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Synthesis and biological evaluation of tricyclic guanidine analogues of batzelladine K for antimalarial, antileishmanial, antibacterial, antifungal and anti-HIV activities

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Abstract

Fifty analogues of batzelladine K were synthesized and evaluated for in vitro antimalarial (*Plasmodium falciparum*), antileishmanial (*Leishmania donovani*), antimicrobial (panel of bacteria and fungi), antiviral (HIV-1) activities. Analogues **14h** and **20l** exhibited potential antimalarial activity against chloroquine-sensitive D6 strain with IC_{50} 1.25 and 0.88 μM and chloroquine-resistant W2 strain with IC_{50} 1.64 and 1.07 μM, respectively. Analogues **12c** and **14c** having nonyl substitution showed the most potent antileishmanial activity with IC_{50} 2.39 and 2.78 μM and IC_{90} 11.27 and 12.76 μM respectively. Three analogues **12c**, **14c** and **14i** were the most active against various pathogenic bacteria and fungi with IC_{50} <3.02 μM and MIC/MBC/MFC <6 μM. Analogue **20l** having pentyl and methyl substituents on tricycle showed promising activities against all pathogens. However, none was found active against HIV-1. Our study demonstrated that the tricyclic guanidine compounds provide new structral class for broad spectrum activity.

Keywords

Batzelladine; tricyclic guanidine; antimalarial; antileishmanial; antimicrobial; anti-HIV

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Supporting information

Supporting Information may be found in the online version of this article:
Experimental procedures for synthesis
Spectral data of intermediates and final compounds
Table 5: Anti HIV activity of compounds

Introduction

Among the parasitic diseases, leishmaniasis and malaria have a high mortality rate affecting millions of people worldwide particularly in developing countries. Malaria ranks third among the major infectious diseases in causing deaths after pneumococcal acute respiratory infections and tuberculosis. Approximately, 1.5-2.5 million people die of malaria every year, accounting for about 5% of all fatalities in the world [1]. Leishmaniasis caused by protozoa of the genus *Leishmania* remains a significant health issue in large part due to the lack of effective and affordable drugs and increasing resistance against existing drugs. More than 2 million new cases of leishmaniasis occur each year, while 15 million people are infected [2-3].

HIV and parasitic infections interact and affect each other mutually. HIV infection may alter the natural history of parasitic diseases, impede rapid diagnosis or reduce the efficacy of antiparasitic treatment, whereas parasitoses may facilitate the infection with HIV and the progression from asymptomatic infection to AIDS. Recently, cause-effect interactions between HIV and malaria were also shown to occur in pregnant women [4]. Leishmania/HIV co-infection has emerged as a result of the increasing overlap of areas in which HIV or leishmaniasis occur, particularly in eastern Africa, India, Brazil and Europe [2]. Multiple immunological mechanisms mediate the impact of HIV infection on visceral leishmaniasis (VL) and vice versa. Both pathogens infect monocytes/macrophages and may establish a latent infection or may accelerate their intracellular multiplication. An increasing number of multidrug-resistant microbial pathogens have become a serious problem particularly during the last decade [5-6]. Consequently, these conditions demand the rapid search and discovery of new broad spectrum antiparasitic agents with novel structural backbone.

The batzelladines, a class of polycyclic marine alkaloids containing a guanidine group, have been isolated from various *Batzella* species. Batzelladines A-N, isolated from various sponges of the genus *Batzella* are of biological interests [7-11]. Batzelladines A and B were found to inhibit the binding of HIV glycoprotein gp-120 to CD4 receptors [7]. Batzelladines F, G and a mixture of H and I were active in the p56lck-CD4 dissociation assay [9]. Few selected batzelladine alkaloids are shown in Figure 1. It has been identified that the tricyclic core of batzelladines is essential for anti-HIV activity [12]. However, antiparasitic potential of this class of compounds has never been explored which led us to synthesize a series of compounds with tricyclic core of batzelladines. Total synthesis of complex batzelladines with many numbers of stereocentres is one of the challenging endeavor for synthetic chemists. We have previously reported the total synthesis of batzelladine K using a biomimetic approach [13]. We report herein, the synthesis of tricyclic guanidine analogues of batzelladines and evaluation of their in vitro antimalarial, antileishmanial, antimicrobial and anti-HIV activities.

Materials and Methods

General

All commercial chemicals and solvents were reagent grade and were used without further treatment unless otherwise noted. Nuclear magnetic resonance spectra were recorded on Brukers avance (400 MHz) with tetramethyl silane (TMS) as internal standard. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to TMS. Mass spectra were recorded on GCMS-QS Shimadzu (QP-500) and LCMS waters (Micromass ZQ). IR spectra were recorded on Nicolet spectrometer. HRMS was recorded on LCMS (Bruker Maxis). TLC was performed on Merck 0.25 mm Kieselgel 60 F254 plates. Column chromatography was performed using either silica gel-60 (60-120 mesh).

Antimalarial activity

In vitro antimalarial activity of all synthesized compounds was evaluated against chloroquine-sensitive (D6) and chloroquine-resistant (W2) clones of *P. falciparum* based on the determination of plasmodial LDH activity [14]. All the analogues were evaluated for in vitro cytotoxicity against mammalian kidney cell line (Vero) up to a highest concentration of 4.76 μg/mL by neutral red assay [15-16]. Selectivity index was calculated for all analogues (Data not shown). S.I. is calculated as the ratio of IC_{50} for cytotoxicity and IC_{50} for antimalarial activity and measures the therapeutic index of the compound under investigation to malaria parasites in comparison to its toxicity to the mammalian cells (if there is any).

Antileishmanial activity

The antileishmanial activity of all analogues was evaluated in vitro against *Leishmania donovani* promastigotes by Alamar Blue assay [17-18]. The activity is reported in terms of IC_{50} and IC_{90} values. Pentamidine and Amphotericin B are used as standards.

Antimicrobial activity

Synthesized analogues were evaluated for their antibacterial properties against *Staphylococcus aureus* ATCC 29213, Methicillin-resistant *S. aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) methods [19-23]. *M. intracellulare* was tested using a modified method of Franzblau et al [24]. Ciprofloxacin was used as standard.

Antifungal activity

The antifungal activities of all analogues against the opportunistic fungi *Candida albicans* ATCC 90028 (Ca), *Cryptococcus neoformans* ATCC 90113 (Cn), *Aspergillus fumigatus* ATCC 204305 (Afu), *Candida glabrata* ATCC 90030 (Cg) and *Candida krusei* ATCC 6258 (Ck) were determined. Amphotericin B was used as positive control.

Anti-HIV activity

Selected synthesized analogues were tested for anti-HIV potential. They were first evaluated for their cytotoxicity in MTT based cell viability assay in CEM-GFP cells [25]. Further non-cytotoxic concentration of each analogue was used for determination of anti-HIV activity. They were evaluated for anti-HIV potential in human CD4+ T cell line CEM-GFP, infected with HIV-$1_{NL4.3}$ virus by p24 antigen capture ELISA assay [26-28]. Zidovudine (AZT) was used as standard.

Results and discussion

Chemistry

Four series of tricyclic guanidine analogues were synthesized using the protocol developed in our laboratory [13]. As shown in Scheme 1, succinaldehyde (**2**) was freshly prepared by hydrolysis of commercially available 2,5-dimethoxytetrahydrofuran (**1**) with 0.6 N HCl [29]. A Wittig reaction of phosphorane **3** with succinaldehyde (0.5 equiv.) at room temperature for 24 hours afforded *E*-isomer of ketone **4** in 60% yield. The Michael addition of guanidine to **4** at 0 °C followed by a reductive imination with sodium borohydride gave **5**.

To identify the role of tricyclic guanidinium core in biological activity, the pyrrolidine ring of tricycle was replaced by piperidine ring. Glutaraldehyde was employed to construct the piperidine ring in previously described synthetic route. As shown in Scheme 1, Wittig

reaction of **3** with glutaraldehyde (0.5 equiv.) gave ketone **7**. Condensation of **7** with guanidine in DMF via sequential double Michael addition, followed by sodium borohydride reduction afforded the **8**.

A series of compounds **12a-l** were synthesized following a strategy shown in Scheme 2 (Series 1). The synthesis was started with alkylation of phosphorane **3** with alkyl halides (**9a-l**) employing butyllithium as a base to obtain phosphorane **10a-l**. Wittig reaction of **10a-l** with succinaldehyde afforded **11a-l** which were identified by NMR experiment. The NMR data showed coupling constants in range of 15-18 Hz for *trans* protons indicating synthesis of *E*-isomeric form of **11a-l**. Condensing guanidine with **11a-l** at 0 °C via Michael addition, followed by a reduction with sodium borohydride accomplished synthesis of compounds **12a-l**. Using the similar synthetic route, compounds **14a-l** (Series 2) were synthesized where glutaraldehyde was employed instead of succinaldehyde as depicted in Scheme 2. In Series 1 and 2, compounds were substituted with identical alkyl chains on both side of tricycle.

Compounds of Series 3 (**17a-l)** were synthesized as shown in Scheme 3, with two different alkyl groups on each side of tricyclic core. Synthesis of phosphorane **10a-l** was carried out as shown in Scheme 2. Wittig reaction of **10a-l** with excess of succinaldehyde afforded ketone **15a-l**. It is noteworthy that excess of succinaldehyde (>3 equiv.) is essential to obtain the ketone (**15a-l**), to avoid the reaction of the phosphorane (**3**) with free aldehyde group of **15a-l** to form di-α, β-unsaturated ketone (symmetrical). Further, **15a-l** were treated with **3** at room temperature to provide **16a-l**, which were reacted with guanidine followed by a reduction with sodium borohydride to accomplish synthesis of compounds **17a-l** as single diastereo-isomers. Similarly, compounds **20a-l** (Series 4) were synthesized using glutaraldehyde in the synthetic Scheme 3. The relative stereochemistry of all derivatives was established by NOE experiments as shown previously [13].

Antimalarial activity

The antimalarial activities of all the compounds are reported as IC_{50} values against D6 and W2 strains of *P. falciparum*. Activity data is shown only for active compounds in Table 1. Compounds having methyl substituents, **5** and **8** did not show any antimalarial activity. In Series 1 and 2 (compounds **12a-l** and **14a-l**), all analogues were active against the *P. falcifarum*. Amongst, compound **14h** was the most active and showed IC_{50} of 1.25 and 1.64 μM against D6 and W2 strains, respectively. Analogue **14b** exhibited IC_{50} of 1.52 and 1.96 μM against D6 and W2 strains, respectively, while **14i** displayed IC_{50} of 1.37 and 2.26 μM against D6 and W2 strains, respectively. Whereas in Series 3 and 4 (compounds **17a-l** and **20a-l**), the most potent analogue, **20l** displayed IC_{50} of 0.88 μM for D6 clone and 1.07 μM for W2 clone. The activity of this compound was much higher in comparison to the natural product, batzelladine K (**17l**) which showed IC_{50} of 14.85 μM for D6 clone and 13.65 μM for W2 clone.

All the analogues were evaluated for in vitro cytotoxicity and Selectivity Index was calculated. S.I. ≥ 10 is generally considered significant, indicating that antimalarial activity is not due to cytotoxicity. None of the compounds were found cytotoxic with the exception of **12c** and **14c**, indicating their selectivity for antimalarial activity.

Antileishmanial activity

Activity data is shown only for active compounds in Table 2. From Series 1 and 2, analogues **12c** and **14c** were the most potent with IC_{50} of 2.39 and 2.78 μM and IC_{90} of 11.27 and 12.76 μM, respectively. Remaining compounds also displayed antileishmanial activity to various extents with the exception of **12g** and **14g**. Among Series 3 and 4, **20l** was

found be to the most active with IC_{50} of 9.52 μM and IC_{90} of 20.95 μM. Natural product, batzelladine K (**17l**) was weakly active with IC_{50} of 48.19 μM and IC_{90} of 124 μM.

Antimicrobial activity

Activity data is shown only for active compounds in Table 3. In Series 1 and 2, thirteen analogues have shown inhibitory activity against *S. aureus* and MRSA with IC_{50} in the range of 2.44-10 μM and MIC in the range of 5.99-20 μM. Of these, **12c** having a nonyl substituent was the most potent having IC_{50} of 3.02 μM and 2.44 μM, and bactericidal activity at 23.98 μM and 12 μM against *S. aureus* and MRSA, respectively. The analogues **14c** and **14h** exhibited IC_{50} values of 3.01 μM and 3.18 μM, and were bactericidal at 5.8 μM and 5.48 μM respectively against *S. aureus*. The **14h** inhibited the growth of *E. coli* with IC_{50} value of 5.96 μM and showed bactericidal activity at 10.96 μM. Only four compounds from Series 2 were active against *P. aeruginosa* (**14c**, **14h**, **14i**, and **14l**) with **14h** being most active with IC_{50} of 10.96 μM and MIC of 21.9 μM. The **14i** was found to be the most effective against *M. intracellulare* with IC_{50} value of 5.95 μM and MBC of 12 μM.

The analogues from Series 3 and 4 were also active against all strains; ten compounds have shown moderate to potent antibacterial activity against both the strains of *S. aureus* with IC_{50} values in the range of 4.44-64 μM and all were bactericidal at <80 μM. The most potent activity was shown by **20l** having IC_{50} of 4.44 μM and 7.07 μM, MIC of 7.93 μM and 15.8 μM and bactericidal at 15.87 μM against *S. aureus* and MRSA respectively. Four analogues showed moderate activity against *E. coli*; **20l** having IC_{50} value of 23.27 μM and MIC of 63.49 μM. None of analogues in Series 3 and 4 were active against *P. aeruginosa*. Ten analogues exhibited the promising activity against *M. intracellulare*; **20l** being the most active with IC_{50} of 6.73 μM, MIC of 15.87 μM and bactericidal at 31.74 μM. In comparison, batzelladine K (**17l**) showed a weak activity against *S. aureus*, MRSA and *M. intracellulare* (IC_{50} 64.09, 56 and 79.5 μM respectively).

Antifungal activity

Activity data is shown only for active compounds Table 4. The tricyclic guanidine analogues showed activities to a variable extent against various strains. In Series 1, **12c** having nonyl substitution produced the most potent activity with IC_{50} of 3.02, 2.18, 2.11 and 5.46 μM, MIC and MFC of 5.99, 2.99, 2.99 and 11.99 μM against *C. albicans, C. glabrata, C. neoformans* and *A. fumigatus* respectively; while in Series 2, analogue **14c** showed IC_{50} of 3.83, 3.75 and 2.39 μM, and fungicidal at 5.80, 5.80 and 2.90 μM against *C. albicans, C. glabrata* and *C. neoformans* respectively. **14c** was the most potent in Series 2 against *C. krusei* (IC_{50} = 1.87 μM, MIC and MFC = 2.90 μM). The most potent activity against *C. neoformans* was observed with analogues **14h** and **14i** (IC_{50} = 0.46 and 1.03 μM, MIC and MFC = 0.68 and 1.51 μM, respectively).

In Series 3 and 4, **17b** was moderately active against *C. albicans* and *A. fumigatus* (IC_{50} = 22 and 46.73 μM) and **17c**, **20c** and **20l** were active against *C. glabrata* (IC_{50} of 17.32-19.30 μM) whereas, they showed promising activity against *C. krusei* (IC_{50} values of 9.57, 6.99 and 8.98 μM respectively, and MIC and MFC value of 15 μM), and *C. neoformans* (IC_{50} of 2.61, 3.66 and 2.12 μM, MIC of 8.17, 7.83 and 3.96 μM, and MFC of 8.17, 15.67 and 3.96 μM respectively). Batzelladine K (**17l**) was inactive against all pathogens except *C. neoformans* (IC_{50} 76.18 μM).

Anti-HIV activity

None of tested analogues were found to inhibit the growth of HIV-1 (supporting information-Table 5). Batzelladine K also demonstrated no anti-HIV activity.

Conclusion

We have synthesized four series of tricyclic guanidine analogues based on batzelladine K structural framework. The analogues were evaluated for antimalarial, antileishmanial, antibacterial, antifungal and anti-HIV activities. The **14h** and **20l** produced the most potent antimalarial activity against chloroquine-sensitive D6 (IC_{50} of 1.25 and 0.88 μM respectively) and chloroquine-resistant W2 (IC_{50} of 1.64 and 1.07 μM respectively) strains of *P. falciparum*. The most potent analogues having nonyl substituent, **12c** and **14c**, not only exhibited antileishmanial activities but also showed potent antibacterial and antifungal activities.

Analogue **20l** having pentyl and methyl substitutions showed promising activities against all pathogens. However, none was active against HIV. The combination of broad spectrum of activities makes these tricyclic guanidines as a promising new structural class of compounds. Further exploration of these tricyclic guanidines can provide lead compounds for further development as antimicrobial agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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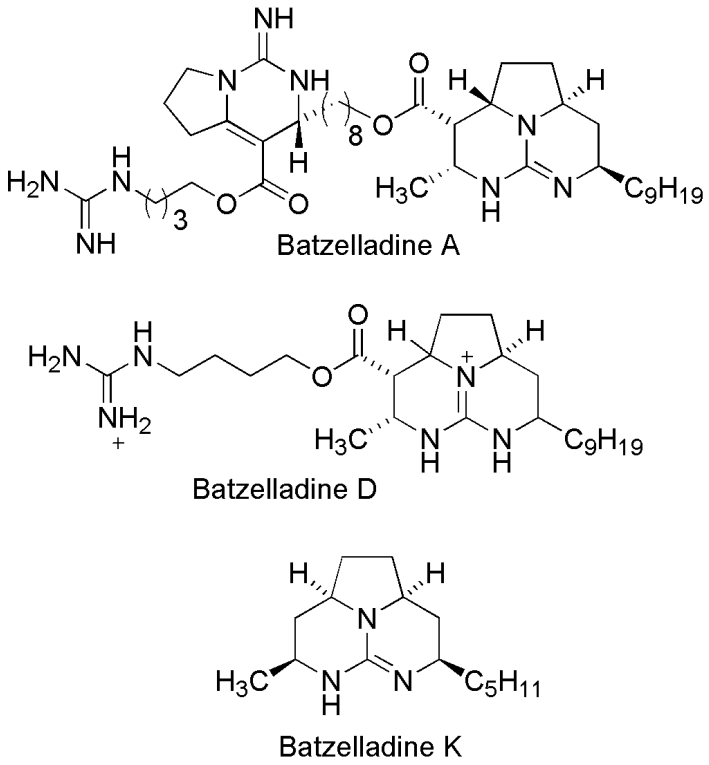
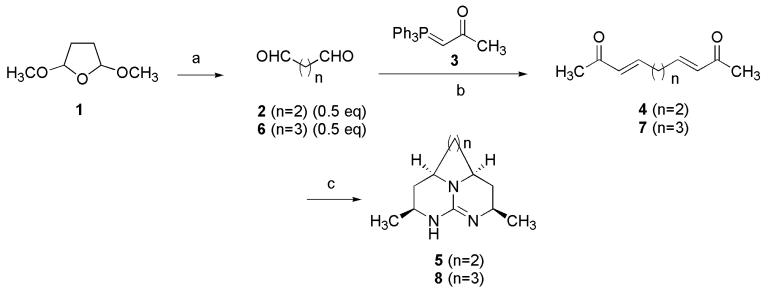
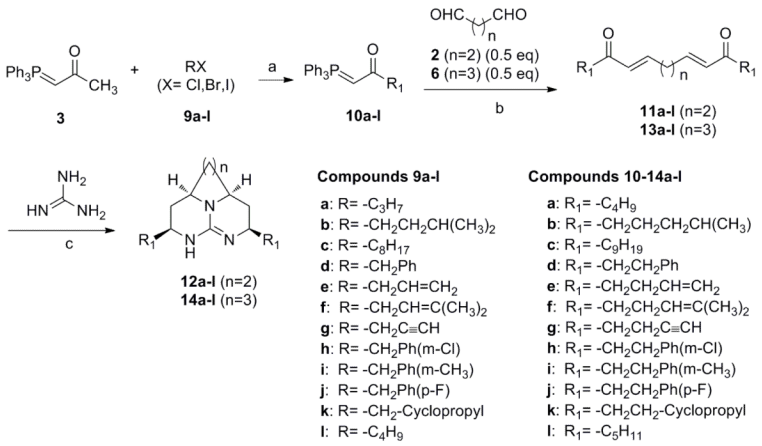


Figure 1.
Selected batzelladine alkaloids



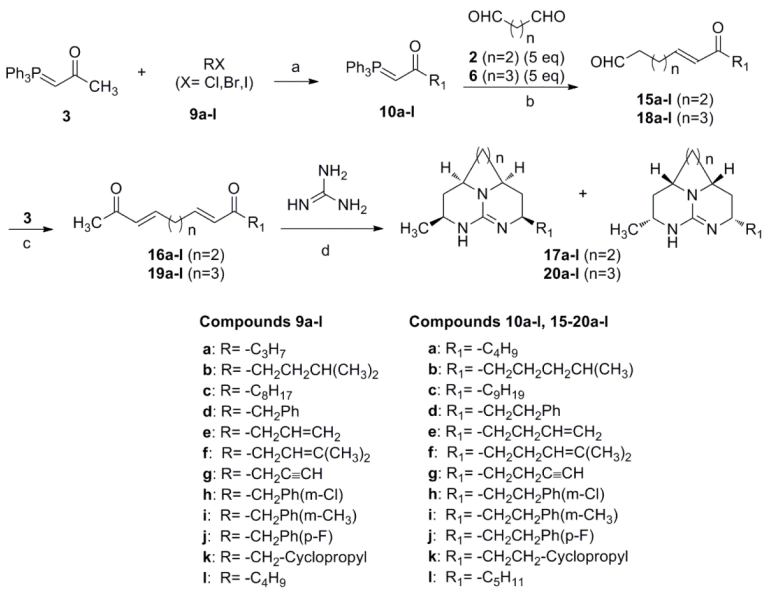
Scheme 1.

Reagents and conditions. a) HCL, Na_2CO_3, 2 h (repeated 5 times), **2**: 70%; b) DCM, 24 h, **4**: 60%, **7**: 65%; c) Guanidine, DMF/0°C-RT/5h, 3:1:3 DMG/H_2O/MeOH, $NaBH_4$/16 h, **5**: 30 % **8**: 25%.



Scheme 2.

Reagents and condition: a) BuLi/−78 °C, RT/16 h, 95-98%; b) DCM/24 h 60-70%; c) DMF, 0 °C-RT/5 h, 3:1:3 DMG/H_2O/MeOH, $NaBH_4$/16 h, 25-40%.



Scheme 3.

Reagents and conditions. a) BuLi/−78 °C, RT/16 h, 95-98%; b) DCM/24 h, 60-70%; c) DCM/24 h, 70-80%; d) DMG/0 °C-RT/5 h, 3:1:3 DMF/H_2O/MeOH, $NaBH_4$/16 h, 25-45%.

Table 1

In vitro antimalarial activity and cytotoxicity of tricyclic guanidine derivatives

Compd No.	*P. falciparum* (D6 Clone) IC_{50} (μM)	*P. falciparum* (W2 Clone) IC_{50} (μM)	Cytotoxicity (Vero) IC_{50} (μM)	Compd No.	*P. falciparum* (D6 Clone) IC_{50} (μM)	*P. falciparum* (W2 Clone) IC_{50} (μM)	Cytotoxicity (Vero) IC_{50} (μM)
12a	6.49	7.22	NC	**14l**	2.25	2.82	NC
12b	1.98	2.70	NC	**17b**	3.04	3.53	NC
12c	1.48	1.43	6.71	**17c**	4.59	6.20	NC
12d	6.16	8.04	NC	**17d**	7.62	9.93	NC
12e	10.62	10.98	NC	**17f**	16.05	-	NC
12f	7.29	7.59	NC	**17g**	20.60	20.60	NC
12g	9.66	5.57	NC	**17h**	10.09	7.25	NC
12h	6.34	7.71	NC	**17i**	9.39	8.05	NC
12i	4.23	4.98	NC	**17j**	15.76	13.24	NC
12j	5.62	7.33	NC	**17l**	14.85	13.65	NC
12l	3.01	3.60	NC	**20a**	3.21	5.62	NC
14a	2.99	4.81	NC	**20b**	4.69	7.22	NC
14b	1.52	1.96	NC	**20c**	3.44	3.76	NC
14c	1.39	1.76	6.96	**20d**	9.24	12.45	NC
14d	6.20	8.26	NC	**20e**	19.27	19.27	NC
14e	6.27	7.66	NC	**20f**	6.54	9.45	NC
14f	1.69	2.18	NC	**20g**	17.14	19.24	NC
14g	14.13	-	NC	**20h**	2.41	3.02	NC
14h	1.25	1.64	NC	**20i**	5.78	7.71	NC
14i	1.37	2.26	NC	**20l** *	0.88	1.07	NC

IC_{50} = sample concentration that causes 50% cell growth inhibition compared to vehicle control; NC = No cytotoxicity upto 4.76 μg/mL; "-" = Not active;

Chloroquine: IC_{50} = 0.052 μM (D6 clone); IC_{50} = 0.46 μM, (W2 clone). Artemisinin: IC_{50} = 0.018 μM (D6 clone); IC_{50} = 0.015 μM (W2 clone).

*S.I.: selectivity index [TC50 (Vero)/IC_{50} (*P. falciparum*)] > 17;

Table 2

In vitro antileishmanial activity (*L. donovani*) of tricyclic guanidine derivatives

Compd. No.	*L. donovani*		Compd. No	*L. donovani*	
	IC_{50} (μM)	IC_{90} (μM)		IC_{50} (μM)	IC_{90} (μM)
12a	64.98	126	**17b**	14.44	29.65
12b	12.31	33.03	**17c**	10.13	23.85
12c	2.39	11.27	**17d**	59.60	112.5
12d	48.25	91.15	**17e**	85.69	>13
12e	73.26	>130	**17f**	61.06	125
12f	28.87	91.18	**17h**	63.09	>100
12h	29.47	72.56	**17i**	63.75	>100
12i	32.41	79.80	**17j**	62.91	>100
12j	29.34	78.24	**17l**	48.19	124.4
12l	13.33	55.73	**20a**	29.31	116
14a	34.02	106.5	**20b**	57.76	119
14b	9.51	19.59	**20c**	10.34	22.25
14c	2.78	12.76	**20d**	57.23	114
14d	46.51	93.02	**20e**	133.6	>100
14e	62.71	128.9	**20f**	50.90	>100
14f	10.20	20.40	**20h**	12.08	45.31
14h	7.23	14.91	**20i**	48.23	106
14i	7.71	15.90	**20l**	9.52	20.95
14l	10.97	21.94			

IC_{50} and IC_{90} = Sample concentrations that kill 50% and 90% cells compared to the solvent controls; Pentamidine: IC_{50} = 2.94 μM, IC_{90} = 5.88 μM; Amphotericin B: IC_{50} = 0.173 μM, IC_{90} = 0.358 μM.

Table 3

In vitro antibacterial activity of tricyclic guanidine derivatives

Compd No.	*S. aureus*			*MRSA*			*E.coli*			*P. aeruginosa*			*M. intracellulare*		
	IC_{50} μM	MIC μM	MBC μM	IC_{50} μM	MIC μM	MBC μM	IC_{50} μM	MIC μM	MBC μM	IC_{50} μM	MIC μM	MBC μM	IC_{50} μM	MIC μM	MBC μM
12b	8.13	15.01	30	6.48	15	-	39.94	-	-	-	-	-	7.74	15	30
12c	3.02	5.99	23.98	2.44	5.99	12	7.02	-	-	-	-	-	7.41	12	23.9
12f	15.80	30.39	60.79	11.67	30.3	-	-	-	-	-	-	-	20.3	30	60
12h	3.26	5.66	45.35	3.28	5.66	22.67	-	-	-	-	-	-	11.1	22.67	45.35
12i	3.44	6.23	49.87	3.39	6.23	25	-	-	-	-	-	-	12	12.46	24.93
12j	6.89	12.22	48.90	11.63	-	-	-	-	-	-	-	-	16.8	24.45	24.45
12l	8.19	16.39	65.57	8.59	16.3	-	-	-	-	-	-	-	13.5	32.78	65.57
14b	6.25	14.40	57.63	4.26	14.4	28.8	14.26	28.8	28.8	-	-	-	8.12	14.40	14.40
14c	3.01	5.80	5.80	3.10	5.8	11.6	6.33	-	-	45	-	-	9.81	11.6	23.20
14f	4.60	7.28	14.57	5.07	14.5	14.5	29.82	58.3	58.3	-	-	-	7.05	14.5	29
14h	3.18	5.48	21.93	2.89	5.48	10.96	5.96	10.96	10.96	10.96	21.9	-	8.26	10.96	43.86
14i	3.20	6.02	24.09	5.54	12	12	12.38	24.09	-	37.88	48.2	-	5.95	12	12
14l	12.32	31.34	31.34	8.27	15.6	15.6	31.91	62.69	62.69	30.31	-	-	11	15.6	31.2
17b	8.21	38.02	38.02	7.90	19.0	-	69.12	-	-	-	-	-	13	19	38
17c	17.28	32.68	65.35	27.38	-	-	57.22	-	-	-	-	-	35.3	65.35	65.35
17h	16.40	31.54	-	8.51	15.7	-	-	-	-	-	-	-	34.1	63.09	31.54
17j	19.0	33.11	-	56.85	-	-	-	-	-	-	-	-	34.9	66.22	66.22
17l	64.09	80.32	-	56.06	80.3	-	-	-	-	-	-	-	79.5	-	-
20a	24.33	40.16	-	26.78	40.1	-	-	-	-	-	-	-	34.4	80.32	80.32
20b	36.49	72.20	-	29.09	36.1	-	-	-	-	-	-	-	44.3	72.20	72.20
20c	8.74	15.67	31.34	11.28	31.3	31.3	-	-	-	-	-	-	25.9	62.6	62.6
20h	8.52	15.10	30.21	8.30	15.1	30.2	47.88	-	-	-	-	-	15.8	30.2	30.2
20l	4.44	7.93	15.87	7.07	15.8	-	23.27	63.49	-	48.19	-	-	6.73	15.87	31.74

IC_{50} = Concentration (μM) that affords 50% growth inhibition; MIC = Minimum Inhibitory Concentration (the lowest concentration in μM that allows no detectable growth); MBC = Minimum Bactericidal Concentration (the lowest concentration in μM that kills the organism); "-" = Not active at the highest test concentration of 20 μg/mL; Ciprofloxacin: IC_{50} = 0.3 μM, MIC = 1.5 μM, MBC = 1.5 μM (Sa); IC_{50} = 0.3 μM, MIC = 0.75 μM, MBC = 1.5 μM (MRSA); IC_{50} = 0.009 μM, MIC = 0.024 μM, MBC = 0.377 μM (Ec); IC_{50} = 0.311 μM, MIC = 3 μM, MBC = "-" (Pa); IC_{50} = 0.69 μM, MIC = 1.5 μM, MBC = 3 μM (Mi).

Table 4

In vitro antifungal activity of tricyclic guanidine derivatives

Compd No.	*Candida albicans*			*Candida glabrata*			*Candida krusei*			*Cryptococcus neoformans*			*Aspergillus fumigatus*		
	IC_{50} μM	MIC μM	MFC μM	IC_{50} μM	MIC μM	MFC μM	IC_{50} μM	MIC μM	MFC μM	IC_{50} μM	MIC μM	MFC μM	IC_{50} μM	MIC μM	MFC μM
12b	18	60	60	-	-	-	19.76	60	60	8.73	15	30	33.54	-	-
12c	3.02	5.99	5.99	2.18	2.99	2.99	2.47	2.99	2.99	2.11	2.99	2.99	5.46	11.99	11.99
12f	-	-	-	-	-	-	33.22	-	-	29.54	60.79	30.39	-	-	-
12h	-	-	-	-	-	-	35.89	45.35	45.35	6.71	11.33	11.33	-	-	-
12j	-	-	-	-	-	-	-	-	-	23.71	48.9	48.9	-	-	-
12l	-	-	-	-	-	-	39.21	-	-	28.68	32.78	32.78	-	-	-
14b	7.03	28.81	57.63	35.96	-	-	13.91	28.81	28.81	4.20	3.60	3.60	35.64	-	-
14c	3.89	5.80	5.80	3.75	5.80	5.80	1.87	2.90	2.90	2.39	5.80	2.90	10.11	23.20	23.20
14f	16.44	29.15	58.30	34.66	58.30	-	11.39	29.15	29.15	12.47	14.57	14.57	35.27	-	-
14h	34.93	-	-	-	-	-	12.34	21.93	43.86	0.46	0.68	0.68	-	-	-
14i	25.90	48.19	-	-	-	-	16.84	24.09	24.09	1.03	1.51	1.51	47.78	-	-
14l	9.09	31.34	62.69	34.16	62.69	62.69	12.94	31.34	31.34	4.29	7.83	7.83	59.34	-	-
17b	22.09	38.0	76	-	-	-	24.37	76	38	17.60	38	38	47.37	-	-
17c	-	-	-	17.32	32.68	65.36	9.57	16.34	16.34	2.61	8.17	8.17	-	-	-
17h	-	-	-	-	-	-	-	-	-	16.49	31.54	31.54	-	-	-
17j	-	-	-	-	-	-	-	-	-	35.49	66.22	66.22	-	-	-
17l	-	-	-	-	-	-	-	-	-	76.18	-	-	-	-	-
20a	-	-	-	-	-	-	-	-	-	19.96	40.16	40.16	-	-	-
20b	-	-	-	-	-	-	-	-	-	15.34	18.0	18.0	-	-	-
20c	-	-	-	19.0	31.34	62.69	6.99	15.67	15.67	3.66	7.83	15.67	-	-	-
20h	-	-	-	-	-	-	-	-	-	3.56	7.55	7.55	-	-	-
20l	16.79	31.74	31.74	19.30	31.74	63.49	8.98	15.87	15.87	2.12	3.96	3.96	34.25	-	-

IC_{50} = Concentration (μM) that affords 50% growth inhibition; MIC= minimum inhibitory concentration (the lowest concentration in μM that allows no detectable growth); MFC = minimum fungicidal concentration (the lowest concentration in μM that kills the organism); "-" = No activity at the highest test concentration of 20 μg/mL. Amphotericin B: IC_{50} = 0.30 μM, MIC = 1.35 μM, MFC = 1.35 μM (Ca); IC_{50} = 0.39 μM, MIC = 0.68 μM, MFC = 1.35 μM (Cg); IC_{50} = 0.56 μM, MIC = 1.35 μM, MFC = 1.35 μM (Ck); IC_{50} = 0.86 μM, MIC = 1.35 μM, MFC = 2.57 μM (Afu); IC_{50} = 0.26 μM, MIC = 0.68 μM, MFC = 0.68 μML (Cn).