Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 845–853
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Phase transfer catalyzed synthesis of bis-quinolines: Antileishmanial activity in experimental visceral leishmaniasis and *in vitro* antibacterial evaluation

Partha Palit [a], Priyankar Paira [a], Abhijit Hazra [a], Sukdeb Banerjee [a], Asish Das Gupta [b], Sujata G. Dastidar [b], Nirup B. Mondal [a,*]

[a] *Steroid and Terpenoid Chemistry Division, Indian Institute of Chemical Biology, Council of Scientific and Industrial Research, 4 Raja SC Mullick Road, Jadavpur, Kolkata 700 032, India*
[b] *Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India*

Received 5 September 2007; received in revised form 14 April 2008; accepted 24 April 2008
Available online 2 May 2008

Abstract

A one-pot synthesis of some novel bis-quinolines has been achieved under phase transfer catalyzed conditions using 8-hydroxy quinoline derivatives as substrates. The synthesized analogues were evaluated for antileishmanial activity against *Leishmania donovani* promastigotes and amastigotes. The entire bis-quinolines showed efficacy in both *in vitro* and *in vivo* studies. Compound **5** (1,1-bis-[(5-chloro-8-quinolyl)oxy] methane) exhibited the most significant activity. Compounds **4** (1,1-bis-[(8-quinolyl)oxy]methane) and **9** (1,5-bis-[(2-methyl-8-quinolyl)oxy] pentane) also demonstrated significant leishmanicidal efficacy against established visceral leishmaniasis in BALB/c model. Ultrastructural studies of promastigotes treated with compound **5**, demonstrated membrane blebbing, chromatin condensation and vacuolization in the parasites and the flagellated parasites became round shaped after treatment. Moreover, *in vitro* antibacterial activity of compound **5** against several bacterial strains revealed its promising efficacy. The findings suggested that 1,1-bis-[(5-chloro-8-quinolyl)oxy]methane (**5**) is a bright candidate to be considered as lead compound for leishmanicidal drug.
© 2008 Elsevier Masson SAS. All rights reserved.

Keywords: Bis-quinolines; *Leishmania donovani*; Antileishmanial activity; Transmission electron microscopy; Antibacterial screening

1. Introduction

Leishmaniasis, a group of tropical diseases is caused by a number of species of protozoan parasites belonging to the genus leishmania [1]. These unicellular organisms present a spectrum of diseases ranging from benign cutaneous lesions through the disfiguring mucocutaneous forms to the fatal visceralizing form, often ending in death if treatment is not provided [2]. The disease currently threatens about 350 million people in 88 countries around the globe, with 2 million new cases affected annually [3]. In recent years, the co-existence of human immunodeficiency virus (HIV) with leishmaniasis has worsened the incidence more and more and contributed a new profile to the disease [4,5]. Its devastating impact is exemplified by the epidemic that occurred in the 1990s in Sudan [6] reminds that the discovery of effective drug for the radical cure of leishmaniasis still remains as one of the last unconquered frontiers in drug discovery.

Chemotherapy of leishmaniasis has been based on the pentavalent antimonials, sodium stibogluconate (pentostam) and meglumine antimonite (glucantime), since their discovery in 1940s [7]. These drugs contain multiple uncharacterized molecular structures with variable efficacies, possess severe side effects and require lengthy treatments with parenteral administration under medical supervision [8]. Also, reports of unresponsiveness to antimony treatment have become

* Corresponding author. Tel.: +91 33 2473 3491/3493/0492; fax: +91 33 2473 5197/0284.
E-mail address: nirup@iicb.res.in (N.B. Mondal).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.04.014

frequent [7,8]. Second line of drugs amphotericine B, a polyene anti-fungal agent, and pentamidine, an aromatic derivative of diamidine, are being used in the treatment of visceral leishmaniasis cases that do not respond to the antimonials. But the efficacies of amphotericine B and pentamidine are inextricably intertwined with their various toxic effects like cardiac abnormalities, hypotension, dysglycemia and renal disfunction [9]. Miltefosine, an oral alkyl phospholipid, has shown promising results in the treatment of visceral leishmaniasis during phase III trial [10,11]. However, reports of its severe side effects are also emerging in some cases [12]. Briefly, they all suffer from limitations of cost, specific toxicities, parenteral administration, emergence and spread of drug resistance or extended treatment regimens. Therefore, there is an urgent need for the development of improved treatments for leishmaniasis that are safe, inexpensive and easily available to the patients.

The quinolines are prevalent in a wide variety of biologically active compounds. Naturally occurring quinoline analogues [13] were well known for their anti-protozoal activity. Earlier reports suggested that several compounds containing quinoline moiety has shown leishmanicidal activity [14–20]. We earlier reported a successful synthesis of indolylquinoline [21] and anilidoquinoline analogues [22], which exhibited encouraging antileishmanial activity in both *in vitro* and *in vivo* studies. As part of our continued search for newer antileishmanial compounds, we contemplated that pentamidine type molecules, prepared with quinoline nuclei may exploit new class of antileishmanial agents. We confined our approach to short preparation strategies using cheaper reagents, which may be amenable for future exploitations as multi-step synthesis are seldom viable for commercialization. Phase transfer catalyzed reactions particularly appealed to us for the preparation of the desired product. We employed 8-hydroxy quinoline (**1**), and its 5-chloro (**2**), and 2-methyl (**3**) analogues as model substrates and 1,ω-dihalo derivatives of methane, ethane, propane, butane and pentane as solvent cum reactant. The reactions were carried out using catalytic amounts of commercially available quaternary ammonium halide as PTC in the presence of an inorganic base. In the present investigation, we report the *in vitro* and *in vivo* evaluation of some bis-quinolines and one oxazinoquinolone analogue against *Leishmania donovani*, a causative agent of visceral leishmaniasis (VL) besides the *in vitro* antibacterial evaluation of bis-quinoline derivative (**5**).

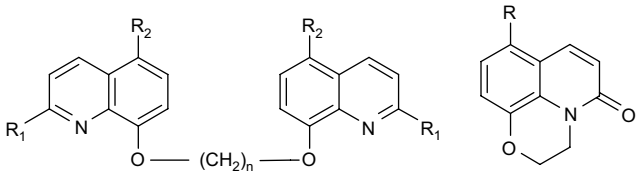
2. Results and discussion

2.1. Chemistry

Initially, dichloromethane was reacted with **1–3** in 10% sodium hydroxide solution in the presence of tetrabutyl ammonium bromide by constant stirring at ambient temperature. The reaction time varied from 24 to 48 h (monitored by TLC). In all cases, the products (**4–6**) were formed in good yield and mono-ethers were not obtained. To test the generality of the method we treated 2-methyl-8-hydroxy quinoline (**3**) with 1,ω-dihalo derivatives of ethane, propane, butane and pentane. The last three reagents indeed produced binuclear compounds of our choice, 1,3-bis-[(2-methyl-8-quinolyl)oxy]propane (**7**), 1,4-bis-[(2-methyl-8-quinolyl)oxy]butane (**8**) and 1,5-bis-[(2-methyl-8-quinolyl)oxy]pentane (**9**) (Fig. 1). The reaction products were characterized from their IR, ^{1}H NMR and ^{13}C NMR and mass spectral analysis. The assignments of ^{13}C signals were made from their chemical shifts, DEPT studies and comparison of their shift data with those of similar compounds and compounds **4–9** appear to be new. But the reaction of **3** with 1,ω-dihalo ethane produced an interesting oxazinoquinolone (**10**) rather than the di-ethers. The other two substrates viz. **1** yielded **10** and **2** produced very small amount of similar tricyclic oxazinoquinolones. Although synthesis of **10** has been reported earlier [23] no bio-evaluation has yet been reported.

2.2. Antileishmanial activity

To determine whether any of the synthesized compounds **4–10** had any effect on the promastigotes of *L. donovani*, graded concentrations of these derivatives (dissolved in DMSO) were added to the promastigote culture individually. DMSO had no effect on the growth of *L. donovani* promastigotes at a final concentration of 0.1% (v/v). After treatment with these compounds, antileishmanial efficacy against promastigotes was evaluated through inhibition of MTT reduction assay. The results were expressed as IC_{50} values of the compounds and summarized in Table 1. The data reveals that 50% lethal concentrations of compounds **5**, **4**, and **9** against promastigotes are 2, 2.4, and 2.8 μg/ml, respectively, which appeared to be very promising. Similar to promastigotes, the leishmancidal efficacy against intracellular amastigotes of these bis-quinolines and oxyzinoquinolone analogues are also quite encouraging. The data (Table 1) entails that the IC_{50} value of the analogues **4**, **5**, **8**, and **9** against intracellular amastigotes is appreciably significant. However, all the compounds elicited antileishmanial activity in a dose-dependent manner. The *in vitro* results against both forms of the parasite suggested that compound **5** is almost equally effective like pentamidine in killing the parasites but less effective in comparison to amphotericin B. The macrophage toxic dose and RBC lytic dose (data not shown) of the tested compounds are much higher than the killing dose against parasites. Hence, the IC_{50} and IC_{90} doses of tested compounds against parasites are safe and devoid of toxicity towards the normal mammalian cells.



4. R_1= R_2= H, n=1; **5**. R_1= H, R_2=Cl, n=1;
6-9. R_1= Me, R_2=H, n=1, 3, 4, 5; **10**. R=H,

Fig. 1.

Table 1
Antileishmanial activity and cytotoxicity towards macrophages of compounds **4**–**10** and reference drugs pentamidine and amphotericin B

Compounds	IC_{50} (μg/ml) against promastigotes	IC_{50} (μg/ml) against amastigotes	MTD[a] (μg/ml)
4	2.4	2.3	>85
5	2.0	2.1	>80
6	3.4	2.9	>100
7	13.5	10	>200
8	3.2	2.7	>90
9	2.8	2.4	>85
10	16.5	14.0	>220
Pentamidine	2.1	2.8	>90
Amphotericitin B	0.16	0.1	>4

[a] MTD are the compound concentrations that destroy the host murine peritoneal macrophages, indicate the toxic dose.

The encouraging results appeared from the *in vitro* studies of compound **4**–**6**, **8**, and **9** prompted us to investigate the antileishmanial activity in established visceral leishmaniasis on BALB/c mice model through intraperitoneal administration.

BALB/c mice were infected with *L. donovani* as described [22]. After one month, groups of 4 mice were treated with PBS (phosphate-buffered saline), SAG (sodium antimony gluconate) or compound **4**–**6**, **8**, and **9** (intraperitoneally). The parasite load in the spleen and in the liver was determined as described [21]. The treatment led to significant reduction of parasite burden in spleen 95% ($P < 0.0001$) and 75% ($P < 0.0001$) and in liver 98.49% ($P < 0.0001$) and 78% ($P < 0.0001$), compared to untreated controls, 30 days post-intraperitoneal treatment with **5** at a dose of 12.5 and 5.5 mg/kg body weight, respectively (Fig. 2). Compounds **4**, **9** and **8** had also rendered appreciable results by reducing 89, 82 and 73.5% splenic and 92, 88.5 and 78% liver parasite load, respectively, in experimental mice. Dose of 250-mg/kg body weight of SAG was able to reduce the parasite burden from spleen and liver only by 57% and 60%, respectively. Hence, both the doses of compound **5**, **4** and **9** were much more effective compared to the standard drug sodium antimony gluconate (20- and 45-fold higher than the two doses of **5**) and other bis-quinoline analogues in eradicating the parasite load from the spleen and liver. Moreover, treatment with **5**, **4** and **9** showed significant decrease in weights of the spleen and liver too, compared to untreated controls (Table 2).

To check the liver and kidney function, the specific serum enzyme and blood urea levels of normal mice receiving treatment with **5** were analyzed and the results are shown in Fig. 3.

The levels of both SGPT and SGOT in mice upon receiving compound **5** approach almost normal values with respect to untreated control. Blood urea level after treatment with compound **5** is within the normal acceptable range. It was evident

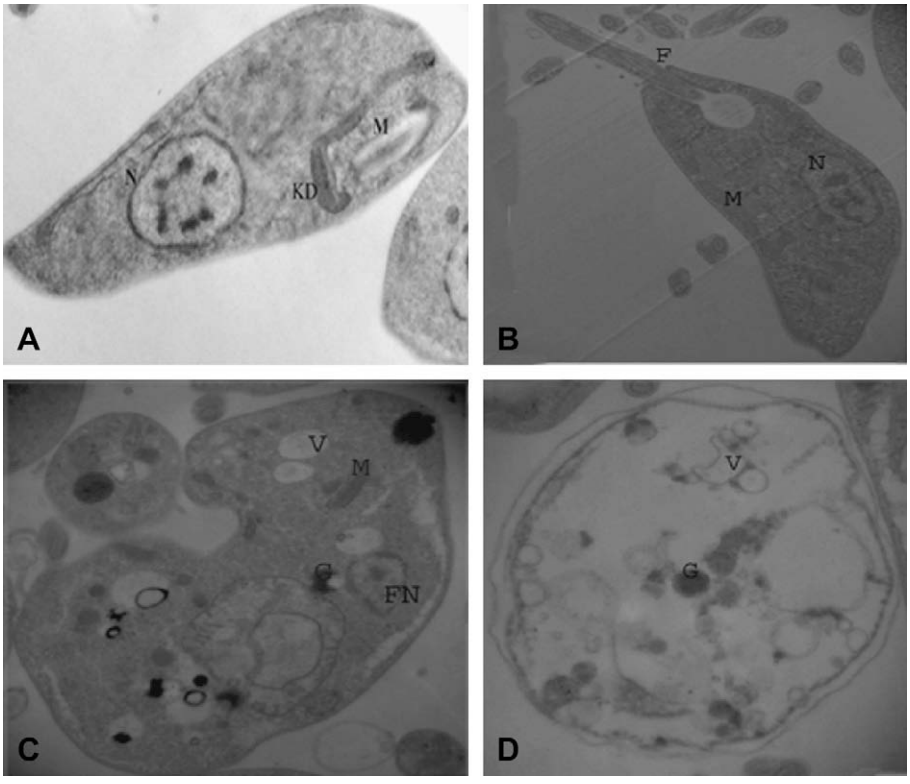


Fig. 2. Electron microscopic analysis of *L. donovani* AG83 cells treated with 0.1% DMSO alone, IC_{50} and IC_{90} doses of compound **5** for 2 h. (A) and (B) Control cells treated with 0.1% DMSO alone, (C) cells treated with IC_{50} (2 μg/ml) of **5** for 2 h, (D) cells treated with IC_{90} (11.5 μg/ml) of **5** for 2 h. (A) and (B) Control promastigotes, note the elongated body and the normal aspect of the intracellular organelles (KD, kinetoplast; F, flagellum; N, nucleus; M, mitochondria), (C) IC_{50} of compound **5** induced formation of vacuolization (V) and alterations at the plasma membrane and fragmented nuclei (FN) were observed, (D) parasites treated with IC_{90} showed total collapse of the intracellular organelles and presence of granules prior to cell death.

Table 2
Effect of compounds **4**–**6**, **8** and **9** on weight of spleen and liver after one-month intraperitoneal treatment

Compound	Dose (mg/kg body wt)	Spleen wt (mg) after treatment (±S.E.)	Liver wt (mg) after treatment (±S.E.)
Untreated infected control	–	480 ± 25	1650 ± 45
4	12.5	110 ± 13.6***	980 ± 61***
5	5.5	175 ± 12***	1120 ± 32***
5	12.5	90 ± 8***	900 ± 45***
6	12.5	232 ± 24***	1205 ± 34.7***
8	12.5	190 ± 25***	1125 ± 17***
9	12.5	135 ± 34***	1020 ± 41***
Sodium antimony gluconate	250	298 ± 10***	1257 ± 24***

Statistical significance compared to untreated mice is indicated as follows: ***$P < 0.0001$ significant versus untreated controls (analyzed by unpaired student *t*-test). Values are means ± standard error of results for 5 mice.

that the values of serum enzyme levels of treated mice are almost lower than the untreated normal mice. The results revealed that compound **5** is non-toxic to kidney and liver up to a dose of 12.5 mg/kg body weight intraperitoneally (Fig. 4). Furthermore, the investigation of antibacterial screening data revealed that the tested compound **5** showed moderate to good bacterial inhibition (Table 3). The results revealed that one strain of *Staphylococcus aureus* could be inhibited at 5 μg/ml concentration of the compound while the other strain of *S. aureus* failed to grow at 10 μg/ml concentration. *Shigella dysenteriae* 7 NCTC 519/66 and *Vibrio cholerae* 569B had the MIC of the compound 20 μg/ml level, while all the other bacteria were totally inhibited at 50 μg/ml dose.

To identify the mode of action of the compound on the parasites, the transmission electron microscopy (TEM) study was carried out with both treated promastigotes (with **5**) and untreated promastigotes. The intracellular damage caused by the compound on *L. donovani* promastigotes after a short-term incubation (Fig. 2) revealed the ultrastructural modifications of the parasites. The images clearly exhibit dose-dependent damage, with most significant changes with ED_{90} doses compared to controls. After 2 h of incubation (Fig. 2C), compound **5** at IC_{50} dose induced an enlargement of the kinetoplast and a considerable enhancement in the number of lipid granules and vacuoles. After the same period of incubation (Fig. 2D), initial changes in promastigotes were observed as an increase in the number of vacuoles and lipid granules, membrane blebbing, which were much more pronounced at IC_{90} dose of **5**.

It is well documented that macrophages are the major target cells for antileishmanial therapy because they are critical for the clearance of intracellular parasites via the production of cytokines and generation of reactive nitrogen oxide metabolites [25,26]. Therefore, it was of interest to analyze the production of pro-inflammatory cytokines by macrophages treated with different bis-quinoline analogues in the present study. Surprisingly, while exposure of *L. donovani* infected macrophages to compounds **4**–**10** alone did not cause significant changes in the levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α), these compounds strongly down-regulated the release of IL-6 and TNF-α in response to bacterial lipo-polysaccharide or the cytokine interferon-γ, two types of macrophage stimulators (data not shown). Moreover, compounds **4**–**10** did not induce the production of nitric oxide by infected macrophages (data not shown). Together, these findings strongly reveal that the significant decrease in

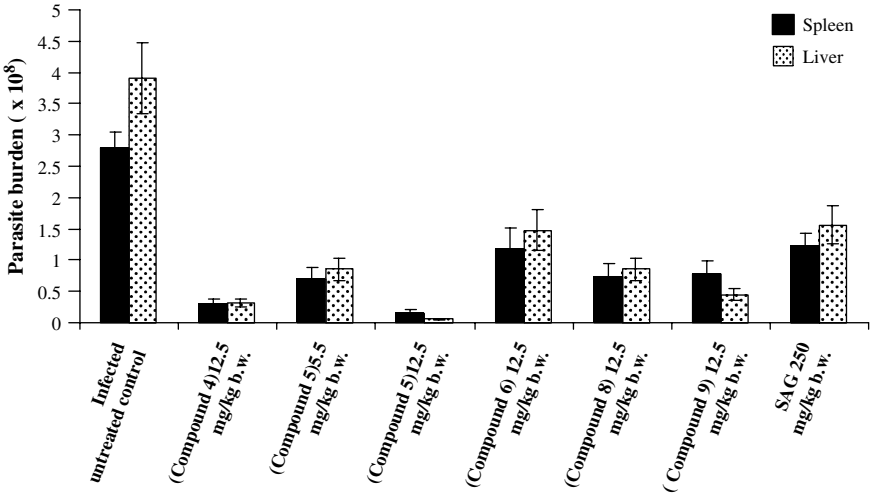


Fig. 3. Antileishmanial activity of compounds **4**–**6**, **8** and **9** *in vivo* against established VL infection by *L. donovani* AG83 in BALB/c mice. One-month post-infection, infected mice were treated with compound **5**, intraperitoneally at a dose of 5.5 and 12.5 mg/kg body weight and compounds **4**, **6**, **8** and **9** at 12.5 mg/kg body weight in 0.2% Tween 80 in PBS (0.02 M), two times weekly for one month. Another group of mice were treated with standard antileishmanial drug sodium antimony gluconate at a dose of 250 mg/kg body weight intraperitoneally two times weekly for one month. Control untreated group received only 0.2% Tween 80 in PBS (0.02 M). Mice were sacrificed 4 weeks post-treatment. Levels of parasite burden in spleen and liver were counted from impression smears after Giemsa staining and expressed as the total parasite load per organ, using the formula (organ weight in mg × the number of amastigotes per cell nucleus × 2×10^5). Values represent the mean ± S.E. of 5 animals.

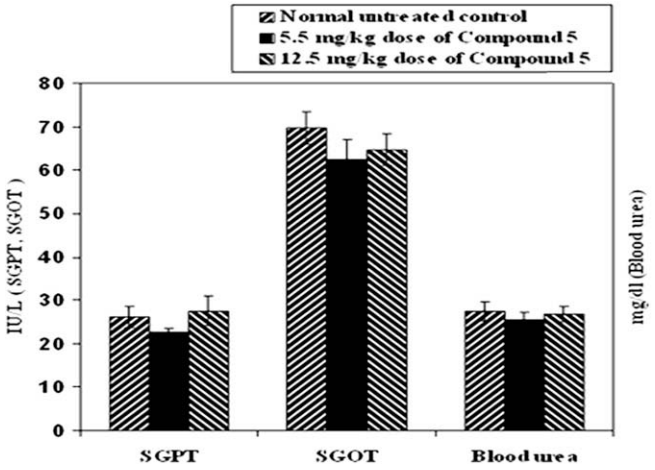


Fig. 4. Specific enzyme levels in sera of normal mice with and without treatment after therapeutic dose at 5.5 and 12.5 mg/kg body weight of compound **5**. Enzyme assays were performed using the kits from Dr. Reddy's Laboratories following the manufacturer's instructions. SGPT and SGOT activities were expressed as IU/l. Blood urea was expressed as mg/dl. Animal received indicated treatment for two times per week for 4 weeks, and bloods were collected one-month post-treatment for estimation of serum enzymes. Data represents mean ± S.E. of 4 animals per group.

macrophage infection rate induced by bis-quinolines are based on direct effect of the compounds on the intracellular parasites and emphasize the importance of examining the mechanism of action of drug candidates. We presume an apoptosis-like death pathway might be the possible mode of action of such type of bis-quinoline-like compounds.

The structural features of the tested compounds **4**–**10** (Fig. 1) revealed that the presence of functional group in the bis-quinolines as well as the length of hydrophobic chain in the ether bridge between quinoline moieties might have played a pivotal role regarding their efficacy. It was presumed that hydrophobic chain length in the ether bridge between quinoline moieties will play a vital role as in pentamidine but our observation from *in vivo* investigation of all compounds suggest that presence of a halogen substituent showed more importance than the hydrophobic chain length as evidenced by compounds **5** and **4**. The absence of halogen group (chlorine) in compound **4** has rendered less activity of this bis-quinoline derivative compared to compound **5** on visceral leishmanisis. On the other hand the activity of compound **4** is higher than **9**, **8** and **6** where elongated hydrophobic chain is present in the ether bridge between quinoline moieties along with a methyl substitution at 2-position of quinolines. However, from the experimental evidences taken together it may be concluded that compound **5** might be a new agent for sole or conjunctive therapy of leishmaniasis with minimal toxicity.

3. Conclusion

We have synthesized and evaluated a series of bis-quinoline analogues and one oxazinoquinoline derivative. To our knowledge, this is the first report of bis-quinoline derivatives, as potential antileishmanial agents, which were effectual

Table 3
In vitro antibacterial activity of compound **5** against different bacterial strains

Bacterial strains	Concentrations of compound **5** (μg/ml)					
	0	5	10	25	50	MIC
S. aureus ML6	+	+	−	−	−	10
S. aureus ML 145	+	−	−	−	−	5
B. licheniformis NCTC 10341	+	+	+	+	−	50
S. typhimurium NCTC 74	+	+	+	+	−	50
S. dysenteriae 6 NCTC 100/54	+	+	+	+	−	50
S. dysenteriae 7 NCTC 519/66	+	+	−	−	−	10
E. coli K12 Row	+	+	+	+	−	50
E. coli ATCC 25938	+	+	+	+	−	50
V. cholerae 569 B	+	+	+	−	−	25
V. cholerae 582	+	−	−	−	−	5
P. mirabilis 7	+	+	+	+	−	50
P. mirabilis 175	+	+	+	+	−	50
P. aeruginosa ATCC 27853	+	+	+	−	−	25

0, Control plate without compound (dimethyl sulphoxide as vehicle); +, growth; −, no growth (100% inhibition). MIC (μg/ml) = minimum inhibitory concentration, i.e., lowest concentration of compound that completely inhibits the growth of bacteria.

intraperitoneally for the reduction of parasite burden of *L. donovani* infected BALB/c mice without showing any toxic manifestation. As far as the *in vitro* antileishmanial screening results are concerned only compounds **4**, **5**, **8**, and **9** displayed profound activity against *L. donovani* AG83, compound **5** showed maximum efficacy against parasite growth *in vitro* and *in vivo*. Ultra-structure study of treated parasite showed certain morphological changes, which is the sign of programmed cell death. At the late stage of apoptosis, the plasma membrane blebs and pinches off to form apoptotic bodies (Fig. 2D). The flagellated promastigotes become round shaped with an increase in vacuoles and lipid bodies. Similar to our study there are reports on Sitamaquine, an 8-amino-quinoline analogue, that induces fine structural alterations in *Leishmania tropica* within human macrophages [27], rapid collapse of the mitochondrial inner membrane potential of *L. donovani* promastigotes [28] and, promotes a fast and extensive alkalinization of the *L. donovani* acidocalcisomes [29]. Few quinolines (imidazo-quinolinamines) such as imiquimod have also been demonstrated to be potent inducers of alpha interferon and cytokines in both *in vitro* and *in vivo* experiments [30]. This will help us to elucidate in detailed mechanism of action of these bis-quinoline derivatives against parasites in future study. Furthermore, *in vitro* antibacterial activity displayed by compound **5** was satisfactory. We exhibited in this investigation that bis-quinoline derivative (compound **5**) showed remarkable activity considering the structure activity relationship against *L. donovani* both *in vitro* and *in vivo* in pharmacologically relevant doses with a higher therapeutic index, without showing any adverse effect.

4. Experimental protocols

4.1. Compound preparation and characterization

All the compounds (Fig. 1) evaluated in this work were synthesized in one-pot sequences. Melting points were

determined in capillaries and are uncorrected. IR spectra were recorded as KBr pellets using a JASCO 410 FTIR spectrometer. The NMR spectra were recorded using a BRUKER 300 DPX spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, in $CDCl_3$ with TMS as internal standard. Mass spectra (positive mode) were obtained on a Micromass Q-TOF micro™ spectrometer in the electro spray mode. Column chromatography was performed on silica gel 60 (E. Merck India Ltd., Mumbai). TLC was performed on Merck $60F_{254}$ pre-coated silica gel 60 plates. Compounds were visualized with UV light or on heating after spraying L.B. reagent as developing agent.

The synthesis, characterization and plausible mechanism of formation of oxazinoquinolone (**10**) has recently been described from our laboratory [23]. Synthetic details and characterization of bis-quinolines (**4**–**9**) are stated below.

4.2. General procedure

Appropriate amounts (0.02, 0.025 or 0.03 mol) of 8-hydroxy quinoline (**1**), its 5-chloro (**2**), and 2-methyl derivatives were dissolved in ~10 ml of the chlorinated solvent (dichloromethane, or 1,ω-dichloro derivatives of ethane, propane, butane and pentane). Aqueous NaOH solution (10%, 50 ml) was added to the solution at ambient temperature followed by 1 mmol of tetrabutyl ammonium bromide. The mixture was stirred continuously for 24–48 h. After completion of the reaction (monitored by TLC), the contents were transferred to a separating funnel and the organic layer was separated, washed free from alkali with water, dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel (E. Merck) using petroleum ether (60–80 °C) and chloroform in different ratios.

4.2.1. 1,1-Bis-[(8-quinolyl)oxy]methane (*4*)

Following the general procedure described above the product **4** was obtained from **1** (0.02 mol) and dichloromethane (0.1 mol) in 42% yield and crystallized from CH_2Cl_2–hexane as fine needles, mp 119 °C; IR: ν_{max} (cm^{-1}) 3180, 3136, 3103, 2949, 2766, 1743, 1621, 1564, 1420, 1385, 1272 and1231; MS: *m/z* (%) 325 $[M + Na, 100]^+$; ^{1}H NMR: δ 6.37 (s, 2H, $-O-CH_2-O-$), 7.43 (m, 6H, H-3, 3′, 6, 6′, 7, 7′), 7.81 (dd, 2H, $J = 3.6$, 5.4 Hz, H-5, 5′), 8.12 (m, 2H, H-4, 4′), 8.96 (m, 2H, H-2, 2′); ^{13}C NMR: δ 93.1 (t, $-O-CH_2-O-$), 114.0 (d, C-7, 7′), 121.5 (d, C-5, 5′), 121.8 (d, C-3, 3′), 126.7 (d, C-6, 6′), 129.5 (s, C-4a, 4′a), 135.4 (d, C-4, 4′), 142.4 (s, C-8a, 8′a), 149.6 (d, C-2, 2′) and 152.7 (s, C-8, 8′). (Anal. Found: C, 75.44; H, 4.70; N, 9.23; $C_{19}H_{14}N_2O_2$ requires C, 75.48; H, 4.67; N, 9.27).

4.2.2. 1,1-Bis-[(5-chloro-8-quinolyl)oxy]methane (*5*)

Following the same procedure, **5** was obtained from **2** (0.02 mol) and dichloromethane (0.1 mol). Crystallization from benzene–hexane gave needle-like crystals (yield 37%), mp 236 °C; IR: ν_{max} (cm^{-1}) 3060, 1589, 1499, 1465, 1363, 1311, 1230, 1119, 1083, 975, 930, 825, and 782; MS: *m/z* (%) 395 $([M + Na]^+$ Cl^{37}, 33), 395 $([M + Na]^+$ Cl^{35}, 100); ^{1}H NMR: δ 6.33 (s, 2H, $-O-CH_2-O-$), 7.54 (m, 4H, H-3, 3′, 6, 6′), 7.68 (m, 2H, H-7, 7′), 8.53 (d, $J = 8.4$ Hz, 2H, H-4, 4′), 8.99 (m, 2H, H-2, 2′); ^{13}C NMR: δ 93.1 (t, $-O-CH_2-O-$), 113.9 (d, C-7, C-7′), 122.3 (d, C-3, 3′), 124.7 (s, C-4a, 4′a), 126.5 (d, C-6, 6′), 127.1 (s, C-5, 5′), 133.1 (d, C-4, 4′), 140.9 (s, C-8a, 8′a), 150.1 (d, C-2, 2′) and 151.6 (s, C-8, 8′). (Anal. Found: C, 61.44; H, 3.25; N, 7.58; $C_{19}H_{12}Cl_2$ N_2O_2 requires C, 61.47; H, 3.26; N, 7.55).

4.2.3. 1,1-Bis-[(2-methyl-8-quinolyl)oxy]methane (*6*)

The product **6** (yield 40%) obtained from **3** (0.025 mol) and dichloromethane (0.1 mol) following the same procedure and crystallization from CH_2Cl_2–hexane had mp 110 °C; IR: ν_{max} (cm^{-1}) 3364, 3062, 2922, 1604, 1565, 1505, 1474, 1233, 1081, 993 and 837; MS: *m/z* 331 $([M + H]^+)$; ^{1}H NMR: δ 2.77 (s, 6H, $2 \times CH_3$), 6.36 (s, 2H, $-O-CH_2-O-$), 7.28 (d, $J = 8.4$ Hz, 2H, H-3, 3′), 7.42 (m, 4H, H-6, 6′, 7, 7′), 7.73 (dd, $J = 1.2$, 7.5 Hz, 2H, H-5, 5′), 8.01 (d, $J = 8.4$ Hz, 2H, H-4, 4′); ^{13}C NMR: δ 25.6 (q, CH_3), 93.9 (t, $-O-CH_2-O-$), 115.4 (d, C-7, C-7′), 121.9* (d, C-5, C-5′), 122.4* (d, C-3, 3′), 125.7 (d, C-6, C-6′), 127.8 (s, C-4a, C-4′a), 136.1 (d, C-4, C-4′), 140.3 (s, C-8a, C-8′a), 152.3 (s, C-8, C-8′) and 158.3 (s, C-2, 2′) [* may be interchanged]. (Anal. Found: C, 76.30; H, 5.51; N, 8.45; $C_{21}H_{18}N_2O_2$ requires C, 76.34; H, 5.49; N, 8.48).

4.2.4. 1,3-Bis-[(2-methyl-8-quinolyl)oxy]propane (*7*)

The product **7** was obtained from **3** (0.025 mol) and 1,3-dichloropropane (0.1 mol) and crystallized from dichloromethane–hexane as fine needles (yield 26%), mp 124 °C; IR: ν_{max} (cm^{-1}) 3367, 2931, 1646, 1428, 1262, 1109 and 746; MS: *m/z* 359 $([M + H]^+)$; ^{1}H NMR: δ 2.58 (m, 2H, $-O-CH_2-CH_2-CH_2-$), 2.78 (s, 6H, $2 \times CH_3$), 4.38 (m, 4H, $2 \times O-CH_2$), 7.09 (m, 2H, H-7, H-7′), 7.35 (m, 6H, H-3, H-3′, H-5, 5′, 6, 6′), 8.02 (d, 2H, $J = 8.4$ Hz, H-4, 4′); ^{13}C NMR: δ 25.9 (q, $2 \times CH_3$), 30.8 (t, $-O-CH_2-CH_2-CH_2-O-$), 67.4 (t, $2 \times -O-CH_2$), 110.3 (d, C-7, 7′), 120.3 (d, C-5, C-5′), 122.9 (d, C-3, 3′), 126.1 (d, C-6, C-6′),128.2 (s, C-4a, C-4′a), 136.6 (d, C-4, C-4′), 140.2 (s, C-8a, C-8′a), 154.4 (s, C-8, C-8′) and 158.6 (s, C-2, 2′). (Anal. Found: C, 77.17; H, 6.25; N, 7.95; $C_{23}H_{22}N_2O_2$ requires C, 77.07; H, 6.19; N, 7.82).

4.2.5. 1,4-Bis-[(2-methyl-8-quinolyl)oxy]butane (*8*)

The product **8** obtained from **3** (0.025 mol) and 1,4-dichlorobutane (0.1 mol) was crystallized from acetonitrile–hexane as needles (yield 28%), mp 174 °C; IR: ν_{max} (cm^{-1}) 3463, 3046, 2925, 1602, 1564, 1503, 1430, 1383, 1263 and 1107; MS: *m/z* 395 $[M + Na]^+$, 373 $[M + H]^+$; ^{1}H NMR: δ 2.30 (m, 4H, $-CH_2-CH_2-$), 2.71 (s, 6H, $2 \times CH_3$), 4.40 (m, 4H, $2 \times -O-CH_2$), 7.08 (dd, $J = 2.1$, 6.3 Hz, 2H, H-7, 7′), 7.33 (m, 6H, H-3, 3′, 5, 5′, 6, 6′), 7.99 (d, $J = 8.4$ Hz, 2H, H-4, 4′); ^{13}C NMR: δ 26.1 (q, $2 \times CH_3$), 26.3 (t, $-(CH_2)_2-$), 69.3 (t, $2 \times -O-CH_2$), 109.6 (d, C-7, 7′), 119.7 (d, C-5, 5′), 122.8 (d, C-3, 3′), 126.1 (d, C-6, 6′), 128.1 (s, C-4a, 4′a), 136.4 (d, C-4, 4′), 140.3 (s, C-8a, 8′a),

154.6 (s, C-8, 8′) and 158.4 (s, C-2, 2′). (Anal. Found: C, 77.42; H, 6.45; N, 7.48; $C_{24}H_{24}N_2O_2$ requires C, 77.39; H, 6.49; N, 7.52).

4.2.6. 1,5-Bis-[(2-methyl-8-quinolyl)oxy]pentane (**9**)

A mixture of **3** (0.02 mol) was allowed to react with 1,5-dichloropentane (0.1 mol) following the general procedure affording **9**. Purification of the residue by chromatography (eluant: benzene–chloroform 3:1) and subsequent crystallization from acetonitrile–hexane gave **9** as needles (yield 32%); mp 180 °C; IR: ν_{max} (cm^{-1}) 3508, 3050, 2946, 1606, 1563, 1261, 1106 and 834; MS *m/z* 409 $[M+Na]^+$, 387 $[M+H]^+$; ^1H NMR: δ 1.80 (m, 2H, O–CH_2–CH_2–CH_2–CH_2–CH_2–O–), 2.17 (m, 4H, O–CH_2–CH_2–CH_2–CH_2–O–), 2.78 (s, 6H, 2 × CH_3), 4.29 (t, $J = 6.9$ Hz, 2 × –O–CH_2), 7.05 (m, 2H, H-7, 7′), 7.34 (m, 6H, H-3, 3′, 5, 5′, 6, 6′), 8.01 (d, *J*, 8.4 Hz, 2H, H-4, 4′); ^{13}C NMR: δ 23.1 (t, O–CH_2–CH_2–CH_2–CH_2–CH_2–O), 25.9 (q, 2 × CH_3), 29.1 (t, –O–CH_2–CH_2–CH_2–CH_2–CH_2–O–), 69.4 (t, 2 × –OCH_2), 109.7 (d, C-7, 7′), 119.7 (d, C-5, 5′) 122.9 (d, C-3, 3′), 126.3 (d, C-6, 6′), 128.1 (s, C-4a, 4a′), 136.8 (d, C-4, 4′), 139.9 (s, C-8a, 8′a), 154.5 (s, C-8, 8′) and 158.5 (s, C-2, 2′). (Anal. Found: C, 77.65; H, 6.81; N, 7.28; $C_{25}H_{26}N_2O_2$ requires C, 77.69; H, 6.78; N, 7.25).

4.3. Other chemicals

8-Hydroxy quinoline, its 5-chloro and 2-methyl derivatives and chlorinated reagents e.g. dichloromethane, or 1,ω-dichloro derivatives of ethane, propane, butane and pentane were purchased from Aldrich–Sigma Corporation (USA). All other solvents and chromatographic absorbents were procured from E. Merck (Germany) and SRL (India) Ltd. unless otherwise indicated.

4.4. Parasite culture and growth conditions

L. donovani strain AG83 (MHOH/IN/1983/AG83) was originally obtained from Indian Kala-azar patient [31] and maintained in golden hamsters. Amastigotes were isolated from spleens of *L. donovani* infected golden hamsters as described [32]. Promastigotes were grown at 22 °C in M199 (Gibco Laboratories, NY, USA) liquid media supplemented with 10% fetal bovine serum (FBS).

4.5. Effects of synthesized compounds on L. donovani promastigotes in vitro

Logarithm phase promastigotes (2 × 10^6/ml) was incubated with or without graded concentrations of 1, 3, 5, 7, 10, and 15 μg/ml of the compounds or standard antileishmanial drugs amphotericin B and pentamidine in Medium-199 supplemented with 10% FBS at 22 °C for 2 h. After treatment, all tubes were washed twice with 0.02 (M) PBS and each pellet finally was dissolved in 100 μl (2 mg/ml) MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solutions [33]. All tubes were incubated at 22 °C for 4 h, centrifuged at 8000 g for 10 min and pellets were dissolved in DMSO. Readings were taken at 570 nm spectrophotometrically and % of lysis of promastigotes caused by the compounds was calculated. The IC_{50} dose was evaluated by linear regression analysis method in Graphpad prism3 software.

4.6. In vitro antileishmanial activity of synthesized compounds on intracellular amastigotes

Cytotoxicity of the compounds was estimated by microscopic counting of the viable amastigotes by Giemsa staining method after treatment with the compounds at graded doses and standard antileishmanial drugs. Antileishmanial activity of the compounds on intracellular *L. donovani* amastigotes was carried out with thioglycolate-elicited peritoneal exudates. Thioglycolate-elicited peritoneal exudate was used as the source of macrophages for better recovery and easier isolation. Approximately 2 × 10^6/ml macrophages were allowed to adhere to glass coverslips (20 mm 3.25 mm) in RPMI-1640 (Gibco Laboratories) supplemented with 10% FBS and cultured for overnight at 37 °C in 5% CO_2 before *in vitro* infection with *L. donovani*. Stationary phase *L. donovani* promastigotes (2 × 10^7/ml) were added to each coverslip and incubated for 6 h at 37 °C in 5% CO_2. Coverslips were washed with 10% FBS-supplemented RPMI-1640 to remove uningested parasites and incubated for 24 h in the presence or absence of graded concentrations (0.5, 1, 3, 5, 10 μg/ml) of compounds. Infected macrophage cultures were washed with PBS, fixed with methanol in slides, stained with Giemsa, and examined microscopically under oil immersion. At least 200 target macrophages were examined for each coverslip under microscope. Antileishmanial activity was determined by calculating the number of amastigotes per 200 macrophages with respect to controls.

4.7. Assessment of antileishmanial activity of compound **5** in vivo

BALB/c mice (4–6 weeks) were injected intravenously with freshly transformed promastigotes of *L. donovani* (2 × 10^7/mouse). One-month post-infection, 4-week-infected BALB/c mice were treated with 1,1-bis-[(5-chloro-8-quinolyl)oxy]methane (**5**) at a dose of 5.5 and 12.5 mg/kg body weight (doses were chosen as 1/22nd and 1/10th, respectively, of LD_{50} value of compound **5**, data not shown) intraperitoneally two times weekly for one month. Each mouse received a total of 8 intraperitoneal administrations of compound **5** for one month. Mice in the untreated group were received vehicle control (0.2% Tween 80 in PBS) by the same route. Therapy was compared with the standard antileishmanial drug sodium antimony gluconate, which was administered to another group of mice at a dose of 250 mg/kg body weight. Mice in all groups were sacrificed on one-month post-treatment. The splenic and liver parasites were determined by impression smear of Giemsa staining. Levels of organ parasite load were determined and expressed as the

total parasite burden per organ, using the formula [24]: (organ weight in mg × the number of amastigotes per cell nucleus × 2 × 10^5).

4.8. Study of parasite ultra-structure by TEM

Transmission electron microscopy (TEM) was carried out with compound **5** treated and untreated cells according to the method described earlier [34]. Parasites were incubated with the compound at the doses of 2 μg/ml (IC_{50}) and 11.5 μg/ml (IC_{90}), respectively, for 2 h. Sections were cut with a Du-point diamond knife in an LKB Ultramicrotome, stained on copper grids with uranyl acetate and lead acetate for 10–15 min, respectively, and examined under JEOL 100CX TEM.53.

4.9. Serum enzyme assay

The blood sera of normal mice and mice receiving treatment with compound **5** at a dose of 5.5 and 12.5 mg/kg body weight two times per week for 4 weeks, were subjected to estimate for the enzymes serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) and blood urea, one-month post-treatment. These enzymes and blood urea were assayed using the kits from Dr. Reddy's Laboratories (Hyderabad, India) following the manufacturer's protocol. Blood urea activity was expressed as mg/dl, whereas SGPT and SGOT activities were expressed as IU/l.

4.10. Determination of antibacterial efficacy

The MIC of compound **5** towards different test bacterial strains was accurately determined by agar dilution method [35]. Compound **5** was dissolved in sterile 0.2% DMSO and final concentration was made up by distilled water and added to molten nutrient agar at concentrations of 5, 10, 25 and 50 μg/ml compared to 0.2% DMSO (control). It was then poured into sterile Petri dishes under aseptic conditions maintaining the pH at 7.2–7.4. Gram-positive bacteria were grown in nutrient broth and Gram-negative bacteria in peptone water for 18 h and bacteria were harvested during the stationary growth phase. A direct suspension of organisms was prepared in 5 ml sterile distilled water. The turbidity of the suspension was adjusted to a 0.5 McFarland standard [36] with a Chemito UV 2600 Double Beam UV spectrophotometer (Chemito Technology Pvt Ltd, Nasik, India) at 625 nm, which corresponded to 2.4 × 10^8 colon forming units (CFU)/ml. The inocula were prepared by further diluting the suspension 1:100 with sterile distilled water in such a manner that a 2 mm (internal diameter) loopful of culture contained 10^5 CFU. These were spot inoculated on the nutrient agar plates containing increasing amounts of the drug, including a control. The plates were incubated at 37 °C and examined for the appearance of growth after 24 h (extended up to 72 h where necessary).

5. Statistical analysis

Data are expressed as mean ± S.E. unless mentioned. Comparisons were made between different treatments using unpaired Student's *t*-test.

Acknowledgements

Financial assistance from the Department of Biotechnology, Govt. of India, for this investigation, is gratefully acknowledged.

References

[1] M.J. Chan-Bacab, L.M. Penia-Rodriguez, Nat. Prod. Rep. 18 (2001) 674–688.
[2] B.C. Walton, American Cutaneous and Mucocutaneous Leishmaniasis, in: W. Peters, R. Killick-Kendrick (Eds.), The Leishmaniasis in Biology and Medicine, Academic Press, London, 1987.
[3] World Health Organization (WHO), Programme for the Surveillance and Control of Leishmaniasis (2002).<http://www.who.int/emc/diseases/leish/index.html>.
[4] D. Wolday, N. Bevhe, H. Akuffo, S. Britton, Parasitol. Today 15 (1999) 182–187.
[5] World Health Organisation, Leishmenia and HIV in Gridlock, UNAIDS, Geneva, Switzerland, 1998.
[6] J. Seaman, A. Mercer, E. Sondorp, Int. J. Epideminol. 25 (1996) 862–871.
[7] J.D. Berman, Clin. Infect. Dis. 24 (1997) 684–703.
[8] P.L. Olliaro, A.D.M. Bryceson, Parasitol. Today 9 (1993) 323–328.
[9] E.A. Vande War, J.W. Tracy, in: C.D. Smith, A.M. Reynard (Eds.), Farmacologia, Medica Panamericana, Argentina, 1993.
[10] T. Jha, S. Sundar, C. Thakur, P. Bachmann, J. Karbwang, C. Fischer, A. Voss, J. Berman, N. Engl. J. Med. 341 (1999) 1795–1800.
[11] S. Sundar, A. Makharia, D. More, G. Agarwal, A. Voss, C. Fischer, P. Bachmann, H. Murray, Clin. Infect. Dis. 31 (2000) 1110–1113.
[12] S. Gupta, R. Sharma, S.C. Srivastava, Acta Trop. 94 (1) (2005) 41–47.
[13] B. Akendengue, E. Ngou-Milama, A. Laurens, R. Hocquemiller, Parasite 6 (1) (1999) 3–8.
[14] A. Fournet, M.E. Ferreira, A.R.D. Arias, S.T. De Ortiz, S. Fuentes, H. Nakayama, A. Schinini, R. Hocquemiller, Antimicrob. Agents Chemother. 40 (1996) 2447–2451.
[15] J. Dade, O. Provot, H. Moskowitz, J. Mayrargue, E. Prina, Chem. Pharm. Bull. 49 (4) (2001) 480–483.
[16] A.F. Mohammed, A. Fournet, E. Prina, J.F. Mouscadet, X. Franck, R. Hocquemiller, B. Figadere, Bioorg. Med. Chem. 11 (2003) 5013–5023.
[17] A.G. Tempone, A.C.M.P. Da Silva, C.A. Brandt, F.S. Martinez, S.E.T. Borborema, M.A.B. da Silveira, H.F. de Andrade Jr., Antimicrob. Agents Chemother. 49 (3) (2005) 1076–1080.
[18] H. Nakayama, P.M. Loiseau, C. Bories, S.T. de Ortiz, A. Schinini, E. Serna, A.R. de Arias, A.F. Mohamed, X. Franck, B. Figadere, R. Hocquemiller, A. Fournet, Antimicrob. Agents Chemother. 49 (12) (2005) 4950–4956.
[19] R. Dietze, S.F.G. Carvalho, L.C. Valli, J. Berman, T. Brewer, W. Milhous, J. Sanchez, B. Schuster, M. Grogl, Am. J. Trop. Med. Hyg. 65 (6) (2001) 685–689.
[20] J.L. Vennerstrom, A.L. Ager Jr., A. Dorn, S.L. Andersen, L. Gerena, R.G. Ridley, W.K. Milhous, J. Med. Chem. 41 (1998) 4360–4364.
[21] G. Chakrabarti, A. Basu, P.P. Manna, S.B. Mahato, N.B. Mandal, S. Bandyopadhyay, J. Antimicrob. Chemother. 43 (1999) 359–366.
[22] N.P. Sahu, C. Pal, N.B. Mandal, S. Banerjee, M. Raha, A.P. Kundu, A. Basu, M. Ghosh, K. Roy, S. Bandyopadhyay, Bioorg. Med. Chem. 10 (2002) 1687–1693.

[23] R. Dutta, D. Mandal, N. Panda, N.B. Mondal, S. Banerjee, S. Kumar, M. Weber, P. Lugar, N.P. Sahu, Tetrahedron Lett. 45 (2004) 9361–9364.
[24] L.A. Stauber, E.N. Franchino, J. Grun, J. Protozool. 5 (1958) 269–273.
[25] P. Scott, Immunol. Res. 27 (2003) 489–498.
[26] A. Ponte-Sucre, R. Vicik, M. Schultheis, T. Schirmeister, H. Moll, Antimicrob. Agents Chemother. 50 (2006) 2439–2447.
[27] S.G. Langreth, J.D. Berman, G.P. Riordan, L.S. Lee, J. Protozool. 30 (1983) 555–561.
[28] A.E. Vercesi, R. Docampo, Biochem. J. 284 (1992) 463–467.
[29] A.E. Vercesi, C.O. Rodrigues, R. Catisti, R. Docampo, FEBS Lett. 473 (2000) 203–206.
[30] S. Buates, G. Matlashewski, Antimicrob. Agents Chemother. 45 (2001) 1137–1142.
[31] A.K. Ghosh, F.K. Bhattacharyya, D.K. Ghosh, Exp. Parasitol. 60 (1985) 404–413.
[32] C.L. Jaffe, G. Grimaldi, D. McMohan-Pratt, in: C.M. Morel (Ed.), Genes and Antigens of Parasites: A Laboratory Manual, second ed. Fundacao Oswaldo Cruz, Rio de Janiero, 1984.
[33] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[34] N. Sen, B.B. Das, A. Ganguly, T. Mukherjee, G. Tripathi, S. Bandyopadhyay, S. Rakshit, T. Sen, H.K. Majumder, Cell Death Differ. 11 (2004) 924–936.
[35] National Committee for Clinical Laboratory Standards, Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. Approved Standard, NCCLS, Wayne, PA, 2001.
[36] J. McFarland, J. Am. Med. Assoc. 49 (1907) 176–178.