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Design and synthesis of novel tetrahydronaphthyl azoles and related cyclohexyl azoles as antileishmanial agents

Vijay K. Marrapu [a], Nagarapu Srinivas [a], Monika Mittal [b], Nishi Shakya [b], Suman Gupta [b], Kalpana Bhandari [a,*]

[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

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ABSTRACT

A novel series of *trans*-2-aryloxy-1,2,3,4,-tetrahydronaphthyl azoles and related cyclohexyl azoles were synthesized and evaluated in vitro against *Leishmania donovani*. Compound **9** identified as most active analog with IC_{50} value of 0.64 μg/mL and SI value of 34.78 against amastigotes, and is several folds more potent than the reference drugs sodium stilbogluconate and paromomycin. It also exhibited significant in vivo inhibition of 83.33%, and provided a new structural scaffold for antileishmanials.

There is a lack of effective, safe, and affordable pharmaceuticals to control neglected tropical diseases (NTDs) such as leishmaniasis that cause high mortality and morbidity among poor people in the developing world. The NTDs are a group of chronic and debilitating conditions, caused by parasitic, bacterial, and other infections.[1]

Leishmaniasis, a parasitic disease caused by organisms of the *Leishmania* genus, comprises three clinical forms: visceral, cutaneous, and mucocutaneous.[2] The lesions may be confined to skin, in the case of cutaneous leishmaniasis, or may invade subcutaneous tissue, as in the potentially fatal visceral form, depending on the infecting organism and the immunological status of the host.[3] Chemotherapeutic treatment of leishmaniasis usually relies on the use of pentavalent antimonials such as sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), but toxic side effects and drug resistance are frequently encountered.[4] The second-line compounds used during the treatment of unresponsive cases generally include pentamidine and amphotericin B and its lipid formulations.[5] The oral anticancer drug miltefosine[6] is an effective drug, but there are side effects and drawbacks related to treatment. Antifungal imidazole and triazole derivatives such as ketoconazole, fluconazole and itraconazole, which block ergosterol biosynthesis at the level of cytochrome P-450-dependent C14α-demethylase,[7] are also effective against different *Leishmania* species both in vitro and in vivo. Various N-substituted azole derivatives have also been reported with promising in vitro antileishmanial activity.[8] *Leishmania* resemble fungi in synthesizing 24-substituted sterols such as ergosterol, while mammals have just cholesterol, it accounts for many antifungal sterol biosynthesis inhibitors (SBIs) to also be leishmanicidal. Ketoconazole and fluconazole have undergone evaluation in VL in India.[9] However, despite reports of their usefulness, the antileishmanial activity was not enough to induce clinical cure by themselves. Thus, the development of new, efficient, and safe drugs for the treatment of this disease is imperative.

Recently, we reported on the synthesis of a series of novel aryloxy alkyl and aryloxy aryl alkyl imidazoles as potential antileishmanial agents.[10] Based on the above report and in continuation of our efforts to generate azole based novel antileishmanial agents,[11] we synthesized a series of novel aryloxy tetrahydronaphthyl azoles (**4**–**13**) and related cyclohexyl azoles (**22**–**28**) (Fig. 1) and evaluated them in vitro against the *Leishmania* parasites. Many of these derivatives were found to possess strong inhibitory activity against *Leishmania donovani* when compared to the standard drugs. After this large in vitro screening, compounds **9** and **10**, identified as the most active analogues of the series, were selected for in vivo analysis and the results are reported in this Letter.

The synthesis of compounds **4**–**13** was carried out according to Scheme 1. The regioselective ring opening of 1,2-epoxy-tetrahydronaphthalene[12] **1** with imidazole in absolute ethanol furnished *trans*-1-imidazol-1-yl-1,2,3,4-tetrahydro-naphthalen-2-ol (**3a**). The **3a** can also be prepared directly from the bromohydrin[12] **2** in low yields (27–28%). The 1,2,3-triazole did not react with epoxide **1** under similar conditions. However, the reaction was achieved in the presence of tetrabutyl ammonium iodide to give the product, 1-[1,2,3]-triazol-1-yl-1,2,3,4-tetrahydro-naphthalen-2-ol (**3b**) in excellent yields (~72%). Further, etherification of the hydroxyl intermediates **3a** and **3b** with substituted aryl/benzyl halides

* Corresponding author. Tel.: +91 522 2262411; fax: +91 522 2623405.
E-mail address: bhandarikalpana@rediffmail.com (K. Bhandari).

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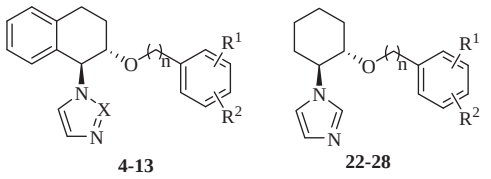
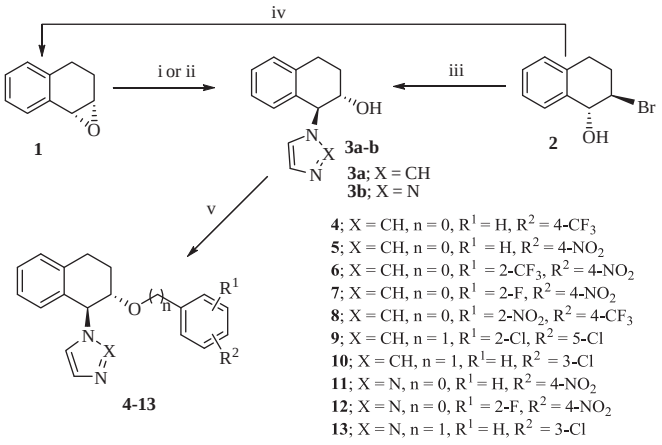


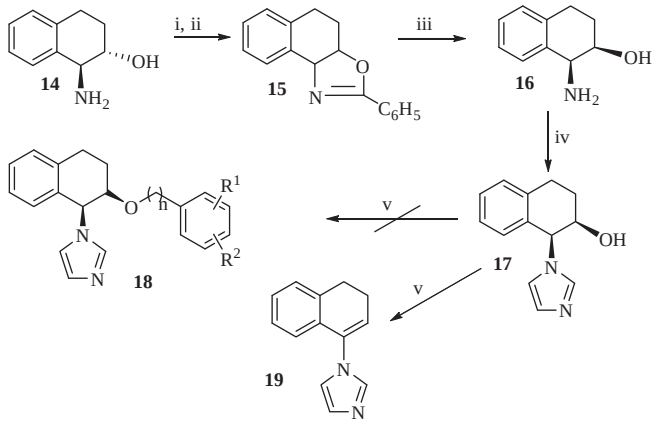
Figure 1. Aryloxy tetrahydronaphthyl and cyclohexyl azoles.



Scheme 1. Reagents and conditions: (i) imidazole, abs. ethanol, 90 °C, 6 h; (ii) 1,2,3-triazole, $(But)_4N^+I^-$, rt, 8 h; (iii) imidazole, acetonitrile, 90 °C, 8 h; (iv) dry THF, NaOMe, 0 °C, 5 h; (v) NaH (60% oil), substituted aryl or benzyl halide, DMF, 0 °C–rt, 4–5 h.

furnished the desired ethers **4–13** (Scheme 1). The ^1H NMR spectra of compounds **4–13** showed a doublet at approximately δ 5.1–5.8 for H-1 proton with J_{1-2} = 7–8 Hz, indicative of a *trans* diaxial orientation.

It was thought interesting to synthesize and evaluate the above ethers with *cis* geometry (Scheme 2). Benzoylation of the *trans*-1-amino-2-hydroxytetrahydronaphthalene (**14**) followed by treatment with thionyl chloride gave the 2-phenyl oxazoline (**15**) which was hydrolyzed with 6 N H_2SO_4 to furnish the required *cis* amino alcohol[13a] **16**. Reaction of the *cis* amino alcohol **16** with ammonium acetate, glyoxal and formaldehyde in methanol[13b] under refluxing condition gave the desired *cis*-1-imidazol-1-yl-1,2,3,4-tetrahydronaphthalen-2-ol (**17**). However, the etherification of *cis*-imidazolyl alcohol **17** with aryl halides did not give the desired *cis* ethers (**18**) but furnished the dehydrated product **19**.



Scheme 2. Reagents and conditions: (i) PhCOCl, KOH, H_2O, rt, 3 h; (ii) $SOCl_2$,CH_2Cl_2, rt, 7 h; (iii) 6 N·H_2SO_4, reflux, 11 h; (iv) glyoxal, NH_4OAc, HCHO, methanol, 80 °C, 8 h; (v) NaH (60% oil), substituted aryl or benzyl halide, DMF, 0 °C–rt, 4–5 h.

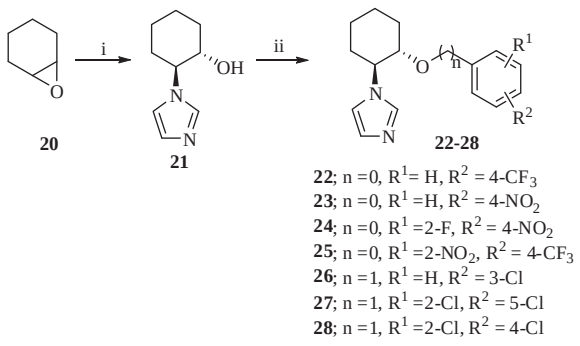
Further, for SAR studies the related aryloxy cyclohexyl imidazoles (**22–28**) were also prepared (Scheme 3). Reaction of imidazole with 1,2-epoxycyclohexane (**20**) under refluxing conditions furnished *trans*-2-imidazolyl cyclohexanol (**21**). Etherification of the hydroxyl intermediate with substituted aryl halides furnished the corresponding ethers **22–28**. All the compounds shown in Schemes 1–3 were obtained as racemic mixtures.

The compounds selected for study (**4–13** and **22–28**) were evaluated in vitro against transgenic *L. donovani* promastigotes and intracellular amastigotes[14] at various concentrations and cytotoxicity responses[15] were assessed using mouse macrophage cell line (J-774-A-1) and taking sodium stibogluconate, paromomycin and podophyllotoxin (for cytotoxicity assay) as controls. Cell viability was determined using the MTT assay.[15] CC_{50} values were estimated through the preformed template 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 equations. Any synthesized analogues with in vitro IC_{50} value exceeding above 15 μg/mL was considered as inactive. Based on IC_{50} and SI values two compounds were further evaluated for in vivo activity intraperitoneally at 50 mg/kg × 5, ip dose against *L. donovani*/Hamster model (*Mesocricetus auretus*).[17] Sodium stibogluconate and paromomycin were used as reference drugs.

The in vitro biological activities of aryloxy tetrahydronaphthyl azoles/aryloxy cycloalkyl azoles (**4–13** and **22–28**) have shown encouraging results against *L. donovani*. Table 1 displays % inhibition (promastigotes), IC_{50} and SI values of the synthesized azoles against intracellular amastigotes. Interestingly, all the compounds (except **4**) exhibited high inhibition of 89–100% at 10 μg/mL concentration against promastigotes. These compounds were further evaluated against amastigotes and IC_{50} and SI values were calculated. Two compounds **9** and **10** of low IC_{50} and SI above 10 were tested further for in vivo antileishmanial activity and the results are presented in Table 1.

The IC_{50} values for amastigotes of the test derivatives indicate that out of 17 synthesized compounds, 13 compounds exhibited high activity against *L. donovani* (IC_{50} 0.64–6.52 μg/mL), better than the reference drugs sodium stibogluconate (IC_{50} = 46.54 μg/mL) and paromomycin (24.79 μg/mL). The hydroxyl intermediates (**3a** and **3b**), showed either low inhibition or no inhibition. Further, conversion of hydroxyl group to aryloxy/benzyloxy moiety (compounds **4–13** and **22–28**, Table 1) resulted in enhancing the activity several folds (IC_{50} 0.64–6.52 μg/mL).

Among the tetrahydronaphthyl azole series (**4–13**) the compounds with benzyloxy moiety (**9**, **10** and **13**) appeared more active compare to the aryloxyl analogs exerting a strong inhibitory effect on the amastigote form of parasite, while three compounds (**9**, **10** and **13**) produced an interesting selective antiamastigote



Scheme 3. Reagents and conditions: (i) imidazole, abs. ethanol, 90 °C, 12 h; (ii) NaH (60% oil), substituted aryl or benzyl halide, DMF, 0 °C–rt, 4–5 h.

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

Sl. No.	Compd No.	In vitro assessment		[a]Cytotoxicity CC_{50} (μg/mL)	Selective index (SI) CC_{50}/IC_{50}	In vivo activity (dose-50 mg/kg × 5, ip) % inhibition ± SE
		Anti promastigote activity (% inhibition at 10 μg/mL)	Anti amastigote activity [#]IC_{50} (μg/mL)			
1	**3a**	60	>50	>100	2	
2	**3b**	90.42	NI	35.59	NA	
3	**4**	60	NI	52.46	NA	
4	**5**	94.89	4.53	21.43	4.73	
5	**6**	91.16	1.59	10.69	6.73	
6	**7**	100	6.52	32.13	4.92	
7	**8**	99.59	3.58	20.08	5.61	
8	[*]**9**	100	0.64	22.26,	34.78	83.33 ± 6.16
9	[*]**10**	100	1.34	15.46	11.53	32.10 ± 8.51
10	**11**	99.62	NI	>100	NA	
11	**12**	100	NI	>100	NA	
12	**13**	100	3.01	30.20	10.03	
13	**22**	99.80	NI	ND	NA	
14	**23**	98.56	2.23	8.27	3.70	
15	**24**	95.69	2.15	14.25	6.62	
16	**25**	99.00	5.07	22.13	4.36	
17	**26**	96.9	1.404	12.76	9.10	
18	**27**	89.57	4.03	27.16	6.73	
19	**28**	99.47	2.44	3.43	1.41	
20	SSG	946.52	46.54	297.38	6.38	92.20 ± 5.25
21	Paromomycin	123.92	24.79	53.33	2.15	46.7 ± 9.82

[#] IC_{50} >15 μg/mL = inactive; IC_{50} >5–15 μg/mL = moderately active; IC_{50} <5 μg/mL = highly active compounds.
[*] Compounds were picked up for in vivo evaluation; NI = no inhibition at 10 μg/mL; ND = not done; NA = not available; SSG = sodium stibogluconate.
[a] CC_{50} (cytotoxic concentration for 50% inhibition) is evaluated against J-774A-1 cell lines.

activity (SI >10). The aryloxy triazole derivatives **11** and **12** were found inactive whereas the benzyloxy triazole derivative **13** expressed good antiamastigote activity (IC_{50} = 3.01 μg/mL) with SI = 10.01, was slightly less active than its imidazole counterpart **10** (IC_{50} = 1.34 μg/mL and SI = 11.53).

Further, to investigate the SAR effect of phenyl ring, we also synthesized and evaluated a set of aryloxy cyclohexyl azoles (**22**–**28**). Though, these compounds displayed a strong inhibitory activity on the intracellular amastigote IC_{50} ranging from 1.40 to 5.07 μg/mL, but the selective index of all the compounds was below 10, revealing the presence of the phenyl rings is crucial for better activity profile.

The overall activity profile of compounds (**4**–**13**, **22**–**28**) 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 (except in compounds **4** and **22** where the CF_3 group at 4-position renders the molecule inactive). However, it is interesting to note that the introduction of NO_2 group at position 2 together with 4-CF_3 imparted increased selectivity (**8** and **25**). Similarly, the NO_2 group at position 4 (**5** and **23**) renders the molecule moderately active, while the presence of a fluorine atom at 2 position together with 4-NO_2 enhances the activity (**7** and **24**). Moreover, the presence of a CF_3 group at 2 position together with 4-NO_2 further confers increased selectivity (**6**). Among the benzyloxy analogues the 2,5-dichloro derivative **9** and 3-chloro-derivatives **10**, **13** and **26** displayed the highest selectivity index (34.78, 11.53, 10.03 and 9.10, respectively).

Two compounds (**9** and **10**) of SI above 10 were tested further for in vivo antileishmanial activity. Compound **9** the 2,5-dichlorobenzyloxy tetrahydronaphthyl imidazole, exhibited significant in vivo activity with 83.33% inhibition of parasite growth while compound **10** (2,5-dichlorobenzyloxy tetrahydronaphthyl triazole) displayed moderate activity with 32.10% inhibition. Thus compound **9** has shown superior in vivo activity than paromomycin and was less active than sodium stibogluconate. It is interesting to note that in both the series the tetrahydronaphthyl azoles (**4**–**13**) and cyclohexyl azoles (**22**–**28**) the highest activity (SI values) were shown by the compounds with either a 2,5-dichloro or a 3-chloro benzyloxy moiety. This finding indicates that benzyloxy moiety with chloro substituents should be investigated for the development of highly selective antileishmanial compounds.

These results clearly indicate that newly synthesized tetrahydronaphthyl azoles reported herein are promising compounds and provide useful model for further structural and biological optimization. Compound **9** displayed not only a lower (38–72 times) IC_{50} value than that of the reference drugs, but also 6- and 16-fold more selective (SI = 34.78) as compared to that of standard drugs sodium stibogluconate and paromomycin, respectively. The study opens up the possibility of advancing this new class of compounds as novel antileishmanial agents. Further studies on these compounds to optimize the efficacy is in progress in our laboratory.

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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.2011.01.026.

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