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Synthesis of a Novel Quinoline Derivative, 2-(2-Methylquinolin-4-ylamino)-*N*-phenylacetamide—A Potential Antileishmanial Agent

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Abstract—Some novel quinoline derivatives were prepared and tested for antileishmanial activity. 2-(2-Methylquinolin-4-ylamino)-*N*-phenylacetamide (**2**) was found to be significantly more active than the standard antileishmanial drug sodium antimony gluconate (SAG) in reducing the parasite load both in the spleen and liver at a much lower concentration in hamster models. The results suggest that the compound could be exploited as an antileishmanial drug.

Introduction

Leishmaniasis is a severe global public health problem, especially in the tropical and sub-tropical countries. Infection by various species and strains of *Leishmania* causes a wide spectrum of disease in humans, with many different clinical manifestations (cutaneous, mucocutaneous, and visceral). The asperity of the disease is largely ordained by the immunological status of the infected individual and by the species of *Leishmania* involved. Approximately 350 million people in 80 countries are estimated to be prone to the disease.[1] The visceral form of leishmaniasis, caused by the parasite *Leishmania donovani*, is commonly known as Kala-azar in which the phagocytic cells of the spleen, liver, and bone marrow are invaded; it is often fatal in more than 90% of the untreated cases.[2] There is still no effective vaccine for Kala-azar, and chemotherapy remains the most effective control measure.

In spite of the discovery of a number of potentially useful antileishmanial compounds, the toxic pentavalent antimonials remain the mainstay of treatment for leishmaniasis. The second line drugs, the bisamidines stilbamidine or pentamidine, and the glycomacrolide amphotericin B, although used clinically, display high liver and heart toxicities,[3–6] develop clinical resistance in the subjects after few weeks of treatment, and contribute to increased co-infections.[7] Therefore, development of more effective and safer chemotherapeutic agents for treating leishmaniasis remains desirable, and rational approaches are needed to identify novel drugs against these protozoal cells. The present paper describes the synthesis and antileishmanial activity evaluation of some quinoline derivatives. Among the quinoline derivatives tested, the compound 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide (**2**), easily prepared in high yield, was found to be significantly more active than the standard antileishmanial drug sodium antimony gluconate both in vitro and in vivo. The results suggest that the compound could be exploited as an antileishmanial drug.

Results and Discussion

Chemistry

In a previous paper,[8] we have reported the preparation of several indolylquinoline derivatives from indole by Friedel–Crafts reaction. One of the compounds,

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2-(2″-dichloroacetamidobenzyl)-3-(3′-indolyl)-quinoline (**1**), was found to be significantly active against *L. donovani* both in vitro and in vivo.[9,10] The carbon skeleton of the indolylquinoline moiety consists of three entities, that is indole, quinoline, and acetanilide. To study the structure–activity relationships, several analogues were prepared and evaluated for their antileishmanial activity.

2-Chloro-*N*-phenylacetamide (**3**), synthesized by reacting chloroacetyl chloride with aniline, was condensed with 4-aminoquinaldine (**4**) in the presence of sodium hydride and dimethyl sulfoxide, giving rise to four products. The compounds were separated by column chromatography over silica gel to furnish *N*-phenyl-2-phenylaminoacetamide (**5**), 2,2′-dimethyl-[3,3′]biquinolinyl-4,4″-diamine (**6**), 1,4-diphenylpiperazine-2,5-dione (**7**), and 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide (**2**). The structures of **2**, **5**, **6**, and **7** were deduced[11] from their spectral data (IR, ^{1}H and ^{13}C NMR, MS) and elemental analysis. Compounds **2**, **5**, and **6** appear to be new. Although synthesis of **7** has been reported by other workers,[12,13] neither ^{1}H nor ^{13}C NMR spectral data was available in the literature.

Preliminary investigation revealed that among the compounds prepared, 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide (**2**) possesses very promising antileishmanial activity against *L. donovani* both in vitro and in vivo. We therefore sought to prepare **2** in better yields. It was found that the desired compound **2** was obtained quantitatively when the reaction was carried out in an inert atmosphere keeping the other parameters the same. This provided us with enough material to carry out the biological activity tests described below.

Antileishmanial activity

Effect of 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide (2) on the growth of *L. donovani* promastigotes (in vitro). To determine whether any of the above compounds (**2**, **5**–**7**) had any effect on the promastigotes, various 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). Of the four compounds (**2**, **5**–**7**) tested, compound **2** was found to inhibit the growth in a dose dependent manner (Fig. 1). At a concentration as low as 5.0 μg/mL, **2** inhibited the growth of the organism by approximately 66% on the second day, and 92 and 95% on the fourth and seventh days of culture, respectively ($p < 0.001$ for each comparison). A lower concentration (1.0 μg/mL) of **2** was also found to be growth inhibitory to the organism. However, the differences were not statistically significant. The other compounds (**5**–**7**) were virtually ineffective in inhibiting the growth up to a concentration of 5.0 μg/mL (Fig. 1) (Scheme 1).

Arrest of cell-cycle progression in *L. donovani* promastigotes by compound 2 leads to apoptosis, but has no appreciable effect on normal human T cell blasts. Cell-cycle analysis demonstrated that after 1 day of culture, **2** at a concentration of 5.0 μg/mL caused *L. donovani* promastigotes to remain as resting G_0/G_1 cells and inhibited their entry into the S phase. The percentage of dead cells did not increase during this incubation period, when growth arrest was visible. After 2 days of culture, **2** caused substantial increase in cell death, compared to DMSO treated cultures (Fig. 2). Of note, **2** induced only barely detectable arrest of normal human T cell blasts at the G_0/G_1 phase after incubation for 3 days.

Treatment of established visceral leishmaniasis in hamsters with 2 (in vivo). Golden hamsters were infected with *L. donovani* as described.[9] After 1 month, groups of four hamsters were treated with phosphate-buffered saline (PBS), sodium antimony gluconate (SAG) or **2**. The parasite load in the spleen and in the liver was determined as described.[9] As shown in Figure 3, compound **2** was significantly more effective than SAG in reducing the parasitic burden in both spleen and liver, lowering the parasite burden by almost 93.2% in both the organs. On the other hand, SAG reduced the parasite burden by 82.5 and 72.6% in the spleen and the liver, respectively. Micrographs of Giemsa-stained liver and splenic smears of *L. donovani* infected golden hamsters after treatment with DMSO, SAG or **2** are shown in Figure 4.

Compound 2 appears non-toxic to liver as judged by specific enzyme levels. To check the liver function, the specific serum enzyme levels of hamsters undergoing experimental visceral leishmaniasis and receiving therapy with compound **2** were analyzed and the results are shown in Figure 5. The level of serum alkaline phosphatase (ALP) was markedly higher in *L. donovani* infected hamsters compared to uninfected controls. The level of ALP decreased in infected hamsters upon treatment with compound **2**. However, the value was still

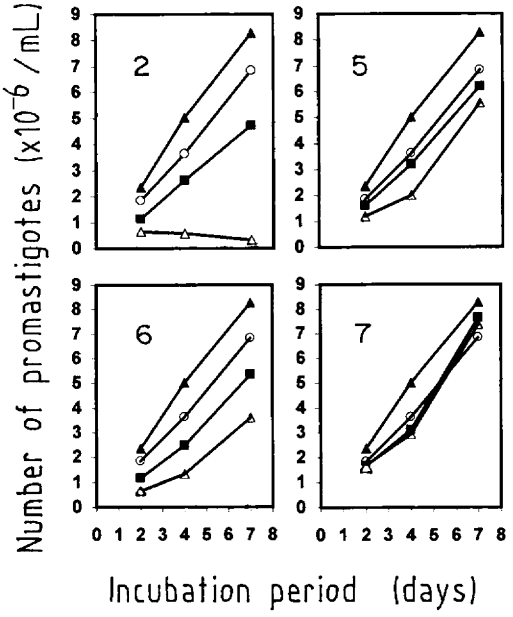
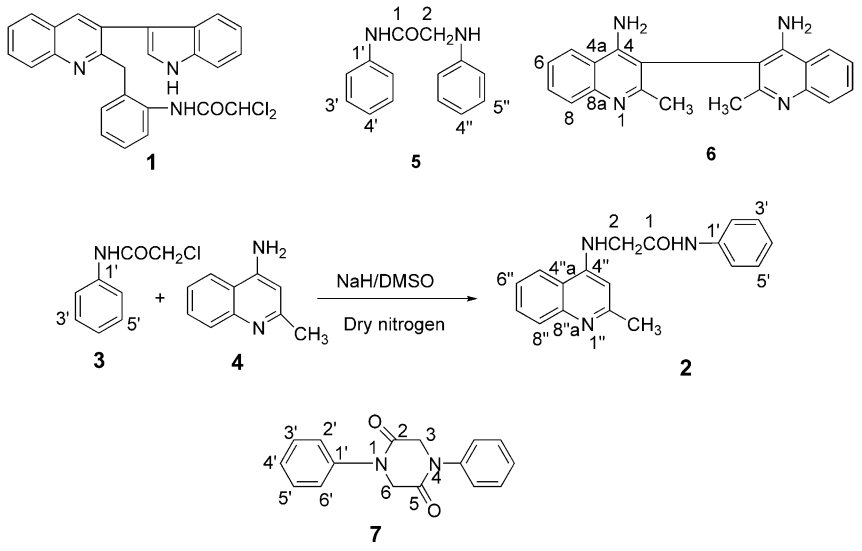


Figure 1. Effect of **2**, **5**, **6**, and **7** on the growth of *L. donovani* promastigotes in vitro. Data are shown for one representative experiment out of three with similar results. Media control (▲); DMSO control (○); 1.0 μg/mL (■); 5.0 μg/mL (△).

higher than normal. On the other hand, *L. donovani* infection reduced glutamate pyruvate transaminase (SGPT) drastically and glutamate oxaloacetate transaminase (SGOT) only marginally. The levels of both SGPT and SGOT increased in infected hamsters upon receiving compound **2** approaching almost normal values. These results suggested that **2** is non-toxic to liver up to a dose of 40 mg/kg body weight.

Conclusion

The results suggest that **2** mediates antileishmanial activity by inducing cell-cycle arrest leading to apoptosis. It is worthy of mention that **2**, which has a molecular weight of 291, log P of 3.5 (calculated via ACD) and only 3 H-bond donors and acceptors, is expected to pass through the Lipinski filter.[14] It therefore promises



Scheme 1.

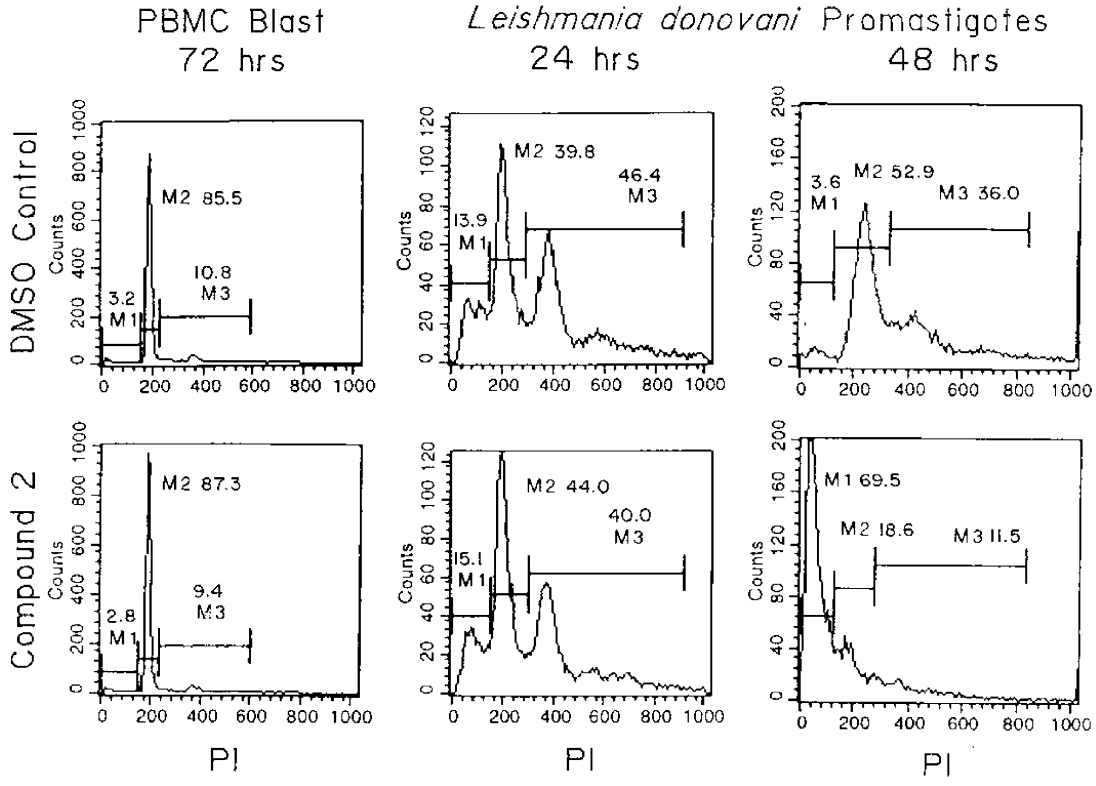


Figure 2. Effect of **2** on the cell-cycle of *L. donovani* promastigotes: *L. donovani* promastigotes (5×10^6/mL) were incubated with DMSO (0.2%), compound **2** (5.0 μg/mL dissolved in DMSO) at 22 °C and analyzed for DNA content by flow cytometry as described in the Experimental. DNA content of T-cell blasts was analyzed in the same way, except that PHA (5.0 μg/mL) was added to normal human peripheral blood mononuclear cell culture and incubation was carried out at 37 °C. Gates were set to assess the percentages of dead (<2n DNA), G_0/G_1 (2n DNA), and $S+G_2+M$ (>2n DNA) cells.

to be the first antileishmanial drug that can be used orally. Moreover, the preparation of **2** is very simple and the yield is nearly quantitative.

Experimental

Melting points (uncorrected) were determined with a capillary melting point apparatus. IR spectra were recorded on a JASCO FT/IR (model 410) in KBr pellets. MALDI-TOF MS (positive) was conducted using Perseptive Bio System Voyager DE-STR Mass spectrometer with 2,5-dihydroxy benzoic acid as matrix. ^{1}H and ^{13}C NMR spectra were taken on a Bruker 300 MHz DPX spectrometer at 300 and 74.99 MHz, respectively, with tetramethylsilane as internal standard. Sodium hydride, 4-aminoquinaldine, aniline and chloroacetyl chloride were obtained from Aldrich. Organic solvents are the products of E. Merck (India). Silica gel for column chromatography was obtained from SRL (India). Thin layer chromatography was performed on precoated silica gel 60 F_{254} aluminium sheets obtained from E. Merck (Germany) using the solvent system 13% MeOH in $CHCl_3$ and spots were developed using Drangendorff's reagent. Medium 199 was purchased from GIBCO BRL, USA.

2-Chloro-*N*-phenylacetamide (3). A solution of chloroacetyl chloride (5.6 g, 0.05 mol) in methylene chloride (20 mL) was added dropwise to a stirred solution of aniline (4.56 g, 0.05 mol) in dry methylene chloride (10 mL) at 0–5 °C during 30 min. The reaction mixture was stirred at the same temperature for another 30 min and at ambient temperature (30 °C) for a further hour. It was then poured into crushed ice. The organic layer was separated, washed successively with $NaHCO_3$ soln and water, and dried over anhydrous sodium sulphate. Evaporation under reduced pressure gave a solid, which was crystallized from ethylacetate–hexane to give **3** (7.61 g, 90%), mp 132 °C (lit.[12] mp 131–132 °C); IR (KBr, major peaks, cm^{-1}) 3267, 1673, 752, 689; ^{1}H NMR ($CDCl_3$) δ 4.18 (2H,–$COCH_2Cl$), 7.17 (1H, 4′-H), 7.35 (2H, 3′ and 5′-H), 7.5 (2H, 2′ and 6′-H), 8.2 (1H, br, NH); ^{13}C NMR ($CDCl_3$) δ 43.6 (t, C-2), 120.8 (d, C-2′ and 6′), 125.9 (d, C-4′), 129.8 (d, C-3′ and 5′), 137.4 (s, C-1′) and 164.5 (s, C-1). (Found: C, 56.68; H, 4.71; N, 8.24; Cl, 20.87; calcd for C_8H_8ClNO, C, 56.65; H, 4.75; N, 8.26; Cl, 20.90%).

Sodium hydride (400 mg) dispersed in oil was washed with dry petroleum ether and then stirred in dry DMSO (5 mL) at 75 °C for 20 min. A solution of 4-aminoquinaldine[15] (**4**) (158 mg, 1 mmol) in dry DMSO (3 mL) was added dropwise to the mixture and stirring continued for 30 min at 75 °C until olive green color appears. 2-Chloro-*N*-phenylacetamide (**3**) (168 mg, 1 mmol) in DMSO was added to the mixture and stirring continued at the same temperature for 8 h. After completion of the reaction (monitored by tlc), the reaction mixture was allowed to cool, poured into crushed

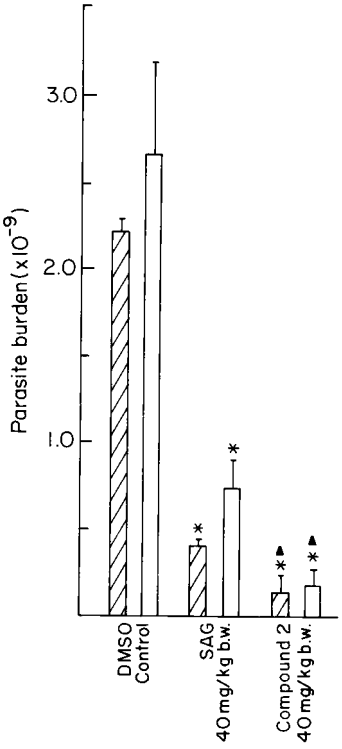


Figure 3. Treatment of established visceral leishmaniasis in hamsters with compound **2**. Indicated treatment was initiated at 4 weeks post-infection, the parasite burden was determined at 10 weeks post-infection as described in the Experimental. Spleen (▨); liver (□). $p < 0.005$ versus DMSO control (*); $p < 0.002$ versus SAG (▲).

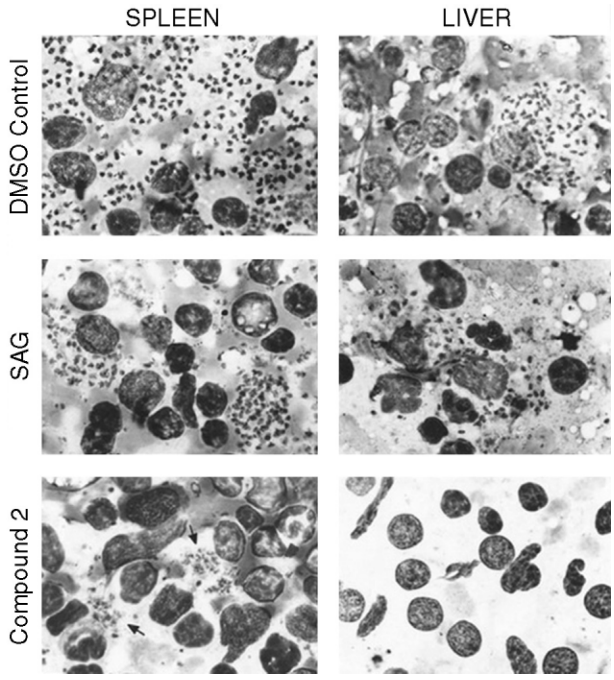


Figure 4. Micrographs of Giemsa-stained spleen and liver impression smears of *L. donovani* infected hamsters after treatment with DMSO, SAG or **2**. Smears are from 10 weeks post-infected hamsters. Treatment protocol was as described in the Experimental. Magnification, ×1000. Note the presence of healthy amastigotes in smears of DMSO-treated animals, reduced number of amastigotes in smears of SAG treated animals and degraded amastigotes (→) or no amastigotes in smears of compound **2** treated animals.

ice, and then extracted with ethyl acetate (3×100 mL). The organic layer was washed with water until free from alkali, evaporated to dryness and chromatographed over silica gel. Graded elution was effected with petroleum ether (60–80 °C), mixture of petroleum ether and chloroform (4:1, 3:2 and 1:4), chloroform and chloroform–methanol (49:1, 19:1 and 9:1). A total of 45 fractions of 50 mL each were collected and mixed on the basis of tlc.

***N*-Phenyl-2-phenylaminoacetamide (5).** Fractions eluted with petroleum ether and chloroform (3:2) yielded a residue which on crystallization from methanol furnished needles (19 mg, 5.9%), mp 110 °C; IR (KBr, major peaks, cm^{-1}) 3325, 1670, 1518, 755 and 695; ^1H NMR ($CDCl_3$) δ 3.9 (d, 2H, $J=5.7$ Hz, H-2), 4.36 (br s, 1H, CH_2NH), 6.69 (d, 2H, $J=7.8$ Hz, H-2″ and 6″), 6.86 (t, 1H, $J=7.5$ Hz, H-4″), 7.11 (t, 1H, $J=7.5$ Hz, H-4′), 7.23 (t, 2H, $J=7.8$ Hz, H-3″ and 5″), 7.31 (t, 2H, $J=7.8$ Hz, H-3′ and 5′), 7.52 (d, 2H, $J=7.8$ Hz, H-2′ and 6′) and 8.59 (br s, 1H, CONH); ^{13}C NMR ($CDCl_3$) δ 49.9 (t, C-2), 113.6 (d, C-2″ and 6″), 119.8 (d, C-4″), 119.9 (d, C-2′ and 6′), 124.5 (d, C-4′), 129.0* (d, C-3′ and 5′), 129.6* (d, C-3″ and 5″), 137.3 (s, C-1′), 147.0 (s, C-1″) and 168.8 (s, C-1) (*values may be interchanged). (Found: C, 74.29; H, 6.27; N, 12.40; $C_{14}H_{14}N_2O$ requires, C, 74.31; H, 6.24; N, 12.38%).

2,2′-Dimethyl-[3,3′]biquinolinyl-4,4′-diamine (6). Fractions eluted with petroleum ether–chloroform mixture (1:4) were combined and rechromatographed to give a residue which on crystallization from methanol furnished needles of **6** (27 mg, 8%), mp 215 °C; IR (KBr, major peaks, cm^{-1}) 3458, 3292, 1642, 1578 and 759; MS MALDI-TOF (positive ion) m/z 313 $[(M+H)-2H]^+$, 311 $[(M+H)-4H]^+$; ^1H NMR ($CDCl_3$) δ 2.73 (6H, s, CH_3-2 and 2′), 5.14 (4H, br s, NH_2-4 and 4′), 7.43 (t, 2H, $J=7.5$ Hz, H-6 and 6′), 7.60 (m, 2H, H-7 and 7′), 7.67 (m, 2H, H-5 and 5′) and 7.92 (d, 2H, $J=8.4$ Hz, H-8 and 8′); ^{13}C NMR ($CDCl_3$) δ 24.0 (q, 2 and 2′-CH_3), 110.2 (s, C-3 and 3′), 117.7 (s, C-4a and 4′a), 120.1 (d, C-6 and 6′), 124.9 (d, C-5 and 5′), 129.2* (d, C-7 and 7′), 129.3* (d, C-8 and 8′), 144.9$^+$ (s, C-8a and 8′a), 145.9$^+$ (s, C-4 and 4′) and 156.2 (s, C-2 and 2′) (assignments marked with similar symbols may be interchanged). (Found: C, 76.44; H, 5.73; N, 17.85; $C_{20}H_{18}N_4$ requires, C, 76.41; H, 5.77; N, 17.82%).

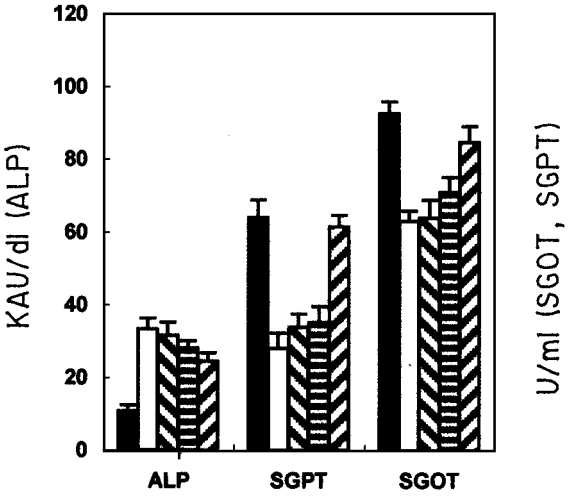


Figure 5. Specific enzyme levels in sera of hamsters undergoing experimental visceral leishmaniasis. Enzyme assays were performed using the kits from Dr. Reddy's Laboratories following the manufacturer's instructions. ALP activity was expressed as KA units/dL; 1 KA unit/dL = 7.1 U/L.[19] SGPT and SGOT activities were expressed as units/mL. Animals received indicated treatment four weeks post-infection, sacrificed 10 weeks post-infection for collection of sera as described in the Experimental. Data represent mean ± SD of 3–5 animals per group. Normal (■); infected animals treated with DMSO (□); infected animals treated with **2** [10.0 mg/kg body weight (▧)]; infected animals treated with **2** [20 mg/kg body weight (▤)]; infected animals treated with **2** [40 mg/kg body weight (▨)].

1,4-Diphenylpiperazine-2,5-dione (7). Fractions eluted with chloroform–methanol (49:1) on further chromatographic purification and subsequent crystallization from methanol gave **7** (15 mg, 5%), mp 267 °C (lit.[11] 266–267 °C); IR (KBr, major peaks, cm^{-1}) 1655, 1496, 1469, 756 and 693; ^1H NMR ($CDCl_3$) δ 4.54 (s, 4H, H-3 and 6), 7.35 (m, 6H, H-3′, 3″, 4′, 4″ and 5′, 5″), 7.46 (m, 4H, H-2′, 2″ and 6′, 6″); ^{13}C NMR (C_5D_5N) δ 53.4 (t, C-3, 6), 125.4 (d, C-2′, 2″ and 6′, 6″), 127.1 (d, C-4′, 4″), 129.2 (d, C-3′, 3″ and 5′, 5″), 140.4 (s, C-1′ and 1″), 164.7 (s, C-2 and 5). (Found: C, 72.19; H, 5.26; N, 10.49; calcd for $C_{16}H_{14}N_2O_2$, C, 72.16; H, 5.30; N, 10.52%).

2-(2-Methylquinolin-4-ylamino)-*N*-phenylacetamide (2). Fractions eluted with chloroform–methanol mixture (9:1) were mixed, purified by rechromatography and crystallized from methanol to furnish needles of **2** (74 mg, 22%), mp 220 °C; IR (KBr, major peaks, cm^{-1}) 3265, 1673, 1596, 1569, 1540, 1439, 1255, and 659; MS (MALDI-TOF, positive ion) m/z 292 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 2.60 (3H, s, 2″-CH_3), 4.08 (2H, d, $J=5$ Hz, 2-H_2), 5.9 (1H, br s, CH_2NH), 6.23 (1H, s, H-3″), 7.13 (1H, t, $J=8$ Hz, H-4′), 7.32 (2H, t, $J=8$ Hz, H-3′ and 5′), 7.42 (1H, br t, $J=8$ Hz, H-6″), 7.53 (2H, br d, $J=8$ Hz, H-2′ and 6′), 7.64 (1H, t, $J=8.5$ Hz, H-7″), 7.83 (1H, br d, $J=8.5$ Hz, H-5″), 7.95 (1H, br d, $J=8.5$ Hz, H-8″) and 8.5 (1H, s, CONH); ^{13}C NMR ($CDCl_3$) δ 25.6 (q, 2″-CH_3), 47.8 (t, C-2), 100.2 (d, C-3″), 117.5 (s, C-4″a), 119.2 (d, C-6″), 120.1 (d, C-2′, 6′), 124.7* (d, C-5″), 125.0* (d, C-4′), 129.2$^+$ (d, C-3′, 5′), 129.6$^+$ (d, C-7″, 8″), 137.1 (s, C-1′), 148.2* (s, C-4″), 148.9* (s, C-8″a), 159.8 (s, C-2″) and 167.1 (s, C-1) (assignments having similar symbols may be interchanged). (Found: C, 74.21; H, 5.86; N, 14.39; $C_{18}H_{17}N_3O$ requires, C, 74.20; H, 5.88; N, 14.42%).

When the same reaction was carried out in dry nitrogen atmosphere keeping all the parameters the same as described above, only the biologically active compound **2** was obtained (312 mg, 95%).[16]

Determination of antileishmanial activity in vitro. Antileishmanial activity of **2**, **5**–**7** was tested on a pathogenic strain of *L. donovani* (strain AG 83), originally obtained from an Indian Kala-azar patient[17] and maintained in golden hamsters. Promastigotes were obtained by transforming amastigotes (isolated from spleens of infected hamsters) and were maintained in vitro in

Medium-199 supplemented with 10% fetal bovine serum (FBS). Promastigotes (1×10^6) were incubated with or without various concentrations of different synthesized compounds in Medium-199 supplemented with 10% FBS at 22 °C. Growth of promastigotes was monitored by counting the number of motile promastigotes microscopically.

Analysis of cell-cycle progression by flow cytometry. In vitro grown *L. donovani* promastigotes and normal human T-cell blasts were analyzed for cell-cycle progression. T-cell blasts were prepared by culturing normal human peripheral blood mononuclear cells (PBMC), isolated from freshly drawn heparinized blood by Ficoll/Hypaque density gradient centrifugation, in RPMI-1640 medium containing 10% FBS and 5.0 μg/mL phytohemagglutinin (PHA) for 3 days at 37 °C in 5% CO_2. Cells were fixed with 40% ethanol, treated with 500 μg/mL RNase A, and then with 69 μM propidium iodide (PI) for analysis of DNA content by flow cytometry as described[18] using FACS Calibur (Becton Dickinson, USA).

Determination of antileishmanial activity of 2 in vivo. Golden hamsters (4–6 weeks old) were injected with freshly transformed promastigotes of *L. donovani* (2×10^7/hamster) by intra-cardiac route. Therapy with **2** and sodium antimony gluconate (SAG) started 1 month after infection. Compound **2** or SAG was injected at the same dose (40 mg/kg body weight) via the same route (intra-muscular) following the same protocol (once a week for 4 weeks). Animals in the control group received 100 μL DMSO intra-muscularly once a week for 4 weeks. Animals of all groups were sacrificed 2 weeks after the last treatment. Parasite load in the spleen and in the liver was determined from impression smears after Giemsa staining. Results were expressed as the total parasite load per organ, using the formula[19] (organ weight in mg×the number of amastigotes per cell nucleus$\times2\times10^5$).

Serum enzyme assays. The blood sera of normal hamsters and *L. donovani*-infected hamsters receiving treatment were subjected to assay for the enzymes alkaline phosphatase (ALP), glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT). These enzymes were assayed using the kits from Dr. Reddy's Laboratories, Hyderabad, India following the manufacturer's instructions. ALP activity was expressed as KA units/dL,[20,21] whereas SGPT and SGOT activities were expressed as units/mL.[21,22]

Statistical analysis. Statistical analyses were performed by Student's *t*-test with the program Tadpole III.[23]

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11. It appears that **7** originated from self-condensation of two units of the anion of **3**, but the origin of **5** and **6** is less obvious. It is, however, possible that **5** originated from an intermediate like **8** through cleavage of the tertiary amide bond via a nucleophilic attack, while **6** could have formed by the dimerisation of two anion radicals derived from **4**. The dimeric nature of **6** was evident from its NMR spectra. The FAB-MS of **6** did not show the expected protonated molecular ion peak at *m*/*z* 315, but displayed prominent peaks at *m*/*z* 313 and 311 corresponding to loss of two or four hydrogen atoms, respectively, from the parent ion. It is presumed that the hydrogen atoms are lost from the two amino groups with the formation of a six-membered ring.

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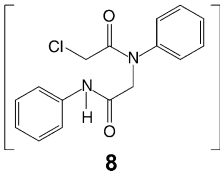

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