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Synthesis of 9-anilinoacridine triazines as new class of hybrid antimalarial agents

Ashok Kumar [a], Kumkum Srivastava [b], S. Raja Kumar [b], S. K. Puri [b], Prem M. S. Chauhan [a,*]

[a] Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226 001, India
[b] Division of Parasitology, Central Drug Research Institute, Lucknow 226 001, India

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ABSTRACT

There is challenge and urgency to synthesize cost-effective chemotherapeutic agents for treatment of malaria after the widespread development of resistance to CQ. In the present study, we synthesized a new series of hybrid 9-anilinoacridine triazines using the cheap chemicals 6,9-dichloro-2-methoxy acridine and cyanuric chloride. The series of new hybrid 9-anilinoacridine triazines were evaluated in vitro for their antimalarial activity against CQ-sensitive 3D7 strain of *Plasmodium falciparum* and their cytotoxicity were determined on VERO cell line. Of the evaluated compounds, two compounds **17** (IC_{50} = 4.21 nM) and **22** (IC_{50} = 4.27 nM) displayed two times higher potency than CQ (IC_{50} = 8.15 nM). Most of the compounds showed fairly high selectivity index. The compounds **13** and **29** displayed >96.59% and 98.73% suppression, respectively, orally against N-67 strain of *Plasmodium yoelii* in swiss mice at dose 100 mg/kg for four days.

The burden of malaria on the health and economics of the subtropical countries is increasing due to the rapid spread of resistance to the most of the classical drugs in use. It is estimated that malaria affects the 500 million people across the world, causes approximately 2.5 million deaths every year especially among the children under the age of five years.[1] *Plasmodium falciparum* is the most virulent form of parasite among the four plasmodium species infecting humans and is responsible for most of mortality.[2] Currently an effective therapeutic option for the treatment of resistant malaria is combination therapy based on the natural endoperoxide artemisinin and its semisynthetic derivatives.[3] The combination therapy is a viable option as the combination of drugs delays the selection of resistance to the parasite but the cost of treatment is much higher than monotherapy.[4] So there is an urgent need for new safer, more efficacious and affordable antimalarial agents as the disease is prevalent mainly in the poor subtropical countries.

The antimalarial drugs based on 4-aminoquinoline scaffold have been the mainstay of the malaria chemotherapy (Fig. 1) but the emergence of resistance to most commonly employed drugs like chloroquine (CQ), quinine, and mefolquine has limited their use in treatment.[5] So it is imperative to investigate other biologically important nucleus for the development of new antimalarial agents. In this context acridine nucleus offers an alternative to quinoline moiety. Quinacrine was the first synthetic antimalarial used before chloroquine. Pyronaridine is a new highly active blood schizonticidal mannich base antimalarial developed in china, effective against chloroquine resistant strain of *P. falciparum*.[6] Over the last few years acridine derivative mainly based on 9-aminoacridine and 9-anilinoacridine scaffold have been reported for antimalarial activity.[7–10] Moreover, acridine moiety possess a wide range of biological activities including antitumor,[11] antiprion,[12] anti-alzheimer,[13] antileishmanial, and antitrypanosomal[14] activity. Various

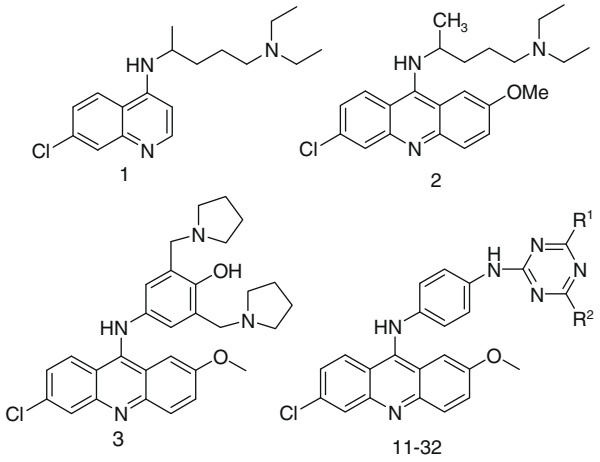

Figure 1. Structure of CQ, quinacrine, pyronaridine and title compounds.

* Corresponding author. Tel.: +91 522 221 2411x4332; fax: +91 522 262 3405.
E-mail addresses: prem_chauhan_2000@yahoo.com, premsc58@hotmail.com (P.M.S. Chauhan).

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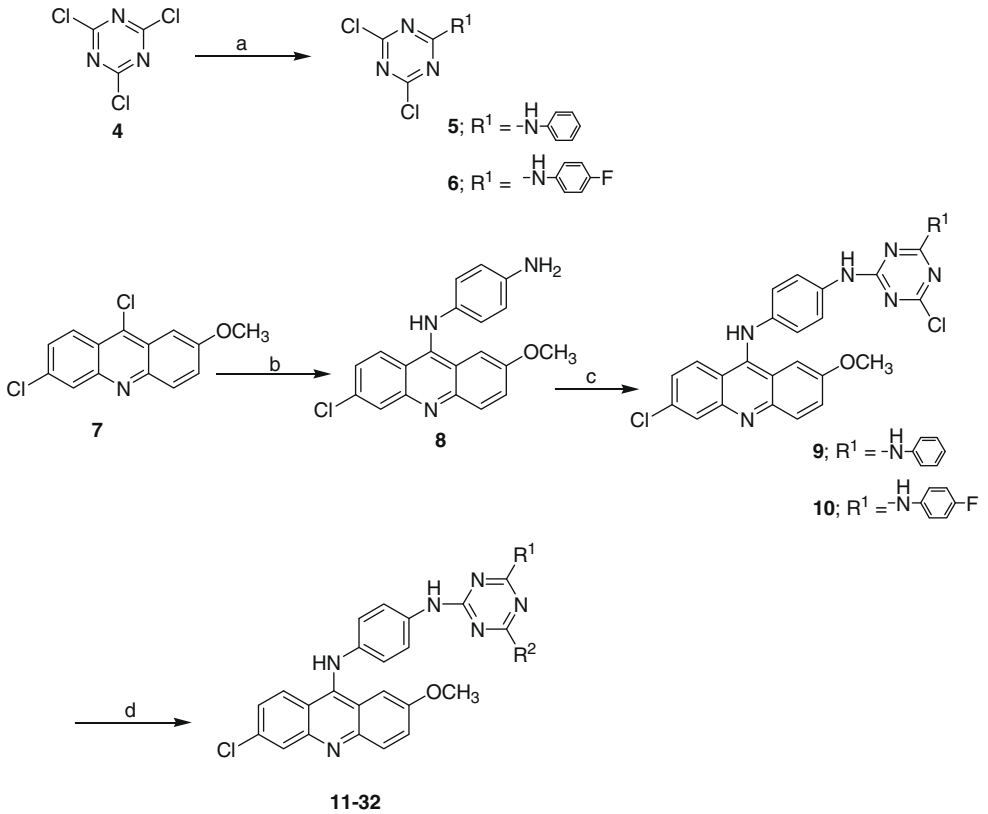
modes of action of acridine derivatives for their antimalarial effect have been proposed including DNA intercalation,[15] binding to heme,[16] and inhibition of the enzyme topoisomerase II.[17]

Designing hybrid drugs with multiple effects is a common strategy in today's search for new treatment of malaria. In recent years, various structurally diverse hybrid molecules were reported for the antimalarial activity. It included the 4-aminoquinoline-based isatin derivatives,[18] the 4-aminoquinoline-based β-carbolines,[19] the peroxide-based trioxaquine derivatives,[20,21] ferrocene–chloroquine analogues,[22] the 4-aminoquinolines based on inhibitors of a neutral zinc aminopeptidase,[23] the 4-aminoquinoline-based pyrazole analogues,[24] the 4-aminoquinoline-based tetraoxanes,[25] and the clotrimazole-based 4-aminoquinolines.[26] More recently, a new dual function acridones as a new antimalarial chemotype having the heme-targeting character of acridones along with a chemosensitizing component able to reverse the CQ-resistance were discovered.[27]

Triazine and dihydrotriazine derivatives have been reported to possess antimalarial activity.[28–31] Today, cycloguanil used for prophylaxis and treatment of malaria lost its efficacy due to emergence of resistance which warrants further investigation for developing new antimalarials. As part of our research efforts devoted to synthesis of nitrogen heterocycles as antimalarial agents, our group have identified trisubstituted triazines, pyrazole based triazines and hybrid 4-aminoquinoline triazines as antimalarials.[32] We envisaged that combining triazine with 9-anilinoacridine would lead to develop more efficacious antimalarial molecules. In this Letter, we report the synthesis and evaluation for in vitro and in vivo antimalarial activity of a series of hybrid 9-anilinoacridine triazines as well as determination of their cytotoxicity.

The synthesis of title compounds **11**–**32** were achieved by a synthetic protocol as depicted in Scheme 1. To begin with 4,6-dichloro-6-substituted-[1,3,5]triazine **5** and **6** were synthesized by nucleophilic substitution of cyanuric chloride with amines. Commercially available 6,9-dichloro-2-methoxyacridine **7** was condensed with *p*-phenylenediamine to give the *N*-(6-chloro-2-methoxy-acridinyl-9-yl)-benzene-1,4-diamine **8** using the *p*-TSA as a catalyst.[33] The compound **8** was refluxed with 4,6-dichloro-6-substituted-[1,3,5]triazines in THF to yield the corresponding 6-chloro-*N*-[4-(6-chloro-2-methoxy-9-acrdinyl-9-ylamino)-phenyl]-*N*′-substituted-[1,3,5]triazine-2,4-diamine **9** and **10**. The compounds **9** and **10** were subjected to nucleophilic substitution with various amine to give the title compounds **11**–**32** (Table 1). All the synthesized compounds were well characterized by IR, mass, NMR, and elemental analysis.[34]

All the synthesized compounds were evaluated in vitro for their antimalarial activity against CQ-sensitive strain 3D7 of *P. falciparum* using the described standard protocol.[35] The activity results are summarized in Table 1. Variations of different substituents on the triazine nucleus around position-4 and position-6 have been explored to ascertain the structure–activity relationship among the synthesized compounds of the series. Among the 22 evaluated compounds of the series, two compounds showed the two times higher antimalarial potency than CQ, three compounds displayed the antimalarial activity comparable to CQ, 13 compounds exhibited the IC_{50} values ranging from 15.08 to 68.39 nM and the four compounds showed the IC_{50} values ranging from 115.8 to 365.29 nM. While keeping the aniline or *p*-fluoroaniline as substituent at position-4, various amines were placed at position-6 to study the effect of substituent on the antimalarial potency. Considering the importance of fluorine in medicinal chemistry, aniline was replaced with *p*-fluoroaniline at position-4 around the triazine nucleus. However, activity results clearly suggest that the compound bearing aniline at position-4 around triazine nucleus were



Scheme 1. Reagents and conditions: (a) aniline/*p*-fluoroaniline, THF, 0 °C temp, 2 h; (b) *p*-phenylenediamine, *p*-TSA, absolute ethanol, reflux, 3 h; (c) monosubstituted triazines, dry THF, reflux, 10 h; (d) various amines, dry THF, 65 °C, 4 h.

Table 1
In vitro antimalarial activity of title compounds against CQ-sensitive strain 3D7 of *P. falciparum* and their cytotoxicity on VERO cell line

Compound	R^1	R^2	IC_{50} (nM)	SI
11	Aniline	*N*-Methyl piperazine	22.10	146.41
12	Aniline	*N*-Ethylpiperazine	19.17	164.46
13	Aniline	4-(2-Aminoethyl)morpholine	6.97	2896.02
14	Aniline	4-(3-Aminopropyl)morpholine	15.08	61.38
15	Aniline	*N,N*-Dimethylethylenediamine	7.95	159.75
16	Aniline	*N,N*-Diethylethylenediamine	21.78	136.85
17	Aniline	*N,N*-Dimethylpropylenediamine	4.21	295.02
18	Aniline	*n*-Butylamine	289.17	138.19
19	Aniline	Cyclopentylamine	17.51	5008.61
20	Aniline	2-Amino-1-ethanol	9.46	754.62
21	Aniline	3-Amino-1-propanol	365.29	17.45
22	Aniline	Hydrazine	4.27	315.39
23	Aniline	Ammonia	68.39	448.48
24	*p*-Fluoro-aniline	*N*-Methyl piperazine	49.44	210.17
25	*p*-Fluoro-aniline	*N*-Ethylpiperazine	65.61	193.17
26	*p*-Fluoro-aniline	*N,N*-Dimethylethylenediamine	40.06	28.12
27	*p*-Fluoro-aniline	*N,N*-Diethylethylenediamine	27.88	155.29
28	*p*-Fluoro-aniline	*N,N*-Dimethylpropylenediamine	35.17	99.82
29	*p*-Fluoro-aniline	4-(2-Aminoethyl)morpholine	20.15	3088.52
30	*p*-Fluoro-aniline	4-(3-Aminopropyl)morpholine	45.13	1558.49
31	*p*-Fluoro-aniline	Ammonia	119.31	37.13
32	*p*-Fluoro-aniline	Methylamine	115.83	335.72
CQ			8.15	8983

IC_{50}: concentration corresponding to 50% growth inhibition of the parasite.
Selectivity index (SI) = IC_{50} values of toxic activity/IC_{50} values of antimalarial activity.

more active than compounds with *p*-fluoroaniline. In case of compounds with aniline as common substituent, the compounds **11** and **12** having *N*-methyl piperazine and *N*-ethylpiperazine at position-6 displayed the much higher antimalarial potency than the compounds **25** and **26** bearing the *p*-fluoroaniline. Compound **13** bearing the aniline and 4-(2-aminoethyl) morpholine showed the three times higher potency than compound **29** having the *p*-fluoroaniline. Replacing the 4-(2-aminoethyl) morpholine with 4-(3-aminopropyl) morpholine, antimalarial activity was reduced two times (**13**, IC_{50} = 6.97 nM; **14**, IC_{50} = 15.08 nM). This activity pattern was also consistent in compounds **29** and **30** with *p*-fluoroaniline (**29**, IC_{50} = 20.15 nM; **30**, IC_{50} = 45.13 nM). Compound **15** bearing aniline and *N,N*-dimethylethylenediamine had an IC_{50} value of 7.45 nM while compound **16** with aniline and *N,N*-diethylethylenediamine displayed an IC_{50} value of 21.78 nM. On substituting the *N,N*-dimethylethylenediamine with *N,N*-dimethylpropylenediamine **17** (IC_{50} = 4.21 nM), the antimalarial activity was significantly increased. Compound **15** was five times more potent than compound **26** while the compound **17** was eight times more active than compound **28**. Compound bearing aniline and cyclopentylamine **19** had an IC_{50} value of 17.51 nM while substituting the cyclopentylamine with *n*-butylamine **18** led the dramatic decrease in antimalarial potency. Compound **22** having aniline and hydrazine as substituents displayed an IC_{50} value of 4.27 nM whereas compound **23** having aniline and ammonia as substituents showed an IC_{50} value of 68.39 nM. This observation indicates that hydrazine being the more basic favors the antimalarial activity. Compound **31** bearing the *p*-fluoroaniline and ammonia as substituents had an IC_{50} value of 119.31 nM while the compound **32** with *p*-fluoroaniline and methylamine exhibited an IC_{50} value of 115.83 nM. Structure–activity relationship studies based on the activity results revealed that aniline at position-4 and substituents *N*-ethylpiperazine, *N,N*-dimethylethylenediamine, *N,N*-dimethylpropylenediamine, 4-(2-aminoethyl)morpholine, 4-(3-aminopropyl)morpholine, 2-amino-1-ethanol, hydrazine and cyclopentylamine at position-6 were well tolerated for antimalarial activity.

The cytotoxicity of the all the synthesized hybrid 9-anilinoacridine triazine were determined against VERO cells using MTT assay.[36] Tested compounds were endowed with selectivity index ranging from 17.45 to 5008.61. The most of the compounds having good in vitro activity have shown fairly high selectivity index. Compound **19** having an IC_{50} value of 17.51 nM have shown the highest selectivity index among the evaluated compounds. Amongst the most potent compounds of the series, compounds **13**, **17**, and **22** with the IC_{50} values of 6.97, 4.21, and 4.27 nM displayed the selectivity index 2896.02, 295.02, and 31.39, respectively, thus demonstrating good activity profile.

Table 2
In vivo antimalarial activity against chloroquine resistant strain N-67 of *P. Yoelii* in swiss mice at dose 100 mg/kg for 4 days by oral route

Compound	% Suppression on day 4
13	96.59
15	49.00
17	95.70
20	58.52
27	95.42
29	98.73
CQ	99.9

The compounds with good in vitro activity were further subjected to in vivo study against CQ-resistant N-67 strain of *Plasmodium yoelii* orally in swiss mice at dose 100 mg/kg for four days (Table 2).[37] The compounds **13**, **17**, **27**, and **29** exhibited >95% suppression but could not provide significant protection to the treated mice in 28 days survival assay. Further structural optimization of 9-anilinoacridine triazine analogues may lead to the development of the more potent molecules.

In conclusion, there is challenge and urgency to synthesize cost-effective chemotherapeutic agents for treatment of malaria after the widespread development of resistance to CQ. As part of our research devoted to develop heterocycles as antimalarial, a series of 22 hybrid 9-anilinoacridine triazine molecules were synthesized. Compounds **13**, **15**, **17**, **20**, and **22** exhibited the good in vitro antimalarial activity with high selectivity index. Moreover, the compounds **13** and **29** displayed 96.59% and 98.73% suppression, respectively, against CQ-resistant N-67 strain of *P. yoelii* by oral

administration. Activity data suggests that fine-tuning of substituent at position-4 and position-6 around the triazine nucleus might yield the more potent antimalarial molecules.

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34. *General procedure for the synthesis of title compounds* (**11**–**32**): The solution of compound (11–32, 1.0 equiv) and different amines (2.0 equiv) in dry THF (25 ml) in the presence of K_2CO_3 was refluxed for 4 h. The solvent was removed under reduced pressure and the resultant residue was dissolved in chloroform (50 ml). The organic phase was washed with water (3×20 ml), dried over anhydrous Na_2SO_4. The solution was concentrated and purified with column chromatography using chloroform/methanol (100:2) to afford the compound (**11**–**32**). Spectroscopic data for **13**: MS: 648 (M+1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.21–8.06 (m, 2H, Ar-H), 7.98 (d, 1H, J = 9.24 Hz, Ar-H), 7.56 (d, 2H, J = 7.72 Hz, Ar-H), 7.46–7.43 (m, 3H, Ar-H), 7.33–7.26 (m, 4H, Ar-H), 7.14 (br s, 1H, NH), 7.04 (t, 1H, J = 7.32 Hz, Ar-H), 6.84 (br s, 2H, NH), 6.81 (d, 2H, J = 8.76 Hz, Ar-H), 5.54 (br s, 1H, NH), 3.77 (s, 3H, OCH_3), 3.72 (t, 4H, J = 4.72 Hz, O–CH_2), 3.43 (t, 2H, J = 5.82 Hz, NH–CH_2), 2.57 (t, 2H, J = 5.82 Hz, N–CH_2), 2.50 (t, 4H, J = 4.72 Hz, N–CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.38, 164.80, 156.93, 148.11, 139.38, 135.45, 133.52, 129.13, 126.01, 125.71, 125.41, 123.38, 122.42, 121.49, 120.79, 119.27, 118.48, 100.52, 57.67, 56.48, 55.80, 37.60, 30.09. Anal. Calcd for $C_{35}H_{34}ClN_9O_2$: C, 64.86; H, 5.29; N, 19.45. Found: C, 64.73; H, 5.17; N, 19.63; **17**: MS: 620 (M+1); ^{1}H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ (ppm) 8.02–7.92 (m, 3H, Ar-H), 7.51–7.44 (m, 4H, Ar-H), 7.37 (dd, 1H, J = 2.58 Hz, J = 9.39 Hz, Ar-H), 7.25–7.18 (m, 4H, Ar-H), 6.99 (t, 1H, J = 6.96 Hz, Ar-H), 6.87 (d, 2H, J = 8.43 Hz, Ar-H), 3.77 (s, 3H, OCH_3), 3.43 (t, 2H, J = 6.3 Hz, NH–CH_2), 2.62 (t, 2H, J = 6.72 Hz, CH_2), 2.42 (s, 6H, CH_3), 1.85 (quint., 2H, J = 6.72 Hz, CH_2); ^{13}C NMR ($CDCl_3$ + CD_3OD, 50 MHz): δ 166.24, 164.52, 156.45, 146.90, 140.71, 139.20, 136.15, 129.00, 126.04, 125.34, 123.43, 122.49, 121.08, 120.26, 119.59, 117.93, 101.29, 56.88, 55.72, 44.67, 29.99, 26.78. Anal. Calcd for $C_{34}H_{34}ClN_9O$: C, 65.85; H, 5.53; N, 20.33. Found: C, 65.98; H, 5.48; N, 20.46; **26**: MS: 624 (M+1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.16–8.05 (m, 2H, Ar-H), 7.98 (d, 1H, J = 9.00 Hz, Ar-H), 7.46–7.43 (m, 5H, Ar-H), 7.33–7.30 (m, 2H, Ar-H), 7.14 (br s, 1H, NH), 6.98 (t, 2H, J = 8.61 Hz, Ar-H), 6.86 (br s, 2H, NH), 6.83 (d, 2H, J = 8.76 Hz, Ar-H), 5.59 (br s, 1H, NH), 3.77 (s, 3H, OCH_3), 3.49 (t, 2H, J = 5.82 Hz, NH–CH_2), 2.50 (t, 2H, J = 5.82 Hz, N–CH_2), 2.26 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 50 MHz): δ 171.27, 169.55, 160.46, 150.41, 144.32, 139.93, 136.24, 134.73, 130.46, 126.98, 126.83, 124.06, 122.92, 121.75, 120.26, 119.82, 101.37, 63.72, 60.67, 50.66, 43.62. Anal. Calcd for $C_{33}H_{31}ClFN_9O$: C, 63.51; H, 5.01; N, 20.20. Found: C, 63.42; H, 5.13; N, 20.03.
35. The compounds were dissolved in DMSO at 5 mg/ml. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 μl of highest starting concentration (500 ng/ml) was dispensed in duplicate wells in row B of 96-well tissue culture plate. The highest concentration for chloroquine was 25 ng/ml. Subsequently twofold serial dilutions were prepared up to row H (seven concentrations). Finally 50 μl of 2.5% parasitized cell suspension containing 0.5% parasitaemia was added to each well except four wells in row A which received non-infected cell suspension. These wells containing non-infected erythrocytes in the absence of drugs served as negative controls, while parasitized erythrocytes in the presence of CQ served as positive control. After 72 h of incubation, 100 μl of lysis buffer [20 mM tris (pH 7.5), 5 mM EDTA, 0.008% (wt/vol) saponin, and 0.08% (vol/vol) Triton X—100] containing 1× concentration of SYBER Green I (Invitrogen) was added to each cell. The plates were re-incubated for one hour at room temperature and examined for the relative fluorescence units (RFUs) per well using the FLUOstar, BMG lab technologies. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose–response curves. Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803.
36. Cytotoxicity of the compounds was determined against VERO cell lines (C-1008; Monkey kidney fibroblast cells) using MTT assay. A total of 1×10^4 cells/well were incubated with varying concentrations of compound for 72 h. The highest concentration of compound was 100 μg/ml. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose–response curves and represented the concentration of compound required to kill 50% of the fibroblast cells. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
37. The in vivo drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ. The mice (22 ± 2 g) were inoculated with 1×10^6 parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0 to 3, once daily. The aqueous suspensions of compounds were prepared with a few drops of Tween 80. The efficacy of test compounds was evaluated at 100 mg/kg/day and required daily dose was administered in 0.1 ml volume via oral route. Parasitaemia levels were recorded from thin blood smears between days 4 and 6. The mean value determined for a group of five mice was used to calculate the percent suppression of parasitaemia with respect to the untreated control group. Mice treated with CQ served as reference controls. Puri, S. K.; Singh, N. *Expl. Parasitol.* **2000**, *94*, 8.