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Research Article

SYNTHESIS AND *In Vivo* ANTI-MALARIAL EVOLUTION OF 4-QUINAZOLINONE DERIVATIVES

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ABSTRACT

Natural product governs an important role in the development of novel drugs against several diseases. A wide range of semi synthetic and synthetic analogues of natural product till used as an anti malarial, anti cancer drugs and so on. It is well known to us that Malaria is becoming a life threatening disorder now a day because of its on going resistant upon conventional antimalarial drugs. In the present study we select a potent anti malarial natural product Febrifugine obtained from *Dichroa febrifuga* and synthesized some amide derivative. After subsequent pharmacological evolution we performed 3D QSAR analysis to determine the structural insight of these type of molecules for there antimalarial property.

Keywords: Febrifugine, Anti malarial, 4-Quinazolinone.

INTRODUCTION

Bioactive molecules from natural sources play an important role to combat against various diseases[1]. Molecules like Morphine, Quinine, Ivermactine, Taxol, Artemisinin etc are the excellent example of some naturally occurring compounds and there derivatives possess enormous clinical effectiveness to combat against various diseases. More over anti microbial resistance against conventional chemotherapy motivate the researchers to discover of develop novel Led molecules for healthier as well as cost effective therapy. For example, first antimalarial drug quinine was isolated from the bark of *cinchona officionalis.* After that various clinically used medicines like Chloroquine was developed based on quinine which till used as a potent anti malarial drugs. But by *falciparum* species day by day develop resistance against conventional anti malarial drugs and malaria becoming a devastating disorders till in this twenty first century. This causes millions of death in every year especially in developing countries. There is a naturally occurring compound Artemisinin shows very good anti malarial properties but it has some draw backs. Its semi synthetic derivatives like sodium artisunate, artiether etc possess superior effectiveness against Chloroquine resistant falciparum species and used a clinically useful medicine[2]. In the present work we focused on a naturally occurring anti malarial compound Febrifugine possess higher level antimalarial affectivity but it can not be a successful clinically used drug candidate due o its toxicity profile[3]. A large number derivative of Febrifugine ware synthesized and tested by various researchers but in every case either they are not showing a promising activity or having typical synthetic methodology for there preparation[4].

Considering this, we are interested to study the effect of some amide compounds based on the structure of Febrifugine using a cost effective and easy synthetic methodology.

MATERIAL AND METHOD

Melting point was determined using a Sturat SMP heating stage microscope and was uncorrected. Nuclear magnetic resonance (NMR) ^{1}H 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. 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.

Ethyl or Methyl (Oxiran-2-ylmethyl pyrrolidine-2-carboxylate) 4: Compound **4** is the key intermediate for the synthesis of all derivatives. It was prepared as per our previously reported method[5].

Compound 5a-e: Compound **5a-e** was synthesized as per our previously reported method .[5]

5a A white solid compound

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.72 (d, *J* = 24.5 Hz, 2H), 7.42 (d, *J* = 17.6 Hz, 2H), 4.37 (s, 1H), 4.06 (s, 1H), 3.91 – 3.73 (m, 4H), 3.22 – 2.91 (m, 4H), 2.45 (s, 1H), 2.19 (s, 1H), 2.06 (s, 1H), 1.87 – 1.57 (m, 3H).

Compound 5b: A yellowish white solid compound. Soluble in Chloroform,

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.66 (s, 1H), 7.42 (s, 1H), 7.35 (s, 1H), 6.41 (s, 1H), 4.85 (s, 1H), 3.92 (s, 1H), 3.73 – 3.66 (m, 3H), 3.18 (s, 1H), 3.02 (s, 1H), 2.82 – 2.63 (m, 4H), 2.45 (s, 1H), 2.10 (d, *J* = 18.1 Hz, 2H), 2.00 (s, 2H), 1.83 (s, 1H), 1.76 (s, 1H), 1.67 (s, 1H).

Compound 5c: A yellowish solid compound soluble in $CHCl_3$

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.70 (s, 1H), 7.65 – 7.55 (m, 2H), 7.45 (d, *J* = 9.8 Hz, 2H), 7.39 – 7.27 (m, 3H), 6.48 (s, 1H), 4.39 (s, 1H), 3.79 – 3.64 (m, 4H), 3.20 (d, *J* = 13.7 Hz, 2H), 2.86 (d, *J* = 23.6 Hz, 2H), 1.92 (dt, *J* = 52.5, 37.9 Hz, 6H).

Compound 5d: A white solid compound soluble in DCM

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.70 (s, 1H), 7.62 – 7.31 (m, 6H), 6.24 (s, 1H), 4.69 (s, 1H), 4.19 (s, 1H), 3.79 – 3.70 (m, 3H), 3.18 (s, 1H), 2.96 (s, 1H), 2.60 – 2.29 (m, 3H), 2.15 – 1.55 (m, 5H).

Compound 5e: A white solid compound soluble in DCM.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.69 (s, 1H), 7.61 – 7.38 (m, 4H), 6.99 – 6.85 (m, 2H), 6.24 (s, 1H), 4.04 (s, 1H), 3.90 – 3.69 (m, 7H), 3.38 (s, 1H), 3.18 (s, 1H), 2.91 (d, *J* = 9.9 Hz, 2H), 2.49 (d, *J* = 21.8 Hz, 2H), 2.06 (s, 1H), 1.94 – 1.53 (m, 3H).

In vitro antimalarial activity against *Plasmodium falciparum* K1[6]

The methods of Trager and Jenson, (1976) were employed for cultivation of Plasmodium culture. Blood sample (group: O+) with mono infection of *Plasmodium falciparum* was collected from malaria patient in citrate dextrose containing vial. The hematocrit of the blood sample was first determined by centrifuging it at 2000 rpm 10 mins. Plasma and the buffy coat containing WBC were removed. RPMI (Rosewell Park Memorial Institute) 1640 medium including HEPES (supplied by Himedia) was prepared and of 7.5% $NaHCO_3$ solution was added (0.2ml in 100ml RPMI 1640) after adjusting the pH of the media to 7.2. Then it was filtered through bacteriological filter (0.22µ). Fresh human serum of same blood group was added to the media 10% by volume. Parasitized RBC was added in the media so that the final hematocrit of the RPMI+RBC mixture gets adjusted to 4%. The solution was transferred to sterile petri dish and kept in desiccator containing two candles so that the air in the desiccator contains 93% N_2, 4% CO_2, 3% O_2. The desiccator was incubated at 37°C in BOD incubator for 48 hrs.

After 48 hrs the culture was collected and 5% D-Sorbitol was added to synchronize the culture. Parasitemia of the culture was observed and percentage of parasitemia was measured. Fresh RBC of O+ group was added to adjust the parasitemia to 1%. Fresh RPMI 1640 media was added finally to raise the hematocrit up to 8%. This solution was used as

the stock solution of parasite. The plant extract solution was prepared in fresh RPMI 1640 medium by concentration of 1mg/ml (Solution 1) and 100µg/ml (Solution 2). The 96 wells microtiter plates were labeled appropriately and different volume of parasite stock solution was added and the drug solution was mixed to make up the volume 100 µl in each well (table 1). Final concentrations of the drug in each well were 5, 8, 15, 30, 50, 80, 100, 120, 150, 180, 200 µg/ml. A control was kept to determine the final parasitemia in untreated parasite culture. Standard drug chloroquine the blood-medium mixture was gently shaken from time to time to ensure that the blood was kept in suspension. The parasite density was estimated as the number of mature schizonts per 200 white blood cells. The obtained data was fitted to a dose-response curve (using the ORIGINE software). The IC_{50} defined as the drug concentration corresponding to 50% inhibition of schizonts growth in the control wells, was resolute by nonlinear regression analysis of log dose/response curves.

RESULT AND DISCUSSION

The in vitro antimalarial assessment of 4-Quinazolinone derivatives was carried out by aforesaid method. This compound shows good P.falciparum inhibitory activity in concentration dependent manner. The pIC50 values of these compounds against p. falciparum were listed in Table 1 represented as the concentrations that can inhibit 50% of the total microbial population in compared with a control which contain no anti malarial compounds. Among all the compound **5a** shows good *in vitro* antimalarial activity. This line of evidence indicate substitution at position 2 of 4-quinazolinone with bulky group dose not improve the anti malarial property. Phenyl, p-Methoxy phenyl substitution at position 2 of 4-Quinazolinone moiety markedly reduces the biological significance. A 3-hydroxy substituted piperidine ring attached with quinazolinone moiety through a three carbon atom chain. The carbon chain act as a linker between 4-quinazolinone and substituted piperidine contains a keto group. Replacement of this 3-hydroxy substituted piperidine ring with 2-methyl ester substituted pyrrolidine ring and a secondary alcohol in the carbon linker reduces the activity. But they are not completely inactive.

CONCLUSION

The antimalarial activity of quinazolin-4(3H)-one derivative has been studied. These are showing anti malarial property in higher concentration. in future we are planning to synthesize more number of 4-Quinazolinone derivatives to develop compounds with promising anti malarial property.

ACKNOWLEDGMENTS

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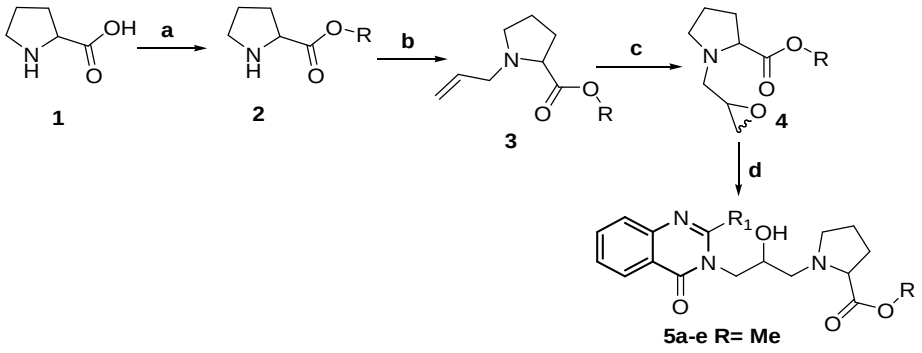
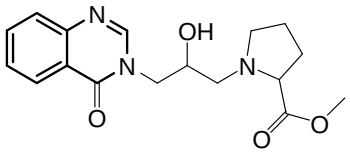
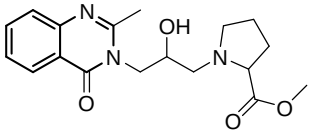
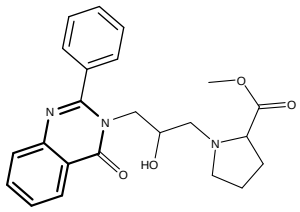
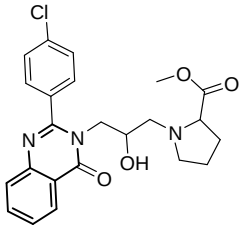
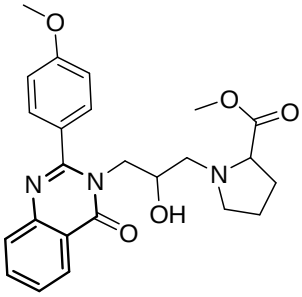
Scheme 1



Scheme 1: a = $SOCl_2$, Dry MeOH, reflux, 4h, **b**= Allyl Bromide, Acetone, K_2CO_3, 12h, **c**= mCPBA, DCM, 6h, **d**= NaH,DMF, Quinazolinones, reflux, 12h,

Table 1: Results of In vitro activity of synthesized compounds

ID	Structure	pIC_{50} (in µmol)	Id	Structure	pIC_{50} (in µmol)
5a		59	**5b**		72
5c		276	**5d**		205
5e		523	**5f**	Chloroquin	1.62

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