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The Effect of Fluorine Substitution on the Haemotoxicity of Primaquine

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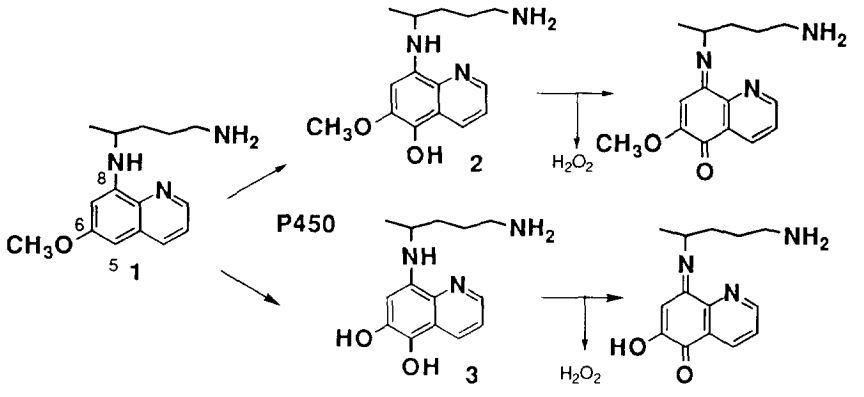
Abstract : 5-Fluoro-6-methoxy-8-nitroquinoline was synthesised by a modified Skraup Reaction and was subsequently converted into 5-fluoroprimaquine in three steps. 5-Fluoroprimaquine **9** is less susceptible to *in vitro* bioactivation than primaquine **1** in a range of different species. However, significant bioactivation of **9** was observed with hepatic microsomes from two species including man.

Introduction

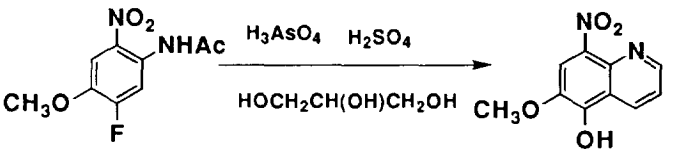
Primaquine PQ, **1**, an 8-aminoquinoline antimalarial, plays a unique role in the treatment of malaria in that it is the only drug capable of eliminating the persistent liver forms of the parasite responsible for relapses in *Plasmodium vivax* and *Plasmodium ovale* infections.[1] The clinical usefulness of primaquine is limited, however, by its toxic side-effects which include methaemoglobinaemia and in certain circumstances haemolytic anaemia.[2-7] The observed side-effects of primaquine are generally accepted to result from oxidative metabolism to a number of potentially toxic metabolites (Scheme 1). In particular ring-hydroxylated metabolites such as 5-hydroxyprimaquine 5-HOPQ **2** and 5,6-dihydroxyprimaquine 5,6-HOPQ **3** have been shown to be significantly more effective than primaquine in oxidising haemoglobin and depleting reduced glutathione (GSH) in human erythrocytes.[6-8] These latter effects can be attributed to the corresponding quinoneimine derivatives[9,10] (Scheme 1) which together with hydrogen peroxide are the main products of the fast autooxidation undergone by these metabolites at neutral pH.[11]



Scheme 1. Oxidative Metabolism of Primaquine to Potentially Toxic Quinoneimine Metabolites

Since 5-hydroxylation results in the formation of potentially toxic quinoneimine metabolites we have attempted to block this pathway by introduction of fluorine at this position. Previous approaches to the related

5-fluoropamaquine failed, since the key intermediate in this synthesis is 5-fluoro-6-methoxy-8-nitroquinoline **5**. Attempts to prepare this compound via the Skraup reaction of 5-fluoro-4-methoxy-2-nitroacetanilide[12] gave 5-hydroxy-6-methoxy-8-nitroquinoline **4** (Scheme 2) .The 8-nitro substituent activates the fluorine atom at position 5 towards nucleophilic displacement and, under the reaction conditions employed, almost complete replacement of the fluorine by hydroxide occurs.



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Scheme 2. Attempted Synthesis of 5-fluoro-6-methoxy-8-nitroquinoline

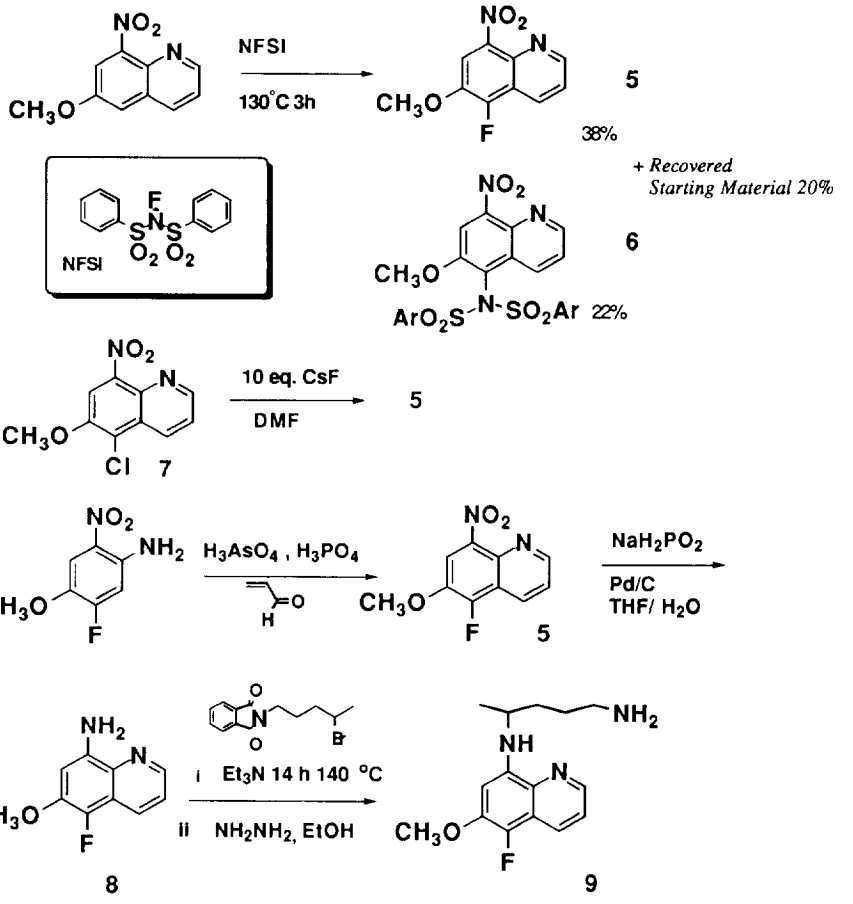
Results and Discussion

In this communication, comparison of three approaches to the synthesis of the required intermediate 5-fluoro-6-methoxy-8-nitroquinoline **5** are made. It was envisaged that **5** would be readily accessible by electrophilic substitution of 6-methoxy-8-nitroquinoline with an appropriate fluorinating agent such as NFSI[13] or NFPT.[14,15] Reaction of 6-methoxy-8-nitroquinoline with NFPT in refluxing 1,1,2 trichloroethane resulted in the formation of polymeric tars with no evidence for product formation. However, treatment with 5 equivalents of NFSI at 130°C resulted in the formation of **5** in 38% yield (Scheme 3). In addition to the required fluoroquinoline, the sulfonimide (22 %) **6** was obtained. This product may have arisen either by nucleophilic aromatic substitution of **5** with the side product dibenzene sulfonamide or via direct amination of the quinoline with NFSI.[16] Thus, it was apparent that formation of **5** was compromised by these unwanted side reactions and an alternative preparation was undertaken.

Nucleophilic aromatic substitution of activated halogenobenzenes with alkali metal fluorides is an important route to fluorinated aromatics.[17] In particular, it has been shown that the chlorine atom in 4-chloronitrobenzene can be replaced routinely by fluorine by treatment with an alkaline metal fluoride in refluxing solvents such as dimethylformamide (DMF), or dimethylsulfoxide (DMSO).[18] By analogy, the chlorine atom at the 5-position of 5-chloro-6-methoxy-8-nitroquinoline **7**[19] is activated and might be expected to undergo replacement by treatment with a suitable source of nucleophilic fluorine. The requisite chloroquinoline **7** was synthesised in two steps from 5, 6-dimethoxy-8-nitroquinoline[20] and it was found that treatment with 10 equiv. cesium fluoride in DMF at 110°C led to a 33% conversion to the required fluoroquinoline (Scheme 3).[21] Major drawbacks in this route were purification[22] of the product and difficulties in scaling the reaction up to obtain suitable quantities for conversion into the required target molecule.

Finally the Skraup Reaction of 5-fluoro-4-methoxy-2-nitroaniline was reinvestigated. The conditions employed by Elderfield involved heating the reaction mixture at high temperatures in 98% sulfuric acid as described. We decided to modify these conditions by employing the procedure of Yale and Bernstein[23] whereby 80% phosphoric acid, acrolein and arsenic acid are employed with a shorter reaction time and a lower

temperature. Using these conditions a yield of 30% was obtained following purification by flash column chromatography. This represents a significant improvement over the earlier procedure where only 5% of product was obtained following optimisation of reaction conditions. The major advantage of this method is that the reaction can be scaled up to multigram quantities.



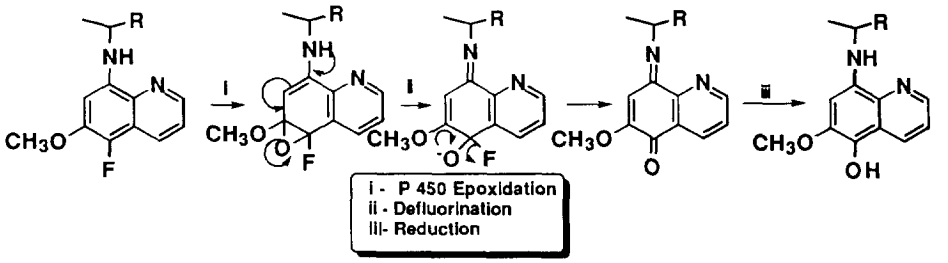
Scheme 3 Synthesis of 5-Fluoroprimaquine

The reaction sequence was completed as shown in Scheme 3. Reduction of the nitro group was performed in quantitative yield by using sodium hypophosphite with 10% palladium as catalyst. The amine **8** was then alkylated with 2-bromo-5-phthalidimidopentane to give the phthaloyl protected primaquine in excellent yield. The synthesis was completed by removal of the phthalimide protecting group with hydrazine hydrate. The final product **9** was recrystallised from aqueous ethanol/phosphoric acid as the monophosphate.[24]

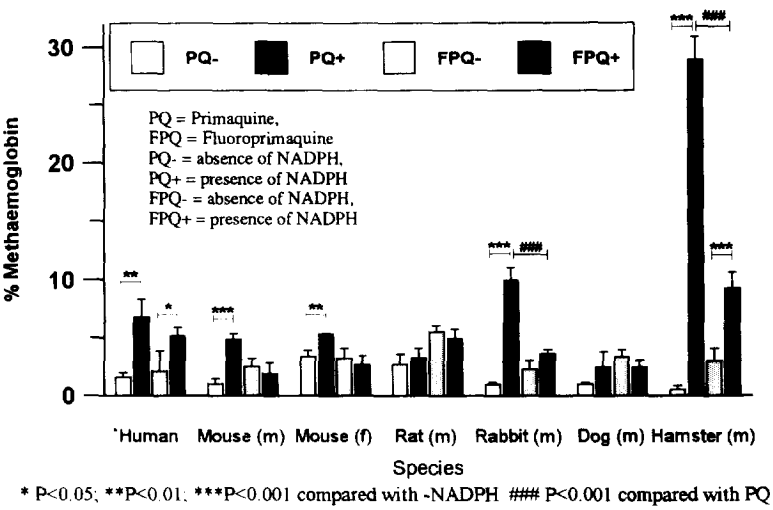
The toxicity of primaquine and its fluorinated analogue were assessed *in vitro* [25], since there is no suitable *in vivo* model for primaquine toxicity. The *in vitro* system used for the measurement of primaquine

toxicity consists of hepatic microsomes from various species and human red blood cells.[26] The methaemoglobin formation, measured by the method of Jollow[27] in the various species is shown in Fig. 1. Primaquine was bioactivated to a haemotoxic metabolite by microsomes prepared from all six species investigated. The extent of bioactivation varied considerably which may reflect the well-established interspecies difference in the expression of hepatic cytochrome P450.[28] It can be seen from Fig.1 that fluoroprimaquine can undergo bioactivation with human and liver hamster microsomes. However, in the case of microsomes from the hamster, the bioactivation was clearly reduced, whereas with the rabbit microsomes bioactivation was blocked. Thus, it is apparent that there is not only species variation in the metabolism of primaquine but also of fluoroprimaquine.

The methaemoglobinaemia associated with primaquine is thought to be a consequence of cytochrome P 450 mediated oxidation of the corresponding 5-hydroxylated metabolites to quinoneimine metabolites. The formation of quinoneimine metabolites and 5-HOPQ from 5FPQ could arise from metabolism according to Scheme 4.



Scheme 4. Defluorination of 5-Fluoroprimaquine



* P<0.05; **P<0.01; ***P<0.001 compared with -NADPH ### P<0.001 compared with PQ (+NADPH)

Fig 1 Methemoglobin Formation of Primaquine and 5-Fluoroprimaquine.

Obviously, identification of metabolites would be required to confirm such a mechanism and it is apparent that any *in vivo* assessment of either the pharmacological or toxicological activity of fluoroprimaquine would require a full analysis of metabolites in order to define the precise chemical mechanisms of activity / toxicity.

The antimalarial activity of primaquine and 5-fluoroprimaquine was assessed against *P. berghei* in female CD1 mice (20-25g). The mice (n = 6 per group) were infected with 10^6 parasites in red cell suspension (100 µl) prior to administration of the two test compounds (0.2 to 80 µmol/kg) i.p. in saline (100 µl/animal) for 4 days.[29] The IC 50 values were 2.20 ± 0.79 and 7.78 ± 0.93 µmol/kg for primaquine and fluoroprimaquine respectively. Therefore, the introduction of fluorine did not result in loss of antimalarial efficacy *in vivo*.

Conclusion

This preliminary study has indicated that the introduction of fluorine into primaquine does not compromise antimalarial activity. Furthermore, from the initial toxicological work, fluorine substitution has produced an analogue which is apparently less susceptible to bioactivation in a range of different species. Nevertheless, significant bioactivation was observed with hepatic microsomes from two species including man. However, to fully ascertain the utility of fluorine as a blocking substituent for 5-hydroxylation, a full metabolic profile of both primaquine and fluoroprimaquine in an appropriate animal model is required. Again, this study has demonstrated the utility of fluorine substitution as a probe to investigate chemical aspects of drug action, but in this particular example the limitation of fluorine as a means of totally blocking oxidative biotransformations has been illustrated.

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21. m.p. = 155°C (lit.[4] m.p. = 156 °C); ^{1}H NMR ($CDCl_3$, 200 MHz); δ 9.02 (1H, dd, J_{H-H} = 3.85 Hz and 1.64 Hz, ArH), 8.45 (1H, dd, J_{H-H} = 8.80 Hz and 1.65 Hz, Ar-H), 8.04 (1H, d, J_{H-F} = 9.00 Hz, Ar-H), 7.57 (1H, dd, J_{H-H} = 8.80 Hz and 3.85 Hz, Ar-H), 4.11 (3H, s, -OCH_3); MS *m/z* 222 (M+, 100 %), 192 (56 %), 133 (33 %), 121 (28 %); HRMS *m/z* 222.04420, ($C_{10}H_7N_2O_3F$ requires 222.177729).
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24. ^{1}H NMR ($CDCl_3$, 200 MHz); δ 8.58 (1H, dd, J_{H-H} = 4.40 Hz and 1.65 Hz, ArH), 8.23 (1H, dd, J_{H-H} = 8.25 Hz and 1.65 Hz, Ar-H), 7.37 (1H, dd, J_{H-H} = 8.25 Hz and 4.40 Hz, Ar-H), 6.38 (1H, d, J_{H-F} = 7.70 Hz, Ar-H), 4.02 (3H, s, -OCH_3), 3.73 (2H, m, $C\underline{H}_2NH_2$), 1.40-1.90 (5H, m, CH_2CH_2N, $\underline{CH}NH$), 1.33 (2H, d, J_{H-H} = 7 Hz, $C\underline{H}_3$-CH); IR (neat) 1590 and 810, cm^{-1}; MS *m/z* 277 (M+, 14 %), 219 (100 %), 204 (23 %); HRMS *m/z* 277.15947, ($C_{15}H_{20}N_3OF$ requires 277.15963).
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26. Microsomes were prepared from human liver samples or the livers of CD 1 mice, Wistar rats, New Zealand White Rabbits or beagle dogs. Primaquine or 5-fluoroprimaquine (100 μM final concentration) in distilled water (10 μl) was incubated with protein (1 mg). NADPH (1 mM omitted from controls) and red cells (500 μl of a 50 % suspension) for 1 h at 37° C. The final incubation volume was 1 ml. Toxicity towards RBC was assessed by methemoglobin formation using the method of Harrison. [27]
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