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SYNTHESIS AND METHEMOGLOBIN TOXICITY OF THE AMIDES OF 6/7 MONO OR DISUBSTITUTED QUINOLONE*

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Abstract : A series of 6/7-mono and disubstituted quinolone-3-carboxamide derivatives **(1-12)** were synthesized and their *in vitro* methemoglobin producing capacity have been delineated. The compounds **5**, **6**, **9** and **10** showed minimum methemoglobin toxicity.

Introduction : In an earlier communication, the influence of substituents in quinoline ring for evoking methemoglobin (MetHb) toxicity has been reported[1]. Global search for non 8-aminoquinolines as antimalarials was aimed at identifying chemical entities which were not associated with MetHb toxicity. This search indicated the possibility of 6,7-disubstituted quinolones as radical curative antimalarials[2,3], but their ability to cause methemoglobinemia were not studied. Quinolone antibacterials as a class is well tolerated even at a higher dose schedule[4].

Quinolines that can be oxidized to imminoquinones or to quinones, cause significant hemoglobin oxidation. On the other hand the peroxides produced by the interaction of oxyhemoglobin and certain drugs or chemicals has been implicated for the MetHb formation, that often accompany the Heinz body formation, lipid peroxidation cell fragility and hemolysis[5,6]. In the light of these observation, the present study was undertaken to ascertain the abilities of test compounds to cause methemoglobin formation at high doses. The outcome of this study is likely to help in ascertaining safety potential of test compounds.

Chemistry : Ethyl 4(1H)-quinolone-3-carboxylates (I) were prepared by literature method[7,8]. Reaction of I with amines such as cyclohexylamine, N-methylpiperazine, N,N-diethyl-2-aminopentane and n-octylamine in pyridine under pressure at 120°C afforded their respective substituted 4(1H)-quinolone-3-carboxamides (**1-12**) as shown in Table-I.

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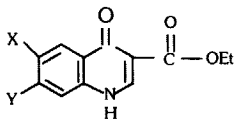

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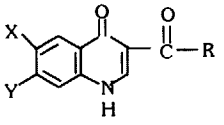
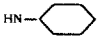
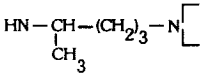
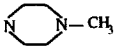
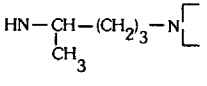
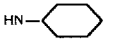
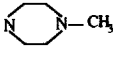
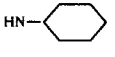
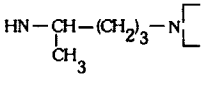
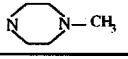


(1- 12)

Table-I

Compound No.	X	Y	R	Compound No.	X	Y	R
1.	NO_2	H		**7.**	F	H	
2.	NO_2	H		**8.**	F	H	$HN-(CH_2)_7-CH_3$
3.	NO_2	H		**9.**	F	Cl	
4.	NO_2	H	$HN-(CH_2)_7-CH_3$	**10.**	F	Cl	
5.	F	H		**11.**	F	Cl	
6.	F	H		**12.**	F	Cl	$HN-(CH_2)_7-CH_3$

Materials and Methods : Stock solutions of 10mM concentration of ciprofloxacin, norfloxacin, ofloxacin and the new synthesized compounds **(1-12)** were prepared in DMSO (dimethylsulfoxide) and triple distilled water.

***In vitro* Incubations** : Blood from a normal beagle dog was collected in acid citrate dextrose containing tubes and 20%(v/v) hemolysate was prepared as described elsewhere[9]. The *in vitro* incubation study was performed follwoing the method of Fletcher *et al* (1988)[10], with slight modifications. Briefly, the hemolysate (0.5 ml) was placed in the individual test tubes and the drugs/compounds were added to give a final concentration of 1 and 5mM in a final volume of 1 ml. The tubes were stoppered and then placed in a shaking water bath and incubated at 37°C for about 90 min in the dark. Aliquots were drawn from each sample tube before and after the incubation for estimation of MetHb level and MetHb reductase activity.

Estimations : Methemoglobin content was determined 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 standard.

Results and Discussion :

Elevated MetHb concentration in blood in response to administration of an oxidant drug or compound represents the equilibrium of drug induced hemoglobin oxidation and enzyme dependent reduction[14]. The *in vitro* effect of ciprofloxacin, norfloxacin, ofloxacin and the synthesized quinolones on methemoglobin level as well as on methemoglobin reductase activity have been summarized in Table II and III.

The present study indicated that ciprofloxacin, norfloxacin and ofloxacin, basically used as antibacterials, were absolutely non toxic as they did not cause hemoglobin oxidation even at 5mM concentration.These antibacterials served as the negative control compounds for the MetHb assay.

Amongst all the synthesized quinolones, compounds **9** and **10** were found to be completly non toxic as they neither raised the MetHb level significantly nor showed any inhibitory effect on methemoglobin reductase activity, both at 1 and 5mM concentrations. Similarly the compounds **5** and **6** were also found to be safe, causing only mild elevation in MetHb level at high concentration. Besides, they also did not affect the reductase activity.

Table-II *In vitro* effect of the synthesized quinolones on methemoglobin formation

S.No.	Additives	% Methemoglobin At 1mM concentration	% Methemoglobin At 5mM concentration
I.	Control	0.72 ± 0.03	0.72 ± 0.03
II	**1**	nil	6.8
III.	**2**	nil	8.6
IV.	**3**	nil	5.17
V.	**4**	nil	7.7
VI	**5**	nil	1.5
VII.	**6**	nil	2.0
VIII.	**7**	1.7	22.4
IX.	**8**	nil	18.9
X	**9**	nil	nil
XI	**10**	nil	nil
XII	**11**	nil	16.9
XIII.	**12**	1.7	29.3

Values are mean ± S.D. of 3-4, separate observations. MetHb toxicity of compounds have been expressed after deleting the control value.Ciprofloxacin, norfloxacin and ofloxacin, used in this assay, were not found to raise the MetHb level both at 1 and 5 mM concentrations.

However, the compounds **1, 2, 3** and **4** showed significant increases in the MetHb level (5.1-8.6%) at 5mM concentration only. But these compounds did not exhibit any inhibitory effect on the repair mechanism (i.e. methemoglobin reductase). On the other hand, the compounds **7, 8, 11** and **12** were found to be highly toxic, depicting about 16-30% increase in the MetHb level at 5mM concentration and a mild increase in MetHb concentration at 1mM concentration too. These compounds were also observed to inhibit the methemoglobin reductase activity upto about 14-31% at high concentration (5mM).

Table-III *In vitro* effect of the synthesized quinolones on methemoglobin reductase activity.

S.No.	Additives	Methemoglobin reductase activity# 33μM (final concentration)	166μM (final concentration)
I.	Control	0.064 ± 0.002	0.064 ± 0.002
II	**1**	0.064 ± 0.002	0.064 ± 0.001
III.	**2**	0.064 ± 0.002	0.064 ± 0.002
IV.	**3**	0.064 ± 0.002	0.064 ± 0.002
V.	**4**	0.064 ± 0.002	0.064 ± 0.003
VI	**5**	0.064 ± 0.002	0.064 ± 0.002
VII	**6**	0.064 ± 0.003	0.064 ± 0.002
VIII	**7**	0.064 ± 0.002	0.052 ± 0.003**
IX	**8**	0.064 ± 0.002	0.055 ± 0.001**
X.	**9**	0.064 ± 0.002	0.064 ± 0.002
XI	**10**	0.064 ± 0.001	0.064 ± 0.002
XII	**11**	0.064 ± 0.002	0.044 ± 0.001***
XIII.	**12**	0.064 ± 0.002	0.047 ± 0.002***

Values are mean ± S.D. of 3-4 separate observations. #Enzyme activity expressed as nmoles of MetHb reduced min^{-1} mg $protein^{-1}$. **P< 0.01, ***P< 0.005. Ciprofloxacin, norfloxacin and ofloxacin, used in this assay, were not found to inhibit the enzyme activity at both the concentrations.

The comparative analysis of the MetHb toxicity produced by Ethyl4(1H)-substituted quinolone-3-carboxylates (I) with different substituents revealed that all the substituents (R) at position-3 in 6-nitroquinolone showed MetHb toxicity to significant level as mentioned above in case of compounds **1-4**. Replacement of nitro group by fluoro group led to the syntheses of compounds **5-8**, in which the compounds **5** and **6** having N-cyclohexylcarboxamido and N-4-methylpiperazine carboxamido function at position-3, respectively were found to exhibit almost no MetHb toxicity, while other analogues of this series (**7**, **8**) were highly toxic.

Amongst the 7-chloro-6-fluoro-4(1H)-quinolone-3-carboxamides (**9-12**), compounds **9** having N-cyclohexylcarboxamido at position-3 and **10** possessing 3-(4'-methylpiperazine)carboxamido were absolutely safe as they did not show significant increases in MetHb content. However, other derivatives (**11**, **12**) were toxic as in the case of 6-fluoro-4(1H)-quinolone series.

This exploratory research work reveals that 6-fluoro or 7-chloro-6-fluoro-4(1H) quinolones having N-cyclohexyl or N-4'-methylpiperazine carboxamido function at position-3 are not able to elevate the MetHb concentration in blood, nor do they affect the methemoglobin reductase activity. Thus, these four compounds (**5**,**6**,**9** and **10**) could be further used for evaluating the *in vitro/in vivo* biological activity.

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