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Design and synthesis of novel substituted quinazoline derivatives as antileishmanial agents

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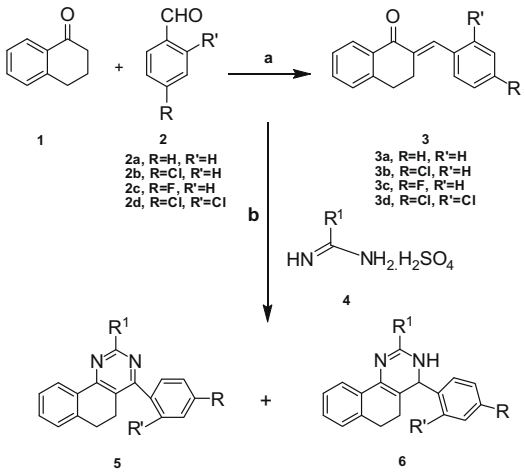
ABSTRACT

4-(Substituted-benzylidine)-2-substituted-5,6-dihydrobenzo[*h*]quinazoline (**5a–p**) and 4-(substituted-benzylidine)-2-substituted-3, 4, 5, 6-tetrahydrobenzo[*h*]quinazoline (**6a–p**) have been synthesized from 2-(substituted-benzylidine)tetralone-1(**3a–d**) and several substituted guanidine sulfates(**4a–d**).These compounds were tested for their in vitro antileishmanial activity. The compounds **6i, 6f, 6g** show promising antileishmanial activity against *Leishmania donovani.*

Leishmaniasis is a parasitic disease caused by different species of genus Leishmania, protozoan which is transmitted by an insect vector phleobotomine sandfly to humans. Leishmania has been classically divided into cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL), which can be fatal when untreated. The disease is endemic in many tropical and subtropical regions of the world, with 350 million people at risk.[1] Around 12 million people are affected by the disease in which 1.0–1.5 million cases per annum of cutaneous leishmaniasis, 0.5 million cases of the fatal visceral leishmaniasis.[2]

With the spread of HIV, visceral leishmaniasis has become increasingly prevalent. Reactivation of asymptomatic or previously healed leishmaniasis is common when there is co infection of AIDS with leishmaniasis. There is still no effective vaccine for Kala-azar and chemotherapy remains the most effective control measure. Meglumine antimoniate (Glucantine) and sodium stibogluconate (Pentostam) are the first line drugs for the treatment of leishmaniasis. Pentamidine and Amphotericin B lipid complex[3] are the second line drugs in which Amphotericin B and its lipid complex are quiet effective. Miltefosine,[4] newly developed first oral drug is effective but can not be given to pregnant women. Although new drugs have been available in recent years for the treatment of visceral leishmaniasis, treatment of leishmaniasis suffers from problems of drug resistance and severe toxicity. In view of the above, the search for the new molecular structures which would have high tolerance limit as well as no toxicity, constitutes the major thrust area of research on leishmaniasis. Natural products are being explored to generate new leads in the chemotherapy of leishmaniasis.[5] Compounds of both synthetic and natural origin comprising a diverse group of chemical structures have been reported as antileishmanial agents. These include mostly the nitrogen heterocycles such as quinolines,[6] acridines,[7] phenothiazines,[8] pyrimidines,[9] purines,[10] bis-benzamidines,[11] pyrazolo[3,4*b*]pyridine,[12] benzothiazoles[13] and imidazolidine.[14] Other classes of compounds include buparvaquone-oxime,[15] bisbenzamidines,[16] chalcones,[17] quinines,[18] amino acid esters and amides,[19] amino



Scheme 1. Reagents and conditions: (a) 5% alcoholic KOH, ethanol, 0 °C, rt, 2 h; (b) substituted guanidine sulfates **4**, *t*-BuOK, methanol, reflux, 24 h.

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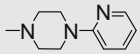
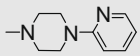
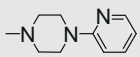
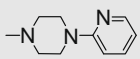
Table 1
Antileishmanial in vitro activity against luciferase–promastigote system of dihydroquinazolines **5a–p** and tetrahydroquinazolines **6a–p** against *L. donovani*

R	R′	R^1	Compd **5**	Mean % inhibition (at 10 μg/ml) ± S.D. (n = 2) promastigote	Compd **6**	Mean % inhibition (at 10 μg/ml) ± S.D. (n = 2) promastigote
H	H		**5a**	71.5 ± 0.84	**6a**	99.9 ± 0.07
H	H		**5b**	91.8 ± 1.48	**6b**	99.8 ± 0.14
H	H		**5c**	74.7 ± 1.20	**6c**	99.8 ± 0.28
H	H		**5d**	62.2 ± .70	**6d**	99.4 ± 0.49
F	H		**5e**	64.8 ± 0.49	**6e**	99.6 ± 0.21
F	H		**5f**	47.8 ± 1.48	**6f**	99.9 ± 0.07
F	H		**5g**	61.8 ± 1.55	**6g**	99.9 ± 0.14
F	H		**5h**	44.9 ± 1.34	**6h**	68.2 ± 0.98
Cl	H		**5i**	74.8 ± 0.84	**6i**	98.7 ± 0.49
Cl	H		**5j**	53.5 ± 1.62	**6j**	99.6 ± 0.42
Cl	H		**5k**	58.3 ± 1.06	**6k**	96.3 ± 0.70
Cl	H		**5l**	51.4 ± 1.13	**6l**	99.9 ± 0.07
Cl	Cl		**5m**	93.1 ± 3.11	**6m**	99.7 ± 0.42
Cl	Cl		**5n**	95.2 ± 0.77	**6n**	99.7 ± 0.35
Cl	Cl		**5°**	77.9 ± 0.84	**6o**	99.8 ± 0.21
Cl	Cl		**5p**	15.7 ± 1.62	**6p**	99.9 ± 0.14
			SSG	[a]		
			Pentamidine	[b]		

[a] SSG (sodium stilbogluconate) shows no inhibition in promastigotes at 10 μg/ml.
[b] Pentamidine shows 100% inhibition in promastigotes at 10 μg/ml, All compounds were confirmed by MS (ES+) analysis.

alcohols,[20] alkyl phospholipids,[21] ether phospholipids[22] sulfanilamides,[23] artemisinin[24] and certain platinum complexes.

Biochemical targets are also under investigation[25] in which Dihydrofolate reductase (DHFR)[26] is being used to design the novel compounds. DHFR has been a successful target for antimalarials (pyrimethamine, cycloguanil), antibacterial (trimethoprim) and anticancer (methotrexate). A number of compounds having pteridine, quinazoline and pyrimidine moieties are reported to be potent inhibitors of DHFR in Leishmania. Based on these observations we hypothesized to synthesize dihydro and tetrahydroqui-

nazolines and screen them for their antileishmanial activity. Coombs group[27,28] investigated some 5-substituted 2,4-diaminopyrimidines that were good inhibitors of *Leishmania mexicana* DHFR in vitro model. However, in a mouse study, the compounds were found toxic. In this communication, we report the quinazoline based novel compounds having antileishmanial activity.

The synthetic strategy for the quinazoline-based compounds has been depicted in Scheme 1. Tetralone-1, on reaction with various aldehydes **2a–d** in alcoholic solution in the presence of sodium or potassium hydroxide furnished benzylidenes[29] **3a–d**. These benzylidenes **3a–d** were then subjected to cyclization by treating them with guanidine sulfates[30] **4a–d**(R^1 = cyclic amines) in the presence of potassium tertiary butoxide in methanol under reflux for 24 h. As confirmed by HPLC and TLC analysis, the crude products were the mixture of two classes of compounds.

Of these two types of product, tetrahydroquinazolines **6** had moderate stability. After purification it had a tendency to undergo slow oxidation when kept in methanolic solution to dihydroquinazoline **5** on exposure to air and high temperature.[†] This led us to treat the crude product mixtures comprising **5** and **6** with DDQ aiming to exclusively get dihydroquinazolines **5**. However, addition of oxidizing agent failed to facilitate the conversion of **6** to **5**. All the synthesized compounds were well characterized by spectroscopic methods such as IR, mass, NMR and elemental analysis.

The in vitro efficacy of the synthesized compounds on promastigotes and amastigotes of *Leishmania donovani* were assessed by a previously described method.[31] The antileishmanial activity of dihydro (**5a–p**) and tetrahydro (**6a–p**) quinazoline derivatives against a clinically derived strain of *L. donovani* is shown in Table 1 while compounds having promastigote inhibition more than 80% were screened against amastigotes and their IC_{50} was calculated as shown in Table 2. The Data suggests that tetrahydroquinazoline derivatives represent interesting leads as antileishmanial agents. All the tetrahydroquinazoline (**6a**–**p**) derivatives showed close or equal to 100% inhibition against promastigotes except **6h** (68.9%). However, the corresponding dihydroqinazoline derivatives (**5a**–**p**) generally showed significantly lower inhibition (14.7–94.7%) with **5n** being the most active compound. It was observed that the compounds having R^1 as pyridyl piperazinyl group **5d, 5h, 5l, 5p, 6d** and **6h** possessed comparatively poor activities in both dihyro and tetrahydroquinazolines. This observation shows that when R and R′ are identical, the bigger ortho- substituent R^1 led to less active compounds **5d, 5p** (as compared 1-pyridin-2-yl-piperazine with other cyclic amino groups) indicating that cyclic amino group R^1 have impact on the activity. It was also noticed that when the para-substituent R is a Cl moiety, the e-withdrawing ortho-substituent (R′ = Cl) resulted in more active compounds than the corresponding unsubstituted analogs (comparing **5m–o** with **5i–k**) suggesting the ortho-position R′ group can also impact the activity of the products. In the absence of ortho-substituent (R′ = H), the ones without para-substituent (R = H) were more active than the ones with e-withdrawing para-substituents (R = F or Cl) (comparing **5a–d** with **5e–h** or **5i–l**) demonstrating the importance of the impact of para-substituent on activity. Overall, for the dihydroquinazoline derivatives (**5a–p**), R, R′ and R^1 all have impact on the activity of the products.

Among dihydroquinazolines compounds **5b, 5h**, **5m** and all of the tetrahydroquinazolines **6a–p** were screened against amastigotes and gave 45–100% inhibition Among them the compounds giving >80% inhibition against amastigotes were selected for the calculation of IC_{50}. All these compounds gave IC_{50} <10 μg/ml with **6l** being the most active one. Among the tetrahydroquinazoline derivatives **6a–p**, **6l** with R^1 as pyridyl piperazinyl group and R as chloro substituent, exhibited least IC_{50} value of 2.65 μg/ml. Replacement of chloro group with more electronegative fluoro group as in **6h** led to increase in IC_{50} value to 3.58 μg/ml. Introduction of another chloro group at ortho position in **6l** decreased the activity by approximately 2.5-fold as in **6p**. Replacement of pyridyl piperazine group with piperidinyl group led to decrease in activity. The compound having no substitution as R and R′ **6b** gave IC_{50} value of 3.48 μg/ml. Introduction of fluoro group as ortho-substituent in **6f** led to slight decrease in IC_{50} value (3.22 μg/ml) .Introducing five-membered pyrolidinyl substituent at R^1 led to increase in IC_{50} values. Compound **6g** having fluoro substituent as R′ showed IC_{50} value of 4.07 μg/ml and surprisingly lesser electronegative R′ substituent chloro as in **6k** resulted in abruptly high IC_{50} value of 8.95 μg/ml. Replacement of pyrolidinyl group with morpholinyl substituent in **6k** again decreased the IC_{50} value to 5.20 μg/ml for **6i**. From this data it can be observed that R^1 and R′ both have considerable effect on the IC_{50} values. All the compounds exhibited higher activities against *L. donovani* as compared to reference drugs Sodium Stibogluconate (IC_{50} = 53.62 μg/ml) and pentamidine (IC_{50} = 12.11 μg/ml) as representing the interesting leads as antileishmanial compounds.

Table 2
Antileishmanial in vitro activity against luciferase–amastigote system

Compd	IC_{50} (μg/ml)
6a	ND*
6b	3.48
6c	ND
6d	ND
6e	ND
6f	3.22
6g	4.07
6h	3.58
6i	5.20
6j	ND
6k	8.95
6l	2.65
6m	ND
6n	ND
6o	ND
6p	6.33
SSG	53.62
Pentamidine	12.11

* ND = not done.

In conclusion, we have identified a new series of quinazoline derivatives as inhibitors of *L. donovani*.The preliminary investigations revealed that these quinazolines has potential as antileishmanial agents and has opened a new avenue for further exploration.

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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.2009.07.081.

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† The assay data is taken by making fresh stock solution for the screening of compounds.

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