Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 21, pp. 2741-2746, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10074-9

SYNTHESIS OF 7-CHLORO-4-SUBSTITUTED AMINOQUINOLINES AND THEIR *IN VITRO* ABILITY TO PRODUCE METHEMOGLOBIN IN CANINE HEMOLYSATE

Sandhya Srivastava[a], Swati Tewari[b], Sanjay K. Srivastava[b], P.M.S. Chauhan[b], A.P. Bhaduri[b], S.K. Puri[c] and V.C. Pandey[a*]

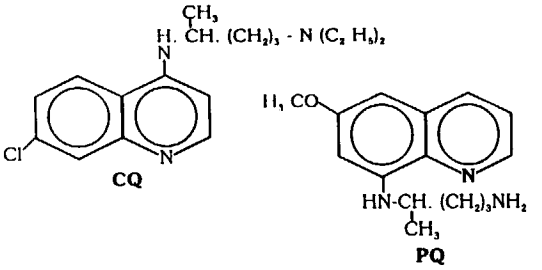
Divisions of Biochemistry[a], Medicinal Chemistry[b] and Microbiology[c]
Central Drug Research Institute, Lucknow-226001 (India)

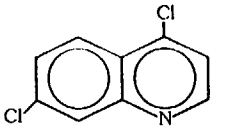
Abstract: Synthesis of aminoquinoline derivatives **(2-15)** and their *in vitro* effects on methemoglobin formation and methemoglobin reductase activity are delineated. Some of the screened compounds have shown considerable methemoglobin toxicity. © 1997 Elsevier Science Ltd.

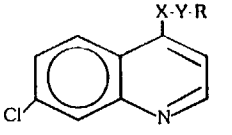
Introduction: Design of the molecular framework of new bioactive compound requires simulation of appropriate bioactive pharmacophore and deletion of side-effect generating sub-structural units in its molecular architecture. The latter normally is not projected in scientific publications and the generation of data for supporting the involvement of a particular pharmacophore for evoking a severe side effect or toxicity is difficult and expensive. This impediment in drug design can be removed provided a simple *in vitro* test system is developed to ascertain the side effect or toxicity. In our search for new compounds for combating parasites other than plasmodia, the need arose for incorporating 7-chloro-4-substituted aminoquinoline as a pharmacophore and during this exercise it was essential to understand whether sub-structural units present in the designed molecules are capable of eliciting hemoglobin oxidation. Extensive work on 4-aminoquinolines as antimalarials has revealed 4-amino-7-chloroquinoline as a safe pharmacophore since drugs of this class of compounds do not cause methemoglobin (MetHb.) toxicity. Yet it is not explicitly clear whether minor structural change of this pharmacophore will elicit MetHb. toxicity. The present investigation aims towards resolving this issue.

Hemoglobin is a readily available major protein in the human blood and carries out a well known functions of oxygen and carbon dioxide transportation. However, the oxidation of its ferrous porphyrin complex into ferric form causes impairment of the physiological functions. About three percent of hemoglobin in the circulating blood is autoxidized into MetHb. per day and reduced again by methemoglobin reductase system[1]. An unusual increase in the MetHb. content occurs when the red blood cells are exposed to certain oxidative stress, such as "oxidant" drugs or compounds having high redox potential or some pathogenic parasites[2,3,4]. The 8-aminoquinolines, administered for eliminating tissue forms of malaria, produce quinone metabolites which are responsible for its major adverse effects, namely hemolytic anemia in glucose-6-phosphate dehydrogenase

#CDRI Communication No. 5722







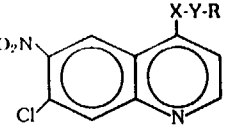
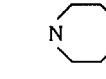
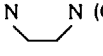
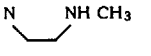
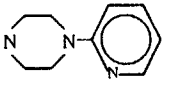
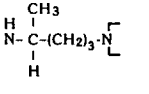
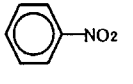
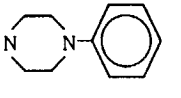
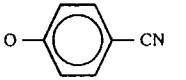
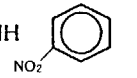
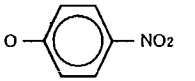


Table : I

Compd. No.	X	Y	R	Compd. No.	X	Y	R	Compd. No.	X	Y	R
2	NH	$(CH_2)_2$	OH	**7**	NH	$(CH_2)_2$		**12**		$(CH_2)_3$	
3	NH	$(CH_2)_2$	Cl	**8**	NH	$(CH_2)_2$		**13**		$(CH_2)_3$	
4	NH	NH		**9**	NH	$(CH_2)_2$		**14**	NH	$(CH_2)_2$	OH
5	NH	CH_2		**10**	NH	$(CH_2)_2$		**15**	NH		
6	NH			**11**	NH	$(CH_2)_2$					

deficient individuals and methemoglobinemia (cyanosis)[5,6]. The mechanism by which hemoglobin is oxidized to MetHb. is incompletely understood. The role of superoxide anion and hydrogen peroxide has been implicated in the methemoglobinemia produced by these compounds[7]. The peroxides produced by the interaction of oxyhemoglobin and certain drugs or chemicals has been held responsible for the heinz body formation, lipid peroxidation, cell fragility and hemolysis that often accompany the MetHb. formation[8]. In the light of these observations, the precise knowledge of sub-structural units capable of eliciting hemoglobin toxicity acquires importance.

Chemistry: The aminoquinolines (**2-15**) were prepared by conventional methods of synthesis[9]. Reactions of 4,7-dichloroquinoline (1) and primary amines such as ethanol amine, nitrophenyl hydrazine, furfuryl amine and p-amino-aceto phenone yielded the compounds **2,4,5** and **6**, respectively. Nitration of compounds **2** and **6** with fuming nitric acid yielded compounds **14** and **15**, respectively. Reactions of compound **3** with piperazine and substituted piperazines, yielded the compounds **7, 8** and **9**, respectively and with different substituted phenols like, p-cyanophenol and p-nitrophenol in DMF/K_2CO_3 yielded compounds **10** and **11** ,respectively. Reactions of 4-piperazinyl-7-chloroquinoline and 1,3-dibromopropane gave their corresponding bromo derivatives which on treatment with 4-methylpiperazine and 2-amino-5-diethyl-aminopentane yielded compounds **12** and **13**, respectively.

Materials and Methods

***In vitro* incubations :** A stock solution of 10 mM concentration of compounds **2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14** and **15** were prepared in DMSO and triple distilled water (TDW). Same concentration of sodium nitrite ($NaNO_2$), chloroquine (CQ) and primaquine (PQ) were also prepared in TDW. Blood from a normal beagle dog was collected in acid citrate dextrose containing tubes and 20% (v/v) hemolysate was prepared as described elsewhere[10].

Aliquots (0.5ml) of the hemolysate were placed in test tubes and the test compounds 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 and incubated at 37^{0}C for about 90 minutes in the dark. Aliquots of each sample were taken 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)[11]. Methemoglobin reductase was assayed according to the method of Hegesh *et al.* (1968)[12]. Protein was measured by following the method of Lowry *et al.* (1951)[13], using bovine serum albumin as a standard.

Results and Discussion

The equilibrium of hemoglobin and MetHb. is maintained by the reduction of excess MetHb. by the pyridine nucleotide dependent reductases present in the

red blood cells[1]. Elevated MetHb. in response to administration of an oxidant represents the equilibrium of drug-induced hemoglobin oxidation and enzyme dependent reduction. An *in vitro* assay of hemoglobin oxidizing potential, distinguish compounds which oxidise hemoglobin at a slow rate from those which have a rapid rate of oxidation. Aminoquinolines that can be oxidized to iminoquinones or to quinones[6] cause significant hemoglobin oxidation.

The Table II shows the profile of MetHb. formed when the red cell lysates were incubated with $NaNO_2$, PQ and CQ at different concentrations. Primaquine and CQ represent the standard 8 aminoquinoline and 4-aminoquinoline respectively. Chloroquine was not found to be toxic even at 5 mM concentration. Sodium nitrite was most toxic, producing 100% MetHb. level at 1 mM concentration and PQ showed 89.9% MetHb at 5 mM concentration (final). The MetHb. concentration was found to increase in a concentration dependent manner with $NaNO_2$ and PQ. Compounds **2, 7, 8,10, 12** and **13** were unable to produce any significant hemoglobin oxidation even at 5 mM concentration, when compared to the control values.

The NADH-dependent methemoglobin reductase plays a vital role in reducing MetHb into its active form. A deficiency in this enzyme results in congenital methemoglobinemia[14]. Keeping in view of these reports, *in vitro* effect of above mentioned compounds on methemoglobin reductase activity were also studied.

Table II *In vitro* effect of sodium nitrite, chloroquine, primaquine and the synthesized aminoquinolines on methemoglobin formation.

S.No.	Additives	% Methemoglobin	
		At 1 mM. concentration	At 5 mM concentration
i	None (control)	0.88±0.05	0.88±0.05
ii	$NaNO_2$	19.21±3.5 (at 250 μM)*	100 (at 1 mM)*
iii	PQ	11.52±2.00	89.9±4.7
iv	CQ	nil	nil
v	**2**	nil	nil
vi	**4**	9.3±0.8	82.56±5.2
vii	**5**	nil	22.2±2.0
viii	**7**	nil	nil
ix	**8**	nil	nil
x	**9**	nil	6.15±0.05
xi	**10**	nil	nil
xii	**11**	nil	11.2±2.8
xiii	**12**	nil	nil
xiv	**13**	nil	nil
xv	**14**	2.7±0.4	21.92±3.0
xvi	**15**	nil	12.84±1.3

Values are mean±S.D. of 3-4 separate observations.

*Concentrations of $NaNO_2$ are given in parenthesis

It is apparent from the results (Table III) that the compound or drug which is able to inhibit the enzyme activity significantly tends to produce greater amount of MetHb. The compound **4** caused about 50% inhibition in the enzyme activity at 133 μM final concentration. However, the rest of the compounds did not cause any significant inhibition. Sodium nitrite, a potent oxidant of hemoglobin did not reveal any noticeable inhibition in the enzyme activity when incubated for the same time period. The above finding indicate a dual mechanism for the oxidative effects on hemoglobin. It implies that a compound or a drug generate MetHb. either by direct interaction of a compound or its metabolite to oxyhemoglobin, or by suppression of methemoglobin reductase activity or both the mechanisms coexist.

Comparative study on the MetHb. toxicity of 7-chloro- 4-substituted aminoquinolines with different substituents, revealed that compounds **2** and **6** having hydroxyl and acetyl group, respectively were not toxic. However, the nitration products, **14** and **15**, exhibited considerable MetHb. toxicity. Another nitro compound (**11**) also caused hemoglobin oxidation and the replacement of the nitro group by a cyano moiety did not produce methemoglobinemia. Among all the synthesized aminoquinolines the compound **4** manifested maximum MetHb. toxicity (82.5%). The compound **5** which does not possess a nitro group was found to cause about 22.2% of hemoglobin oxidation.

Table III. *In vitro* effect of Sodium nitrite, primaquine, chloroquine and the synthesized aminoquinolines on methemoglobin reductase activity.

S.No.	Additives	Methemoglobin reductase activity	
		At final concentration 33 μM	At final concentration 166 μM
i	None(Control)	0.065±0.002	0.065±0.002
ii	$NaNO_2$	0.065±0.002	0.065±0.002
iii	CQ	0.065±0.002	0.065±0.002
iv	PQ	0.065±0.002	0.056±0.003**
v	**2**	0.065±0.001	0.065±0.001
vi	**4**	0.065±0.002	0.032±0.003***
vii	**5**	0.065±0.005	0.057±0.012
viii	**7**	0.065±0.001	0.065±0.001
ix	**8**	0.065±0.003	0.065±0.001
x	**9**	0.065±0.002	0.065±0.001
xi	**10**	0.065±0.001	0.005±0.001
xii	**11**	0.065±0.002	0.059±0.007
xiii	**12**	0.065±0.001	0.065±0.001
xiv	**13**	0.065±0.003	0.065±0.005
xv	**14**	0.065±0.007	0.065±0.0618
xvi	**15**	0.065±0.002	0.065±0.002

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

These observations indicated that the nitro and furfuryl groups present as substituents in 7-chloro- 4-aminoquinolines were responsible for producing MetHb. toxicity and none of the compounds (except 4) synthesized had noticeable effect on the repair mechanism namely methemoglobin reductase.

The present study clearly suggests that minor changes in the substructural units of the compounds may elicit the oxidative effect on hemoglobin. It is therefore suggested that simple *in vitro* assays for MetHb. toxicity mentioned in this paper should be undertaken before selecting substructural units for designing new molecules and also for optimising the biological activity of a lead compound. This simple *in vitro* test system reported here may be adopted for screening associated toxicity of compounds, if any, in all drug developmental programmes.

Acknowledgement: The authors are grateful to the Director, CDRI and the Head of the Biochemistry Division for providing the necessary facilities. Three of us (SS, SKS and ST) are thankful to WRAIR, USA for the award of Research Fellowship.

References and Notes:

1. Hsiech H.S. and Jaffe E.R. In: The Red Blood Cell D.M.N. Surgenor, ed. New York, Academic Press II (1972), 799.
2. Bodansky, O. Pharmacol. Rev. (1951) 3, 144.
3. Biswas T., Pal, J.K., Naskar, K., Ghosh, D.K. and Ghoshal, J. Mol. Cell. Biochem. (1995) 146, 99.
4. Saxena N., Puri, S.K., Sahni, S.K., Dutta, G.P. and Pandey, V.C. Clin. Chem. Enzymol. Commun. (1987) 2, 55.
5. Thompson P.E. and Werbel L.M. In: Antimalarial Agents, Chemistry and Pharmacology (1972), Academic Press, New York.
6. Lopezshirley, K., Zhang, F., Gosser, D., Scott, M. and Meshnick, S.R. J. Lab. Clin. Med. (1994). 123, 126.
7. Link, C.M., Theoharides, A.D., Anders, J.C., Chung, H. and Canfield, C.J. Toxicol. and App. Pharmacol. (1985) 81, 192.
8. Hjelm, M. and Verdier, C.H. de Biochem. Pharmacol. (1965) 14, 1119.
9. Chauhan, P.M.S., Sharma, S. and Bhakuni, D.S. Ind. J. Chem. (1986) 25B, 828.
10. Rossi-Fanelli, A., Antonini, E. and Caputo, A. J. Biol. Chem. (1961) 236, 391.
11. Evelyn, K.A. and Malloy, N.T. J. Biol. Chem. (1938) 126, 655.
12. Hegesh, E., Calmanovici, N. and Avron, M.J. Lab. Clin. Med. (1968) 72, 339.
13. Lowry, O.H., Rosebrough, J., Farr, A.L. and Randall, F. J. Biol. Chem. (1951) 193, 265.
14. Gotlieb, A., Novden, U.Z., Hegesh, E. Isr. J. Med. (1973), 9, 909.

(*Received in Japan* 18 *July* 1997; *accepted* 29 *September* 1997)