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Synthesis and biological evaluation of novel 4-(hetero) aryl-2-piperazino quinazolines as anti-leishmanial and anti-proliferative agents ☆

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ABSTRACT

A series of new class of 4-(hetero)aryl-2-piperazino quinazolines were synthesized and assessed for in vitro activity against extracellular promastigotes and intracellular amastigotes of *Leishmania donovani*. Among the compounds evaluated, compound **4bb** and **4cb** showed the selectivity index (SI) value > 8.03 and 4.21, respectively, which is promising as compared with sodium stilbogluconate (SSG) and pentamidine with the SI of 6.38 and 2.07, respectively. The synthesized compounds were also tested for anti-proliferation activity in a panel of mammalian cell lines. Compound **4aa** which is quite inactive and **4ab** which is hardly selective in anti-leishmanial assay are found to have significant activity in anti-proliferative assay.

Diseases caused by protozoal parasites such as leishmaniasis have an overwhelming impact on public health throughout the world, particularly in the tropics and subtropics. Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* presenting several forms of the disease such as cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL). The later form caused by the parasite *Leishmania donovani* can be fatal if untreated. The disease is transmitted in vertebrate after inoculation of promastigotes by the bite of the infected female phlebotomine sandfly.[1] Subsequently, the promastigotes are quickly phagocytized by the macrophages of the host and further changes to the amastigotes form. The clinical manifestation of the disease is a consequence of the multiplication of the amastigotes inside the macrophages.

According to the World Health Organization's recent reports 88 countries are affected by leishmaniasis, comprehending 12 million infected people worldwide, with approximately 350 million people at risk. The incidence is increasing worldwide, with 1–2 million new cases registered annually.[2] For many decades, the first choice of drug treatment for leishmaniasis has been the pentavalent antimonials, sodium stilbogluconate (Pentostam®) and meglumine antimonate (Glucantime®) despite the fact that they exhibit renal and cardiac toxicity. Alternative drugs pentamidine and amphotericin B, have not experienced widespread use due to toxicity and cost.[3] Miltefosine, originally developed as an anticancer agent, has been found to be highly effective against leishmaniasis but possess severe gastrointestinal problems.[4] As the leishmaniasis chemotherapy is still inefficient, therefore the immediate action is to develop new agents, more potent and selective for treating this increasing parasitosis. A great number of natural and synthetic compounds comprising divergent chemical structures have been tested in the past few years in antileishmanial assays and among these, quinazoline class of compounds have also been reported to possess antiprotozoal activities through DHFR inhibition.[5]

Biaryls and biheteroaryls are significant building blocks in a large number of natural products[6] and pharmocophores in a variety of biologically active compounds.[7] Naturally occurring napthylisoquinoline alkaloids ancistrotanzanine B and ancistroealaine A are potent antileishmanial agents.[8] These important biheteroaryls were earlier synthesized by metal catalyzed reactions which require multistep reactions,[9] involving toxic or expensive catalysts. Alternately, anhydrous $AlCl_3$ mediated heteroarylation of arenes and heteroarenes could be a cheap, convenient and straightforward synthetic methodology to access the biheteroaryls. In our ongoing program devoted to the synthesis of bioactive molecules, we had focused on quinazolines and chalcones derivatives. Based on these observations we hypothesized and synthesized some substituted biheteroaryl compounds containing quinazoline motif in search of potential antileishmanial agents. In the continuation of our studies,[10] herein we described $AlCl_3$ mediated synthesis of some novel 4-(hetero) aryl-2-piperazino quinazolines and their in vitro evaluation against promastigotes and amastigotes of *Leishmania donovani* and for anti-proliferative activity against various

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cancer cell lines. 2-chloro-4-(hetero)aryl quinazolines, 2-chloro-4-(1*H*-indol-3-yl)-quinazoline (**3a**), 2-chloro-4-(2,4,6-trimethoxy-phenyl)-quinazoline (**3b**), 2-chloro-4-(2,4-dimethoxy-phenyl)-quinazoline (**3c**) and 2-chloro-4-(1-methyl-1*H*-indol-3-yl)-quinazoline (**3d**) were prepared by a novel method of $AlCl_3$ mediated selective arylation by different arenes and heteroarenes at position 4 of 2,4-dichloroquinazoline **1**.

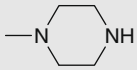
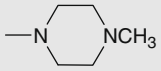
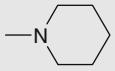
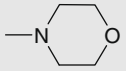
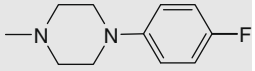
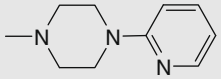
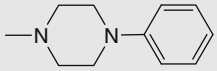
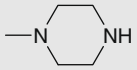
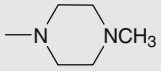
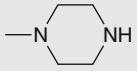
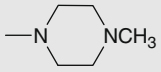
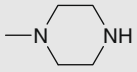
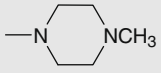
When 1 equiv of arene/heteroarene was reacted with 1 equiv of 2,4-dichloroquinazoline **2**[11] in the presence of 1.2 equiv of anhydrous aluminum chloride using dichloroethane as solvent 2-chloro-4-(hetero)aryl quinazolines **3**[12] were formed exclusively in excellent yields (Scheme 1). To synthesized 2,4-disubstituted quinazolines compounds (**4aa–db**),[13] 2-chloro-4-(hetero)aryl quinazolines were further reacted with different cyclic amines in 1,4-dioxane in presence of triethylamine to afford the desired compounds (Table 1). Reactions in dioxane proceeds much more smoothly and gave the desired product in quantitative yield unlike previously reported methods.[14]

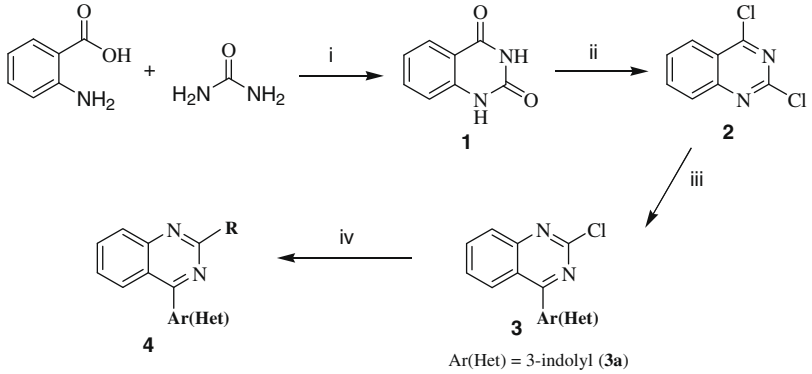
Antileishmanial activity. The *L. donovani* promastigotes (MHOM/IN/Dd8; originally obtained from Imperial college, London) were transfected with firefly luciferase gene and the transfectants were maintained in medium 199 (Sigma chemical Co., USA) supplemented with 10% fetal calf serum (GIBCO) and 1% penicillin (50 U/ml), streptomycin (50 μg/mL) solution (Sigma) under pressure of G418 (Sigma).[15] The compounds were assessed in vitro against transgenic *L. donovani* promastigotes and intracellular amastigotes as described by Ashutosh et al.[15] at various concentrations using sodium stilbogluconate and pentamidine as a reference drugs. Cytotoxicity responses were assessed by MTT assay using mouse macrophage cell line (J-774-A-1).[16] IC_{50} of antileishmanial activity was calculated by Probit analysis.[17]

Anti-proliferative activity. The anti-proliferative activity of the compounds were determined in twofold serial dilutions against four human cancer cell lines, DU145 (Prostate carcinoma), MCF-7 (Breast adenocarcinoma), KB (Oral epidermal carcinoma) and C33A (Cervical carcinoma) and a non cancerous VERO mammalian cell line upto a highest concentration of 50 μg/mL in an MTT assay.[18]

Results and discussion. The in vitro biological activities of 2,4-disubstituted quinazolines have shown encouraging results. IC_{50} and SI values of synthesized quinazolines against promastigotes and intracellular amastigotes have been displayed in Table 2. Amongst the 4-indolyl quinazolines **4ab–4ag**, **4ab** with *N*-methyl piperazine as substituent at 2-position of quinazoline motiff exhibited least IC_{50} value of 0.68 μg/mL in the series. Surprisingly, replacement of *N*-methyl piperazine with more lipophilic piperidine moiety as in **4ac** led to decrease in anti-promastigotic activity

Table 1

Compound	Ar(Het)–	R–
4aa	Indole	
4ab	Indole	
4ac	Indole	
4ad	Indole	
4ae	Indole	
4af	Indole	
4ag	Indole	
4ba	2,4,6-Tri OCH_3–C_6H_2	
4bb	2,4,6-Tri OCH_3–C_6H_2	
4ca	2,4-DiOCH_3–C_6H_3	
4cb	2,4-DiOCH_3–C_6H_3	
4da	1-CH_3–indole	
4db	1-CH_3–indole	



Scheme 1. Reaction conditions: (i) 135–140 °C, 3 h; (ii) $POCl_3$, *N*,*N*-dimethyl aniline, reflux, 5 h; (iii) **Ar(Het)**-H, $AlCl_3$, dry DCE, N_2, 75–80 °C, 3 h; **3a** (80.3%), **3b** (85.0%); 2 h, **3c** (83.0%), **3d** (80.6%); (iv) **R**–H (1.2 mmol), **3a–d** (1 mmol), TEA (2 mmol), dioxane (5 mL), 90–95 °C.

Table 2
In vitro anti-leishmanial activity of compounds **4aa–db**

Compounds	In-vitro screening		Cytotoxicity CC_{50} (μg/mL)	Selectivity index[a] (SI)
	Anti-promastigote activity IC_{50} (μg/mL)	Anti-amastigote activity (MQ/amast. model) IC_{50} (μg/mL)		
4aa	0.56	Toxic	3.11	—
4ab	0.68	4.17	7.27	1.74
4ac	>10	ND	—	—
4ad	1.80	25.14	50.07	1.99
4ae	1.72	12.32	23.41	1.90
4af	2.66	8.02	1.35	0.17
4ag	2.37	4.73	5.67	1.19
4ba	0.25	Toxic	—	—
4bb	6.20	12.45	>100	>8.03
4ca	0.624	5.11	3.98	0.78
4cb	3.79	4.28	18.00	4.21
4da	0.28	12.50	16.01	1.28
4db	0.62	Toxic	1.03	—
Sodium stilbogluconate (SSG)	940	53.62	297.38	6.38
Pentamidine	0.643	12.11	25.15	2.07

ND: not determined.
[a] Selectivity index (SI) defined by the ratio $CC_{50\,(J\text{-}774\ A\text{-}1\ cells)}/IC_{50\,(\textit{Leishmania}\ amastigotes)}$.

with IC_{50} value more than 10 μg/mL. With 4-(2,4,6-trimethoxyphenyl), 4-(2,4-dimethoxyphenyl) and 4-(1-methyl indole) quinazolines reverse trend were exhibited in the anti-promastigote activity with variation of substituents at 2-position as 4-methyl piperazinyl derivatives (**4bb**, **4cb** and **4db**) have lower anti-promastigote activity compared to piperazinyl derivative (**4ba, 4ca** and **4da**), but no similar trends were exhibited in their anti-amastigote activities with variation of substituent in general at 2-position of 4-aryl quinazolines. The compounds **4af** and **4db** exhibited least CC_{50} values at 1.35 μg/mL and 1.03 μg/mL respectively. **4bb** with least cytotoxicity (CC_{50} value above 100 μg/mL) has selectivity index above 8.03 which is comparable with that of sodium stilbogluconate. From the IC_{50} and SI values for intracellular amastigotes of the test derivatives indicate that two compounds, 2-(4-methyl-piperazin-1-yl)-4-(2,4,6-trimethoxyphenyl)-quinazoline **4bb** and 4-(2,4-dimethoxy-phenyl)-2-(4-methyl-piperazin-1-yl)-quinazoline **4cb** exhibited higher activity against *L. donavani* (IC_{50} = 12.45 and 4.28 μg/mL, respectively) as compared to reference drugs sodium stilbogluconate (IC_{50} = 53.62 μg/mL) and pentamidine (IC_{50} = 12.11 μg/mL) and thus represent the interesting leads as antileishmanial agents.

In anti-proliferative assay, compounds **4aa** and **4ab** showed the proliferative inhibition in KB (Oral squamous cell carcinoma) cell line. The compound **4aa** showed the IC_{50} values of 4 μg/mL and 8.2 μg/mL in KB and C-33A respectively, and compounds **4ab** showed the IC_{50} values of 4.9 μg/mL and 6.0 μg/mL in KB and MCF-7, respectively. The activity profile is shown in Table 3.

From the structural activity relationship it is found that the presence of free NH group in form of 1-H-indole as the substituent at position 4 together with piperazine or 4-methyl-piperazine at position 2 of quinazoline enhances the anti-proliferative activity. Compound **4aa** which is quite inactive and **4ab** which is hardly selective in anti-leishmanial assay are found to have significant IC_{50} values in anti-proliferative assay. However, the replacement of indole moiety with an aryl ring in form of 2,3-dimethoxy benzene (**4cb)** and 2,3,5-trimethoxy benzene (**4bb)** together with *N*-methyl group remarkably enhances the antileishmanial activity.

In conclusion, 4-(hetero) aryl-2-substituted piperazino quinazolines were synthesized for the first time and their in vitro antileishmanial and antiproliferative activities were evaluated. Most of the synthesized compounds exhibited moderated to good activity

Table 3
Inhibition of proliferation of the compounds **4aa–db**[a]

Compounds	IC_{50} (μg/mL)				
	DU145	MCF-7	C-33A	KB	Vero
4aa	12.5	14.1	8.2	4.0	8.9
4ab	20.8	6.0	11.5	4.9	22.3

[a] Compounds **4ac–db** were also tested against these cell lines but had an IC_{50} value superior to 50 μg/mL.

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References and notes

1. (a) Desjeux, P. *World Health Stat. Q* **1992**, *45*, 267; (b) Desjeux, P. *Trans. R. Soc. Trop. Med. Hyg.* **2001**, *9*, 239; (c) Guerin, P. J.; Olliaro, P.; Sundar, S.; Boelaert, M.; Croft, S. L.; Desjeux, P.; Wasunna, M. K.; Bryceson, A. D. *Lancet Infect. Dis.* **2002**, *2*, 494.
2. (a) *Tropical Disease Research: Progress 1999–2000*; World Health Organization: Geneva, **2001**.; (b) Gelb, M. H.; Hol, W. G. J. *Science* **2002**, *297*, 343; (c) Trouiller, P.; Torreele, E.; Olliaro, P.; White, N.; Foster, S.; Wirth, D.; Pécoul, B. *Trop. Med. Int. Health* **2001**, *6*, 945.
3. Mcgregor, A. *Lancet* **1998**, *351*, 575.
4. Sangraula, H.; Sharma, K. K.; Rijal, S.; Dwivedi, S.; Koirala, S. *J. Assoc. Physians India.* **2003**, *51*, 686.
5. Berman, J. D.; King, M.; Edwards, N. *Antimicrob. Agents Chemother.* **1989**, *33*, 1860.
6. Bringmann, G.; Ochse, M.; Schupp, O.; Tasler, S. *Progress in the Chemistry of Organic Natural Products*; Springer: Wien, 2001. pp. 82.
7. (a) Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Chem. Rev.* **2003**, *103*, 893; (b) Wang, G. T.; Wang, S.; Gentles, R.; Sowin, T.; Leitza, S.; Reilly, E. B.; von Geldern, T. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 195.
8. Bringmann, G.; Hamm, A.; Schraut, M. *Org. Lett.* **2003**, *5*, 16, 2805.
9. (a) Liu, W. J.; Xie, Y. X.; Liang, Y.; Li, J. H. *Synthesis* **2006**, 860; (b) Marion, N.; Navarro, O.; Mei, J.; Stevens, E. D.; Scott, N. M.; Nolan, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 4101; (c) Lützen, A.; Hapke, M. *Eur. J. Org. Chem.* **2002**, 2292; (e) Mee, S. P. H.; Lee, V.; Baldwin, J. E. *Angew. Chem.* **2004**, *116*, 1152.
10. Diwedi, A. P.; Kumar, S.; Varshney, V.; Singh, A. B.; Srivastava, A. K.; Sahu, D. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2301.
11. Curd, F. H. S.; Landquist, J. K.; Rose, F. L. *J. Chem. Soc.* **1947**, 775.
12. *Typical procedure for 4-(hetero)aryl quinazolines* **3a–d**: To the solution of 2,4-dichloroquinazoline (1 mmol) in dichloroethane (10 mL), was added $AlCl_3$ (1.2 mmol) under N_2 atmosphere and allowed to stirred for 2–5 min. Nucleophilic substrates, Ar(Het)H (1 mmol) were added and allowed to stirred for 2–3 h. After completion of the reaction, the reaction mixture was cooled at room temperature and poured into ice-cold water (100 mL) with continuous stirring for 15–20 min. The crude products were isolated by extracting with ethyl acetate (10 mL × 3). The organic phase was separated and washed with brine, dried over sodium sulphate and evaporated under vacuum. The crude products obtained were further purified by silica gel column chromatography using ethyl acetate–hexane mixture; Compound **3c**: Yield: 83.0%; mp: 140–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (s, 3H), 3.90 (s, 3H), 6.61 (d, *J* = 2.2 Hz, 1H), 6.67 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.49–7.55 (m, 1H), 7.77 (dd, *J* = 8.0 and 0.8 Hz, 1H), 7.85–7.90 (m, 1H), 7.99 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.89, 56.01, 99.23, 105.64, 123.45, 127.70, 128.03, 128.65, 132.68, 135.00, 152.52, 158.66, 163.26, 171.21; ν_{max} (KBr, cm^{-1}): 1611, 1209, 1161; FAB (*m*/*z*): 300, $[M+H]^+$ 301; HRMS-EI: found: 300.0662, calcd: 300.0665. Compound **3d**: Yield: 80.3%; mp: 142–144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 3H), 7.28–7.36 (m, 2H), 7.38–7.43 (m, 1H), 7.55–7.61 (m, 1H), 7.78 (s, 1H), 7.84–7.89 (m, 1H), 7.97 (dd, *J* = 8.3 and 0.7 Hz, 1H), 8.17–8.20 (m, 1H), 8.41 (dd, *J* = 8.6 and 0.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 33.68 (CH_3), 109.96 (CH), 112.19 (C), 121.53 (C), 122.04 (CH), 122.12 (CH), 123.52 (CH), 127.00 (C), 127.29 (CH), 127.46 (CH), 128.05 (CH), 133.93 (CH), 134.33 (CH), 137.65 (C), 152.90 (C), 157.37 (C), 166.15 (C); ν_{max} (KBr, cm^{-1}): 1563, 1347, 735; ES–MS (*m*/*z*): $[M+H]^+$ 294; HRMS-EI: found: 293.0689, calcd: 293.0719.
13. *General procedure for 2,4-disubstituted quinazolines* **4aa–db**: A mixture of 2-chloro-4-(hetero)aryl quinazolines **3a–d** (1 mmol), amines, RH (1.2 mmol) and triethylamine (2 mmol) in dioxane (5 mL) was heated at 90–95 °C for 2–3 h. After completion of the reaction, the solvent was evaporated and added water

(5 mL) and extracted with ethyl acetate (5 mL × 3) to afford the crude product which was further purified by silica gel column chromatography using 3–5% methanol–chloroform mixture. Compound **4db**: Yield: 96.3%; mp: 212–214 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.54–2.58 (t, 4H), 3.92 (s, 3H), 4.07–4.10 (t, 4H), 7.14–7.19 (m, 1H), 7.24–7.29 (m, 1H), 7.32–7.37 (m, 1H), 7.42 (d, J = 7.9 Hz, 1H,), 7.63–7.65 (m, 3H), 8.12–8.18 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 33.53, 44.19, 46.49, 55.44, 109.83, 113.43, 118.43, 121.36, 122.03, 122.16, 123.08, 126.45, 127.29, 127.49, 132.66, 133.31, 137.70, 153.83, 159.11, 164.03; ν_{max}(KBr, cm^{-1}): 1545, 754; ES–MS (m/z): [M+H]$^+$ 358; HRMS-EI: found: 357.1993, calcd: 357.1953; Compound **4bb**: Yield: 96.6%; mp: 139–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, NCH_3), 2.51 (t, 4H), 3.64 (s, 6H, 2 × OCH_3), 3.88 (s, 3H, OCH_3), 4.00 (t, 4H), 6.25 (s, 2H), 7.01–7.06 (m, 1H), 7.33 (d, 1H, J = 8.5 Hz), 7.54- 7.61 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 44.25 (2 × CH_2), 46.51 (2 × CH_2), 55.36 (NCH_3), 55.66 (OCH_3), 56.19 (2 × OCH_3), 91.37 (2 × CH), 109.03 (C), 120.49 (C), 122.06 (CH), 125.97 (CH), 127.32 (CH), 133.32 (CH), 152.59 (C), 159.24 (2 × C), 159.41 (C), 162.18 (C), 166.63 (C); ν_{max} (KBr, cm^{-1}): 1605, 1546, 1132; ES–MS (m/z): [M+H]$^+$ 395; HRMS-EI: found: 394.2006, calcd: 394.2005.

14. Ram, V. J.; Farhanullah; Tripathi, B. K.; Srivastava, A. K. *Bioorg. Med. Chem.* **2003**, *11*, 2439.
15. Ashutosh, G. S.; Ramesh, S. S.; Goyal, N. *Antimicrob. Agetns Chemother.* **2005**, *49*, 3776.
16. Huber, W.; Koella, J. C. *Acta Tropica.* **1993**, *55*, 257.
17. Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press, 1971.
18. Mosmann, T. *J. Immunol Methods* **1983**, *6*, 55.