exceeding 15 μg/mL was considered as inactive. Based on IC_{50} and SI values four compounds were further evaluated for in vivo activity intraperitoneally at 50 mg/kg × 10 ip dose against *L. donovani*/Hamster model (*Mesocricetus auretus*).[17]

The in vitro biological activities of aryloxy alkyl azoles/aryloxy aryl alkyl azoles (**4**– **14, 18–21**) and diaryloxy alkyl imidazoles (**22**–**25**) have shown encouraging results against *L. donovani*. Table 1 displays IC_{50} and SI values of synthesized imidazoles against intracellular amastigotes. Among all the synthesized compounds, 12 compounds (**4**– **9, 18–21, 23** and **25**) have shown IC_{50} in the range of 0.47–4.83 μg/mL against amastigote model. Four compounds (**4, 6, 18** and **23**) showed the maximum SI (selectivity index) value of 36.68, 16.58, 13.85 and 31.89, respectively, which is several folds better than the standard drugs pentamidine and also with sodium stibogluconate.

Among the aryloxy aryl alkyl series (**4**–**7** and **18**–**21**) all the compounds appeared highly active exerting a strong inhibitory effect on the amastigote form of parasite with IC_{50} in the range of 0.47–2.27 μg/mL, while three compounds (**4, 6** and **18**) produced an interesting selective amastigote activity (SI >13). Further, the compounds with three carbon spacer between the phenyl and imidazole rings (**4–9**) were found more potent than the corresponding two carbon derivatives **18–21**, revealing the presence of three carbon spacer for better activity profile. Surprisingly, the related aryloxy alkyl imidazoles (**10**–**14**), though the tested derivatives displayed a strong inhibitory activity on the extracellular promastigotes with 100% inhibition at 10 μg/mL, but all the compounds were found inactive against amastigote model. It is apparent from the activity results (Table 1) that the aryl ring is crucial for the activity as on replacement of phenyl moiety with methyl (**10**–**14**) the antiamastigote activity decreased several folds leading to inactive compounds. Furthermore, the biological activity was also noticeably influenced by the presence of a second aryloxy group (**22**–**25**) as there is a significant increase in their IC_{50} values (from 0.47–2.83 to 4.82–5.62 μg/mL) except in compound **23** which exhibited an IC_{50} of 0.56 μg/mL.

The overall activity profile of the test compounds demonstrated that the biological activity was also influenced by the type of substituent attached at the 2- and 4-position of the aryloxy nucleus. Compounds (**4**, **8**, **18** and **23**) consisting a trifluoromethyl group at 4-position of aryloxy moiety showed the lowest IC_{50} (0.47, 1.85, 2.05 and 0.56 μg/mL) and the maximum SI values (36.68, 11.75, 13.85 and 31.89) except compound **10** of aryloxy alkyl series where all the compounds were inactive. It is interesting to note that while the NO_2 group at position-4 (**5, 11, 19, 22**) renders the molecule moderately active, the same group at position-2 enhances the activity (**6, 13, 21, 25**). Moreover, the presence of a fluorine atom at 2-position together with 4-NO_2 further confers increased selectivity (**7, 12, 20 and 24**).

Structure–activity relationship (SAR) shows that the trifluoromethyl group at position-4 of aryloxy moiety enhances the antileishmanial activity. Four compounds (**4**, **6**, **18** and **23**) of SI above 13 were tested further for in vivo leishmanicidal activity and the results are presented in Table 1. Compounds **4** and **23** with 4-CF_3 moiety exhibited medium in vivo activity with 60% and 58% inhibition of parasite growth, respectively, and compounds **6** and **18** displayed moderate inhibition of 52% and 46%, respectively. This finding indicates that aryloxy moiety with 4-CF_3 substituent

Table 1
In vitro and in vivo antileishmanial activity of synthesized imidazoles

Sl. No.	Compd No.	In vitro assessment		Cytotoxicity CC_{50}[c] (μg/mL)	Selective index (SI)[b] CC_{50}/IC_{50}	In vivo activity (dose—50 mg/kg × 10 ip) percent inhibition
		Anti-promastigote activity (% inhibition at 10 μg/mL)	Anti-amastigote activity IC_{50}[a] (μg/mL) (C.I)			
1	**4**	100	0.47	17.24	36.68[b]	60
2	**5**	98.97	3.82	25.1	6.57	
3	**6**	100	0.48	17.96	16.58[b]	52
4	**7**	96.26	2.83	25.1	8.86	
5	**8**	100	1.85	21.74	11.75	
6	**9**	95.05	2.27	22.15	9.75	
7	**10**	100	8.08	7.5	0.88	
8	**11**	100	NI	24.1	NA	
9	**12**	100	NI	15.0	NA	
10	**13**	100	8.43	>100	11.86	
11	**14**	100	9.97	7.73	0.97	
12	**18**	98.60	2.05	28.45	13.85[b]	46
13	**19**	95.47	3.71	25.21	6.79	
14	**20**	94.63	2.76	21.23	7.69	
15	**21**	97.46	2.25	25.15	11.17	
16	**22**	93.82	5.62	28.14	5.00	
17	**23**	100	0.56	17.86	31.89[b]	58
18	**24**	98.18	5.13	29.57	5.76	
19	**25**	95.53	4.82	36.23	7.51	
20	SSG	946.52 (IC_{50})	46.54	297.38	6.38	84.10 (20 mg/kg)
21	Pentamidine	0.643 (IC_{50})	12.11	31.31	2.58	92 (40 mg/kg)
22	Amphotericin B	0.008 (IC_{50})	0.05	7.24	144.8	92.22 (8 mg/kg × 5, iv)

[a] IC_{50} >15 μg/mL = inactive; IC_{50} >5–15 μg/mL = moderately active; IC_{50} <5 μg/mL = highly active compounds.
[b] Compounds having IC_{50} <5 μg/mL (in vitro antiamastigote activity) and SI >13 were picked up for in vivo evaluation.
[c] CC_{50} (cytotoxic concentration for 50% inhibition) is evaluated against J-774A-1 cell lines; NA = not available; NI = no inhibition; SSG = sodium stibogluconate.

should be investigated for the development of highly selective antileishmanial compounds.

In conclusion, this study has identified aryloxy aryl alkyl imidazole and diaryloxy alkyl imidazoles as a new structural class of azoles with antileishmanial activity both in vitro and in vivo. The potent activity and simple synthesis of these imidazoles suggest that they are potential candidates for the development of new antileishmanial drugs. Further work is in progress to improve the potency of these compounds.

Acknowledgements

N.S. and V.K.M. thank the University Grants Commission (India) and Council of Scientific and Industrial Research (India) for the award of SRF and JRF, respectively. The authors are thankful to S.A.I.F. Division, CDRI, Lucknow for providing spectroscopic data and Mr. Anoop K. Srivastava for providing technical assistance. The work is partially funded by SIP.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.117.

References and notes

1. Sharma, U.; Singh, S. J. *Vector Borne Dis.* **2008**, *45*, 255.
2. (a) Ashford, R. W. *Int. J. Parasitol.* **2000**, *30*, 1269; (b) TDR-Leishmaniasis, 2007.
3. Desjeux, P. *Comp. Immunol. Microbiol Infect. Dis.* **2004**, *27*, 305.
4. Bermudez, H.; Rojas, E.; Garcia, L.; Desjeux, P.; Dujardin, J. C.; Boelaert, M.; Chappuis, F. *Ann. Trop. Med. Parasitol.* **2006**, *100*, 591.
5. (a) Serrano, M.; Hannon, G. J.; Beach, D. *Nature* **1993**, *366*, 704; (b) Balana-Fouce, R.; Reguera, R. M.; Cubria, J. C.; Ordonez, D. *Gen. Pharmacol.* **1998**, *30*, 435; (c) Croft, S. L.; Coombs, G. H. *Trends Parasitol.* **2003**, *19*, 502.
6. (a) Berman, J.; Bryceson, A. D. M.; Croft, S. L.; Engel, J.; Gutteridge, W.; Karbwang, J.; Sindermann, H.; Soto, J.; Sundar, S.; Urbina, J. A. *Trans. R. Soc. Trop. Med. Hyg.* **2006**, *100*, S41; (b) Sundar, S.; Jha, T. K.; Thakur, C. P.; Bhattacharya, S. K.; Rai, M. *Trans. R. Soc. Trop. Med. Hyg.* **2006**, *100*, S26.
7. (a) Saenz, R. E.; Paz, H.; Berman, J. D. *Am. J. Med.* **1990**, *89*, 147; (b) Kubba, R.; Al-Gindan, Y.; El-Hassan, A. M.; Omer, A. H. S. *Saudi Med. J.* **1986**, *7*, 596; (c) Berman, J. D. *Clin. Infect. Dis.* **1997**, *24*, 684; (d) Pearson, J. K.; Sawick, S. *Arch. Dermatol.* **1975**, *111*, 1343; (e) Beltran, H. F.; Gutierrez, F. M.; Biagi, F. F. *Bull. Soc. Pathol. Exot.* **1967**, *70*, 61; (f) Belehu, A.; Naffs, B.; Touw-Langendijk, E. *Br. J. Dermatol.* **1978**, *99*, 421; (g) Regina, G. L.; D'Auria, D. F.; Tafi, A.; Piscitelli, F.; Olla, S.; Caporuscio, F.; Nencioni, L.; Cirilli, R.; La Torre, F.; De Melo, N. R.; Kelly, S. L.; Lamb, D. C.; Artico, M.; Botta, M.; Palamara, A. T.; Silvestri, R. *J. Med. Chem.* **2008**, *51*, 3841.
8. (a) Martínez, M. D. N.; Herrera, J. C.; López, J. N. R. *Int. J. Antimicrob. Agents* **2006**, *28*, 560; (b) Urbina, J. A. *Parasitology* **1997**, *114*, S91.
9. (a) Minodier, P.; Parola, P. *Travel Med. Infect. Dis.* **2007**, *5*, 150; (b) Kelly, S. L.; Lamb, D. C.; Taylor, M. *Biochem. Pharmacol.* **1992**, *44*, 2199.
10. (a) Srinivas, N.; Palne, S.; Nishi; Gupta, S.; Bhandari, K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 324; (b) Bhandari, K.; Srinivas, N.; Palne, S.; Nishi.; Gupta, S. *Ind. Pat.* 610 DEL, 2008.
11. Ibrahem, I.; Casas, J.; Cordova, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 6528.
12. Srinivas, N.; Bhandari, K. *Tetrahedron Lett.* **2008**, *49*, 7070.
13. Srivastava, S.; Bhandari, K.; Shankar, G.; Singh, H. K.; Saxena, A. K. *Med. Chem. Res.* **2004**, *13*, 631.
14. Ashutosh; Gupta, S.; Ramesh; Sundar, S.; Goyal, N. *Antimicrob. Agents Chemother.* **2005**, *49*, 3776.
15. Mossman, T. J. *J. Immunol. Methods* **1983**, *65*, 55.
16. Huber, W.; Koella, J. C. *Acta Trop.* **1993**, *55*, 257.
17. Gupta, S.; Tiwari, S.; Bhaduri, A. P.; Jain, G. K. *Acta Trop.* **2002**, *84*, 165.