Isoquine and Related Amodiaquine Analogues: A New Generation of Improved 4-Aminoquinoline Antimalarials

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Amodiaquine (AQ) (**2**) is a 4-aminoquinoline antimalarial that can cause adverse side effects including agranulocytosis and liver damage. The observed drug toxicity is believed to involve the formation of an electrophilic metabolite, amodiaquine quinoneimine (AQQI), which can bind to cellular macromolecules and initiate hypersensitivity reactions. We proposed that interchange of the 3′ hydroxyl and the 4′ Mannich side-chain function of amodiaquine would provide a new series of analogues that cannot form toxic quinoneimine metabolites via cytochrome P450-mediated metabolism. By a simple two-step procedure, 10 isomeric amodiaquine analogues were prepared and subsequently examined against the chloroquine resistant K1 and sensitive HB3 strains of *Plasmodium falciparum* in vitro. Several analogues displayed potent antimalarial activity against both strains. On the basis of the results of in vitro testing, isoquine (ISQ1 (**3a**)) (IC_{50} = 6.01 nM ± 8.0 versus K1 strain), the direct isomer of amodiaquine, was selected for in vivo antimalarial assessment. The potent in vitro antimalarial activity of isoquine was translated into excellent oral in vivo ED_{50} activity of 1.6 and 3.7 mg/kg against the *P. yoelii* NS strain compared to 7.9 and 7.4 mg/kg for amodiaquine. Subsequent metabolism studies in the rat model demonstrated that isoquine does not undergo in vivo bioactivation, as evidenced by the complete lack of glutathione metabolites in bile. In sharp contrast to amodiaquine, isoquine (and Phase I metabolites) undergoes clearance by Phase II glucuronidation. On the basis of these promising initial studies, isoquine (ISQ1 (**3a**)) represents a new second generation lead worthy of further investigation as a cost-effective and potentially safer alternative to amodiaquine.

Introduction

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

Paracetamol (4-hydroxyacetanilide) contains a *p*-hydroxyanilino moiety, which is believed to undergo P-450-catalyzed oxidation to a chemically reactive quinoneimine (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).[5] This observation indicates that the parent drug undergoes extensive bioactivation in vivo to form amodiaquine quinoneimine (AQQI) or semiquinoneimine (AQSQI) with subsequent conjugate addition of glutathione [6]

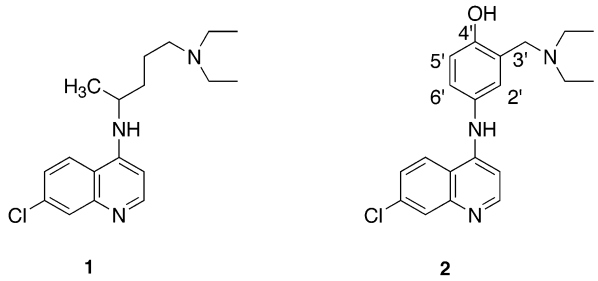

Figure 1. Structures of chloroquine and amodiaquine.

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] In the case of paracetamol it has been shown that introduction of fluorine into the aromatic nucleus increases the oxidation potential of the molecule and thereby blocks the in vivo oxidation of the molecule to a cytotoxic quinoneimine.[8]

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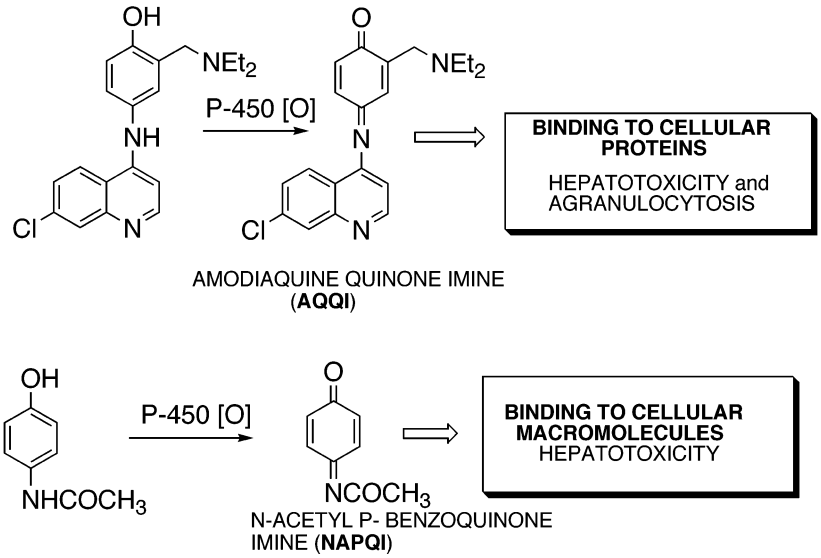
Scheme 1. Bioactivation of Amodiaquine and Paracetamol to Toxic Quinoneimines by P450



Further studies by our group demonstrated that, in a manner similar to paracetamol, the incorporation of fluorine atoms into the 4-hydroxyanilino side-chain of amodiaquine produces compounds with greater oxidative and metabolic stability.[9] From this earlier work, we demonstrated that the 4′-hydroxyl group could be replaced with a 4′-fluorine atom to produce an amodiaquine analogue, fluoroamodiaquine, with antimalarial activity in the low nanomolar range. Despite these promising observations, activity at the level of the parent drug amodiaquine was never achieved with the fluorinated derivatives and, on the basis of cost considerations, it was decided that an alternative approach to producing more metabolically robust analogues, retaining the key pharmacophoric groups, should be sought.

From our previous SAR work,[10,11] we have noted that in the amodiaquine and tebuquine series of 4-aminoquinoline analogues, the presence of the 4′ hydroxyl group within the aromatic ring imparts greater inherent antimalarial activity against chloroquine resistant parasites than the corresponding deoxo analogues. Interchange of the hydroxyl group and the Mannich side-chain provides a means of preventing oxidation to toxic metabolites while retaining possible important bonding interactions with the aromatic hydroxyl function. In this paper, we will describe the synthesis, antimalarial activity, and metabolism of the prototype isoquine (**3a**, ISQ 1), an amodiaquine regioisomer that cannot form toxic metabolites by simple oxidation and which is potent against chloroquine resistant parasites in vitro (Scheme 2). The antimalarial activity of isoquine (**3a**, ISQ 1) will be compared with nine other analogues in this series (Chart 1, **4a**−**12a**). Apart from an excellent antiparasitic profile, isoquine and its side-chain analogues are extremely cheap antimalarials to synthesize and on the basis of initial data reported in this paper, may represent new leads for development of a safe, cheap, affordable, and effective antimalarial for both prophylaxis and treatment of malaria.

Chemistry

The preparation of isoquine and its analogues involves a two-step procedure from commercially available starting materials according to a method originally utilized by Burkhalter and co-workers (Scheme 3).[12] Thus, step 1 involves a Mannich reaction of the commercially available 3-hydroxyacetanilide to provide the Mannich product in yields ranging from 50 to 90% (Table 1). Stage 2 of the sequence involves hydrolysis of the amide function to provide the corresponding Mannich-substituted 3-aminophenol that is subsequently coupled with 4,7-dichloroquinoline to provide target molecules shown in Chart 1. The main difference between the synthesis of this isomeric series and the amodiaquine analogues we have previously prepared is that after amide hydrolysis, the reaction should not be buffered to pH = 6. The intermediate 3-aminophenols have been shown to be quite unstable at neutral pH but are sufficiently nucleophilic to couple with 4,7-dichloroquinoline at lower pHs, i.e., after hydrolysis of the amide, the sequential reaction can be carried out in ethanolic solvent by addition of 4,7-dichloroquino-

Scheme 2. Redesign of Amodiaquine

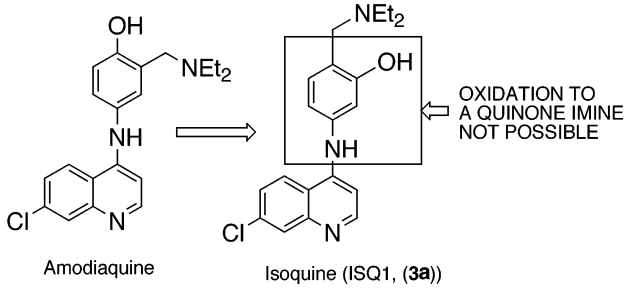
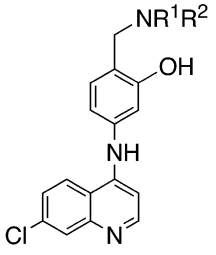


Chart 1. Isoquine ISQ1 (**3a**) and Analogues **4a**−**12a**



ISQ 1 (**3a**) R^1 = Et, R^2 = Et
(**4a**) R^1 = H, R^2 = *t*-Bu
(**5a**) R^1 , R^2 = Me
(**6a**) R^1 , R^2 = *n*-propyl
(**7a**) R^1 , R^2 = *n*-butyl
(**8a**) R^1 , R^2 = $-(CH_2)_4-$
(**9a**) R^1 , R^2 = $-(CH_2)_2O(CH_2)_2-$
(**10a**) R^1 , R^2 = $-(CH_2)_5-$
(**11a**) R^1 = H, R^2 = *i*-Pr
(**12a**) R^1 = H, R^2 = Et

Scheme 3. Synthesis of Analogues **3a**−**12a**

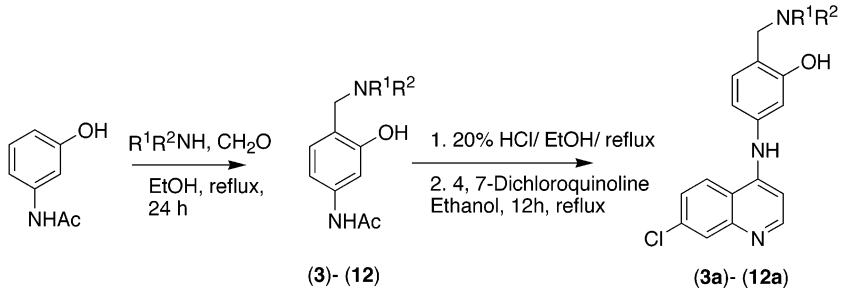


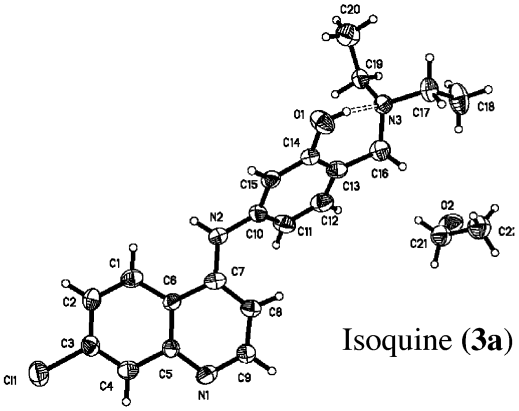
Table 1. Yields for the Synthesis of Isoquine (**3a**) and Analogues **4a**−**12a**

intermediate amide	yield, %	product	yield, %
(**3**) R^1 = Et, R^2 = Et	69	ISQ 1 (**3a**) R^1 = Et, R^2 = Et	71
(**4**) R^1 = H, R^2 = *t*-Bu	70	(**4a**) R^1 = H, R^2 = *t*-Bu	69
(**5**) R^1, R^2 = Me	74	(**5a**) R^1, R^2 = Me	69
(**6**) R^1, R^2 = *n*-propyl	63	(**6a**) R^1, R^2 = *n*-propyl	61
(**7**) R^1, R^2 = *n*-butyl	52	(**7a**) R^1, R^2 = *n*-butyl	58
(**8**) R^1, R^2 = $(CH_2)_4$	89	(**8a**) R^1, R^2 = $(CH_2)_4$	80
(**9**) R^1, R^2 = $(CH_2)_2O(CH_2)_2$	72	(**9a**) R^1, R^2 = $(CH_2)_2O(CH_2)_2$	60
(**10**) R^1, R^2 = $(CH_2)_5$	80	(**10a**) R^1, R^2 = $(CH_2)_5$	71
(**11**) R^1 = H, R^2 = *i*-Pr	51	(**11a**) R^1 = H, R^2 = *i*-Pr	67
(**12**) R^1 = H, R^2 = Et	55	(**12a**) R^1 = H, R^2 = Et	68

Table 2. In Vitro Antimalarial Activities of Chloroquine, Amodiaquine, and Analogues **3a**−**12a**

drug[a]	IC_{50} (nM) HB3	SD ± mean	IC_{50} (nM) K1	SD ± mean
chloroquine (**1**)	14.98 (6)	3.98	183.82 (6)	11.13
amodiaquine (**2**)	9.60 (9)	3.73	15.08 (9)	9.36
isoquine (ISQ-1) (**3a**)	12.65 (9)	4.75	17.63 (9)	7.00
isoquine diphosphate (**3b**)	9.02 (3)	4.06	6.01 (3)	8.00
(4a)	30.03 (3)	17.67	32.75 (4)	13.16
(5a)	14.76 (9)	12.30	18.65 (9)	9.08
(6a)	19.78 (9)	15.30	30.63 (4)	16.53
(7a)	51.88 (4)	19.77	37.21 (4)	12.86
(8a)	28.37 (4)	9.03	21.75 (3)	2.65
(9a)	97.20 (4)	15.31	112.37 (4)	36.99
(10a)	9.07 (4)	0.30	20.28 (3)	4.99
(11a)	20.22 (4)	4.34	26.22 (4)	8.03
(12a)	16.24 (4)	11.24	32.42 (4)	14.70

[a] Amodiaquine was tested as the hydrochloride salt, ISQ-1 and **3a**−**12a** were all tested as free bases. Chloroquine was tested as the diphosphate.



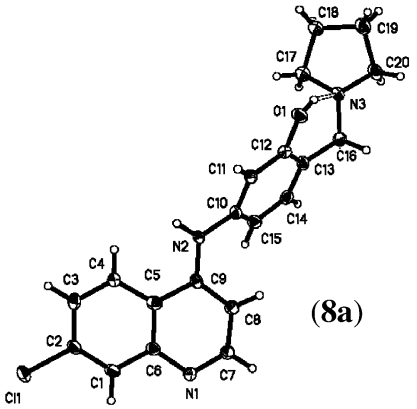


Figure 2. X-ray cystal structures of ISQ-1 (**3a**) and **8a**.

line.[13] For purification, analogues were chromatographed as their hydrochloride salts using methanol/dichloromethane (10−20% MeOH/ dichloromethane) as eluent. The free bases could be conveniently obtained by dissolving pure columned solid hydrochloride product in distilled water and adding saturated sodium bicarbonate solution. The precipitated free base could then be dried and recrystallized from either 2-propanol or methanol. Compounds were analyzed by HPLC and full spectroscopic details are included in the Experimental Section. For ISQ 1 and its pyrrolidinyl analogue (**8a**), X-ray crystallography studies demonstrate that there is an internal hydrogen bond between the hydroxyl function (OH as donor) and the side-chain nitrogen (Figure 2). This may lead to a subtle effect on the pK_a of the side-chain Mannich nitrogen atom and a reduction in basicity.

Antimalarial Activity. Analogues were initially tested in vitro against the chloroquine sensitive HB3 strain and against the highly chloroquine-resistant K1 strain of *P. falciparum* (Table 2). Against the HB3 chloroquine sensitive isolate, isoquine (**3a**), its diphosphate salt (**3b**), and compounds **5a**, **6a**, **10a**, and **12a** all express activity below 20 nM. In line with previous SAR studies on 4-aminoquinoline analogues, the morpholinyl analogue (**9a**) is a poor antimalarial with activity close to 100 nM.[32] Clearly, the two most potent compounds tested against the HB3 strain were isoquine free base and the diphosphate salt (**3b**) and the piperidinyl analogue (**10a**). The most potent compound against the chloroquine resistant strain was again the diphosphate salt of isoquine although the free base is also as potent as amodiaquine in this strain. Isoquine and its salt are both about 20 times more potent than chloroquine diphosphate. Compounds **5a**, **8a**, **10a**, and **11a** also express excellent activity against this resistant strain. It is clear that in addition to isoquine, compounds **5a** and **10a** are additional leads worthy of further investigation. Isoquine diphosphate was subsequently tested in vivo against the murine *Plasmodium yoelii* NS strain. Data recorded in Table 3 suggests that isoquine has superior antimalarial activity to amodiaquine in vivo. Indeed, by the oral route, **3a** is almost three times more potent than AQ.

Table 3. In Vivo Antimalarial Activity of Isoquine and Amodiaquine versus *P. yoelii* NS Strain in the Peters 4-Day Test

compound	isoquine	amodiaquine
ED_{50} (mg/kg)	2.65	7.65
SD	± 1.48	± 0.35

[a] Groups of male CD-1 mice (n = 5 per dose group) were inoculated by ip injection with 10^6 parasitized erythrocytes in phosphate buffered saline. Drug was administered by oral gavage at time = 0 (equivalent to 2 h post parasite inoculation) and on days 1, 2, and 3. Blood films were prepared from tail snips on day 4 and stained with Giemsa, and parasite density was counted microscopically. Drug doses used were 25, 10, 3, 1, 0.3, and 0.1 mg/kg.

Metabolism Studies. To determine whether the side chain modifications introduced would alter the comparative metabolic fate of metabolism, studies were carried out in the rat. Rearrangement of the hydroxyl and diethylamino side chains to a 1,3-aminophenol suggest that this compound would be unable to form a quinonimine, as it is not chemically feasible to lose two hydrogens via the same oxidative mechanism as AQ.[14]

In addition to being capable of forming toxic metabolites, internal hydrogen bonding between the *p*-hydroxyanilino moiety and the nitrogen of the diethylamino side chain could be responsible for preventing AQ from undergoing the *O*-sulfation and/or *O*-glucuronidation experienced by other structurally related compounds.[15] This results in the failure of AQ to undergo phase II detoxication. Removal of the diethylamino side chain has been shown to allow AQ to undergo excessive *O*-sulfation in the rat. In the context of the present study, we were interested to see if the 3′-OH function in **3** would be capable of undergoing metabolic phase II conjugation reactions.

Using radiolabeled[3][H] isoquine, initial in vivo dispositional studies indicated that there is no alteration in the rate of excretion of ISQ1 into bile and urine after 5 h compared to AQ. The main site for accumulation of ISQ was found to be the liver, a primary target organ for drug metabolism. 6.85% of the AQ dose remained in the liver, 24 h after administration to rats compared to 20.82% of the dose at 5 h. This was coupled with a 3% increase in radioactivity excreted into urine between 5 h and 24 h. Only 5.48% of the ISQ1 dose remained in the liver after 24 h compared to 32.93% after 5 h, suggesting almost complete clearance of the compound at 24 h.

For isoquine (**3a**), during the 5h experiment, a 3-fold increase in plasma levels compared to AQ was witnessed. This increase in plasma levels for isoquine (0.130%, ±0.018) was significantly different to amodiaquine levels (0.05%, ±0.012 $P > 0.05$). This observation may be important since the blood is the primary site of action for 4-aminoquinoline antimalarials. Using LCMS analysis, the main circulating metabolite for isoquine in the plasma was the parent compound, in contrast to amodiaquine where the only metabolite detected was the desethyl metabolite.[16]

After 5 h, 87.17% ISQ1 dose was accounted for in the tissues, bile plasma, and urine. Similarly, 78.56% of the AQ dose could be accounted for after 5 h. This suggests that there are other sites for the accumulation of the compounds which were not analyzed during this study. Glutathione conjugates were only detected in rats administered AQ. Evidence to support this metabolite was found with the presence of a mercapturate metabolite in urine. In direct contrast there was no evidence to suggest the formation of an ISQ−glutathione conjugate in the bile of rats dosed with ISQ 1. This suggests that bioactivation has been blocked; furthermore, and in sharp contrast to amodiaquine, there is evidence to suggest that relocation of the phenolic OH from the 4′ position in AQ to the 3′ position provides a route of metabolic escape. LCMS analysis revealed the presence of glucuronide conjugates in the bile and urine of isoquine-dosed rats. Direct glucuronidation of parent compound (ISQ 1) was also seen, suggesting that the hydroxyl group is no longer restricting Phase II conjugation.

Scheme 4 summarizes the main metabolites from isoquine that were identified in this initial study. Note that amodiaquine or its metabolites do not form glucuronides (Scheme 5) in sharp contrast to isoquine.

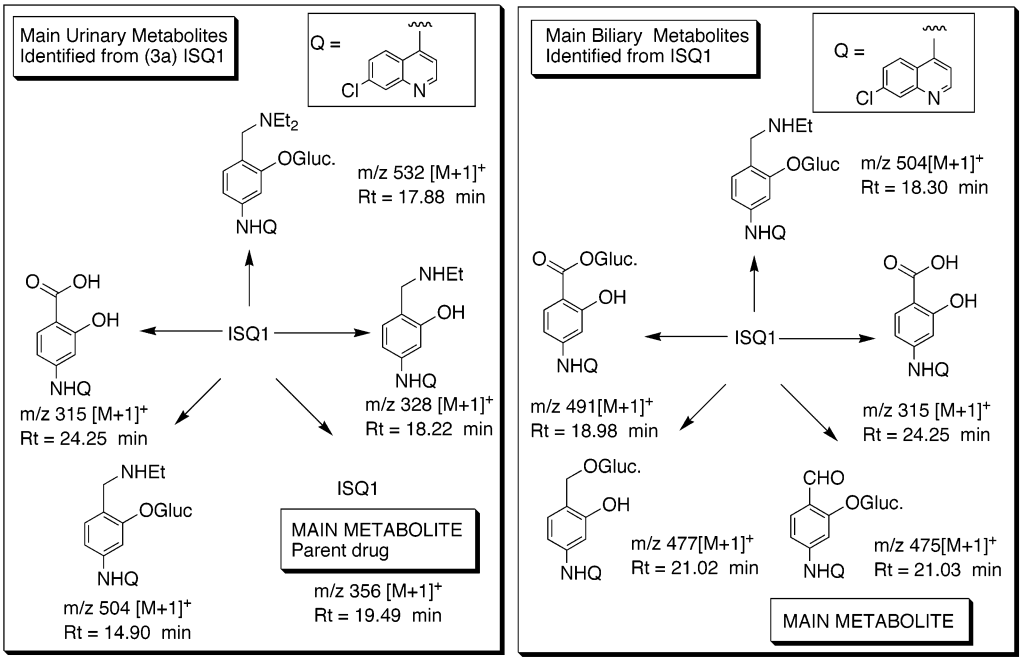
Results from this initial metabolism study have demonstrated that interchange of the diethylamino side chain with the hydroxyl group of AQ as in ISQ1 (**3a**) can prevent the bioactivation of this compound in vivo and can influence the critical balance between bioactivation and detoxication. We propose that this compound may not produce the adverse reactions seen with AQ on the basis that no evidence of chemically reactive metabolites could be obtained. Furthermore, interchange also provides altered routes of metabolism whereby the hydroxyl group can facilitate "metabolic escape". Full details of metabolite identification, LCMS traces, and graphs of tissue distribution have been included as Supporting Information.

Discussion

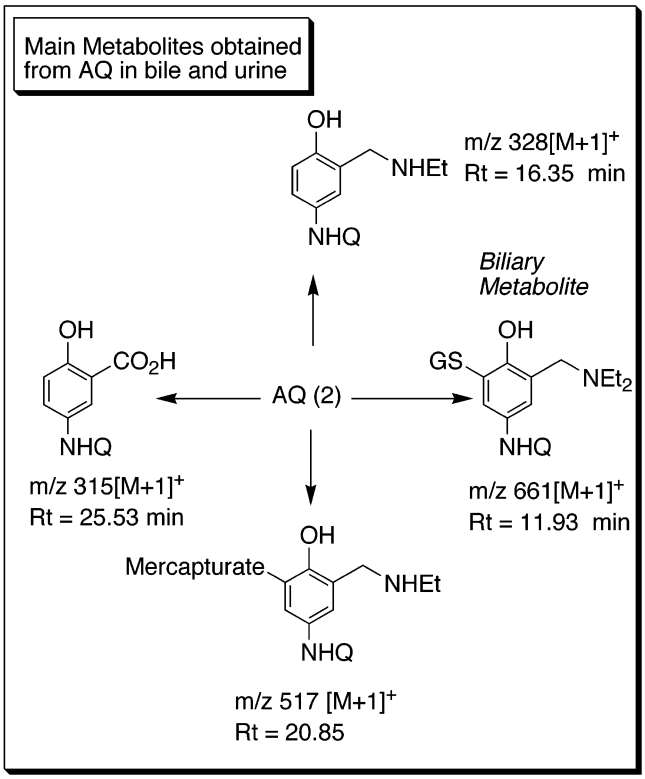
The hemoglobin degradation pathway in *P. falciparum* is a specialized parasite process with a proven history as an exploitable therapeutic target as exemplified by the 4-aminoquinolines and the endoperoxide derivatives.[17] Furthermore, unlike parasite encoded enzymes[18] and transporters,[19] that are currently under investigation, the parasite has difficulty in developing resistance to these two classes of drug (compare the speed of resistance development to chloroquine with that for the antifolates or atovaquone).[20] Resistance to chloroquine (CQ) was first reported in the late 50's, and by the 70's there were examples of culture adapted strains with IC_{50}s of 200−300 nM. Despite the continued widespread exposure of parasite populations to CQ in many parts of the world, resistance beyond this level is rarely observed. Attempts to increase this level of resistance in a laboratory setting have failed, suggesting the parasite may have difficulty in developing resistance strategies beyond this point. Based on this and our understanding of 4-aminoquinoline action, the development of a new 4-aminoquinoline derivative effective against these highly CQ resistant isolates should pose the parasite major difficulties in terms of resistance acquisition and would therefore have an expected useful therapeutic lifespan in excess of many of the other drugs currently under development.

For the past 15 years we have been involved in research aimed at understanding the mechanism(s) of action and toxicity of and the basis of parasite resistance

Scheme 4. Metabolic Scheme for Isoquine ISQ-1 (**3a**)



Scheme 5. Metabolic Scheme for Amodiaquine (**2**)



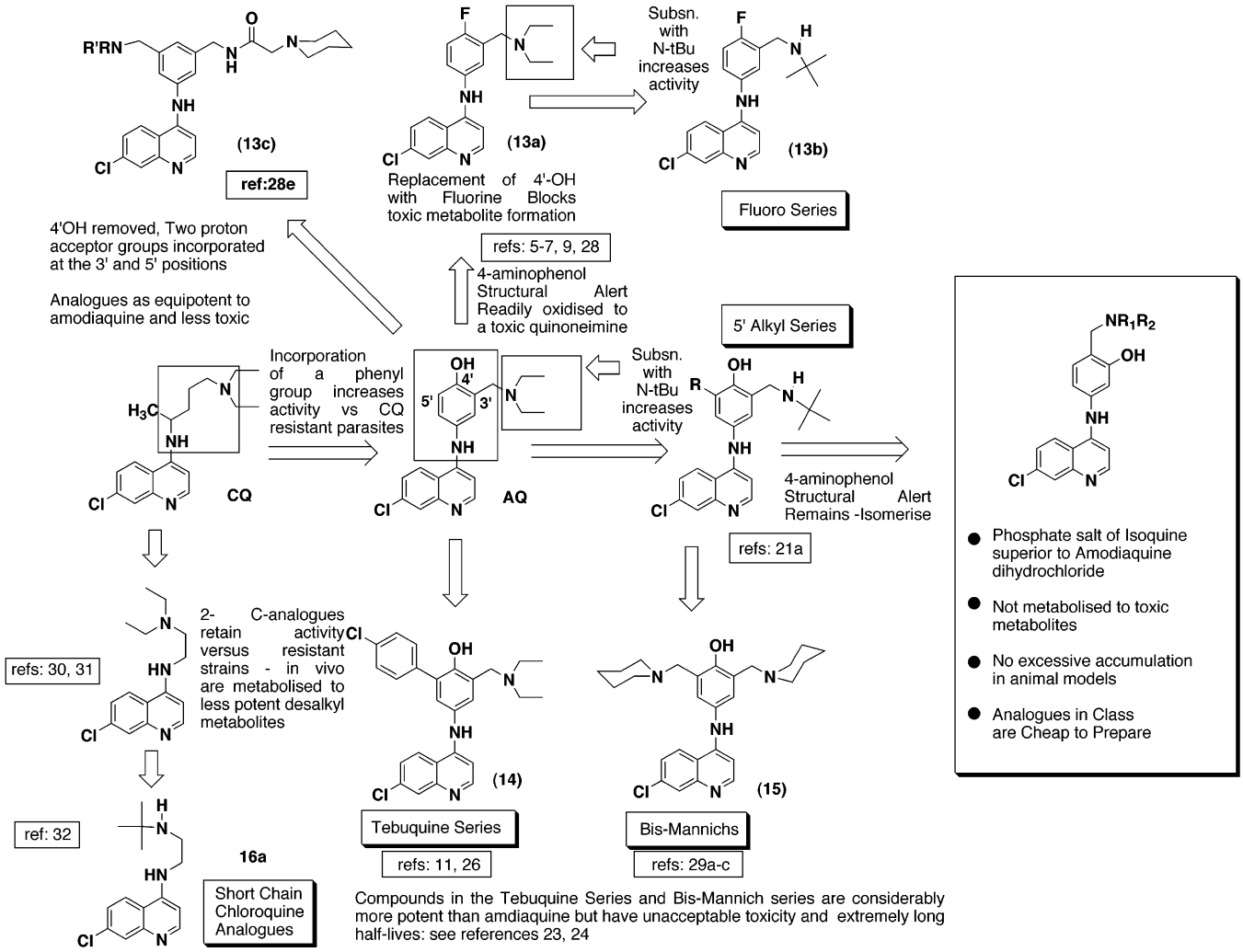
to the 4-aminoquinolines.[21,28a−d] Integral to our studies of the basic biology has been the synthesis of novel 4-aminoquinoline analogues that have been used as chemical probes to investigate structure−activity and structure−toxicity relationships (see Chart 2). This subsequently resulted in the establishment of a rational drug design program that has now generated more than 100 chemical entities.

Chart 2 summarizes the different classes of 4-aminoquinoline that we have investigated in our drug development program. On the basis of the important observation that amodiaquine retains antimalarial activity against chloroquine resistant parasites,[27b] our initial studies involved the design and synthesis of fluoroamodiaquine (**13a**) as a safer alternative to AQ. On the basis of metabolism studies, **13a** was chemically modified to produce a new lead compound (**13b**) which expressed activity in vitro at about half the level of amodiaquine versus CQ-resistant strains, but with equivalent oral in vivo potency versus *Plasmodium berghei*.[9] While analogues in this series initially looked promising, concern about cost led us to consider three other series of synthetically more accessible analogues; the tebuquine series (**14**),[11] the bis-Mannich series (**15**),[29] and the 5′-alkyl series class of 4-aminoquinoline.[21] Compounds in the tebuquine and bis-Mannich series are considerably more potent than AQ or CQ in vitro and in vivo but have subsequently been shown to have unacceptable toxicity profiles and extremely long half-lives.[23] On the basis of initial toxicological evaluation and the fact that all three sets of amodiaquine derivative retain the 4-aminophenol "structural alert" further investigations were not pursued. Recent studies by the Sergheraert group have also examined analogues where the 4′-aminophenol alert has been modified by removal of the 4′ hydroxyl function. Compounds of the general class (**13c**) where shown to have excellent in vitro and in vivo potencies.[28e]

Our most recent studies on 4-aminoquinoline SAR have revealed, like others,[30,31] that short chain two-carbon side-chain chloroquine analogues retain activity against chloroquine resistant plasmodia. Our efforts[32] were directed toward compounds less likely to undergo metabolic *N*-terminal dealkylation, a process that produces *N*-desalkyl metabolites that are considerably less potent against chloroquine resistant strains. Some of these 2-C analogues, e.g. **16a**, display good antiparasitic profiles.[30−32]

From our SAR studies, it was clear that the presence of a 4-arylamino moiety provides analogues with supe-

Chart 2. Approaches Leading to the Discovery of the Isoquine Class of 4-Aminoquinoline Antimalarial



rior activity against CQ resistant strains, and it was also apparent that the presence of an aromatic hydroxyl function appears to be important for additional levels of antiparasitic activity. [11a] It therefore seemed reasonable that interchange of the 3′-Mannich side chain with the 4′-OH function would provide a new template capable of delivering a series of compounds chemically incapable of forming potentially toxic quinoneimine metabolites. Furthermore, it was proposed that analogues in this series would not only be potent against resistant strains, but would also be as cheap to prepare as AQ on an industrial scale.

A potential drawback with any new 4-aminoquinoline antimalarials is the possibility of cross-resistance with chloroquine. Clearly from Table 2, the data presented here demonstrates that for isoquine and several analogues, there is minimal cross-resistance with chloroquine in the highly CQ resistant K1 strain (not shown to be statistically significant by the Mann−Whitney U test). These observations are supported by the recent demonstration that mutations in the pfcrt gene have a minimal effect on the activity and accumulation of amodiaquine compared with chloroquine. Although the results of in vitro testing are encouraging, it is clear that further studies will be required to include a wider panel of chloroquine resistant isolates to fully determine the potential utility of this new class of 4-aminoquinoline.

Table 4. Comparison of Calculated Log *P* (ClogP) versus Antimalarial Activity

drug[a]	IC_{50} (nM) HB3	ClogP[a]
chloroquine (**1**)	14.98	5.04
amodiaquine (**2**)	9.60	4.51
isoquine (ISQ-1) (**3a**)	12.65	4.51
isoquine diphosphate (**3b**)	9.02	NA
(4a)	30.03	4.22
(5a)	14.76	3.45
(6a)	19.78	5.57
(7a)	51.88	6.62
(8a)	28.37	4.08
(9a)	97.20	3.36
(10a)	9.07	4.63
(11a)	20.22	3.82
(12)	16.24	3.51

[a] ClogP values calculated using the Biobyte Mac log P 4 Program.

As shown in Table 3, isoquine is orally active in the mouse model of malaria with a superior ED50 to amodiaquine. From Table 4 there is no correlation between the calculated log *P*'s (ClogPs)[11b] of these derivatives and in vitro antimalarial activity against the chloroquine sensitive HB3 strain. The same applies to the chloroquine resistant strain, indicating that other factors apart from lipophilicity have a role in determining the expression of in vitro antimalarial activity.

Chloroquine and to a lesser extent AQ are the only 4-aminoquinoline antimalarials with which we have any

real clinical experience. CQ is considered a safe drug when used as recommended, although cardiovascular and CNS toxicities are seen in overdose and prolonged treatment is associated with retinopathy. However, AQ has been shown to carry an unacceptable risk of agranulocytosis and hepatitis when used for prophylaxis, this has resulted in deaths.[3,4] The toxicological concerns are that as AQ is used more extensively, possibly as a component of an artemisinin combination, the incidence of these adverse drug reactions will increase as pediatric African populations are exposed to the drug on multiple occasions per year.

There are two chemical features of AQ that are considered to be relevant to its toxicity. First, because it is a lipophilic weak base, it is lysosomotropic and is therefore readily taken up by specific white cell populations. Second, as described earlier, the side-chain contains a 4-aminophenol group. This is a substructure now widely recognized as a structural alert for toxicity by medicinal chemists, because of metabolic oxidation to quinonimines.[6,8] For example, as described earlier, paracetamol undergoes oxidation in the liver by cytochrome P450 enzymes to *N*-acetyl *p*-benzoquinonimine, the cause of massive irreversible hepatic necrosis when the drug is taken in overdose. We have shown that AQ readily undergoes oxidation to a quinoneimine.[5] Oxidation is more likely to occur with AQ than paracetamol because of its lower oxidation potential.[9] Extensive metabolic studies, in a variety of model systems, have shown that the oxidation of AQ can be catalyzed by myeoloperoxidase, hypochlorous acid (both released by activated white cells), and cytochrome P450 enzymes. The quinoneimine has been trapped and characterized as a glutathione conjugate which provides a biomarker for the in vivo bioactivation of the drug. AQ undergoes extensive bioactivation in the mouse and the rat.[5−7] One reason for the extensive bioactivation of AQ is that the phenolic group is refractory to *O*-glucuronidation, the normal pathway of biochemical detoxication that one would anticipate for such a molecule. We attribute this to a combination of the pK_a of the group and strong internal hydrogen bonding with the *O*-diethylamino group. Removal of the Mannich side-chain permits phase II detoxication reactions. AQ also undergoes bioactivation to a quinoneimine in human neutrophils, and a similar metabolic process was observed for pyronaridine and amopyroquine.[24]

What are the toxicological consequences of bioactivation? It should first be stated that the formation of glutathione conjugates of AQ does represent detoxication. Nevertheless, at doses of AQ that are not acutely toxic, covalent modification of hepatic proteins has been demonstrated in the rat after only a single dose of the drug. Furthermore we have demonstrated that AQ is weakly immunogenic and AQ quinoneimine is extremely immunogenic in an animal model.[5] More importantly, we have demonstrated the presence of specific antidrug (metabolite) antibodies in a cohort of patients with serious adverse drug reactions to AQ.[7] Thus the mechanism is consistent with that of other drugs such as penicillin and aminopyrine which cause type II hypersensitivity reactions in man.[15] There are no animal models currently available to test for such reactions in preclinical screens. Nevertheless, there are now clearly defined structural alerts in minor drug metabolites for such immune-mediated toxicities.

The strategy we have adopted is one in which we have designed the chemical alert out of the drug structure while retaining antimalarial activity. A similar strategy has been successfully employed in the redesign of general anesthetics and β-blockers currently in clinical use. This modification has resulted in a marked shift in the pattern of metabolism in the rat, which carries pharmacological benefits; thus in the rat model, it was observed that for isoquine there is a complete absence of glutathione conjugates in the bile. This clearly illustrates the lack of bioactivation of the 4-amino arylalkyl side-chain. Cleavage of the side-chain by P450 enzymes is the primary Phase 1 biotransformation. This biotransformation reduces steric hindrance around the phenolic hydroxyl group and enables efficient Phase II glucuronidation. The *O*-glucuronide is rapidly excreted in the bile and does not accumulate in tissues. This metabolic pathway sharply contrasts with that of AQ where the major dealkylated metabolite, desethyl AQ, accumulates in the liver.

One of the primary biotransformations of ISQ1 involves cleavage of the dialkylamino side-chain. We (and others) have shown that this side chain is essential for pharmacological activity. Therefore, we can propose that the in vivo pharmacological response of isoquine will be related to plasma/tissue concentrations of parent drug or the *N*-desethyl metabolite (**12a**), as is the case for amodiaquine. This may be important in terms of cross-resistance patterns with CQ since the desethyl metabolite of AQ rather than parent drug is the main circulating metabolite in man[16b] and this metabolite demonstrates more cross resistance to CQ than the parent drug which may have clinical consequences.[16] This may be a potential concern with isoquine, and a much more detailed study on the identity and antimalarial activity of main plasma metabolites will have to be conducted in future work.[16d]

As with all new entities, it is true that until we have clinical experience, toxicity cannot be ruled out. However, we can be certain that the chemical rearrangement in isoquine and its analogues precludes the formation of a reactive quinonimine. Furthermore, initial studies described here in rodents indicate that isoquine is eliminated at least as quickly as AQ; therefore, we can anticipate that toxicity due to accumulation will not be an issue. The 4-aminoquinolines as a drug class give no other obvious cause for concern based on clinical experience. As outlined above, the type of toxicity that has been associated with AQ is a type II idiosyncratic response characterized, as described above, as nonpredictable. Therefore, there will always remain a real concern that AQ can illicit this form of toxicity when used clinically. The more widespread use of AQ both as monotherapy and in combination, the exposure to multiple doses in high transmission areas, and the use of the drug in HIV +ve individuals suggest it may be unwise to give a safety recommendation based on the earlier retrospective analysis of prophylactic subjects. It is clear that the presence of this metabolic alert in a new drug entity would terminate its further development by the pharmaceutical industry today.

In summary, our initial studies suggest that the isomeric series of amodiaquine analogues presented in this publication are worthy of further investigation as potential, safer alternatives to amodiaquine. In particular, isoquine ISQ1 (**3a**) appears to have many advantages over the clinically used derivatives in this class. As such, isoquine[36] and other members of this isomeric class are currently the subject of preclinical evaluation in a partnership between the Malaria for Medicines Venture (MMV) and Glaxo Smithkline Pharmaceuticals.

Experimental Section

Chemistry. Unless otherwise noted, all solvents and reagents were obtained from commercial suppliers and used without further purification. The 3-hydroxyacetoamidophenol and all of the corresponding amines used in the experiments were purchased from Aldrich Chemical Co. Analytical thin-layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel obtained from Merck. Visualization was accomplished by UV light (254 nm). 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. Solid samples were run as Nujol mulls and liquids neat on sodium chloride disks, as indicated in text. Proton NMR spectra were recorded using Brucker (400, 250, and 200 MHz) NMR spectrometers as clarified in text. Spectra were referenced to the residual solvent peak and chemical shifts expressed in ppm from the internal reference peak. Significant ^{1}HNMR data are written in order: number of protons, multiplicity (b, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; bs, broad-singlet, bm, broad-multiplet), coupling constants in hertz, assignment. Mass spectra were recorded at 70 eV using a VG7070E and/or micromass LCT mass spectrometers. The molecular ion M^+, with intensities in parentheses, is given followed by peaks corresponding to major fragment losses. Melting points were performed using a Gallemkamp melting point apparatus and are reported uncorrected. Elemental analyses were performed in the microanalysis laboratory in the Department of Chemistry, University of Liverpool.

***N*-(4-Diethylaminomethyl-3-hydroxyphenyl)acetamide (3).** 3-Hydroxyacetanilide (5 g, 33.1 mmol) was added to 100 mL round-bottom flask followed by ethanol (23.6 mL). One equivalent of diethylamine (3.42 mL, 33.1 mmol) and aqueous formaldehyde (2.46 mL) was added and the solution was allowed to heat under reflux for 24 h. After this reflux period, the solvent was removed under reduced pressure and the crude material was purified by silica gel flash column chromatography using 20−80% MeOH/dichloromethane as eluent. This gave the desired product as a pale brown oily residue (5.31 g, 69%); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.15 (bs, 1H, O*H*), 7.05 (dd, 1H, J = 8.2, 1.92 Hz, Ar-*H*), 6.88 (d, 1H, J = 8.2 Hz, Ar-*H*), 6.80 (d, 1H, J = 1.92 Hz, Ar-*H*), 3.71 (s, 2H, CH_2), 2.59 (q, 4H, J = 7.20 Hz, NCH_2CH$_3$), 2.13 (s, 3H, COCH_3), 1.07 (t, 6H, J = 7.20 Hz, NCH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.03, 160.17, 140.76, 130.33, 119.83, 112.40, 109.35, 72.04, 57.48, 47.92, 24.28, 11.94; MS (CI) *m/z* 237 [M + H]$^+$ (100), 164 (28), 122 (12), 74 (61), 58 (32); IR (neat): 3500−2800 (broad-OH band), 1668, 1614, 1538, 1454, 1386, 1273, 1194, 1166, 1114, 1032, 863, 773 and 736 cm^{-1}; HRMS *m/z* calcd for $C_{13}H_{21}N_2O_2$ [M^+ + 1] 237.16029 found, 237.16042. Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

***N*-[4-(*tert*-Butylaminomethyl)-3-hydroxyphenyl]acetamide (4).** Compound **4** was prepared in a manner similar to Mannich base **3** to give the product as a white solid (70% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15 (s, 1H, Ar-*H*), 6.95 (d, 1H, J = 8.30 Hz, Ar-*H*), 6.51 (d, 1H, J = 8.30 Hz, Ar-*H*), 3.72 (s, 2H, CH_2), 2.17 (s, 3H), 1.18 (s, 9H, *t*-Bu). Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

***N*-(4-Dimethylaminomethyl-3-hydroxyphenyl)acetamide (5).** Compound **5** was prepared in a manner similar to Mannich base **3** to give the product as an off-white solid (74%): mp = 135−136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, 1H, J = 1.80 Hz, Ar-*H*), 6.95 (d, 1H, J = 8.13 Hz, Ar-*H*), 6.91 (dd, 1H, J = 8.13, 1.80 Hz, Ar-*H*), 3.58 (s, 2H, CH_2), 2.30 (s, 6H, NCH_3), 2.08 (s, 3H, COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.05, 138.43, 128.66, 110.58, 107.47, 62.47, 53.41, 50.88, 44.43, 24.66; MS (CI) *m/z* 209 [M + H]$^+$ (100), 164 (10), 122 (7), 58 (2); IR (Nujol mull): 3270, 1699, 1614, 1549, 1304, 1271, 1196, 1123, 1015, 975, 870, 824, 802, 763, and 727 cm^{-1}; HRMS *m/z* calcd for $C_{11}H_{17}N_2O_2$ [M^+ + 1] 209.12901; found, 209.12938. Anal. ($C_{11}H_{16}N_2O_2$) C, H, N.

***N*-(4-Dipropylaminomethyl-3-hydroxyphenyl)acetamide (6).** Compound **6** was prepared in a manner similar to Mannich base **3** to provide the product as a pale brown foamy residue (63%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.04 (dd, 1H, J = 7.97 Hz, 1.92, Ar-*H*), 6.86 (d, 1H, J = 7.97 Hz, Ar-*H*), 6.79 (d, 1H, J = 1.92 Hz, Ar-*H*), 6.58 (s, 1H, O*H*), 3.68 (s, 2H, CH_2), 2.43 (t, 4H, J = 7.68 Hz, NCH_2CH$_2$CH$_3$), 2.12 (s, 3H, COCH_3), 1.50 (m, 4H, NCH$_2$CH_2CH$_3$), 0.85 (t, 6H, J = 7.42 Hz, NCH$_2$CH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.96, 158.71, 140.25, 131.116, 119.692, 111.97, 108.77, 56.53, 49.66, 23.86, 20.60, 12.10; IR (neat) 3316, 2963, 2779, 1739, 1694, 1668, 1614, 1538, 1466, 1373, 1272, 1167, 1115, 1082, 1059, 978, 863 and 757 cm^{-1}; MS (CI) *m/z* 265 [M + H]$^+$(100), 164 (13), 102 (98) 72 (26); HRMS *m/z* calcd for $C_{15}H_{25}N_2O_2$ [M^+ + 1] 265.19159, found 265.19116. Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

***N*-(4-Dibutylaminomethyl-3-hydroxyphenyl)acetamide (7).** Compound **7** was prepared in a manner similar to Mannich base **3** to provide the product as a pale brown foamy residue (52%); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (s, 1H, O*H*), 7.03 (d, 1H, J = 7.90 Hz, Ar-*H*), 6.85 (d, 1H, J = 7.90 Hz, Ar-*H*), 6.78 (d, 1H, Ar-*H*), 3.67 (s, 2H, CH_2), 2.45 (t, 4H, J = 7.12 Hz, NCH_2CH$_2$CH$_2$CH$_3$), 2.10 (m, 4H, NCH$_2$CH_2-CH$_2$CH$_3$), 1.47 (m, 4H, NCH$_2$CH$_2$CH_2CH$_3$), 0.86 (t, 6H, J = 7.14 Hz, NCH$_2$CH$_2$CH$_2$CH_3 ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.85, 159.89, 140.71, 130.20, 119.98, 112.35, 109.15, 54.72, 50.08, 30.51, 21.99, 14.69; IR (Nujol mull): 3200, 1714, 1696, 1657, 1614, 1578, 1538, 1330, 1258, 1200, 1179, 1018, 980, 870, and 760 cm^{-1}); MS (CI) *m/z* 293 [M + H]$^+$ (95), 130 (100), 86 (25); HRMS *m/z* calcd for $C_{17}H_{29}N_2O_2$ [M^+ + 1] 293.12801, found, 293.12837. Anal. ($C_{17}H_{28}N_2O_2$) C, H, N.

***N*-(3-Hydroxy-4-pyrrolidin-1-ylmethylphenyl)acetamide (8).** Compound **8** was prepared in a manner similar to Mannich base **3** to provide the product as a brown oily residue (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (bs, 1H, O*H*), 7.04 (dd, 1H, J = 7.92, 2.00 Hz, Ar-*H*), 6.91 (d, 1H, J = 7.92 Hz, Ar-*H*), 6.84 (d, 1H, J = 2.00 Hz, Ar-*H*), 3.78 (s, 2H,-CH_2), 2.62 (bs, 4H, pyrrolidinyl-H), 2.15 (s, 3H, COCH_3), 1.84 (bm, 4H, pyrrolidinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.49, 158.94, 138.60, 129.11, 118.18, 110.96, 107.88, 62.01, 54.20, 24.36, 23.01, 23.00; IR (Nujol mull): 3250, 1668,1606, 1167, 1116, 1013, 863, and 722 cm^{-1}; MS (CI) *m/z* 235 [M + H]$^+$ (94), 152 (18), 72 (100), 70 (19); HRMS *m/z* calcd for $C_{13}H_{19}N_2O_2$ [M^+ + 1] 235.30200, found 235.30232. Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

***N*-(3-Hydroxy-4-morpholin-4-ylmethylphenyl)acetamide (9).** Compound **9** was prepared in a manner similar to Mannich base **3** to provide the product as a white solid (72%): mp 150 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.25 (bs, 1H, O*H*), 6.99 (dd, 1H, J = 8.24, 1.92 Hz, Ar-*H*), 6.87 (d, 1H, J = 8.24 Hz, Ar-*H*), 6.82 (d, 1H, J = 1.92 Hz, Ar-*H*), 3.71 (bt, 4H, J = 4.66 Hz, morpholinyl-H) 3.62 (s, 2H, CH_2), 2.51 (bs, 4H, morpholinyl-H), 2.11 (s, 3H, COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.28, 157.99, 138.77, 129.17, 116.77, 110.99, 107.66, 66.80, 61.45, 52.91; IR (Nujol mull): 3334, 1692, 1654, 1627, 1604, 1534, 1512, 1307, 1278, 1245, 1167, 1109, 1067, 1004, 981, 863, 847, and 716 cm^{-1}; MS (CI) *m/z* 250 [M + H]$^+$ (68), 164 (91), 122 (100), 86 (95); HRMS *m/z* calcd for $C_{13}H_{19}N_2O_3$ [M^+ + 1] 250.13174, found 250.13208. Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

***N*-(3-Hydroxy-4-piperidin-1-ylmethylphenyl)acetamide (10).** Compound **10** was prepared in a manner similar to Mannich base **3** to give the product as a white solid (89%): mp 172−173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (bs, 1H, O*H*), 7.03 (dd, 1H, J = 8.11, 1.90 Hz, Ar-*H*), 6.89 (d, 1H, J =

8.11 Hz, Ar-*H*), 6.85 (d, 1H, J = 1.90 Hz, Ar-*H*), 3.63 (s, 2H, CH_2), 2.49 (bs, 4H, piperidinyl-H), 2.14 (s, 3H, COCH_3), 1.62 (bm, 4H, piperidinyl-H), 1.49 (bs, 2H, piperidinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$); δ 168.50, 158.95, 138.66, 129.12, 118.18, 110.18, 110.97, 107.88, 62.09, 54.21, 26.20, 24.96, 24.96, 24.37; IR (Nujol mull): 3307, 1669, 1609, 1325, 1305, 1296, 1191, 1102, 1037, 980, 900, 861 and 815 cm^{-1}; MS (CI) *m*/*z* 249 [M + H]$^+$ (100), 166 (19), 86 (41), 84 (9); HRMS *m*/*z* calcd for $C_{14}H_{21}N_2O_2$ [M^+ + 1] 249.16029 found 249.16061. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

***N*-[3-Hydroxy-4-(isopropylaminomethyl)phenyl]-acetamide (11).** Compound (**11**) was prepared in a manner similar to Mannich base **3** to give the product as a pale brown residue (51%); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.15 (bs, 1H, O*H*), 7.05 (dd, 1H, J = 8.08 Hz, 2.05, Ar-*H*), 6.87 (d, 1H, J = 8.08 Hz, Ar-*H*), 6.80 (d, 1H, J = 2.05 Hz, Ar-*H*), 3.91 (s, 2H, CH_2), 2.24 (m, 1H, isopropyl-H), 2.11 (s, 3H, COCH_3), 1.11 (d, 6H isopropyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.79, 151.99, 139.95, 121.18, 119.75, 112.93, 110.58, 49.79, 48.28, 22.52, 16.12; IR (neat): 2982, 1687, 1682, 1614, 1539, 1427, 1276, 1203, 1135, 1026, 981, 964, 834, 799, 720 and 660 cm^{-1}; MS (CI) *m*/*z* 223 [M + H]$^+$ (100), 164 (20), 60 (90); HRMS *m*/*z* calcd for $C_{12}H_{19}N_2O_2$ [M^+ + 1] 223.14465 found 223.14532. Anal. ($C_{12}H_{18}N_2O_2$) C, H, N.

***N*-(4-Ethylaminomethyl-3-hydroxyphenyl)acetamide (12).** Brown oily residue (55%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 1.89 Hz, Ar-*H*), 7.11 (dd, 1H, J = 8.18 Hz, Ar-*H*), 6.90 (dd, 1H, J = 8.18, 1.89 Hz, Ar-*H*), 3.99 (s, 2H, CH_2), 2.89 (q, 2H, NHCH_2CH$_3$), 2.07 (s, 3H, COCH_3), 1.08 (t, 3H, NHCH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.73, 148.89, 141.67, 131.66, 117.21, 112.07, 111.86, 108.54, 43.47, 23.91, 12.69; MS (CI) *m*/*z* 209 [M + H]$^+$ (72), 166 (100), 152 (80); HRMS *m*/*z* calcd for $C_{11}H_{17}N_2O_2$ [M^+ + 1] 209.12900 found 209.12918. Anal. ($C_{11}H_{16}N_2O_2$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-diethylaminomethylphenol (3a). Aqueous hydrochloric acid (20%) (25 mL) was added to a round-bottom flask containing the amide **2a** (4.47 g, 18.9 mmol) and the solution heated under reflux for 6 h. The solvent was then removed in vacuo and the resulting residue coevaporated with ethanol to give the corresponding hydrochloride salt. 4,7-Dichloroquinoline (4.12 g, 20.8 mmol) and ethanol (30 mL) were added, and the reaction was heated under reflux for 12 h until completion of the reaction (determined by TLC). A pale yellow solid was obtained upon removing the solvent under reduced pressure; this was subsequently purified by flash column chromatography using 20−80% MeOH/dichloromethane as eluent to yield the quinoline hydrochloride salt as a yellow foamy solid (6.47 g, 80%). To liberate the free base compound, this solid was dissolved in distilled water (18 mL) and the solution basified by careful dropwise addition of saturated sodium bicarbonate (added until no more precipitate formed). Dichloromethane (100 mL) was added, and the free base was extracted into the organic layer. Subsequent drying and removal of solvent in vacuo afforded the desired product as a pale off-white solid (3.76 g, 71%): mp 134−135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, 1H, J = 5.24 Hz, quinoline-H), 8.02 (d, 1H, J = 2.20 Hz, quinoline-H), 7.83 (d, 1H, J = 8.92 Hz, quinoline-H), 7.44 (dd, 1H, J = 8.96, 2.16 Hz, quinoline-H), 7.04 (d, 1H, J = 5.24 Hz, quinoline-H), 6.98 (d, 1H, J = 7.96 Hz Ar−H), 6.74 (d, 1H, J = 2.08 Hz, Ar−H), 6.68 (dd, 1H, J = 7.96, 2.22 Hz, Ar−H), 6.53 (bs, 1H, O*H*), 3.79 (s, 2H, CH_2), 2.65 (q, 4H, J = 7.14 Hz, NCH_2CH$_3$), 1.14 (t, 6H, J = 7.14 Hz, NCH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.44 152.35, 150.11, 147.84, 140.22, 135.57, 129.58, 129.41, 126.38, 121.54, 119.27, 118.53, 113.17, 110.54, 103.28, 56.99, 46.72, 11.57; IR (Nujol mull) 2930, 2858, 1668, 1612, 1575, 1529, 1459, 1424, 1378, 1327, 1277, 1192, 1178, 1159, 1115, 1079, 992, 974, 907, 873, 855, 814 and 772 cm^{-1}; MS (CI) *m*/*z* 356 [M + H]$^+$ (100), 285 (24), 271 (92), 110 (100); HRMS *m*/*z* calcd for $C_{20}H_{23}ClN_3O$ [M^+ + 1] 356.15292 found, 356.15169. Anal. ($C_{20}H_{22}ClN_3O$) C, H, N.

2-(*tert*-Butylaminomethyl)-5-(7-chloroquinolin-4-ylamino)phenol (4a). This compound was prepared in a manner similar to **3a** to provide a pale yellow solid (69%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 5.30 Hz, quinoline-H), 8.01 (d, 1H J = 2.14 Hz, quinoline-H), 7.85 (d, 1H, J = 8.90 Hz, quinoline-H), 7.43 (dd, 1H, J = 8.90, 2.14 Hz, quinoline-H), 7.02 (d, 1H, J = 5.32 Hz, quinoline-H), 6.99 (d, 1H, J = 7.98 Hz, Ar−H), 6.76 (d, 1H, J = 2.20 Hz, Ar−H), 6.68 (dd, 1H, J = 7.96, 2.20 Hz, Ar−H), 6.63 (bs, 1H, O*H*), 3.99 (s, 2H, CH_2), 1.20 (s, 9H, *t*-Bu). Anal. ($C_{20}H_{22}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-dimethylaminomethylphenol (5a). This compound was prepared in a manner similar to **3a** to provide a pale yellow solid (69%). mp 176.6−177.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, 1H, J = 5.40 Hz, quinoline-H), 8.04 (d, 1H, J = 2.06 Hz, quinoline-H), 7.84 (d, 1H, J = 9.06 Hz, quinoline-H), 7.45 (dd, 1H, J = 9.06, 2.06 Hz quinoline-H), 7.05 (d, 1H, J = 5.40 Hz, quinoline-H), 6.98 (d, 1H, J = 7.95, Ar−H), 6.76 (d, 1H, J = 2.22 Hz, Ar−H), 6.69 (dd, 1H, J = 7.95, 2.22 Hz, Ar−H), 6.51 (bs, 1H, O*H*), 3.67 (s, 2H, CH_2), 2.37 (s, 6H, NCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.78, 152.37, 149.50, 149.4, 146.60, 134.60, 129.59, 129.47, 126.42, 121.50, 119.04, 113.18, 110.39, 103.35, 62.86, 44.83, 31.24; IR (Nujol mull) 3194, 2376, 2306, 1702, 1684, 1653, 1458, 1375, and 1107 cm^{-1}; MS (CI) *m*/*z* 328 [M + H]$^+$ (76), 285 (100), 251 (20), 63 (20); HRMS *m*/*z* calcd for $C_{18}H_{19}ClN_3O$ [M^+ + 1] 328.12164 found, 328.12123. Anal. ($C_{18}H_{18}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-dipropylaminomethylphenol (6a). This compound was prepared in a manner similar to **3a** to provide a pale yellow solid (61%). mp 183−184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, 1H, J = 5.40 Hz, quinoline-H), 8.03 (d, 1H, J = 2.20 Hz, quinoline-H), 7.84 (d, 1H, J = 8.90 Hz, quinoline-H), 7.45 (dd, 1H, J = 8.90, 2.22 Hz, quinoline-H), 7.06 (d, 1H, J = 5.41 Hz, quinoline-H), 6.98 (d, 1H, J = 7.95 Hz, Ar−H), 6.74 (d, 1H, J = 2.22 Hz, Ar−H), 6.69 (dd, 1H, J = 7.95, 2.22 Hz, Ar−H), 6.55 (bs, 1H, O*H*), 3.79 (s, 2H, CH_2), 2.60 (t, 4H, J = 7.63 Hz, NCH_2CH$_2$), 1.59 (m, 4H, NCH$_2$CH_2), 0.92 (t, 6H, J = 7.31 Hz, CH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.87, 152.37, 150.14, 149.4, 147.76, 140.24, 135.57, 129.57, 129.46, 126.39, 121.50, 119.41, 113.13, 110.42, 103.30, 58.28, 55.83; IR (Nujol mull) 3150, 1733, 1699, 1657,1578, 1538, 1331, 1258, 1181, 1159, 980, 855, 807 and 721 cm^{-1}; MS (ES+) *m*/*z* 384.2 [M + H]$^+$ (100), 283 (82), 192.6 (34); HRMS *m*/*z* calcd. for $C_{22}H_{27}N_3$-OCl (M^+ + 1) 384.1843 found 384.1845. Anal. ($C_{22}H_{26}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-dibutylaminomethylphenol (7a). This compound was prepared in a manner similar to **3a** to provide a pale brown solid (58%). mp 153 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 5.22 Hz, quinoline-H), 8.00 (d, 1H, J = 2.20 Hz, quinoline-H), 7.83 (d, 1H, J = 9.08 Hz, quinoline-H), 7.42 (dd, 1H, J = 9.0, 2.22 Hz, quinoline-H), 7.03 (d, 1H, J = 5.50 Hz, quinoline-H), 6.96 (d, 1H, J = 7.98 Hz, Ar−H), 6.72 (d, 1H, J = 2.00 Hz, Ar−H), 6.67 (dd, 1H, J = 7.98, 2.00 Hz Ar−H), 6.54 (bs, 1H, O*H*), 3.75 (s, 2H, CH_2), 2.52 (t, 4H, J = 7.14 Hz, NCH_2CH$_2$), 1.50 (m, 4H, CH$_2$CH_2CH$_2$), 1.30 (m, 4H, CH$_2$CH_2CH$_3$) 0.90 (t, 6H, J = 7.31 Hz, CH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.55, 152.03, 149.85, 149.79, 147.44, 135.21, 129.24, 129.11, 127.93, 126.02, 121.16, 118.19, 112.82, 110.11, 102.94, 57.85, 53.23, 28.45, 20.62, 13.96; IR (Nujol mull) 3190, 2354, 1739, 1733, 1714, 1699, 1696, 1657, 1654, 1614, 1578, 1538, 1330, 1258, 1200, 1179, 1159, 1118, 980, 909, 870, 856, 818 and 760 cm^{-1}; MS (ES+) *m*/*z* 412.2 [M + H]$^+$ (100), 283 (92), 206 (65); HRMS *m*/*z* calcd for $C_{24}H_{31}N_3OCl$ [M^+ + 1] 412.2156 found 412.2150. Anal. ($C_{24}H_{30}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-pyrrolidin-1-ylmethylphenol (8a). This compound was prepared in a manner similar to **3a** to provide an off-white solid (80%): mp 163.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, 1H, J = 5.41 Hz, quinoline-H), 8.02 (d, 1H, J = 2.07 Hz, quinoline-H), 7.84 (d, 1H, J = 9.06 Hz, quinoline-H), 7.44 (dd, 1H, J = 9.06, 2.07 Hz, quinoline-H), 7.03 (d, 1H, J = 5.41 Hz, quinoline-H), 6.99 (d, 1H, J = 7.95 Hz, Ar−H), 6.75 (d, 1H, J = 2.06 Hz, Ar−H), 6.68 (dd, 1H, J = 7.95, 2.07 Hz, Ar−H), 6.59 (bs, 1H, O*H*), 3.85 (s, 2H, CH_2), 2.65 (bm, 4H, pyrrolidinyl-H), 1.90 (bm, 4H, pyrrolidinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.41,

151.98, 149.75, 147.54, 139.92, 135.24, 129.04, 128.77, 126.03, 121.24, 119.31, 118.19, 112.84, 110.07, 102.92, 58.50, 53.55, 23.72; IR (Nujol mull) 3175, 1667, 1652, 1615, 1576, 1536, 1512, 1457, 1427, 1376 cm^{-1}; MS (CI) *m/z* 354 $[M + H]^+$ (65), 285 (100), 271 (35), 72 (93); HRMS *m/z* calcd for $C_{20}H_{21}ClN_3O$ $[M^+ + 1]$ 354.13730 found 354.13713 Anal. ($C_{20}H_{20}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-morpholin-4-ylmethylphenol (9a). This compound was prepared in a manner similar to **3a** to provide the desired product an off-white solid (60%): mp 185−186°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, 1H, J = 5.25 Hz, quinoline-H), 8.04 (d, 1H, J = 2.06 Hz, quinoline-H), 7.84 (d, 1H, J = 8.91 Hz, quinoline-H), 7.45 (dd, 1H, J = 8.91, 2.06 Hz, quinoline-H), 7.04 (d, 1H, J = 5.25 Hz, quinoline-H), 7.01 (d, 1H, J = 7.95 Hz, Ar−H), 6.77 (d, 1H, J = 2.22 Hz, Ar−H), 6.72 (dd, 1H, J = 7.95, 2.22 Hz, Ar−H), 6.52 (bs, 1H, O*H*), 3.78 (bm, 4H, morpholinyl-H), 3.73 (s, 2H CH_2), 2.60 (bm, 4H, morpholinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.19, 152.33, 150.13, 147.63, 140.80, 135.66, 130.16, 129.46, 126.50, 121.51, 117.64, 113.46, 110.34, 103.44, 102.23, 67.16, 61.87, 53.32; IR (Nujol mull) 3200, 1699, 1654, 1575, 1533, 1346, 1335, 1249, 1118, 862 and 811 cm^{-1}; MS (CI) *m/z* 370 $[M + H]^+$ (100), 285 (71), 251 (30), 208 (34) 164 (26); HRMS *m/z* calcd for $C_{20}H_{21}ClN_3O_2$ $[M^+ + 1]$ 370.13223 found 370.13276. Anal. ($C_{20}H_{20}ClN_3O_2$) C, H,N.

5-(7-Chloroquinolin-4-ylamino)-2-piperidin-1-ylmethylphenol (10a). This compound was prepared in a manner similar to **3a** to provide the desired product as an off-white solid (71%): mp 182.6; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 5.22 Hz, quinoline-H), 8.00 (d, 1H, J = 1.92 Hz, quinoline-H), 7.83 (d, 1H, J = 9.06 Hz, quinoline-H), 7.42 (dd, 1H, J = 9.06, 1.92 Hz, quinoline-H), 7.03 (d,1H, J = 5.22 Hz, quinoline-H), 6.97 (d, 1H, J = 7.98 Hz, Ar−H), 6.73 (d, 1H, J = 2.06 Hz, Ar−H), 6.67 (dd, 1H, J = 7.98, 2.06 Hz, Ar−H), 6.58 (bs, 1H, O*H*), 3.67 (s, 2H, CH_2), 2.51 (bm, 4H, piperidinyl-H), 1.64 (bm, 6H, pyrrolidinyl-H); ^{13}C NMR (100 MHz, $CDCl_3$)- 159.81, 152.31, 150.06, 147.85, 140.26, 135.61, 129.74, 129.39, 126.40, 121.53, 118.78, 118.52, 113.21, 110.48, 103.29, 62.14, 54.28, 26.22, 24.35; MS (CI) *m/z* 368 $[M + H]^+$ (70), 285 (100), 271 (37), 86 (93); IR (Nujol mull) 3200, 1614, 1575, 1455, 1377, 1249, 1198 and 822 cm^{-1}; HRMS *m/z* calcd for $C_{21}H_{23}ClN_3O$ $[M^+ + 1]$ 368.15292 found 368.15403. Anal. ($C_{21}H_{22}ClN_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-(isopropylaminomethyl)phenol (11a). This compound was prepared in a manner similar to **3a** to provide the product as a pale yellow solid (67%): mp 157.6−158.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, 1H J = 5.32 Hz, quinoline-H), 8.03 (d, 1H, J = 2.08 Hz, quinoline-H), 7.84 (d, 1H, J = 8.90 Hz, quinoline-H), 7.49 (dd, 1H, J = 8.90, 2.08 Hz, quinoline-H), 7.02 (d, 1H, J = 5.30 Hz, quinoline-H), 6.99 (d, 1H, J = 7.92 Hz, Ar−H), 6.75 (d, 1H, J = 2.24 Hz, Ar−H), 6.69 (dd,1H, J = 7.92, 2.24 Hz, Ar−H), 6.51 (bs, 1H, O*H*), 4.02 (s, 2H, CH_2), 2.93 (septet, J = 6.36 Hz, 1H, isopropyl-H), 1.19 (d, 6H, J = 6.36 Hz, isopropyl-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.79, 151.99, 149.76, 147.49, 139.95, 135.24, 129.08, 128.98, 126.04, 121.18, 119.75, 118.18, 112.93, 110.58, 102.91, 49.79, 48.28, 22.52. IR (Nujol mull) 3280, 1600, 1576, 1429, 1333, 1280, 1179, 1159, 1118, 814 and 761 cm^{-1}; MS *m/z* 342.1 $[M + H]^+$ (100), 283 (30), 171 (76); HRMS (ES+) *m/z* calcd for $C_{19}H_{21}ClN_3O$ $[M^+ + 1]$ 342.1373 found 342.1377. Anal. ($C_{19}H_{20}Cl\ N_3O$) C, H, N.

5-(7-Chloroquinolin-4-ylamino)-2-ethylaminomethylphenol (12a). This compound was prepared in a manner similar to **3a** to provide the product as a pale brown solid (68%). mp 136.3−131.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 8.54 (d, 1H, J = 5.32 Hz, quinoline-H), 8.01 (d, 1H, J = 2.14 Hz, quinoline-H), 7.85 (d, 1H, J = 8.95 Hz, quinoline-H), 7.43 (dd, 1H, J = 8.95, 2.14 Hz, quinoline-H), 7.02 (d, 1H, J = 5.32 Hz, quinoline-H), 6.99 (d, 1H, J = 7.98 Hz, Ar−H), 6.76 (d, 1H, J = 2.20 Hz, Ar−H), 6.68 (dd, 1H, J = 7.96, 2.20 Hz, Ar−H), 6.63 (bs, 1H, O*H*), 4.02 (s, 2H, CH_2), 2.76 (q, 2H, J = 7.17 Hz NHCH_2CH$_3$), 1.19 (t, 3H, J = 7.17 Hz, NHCH$_2$CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 159.70, 151.97, 149.74, 147.53, 139.99, 135.23, 129.16, 129.01, 126.01, 121.26, 119.32, 118.20, 112.93, 110.48, 102.92, 52.10, 43.08, 14.83; IR 3274, 1615, 1573, 1542, 1461, 1336, 1282, 1113, 1082, 886, 909, 818 and 764 cm^{-1}; MS *m/z* 328 $[M + H]^+$ (10), 285 (29), 271 (100), 207 (89) 91 (22), 58 (14); HRMS (CI) *m/z* calcd for $C_{18}H_{19}ClN_3O$ $[M^+ + 1]$ 328.1216 found 328.12155. Anal. ($C_{18}H_{18}ClN_3O$) C, H, N.

Biology. In Vitro Testing Protocol. Antimalarial Activity. Two strains of *P. falciparum* were used in this study: (a) The K1 strain which is known to be CQ resistant and (b) the HB3 strain which is sensitive to all antimalarials. Parasites were maintained in continuous culture using the method of Jensen and Trager.[33] Cultures were grown in flasks containing human erythrocytes (2−5%) with parasitemia in the range of 1% to 10% suspended in RPMI 1640 medium, supplemented with 25 mM HEPES and 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 with an adaption of the 48-h sensitivity assay of Desjardins et al. using [^{3}H]-hypoxanthine incorporation as an assessment of parasite growth.[34] Stock drug solutions were prepared in 100% dimethyl sulfoxide (DMSO) and diluted to the appropriate concentration using complete medium. Assays were performed in sterile 96-well microtiter plates, and each plate contained 200 μL of parasite culture (2% parasitemia, 0.5% haematocrit) with or without 10 μL drug dilutions. Each drug was tested in triplicate and parasite growth compared to control wells (which consituted 100% parasite growth). After 24-h incubation at 37 °C, 0.5 μCi hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filter-mats, dried for 1 h at 55 °C, and counted using a Wallac 1450 Microbeta Trilux Liquid scintillation and luminescence counter. IC_{50} values were calculated by interpolation of the probit transformation of the log dose−response curve.

In Vivo Testing Protocol.[35] Male, random Swiss albino mice weighing 18−22 g were inoculated intraperitoneally with 10^7 parasitized erythrocytes with *P. yoelii* NS strain. Animals were then dosed daily via two routes (intraparential or oral) for four consecutative days beginning on the day of infection. Compounds were dissolved or suspended in the vechile solution consisting of methanol, phosphate-buffered saline, and DMSO (2:5:3 v/v). The parasitemia was determined on the day following the last treatment and the ED_{50} (50% suppression of parasites when compared to vehicle only treated controls) calculated from a plot of log dose against parasitemia.

Metabolism Studies. Materials. Opti-solv tissue solubilizer was a product of Wallac (Loughborough U.K). Ultima-gold liquid scintillation fluid was purchased from Packard bioscience. Glacial acetic acid was a Merck product. [^{3}H]AQ and [^{3}H]ISQ were synthesized by the University of Liverpool. Heparin was a product of CP pharmaceuticals (Wrexham UK). All other chemicals used were purchased from Sigma (Poole, U.K.)

Statistics. All values are given as the mean ± SEM. All statistical analyses were carried out using a Mann−Whitney test, and the differences were deemed significant at $p < 0.05$

Investigation of Biliary and Urinary Excretion of [^{3}H] AQ and [^{3}H] ISQ after Administration to Male Wistar Rats. Male Wistar rats (200−300 g) were anesthetized with urethane (1.4 g/mL in 20 mL in 0.9% saline, 20 mL/kg) and their state of consciousness determined, using the cornea reflex test and the limb retraction test. The rats were carefully monitored throughout the procedure to ensure that anesthesia was maintained.

A small incision was made in the throat, and the trachea was located, via blunt dissection of the surrounding connective tissue. A 1.57 mm (I.D.) polythene tube cannula was inserted and securely fastened. The jugular vein was also cannulated with 0.58 mm (I.D.) tubing to allow iv administration of the compounds. A syringe containing saline was attached to the jugular cannula to act as a seal preventing air bubbles from entering the vein. An incision was made along the midline of the abdomen. The common bile duct was located and allowed to dilate before a 0.58 mm (I.D.) cannula was inserted. Control

bile was obtained. The penis was ligated, to allow urine to be collected during the experiment.

The radiolabeled compounds had been made up in a vehicle composed of 50% dimethyl sulfoxide (DMSO)−49% water−1% citric acid. DMSO replaced the saline in the jugular cannula to prevent the drug precipitating out of solution during dosing.

A 500 μL Hamilton syringe was filled with either [^{3}H]AQ or [^{3}H]ISQ (54 μmol/kg, 25 μCi/kg) and connected to the cannula. The radiolabeled compounds were infused via the jugular vein over a period of 30 min to prevent respiratory depression caused by DMSO. Bile fractions were collected at hourly intervals for 5 h from the start of dosing, All samples were weighed and their weight recorded.

After 5 h, any remaining urine was aspirated from the bladder, and blood was collected via cardiac puncture with a heparinized needle into a heparinized tube. The sample was centrifuged to allow the plasma and red blood cells to be separated (4000 rpm for 5 min). The volume of plasma was recorded. Tissues (brain, heart, kidney, liver, lung, spleen, and skin) were removed from the animal, rinsed in saline before being placed in vials, and weighed. All samples were stored at −80 °C until they were required for analysis.

Investigation of [^{3}H] AQ and [^{3}H] ISQ Remaining in the Plasma after Dosing. The animal was anesthetized and the jugular vein cannulated as described above. To allow blood samples to be taken regularly, the carotid artery was cannulated with 0.58 mm I.D. polythene tubing. The cannula was attached to a heparinized saline syringe to prevent blood escaping from the cannula and to prevent air bubbles from entering the blood stream. The animals were dosed as described above, and the clock was started at the end of the dosing period. Blood samples (300 μL) were collected at 15 min, 30 min, and hourly postdosing. Heparinized saline was flushed through the cannula after every blood collection to prevent clotting within the tubing. The saline was allowed to drain out of the cannula prior to every collection point.

After the fifth hour sample had been collected, all remaining blood within the rat was allowed to drain from the cannula into a heparinized tube. All samples were centrifuged (4000 rpm for 5 min) immediately after collection, and the pellet and supernatant were separated. The volume of the plasma was recorded. The tissues were also removed and weighed, and all samples were stored at −80 °C until analyzed. The active components of the plasma were removed via extraction with ether for LC/MS analysis.

24 h Metabolism Study. [^{3}H]AQ (54 μmol/kg) was administered ip to male Wistar rats (200−300 g). Each animal was placed in a wire-bottom metabolism cage with access to food and water. Urine was collected over 24 h, and the cage was rinsed with distilled water (10 mL) at the end of the collection period. After 24 h, the animals were anesthetized with phenobarbitone (60 mg/kg in 0.9% saline) and their tissues removed and blood collected via cardiac puncture with a heparinized needle. All samples were stored at −80 °C until analyzed. Previous ISQ data was obtained for comparison with AQ. These data was produced following the same procedure used for AQ.

Analysis of the Radioactivity Excreted into Urine, Bile, and Plasma. Aliquot of bile (2 × 10 μL), urine (2 × 50 μL), and plasma (2 × 50 μL) from animals dosed with [^{3}H]AQ or [^{3}H]ISQ were added to 4 mL of liquid scintillant and vortexed thoroughly. Samples were left in darkness overnight to prevent chemiluminescence. Radioactivity was then determined using a Packard 1500 Liquid Scintillation Analyzer.

Investigation of the Tissue Distribution of [^{3}H]AQ or [^{3}H]ISQ over 5 h. Portions of each tissue (50−100 mg) and aliquots of red blood cells (50−60 mg) were taken in duplicate, and tissue solubilizer (0.5 mL) was added to each sample and left overnight at 50 °C. The samples were cooled to room temperature before being decolorized with hydrogen peroxide (200 μL) and left for 1 h. The mixture was then neutralized with glacial acetic acid (30 μL) and 12 mL of scintillation fluid was added. The mixture was mixed thoroughly and left overnight in the dark. The samples were assayed for radioactivity (all volumes of chemicals to be added were doubled for solubulizing the red blood cells).

Analysis of Urinary, Biliary, and Plasma Metabolites of Male Wistar Rats Dosed with [^{3}H]AQ or [^{3}H]ISQ. Aliquots (50−100 μL) of bile, urine, and plasma ether extracts were eluted from a Zorbax SB-18 column with a slow acetonitrile gradient (10−50% over 30 min) in ammonium acetate (5.0 mM, pH 3.8) at 0.9 mL/min. Two Jasco PU-980 pumps were linked to a mixing module, allowing effluent to mix with scintillation fluid prior to reaching the Flo-One A250 beta radioactive flow detector or allowing the effluent to be delivered to the Quattro II mass spectrometer. Nebulizing and drying gas were delivered at a rate of 13 L/h and 300 L/h, respectively. The temperature of the LC/MS interface was 70 °C, and the capillary voltage was 3.7×10^{-2} V. The extent of fragmentation was modulated via altering the cone voltage.

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Supporting Information Available: X-ray crystallographic data and further details of metabolism studies on isoquine. This material is available free of charge via the Internet at http://pubs.acs.org.

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