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SYNTHESIS OF BISQUINOLINES AND THEIR IN VITRO ABILITY TO PRODUCE METHEMOGLOBIN IN CANINE HEMOLYSATE#.

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Abstract : Synthesis of a number of derivatives of bisquinolines (**3-9**) have been reported here. Effect of these compounds on *in vitro* methemoglobin formation and methemoglobin reductase activity has resulted in the identification of two potential compounds (**5** & **7**), showing negligible methemoglobin toxicity.

Introduction

The spread of drug resistant Plasmodium has made the prophylaxis and treatment of malaria increasingly difficult. Chloroquine (CQ) that was once the first line antimalarial drug because of its efficacy and low toxicity, now frequently fails against malaria. The molecular basis for CQ resistance has not been definitively established but it appears to involve a decreased accumulation of the drug by resistant parasite[1] The alarming spread of drug resistance has led the WHO to predict that in the absence of new antimalarial strategies, the number of people suffering from malaria will be doubled by the year 2010[2]. Thus, it is imperative that novel drugs are developed to keep ourselves one step ahead of the parasite. Recently a series of bisquinolines were reported as potential agents against chloroquine resistant malaria[3], but their ability to cause methemoglobinemia were not studied. Although the exact mechanism of action is still unknown, this class of compounds have been shown to interfere with the heme polymerization in the parasite food vacuole. Since these bisquinolines are bulky in structure, they are less efficiently extruded by chloroquine resistant parasite in comparison to chloroquine[3].

In our earlier report[4], we have shown that certain minor changes in the structural units of 7-chloro, 4-substituted aminoquinolines may lead to the oxidative effect on hemoglobin. Besides, 8-aminoquinolines are also known to be the potent inducer of methemoglobin (Met Hb)[5] . It was, therefore, suggested that simple

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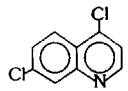

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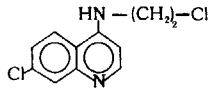

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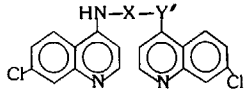

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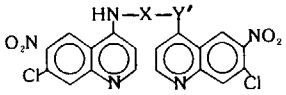

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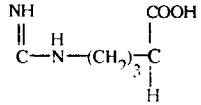
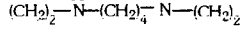
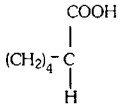
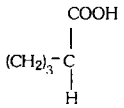
Table 1

Compound No.	Yield (%)	X	Y′	Compound No.	Yield (%)	X	Y′
3.	86	$(CH_2)_4$	NH	**7.**	87	$(CH_2)_2$	
4.	70		NH	**8.**	65		NH
5.	62		NH	**9.**	86	$(CH_2)_4$	NH
6.	76		NH				

in vitro assay for methemoglobin toxicity must be performed before selecting the structural units for designing new molecules and also for optimising the biological activity of a lead compound[4].

Methemoglobin level above the steady state concentration in blood hampers the normal physiological functions of hemoglobin[6]. The exact mechanism of MetHb formation is not completely understood. The role of superoxide anion and hydrogen peroxide has been implicated in the methemoglobinemia produced by certain quinoline compounds. The peroxides produced by the interaction of certain drugs and oxyhemoglobin have also been held responsible for the methemoglobin formation, that often accompany the heinz body formation, lipid peroxidation, cell fragility and hemolysis[7]. In the light of these observations, it becomes essential to identify the substructural units capable of eliciting hemoglobin oxidation.

Chemistry

The bisquinolines (**3-9**) were prepared by using conventional methods of synthesis[3] Reactions of 4, 7 dichloroquinoline (**1**) and primary amines, such as 1,4 diaminobutane, arginine, lysine and ornithine yielded the compounds **3, 4, 5** and **6** respectively. Reaction of compound **2** with piperazine and 4, 7 - dichloroquinoline yielded the compound **7**, in the presence of dry DMF/K_2CO_3. Reaction of compound **2** with 1, 4,-diaminobutane in 2:1 ratio yielded the compound **8**. Nitration of compound **3** with fuming nitric acid yielded the compound **9**.

Materials and Methods

***In vitro* Incubations :** A stock solution of 10mM concentration of compounds **3**, **4**, **5**, **6**, **7**, **8** and **9** were prepared in DMSO (dimethyl sulfoxide) and triple distilled water (TDW). Same concentration of chloroquine (CQ) and primaquine (PQ) were also prepared in TDW. Blood from normal beagle dog was collected in acid citrate dextrose (ACD) containing tubes and 20% (v/v) hemolysate was prepared as described elsewhere[8]. The *in vitro* incubation was performed following the method of Fletcher *et al* (1988)[9] with slight modifications. The hemolysate (0.5 ml) was placed in test tubes and the test compounds/drugs were added to give a final concentration of 1 and 5 mM in a total volume of 1 ml. The tubes were stoppered and then placed in a shaking water bath at 37°C for about 90 min in the dark. Aliquots were taken from each sample before and after the incubation for estimation of MetHb and methemoglobin reductase activity.

Estimations : Methemoglobin content was estimated according to the method of Evelyn and Malloy (1938)[10]. Methemoglobin reductase was assayed according to the method of Hegesh *et al* (1968)[11]. Protein was measured by following the method of Lowry *et al* (1951)[12], using bovine serum albumin as a standard.

Results and Discussion

Increased MetHb level in response to the administration of an oxidant represents the equilibrium of drug induced hemoglobin oxidation and enzyme dependent reduction. The *in vitro* effect of compounds on methemoglobin formation and MetHb reductase activity may also prove to be helpful in understanding the mechanism of their action. The MetHb producing capacity of a drug/compound is often governed by its metabolite rather than the parent compound. Aminoquinolines that can be metabolized into quinones or iminoquinone cause significant hemoglobin oxidation[13]. The Table II depicts the profile of MetHb formed when the red cell lysate was individually incubated with the synthesized bisquinolines and with the standard 4-aminoquinoline (CQ) and 8-aminoquinoline (PQ) compounds. The data obtained show that chloroquine was not able to form methemoglobin even at 5mM concentration.

Table II : *In vitro* effect of chloroquine, primaquine and the synthesized bisquinolines on methemoglobin formation.

		% Methemoglobin	
S.No.	**Additives**	**At 1mM concentration**	**At 5mM Concentration**
i.	Control	0.65±0.23	0.65±0.23
ii.	Chloroquine	nil	nil
iii.	Primaquine	11.52±2.0	87.6±4.5
iv.	3	29.5±3.7	90.8±2.6
v.	4	nil	1.50±0.40
vi.	5	nil	nil
vii.	6	nil	6.89±1.00
viii.	7	nil	nil
ix.	8	nil	3.0±0.22
x.	9	nil	6.42±0.74

Values are mean ± S.D. of 3-4 separate observations. MetHb toxicity of all the compounds/drugs have been expressed after deleting the control value.

However, the 8-aminoquinoline (PQ) showed considerable MetHb toxicity both at 1 and 5 mM concentrations. Amongst all the synthesized bisquinolines, compounds **5** and **7** were detected to be absolutely safe showing no sizeable toxicity of MetHb both at 1 and 5 mM concentrations. All the bisquinoline compounds (except compound **3**) were found to be non toxic at 1mM concentration. However, mild increase in the MetHb level (3.0–6.8%) was noticed with compounds **6, 8** and **9** at 5mM concentration. The compound **3** was determined to be highly toxic showing about 30 and 90% MetHb toxicity at 1 and 5 mM concentration, respectively.

The NADH-dependent methemoglobin reductase plays key role in maintaining hemoglobin in its reduced and active state. Table III summarizes the *in vitro* effect of the above mentioned compounds/drugs on the methemoglobin reductase activity. Maximum inhibition (100%) in the enzyme activity was observed with the compound **3** and about 22% inhibition was noticed with primaquine at 166 μM concentration. However, rest of the compounds did not cause any significant inhibition in the enzyme activity. The present finding implies that the drug/compound induced suppression in the enzyme activity does contribute to the increased methemoglobin toxicity of the same.

Table III : *In vitro* effect of primaquine, chloroquine and the new synthesized bisquinolines on MetHb reductase activity.

S.No.	Additives	Methemoglobin reductase activity[a]	
		At 33μM concentration	At 166μM Concentration
i.	Control	0.067±0.003	0.067±0.003
ii.	Chloroquine	0.067±0.003	0.067±0.003
iii.	Primaquine	0.067±0.002	0.052±0.001'
iv.	3	0.009±0.001'''	0.0'''
v.	4	0.067±0.003	0.067±0.002
vi.	5	0.067±0.003	0.066±0.003
vii.	6	0.067±0.002	0.067±0.001
viii.	7	0.067±0.002	0.067±0.002
ix.	8	0.065±0.004	0.066±0.002
x	9	0.067±0.003	0.067±0.003

Values are mean ± S.D. of 3-4 separate observations ''P < 0.01, '''P<0.001
[a]Enzyme activity expressed as nmoles of MetHb reduced min^{-1} mg $protein^{-1}$.

The comparative analysis of the MetHb toxicity produced by bisquinoline derivatives with their substructural units, revealed that the compound **3** having 1, 4-

diaminobutane group was highly toxic. However, the nitration product, **9** exhibited quite decreased methemoglobin level. In compound **8**, the MetHb toxicity is further reduced, where in addition to 1, 4- diaminobutane group there is NH- $(CH_2)_2$ group attached to it. Due to this addition, the chain between the two quinoline group has increased, and so has the MetHb level. Certain amino acids (viz., arginine, lysine and ornithine) were also used in the structure of compounds **4, 5** and **6** respectively. It was found that the compound **4 & 5** having arginine and lysine group showed almost no toxicity. While the compound **6**, having ornithine group manifested considerable MetHb toxicity. On the other hand, compound **7** having piperazinyl group attached to NH-CH_2-CH_2 group was not toxic.

Thus the present study was found to be helpful in selecting the synthesized compound for optimising their biological activity in the *in vitro* or *in vivo* system.

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