

Synthesis and antimalarial evaluation of some 4-quinazolinone derivatives based on febrifugine

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J. Adv. Pharm. Tech. Res.

ABSTRACT

A series of 2-substituted and 2,3-substituted quinazolin-4(3H)-one derivatives were designed and synthesized based on the structure of febrifugine. The structures of the new compounds were confirmed by spectral analysis. The *in vivo* biological activity test results indicated that those compounds exhibited antimalarial activities against *Plasmodium berghei* in mice, at a dose of 5 mg/kg. Compared to Chloroquine and Artemisinin, these compounds have the advantages of shorter synthetic routes and consequently are highly cost effective in nature.

Key words: Antimalarial activity, febrifugine, Plasmodium berghei, 4-quinazolinone

INTRODUCTION

Malaria is a life-threatening disease even in this decade. Studies have shown that every year throughout the world more than 300 million people suffer from malaria infections and millions of people including children die due to this disease.^[1-3] Due to the ongoing development of resistance of *Plasmodium falciparum* to conventional antimalarial drugs, malaria has become a major global problem and efforts to discover new agents against multi-drug resistant *Plasmodium* strains are long-term and essential tasks for researchers.^[4] Medicinal agents based on novel mechanisms of action are, therefore, required to overcome the increasing resistance and to control an ever increasing number of epidemics caused by the malaria parasite.^[5]

In order to find new and effective antimalarial drugs with a simpler structure and with a low cost synthetic methodology, few 2,3-substituted quinazolin-4(3H)-one derivatives were designed and synthesized according to

the structural features of febrifugine [Figure 1a] which is obtained from Chinese plant roots of *Dichroa febrifuga* Lour. (Chinese name: Cha'ng Shan), a saxifragaceous plant that has been employed against malaria fevers, and no parasite resistant to *D. febrifuga* has been reported. Two alkaloids, i) febrifugine and ii) isofebrifugine [Figure 1b], were isolated from *D. febrifuga* as active principles against malaria.^[6]

Due to numerous toxic effects, Febrifugine is not a successful drug candidate.^[7] The synthetic derivative of febrifugine, 3''-ketofebrifugine [Figure 2] (Kikuchi *et al.* 2002), is cited as a good candidate for clinical trials ($EC_{50}=2.0 \times 10^8$). To assess the biological properties of these alkaloids in detail, studies were carried out on the antimalarial properties of some chemical entities based on febrifugine. A lot of work on the antimalarial activity of many febrifugine analogues has been reported previously.^[8-11] But in all cases, their synthetic methodologies are either costlier or the compounds do not possess promising

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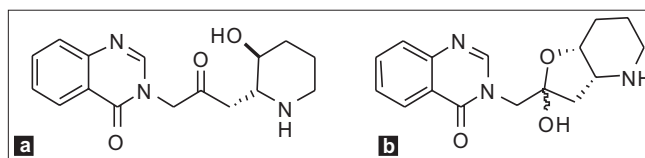


Figure 1: (a) Febrifugine and (b) Iso-febrifugine

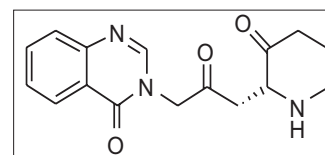


Figure 2: Ketofebrifugine

antimalarial activity. It has been demonstrated that most febrifugine analogues bearing a modified or non-modified 4-quinazolinone moiety are active,^[12] from this line of evidence; it can easily be assumed that the 4-quinazolinone moiety of natural febrifugine possesses activity against the malaria parasite. The 3-hydroxy piperidin-2-yl-propane-2-one at N3 position of the 4-quinazolinone ring was identified as an important fragment for producing biological activity. These results suggest that the 4-quinazolinone moiety, the nitrogen atom of the piperidine ring and propyl chain are necessary for the antimalarial activity. In the present work, some 2-substituted quinazolinones with 1-(2-hydroxy propyl) pyrrolidine-2-carboxylic acid substitution at N3 position of quinazolinone ring were synthesized and the antimalarial property of those compounds was investigated using *in vivo* screening method.

MATERIALS AND METHODS

Melting point was determined using a Sturat SMP heating stage microscope and was uncorrected. Nuclear magnetic resonance (NMR) ¹H spectra were recorded on a Bruker AV300 Supercon NMR System with chemical shifts being represented in parts per million (ppm) and with tetramethylsilane (TMS) as an internal standard. Electron impact Mass spectra (EI-MS), High resolution Mass spectra (HRMS) and Fast atom bombardment mass spectroscopy (FAB MS) were recorded on an Autospec-Ultima ETOF MS spectrometer at an ionization voltage of 70 eV. Reactions were monitored by thin layer chromatography (TLC) and the spots were visualized by spraying the TLC plates with 2% ninhydrin/acetic acid w/v solution. The TLC employed pre-coated silica gel plates (aluminum sheets 20×20 cm, silica gel 60 F254 of Merck K GaA). All solvents and reagents used were of analytical grade and obtained from Merck, India. All solvents used were of spectral grade or distilled prior to use.

As shown in Figure 3, the important intermediate 4 was synthesized by the reaction of commercially available 4-piperidone (3) with epibromohydrin in an anhydrous condition. Compounds 5a–h were prepared using simple coupling reaction of 4 with various quinazolinones using NaH in dry (Di methyl formamide) DMF.

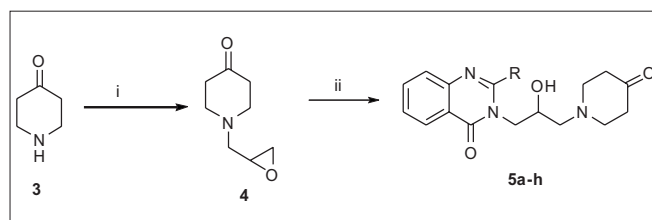


Figure 3: Reagent and condition: i) Epibromohydrine; K_2CO_3 , Acetone, ii) Quinazolinone; NaH, DMF R=a) H, b) Me, c) Ph, d) *p*Cl-Ph, e) *p*Me-Ph, f) *p*Br-Ph, g) *p*F-Ph, h) 2-Br-Ph

1-(oxiran-2-ylmethyl) piperidin-4-one (4)

To an ice-cold solution of 4-piperidone (0.5 g) in dry acetone and K_2CO_3 (0.53 g, 1.2 eq), epibromohydrin (0.68 ml) was added dropwise in nitrogen atmosphere. It was stirred overnight at room temperature. Then, the reaction mixture was filtered off. After evaporation of the solvent under vacuum, it was directly loaded into a column of silica gel and eluted with 0.2–1% EtOAc/hexane. Compound 4 was isolated as a colorless liquid.

¹H NMR (300 MHz, $CDCl_3$) δ 3.30–3.28 (m, 1H), 3.01 (t, J = 5.7 Hz, 2H), 2.84–2.72 (m, 3H), 2.66–2.51 (m, 6H), 2.31 (dd, J = 12.4, 4.9 Hz, 1H).

2,3-substituted quinazolin-4(3H)-one derivative general method of preparation

To an ice-cooled suspended solution of sodium hydride (1.5 eq) in dry DMF, various quinazolin-4(3H)-ones^[13] was added. The reaction mixture was then stirred at 0°C for 30 min. This was followed by dropwise addition of corresponding oxiranes (1.2 eq) in dry DMF. The mixture was then refluxed at 75–80°C for 5 hours and was stirred overnight at room temperature.^[9] Then, the reaction mixture was quenched with 30% HCl solution and extracted with DCM, dried over brine and anhydrous sodium sulfate and evaporated by distillation. The crude mixture was purified by column chromatography over silica gel.

Typical synthesis of 3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one (5a)

To an ice-cooled suspended solution of sodium hydride (0.266 g) in dry DMF (5 ml), quinazolin-4(3H)-one (0.2 g) was added. The reaction mixture was then stirred at 0°C for 30 min. Then, ethyl 1-(oxiran-2-ylmethyl) pyrrolidine-2-carboxylate (0.326 g) in dry DMF was added dropwise. The mixture was then refluxed at 75–80°C for 5 hours and was stirred overnight at room temperature. Then, the reaction mixture was quenched with 30% HCl solution (1.3 ml) and extracted with DCM (10×3 ml), dried over brine and anhydrous sodium sulfate and evaporated by distillation. The crude mixture was purified by column chromatography over silica gel (2% MeOH/ $CHCl_3$) to furnish 3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one. After re-crystallization with DMF, a white crystalline solid was obtained with MP 207°C.

¹H NMR (300 MHz, DMSO) δ 7.96 (s, 1H), 7.72 (d, J = 24.4 Hz, 2H), 7.43 (d, J = 15.4 Hz, 2H), 4.04 (s, 1H), 3.70 (s, 1H), 3.56 (s, 1H), 3.24–3.10 (m, 3H), 3.04 (s, 1H), 2.67–2.47 (m, 7H).

3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl)-2-methylquinazolin-4(3H)-one (5b)

¹H NMR (300 MHz, DMSO) δ 7.93 (s, 1H), 7.67 (s, 1H), 7.39 (d, J = 18.8 Hz, 2H), 6.30 (s, 1H), 4.51 (s, 1H), 3.72 (s, 1H), 3.25–3.03 (m, 3H), 2.70–2.44 (m, 10H), 2.29 (s, 1H).

3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl)-2-phenylquinazolin-4(3H)-one (5c)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.78–7.25 (m, 8H), 6.17 (s, 1H), 3.88 (d, J = 32.9 Hz, 2H), 3.30–3.21 (m, 2H), 3.17–2.90 (m, 3H), 2.76–2.69 (m, 2H), 2.64–2.46 (m, 4H).

2-(4-chlorophenyl)-3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one (5d)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.59–7.38 (m, 4H), 7.39–7.28 (m, 2H), 6.16 (s, 1H), 3.93 (s, 1H), 3.79 (s, 1H), 3.28–3.19 (m, 2H), 3.15–2.90 (m, 3H), 2.78–2.68 (m, 2H), 2.62–2.42 (m, 4H).

3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl)-2-*p*-tolylquinazolin-4(3H)-one (5e)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.71 (s, 1H), 7.62–7.42 (m, 4H), 7.26–7.09 (m, 2H), 6.15 (s, 1H), 4.33 (s, 1H), 3.93 (s, 1H), 3.16–2.90 (m, 4H), 2.73 (t, J = 16.1 Hz, 3H), 2.55–2.37 (m, 4H), 2.36–2.30 (m, 3H).

2-(4-bromophenyl)-3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one (5f)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.54–7.41 (m, 6H), 6.15 (s, 1H), 3.93 (s, 1H), 3.78 (s, 1H), 3.28–3.21 (m, 2H), 3.07 (d, J = 11.2 Hz, 2H), 2.96 (s, 1H), 2.76–2.69 (m, 2H), 2.69–2.48 (m, 4H). m/z 455.02.

2-(4-fluorophenyl)-3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one (5g)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.63–7.54 (m, 2H), 7.46 (d, J = 7.4 Hz, 2H), 7.13–6.98 (m, 2H), 6.16 (s, 1H), 3.95 (s, 1H), 3.80 (s, 1H), 3.28–3.20 (m, 2H), 3.16–2.88 (m, 3H), 2.79–2.66 (m, 2H), 2.61–2.45 (m, 4H). m/z 395.42

2-(3-bromophenyl)-3-(2-hydroxy-3-(4-oxopiperidin-1-yl) propyl) quinazolin-4(3H)-one (5h)

^1H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.84 (s, 1H), 7.70 (s, 1H), 7.59–7.40 (m, 4H), 7.21 (s, 1H), 6.16 (s, 1H), 3.95 (s, 1H), 3.77 (s, 1H), 3.31–3.18 (m, 2H), 3.16–2.88 (m, 3H), 2.76–2.66 (m, 2H), 2.61–2.44 (m, 4H).

Protocol for Evaluating Antimalarial Activity of Analogues Against *Plasmodium berghei* and *Plasmodium yolli* Strains

The method used for screening of the synthesized compounds for their blood schizonticidal activity^[14] is based on a comparison of responses by groups of treated and control mice, six in each group, after infection with *P. berghei* (obtained from NIMR, New Delhi, India). Using standard inoculums of *P. berghei* (1×10^7 infected red cells), it is possible to produce a uniform disease that is fatal to 100% of untreated animals, within 6–8 days, with a mean survival time of 6.2 days. Test animals (Swiss mice of either sex, approximately 29 ± 2 g and same age) were housed in metal-topped cages, given a standard laboratory diet and water *ad libitum*. In order to check factors such as changes in the infectivity of the strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with chloroquine diphosphate and sodium artesunate at dose levels 10 mg/kg/day for 4 days, producing definite increase in survival time, is included as a positive control in every experiment. In each experiment, the test compounds were administered in graded doses of 100, 50, 25, 10 and 5 mg/kg. The compounds showing positive biological activity at 50 mg/kg were further selected for screening at lower doses. On day 0, groups of six mice each were inoculated intraperitoneally with 1×10^6 infected erythrocytes in PBS solution from a donor mouse. Four hours later, mice were administered test compounds/ Chloroquine (CQ)/sodium artesunate/vehicle orally. A total of four doses were given orally on days D0, D1, D2, and D3. The thick and thin films blood smears (blood collected from tail vein) were made on D4 and D7, fixed with methanol and stained with 4% Giemsa at pH 7.2 for 45 min and examined microscopically [Figure 4] in agreement with study results of Peters. One group was left untreated as a positive control. Three different fields were examined on each slide and the number of infected and uninfected red blood cells (RBC) counted and the mean taken. The experimental design was similar to the 4-day suppressive test.^[15]

Percent parasitemia was calculated using the following formula:

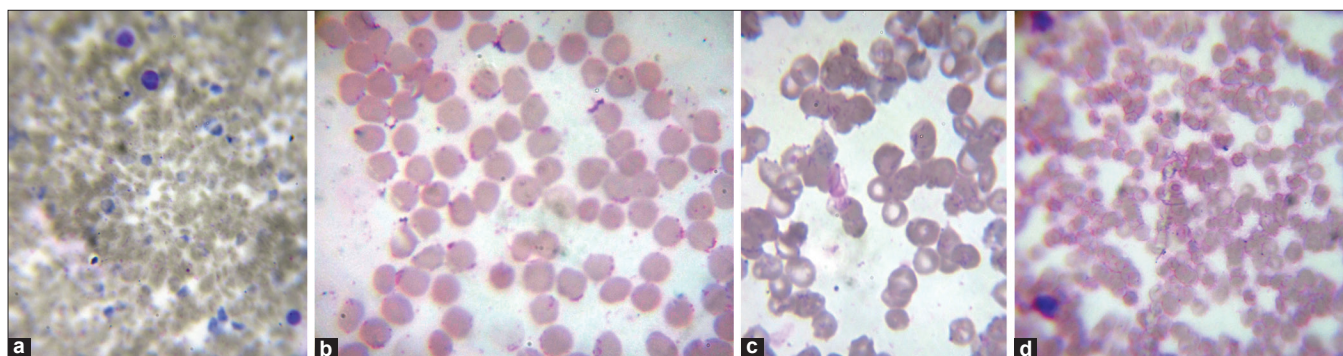


Figure 4: (a) Untreated; (b) treated with chloroquine; (c) treated with sodium artesunate; (d) treated with compound 5a

$$\% \text{ paristemia} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times 100$$

where PRBC is parasitized red blood cells.

Average percentage of parasitemia suppression was calculated with the following formula:^[16]

$$\text{Average percentage of paristemia} = \frac{\text{Av. \% parasitaemia in test}}{\text{Av. \% parasitaemia in control}} \times 100$$

The minimum dose that completely suppressed parasitemia on D4 and D7 was termed as effective suppressive dose (ESD), and the minimum dose that cleared the parasitemia for up to the next 30 days was termed as therapeutically effective dose (TED).

Acute toxicity study was conducted for the active extracts (single dose 2000 mg/kg) using the method of Weil.^[17] Albino mice weighing 20–25 g of either sex were divided into groups of five mice each. Each group received the test compounds orally. Signs of toxicity were observed for the first 2 hours and in 2 hours interval for 6 hours. Mortality was assessed after 24 hours.

RESULTS AND DISCUSSION

The results of acute toxicity studies in Swiss mice show that all the synthesized compounds were safe and non toxic up to 1000 mg/kg as compared to 88 mg/kg for febrifugine. All of the synthesized compounds were tested for *in vivo* blood schizonticidal activity against *P. berghei* infected mice model. The dose tested orally were 100, 50, 25, 10 and 5 mg/kg/day for 4 days. The results for all analogues were compared to a positive control group and negative control group (in this case, all animals usually died by D15) of mice treated with CQ and sodium artesunate (Sod.A^{te}) at the concentration of

10 mg/kg/day × 4 days [Table 1], with mice showing negative parasitemia on D4 and D7.

The term “therapeutically effective” indicates complete elimination of malaria parasites from the body so that relapse will not occur up to day D30. The term “active” or ESD indicates that the treated animals show negative parasitemia up to D7. However, by D28 to D29, some mice showed negative and some mice showed positive test results for parasitemia. The term “inactive” indicates that the treated animals showed positive test result for parasitemia either on D4 or D7 or on both D4 and D7. All of the 2,3-substituted quinazolin-4(3H)-one derivatives (5a–f) were found to be more or equally active in the *P. berghei* test. The most effective compound 5a produced 100% cures at the preliminary tested dose of 50 mg/kg, but exhibited no curing result at 5 mg/kg.

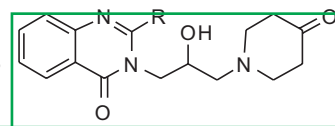
CONCLUSION

In summary, the antimalarial activity of 2,3-substituted quinazolin-4(3H)-one derivative has been identified. Substitution of 2 position of quinazolinone ring does not improve the activity markedly, thereby necessitating further exploration of the most suitable substitution at the N3 positions of quinazolinone. The biological evaluations of these are under process. The results of this study clearly establish that the potent antimalarial activity displayed by analogue 5a is attributed to the incorporation of quinazolinone group of febrifugine, and its 2-aryl substituted analogues do not alter the antimalarial activity.

ACKNOWLEDGEMENT

The authors acknowledge Mr. Subir Pal, CEO/President of Bengal Institute of Pharmaceutical Sciences, Kalyani and Jadavpur

Table 1: *In vivo* antimalarial activity of synthesized compounds



Compound ID	R	Mice (six mice for each dose) alive up to D7 after 4 days treatment					% Parasitemia after 4 days treatment
		5 mg/kg/day ×4, oral	10 mg/kg/day ×4, oral	25 mg/kg/day ×4, oral	50 mg/kg/day ×4, oral	100 mg/kg/day ×4, oral	
5a	H	0/6	0/6	0/6	6/6	6/6	5.925926±0.76
5b	Me	0/6	0/6	0/6	5/6	6/6	7.407407±0.93
5c	Ph	0/6	0/6	0/6	2/6	5/6	13.33333±1.8
5d	Cl	0/6	0/6	0/6	5/6	6/6	7.407407±1.25
5e	Me	0/6	0/6	0/6	3/6	4/6	13.33333±1.56
5f	Br	0/6	0/6	0/6	2/6	3/6	8.888889±1.30
5g	F	0/6	0/6	0/6	5/6	5/6	7.407407±1.27
5h	2-Br	0/6	0/6	0/6	1/6	2/6	11.85185±0.91
CQ	—	—	6/6	—	—	—	0
Sod.Arte	—	—	6/6	—	—	—	0

CQ=Chloroquine

University, Kolkata, for providing financial and technical support for this work. One of the authors is also grateful to Dr. Pellai (NIMR, New Delhi, India), Dr. Ajit S. Singh (Scientist, DRDO, Kanpur, India) and Dr. Arup Mukherjee (Calcutta University, Kolkata, India) for their invaluable technical advice for finishing the project in time.

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Source of Support: Bengal Institute of Pharmaceutical Sciences, Kalyani and Jadavpur University, Kolkata,, **Conflict of Interest:** Nil.

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