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 (EC₅₀ = 2.0×10⁸). 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...
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 (Dmethyl formamide) DMF.

![Figure 3](image-url)

**Figure 3:** Reagent and condition: i) Edpibromohydrine; K$_2$CO$_3$, Acetone, ii) Quinazolinone; NaH, DMF R=a) H, b) Me, c) Ph, d) pCl-Ph, e) pMe-Ph, f) pBr-Ph, g) pF-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$_2$CO$_3$ (0.53 g, 12 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 dydride (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, 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)

$^1$H 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)

$^1$H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.59–7.28 (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)

$^1$H 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)

$^1$H 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), 3.28–3.21 (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)

$^1$H NMR (300 MHz, DMSO) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.63–7.54 (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 395.42.

Protocol for Evaluating Antimalarial Activity of Analogues Against Plasmodium berghei and Plasmodium yoelli 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 ($1 \times 10^7$ 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 $1 \times 10^6$ 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 vain) 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:

\[
\text{Percent parasitemia} = \frac{\text{Number of infected RBC}}{\text{Total RBC}} \times 100
\]

Figure 4: (a) Untreated; (b) treated with chloroquine; (c) treated with sodium artesunate; (d) treated with compound 5a
% parasitemia = \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]

\frac{\text{Av. % parasitaemia in control}}{\text{Av. % parasitaemia in test}} \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.Arte) at the concentration of 10 mg/kg/day \times 4 \text{ 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.

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