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Design, synthesis and biological evaluation of aryl pyrimidine derivatives as potential leishmanicidal agents ☆

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ABSTRACT

A series of substituted aryl pyrimidine derivatives was synthesized and evaluated in vitro for their antileishmanial potential against intracellular amastigotes of *Leishmania donovani* using reporter gene luciferase assay. Among all, 8 compounds showed promising IC_{50} values ranging from 0.5 to 12.9 μM. Selectivity indices (S.I.) of all these compounds are far better than reference drugs, sodium stibogluconate (SSG) and miltefosine. On the basis of good S.I., compounds were further screened for their in vivo antileishmanial activity against *L. donovani*/hamster model. Compounds **2d**, **4a** and **4b** have shown significant inhibition of parasitic multiplication that is 88.4%, 78.1% and 78.2%, respectively at a daily dose of 50 mg/kg × 5 days, when administered intraperitoneally. Compound **2d** is most promising one, which may provide a new lead that could be exploited as a new antileishmanial agent.

Leishmaniasis, a vector-borne parasitic disease, is a major cause of concern in developing countries. The disease is caused by more than 20 species of protozoan *Leishmania* and transmitted by the bite of female phlebotomine sand flies. Leishmaniasis has traditionally been classified into three major clinical forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) which differs in immunopathologies and degree of morbidity and mortality. VL caused by *Leishmania donovani* is the most severe form of leishmaniasis and is usually fatal in the absence of treatment.[1] Most of the first line drugs available for the treatment of leishmaniasis such as sodium stibogluconate, meglumine antimoniate, pentamidine, etc. cause serious side effects and toxicity.[2–4] Although drugs like amphotericin B and its lipid complex[5] are quite effective but they are expensive and out of reach of poor people.[6] Newly introduced first orally active drug miltefosine[7] is quite effective but shows teratogenic effects therefore its use is strictly prohibited in pregnant women. Moreover, resistance developing to first line agents is now increasing even up to an extent of >60% of the patient in some endemic areas.[1] Thus, new, safer, affordable drugs are urgently required for the treatment of leishmaniasis. Various classes of natural products such as licochalcone[8] A, quinoline alkaloids[9] have shown promising antileishmanial activity. Some biochemical targets trypanothione reductase,[10] cysteine peptidases,[11] sterol biosynthesis,[12] dihydrofolate reductase[13] (DHFR) and ornithine decarboxylase[14] are also under investigation to generate novel small molecules as lead compounds.[15] Pyrimidine[16] derivatives are known DHFR inhibitors[13] and have been evaluated for the design of novel antileishmanial agents. In continuation of our efforts to generate novel synthetic molecules as antileishmanial agents coupled with encouraging results of **1**, **2** and **3** (Fig. 1), we have designed and synthesized novel pyrimidine derivatives. The synthesized compounds were evaluated for their in vitro/in vivo antileishmanial activity against amastigote form of *L. donovani* and the results are reported in this manuscript.

α-Oxoketene dithioacetals of aromatic substrates are very useful synthons in the synthesis of variety of heterocyclic and carbocyclic compounds.[17] Considering their importance in the synthesis of various heterocycles, a novel series of 4-S-substituted pyrimidine derivatives (**2a**–**i**) and 4-N-substituted pyrimidine derivatives (**4a**, **b**) has been synthesized to assess their antileishmanial potential. The protocol for the synthesis of pyrimidine derivatives begins with the synthesis of substituted aryl ketene dithioacetals as described in our previous articles.[18a,b] The ethanolic solution of these aryl dithioacetals (**1a**–**i**) on reaction with guanidine hydrochloride at 130 °C gave the desired compounds (**2a**–**i**) in quantitative yield (Scheme 1). Synthesis of 4-N-substituted pyrimidine derivatives (**4a**, **b**) was performed in two steps. In the first step, dithioacetals (**1d**, **1g**) were

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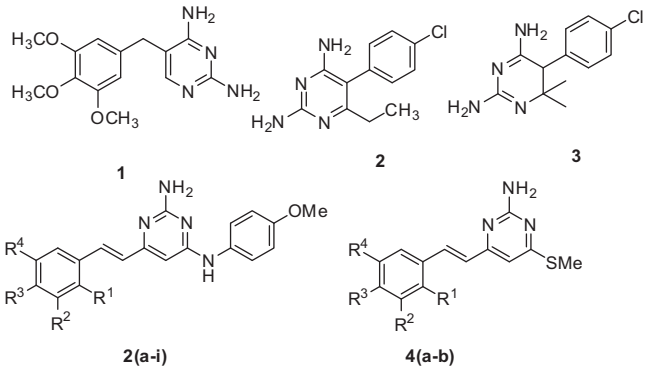
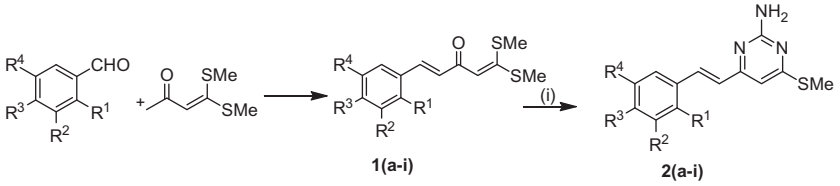


Figure 1. Structures of pyrimidines: trimethoprim **1**, pyrimethamine **2**, cycloguanil **3** and synthesized compounds **2**(**a**–**i**) & **4**(**a**–**b**) as antileishmanial agents.

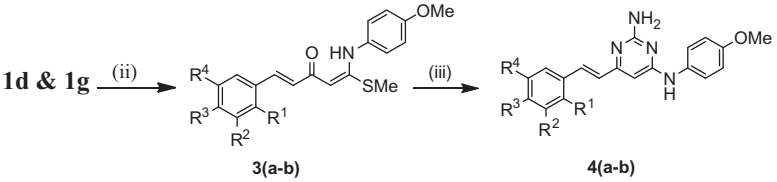
reacted with anisidine to yield intermediate compound (**3a**, **b**). In second step, these intermediates were allowed to react with guanidine hydrochloride at 135 °C to give the target compounds (**4a**, **b**) (Scheme 2).The structures of all the synthesized compounds were determined on the basis of their spectroscopic data and microanalysis. The IR spectra of the compounds, in general, exhibited the absorption band at around 3451 cm^{-1} indicating the presence of heterocyclic amino group, absorption band of methyl group appeared at around 2918 cm^{-1}. The ^1H & ^{13}C NMR spectra are consistent with the proposed structure. The ^1H NMR spectra of a prototype molecule **2d** displayed exchangeable N–H proton signal as a singlet at δ 4.90 ppm. The two proton of benzyloxy methylene showed a singlet at 5.09 ppm. and one multiplet at 7.39–7.47 (5H, C_6H_5) the aromatic protons showed two doublets at 6.98 and 7.49 ppm with coupling constant 9.00 Hz. The two *trans* olefinic protons showed doublet at 6.69 and 7.66 ppm with coupling constant 16.00 Hz. ^{13}C NMR spectra, exhibited the three aromatic carbons appeared as signals at 128 and 129 ppm for aromatic carbons and styryl carbons at 124 and 134 ppm. Almost similar patterns were observed in ^1H NMR and ^{13}C NMR spectra of the rest of the compounds of series.

All the synthesized compounds were screened for their in vitro antileishmanial activity against intramacrophagic *L. donovani* amastigotes[20,21]. Antileishmanial in vitro screening results of all the synthesized compounds (**2a**–**i** and **4a**, **b**) have been reported in Table 1. Among the 4-S-substituted pyrimidine derivatives (**2a**–**i**), compound **2d**, having benzyloxy aryl substitution, was found the most promising one with IC_{50} value of 2.0 μM and selectivity index (S.I.) of 188. Among the mono-, di- and tri-methoxy substituted aryl ring containing 4-S-substituted pyrimidine derivatives (**2a**–**c**), compound **2b**, having dimethoxy aryl substitution, have shown better antileishmanial in vitro activity with IC_{50} 2.0 μM. No regular order of decrease or increase in the activity among these three derivatives was observed. Rest of the compounds (**2e**, **g** and **i**) have shown good to moderate in vitro activity (IC_{50} value 12.9, 3.6, 6.3 μM, respectively). Almost, all the 4-S-substituted pyrimidine derivatives were found better than reference drugs against intracellular amastigotes in vitro. In order to assess the effect of –SMe group on the activity of 4-S-substituted pyrimidine derivatives, we undertook some modification on the pyrimidine ring. 4-O-substituted terpenyl pyrimidines displayed moderate antileishmanial activity profile.[19] Therefore, we decided to introduce a nitrogen group at the 4-position of pyrimidine ring which is required for the DHFR inhibition. The synthetic strategy adopted to have these changes is depicted in Scheme 2. The synthesized 4-N-substituted pyrimidine derivatives (**4a**, **b**) were screened for their antileishmanial in vitro activity. These 4-N-substituted pyrimidine derivatives displayed far better in vitro activity as compared to their 4-S-substituted analogs. All the synthesized compounds were found non-toxic to mammalian cell line (CC_{50} ranging from 57.8 to 375.9 μM) at activity concentration. All the synthesized 4-S- and 4-N-substituted pyrimidine derivatives, screened for their in vitro potential were also tested in vivo against *L. donovani*/hamster model at dose of 50 mg/kg × 5 days by intraperitoneal route. There were no toxic symptoms observed during treatment of leishmania infected animals. Among all, compound **2d** has shown very promising in vivo efficacy[22] with 88.4% inhibition of parasite multiplication which is comparable to the reference drug sodium stibogluconate (88.5% inhibition) and slightly less to the miltefosine (98.1% inhibition). However, compounds **2b**, **2c**, **2e, 2g** and **2i** have shown moderate in vivo inhibition (in the range of 33–55%) of parasitic growth as compared to the untreated control. Although majority of the synthesized compounds have shown better in vitro activity profile as compared to reference drugs but the same trend could not be resulted in in vivo efficacy of these compounds. To understand this discrepancy in both in vitro and in vivo results, further pharmacokinetic and pharmacodynamic studies are in progress in our lab and shall be communicated in our future communication.

In summary, synthesis and biological evaluation of these 4-S-substituted and 4-N-substituted pyrimidine derivatives led us to discover compound **2d** which showed very promising in vitro

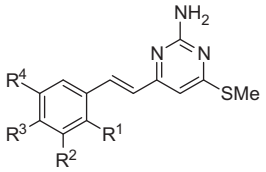


Scheme 1. Synthesis of pyrimidine derivatives (**2a**–**i**). Reagents and conditions: (i) guanidine hydrochloride in isopropanol, ethanol, heat in steel bomb, 130 °C for 24 h.

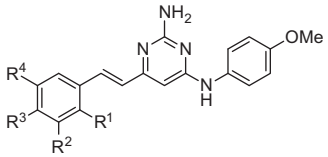


Scheme 2. Synthesis of pyrimidine derivatives (**4a** and **b**). Reagents and conditions: (i) prim. amines, Ethanol, reflux, 9h; (ii) guanidine hydrochloride, isopropanol, heat in steel Bomb at 135 °C, for 18 h.

Table 1
In vitro and in vivo antileishmanial activity and cytotoxicity results of synthetic pyrimidine derivatives **2a–i** and **4a**, **b**



2(a-i)



4(a-b)

Compd. No.	R^1	R^2	R^3	R^4	In vitro assessment		Selectivity Index (S.I.) CC_{50}/IC_{50}	In vivo activity (Dose- 50 mg/kg × 5 days, ip[b]) % Inhibition ± SD
					IC_{50} (μM)	CC_{50} (μM)		
2a	H	OCH3	OCH3	OCH3	>40 ± 0	ND*	NA*	NI*
2b	H	OCH3	OCH3	H	2.0 ± 0.2	124.8 ± 14.8	62	47.1 ± 28.7
2c	H	H	OCH3	H	9.1 ± 0.8	249.7 ± 7.0	27	54.4 ± 12.5
2d	H	H	OBn	H	2.0 ± 0.1	375.9 ± 5.1	188	88.4 ± 10.6
2e	H	H	OTHP	H	12.9 ± 1.2	248.2 ± 7.9	19	33.5 ± 29.4
2f	H	OCH3	OH	H	ND	ND	NA	ND
2g	H	H	OTHP	H	3.6 ± 0.7	121.8 ± 4.8	34	54.4 ± 13.2
2h	H	H	OH	H	ND	ND	NA	ND
2i	H	H	OSO_3Na	H	6.3 ± 0.6	301.1 ± 10.9	48	55.1 ± 10.5
4a	H	H	OBn	H	0.5 ± 0.1	57.8 ± 5.9	116	78.1 ± 17.7
4b	H	H	OTHP	H	2.7 ± 0.5	345.4 ± 19.6	128	78.2 ± 4.4
SSG[a]	—	—	—	—	59.8 ± 7.5	>400 ± 0	>7	88.5 ± 4.4
Miltefosine[c]	—	—	—	—	12.5 ± 0.9	54.7 ± 6.9	4	98.1 ± 1.0

IC_{50} and CC_{50} values are the mean ± SD of two independent experiments.
See References and notes section for experimental details.
The selectivity index (S.I.) is defined as the ratio of CC_{50} on vero cells to IC_{50} on *L. donovani* intramacrophagic amastigotes.
ND* = not done, NA* = not available, NI* = no inhibition.
[a] SSG = Sodium stibogluconate (40 mg/kg × 5 d, ip).
[b] ip = Intraperitonial.
[c] Miltefosine (30 mg/kg × 5 d, po) used as a reference drugs.

antileishmanial activity against intracellular amastigotes and have potential in vivo efficacy in the golden hamster model of VL. Moreover, it displayed no toxicity for macrophages and vero cells, and its high value of selectivity index was much better than other current antileishmanials. These findings revealed that these 4-S-substituted and 4-N-substituted pyrimidine derivatives can be very useful for further optimization work in antileishmanial chemotherapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.060.

References and notes

1. Singh, N.; Kumar, M.; Singh, R. K. *Asian Pacific J. Trop. Med.* **2002**, 485.
2. Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J.; Boelaert, M. *Nat. Rev. Microbiol.* **2007**, *5*, 873.
3. Graebin, C.; Uchoa, F. D.; Bernardes, L. S. C.; Campo, V. L.; Carvalho, I.; EiflerLima, V. L. *Anti-infective Agents Med. Chem.* **2009**, *8*, 345.
4. Coler, R. N.; Reed, S. G. *Trends Parasitol.* **2005**, *21*, 244.
5. Goldsmith, D. R.; Perry, C. M. *Drugs* **1905**, *2004*, 64.
6. Online Available from: http://whqlibdoc.who.int/trs/WHOTRS_949_eng.pdf.
7. Croft, S. L.; Seifert, K.; Duchene, M. *Mol. Biochem. Parasitol.* **2003**, *126*, 165.
8. Chen, M.; Zhai, L.; Christensen, S. B.; Theander, T. G.; Kharazmi, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2023.
9. Yates, C. *Curr. Opin. Invest. Drugs* **2002**, *3*, 1446.
10. Fries, D. S.; Fairlamb, A. H., In D. J. Abraham (Ed.), *Burger's Medicinal Chemistry and Drug Discovery*, Vol. 5, 6th ed., Wiley, pp 1033–1087.
11. Sajid, M.; Mckerro, J. R. *Mol. Biochem. Parasitol.* **2002**, *120*, 1.
12. Roberts, C. W.; Leod, R. M.; Rice, D. W.; Ginger, M.; Chance, M. L.; Goad, L. J. *Mol. Biochem. Parasitol.* **2003**, *126*, 129.
13. Gilbert, I. H. *Biochem. Biophys. Acta* **2002**, *1587*, 249.
14. Muller, S.; Coombs, G. H.; Walter, R. D. *Trends Parasitol.* **2001**, *17*, 242.
15. Croft, S. L.; Coombs, G. H. *Trends Parasitol.* **2003**, *11*, 502.
16. Chandra, N.; Ramesh; Ashutosh; Goyel; Suryawanshi, S. N.; Gupta, S. *Eur. J. Med. Chem.* **2005**, *40*, 552.
17. Suresh, J. R.; Kumar, U. K. S.; Junjappa, H. I.; Junjappa, H. *Tetrahedron* **2001**, *57*, 781.
18. (a) Pandey, S.; Surawanshi, S. N.; Gupta, S.; Srivastava, V. M. L. *Eur. J. Med. Chem.* **2005**, *40*, 751; (b) Kumar, S.; Tiwari, A.; Suryawanshi, S. N.; Mittal, M.; Vishwakarma, P.; Gupta, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6728.
19. Pandey, S.; Surawanshi, S. N.; Gupta, S.; Srivastava, V. M. L. *Eur. J. Med. Chem.* **2004**, *39*, 969.
20. *In vitro antileishmanial assay:* In order to assess the activity of aryl pyrimidines against amastigote stage of the parasite, the murine macrophage cell line (J-774A.1) infected with luciferase-transfected promastigotes was used. Briefly, cells (4×10^3/100 μL) were seeded in 96-well plates. After 24 h incubation in CO_2 incubator, cells were infected with promastigotes (4×10^4/100 μL). Promastigotes invade the macrophage and are transformed into amastigotes. The test compounds in appropriate concentration (0.62–40 μM) were added and plates were incubated in CO2 incubator for 72 h. After incubation, the compound-containing medium was aspirated and 50 μL PBS was added in each well and mixed with an equal volume of steady Glo® reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer (Pandey et al., 2007). The values are expressed as relative luminescence units (RLU). Data were transformed into graphical program (Excel) and the inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls. (Pandey, Susmita; Suryawanshi, S.N.; Nishi; Goyal, Neena; Gupta, Suman. *Eur. J. Med. Chem.* **2007**, *42*, 669).
21. *Cytotoxicity assay:* The cytotoxicity of compounds was determined by following the method of Mosmann (1983) with some modifications. The monkey kidney fibroblast cells (Vero cell line) were incubated with test compounds for 72 h and MTT was used as reagent for detection of viable cells. Fifty percent cytotoxic concentration (CC_{50}) values were estimated as described by Huber & Koella (1993). (Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55) (Huber, W.; Koella, J. C.; *Acta Trop.* **1993**, *55*, 257).
22. *In vivo antileishmanial assay:* The in vivo antileishmanial activity was carried out in golden hamsters infected with *L. donovani* as described by Kumar et al. (2012). Briefly, golden hamsters (inbred strain) of either sex weighing 40–45 g were infected intracardiacally with 1×10^7 amastigotes per animal. Pre-treatment spleen biopsies were performed after 15–20 days to assess the degree of infection. The animals with +1 infection (5–15 amastigotes/100 liver

cell nuclei) were included in the in vivo trials. After establishment of infection, drug treatment by either intraperitoneal (ip) or per oral (p.o.) route was initiated for 5 consecutive days. Sodium stibogluconate (SSG) and miltefosine are used as reference drugs. Post-treatment biopsies were done on day 7 after the last dose of drug administration and amastigote counts are assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, and also the initial count in treated animals was compared and the efficacy was expressed in terms of per cent inhibition (PI) (Kumar, S; Tiwari, A; Suryawanshi, S.N.; Mittal, M; Vishwakarma, P. and Gupta, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6728).