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Aryloxy cyclohexyl imidazoles: A novel class of antileishmanial agents ☆

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ABSTRACT

Thirteen novel aryloxy cyclohexane-based mono and bis imidazoles were synthesized and evaluated in vitro as antileishmanials against *Leishmania donovani* and cytotoxicity assessed. These compounds were better than the existing drugs, sodium stibogluconate and pentamidine in respect to IC_{50} and SI values. Promising compounds were tested further in vivo. Among all, the bis methylimidazole with 2-fluoro, 4-nitro aryloxy group (**9**) exhibited significant in vivo inhibition of 77.9%, thus providing new structural lead for antileishmanials.

Leishmaniasis is a group of parasitic diseases of global distribution transmitted by the bite of the infected female phlebotomine sandfly[1] and manifest with visceral, cutaneous, and mucocutaneous forms. This disease is currently prevalent in four continents, being endemic in 88 countries, threatening 350 millions worldwide.[2] Chemotherapy for these parasitic diseases is generally ineffective mainly due to the emergence of drug-resistant strains, significant toxicity, variable efficacy, lack of oral bioavailability and high cost of the therapeutic agents.[3,4] The pentavalent antimonials are widely used as primary therapy whereas alternative drugs include amphotericin B, pentamidine, paromomycin, miltefosine and azoles,[5,6] all suffer from one or more of the above deficiencies. Drug resistance, high toxicity and high treatment costs necessitate the need for novel therapeutics.[7]

Among potential orally active drugs for the treatment of these complex diseases, sterol biosynthesis inhibitors offer an attractive possibility, as *Leishmania* parasites synthesize specific sterols which seem to be essential for cell proliferation and viability.[8,9] Azole antifungal agents, have been used as antileishmanial agents since 1980s,[10–12] inhibit the growth of *Leishmania* amastigotes in culture systems by inhibiting the cytochrome P-450-mediated 14a-demethylation of lanosterol, blocking ergosterol synthesis, and causing accumulation of 14a-methyl sterols.[8,10] Metronidazole and *N*-substituted azoles (ketoconazole, miconazole, fluconazole and itraconazole) are well-tolerated drugs[13,14] that are potentially active against *Leishmania*, but their use in the treatment of cutaneous and visceral leishmaniasis has produced conflicting results.[15,16]

Based on above report, we recently prepared a series of novel aryloxy benzocycloalkyl azoles and found that they were highly active in vitro against *Leishmania donovani* and also exhibited significant in vivo activity in *L. donovani*/Hamster model. In view of above and our continuation of studies on chemotherapy of *Leishmania*, we decided to synthesize an expanded series of aryloxy cycloalkyl azoles, and investigated their biological effects against the *Leishmania* parasites and the results are reported in this communication.

The compounds used in the present study were prepared from cyclohexanone. The synthetic route for the preparation of 2,6-bisimidazolyl-methyl-1-aryloxycyclohexane (**5–9**) is outlined in Scheme 1. Mannich reaction on cyclohexanone with 2 moles of pyrrolidine gave 2,6-bis-pyrrolidin-1-ylmethyl-cyclohexanone (**2**) which on reaction with imidazole in presence of ethanol/water resulted in the replacement of pyrrolidine moiety with imidazole (**3**). The keto intermediate **3** was then transformed to corresponding hydroxy derivative **4** in a single diasteriomeric form, which on reaction with proper aryl halides furnished the desired 2,6-bis-imidazolylmethyl-cyclohexyl aryl ethers (**5–9**) (Scheme 1).

For the synthesis of 1-(2-aryloxy-cyclohexylmethyl)-1*H*-imidazoles **13–17**, cyclohexanone was reacted with pyrrolidine under Mannich conditions in the presence of L-proline[17] to give 2-pyrrolidin-1-ylmethyl-cyclohexanone (**10**). Subsequent replacement of the pyrrolidine with imidazole followed by reduction led to the *cis*/*trans* mixture of 2-imidazol-1-ylmethyl-cyclohexanol **12a**/**12b**.[18] The *trans* isomer **12b** (major product) was condensed with substituted aryl halides to obtain the corresponding ethers **13–17** (Scheme 1). For SAR studies we also synthesized the directly connected imidazole derivatives viz: 1-aryloxy-2, 6-bisimidazolyl cyclohexane **21–23** (Scheme 2). Bromination of cyclohexanone[19]

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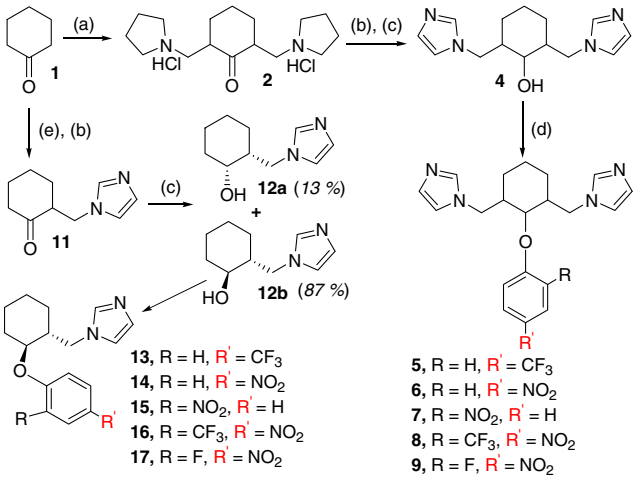
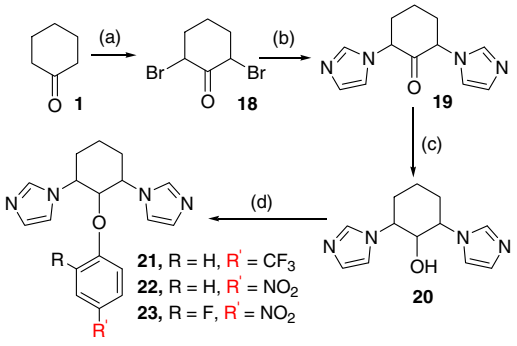


Scheme 1. Reagents and conditions: (a) $(HCHO)_3$, pyrrolidine hydrochloride (2.0 equiv), isopropanol; (b) Imidazole, ethanol: water (2:3); (c) $NaBH_4$; (d) K(*t*-OBu), DMSO, substituted aryl halides (a–c); (e) $(HCHO)_3$, pyrrolidine (1.0 equiv), L-proline (0.3 equiv), DMSO.

gave the 2,6-dibromo derivative **18** which on reaction with imidazole followed by reduction with $NaBH_4$ gave the 2,6-di-imidazol-1-yl cyclohexanol **20**. Condensation of this hydroxy compound with substituted aryl halides gave the required aryloxy bisimidazolyl cyclohexanes **21–23**.

All the compounds shown in Schemes 1 and 2 were obtained as racemic mixtures. Their structures, including the relative configurations depicted in Scheme 1. The conformations were established through the complete analysis of their 1D and 2D NMR, NOESY and NOE enhancement studies, MS and IR spectra. In representative cases the unambiguous assignment of all significant ^1H and ^{13}C NMR signals was performed. Careful examination of the ^1H, ^{13}C NMR of bisimidazol-1-yl-methyl-cyclohexanol **4** suggested it as symmetrical conformation. The 2D NMR (Dept-90, 135, COSY, HMBC and HSQC) also supported the same. Inharmoniously the C-1 proton appeared as singlet at δ 3.36 (instead of a multiplet or triplet). After etherification (**5-9**) the same singlet shifted down field, δ 4.41–4.54 (no change in symmetry). The ^1H–^1H COSY spectrum of **4** also revealed that there are no characteristic correlations with adjacent protons (H-2, H-6). However, the conformation of cyclohexyl ethers **5–9** and **21–23** was determined from the analysis of the splitting patterns and shifting of the ^1H NMR signals together with a 2D NOESY and NOE enhancement studies. Previously Claudon and his co workers have established the thermodynamically stable conformation of 2,6 di-benzyl substituted cyclohexanols.[20] They affirmed that both 2_{eq}, 6_{eq}, 1_{ax} and 2_{eq}, 6_{eq}, 1_{eq} conformers were 100% stable and the reactivity varies



Scheme 2. Reagents and conditions: (a) Br_2, CCl_4; (b) Imidazole, DMF; (c) $NaBH_4$; (d) NaH, DMF, substituted aryl halides.

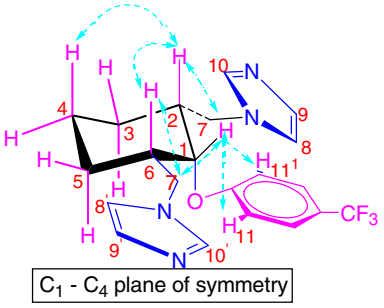


Figure 1. Characteristic and key NOESY correlations for **5**.

(~40% more for equatorial OH). The significant correlations between H-1 and its α-protons in the NOESY spectra of **5** supported the fact that the aryloxy function is at axial position. Hence the plane of symmetry (σ) is possible in chair as well as in boat conformations. Further, NOE correlations from H-1 to H-2_{ax}, H-7a, H-7b and also with H-8, H-10 and H-11 (considering symmetrical) (Fig. 1) were observed but no interaction was found between H-1 and H-4 (these 1, 4 flag pole interactions possible in boat conformation). An examination of Drieding models also revealed that these correlations were possible only if the cyclohexyl ring occurs in a chair conformation with two imidazole groups in equatorial and aryloxy at axial position (Fig. 1).

The ^1H NMR of 2, 6-Di-imidazol-1-yl-cyclohexanol **20** was quite different from bisimidazol-1-yl-methyl-cyclohexanol **4**. The H-1 proton appearing as double doublet (J = 8.82, 3.66 Hz) at δ *4.48–4.52* and H-2, H-6 protons were found as two individual multiplets at δ 2.5, δ *2.9,* respectively. After etherification (**21–23**), the same pattern of ^1H signals multiplicity was maintained (with little down field shifting of H-1, H-2 and H-6). In ^{13}C all the carbons showed individual signals (no σ is found). The H-7 and H-8 (imidazole protons) appeared at δ *8.01–8.09* and 7.65–7.66 rather than its regular position, that is, δ 7.48–7.51 and 7.05–7.09, respectively).[21] These can interact (di-pole interaction) with phenoxy oxygen only if the cyclohexyl ring is in skew boat conformation (by Drieding models). This conformation is highly energetic and the key NOE enhancement was found between H-1 and H-2 (Fig. 2) (**21**).

The compounds selected for study were evaluated in vitro against transgenic *L. donovani* promastigotes[22] and intracellular amastigotes[22] at various concentrations taking sodium stibogluconate and pentamidine as a control and cytotoxicity responses[23] were assessed using mouse macrophage cell line (J-774-A-1). All the compounds killed promastigotes and amastigotes in concentration dependent manner and showed 100% inhibition of parasites at a maximum concentration of 10 μg/ml. IC_{50} of antileishmanial activity was calculated by Probit analysis.[24] Based on IC_{50} and SI values six compounds were further evaluated for in vivo activity

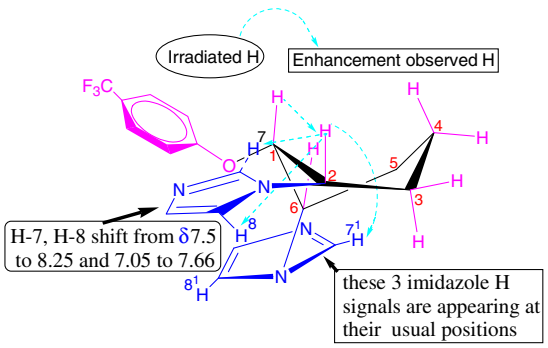


Figure 2. NOE enhancements for **21**.

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

Sl.No.	Compound	In vitro assessment[@]				
		Anti promastigote activity IC_{50} (μg/ml)	Anti amastigote activity.[#] IC_{50} (μg/ml) (C.I)	[a] Cytotoxicity CC_{50} (μg/ml)	Selective index (SI)	In vivo activity (dose-50 mg/kg × 10 i.p.) percent inhibition
1	**4**	9.4	10.77	>100	9.28	
2	**5**	3.284	1.13	94.47	[*]83.60	51.00
3	**6**	5.524	1.97	73.88	[*]37.51	52.00
4	**7**	9.57	2.437	69.39	28.47	
5	**8**	6.725	0.588	14.44	24.557	
6	**9**	3.57	1.17	39.37	[*]33.64	77.90
7	**13**	0.81	3.83	35.59	9.292	
8	**14**	0.82	4.57	44.59	9.757	
9	**15**	4.23	6.72	63.11	9.39	
10	**16**	2.37	2.13	13.84	6.49	
11	**17**	1.08	0.71	>100	[*]140.84	55.35
12	**21**	2.06	3.0	96.43	[*]32.143	36.8
13	**22**	4.67	8.76	>100	11.4	
14	**23**	6.13	3.02	90.6	[*]30	56.69
15	Sodium Stibogluconate	946.52	46.54	297.38	6.38	84.10 (20 mg/Kg)
16	Pentamidine	0.643	12.11	31.31	2.58	92 (40 mg/Kg)

[@] All the compounds showed concentration dependent response against extra cellular promastigotes and intracellular amastigotes.
[#] IC_{50} = >15 μg/ml = Inactive; IC_{50} = 5–15 μg/ml = moderately active; IC_{50} = <5 μg/ml = highly active compounds.
[*] Compounds having IC_{50} = <5 μg/ml (in vitro antiamastigote activity) and SI = >30 were picked up for in vivo evaluation.
[a] CC_{50} (cytotoxic concentration for 50% inhibition in cell viability) was evaluated against J-774A-1 cell line.

intraperitoneally at 50 mg/kg × 10 i.p dose against *L.donovani*/Hamster model.[25]

The in vitro biological activities of bis and mono imidazoles have shown encouraging results. Table 1 displays IC_{50} and SI values of synthesized bis and mono imidazoles against promastigotes and intracellular amastigotes. The IC_{50} and SI values for amastigotes of the test derivatives indicate that all compounds exhibited high activity against *L. donavani* (IC_{50} 0.58–8.76 μg/ml), better than the reference drugs sodium stibogluconate (IC_{50} = 46.54 μg/ml) and Pentamidine (IC_{50} = 12.11 μg/ml). Among the bisimidazolyl methyl series (**5–9**) all the compounds appeared highly active exerting a strong inhibitory effect on the amastigote form of parasite with IC_{50} in the range of 0.588 to 2.437 μg/ml, while three compounds (**5**, 6 and **9**) produced an interesting selective antiamastigote activity (SI > 30). Concerning monoimidazolyl methyl analogues **13–17**, though the tested derivatives displayed a strong inhibitory activity on the intracellular amastigote IC_{50} ranging from 0.71 to 4.57 μg/ml, but the selective index of all the compounds was below 10 except **17** which showed a high SI value of 140.84. Among the corresponding bisimidazolyl derivatives (**21–23**), two compounds **21** and **23** expressed interesting antiamastigote activity (IC_{50} of 3.00 and 3.02 μg/ml), with SI > 30.

The hydroxyl intermediate (4), showed an IC_{50} 10.77 and SI 9.28. It is apparent from activity results (Table 1) that on introduction of aryl moiety the activity increased several folds (0.588–2.437 μg/ml). Further the compounds of bisimidazolyl series **5–9** with one carbon spacer were found more potent than the corresponding bisimidazolyl derivatives **21–23** as well as monoimidazolyl analogues **13–16** (except **17**), revealing the presence of a carbon spacer between the cyclohexane and imidazole rings for better activity profile. The overall activity profile of compounds (**5–9, 21–23, 13–17**) demonstrated that there is a small difference in their IC_{50} values. Thus, the biological activity was slightly influenced by the type of substituent attachment at the 2 and 4-position of the aryloxy nucleus. However, it is interesting to note that while the NO_2 group at position 2 (**7, 15**) renders the molecule moderately active, the same group at position 4 enhances the activity (**6, 14**). Moreover, the presence of a fluorine atom at 2 position together with 4-NO_2 further confers increased selectivity **9, 17** and **23**.

Six compounds (**5, 6, 9, 17, 21** and **23**) of SI above 30 were tested further for in vivo leishmanicidal activity and the results are presented in Table 1. Compound **9** with bis methylimidazolyl moiety and with a 2-fluoro, 4-nitro aryloxy group exhibited significant in vivo activity with 77.9% inhibition of parasite growth. Compound **17** and **23** displayed medium activity with 55.35% and 56.69% inhibition, respectively, while other three showed moderate activity. It is interesting to note that in all the three series (**5–9, 13–17** and **21–23**) the highest activity (in vitro as well as in vivo) was shown by the compounds with a 2-fluoro and 4-NO_2 aryloxy moiety. This finding indicates that aryloxy moiety with a 2-fluoro and 4-NO_2 substituent should be investigated for the development of highly selective antileishmanial compounds.

In conclusion, this study has identified aryloxy cyclohexyl imidazoles as an entirely 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 studies on these compounds and optimization of its structure leading to novel analogues with superior biological properties are on going in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.094.

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