The Effect of Fluorine Substitution on the Metabolism and Antimalarial Activity of Amodiaquine

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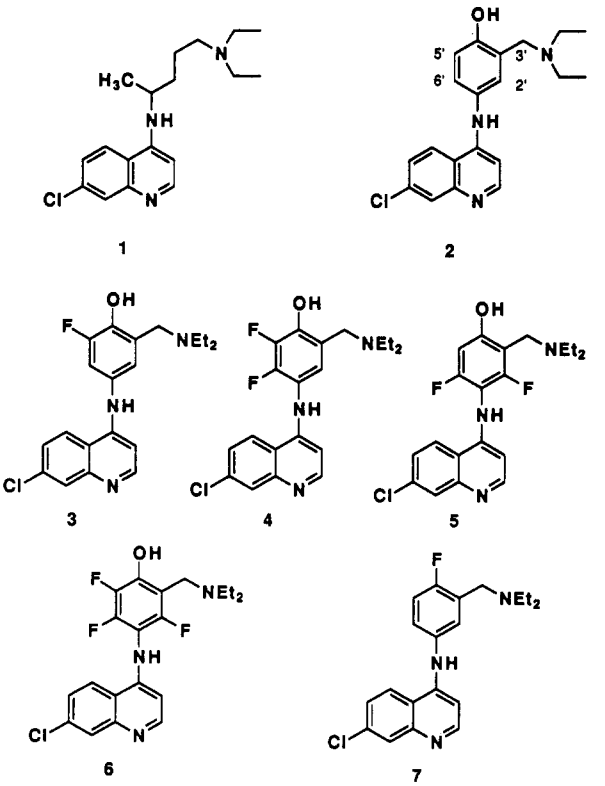
Amodiaquine (AQ) (2) is a 4-aminoquinoline antimalarial which causes adverse side effects such as agranulocytosis and liver damage. The observed drug toxicity is believed to be related to the formation of an electrophilic metabolite, amodiaquine quinone imine (AQQI), which can bind to cellular macro-molecules and initiate hypersensitivity reactions. 5'-Fluoroamodiaquine (5'-FAQ, 3), 5',6'-difluoroamodiaquine (5',6'-DIFAQ, 4), 2',6'-difluoroamodiaquine (2',6'-DIFAQ, 5), 2',5',6'-trifluoroamodiaquine (2',5',6'-TRIFAQ, 6) and 4'-dehydroxy-4'-fluoroamodiaquine (4'-deOH-4'-FAQ, 7) have been synthesized to assess the effect of fluorine substitution on the oxidation potential, metabolism, and *in vitro* antimalarial activity of amodiaquine. The oxidation potentials were measured by cyclic voltammetry, and it was observed that substitution at the 2',6'- and the 4'-positions (2',6'-DIFAQ and 4'-deOH-4'-FAQ) produced analogues with significantly higher oxidation potentials than the parent drug. Fluorine substitution at the 2',6'-positions and the 4'-position also produced analogues that were more resistant to bioactivation. Thus 2',6'-DIFAQ and 4'-deOH-4'-FAQ produced thioether conjugates corresponding to 2.17% (SD: ±0.27%) and 0% of the dose compared with 11.87% (SD: ±1.31%) of the dose for amodiaquine. In general the fluorinated analogues had similar *in vitro* antimalarial activity to amodiaquine against the chloroquine resistant K1 strain of *Plasmodium falciparum* and the chloroquine sensitive T9-96 strain of *P. falciparum* with the notable exception of 2',5',6'-TRIFAQ (6). The data presented indicate that fluorine substitution at the 2',6'-positions and replacement of the 4'-hydroxyl of amodiaquine with fluorine produces analogues (5 and 7) that maintain antimalarial efficacy *in vitro* and are more resistant to oxidation and hence less likely to form toxic quinone imine metabolites *in vivo*.

Introduction

Resistance to chloroquine (1) in *Plasmodium falciparum* malaria has become a major health concern of the developing world.[1] This resistance has prompted a reexamination of the pharmacology of alternative antimalarials that may be effective against resistant strains. Amodiaquine (2) is a 4-aminoquinoline antimalarial which is effective against chloroquine-resistant strains of *P. falciparum*[2] (Chart 1). However, clinical use has been severely restricted because of associations with hepatotoxicity[3] and agranulocytosis.[4]

Acetaminophen (4-hydroxyacetanilide) contains a *p*-hydroxyanilino moiety which is believed to undergo P-450 catalyzed oxidation to a chemically reactive quinone imine (Scheme 1). Amodiaquine also contains this functionality and might be expected to undergo enzymic oxidation to a reactive metabolite. Studies in this laboratory have shown that in the rat amodiaquine is excreted in bile exclusively as the 5'-thioether conjugates (glutathione and cysteinyl). This observation indicates that the parent drug undergoes extensive bioactivation *in vivo* to form amodiaquinequinone imine (AQQI) or -semiquinone imine (AQSQI) with subsequent conjugate addition of glutathione.[5,6] Formation of one of these reactive species *in vivo* and subsequent binding to cellular macromolecules could affect cell function either directly or by immunological mechanisms. Indeed IgG antibodies which recognize the 5'-cysteinyl group have been detected in patients with adverse reactions to amodiaquine.[7] Previous studies have shown that introduction of fluorine into the aromatic nucleus of acetaminophen increases the oxidation potential of the molecule and thereby blocks the *in vivo*

Chart 1. Structures of Chloroquine, Amodiaquine, and Fluorinated Analogues of Amodiaquine



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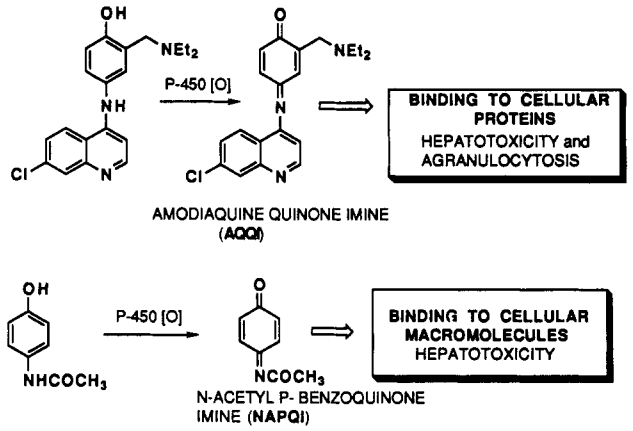
Scheme 1. Oxidative Pathways for Amodiaquine and Paracetamol



oxidation of the molecule to a cytotoxic quinone imine.[8] It was therefore proposed that the introduction of fluorine into the aromatic nucleus of amodiaquine would decrease formation of the ultimate toxin (AQQI or AQSQI) by increasing the stability of the aryl ring to oxidation. Furthermore, it was hoped that the resulting decrease in toxicity would be complemented by maintained antimalarial efficacy.

Thus, to investigate further the pharmacology of amodiaquine, five fluorinated analogues (Chart 1) have been synthesized in order to determine the effect of fluorine substitution on the metabolism, antimalarial activity, and physicochemical properties of the parent drug.

Chemistry

The synthesis of the novel fluorinated analogues (**3–7**) is based on the original synthesis of amodiaquine by Burckhalter,[9] whereby 4-hydroxyacetanilide (**1a**) is subjected to a Mannich reaction in order to introduce the appropriate aminoalkyl side chain. The amide function is subsequently hydrolyzed to generate the amine which is condensed *in situ* with 4,7-dichloroquinoline to give the desired product in good yield (Scheme 2).

Preparation of the fluorinated analogues of 4-hydroxyacetanilide was therefore crucial in the synthetic approach used. The synthesis of the monofluorinated analogue (**3**) is outlined in Scheme 4. Acetylation of the amino function and demethylation[12] of the methoxyacetanilide proceeded in high yield to give 3-fluoro-4-hydroxyacetanilide.

The dialkylamino side chain was introduced by means of the Mannich reaction, and following hydrolysis of the amide function, the intermediate aminophenol from **2c** was coupled with 4,7-dichloroquinoline to give the monofluorinated target molecule **3**.

Scheme 5 shows the synthesis of the 5,6-difluorinated analogue **4**. The reaction of sodium methoxide with 2,3,4-trifluoronitrobenzene at room temperature gave three products which were easily identified as **3a–c** by the NMR spectrum of the mixture. These isomers could not be separated by chromatography or by recrystallization. Treatment of the mixture with boron tribromide at room temperature selectively cleaved the isomers containing *o*-methoxynitrobenzene functions. The resulting *o*-nitrophenols could be easily removed by washing with 2 M NaOH. The resulting monomethoxynitrobenzene **3c** was reduced, acetylated, and demethylated as shown. The synthesis was completed by means of a Mannich reaction on 2,3-difluoro-4-hydroxyacetanilide and subsequent hydrolysis and coupling of the product **3i** to 4,7-dichloroquinoline.

The synthesis of the third target is shown in Scheme 6. Compound **5** was synthesized from 1,3,5-trifluoronitrobenzene in eight steps in an overall yield of approximately 30%. The major problem with this synthesis was in the reaction of 2,4,6-trifluoronitrobenzene with sodium methoxide since fluorine can be displaced from the positions ortho and para to the nitro group. The required 2,6-difluoro-4-methoxynitrobenzene was formed as the minor product, the ratio of the two products **4a**:**4b** being approximately 2:1. Kinetic studies[10] on the methoxydefluorination of polyfluoroaromatics showed that the second-order rate constants for methoxydefluorination at the ortho and para positions are similar, i.e., 17×10^{-3} and 19×10^{-3} mol^{-1} s^{-1}. Thus, in the reaction of 2,4,6-trifluoronitrobenzene with sodium methoxide 2,4-difluoro-6-methoxynitrobenzene is expected to be the major isomer as there are two ortho positions in which fluorine can be displaced. The two isomers (**4a** and **4b**) obtained in the reaction could not be separated by chromatography or by crystallization (as in the case of **3a–c**). However, in the reaction of the two isomers with boron tribromide, 2,4-difluoro-6-methoxynitrobenzene (**4a**) was demethylated more readily, giving 3,5-difluoro-6-nitrophenol (**4c**) which could again be removed by a basic wash. The 2,6-difluorinated derivative was eventually converted into the desired product as depicted in Scheme 6.

The synthesis of the trifluorinated derivative (**6**) is also shown in Scheme 6 and follows essentially the same steps described for the two difluorinated analogues.

The dehydroxy-1-fluoro analogue (**7**) was synthesized from 2-fluoro 5-nitrotoluene by free-radical bromination of the methyl group. The resulting benzyl bromide **6a** was allowed to react with diethylamine in toluene to give the substitution product **6b** in high yield (Scheme 7). The reduction could be achieved either using PtO_2 as catalyst in a catalytic hydrogenator or via stannous chloride reduction. The final stage simply involved coupling the amine **6c** with 4,7-dichloroquinoline as before.

Antimalarial Activity. The antimalarial activity of amodiaquine, chloroquine, and the fluorinated analogues was measured against two strains of *P. falciparum*: (a) the uncloned K1 strain (chloroquine resistant) and (b) the cloned T9-96 strain (chloroquine sensitive). The results are summarized in Tables 1 and 2 and demonstrate that all the analogues, apart from the trifluorinated analogue **6**, demonstrate appreciable antimalarial activity *in vitro*.

Discussion

The purpose of the study was to investigate the effect of fluorine substitution on the metabolism and antimalarial efficacy of amodiaquine. The introduction of fluorine can have a profound effect on the biological activity and the physicochemical properties of aromatic compounds. These changes are a consequence of the enhanced oxidative stability, increased lipid solubility, increased hydrogen-bonding potential, and alterations in the pK_a of acidic and basic functional groups.[14]

The drug toxicity associated with amodiaquine is believed to be a consequence of oxidation to a quinone imine (AQQI), which undergoes nucleophilic attack by cellular macromolecules. The resulting drug–protein conjugate may then initiate hypersensitivity reactions.

Scheme 2. Synthesis of Amodiaquine

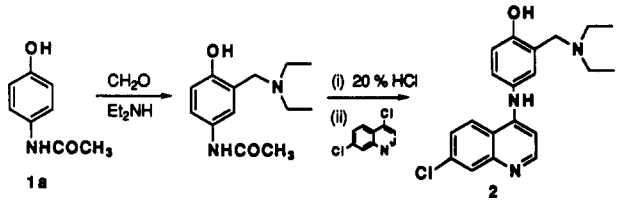


Table 1. Comparison of the Oxidation Potential, pK_a, Bioactivation, and N-Deethylation of Amodiaquine and Five Fluorinated Analogues

analogue	oxidation potential[a]	pK_a[b]	bioactivation (% thioether conj)	N-deethylation (% of dose)
2	0.97	4.95	11.87 (SD 1.31)	14.35 (SD 2.53)
3	0.73	3.96	7.56 (SD 2.14)	8.91 (SD 1.35)
4	0.88	3.42	20.13 (SD 10.14)	5.26 (SD 1.41)
5	1.34	2.59	2.17 (SD 0.27)	4.77 (SD 0.51)
6	1.25	2.17	13.91 (SD 4.45)	
7	>1.5	4.39		10.67 (SD 2.03)

[a] Solvent = DMF. Solutions of test sample were 1 mM. Reference electrode = saturated calomel electrode (platinum counter). Scan rate = 400 mV s^{-1}. Scans were initiated at 0.0 V in the positive direction. [b] pK_a refers to the 4-amino function.

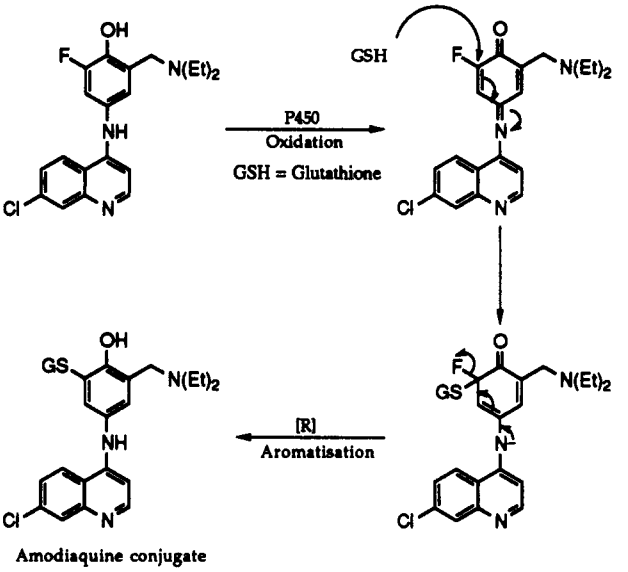
Acetaminophen also contains a *p*-hydroxyanilino moiety that can undergo oxidation to an electrophilic quinone imine intermediate (Scheme 2). In the case of acetaminophen, such a metabolite is believed to be responsible for the toxicity associated with this drug in overdose.[15-18]

We have previously shown that fluorine substitution can block the *in vivo* oxidation of acetaminophen provided that the oxidation potential of the derivative is sufficiently greater than that of the parent drug.[19] Thus, substitution at the 2,6- and 2,3,5,6-positions of acetaminophen significantly reduced hepatotoxicity as shown by measurement of serum transaminase and hepatic glutathione levels following administration of the drug to mice. As a result of these observations, it seemed reasonable that similar fluorine substitution into the aromatic nucleus of amodiaquine would provide novel analogues that were resistant to bioactivation.

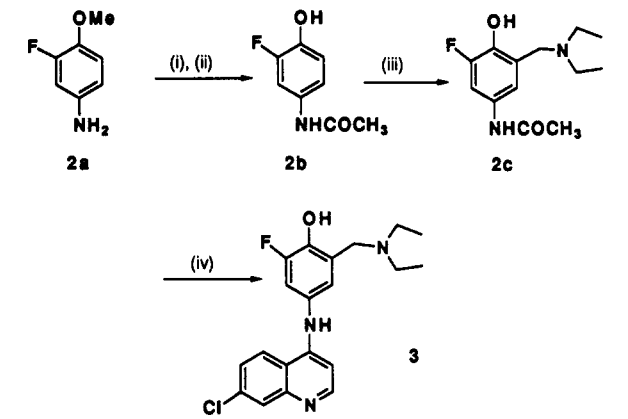
The effect of fluorine substitution on the *in vivo* oxidative metabolism of amodiaquine could be determined by quantification of drug–thioether conjugates excreted into the bile following administration of the appropriate analogue. Previous studies[5] using radiolabeled amodiaquine have shown that amodiaquine does not undergo direct conjugation with either glucuronic or sulfuric acids, but does undergo deethylation to deethylamodiaquine, which accumulates in the liver and is excreted slowly in urine. Thioether conjugates are excreted exclusively in bile, and therefore identification and quantification of biliary metabolites provide evidence for, and measurement of, the bioactivation of amodiaquine and its fluorinated analogues *in vivo*. The oxidation potential, bioactivation, and *N*-deethylation of amodiaquine and its fluorinated analogues is presented quantitatively in Table 1.

The oxidation potential of 2 and the fluorinated analogues were measured by cyclic voltammetry. This indicated that the oxidation was irreversible and involved two electrons. Although irreversible oxidation potentials should be treated with caution, we feel that in this case they do reflect the relative ease of oxidation of the derivatives (2–7). Discussions of the effect of fluorine substitution on the ease of oxidation of dopamine, norepinephrine,[23] 5,7-dihydroxytryptamine,[24] and acetaminophen[19] have also relied on irreversible oxidation potentials. Furthermore the effect of methyl substitution on the irreversible oxidation of acetaminophen has been reported,[25] and the relative ease of oxidation in this series was compared with hepatotoxicity.[26] From Table 1 it is apparent that introduction of fluorine does not uniformly increase the oxidation potential as initially expected. Fluorine ortho to the hydroxyl function lowers the oxidation potential, whereas fluorine meta to this group has the opposite effect. This may be related to the mesomeric electron releasing and inductive electron-withdrawing effects of fluorine on the removal of an electron from the phenolic hydroxyl function.

Scheme 3. Metabolic Defluorination of 5′-Fluoroamodiaquine



Scheme 4[a]

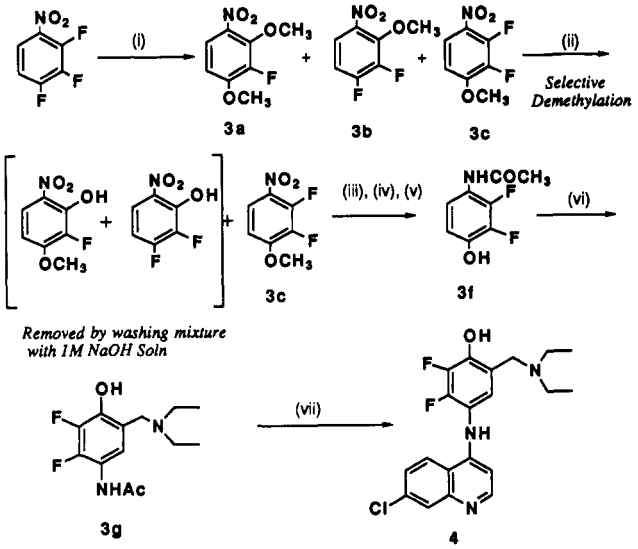


[a] Reagents: (i) Ac_2O, $CHCl_3$ Δ; (ii) BBr_3, CH_2Cl_2, 24 h; (iii) CH_2O, Et_2NH, Δ, 4h; (iv) 20% HCl, 4,7-dichloroquinoline.

5′-Fluoroamodiaquine (5′-FAQ, 3) had a lower oxidation potential than amodiaquine, suggesting that this compound might be more easily oxidized *in vivo*. Bioactivation was somewhat less than in the parent drug. The major biliary metabolite of 5′-FAQ was the 5′-glutathione conjugate,[5] formation of which must have involved cleavage of the carbon–fluorine bond (Scheme 3). Analysis of livers revealed that 5′-FAQ underwent deethylation, although to a lesser extent than the parent drug.

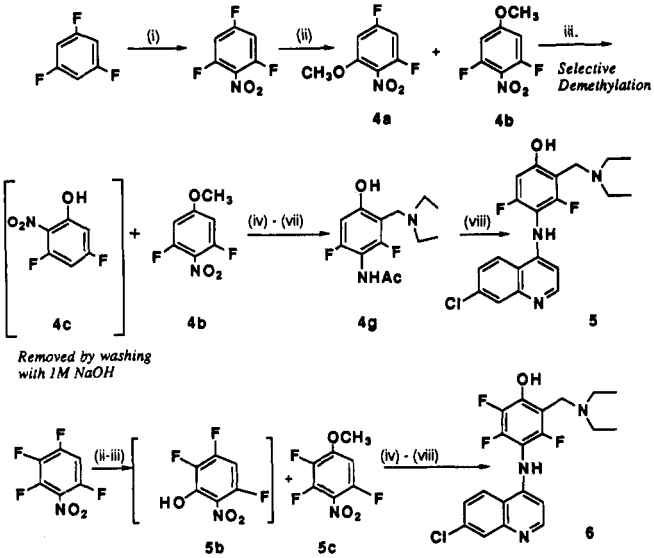
5′,6′-Difluoroamodiaquine (5′,6′-DIFAQ, 4) also had a lower oxidation potential than amodiaquine and was shown to undergo extensive bioactivationi *in vivo*. Glutathione

Scheme 5[a]



[a] Reagents: (i) NaOMe, MeOH; (ii) BBr_3, CH_2Cl_2; (iii) $NaBH_4$, Pd/C, MeOH; (iv) Ac_2O, $CHCl_3$; (v) BBr_3, CH_2Cl_2; (vi) CH_2O, Et_2NH; (vii) 20% HCl, 4,7-dichloroquinoline.

Scheme 6[a]



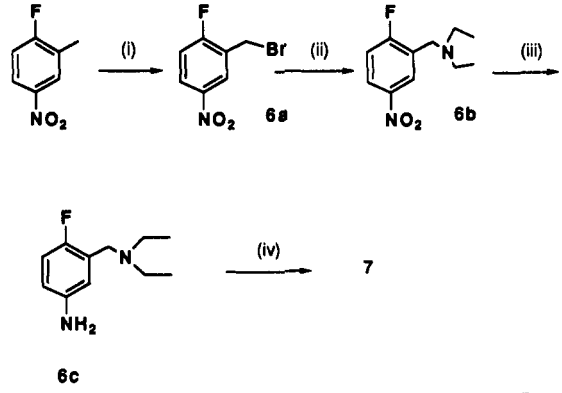
[a] Reagents: (i) HNO_3, H_2SO_4; (ii) NaOMe, MeOH; (iii) BBr_3, CH_2Cl_2; (iv) $NaBH_4$, Pd/C, EtOH; (v) Ac_2O; (vi) BBr_3, CH_2Cl_2; (vii) CH_2O, Et_2NH; (viii) 20% HCl, 4,7-dichloroquinoline.

and thioether conjugates of a monofluoro derivative of amodiaquine were the major biliary metabolites. These two metabolites could arise from conjugation of glutathione at the 5′-position, followed by defluorination, as in the bioactivation of 5′-FAQ. Deethyl-5′,6′-DIFAQ was observed in the livers at the end of the experiment.

The 2′,6′-difluoroamodiaquine analogue (2′,6′-DIFAQ, 5) showed a marked increase in the oxidation potential in comparison with amodiaquine (1.34 and 0.97 V, respectively). This was reflected in the bioactivation of 2′,6′-DIFAQ being reduced to only 2.1% of the dose (cf. amodiaquine, 11.9% of the dose), as measured by a thioether conjugate of 2′,6′-DIFAQ. 2′,6′-DIFAQ was deethylated to a similar extent to 5′,6′-DIFAQ.

Despite the increase in oxidation potential of 2′,5′,6′-TRIFAQ (6) (1.25 compared with 0.97 V for AQ), this derivative underwent comparable bioactivation to amodiaquine. As with the bioactivation of 5′-FAQ and 5′,6′-DIFAQ, 2′,5′,6′-TRIFAQ underwent defluorination and glutathione conjugation. No deethyl-2′,5′,6′-TRIFAQ could be detected in the livers at the end of the experiment.

Scheme 7[a]



[a] Reagents: (i) Br_2, UV, reflux; (ii) Et_2NH, toluene, reflux; (iii) PtO_2, H_2; (iv) 4,7-dichloroquinoline, EtOH, reflux.

Table 2. Antimalarial Activity[a] against the K1 Strain of *Plasmodium falciparum*

drug	IC50 (nmol)	SD
1 (chloroquine)	300	±21.53
2 (amodiaquine, AQ)	24	±5.00
3 (5′-FAQ)	42	±1.00
4 (5′,6′-DIFAQ)	56	±6.36
5 (2′,6′-DIFAQ)	41	±6.36
6 (2′,5′,6′-TRIFAQ)	500	±27.06
7 (4′-deOH-4′-FAQ)	59	±5.66

[a] Data are presented as the mean (n = 3) ● SD.

Table 3. Antimalarial Activity[a] against the T9.96 Strain of *Plasmodium falciparum*

drug	IC50 (nmol)	SD
1 (chloroquine)	27	±3.46
2 (amodiaquine, AQ)	20	±3.46
3 (5F′-AQ)	37	±5.00
4 (5′,6′-DIFAQ)	40	±2.65
5 (2′,6′-DIFAQ)	34	±2.65
6 (2′,5′,6′-TRIFAQ)	291	±23.89
7 (4-deOH-4FAQ)	40	±4.51

[a] Data are presented as the mean (n = 3) ± SD.

The final compound in the series was the dehydroxy-4′-fluoroamodiaquine (deOH-4′-FAQ, 7). This compound was not oxidized over the range used for the oxidation potential measurements (0–1.5 V). Furthermore, this compound gave no thioether metabolites, indicating that replacement of hydroxyl with fluorine precludes bioactivation *in vivo*. DeOH-4′-FAQ underwent deethylation to a similar extent to 5′-FAQ. Further studies using radiolabeled 4′-deOH-4′-FAQ have confirmed the lack of bioactivation of this compound in the rat.[22]

The antimalarial activity for the novel fluorinated analogues was measured against two strains of *P. falciparum*: (a) the uncloned K1 strain (chloroquine resistant) and (b) the cloned T9-96 strain (chloroquine sensitive). All of the compounds with the exception of 2′,5′,6-TRIFAQ demonstrated no significant loss in activity compared with amodiaquine against the K1 strain (Table 2). Similarly, all compounds, again with the exception of TRIFAQ, demonstrated comparable antimalarial activity to amodiaquine when tested against the T9-96 strain (Table 3). Notably, TRIFAQ lost some activity against both strains when compared with amodiaquine. This may be a direct consequence of the increased ionization of the hydroxyl group due to polyfluorine substitution,[19] resulting

in decreased penetration into the parasite vacuole. The slight differences in activity within the series as a whole can possibly be explained by the predicted effects of fluorine substitution on the basicity of the 4-amino group, with the less basic analogues being less potent as a result of decreased accumulation.[20]

This study suggests that the antimalarial activity of amodiaquine is not a function of oxidation to a quinone imine and underlines the possibility that antimalarial efficacy should be readily separated from toxicity in other 4-aminoquinolines containing the *p*-aminophenol functionality.

In summary, we have shown that fluorine substitution into the 4-aminophenol moiety of amodiaquine can have the desired effect of reducing bioactivation of the drug while having little or no effect on its efficacy. The position of substitution of fluorine within the 4-aminophenol ring is critical. There is no simple correlation between ease of oxidation of the amodiaquine analogues and the degree of bioactivation, but the observed metabolism can be rationalized if the effect of fluorine on *both* the ease of formation *and* the reactivity of the quinone imine intermediates is taken into account. Thus efficient bioactivation depends upon ready formation of the quinone imine and on the susceptibility of the latter to attack by glutathione rather than its participation in other redox reactions. Clearly the 4-dehydroxyfluoro derivative **7** cannot give a quinone imine by simple oxidation, and this is reflected in a high oxidation potential and no bioactivation. Compounds **3–6** can all lead to quinone imines. The oxidation potentials indicate that 2′,6′-DIFAQ (**5**) is more resistant to electrochemical oxidation than amodiaquine, and this is consistent with the observed decrease in bioactivation (2.1% of the dose compared with 11.8%, respectively). Interestingly, the corresponding acetaminophen analogue, 2,6-difluoroacetaminophen, also had a high oxidation/low bioactivation profile, and this may indicate a common mechanism of oxidation for these two drugs.

Nucleophilic attack on the quinone imines derived from both amodiaquine and acetaminophen occurs preferentially at the 5-position. Compounds **3**, **4**, and **6** all have a fluorine in the 5′-position so that the corresponding quinone imines can form conjugates by an addition–elimination sequence and a subsequent reduction (Scheme 3). This type of nucleophilic substitution is well established with β-halo-α,β-unsaturated carbonyl compounds. Reactivity toward this nucleophilic addition–elimination pathway would be increased by the electron-withdrawing inductive effects of additional fluorines at the 6′-position as in **4** and **6**, which both show increased bioactivation (20.1% and 13.9% of the dose, respectively) compared with **3** and amodiaquine itself. In the case of **6**, the comparatively more difficult oxidation is offset by efficient scavenging of the highly reactive quinone imine by glutathione.

Conclusion

In conclusion, these studies have demonstrated that introduction of fluorine into the antimalarial amodiaquine can influence the critical balance between drug activation and drug detoxification by reducing the process of oxidative bioactivation. In addition, it has been shown that such modifications can be achieved without any significant loss of activity against chloroquine-sensitive and chloroquine-resistant parasites. This dissociation of activity and toxicity by chemical modification is most striking for deOH-4′-FAQ (**7**), which does not undergo bioactivation to a reactive metabolite *in vivo* but has similar antimalarial activity to amodiaquine and is, therefore, a promising candidate for further studies.

Experimental Section

Antimalarial Testing. Two strains of *P. falciparum* from Thailand were used in this study: (a) the uncloned K1 strain which is known to be chloroquine resistant and (b) the cloned T9.96 strain which is sensitive to all antimalarials.

Parasites were maintained in continuous culture using a method derived from that of Jensen and Trager.[11] Cultures were maintained in culture flasks containing human erythrocytes (2–5%) with parasitaemia ranging from 0.1 to 10% suspended in RPMI 1640 medium supplemented with 25 mM HEPES buffer, 32 mM $NaHCO_3$, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O_2, 4% CO_2, and 93% N_2.

Antimalarial activity was assessed using an adaptation of the 48-h sensitivity assay of Desjardins et al.[13] using [^{3}H]hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were dissolved in 100% ethanol and diluted to an appropriate concentration with complete medium (final concentrations contained less than 1% ethanol). Assays were performed in sterile 96-well microtitre plates, each well containing 100 μL of medium which was seeded with 10 μL of a parasitized red blood cell mixture to give a resulting initial parasitaemia of 1% with a 5% haematocrit. Control wells (which constituted 100% parasite growth) consisted of the above, with the omission of the drug.

After 24 h of incubation at 37 °C, 0.5 μCi of hypoxanthine was added to each well. After a further 24-h incubation, the cells were harvested onto filter mats, dried overnight, placed in scintillation vials with 4 mL of scintillation fluid, and counted on a liquid scintillation counter.

IC50 values were calculated by interpolation of the probit transformation of the log dose–response curve. Each compound was tested against both strains to ensure reproducibility of the results.

Metabolism Studies. Male Wistar rats (n = 4–7, 230–270 g) were anaesthetized with urethane (7% w/v in 0.9% saline, 20 mL/kg). Bile duct cannulas were inserted, and blank bile was collected for 15 min. The fluorinated analogue was then administered to the rats (54 μmol/kg, in PEG 200/saline/EtOH, 6:2.5:1, injection volume 2.7 mL/kg) via the hepatic portal vein. Bile and urine were collected over 5 h, after which the animals were killed by exsanguination, and the livers were removed. Livers were homogenized (25% w/v) in sodium phosphate buffer (0.1 M, pH 7.4). Liver homogenates, bile, and urine were stored at −20 °C until required for analysis.

Analysis of Bile. Aliquots (25 μL) of blank and pooled bile (0–5 h) were analyzed by HPLC (10–25% CH_3CN in $NH_4H_2PO_4$, 0.01 M, pH 4.6, over 30 min, flow rate 1.5 mL/min). Quantification of metabolites was carried out using an external calibration curve of peak area vs mass (1–50 nmol) of a synthetic glutathione adduct of amodiaquine.

Analysis of Liver Homogenates. Aliquots from each liver homogenate (25% w/v, 2 × 0.5 mL) were mixed with MeOH (3 mL) and left overnight at 5 °C. The resulting precipitated protein was removed by centrifugation (1000*g*, 10 min). The protein pellet was washed with MeOH (4 × 3 mL). The supernatants from protein precipitation and from four washes were combined and evaporated to dryness using nitrogen. Each sample was reconstituted in MeOH (200 μL), and after vigorous mixing the samples were centrifuged (1000*g*, 10 min). Aliquots of the resulting supernatants (25 μL) were analyzed by HPLC (10–20% CH_3CN in NH_4OCOCH_3, 0.1 M, pH 3.8, over 18 min, flow rate 1.5 mL/min). Liver extracts from 2′,5′,6′-trifluoroamodiaquine-treated rats were analyzed using a gradient of 10–25% over 18 min). Quantification of metabolites was carried out using external calibration curves of peak area vs mass (0.5–10.0 nmol) for each individual analogue.

Characterization of Biliary Metabolites. Metabolites were isolated and purified from bile using HPLC (10–25% CH_3CN in

$NH_4H_2PO_4$, 0.01 M, pH 4.6, over 30 min, flow rate 1.5 mL/min). Bile collected during the first 90 min after administration of the analogue was injected onto the HPLC column, and the relevant fractions of eluent were collected. The organic component of the eluent was evaporated using nitrogen, and the resulting aqueous solution was applied to a SEP-PAK C_{18} cartridge. The cartridge was washed through with water (20 mL), and the metabolite was eluted with MeOH (ca. 3 mL). The eluent from the SEP-PAK cartridge was evaporated using nitrogen, and the residue was reconstituted in water (200 μL). The metabolite was purified from this solution using a second HPLC system (10–20% CH_3CN in 0.1 M NH_4OCOCH_3, pH 3.8, over 18 min, flow rate 1.5 mL/min). Eluents were processed as above, and the resulting material was analyzed by FIB +ve (or API –ve) mass spectrometry.

Characterization of Liver Metabolites. Liver metabolites were isolated and purified from the reconstituted extracts, as prepared above, by HPLC (10–20% CH_3CN in 0.1 M NH_4OCOCH_3, pH 3.8, over 18 min, flow rate 1.5 mL/min). Liver extracts from TRIFAQ-treated rats were processed using a gradient of 10–25% over 18 min. The extracts were injected onto the HPLC column, and the relevant fractions of eluent were collected. The organic component of the eluent was evaporated using nitrogen, and the resulting aqueous solution was processed using a SEP-PAK C_{18} cartridge as described above. The eluent from the SEP-PAK cartridge was evaporated to dryness using nitrogen and reconstituted in MeOH (ca. 200 μL). The metabolite was purified from this solution by repeating the HPLC and SEP-PAK processing. The metabolite was then analyzed by EI +ve mass spectrometry.

Metabolism Studies: Analysis and Characterization of Biliary Metabolites of the Fluorinated Analogues. All HPLC analysis was carried out on pooled bile samples (0–5 h), in comparison with blank bile collected prior to administration of each analogue. The *in vivo* metabolism of amodiaquine in the rat has been reported previously.[5] The amount of thioether and N-deethylated metabolites are recorded in Table 1 for comparison.

5′-Fluoroamodiaquine (3). A peak was observed (t_R 10.5 min) which cochromatographed with the synthetic amodiaquine–C5′-glutathione conjugate. The peak underwent complete degradation after treatment of the bile with γ-glutamyl transpeptidase. FIB +ve mass spectrometry gave unambiguous characterization of the metabolite as a glutathione conjugate of amodiaquine [m/z 661 $(M + 1)^+$, 588, 315]. Cochromatography with an authentic standard revealed the position of conjugation to be C5′. The metabolite accounted for 7.56 ± 2.14% of the dose. Enzymic hydrolysis with β-glucuronidase or sulfatase had no degradatory effect on the metabolite peak and produced no material which cochromatographed with parent compound.

5′,6′-Difluoroamodiaquine (4). Three components were observed in pooled bile which were not present in blank bile (t_R 3.5, 10, and 14 min, peaks 1, 2, and 3, respectively). Component 1 remains uncharacterized, despite isolation and analysis by mass spectrometry (EI +/–, FIB +/–), and was resistant to hydrolysis by β-glucuronidase, sulfatase, and γ-glutamyl transpeptidase. Peak 2 underwent complete degradation by γ-glutamyl transpeptidase and was characterized as a glutathione conjugate of a monofluoro-substituted amodiaquine analogue, by FIB +ve mass spectrometry [m/z 679 $(M + 1)^+$, 607, 333]. This metabolite accounted for 14.27 ± 6.22% of the dose. Peak 3 was resistant to hydrolysis by γ-glutamyl transpeptidase, sulfatase, and β-glucuronidase. Negative mode atmospheric pressure (API –ve) mass spectrometry characterized the metabolite as a thioether conjugate of a monofluoro-substituted amodiaquine analogue [m/z 404 (parent + S – 1), 333, 311, 283, 251]. This metabolite accounted for 5.86 ± 4.03% of the dose.

2′,6′-Difluoroamodiaquine (5). One metabolite peak was observed (t_R 10.0 min), accounting for 2.17% of the dose. API –ve mass spectrometry characterized the metabolite as a thioether conjugate of 2′,6′-difluoramodiaquine [m/z 422 (parent + S – 1), 350, 328, 280]. Sulfatase and β-glucuronidase produced no parent compound from metabolites in the bile.

2′,5′,6′-Trifluoroamodiaquine (6). Three major peaks were observed (t_R 8.5, 10.5, and 13.0 min, components 1, 2, and 3, respectively). Peak 3 corresponded to the main metabolite which underwent complete degradation following γ-glutamyl transpeptidase hydrolysis. The metabolite was unambiguously characterized by API +ve mass spectrometry as a glutathione conjugate of a difluorinated amodiaquine analogue [m/z 697 $(M + 1)^+$, 624, 351]. This metabolite accounted for 13.91 ± 4.45% of the dose. Components 1 and 2 were resistant to hydrolysis by γ-glutamyl transpeptidase but remain uncharacterized, despite HPLC–MS analysis of the pooled bile sample. Neither sulfatase nor β-glucuronidase hydrolysis produced any parent compound from metabolites in bile.

4′-Dehydroxy-4′-fluoroamodiaquine (7). No metabolite peaks were evident in the bile.

Analysis and Characterization of Liver Metabolites of the Fluorinated Analogues. 5′-Fluoroamodiaquine (3). A peak (t_R 9.0 min) was identified as the parent compound by cochromatography and accounted for 2.36 ± 0.68% of the dose. A second peak (t_R 7.5 min), accounting for 8.91 ± 1.35% of the dose, was characterized by EI +ve mass spectrometry as deethyl-5′-fluoroamodiaquine [m/z 345 (M^+), 300, 271, 236].

5′,6′-Difluoroamodiaquine (4). No parent compound was present. Deethyl-5′,6′-difluoroamodiaquine accounted for 5.26 ± 1.41% (t_R 12.0 min) of the dose and was characterized by EI +ve mass spectrometry [m/z 363 (M^+), 318, 283, 254].

2′,6′-Difluoroamodiaquine (5). No parent compound was present. A component at t_R 10.0 min was observed, which was characterized by EI +ve mass spectrometry as deethyl-2′,6′-difluoroamodiaquine [m/z 363 (M^+), 318]. A second component (t_R 8.0 min) was also detected. This peak had the anticipated retention time of a bisdeethylated metabolite of the parent compound, and as such would account for 3.42 ± 1.44% of the given dose. However, mass spectrometry yielded no evidence of such a metabolite, and the component remains uncharacterized.

2′,5′,6′-Trifluoroamodiaquine (6). A peak (t_R 13.7 min) was identified by cochromatography as the parent compound. This material accounted for 7.10 ± 0.99% of the dose. No other metabolite peaks were observed.

4′-Dehydroxy-4′-fluoroamodiaquine (7). A peak (t_R 12.0 min) was observed. The peak was characterized by EI +ve mass spectrometry as parent compound [m/z 357 (M^+), 342, 286, 270, 250] and accounted for 4.22 ± 1.33% of the dose. A second peak (t_R 10.0 min) was also detected and was characterized by EI +ve mass spectrometry as deethyl-4′-dehydroxy-4′-fluoroamodiaquine [m/z 329 (M^+), 314, 286, 270, 250]. This metabolite accounted for 10.67 ± 2.03% of the dose.

Analysis of Urine. Enzyme hydrolysis using either sulfatase or β-glucuronidase did not result in the appearance of the parent compound for any of the analogues tested.

Electrochemistry: Oxidation Potentials. Cyclic voltammetry experiments were performed using a Hi-Tek PPRI waveform generator connected to a Philips PM 8277 XYE recorder to record current–potential profiles. A Hi-Tek DT 11005 potentiostat was used for controlled potential runs. The reference electrode was the saturated calomel electrode (SCE), and the working and counter electrodes were platinum. Anhydrous DMF was stored under N_2. Tetrabutylammonium hexafluorophosphate (supporting electrolyte) was dried under vacuum at 100 °C for 24 h prior to use. DMF was chosen as solvent due to the poor solubility of several of the analogues in a range of alternative solvents. A solution of the supporting electrolyte (0.1 M) in dry DMF was prepared and degassed with argon for 10 min, and the solution was stored under argon. The concentrations of samples analyzed were 1 mmol in the supporting electrolyte solution. Scans were initiated in the positive direction at a rate of 400 mV s^{-1}. The oxidation potential was determined from the first oxidation wave recorded during the cyclic voltammetric scanning. All samples were degassed with nitrogen for 5 min prior to running scans.

pK_a Values. pK_a values (Table 1) refer to the quinoline nucleus and were determined using the method of Irvin et al.[21]

Experimental Data. 3-Fluoro-4-methoxyaniline, 1,3,5-trifluoronitrobenzene, 2,3,4-trifluoronitrobenzene, 2,3,4,6-tetrafluoronitrobenzene, and 2-fluoro-5-nitrotoluene were obtained from the Aldrich Chemical Co., Gillingham, Dorset, England. Merck Kieselgel 60 F 254 pre-coated silica plates for TLC were obtained from BDH, Poole, Dorset, U.K. Column chromatography was carried out on Merck 938S silica gel. Infrared (IR) spectra were recorded in the range 4000–600 cm^{-1} using a Perkin-Elmer 298

infrared spectrometer. Spectra of liquids were taken as films. Sodium chloride plates (Nujol mull) and solution cells (dichloromethane) were used as indicated. Proton NMR spectra were recorded using a Perkin-Elmer R34 (220 MHz) and Bruker (400 MHz, 250 and 200 MHz) NMR spectrometers. Solvents are indicated in the text, and tetramethylsilane was used as an internal reference. Mass spectra were recorded at 70 eV using a VG7070E mass spectrometer. The samples were introduced using a direct insertion probe. In the text the molecular ion (M^+) is given, followed by peaks corresponding to major fragment losses with intensities in parentheses. Melting points (mp) were determined on a Koffler hot stage apparatus and are uncorrected. Unless otherwise stated, petroleum ether refers to petroleum spirit bp 40–60 °C. Solvents were purified where necessary by standard procedures. Microanalysis was carried out by the University of Liverpool Microanalysis laboratory.

3-Fluoro-4-methoxyacetanilide. 3-Fluoro-4-methoxyaniline (**2a**) (3 g, 21.3 mmol) was acetylated with acetic anhydride (4.34 g, 42.6 mmol) by heating under reflux in toluene for 45 min. The toluene was removed under reduced pressure, and the residue was purified by flash column chromatography using ethyl acetate/petroleum ether (1:3) as eluent to give the product as a white crystalline solid: mp 113 °C;[17] ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.84 (1H, br exch, NH), 7.37 (1H, dd, J_{H-F} = 12.9 Hz and J_{H-H} = 2.4 Hz, ArH), 7.14 (1H, dd, J_{H-H} = 8.9 Hz and 2.4 Hz, Ar-H), 6.87 (1H, t, J_{H-H} and J_{H-F} = 8.9 Hz, Ar-H), 3.93 (3H, s, OCH_3) and 2.13 (3H, s, $COCH_3$); IR (Nujol mull) 3270, 1665, 1260, 1230, 1125, 1032, 815, and 760 cm^{-1}; MS m/z 183 (M^+, 47), 141 (65), 126 (100), 43 (49); HRMS m/z 183.069 45 ($C_9H_{10}FNO_2$ requires 183.069 56). Anal. ($C_9H_{10}FNO_2$) C, H, N.

3-Fluoro-4-hydroxyacetanilide (2b). Boron tribromide (1 M in dichloromethane, 16.8 mL, 16.8 mmol) was added to a stirred suspension of 3-fluoro-4-methoxyacetanilide (1.53 g, 8.4 mmol) in dry dichloromethane (50 mL) under nitrogen, and the resulting mixture was allowed to stir at room temperature overnight. Water (75 mL) was added to hydrolyze the excess of boron tribromide, and the solution was allowed to stir for a further 30 min. The dichloromethane layer was separated and the water layer extracted three times with ethyl acetate (3 × 75 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The product was purified by flash column chromatography using acetone–ethanol (9:1, R_f 0.72) as eluent to give 3-fluoro-4-hydroxyacetanilide as off-white crystals, 1.17 g (83%): mp 190–191 °C; ^{1}H NMR (d_6-DMSO, 250 MHz) δ 9.95 (1H, sharp, exch), 9.63 (1H, br, exch), 7.6 (1H, dd, J_{H-F} = 12.6 Hz and J_{H-H} = 2.2 Hz, Ar-H), 7.14 (1H, dd, J_{H-H} = 8.9 and 2.1 Hz, Ar-H), 6.94 (1H, t, J_{H-H} and J_{H-F} = 8.9 Hz, Ar-H) and 2.08 (3H, s, $COCH_3$); MS m/z 169 (M^+, 33), 127 (100), 79 (15), 43 (36); HRMS m/z 169.0542 ($C_8H_8FNO_2$ requires 169.0539). Anal. ($C_8H_8FNO_2$) C, H, N.

3-[(Diethylamino)methyl]-5-fluoro-4-hydroxyacetanilide (2c). 3-Fluoro-4-hydroxyacetanilide (1.2 g, 7.1 mmol) (**2b**) was subjected to a Mannich reaction with aqueous formaldehyde (0.60 g) solution and diethylamine (0.64 g, 1.2 molar equiv) in ethanol (5 mL). The solution was heated under reflux for 5 h, and the volatile components were removed under reduced pressure to give a yellow gum. Chromatography using dichloromethane/methanol (9:1) gave a pale yellow solid (1.30 g, 77%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.10 (1H, s, NH), 7.7 (1H, s, OH exch), 7.18 (1H, dd, J_{H-F} = 10 Hz, J_{H-H} = 2.2 Hz, Ar-6H), 7.0 (1H, s, Ar-2H), 3.8 (2H, s, CH_2N), 2.6 (4H, q, $N(CH_2CH_3)_2$), 2.2 (3H, s, $COCH_3$), 1.1 (6H, t, NCH_2CH_3); IR (Nujol mull) 3500-2500 (broad), 1670-1610, 1565, 1510, 1480, 1400, 1360, 1300, 1240, 1050, and 740 cm^{-1}; MS m/z 236 (M^+, 55), 221 (42), 210 (5), 164 (56), 121 (90), 72 (26), 58 (100), 43 (18). Anal. ($C_{13}H_{19}N_2O_2F$) C, H, N.

7-Chloro-4-[3′-[(diethylamino)methyl]-5′-fluoro-4′-hydroxyanilino]quinoline (3). Amide **2c** (1.2 g) was hydrolyzed with of 20% HCl (2.80 mL) by heating under reflux for 2 h. 4,7-Dichloroquinoline (1.0 g) was added together with ethanol (10.0 mL), and the reaction mixture was refluxed for a further 6 h. The solvent was removed under reduced pressure, and the residue was dissolved in distilled water (5 mL). Careful addition of a dilute ammonium hydroxide (2%) liberated the free base as a bright yellow solid. The solid was filtered off, washed with water, and dried for 36 h under vacuum. Purification was achieved by flash column chromatography using 5–20% MeOH/dichloromethane as eluent to give the quinoline **3** (1.2 g): ^{1}H NMR (methanol-d_4, 200 MHz) δ 8.42 (1H, d, J_{H-H} = 5.3 Hz, Ar-quin. H2), 8.40 (1H, d, J_{H-H} = 8.9 Hz, Ar-quin. H5), 8.5 (1H, d, J = 2.4 Hz, Ar-quin. H8), 7.60 (1H, d, J = 8.9 Hz, Ar-quin. H6), 7.35 (1H, d, J_{H-F} = 10 Hz, Ar-6′H), 7.32 (1H, s, Ar-2′H), 6.9 (1H, d, Ar-quin. H), 4.15 (2H, s, CH_2N), 3.2 (4H, q, NCH_2CH_3), 1.38 (6H, t, NCH_2CH_3); IR (Nujol mull) 3600–2420, 1610, 1590, 1445, 1236, 1050, 910, and 610 cm^{-1}; MS m/z 373 (M^+, 42), 300 (64), 271 (12), 236 (15), 162 (5), 74 (32), 58 (100); HRMS m/z 373.136 25 ($C_{20}H_{21}N_3FCl$ requires 373.135 72).

2,3-Difluoro-4-methoxynitrobenzene (3c). Sodium (0.76 g, 33 mmol) in dry methanol (35 mL) was added to 2,3,4-trifluoronitrobenzene (5.0 g) in dry methanol (75 mL) during 3 h. The reaction mixture was allowed to stir for 17 h, and the methanol was then removed under reduced pressure to give a yellow residue which was triturated with water. The product was extracted into diethyl ether, the organic extracts were dried ($MgSO_4$), and the solvent was removed to give a yellow oil. NMR analysis of the oil showed clearly that two major products had been formed (peaks at δ 4.12 and 4.04 ppm from the ortho and para isomers, respectively). A third isomer accounting for 5% was assigned as 3-fluoro-2,4-dimethoxynitrobenzene. These isomers could not be separated by recrystallization or by chromatography. However, treatment of the mixed isomers with boron tribromide selectively cleaved the isomers containing the *o*-methoxy functions. Thus boron tribromide (40.0 mL, 1.5 molar equiv) was added to the mixed isomers (4.95 g in 400 mL of dry dichloromethane) under nitrogen. The resulting deep red solution was allowed to stir for 24 h, water was added, and the organic layer was separated. The water layer was extracted three times with diethyl ether, and the combined organic extracts were evaporated under reduced pressure. The resulting residue was taken into 200 mL of diethyl ether and washed with 2 M NaOH to remove the phenols. The ether layer was separated, washed with water, dried ($MgSO_4$), and evaporated to give the product as a yellow solid (3.10 g, 58%). The product was purified by recrystallization from dichloromethane/cyclohexane: mp 92–94 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.00–8.05 (1H, ddd, Ar-H, J_{H-H} = 9.5 Hz, J_{H-F} = 7.33 and 2.15 Hz), 6.80–6.95 (1H, ddd, Ar-H, J_{H-H} = 9.5 Hz, J_{H-F} = 7.33 and 2.15 Hz), 4.04 (3H, s, OCH_3); IR (CH_2Cl_2) 3067, 2951, 1618, 1533, 1503, 1350, 1300, 1190, 1094, and 808 cm^{-1}; MS m/z 189 (M^+, 100), 159 (66), 128 (45), 113 (33), 100 (43); HRMS m/z 189.024 00 ($C_7H_5F_2NO_3$ requires 189.0275). Anal. ($C_7H_5F_2NO_3$) C, H, N.

2,3-Difluoro-4-methoxyaniline (3d). 2,3-Difluoro-4-methoxynitrobenzene (3.0 g, 0.0158 mol) was reduced with sodium borohydride (1.17 g, 2 equiv) and Pd/C (0.40 g) as catalyst in 50% aqueous ethanol. The reaction was followed by TLC and was complete after 40 min. The excess borohydride was destroyed with 2 M HCl, and the solution was then neutralized with a weak sodium hydroxide solution. The resulting liquid was extracted three times with 75-mL portions of ether. The solvent was removed under reduced pressure and taken back into ether which was then dried ($MgSO_4$). The solvent was removed to give a brown solid, which was purified by sublimation (5 mmHg, 100 °C) to give **3d** (2.30 g, 91%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.40–6.70 (2H, m, Ar-H), 3.85 (3H, s, OCH_3), 3.4 (2H, broad, NH_2); IR (Nujol mull) 3684, 3089, 1604, 1510, 1231, 1082, and 935 cm^{-1}; MS m/z 159 (M^+, 51), 144 (100), 116 (32); HRMS m/z 159.049 42 ($C_7H_7NOF_2$) requires 159.049 57). Anal. ($C_7H_7F_2NO$) C, H, N.

2,3-Difluoro-4-methoxyacetanilide (3e). 2,3-Difluoro-4-methoxyaniline (3.3 g) was acetylated as described for **2a**. The product was purified by sublimation (1 mmHg, 200 °C) to give **3e** as white needles (96%): mp 116–118 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.90 (1H, dt, J_{H-H} and J_{H-F} = 8.5 Hz, J_{H-H} = 2.2 Hz, Ar-5H), 6.95 (1H, dt, J_{H-H} and J_{H-F} = 8.5 Hz, J_{H-H} = 2.2 Hz, Ar-6H), 4.10 (3H, s, OCH_3), 2.4 (3H, s, $NHCOCH_3$); IR (CH_2Cl_2) 3684, 3429, 3044, 2971, 1696, 1605, 1511, 1457, 1232, 1091, and 1047 cm^{-1}; MS m/z 201 (M^+, 32), 159 (62), 144 (100), 43 (37); HRMS m/z 201.060 19 ($C_9H_9NO_2F_2$ requires 201.060 13). Anal. ($C_9H_9F_2NO_2$) C, H, N.

2,3-Difluoro-4-hydroxyacetanilide (3f). Boron tribromide (10.0 mL, 9.0 × 10^{-3} mol) was added via syringe to the methoxy compound **3e** (900 mg, 4.5 × 10^{-3} mol) in anhydrous dichloromethane (90 mL) under nitrogen. The solution was left for 24 h, poured onto water and extracted with ethyl acetate. Chro-

matography with petroleum ether/ethyl acetate (1:1) as eluent gave product as a white solid (750 mg, 90%): mp 132–134 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 9.00 (1H, s, NHCOCH$_3$), 8.95 (1H, s, NHCOCH$_3$), 8.90 (1H, s, OH), 7.55 (1H, tt, J_{H-H} and J_{H-F} = 8.95 Hz, J_{H-H} and J_{H-NH} = 2.69 Hz, Ar-6H), 6.65 (1H, dt, J_{H-H} and J_{H-F} = 9.15 Hz, 2.69 Hz, Ar-5H), 2.08 (3H, s, NHCOCH$_3$); IR (CH_2Cl_2) 3683, 3083, 1696, 1605, 1512, 1457, 1223, 1180, 1050, and 1013 cm^{-1}; MS *m/z* 187 (M$^+$, 20), 145 (100), 43 (26); HRMS *m/z* 187.04434 ($C_7H_7NOF_2$ requires 187.04448). Anal. ($C_7H_7F_2NO$) C, H, N.

3-[(Diethylamino)methyl]-5,6-difluoro-4-hydroxyacetanilide (3g). The amide **3f** (750 mg, 4.01 × 10^{-3} mol) was allowed to react with a solution of diethylamine (0.354 g) and formaldehyde (0.38 g) as described for **2b**. Removal of the volatile compound have a thick gum which was chromatographed using dichloromethane/methanol (9:1 through 4:1) to give the product as a white solid (0.90 g, 82.5%): mp 89–92 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 8.8 (1H, NHCOCH$_3$), 7.45 (1H, d, Ar-H, J_{H-F} = 4 Hz), 3.85 (2H, s, CH$_2$N), 2.7 (4H, q, N(CH$_2$CH$_3$)$_2$), 2.1 (3H, s, NHCOCH$_3$), 1.15 (6H, t, N(CH$_2$CH$_3$)$_2$); IR (CH_2Cl_2) 3428, 2965, 2938, 1691, 1545, 1490, 1459, 1228, 1192, 1113, 1055, 994, and 912 cm^{-1}; MS *m/z* 272 (M$^+$, 15), 257 (13), 200 (20), 157 (21), and 58 (100); HRMS *m/z* 272.13353 ($C_{13}H_{18}N_2O_2F_2$ requires 272.13363). Anal. ($C_{13}H_{18}N_2O_2F_2$) C, H, N.

7-Chloro-4-[3′-[(diethylamino)methyl]-5′,6′-difluoro-4′-hydroxyanilino]quinoline (4). The amide function of **3g** was hydrolyzed and coupled with 4,7-dichloroquinoline as for **3** with the following modification. Following hydrolysis with 6 M aqueous HCl, ethanol was added to the solution which was then evaporated to dryness. The residue was triturated with ether to give the dihydrochloride of the aminophenol derived from **3g**. The dihydrochloride salt (0.503 g) was added to 4,7-dichloroquinoline (0.435 g) in ethanol (20 mL) and the solution heated under reflux for 4 h. The volume was reduced to about 5 mL under reduced pressure and poured onto iced water (100 mL) containing concentrated ammonia solution (5 mL). The bright yellow solid was filtered off, washed with water, and purified by flash column chromatography with 10% MeOH/90% dichloromethane as eluent to give the desired product (78%): ^{1}H NMR (methanol-d_4) δ 8.5 (1H, s, Ar-quin. H2), 8.45 (1H, d, J_{H-H} = 8.8 Hz, Ar-quin. H8), 7.65 (1H, dd, J_{H-H} = 8.8 Hz, J_{H-H} = 2.2 Hz, Ar-quin. H6), 6.55 (1H, dd, J_{H-H} = 5.5 Hz, J_{H-H} = 2.20 Hz, Ar H2′), 4.15 (2H, s, CH$_2$N), 3.2 (4H, q, N(CH$_2$CH$_3$)$_2$), 1.5 (6H, t, N(CH$_2$CH$_3$)$_2$); IR (CH_2Cl_2) 3428, 3033, 2966, 2939, 2880, 1610, 1583, 1568, 1488, 1465, 1377, 1366, 1317, 1106, 1061, and 978 cm^{-1}; MS *m/z* 391 (M$^+$, 19), 318 (22), 73 (24), 58 (100); HRMS 391.125 99 ($C_{20}H_{20}N_3OClF_2$ requires 391.126 330). Anal. ($C_{20}H_{20}N_3OClF_2$) C, H, N.

2,6-Difluoro-4-hydroxyacetanilide (4f). This was prepared from 1,3,5-trifluorobenzene by the method of Barnard et al.[19] by the route shown in Scheme 6. The product was purified by flash column chromatography, with 10% methanol/90% dichloromethane as eluent, to give the product as off-white crystals: mp 157 °C;[19] ^{1}H NMR (DMSO-d_6, 250 MHz) δ 10.33 (1H, exch), 9.31 (1H, s, exch), 6.51 (2H, d, J_{H-F} = 9.5 Hz, Ar-H), 2.03 (3H, s, NHCOCH$_3$).

3-[(Diethylamino)methyl]-2,6-difluoro-4-hydroxyacetanilide (4g). 2,6-Difluoro-4-hydroxyacetanilide (1.3 g, 6.95 mmol) was subjected to a Mannich reaction with diethylamine (0.64 g) and formaldehyde (0.60 g) in ethanol (5 mL) as described for **2c**. Purification was achieved using dichloromethane/methanol (9:1) to give the product as a white solid (1.40 g, 74%): mp 110–112 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.75 (1H, s, NHCOCH$_3$), 6.39 (1H, dd, J_{H-F} = 11 Hz and J_{H-H} = 1.65 Hz, Ar-5H), 3.81 (2H, s, CH$_2$N), 2.72 (q, 4H, N(CH$_2$CH$_3$)$_2$), 2.18 (3H, s, NHCOCH$_3$), 1.12 (6H, t, N(CH$_2$CH$_3$)$_2$); IR (CH_2Cl_2) 3422, 3035, 2967, 1695, 1611, 1500, 1352, 1231, 1193, 1159, 1131, 1047, 1006, 889, and 647 cm^{-1}; MS *m/z* 272 (M$^+$, 12), 257 (11), 200 (14), 157 (8), 119 (8), 58 (100); HRMS 272.133 81 ($C_{13}H_{18}N_2O_2F_2$ requires 272.133 63). Anal. ($C_{13}H_{18}N_2O_2F_2$) C, H, N.

7-Chloro-4-[3′-[(diethylamino)methyl]-2′,6′-difluoro-4′-hydroxyanilino]quinoline (5). The amide function of **4h** was hydrolyzed and the intermediate aminophenol coupled with 4,7-dichloroquinoline as for **3f** to give the desired product (58%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.51 (1H, d, J_{H-H} = 5.38 Hz, Ar-quin. 2H), 8.05 (1H, d, J_{H-H} = 2.15 Hz, Ar-quin. 8H), 7.96 (1H, d, J_{H-H} = 8.9 Hz, Ar-quin. 5H), 7.46 (1H, dd, J_{H-H} = 8.9 and 2.15 Hz, Ar-quin. 6H), 6.5 (1H, dd, J_{H-F} = 11 and 2.05 Hz, Ar-5′H), 6.33 (1H, d, J_{H-H} = 5.37 Hz, Ar-quin. 3H), 3.85 (2H, s, CH$_2$N), 2.64 (4H, q, N(CH$_2$CH$_3$)$_2$) 1.15 (6H, t, N(CH$_2$CH$_3$)$_2$); IR (CH_2Cl_2) 3420, 2966, 2930, 1600, 1580, 1555, 1435, 1377, 1317, 1100, and 1055 cm^{-1}; MS (FABS +ve) *m/z* 392 (M$^+$, 100), 319 (66), 299 (6.60), 74 (16), 58 (33). Anal. ($C_{20}H_{20}N_3OClF_2$) C, H, N.

2,3,6-Trifluoro-4-methoxynitrobenzene (5c). Sodium (0.68 g) in methanol (50 mL) was added dropwise to a solution of 2,3,4,6-tetrafluoronitrobenzene (5.0 g) in dry methanol (75 mL) as described for 2,3-difluoro-4-methoxynitrobenzene, **3c**. Following workup and treatment with boron tribromide, the product **5c** (3.95 g, 67%) was obtained as a yellow solid: mp 40–41 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.69 (1H, ddd, J_{H-F} = 11.55, 6.05, and 2.2 Hz, Ar-H), 4.0 (3H, s, OCH$_3$); IR (CH_2Cl_2) 3071, 1631, 1541, 1512, 1456, 1345, 1204, 1132, 1081, 907, 836, and 806 cm^{-1}; MS *m/z* 207 (M$^+$, 100), 177 (82), 161 (29), 146 (42), 118 (49), 99 (47); HRMS 207.013 89 ($C_7H_4NO_3F_3$ requires 207.014 33).

2,3,6-Trifluoro-4-methoxyaniline (5d). 2,3,6-Trifluoro-4-methoxynitrobenzene (3.20 g) was reduced as described for **3d** using sodium borohydride, Pd/C in 50% aqueous ethanol. Following sublimation, product **5c** was obtained (2.68 g, 98%): mp 60–64 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.52 (1H, ddd, J_{H-F} = 12.10, 7.15, and 2.75 Hz, Ar-H), 3.82 (3H, s, OCH$_3$), 3.5 (2H, s, NH$_2$); IR (CH_2Cl_2) 2969–2700 (broad), 1623, 1507, 1242, and 1093 cm^{-1}; MS *m/z* 177 (M$^+$, 59), 162 (100), 134 (22); HRMS 177.040 29, ($C_7H_6NOF_3$ requires 177.040 15).

2,3,6-Trifluoro-4-methoxyacetanilide (5e). 2,3,6-Trifluoro-4-methoxyaniline was acetylated as described for **3e**. Purification was achieved by recrystallization from cyclohexane and dichloromethane to give the desired product (2.4 g, 80%): mp 130–132 °C; ^{1}H NMR δ 7.4 (1H, s, NHCOCH$_3$), 6.95 (1H, ddd, J_{H-F} = 10.95, 6.93, and 2.2 Hz, Ar-H), 4.30 (3H, s, OCH$_3$), 2.62 (3H, s, NHCOCH$_3$); IR (CH_2Cl_2) 3420, 3042, 2977, 1703, 1648, 1522, 1414, 1373, 1236, 1201, 1145, 1087, 889, and 668 cm^{-1}; MS *m/z* 219 (M$^+$, 9), 177 (76), 162 (100), 43 (47); HRMS 219.050 67 ($C_9H_8NO_2F_3$ requires 219.050 71).

2,3,6-Trifluoro-4-hydroxyacetanilide (5f). This preparation was carried out as described for **4f**. The product (1.60 g, 85%) was purified by chromatography using ethyl acetate–ethanol (9:1): mp 150–152 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 9.9 (s, 1H, NHCOCH$_3$), 8.95 (1H, s, OH), 6.87 (1H, ddd, J_{H-F} 11.0, 5.15, and 2.2 Hz, Ar-H), 2.3 (3H, s, NHCOCH$_3$); IR (CH_2Cl_2) 3400–3000 (broad), 2928, 2853, 1629, 1545, 1511, 1492, 1466, 1377, 1061, and 834 cm^{-1}; MS *m/z* 205 (M$^+$, 4.27), 163 (100), 115 (16), 43 (48); HRMS 205.035 77 ($C_8H_6NO_2F_3$ requires 205.035 06).

3-[(Diethylamino)methyl]-2,5,6-difluoro-4-hydroxyacetanilide (5g). This reaction was carried out as for **3g** using acetanilide **5f** (2.36 g), diethylamine (1.05 g), and aqueous formaldehyde (0.974 g). Purification was achieved using flash column chromatography with 9:1 dichloromethane/methanol as eluent to give **5g** (3.0 g, 90%) as a cream-colored solid: mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.85 (1H, s, OH), 7.2 (1H, s, NHCOCH$_3$), 3.93 (2H, s, CH$_2$N), 2.87 (4H, q, N(CH$_2$CH$_3$)$_2$), 1.2 (6H, t, N(CH$_2$CH$_3$)$_2$); IR (CH_2Cl_2) 3420, 3041, 2967, 2942, 2897, 1701, 1649, 1620, 1492, 1470, 1360, 1231, 1191, 1154, 1103, and 1027 cm^{-1}; MS *m/z* 290 (M$^+$, 16), 275 (34), 218 (40), 176 (15), 72 (19), 58 (100); HRMS 290.123 61, ($C_{13}H_{17}N_2O_2F_3$ requires 290.124 21).

7-Chloro-4-[2′-[(diethylamino)methyl]-2′,5′,6′-trifluoro-4′-hydroxyanilino]quinoline (6). The amide **5g** was hydrolyzed and the resulting aminophenol coupled with 4,7-dichloroquinoline as for **3g** except that the reaction mixture was refluxed for 30 h. The volume of solvent was then reduced to about 3 mL, and the mixture was poured onto iced water (10 mL). Using a pH meter the pH was slowly adjusted to 6.5 with ammonia solution. The water was then removed and the product absorbed onto silica and dried. Chromatography using 20% MeOH/90% dichloromethane as eluent gave the product (53%) as a yellow solid. The hydrochloride was obtained by dissolving a small portion (10%) in ethanolic HCl and adding ether to precipitate the required salt as a pale yellow solid: mp 182–185 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.68 (1H, d, J_{H-H} = 8.8 Hz, Ar-quin. 5H), 8.47 (1H, d, J_{H-H} = 6 Hz, Ar-quin. 2H), 8.04 (1H, d, J_{H-H} = 2.0 Hz, Ar-quin. 8H), 7.79 (1H, dd, J_{H-H} = 8.8 Hz and J = 2.0 Hz), 6.48 (1H, d, J = 6 Hz, Ar-quin. 3H), 4.22 (2H, s, CH$_2$N), 3.05 (4H,

q, $N(CH_2CH_3)_2$), 1.25 (6H, t, $N(CH_2CH_3)_2$); MS m/z 409 (M^+, 17), 338 (12), 97 (15), 83 (10), 73 (44), 58 (100), 44 (29); HRMS 409.116 870 ($C_{20}H_{19}ClF_3N_3O$ requires 409.116 87). Anal. ($C_{20}H_{19}ClF_3N_3O\cdot 2H_2O\cdot 2HCl$) C, H, N.

2-Fluoro-5-nitrobenzyl Bromide (6a). 2-Fluoronitrotoluene (9.0 g) was placed in a three-necked flask together with a reflux condenser, stirrer bar, and a dropping funnel with stem reaching the surface of the reactants. A gas absorption trap was fitted to the top of the condenser. The flask was heated to 150 °C, and bromine (3.25 mL) was added carefully during 1.5 h. The reaction vessel was exposed to UV light during addition of the bromine and for a further 1 h after addition was complete. After 2.5 h the contents of the flask was poured into a 500-mL round-bottomed flask containing 150 mL of boiling petroleum ether and 1 g of decolorizing charcoal. The solution was heated under reflux for 10 min and subsequently filtered while hot through a preheated sintered-glass funnel. The solution was left to crystallize overnight in a fridge to give the product (10.10 g, 73%) as pale yellow crystals: mp 77–79 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.36 (1H, dd, J_{H-H} = 2.75 Hz, J_{H-F} = 6.5 Hz, Ar-H), 8.25 (1H, ddd, J_{H-H} = 9.35 Hz, J_{H-F} = 4.4 Hz, and J_{H-H} = 2.75 Hz, Ar-H), 7.2–7.3 (1H, 1, J_{H-H}, J_{H-F} = 8.8 Hz, Ar-H), 4.55 (2H, s, CH_2Br); IR (Nujol mull) 2950, 1635, 1595, 1530, 1470, 1360, 1250, 1100, 930, 740, and 640 cm^{-1}; MS m/z (M^+, 42), 187 (7), 155 (100). Anal. ($C_7H_5NO_2FBr$) C, H, N.

7-Chloro-4-[4′-fluoro-5′-[(diethylamino)methyl]anilino]-quinoline (7). The benzyl bromide **6a** (4 g, 0.017 mol) and diethylamine (2.95 g, 0.034 mol) were heated under reflux in toluene for 4 h. The hydrobromide salt of the excess of diethylamine was filtered off and the solvent removed under reduced pressure. The residue was dissolved in about 10 mL of dry ether, and a solution of 20% ethanolic HCl was added dropwise to give 4-fluoro-3-[(diethylamino)methyl]nitrobenzene as the hydrochloride salt: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.2 (1H, d, J_{H-F} = 6.0 Hz, Ar-H), 8.4 (1H, dd, J_{H-H} = 9.35 Hz, J_{H-F} = 4.4 Hz, Ar-H), 7.4 (1H, dd, J_{H-F} and J_{H-H} = 8.8 Hz), 4.5 (2H, s, CH_2N), 3.15 (4H, broad s, $N(CH_2CH_3)_2$), 1.5 (6H, t, $N(CH_2CH_3)_2$). Without further purification, this hydrochloride (2.0 g) was reduced in ethanol (25 mL) with hydrogen (600 mL) and platinum oxide in a catalytic hydrogenator (STP). The reduction was terminated when uptake of hydrogen was complete, the catalyst was filtered off, and 4,7-dichloroquinoline (8.84 mmol) was added to the filtrate. The solution was heated under reflux for 4 h, and a solution of ammonia was added until precipitation of the product occurred. The product was dried and purified by means of flash column chromatography with 5% MeOH/95% dichloromethane as eluent: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.51 (1H, d, J_{H-H} = 5 Hz, Ar-quin. 2H), 8.07 (1H, d, J_{H-H} = 8.8 Hz, Ar-quin. 5H), 7.83 (d, 1H, J_{H-H} = 2.2 Hz, Ar-quin. 8H), 7.49 (1H, d, J_{H-H} = 8.8 Hz, J_{H-H} = 2.2 Hz, Ar-quin. 5H), 7.40 (d, 1H, J_{H-F} = 5 Hz, ArH) 7.05–7.20 (2H, m, J_{H-F} = 10.5 Hz, ArH), 6.75 (1H, d, J = 5 Hz, Ar-quin. 3H), 3.7 (2H, s, CH_2N), 2.6 (4H, q, $N(CH_2CH_3)_2$), 1.1 (6H, t, $N(CH_2CH_3)_2$); IR (Nujol mull) 2970, 1595, 1590, 1560, 1510, 1470, 1390, 1240, 1210, 1100, 850, and 809 cm^{-1}; MS m/z 357 (M^+, 20), 344 (35), 342 (100), 286 (63), 270 (37), 250 (81), 86 (40). Anal. ($C_{20}H_{21}N_3FCl$) C, H, N.

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References

(1) World Health Org. Severe and Complicated Malaria. W.H.O. Malaria Action Programme. *Trans. R. Trop. Med. Hyg. 80*, 1–50.
(2) Watkins, W. M.; Sixsmith, D. G.; Spencer, H. G.; Boriga, D. A.; Karjuki, D. M.; Kipingor, T.; Koech, D. K. Effectiveness of Amodiaquine as a Treatment for Chloroquine Resistant Plasmodium Falciparum. *Lancet* **1984**, No. 1, 357–359.
(3) Neftel, K. A.; Woodtly, W.; Schmid, M. Amodiaquine Induced Agranulocytosis and Liver Damage. *Br. Med. J.* **1986**, *292*, 721–723.
(4) Lind, D. E.; Levi, J. A.; Vincent, P. C. Amodiaquine Induced Agranulocytosis; Toxic Effects of Amodiaquine in Bone Marrow Culture In Vitro. *Br. Med. J.* **1973**, *1*, 458–460.
(5) Harrison, A. C.; Kitteringham, N. R.; Clarke, J. B.; Park, B. K. The Mechanism of Bioactivation and Antigen Formation of Amodiaquine in the Rat. *Biochem. Pharmacol.* **1992**, *43*, 1421–1430.
(6) Maggs, J. L.; Kitteringham, N. R.; Park, B. K. Drug Protein Conjugates-XIV. Mechanism of Formation of Protein Arylating Intermediates From Amodiaquine a Myelotoxin and Hepatotoxin in Man. *Biochem. Pharmacol.* **1988**, *37*, 303–311.
(7) Clarke, J. B.; Maggs, J. L.; Kitteringham, N. R.; Park, B. K. Detection of IgG Antibodies in Patients with Adverse Drug Reactions to Amodiaquine. *Int. Arch. Allergy Immunol.* **1990**, 1335–1342.
(8) Barnard, S.; Kelly, D. F.; Storr, R. C.; Park, B. K. The Effect of Fluorine Substitution On the Hepatotoxicity and Metabolism of Paracetamol in the Mouse. *Biochem. Pharmacol.* **1993**, *46*, 841–849.
(9) Burckhalter, J. H.; Tendwick, J. H.; Jones, F. H.; Jones, P. A.; Holcombe, W. F.; Rawlins, A. L. Aminoalkylphenols as Antimalarials II (Heterocyclic-amino)-α-amino-o-cresols; The Synthesis of Camoquine. *J. Am. Chem. Soc.* **1948**, *70*, 1363–1373.
(10) Bolton, R.; Sandall, J. P. B. Nucleophilic Displacement in Polyhalageno-aromatic Compounds. Part 7; Kinetics of Methoxydefluorination of Polyfluoronitrobenzenes. *J. Chem. Soc., Perkin Trans. 2* **1978**, 141–144.
(11) Jensen, J. B.; Trager, W. Plasmodium Falciparum in Culture; Use of Outdated Erythrocytes and Description of the Candle Jar Method. *J. Parasitol.* **1977**, *63*, 883–886.
(12) Mc Omie, J. F. W.; Watts, M. L.; West, D. E. Demethylation of Aryl Methyl Ethers By Boron Tribromide. *Tetrahedron* **1968**, *24*, 2289–2292.
(13) Desjardins, R. E.; Canfield, J.; Haynes, D.; Chulay, D. J. Quantitative Assessment of Antimalarial Activity In Vitro by a Semi-automated Microdilution Technique. *Antimicrob. Chemother.* **1979**, *16*, 710–718.
(14) Park, B. K.; Kitteringham, N. R. Effects of Fluorine Substitution on Drug Metabolism: Pharmacological and Toxicological Implications. *Drug Metab. Rev.* **1993**, in press.
(15) Vermeulen, N. P. E.; Bessems, J. G. M.; van de Stratt, R. Molecular Aspects of Paracetamol-Induced Hepatotoxicity and its Mechanism Based Prevention. *Drug Metab. Rev.* **1992**, *24*, 367–407.
(16) Dahlin, D. C.; Nelson, S. D. Synthesis, Decomposition Kinetics and Preliminary Toxicological Studies of Pure NAPQUI, a Proposed Toxic Metabolite of Acetaminophen. *J. Med. Chem.* **1982**, *25*, 885.
(17) Streeter, A. J.; Dahlin, D. C.; Nelson, S. D.; Baillie, T. A. The Covalent Binding of Acetaminophen to Protein. Evidence for Cysteine Residues as Major Sites of Arylation In Vitro. *Chem. Biol. Interact.* **1984**, *48*, 349.
(18) Novak, M.; Pelecanou, M.; Pollack, L. Hydrolysis of the Model Carcinogen N-(pivaloyloxy)-4-methoxyacetanilide: Involvement of N-acetyl-p-benzoquinoneimine. *J. Am. Chem. Soc.* **1986**, *108*, 112.
(19) Barnard, S.; Storr, R. C.; O'Neill, P. M.; Park, B. K. The Effect of Fluorine Substitution on the Physicochemical Properties and the Analgesic Activity of Paracetamol. *J. Pharm. Pharmacol.* **1993**, *45*, 736–744.
(20) Ginsburg, H.; Krugliak, M. Quinoline Containing Antimalarials: Mode of Action, Drug-Resistance and Its Reversal. An Update with Unresolved Puzzles. *Biochem. Pharmacol.* **1992**, *43*, 63–70.
(21) Irvin, J. L.; Irvin, E. M. Spectrophotometric and Potentiometric Evaluation of Apparent Acid Dissociation Exponents of Various 4-Aminoquinolines. *J. Am. Chem. Soc.* **1947**, *69*, 1091–1099.
(22) Harrison, A. Ph.D. Thesis, Liverpool University.
(23) Rice, E. R.; Moghaddam, B.; Creveling, C. R.; Kirk, K. L. Effect of Fluorine Substitution on the Anodic Oxidation of Catecholamines and Amino Acids. *Anal. Chem.* **1987**, *59*, 1534–1538.
(24) Kawase, M.; Sinhababu, A. K.; Bochardt, R. T. Mechanism of Autooxidation of 5,7-Dihydroxytryptamine: Effect of fluorine substitution at Positions 4 and/or 6. *Chem. Pharm. Bull.* **1990**, *38*, 2939–2946.
(25) Miner, D. J.; Rice, E. R.; Riggin, R. M.; Kissinger, P. T. Voltammetry of Acetaminophen and Its Metabolites. *Anal. Chem.* **1981**, *53*, 2258–2263.
(26) Harvison, P. J.; Forte, A. J.; Nelson, S. D. Comparative Toxicities and Analgesic Activities of Three Monomethylated Analogues of Acetaminophen. *J. Med. Chem.* **1986**, *29*, 1737–1743.