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Synthesis and antitrypanosomal evaluation of derivatives of *N*-benzyl-1,2-dihydroquinolin-6-ols: Effect of core substitutions and salt formation

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ABSTRACT

Analogs of the trypanocidal lead compound 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate were prepared to extend the structure–activity relationship in this series of molecules, improve the in vivo antitrypanosomal activity of the lead, and determine whether ester prodrugs are needed to overcome the instability of the dihydroquinolin-6-ols. Two of the most active compounds identified in this study were 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride and 1-(2-methoxy)-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride. These stable solids possessed low nanomolar IC_{50} values against *Trypanosoma brucei rhodesiense* STIB900 in vitro and provided cures in an early treatment acute mouse model of African trypanosomiasis when given ip at 50 mg/kg/day for four consecutive days.

1. Introduction

There is a clear need for new drugs against human African trypanosomiasis (HAT). The parenteral agents pentamidine and suramin are used for treating the first stage of the disease when the causative *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* parasites, respectively, are limited to the hemolymphatic system of the human host. Since only parenteral drugs are currently available, an oral treatment is desirable for this disease stage. New drugs for second stage HAT, where the parasites invade the CNS, are more urgently needed. Pentamidine and suramin are not used against second stage HAT. Melarsoprol, an antiquated organoarsenical agent, has been used as the first line drug for second stage disease since 1949 despite the occurrence of fatal reactive encephalopathies in up to 5% of those who receive the drug.[1] Eflornithine was registered for the treatment of second stage HAT caused by *T. b. gambiense* in 1990. In addition to its limited spectrum of antitrypanosomal activity, eflornithine must also be given in large intravenous doses four times per day for 2 weeks.[2] Results from a phase III trial were reported in 2009 which demonstrated that combination therapy consisting of eflornithine and the anti-Chagas disease drug nifurtimox (the NECT regimen) showed non-inferior clinical efficacy against second stage HAT caused by *T. b. gambiense* compared to eflornithine monotherapy.[3] This treatment regimen against second stage HAT has been added to the World Health Organization's Essential Medicines List.[4] The implementation of the NECT regimen is an important step forward in the treatment of second stage HAT because it significantly reduces the amount of eflornithine required for curing the infection. However, this regimen does not apply to patients with *T. b. rhodesiense* infections, multiple infusions of eflornithine are still required, and parasite resistance threatens the NECT regimen due to the increasing use of eflornithine monotherapy.[3] Several other classes of compounds have recently shown promise against African trypanosomes,[5–7] but none of these agents has entered clinical trials to date. Thus, the identification and development of new candidates for the treatment of HAT should remain a high priority.

We recently reported the synthesis of a series of 2,2,4-trimethyl-1,2-dihydroquinolin-6-yl acyloxy compounds and related congeners which possess potent and selective activity against African trypanosomes in vitro.[8] The ability of these compounds to form

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the corresponding dihydroquinolin-6-ol appeared to be essential for activity against *Trypanosoma brucei*, as derivatives that possessed either an ether substitution at the 6-position of the dihydroquinoline ring system or lacked an oxygen atom at this position were much less active against the parasites in vitro. We hypothesized that antitrypanosomal activity in this series of molecules required ester hydrolysis to provide the corresponding dihydroquinolin-6-ol, followed by oxidation to a quinone imine that could undergo redox cycling in the parasite.[8] Since quinones are known to be substrates of the key trypanosome antioxidant enzyme trypanothione reductase, in the process generating superoxide,[9] the proposed quinone imine may be the ultimate trypanotoxic species produced through exposure of trypanosomes to 2,2,4-trimethyl-1,2-dihydroquinolin-6-yl acyloxy compounds. We also demonstrated that 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**4a**) significantly prolonged the lifespan of mice infected with *T. brucei brucei* STIB795 parasites when this agent was given at an ip dose of 4×50 mg/kg/day. In the present work, we report the synthesis and antitrypanosomal evaluation of additional dihydroquinolines in an attempt to provide a more complete understanding of the SAR of this series. In the process, three molecules were identified that show in vivo antitrypanosomal activity superior to that of **4a**.

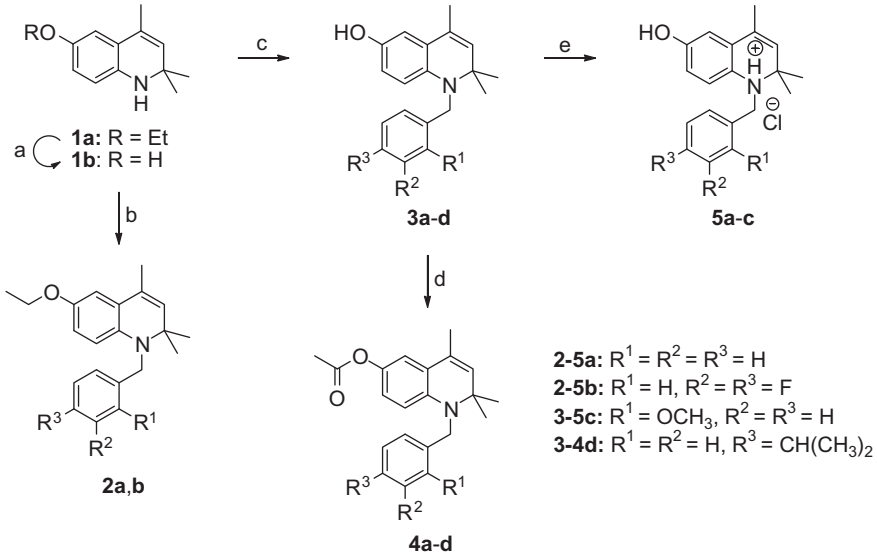
2. Chemistry

The synthesis of target compounds **2a**,**b**, **4a**–**d**, and **5a**–**c** is shown in Scheme 1. 2,2,4-Trimethyldihydroquinolines possessing 6-alkyl ethers **2a**,**b** were synthesized by N-benzylation of commercially available ethoxyquin (**1a**). *N*1-Benzylated-6-*O*-acetyl dihydroquinolines **4a**–**d** were prepared by the route described by Fotie et al.[8] Compound **1a** was O-dealkylated to provide **1b**, which was N-benzylated to give dihydroquinolin-6-ols **3a**–**d**. Acetylation of **3a**–**d** provided target compounds **4a**–**d**, while treatment of **3a**–**c** with aqueous or methanolic HCl afforded hydrochloride salts **5a**–**c**. Unlike dihydroquinolin-6-ols **3a**–**c**, which are oily materials that turn black upon standing,[8] their corresponding hydrochloride salts **5a**–**c** are stable as crystalline materials. For example, **5a** has been stored at room temperature for over 1 year without any evidence of decomposition as assessed by HPLC. The proton NMR spectra of the hydrochloride salts **5a–c** were substantially different from those of the corresponding free bases **3a–c** or acetate esters **4a–c**. In contrast to the spectra of neutral compounds, spectra of the salts recorded in DMSO-d_6 or CD_3OD gave extremely broad signals. Spectra of the salts recorded in $CDCl_3$ were more discernible although with some signal broadening. In the neutral molecules the *gem*-dimethyl and benzylic protons were chemically equivalent, giving rise to sharp singlets integrating for six and two protons, respectively. The corresponding protons were not chemically equivalent in the hydrochloride salts, however, giving rise to separate singlets for the methyl group and separate doublets or multiplets for the benzylic protons.

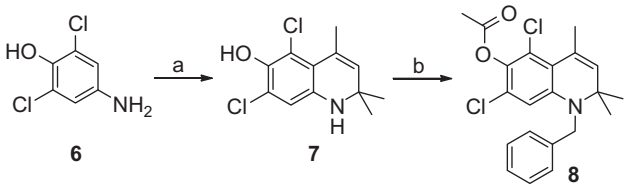
$BF_3{\cdot}OEt_2$ was a useful cyclization catalyst when 4-amino-2,6-dichlorophenol (**6**) was reacted with excess acetone in toluene to form the corresponding dihydroquinoline **7**, although the reaction proceeded in low yield. Benzylation followed by acetylation provided **8** as shown in Scheme 2.

The $BF_3{\cdot}OEt_2$ promoted cyclization was unsuccessful when other aminophenols were used, so 4-methoxy anilines **9a**–**c** were instead employed as starting materials for the synthesis of target compounds containing substitutions at the 7 and 8 positions of the dihydroquinoline ring system. These compounds were prepared using the iodine-catalyzed Skraup cyclization[8,10,11] as the key step, although the yields of the reactions providing dihydroquinolines **10a**–**c** remained poor. Aqueous HBr was then used to demethylate the 6-methoxy group present in **10a**–**c**, with the reaction being selective in the case of dimethoxyquinoline **10b**. Confirmation of the selective demethylation of **10b** to give **11b** was provided through NOE difference spectroscopy. Irradiation of the methoxy group of **11b** at δ 3.70 ppm resulted in enhancement of only the aromatic proton H-7 at δ 6.25 ppm. When the OH group at δ 8.44 ppm was irradiated, however, signals for both H-5 and H-7 were enhanced. Benzylation of **11a**–**c** at *N*1 followed by O-acetylation provided **12a**–**c** (Scheme 3).

Other target compounds substituted at the 7-position (Scheme 4) were prepared from 3-bromo-4-methoxyaniline (**13**), which was synthesized by reduction of 2-bromo-4-nitroanisole with $SnCl_2$.[12] Skraup cyclization of **13** provided 6-methoxy-7-bromodihydroquinoline **14** in modest yield. We observed only the 7-substituted dihydroquinoline product in reactions between either **9c** or **13** with acetone, consistent with literature reports of Skraup cyclizations employing other *m*-substituted anilines as starting



Scheme 1. Reagents and conditions: (a) 48% HBr, reflux, overnight (53%); (b) $ArCH_2Br$, NaH, DMF, overnight (87–89%); (c) $ArCH_2Br$ or $ArCH_2Cl$, Et_3N, toluene, reflux, overnight (67–71%); (d) CH_3COCl, Et_3N, DCM, rt, overnight (66–71%); (e) aqueous or alcoholic HCl (35%).

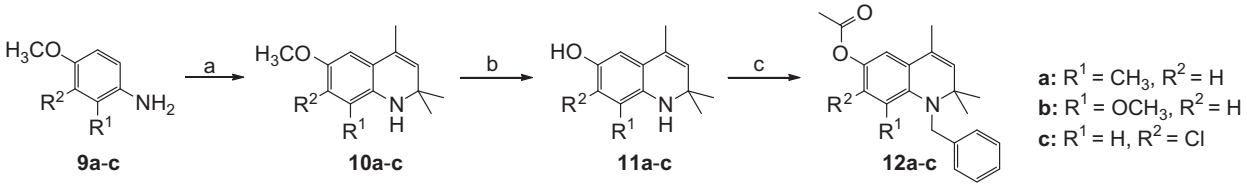


Scheme 2. Reagents and conditions: (a) acetone, $BF_3{\cdot}OEt_2$, toluene, reflux, 24 h (23%); (b) 1-benzyl bromide, Et_3N, reflux, overnight; 2-CH_3COCl, Et_3N, DCM, rt, overnight (75% after two steps).

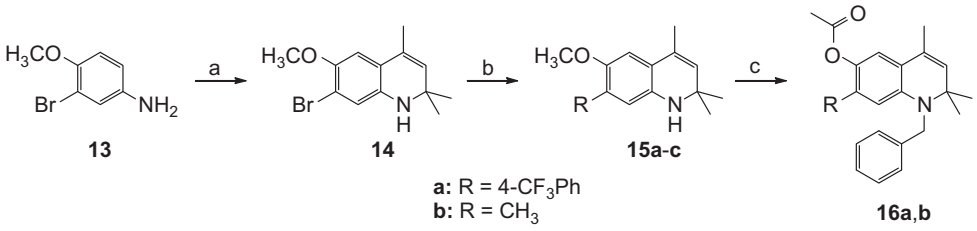
materials.[13,14] Suzuki coupling between **14** and 4-(trifluoromethyl)phenylboronic acid in the presence of catalytic $Pd(PPh_3)_4$ in aqueous potassium carbonate/toluene gave the desired 7-aryldihydroquinoline intermediate **15a** in excellent yield, while the reaction between **14** and methylboronic acid provided **15b** in a more modest yield. Compounds **15a** and **15b** were demethylated, benzylated, and acetylated as described previously to yield the 7-substituted target compounds **16a** and **16b** (Scheme 4).

Synthesis of the 4-phenyl dihydroquinoline target compound **22** (Scheme 5) was accomplished using Sonogashira coupling[15] and Cu-catalyzed cyclization[16] as key steps. Reaction between *p*-anisidine (**17**) and 3-chloro-3-methyl-1-butyne (**18**) gave the *N*-propargyl adduct **19**. A Sonogashira reaction between **19** and bromobenzene according to the procedure described by Roesch and Larock[17] resulted in homo-coupling of the alkyne, but a similar reaction between **19** and iodobenzene gave the desired product **20** in 81% yield. HPLC was used to monitor this reaction, as **19** and **20** were indistinguishable by TLC. CuCl-catalyzed ring closure of **20** gave the expected product **21** in 59% yield. The latter compound underwent the previously described dealkylation, benzylation, and acetylation reactions to yield the 4-phenyl target compound **22**.

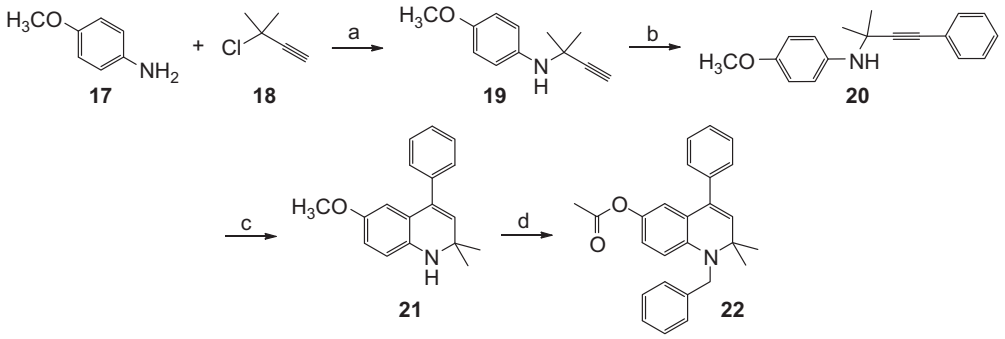
We also envisioned the synthesis of 1,2-dihydroquinolines analogs lacking the 2,2-dimethyl substituents by the preparation of 6-methoxy-1,2-dihydroquinolines followed by demethylation, benzylation, and acetylation steps. However, these efforts proved to be unsuccessful due to the instability of the methoxy dihydroquinolines. The CuCl-catalyzed cyclization of *N*-propargyl-*p*-anisidine gave 6-methoxyquinoline rather than the expected dihydroquinoline, analogous to the cyclization of *N*-propargyl-*p*-toluidine to 6-methylquinoline.[18] Lithium aluminum hydride reduction of 6-methoxyquinoline in our lab gave not the reported dihydroquinoline product[19] but a 4:1 mixture of starting material and tetrahydroquinoline product, although TLC analysis of the reaction mixture showed a faster eluting major component that could not be isolated by column chromatography. A similar reduction of 6-methoxy-4-methylquinoline (prepared from *p*-anisidine and methyl vinyl ketone[20]), resulted only in the recovery of starting



Scheme 3. Reagents and conditions: (a) acetone, I_2, toluene, reflux, 24 h (13–27%); (b) 48% HBr, reflux, overnight (31–53%); (c) 1-benzyl bromide, Et_3N, reflux, overnight (53–72%); 2-CH_3COCl, Et_3N, DCM, rt, overnight (84–90%).



Scheme 4. Reagents and conditions: (a) acetone, I_2, toluene, reflux, 24 h (48%); (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, aq K_2CO_3, toluene (90%) or $CH_3B(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3, THF/H_2O (59%); (c) 1–48% HBr, reflux, 3 h (23–49%); 2-benzyl bromide, Et_3N, reflux, overnight (75–88%); 3-CH_3COCl, Et_3N, DCM, rt, overnight (63–91%).



Scheme 5. Reagents and conditions: (a) CuCl, Cu, Et_3N, overnight (72%); (b) iodobenzene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 60 °C, 3 h (81%); (c) CuCl, toluene, reflux, overnight (59%); (d) 1–48% HBr, reflux, 1 h (49%); 2-benzyl bromide, Et_3N, toluene, reflux, overnight (99%); 3-acetyl chloride, Et_3N, CH_2Cl_2 (87%).

material, despite TLC evidence of the formation of a more lipophilic product. These findings demonstrate the importance of two methyl (or other) substituents at the 2-position to protect the dihydroquinoline core from spontaneous oxidation to a quinoline system.

3. Biological results

3.1. In vitro data

Compounds **4b**–**d** all possess substitutions on the aromatic ring of the *N*1-benzyl group. As observed previously with **4a** and other *N*1-benzylated-6-*O*-acetyl dihydroquinolines,[8] **4b**–**d** possess potent activity against *T. brucei* in vitro, with IC_{50} values ranging from 11 to 290 nM versus *T. b. rhodesiense* and from 41 to 970 nM versus *T. b. brucei* (the observed differences in sensitivity between *T. b. rhodesiense* STIB900 and *T. b. brucei* s427 should not be assigned to the taxonomic distinction but rather to the different assay protocols used in the two laboratories). Compound **4d**, which contains a *p*-isopropyl group on the *N*1-benzyl substituent, is the least active against both parasites, consistent with our earlier observation that bulky substitutions on the aryl ring of the *N*1-benzyl group diminish in vitro antitrypanosomal potency.[8] Like the 6-benzyloxy dihydroquinolines reported earlier, 6-ethoxy derivatives **2a** and **2b** display little in vitro antitrypanosomal activity, as do congeners **12a** and **12b**, which bear 8-methyl and 8-methoxy substituents on the dihydroquinoline core, respectively. In contrast, compounds possessing small substituents at the 7-position of the dihydroquinoline core, as in the 7-chloro dihydroquinoline **12c** and the 7-methyl dihydroquinoline **16b**, exhibit potent in vitro antitrypanosomal activity. The 5,7-dichlorodihydroquinoline **8** also displays sub-micromolar antitrypanosomal activity, although this compound is fivefold less potent than **4a** against *T. b. rhodesiense* and over eightfold less potent than **4a** against *T. b. brucei* in vitro. When the substituent at the 7-position of the dihydroquinoline ring becomes larger, as in the 7-aryl derivative **16a**, in vitro potency against African trypanosomes decreases dramatically compared to the 7-chloro and 7-methyl derivatives **12c** and **16b**. Replacement of the 4-methyl group present in **4a** with a phenyl group as in **22** results in an approximately 340-fold decrease in potency against both *T. b. rhodesiense* and *T. b. brucei*. However, dihydroquinolin-6-ol hydrochloride salts **5a**–**c** all displayed in vitro antitrypanosomal potency similar to that of **4a**. Dihydroquinolines that showed low- to mid-nanomolar potency against trypanosomes exhibited excellent selectivity for the parasites compared to L6 rat myoblasts, with compounds **4c**, **5a**, and **5c** displaying IC_{50} values 1500, 850, and 860 times lower, respectively, against *T. b. rhodesiense* than against these mammalian cells.

3.2. In vivo data

Based on the in vitro antitrypanosomal data shown in Table 1, **4c**, **5a**, and **5c** were selected for in vivo antitrypanosomal evaluation and were compared to **4a**, which had previously shown efficacy against *T. b. brucei* STIB795 in vivo.[8] Despite their lack of in vitro potency, **2a** and **2b** were also selected for in vivo evaluation in the hope that metabolic dealkylation would generate the active dihydroquinolin-6-ol within the host animal. These agents were administered to mice infected with *T. b. rhodesiense* STIB900 starting 1 day after infection by ip injection at a daily dose of 50 mg/kg given for four consecutive days (Table 2). Neither **2a** nor **2b** caused any suppression of parasitemia compared to untreated controls as assessed by microscopic examination of tail blood 5 days post infection. While **4a** suppressed parasitemia compared to untreated controls 5 days after infection, animals that received this compound relapsed on average 12.75 days post infection. Ester prodrug **4c** and hydrochloride salts **5a** and **5c** affected cures in this model, however. The control compounds diminazene and pentamidine gave cures when administered ip at daily doses of 10 mg/kg and 5 mg/kg, respectively, for four consecutive days.

4. Discussion

*N*1-Benzylated-6-*O*-acyloxy dihydroquinolines emerged as a promising new class of antitrypanosomal molecules based on their potent and selective in vitro trypanocidal activity, the simplicity of their structure, and the demonstration of the modest in vivo efficacy of lead compound **4a**.[8] The goals of the studies reported here were to further define the antiparasitic SAR of the dihydroquinolines, to improve the in vivo antitrypanosomal activity of lead **4a**, and to determine whether the instability of the dihydroquinolin-6-ol free base demanded the use of acyloxy prodrugs. These studies resulted in the identification of three new dihydroquinolines that provide cures in the *T. b. rhodesiense* STIB900 murine trypanosomiasis model, including two that do not possess a metabolically labile ester group.

A major focus of the current SAR investigation was to examine the effect of dihydroquinoline core substitutions on in vitro antitrypanosomal potency. We previously showed that replacement of the methyl group at the 4-position of **4a** with a hydrogen decreased in vitro trypanocidal potency by 14-fold.[8] Here, we demonstrate that insertion of a phenyl group at the 4-position (compound **22**) results in a more dramatic reduction in potency. When a chlorine atom or a methyl group is placed at the 7-position (**11c** or **16b**, respectively), the molecules show in vitro trypanocidal potency similar to that of **4a**. However, **16a**, which contains an aryl substituent at the 7-position, is over two orders of magnitude less potent than **4a**. The 5,7-dichloro compound (**8**) retains sub-micromolar in vitro antitrypanosomal potency, but is approximately twofold less potent than the 7-chloro compound **12c**. While compounds bearing small groups at the 7-position of the dihydroquinoline core are potent trypanocides, congeners possessing a methyl or methoxy group at the 8-position of the dihydroquinoline core (**12a** and **12b**, respectively) are over two orders of magnitude less potent against *T. b. brucei* than **4a**. Given that the 7-methyl derivative **16b** is 300 times more potent against *T. b. brucei* in vitro than the 8-methyl derivative **12a** despite the fact that these molecules display identical *C* log *P* values of 4.8 (as calculated by ChemDraw Pro version 11.0.1), the 8-substituted compounds are likely to possess steric or electronic features that interfere with antitrypanosomal activity. Consistent with our previous results with 6-benzyloxy compounds, 6-alkoxy dihydroquinolines **2a** and **2b** are essentially inactive against trypanosomes in vitro. Interestingly, the dihydroquinolin-6-ol hydrochloride salts **5a–c**, which are expected to generate the corresponding free bases at physiological pH, are all comparable in potency to their corresponding 6-*O*-acetyl derivatives **4a**–**c**. This observation is consistent with the hypothesis that the 6-acyloxy group present in the latter molecules is rapidly cleaved in vitro to provide the corresponding dihydroquinolin-6-ols. Earlier we proposed that the dihydroquinolin-6-ol is oxidized to a trypanocidal quinone imine species, which is likely to participate in redox cycling within the parasite.[8] Based on the compounds that have been synthesized and tested in the present work and in our previous study,[8] a summary of the in vitro antitrypanosomal SAR of these dihydroquinolines is presented in Figure 1.

Given their trypanocidal potency and stability, dihydroquinolin-6-ol hydrochloride salts may possess significant advantages over their corresponding neutral 6-acyloxy esters as candidates against HAT. We previously reported that the active dihydroquinolin-6-ol free bases we prepared quickly turned to dark, gummy materials, most likely due to auto-oxidation, and that acylation

Table 1
Activity and toxicity of dihydroquinolines against African trypanosomes and L6 rat myoblasts in vitro[a]

Compound	IC_{50} (μM) versus *T. b. rhodesiense* in vitro	IC_{50} (μM) versus *T. b. brucei* in vitro	IC_{50} (μM) versus L6 rat myoblasts in vitro
2a	41 ± 21	ND	97 ± 2
2b	140 ± 20	ND	90 ± 2
4a	0.012 ± 0.001	0.058 ± 0.008	18 ± 4
4b	0.025 ± 0.006	0.079 ± 0.014[b]	17 ± 1
4c	0.011 ± 0.001	0.041 ± 0.008	17 ± 1
4d	0.28 ± 0.05	0.97 ± 0.15	10 ± 0
5a	0.013 ± 0.004	0.038 ± 0.007	11 ± 2
5b	0.051 ± 0.016	0.068 ± 0.030	11 ± 2
5c	0.014 ± 0.004	0.045 ± 0.006	12 ± 1
8	0.072 ± 0.013	0.49 ± 0.17	110 ± 30
12a	43 ± 17	9.2 ± 2.8	48 ± 13
12b	ND	>100	ND
12c	0.042 ± 0.008	0.22 ± 0.04	22 ± 3
16a	9.0 ± 2.9	15 ± 1	>190
16b	ND	0.031 ± 0.002	ND
22	4.5 ± 0.6	20 ± 0	49 ± 2
Melarsoprol	0.0099 ± 0.0026	ND	ND
Diminazene	0.0058 ± 0.0008	ND	5.0 ± 1.6
Pentamidine	0.0013 ± 0.0001	0.012 ± 0.001	25 ± 11
Suramin	0.12 ± 0.01	0.11 ± 0.01	ND
Podophyllotoxin	ND	ND	0.012 ± 0.001

[a] Mean ± standard error of at least three independent measurements unless otherwise indicated.
[b] Mean ± range of two independent measurements.

Table 2
In vivo activity of **2a**, **2b**, **4a**, **4c**, **5a**, and **5c** against *T. b. rhodesiense* STIB900

Compound	Dose (mg/kg)	Cured/treated	Mean relapse (days)
Control	Untreated	Mean survival: 12.25 ± 0.95 days[a]	
2a	4 × 50 ip	0/4	Inactive
2b	4 × 50 ip	0/4	Inactive
4a	4 × 50 ip	0/4	12.75 ± 1.75[a]
4c	4 × 50 ip	4/4	–
5a	4 × 50 ip	4/4	–
5c	4 × 50 ip	4/4	–
Diminazene	4 × 10 ip	4/4	–
Pentamidine	4 × 5 ip	4/4	–

[a] Mean ± standard error.

of the oxygen atom at the 6-position was required to prevent the color change and allow for a satisfactory elemental analysis.[8] Fortunately, hydrochloride salts **5a**–**c** are stable crystalline materials. Compound **5a**, for example, shows no sign of decomposition after being stored at ambient room temperature for over a year. Since **5a** and **5c** also show excellent in vivo antitrypanosomal efficacy, it may be possible to avoid the difficulties that would result from attempting to develop an ester prodrug for treating HAT. 6-Acyloxy compounds may still offer advantages, however, such as the opportunity to deliver a trypanocidal dihydroquinolin-6-ol directly to the site of infection through selective ester hydrolysis by a parasite esterase. Considering that both hydrochloride salt **5c** and its corresponding 6-*O*-acetyl derivative **4c** cure STIB900 infections at the same dose when given ip (Table 2), both classes of dihydroquinolines deserve further study to assess their advantages and disadvantages as antitrypanosomal candidates.

Compound **4a** served as a lead molecule in this series based on its selective in vitro trypanocidal potency and its moderate efficacy in the STIB795 murine model of *T. b. brucei* infection. We show in the current study that **4a** is only modestly effective against *T. b. rhodesiense* strain STIB900 in vivo when given ip at a dose of 50 mg/kg/day × 4 (see Table 2). However, **4a** is the neutral 6-*O*-acetyl derivative of hydrochloride salt **5a**, which cures STIB900 infected mice when administered at the same dose as **4a**. A preliminary iv pharmacokinetics study in rats also revealed that similar levels of **3a** were observed in plasma within 2 min after iv administration of either **4a** or **5a**,[21] indicating that ester hydrolysis occurs rapidly in the blood. Nevertheless, we offer two possible explanations for the in vivo efficacy differences between compounds **4a**

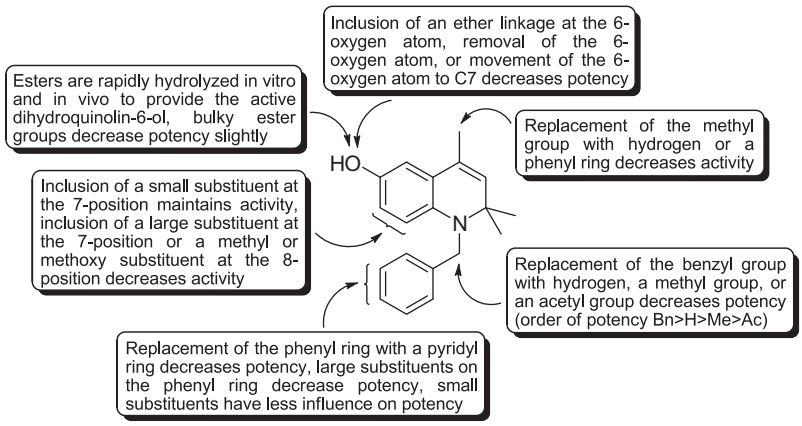


Figure 1. Activity map for dihydroquinolin-6-ols against African trypanosomes in vitro.

and **5a** despite their similar in vitro antitrypanosomal potencies. First, compounds administered by the ip route are quickly delivered to the systemic circulation through the liver.[22] Oxidative metabolism of **4a** by cytochrome P450 enzymes could occur in the liver, which may result in conversion of **4a** to an inactive form before it appears in the bloodstream and is transformed by ester hydrolysis to **3a**. Second, examination of the stability of **4a**, **4c**, **5a**, and **5c** in a phosphate buffer system unexpectedly revealed that esters **4a** and **4c** are less stable in this aqueous environment than the corresponding alcohols **3a** and **3c** derived from hydrochloride salts **5a** and **5c** (see Supplementary Fig. 1). In this experiment, the disappearance of **4a** and **4c** did not result in the appearance of **3a** and **3c**. Thus, we cannot exclude the possibility that degradation of **4a** in the peritoneal cavity is responsible for the lower in vivo antitrypanosomal efficacy of this compound compared to **5a**. Detailed pharmacokinetics studies and the investigation of in vivo trypanocidal action of **4c**, **5a**, and **5c** at different doses and by the oral route will be required to provide additional clues regarding the classes of molecules that should be pursued as HAT candidates.

The in vitro and in vivo results described here are consistent with the mechanistic hypothesis for the dihydroquinolines that we outlined earlier.[8] Both 6-*O*-acyloxy dihydroquinolines and dihydroquinolin-6-ol hydrochloride salts are capable of producing the corresponding dihydroquinolin-6-ol free base in vitro and in vivo, either by ester hydrolysis or by deprotonation, respectively. Considering that (1) the trypanotoxic *N*1-benzyl-dihydroquinolin-6-ols darken spontaneously and (2) dihydroquinolines either lacking a 6-*O* atom or containing a 6-alkoxy or 6-benzyloxy substituent are both stable and inactive against African trypanosomes, the production of a trypanotoxic quinone imine species remains the most likely explanation for the potent activity of these compounds against *T. brucei*. Evidence for the production of such quinone imine species has been obtained for several drugs including acetaminophen,[23,24] diclofenac,[25] thiabendazole,[26] and lumiracoxib,[27] but remains to be demonstrated for the antitrypanosomal dihydroquinolines. Once formed, the proposed quinone imine could display an antitrypanosomal effect and/or toxicity to the host either by acting as an electrophile and reacting with key cellular nucleophiles or by participating in redox cycling and causing oxidative stress. Given the potential for hepatotoxicity arising from the generation of a quinone imine species in vivo, the host toxicity of candidate agents from this class must be carefully monitored during the discovery and development process.

5. Conclusions

The work described here more clearly defines the antitrypanosomal SAR for dihydroquinolines and details the synthesis and curative in vivo antitrypanosomal efficacy of three new dihydroquinolines, including two dihydroquinolin-6-ol hydrochloride salts. Future studies must evaluate the oral bioavailability and more clearly characterize the in vivo antitrypanosomal efficacy of **4c**, **5a**, and **5c**, the potential of the trypanocidal dihydroquinolines to generate a quinone imine intermediate, and more precisely define the mechanism of trypanocidal action of these compounds. Such studies are either planned or in progress, and further results concerning these intriguing candidates against HAT will be reported in due course.

6. Experimental

6.1. General experimental

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data were recorded on a Bruker 300 UltraShield™ or a Varian Gemini 2000 spectrometer. Electrospray Ionization Mass Spectrometry (ESI-MS) was performed on an Agilent Technologies 1100 Series LC/MSD Trap spectrometer or by The Ohio State University Mass Spectrometry and Proteomics facility, which also performed High Resolution ESI-MS (HRESI-MS). Melting points were measured on a Thomas Hoover capillary melting points apparatus and are uncorrected. THF was distilled over Na metal and used immediately after collection or was dispensed directly from a Sure-seal® container. Reaction mixtures were monitored using TLC silica gel 60 F_{254} plates from Dynamic Adsorbents, Inc. or Whatman Paper Ltd or by reverse phase HPLC using a Zorbax SB-C8 column. Gravity and flash column chromatography were performed using type 60A silica gel (60–230 mesh) from Dynamic Adsorbents, Inc. or Fisher Scientific. Select compounds were further purified using silica gel GF preparative 1000 μm UV_{254} plates from Analtech. Elemental analyzes were performed by Atlantic Microlab, Norcross, GA.

6.2. Materials

All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific or Acros Organics and were used without further purification unless stated otherwise.

6.2.1. 1-Benzyl-6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (2a)

A mixture of **1a** (500 mg, 2.3 mmol), benzyl bromide (410 μL, 3.4 mmol), and NaH (110 mg, 4.6 mmol) in DMF (15 mL) was allowed to stir overnight under nitrogen. The solvent was then evaporated under reduced pressure. The residue was re-dissolved in ethyl acetate, washed twice with distilled water, brine solution and further purified on a silica gel column using hexanes–ethyl acetate (9:1) as the mobile phase to yield 1-benzyl-6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (**2a**, 615 mg, 87%) as a brown sticky oil that became solid after standing overnight, mp 50–52 °C. ^{1}H NMR (DMSO-d_6) δ 1.21–1.29 (9H, m), 1.94 (3H, s), 3.82–3.88 (2H, m), 4.41 (2H, s), 5.47 (1H, s), 6.10 (1H, d, J = 8.7 Hz), 6.46 (1H, d, J = 8.7 Hz), 6.60 (1H, s), 7.17–7.35 (5H, m); ^{13}C NMR (DMSO-d_6) δ 14.8, 18.3, 27.3, 47.4, 56.3, 63.1, 110.7, 112.1, 113.4, 123.7, 126.1, 127.0, 128.2, 128.8, 130.9, 137.8, 140.1, 149.7; HRESI-MS: $[M+H]^+$ *m/z* 308.2019 (calcd 308.2014) corresponding to $C_{21}H_{25}NO$. Anal. ($C_{21}H_{25}NO$) Calcd C, 82.04; H, 8.20; N, 4.56. Found: C, 82.20; H, 8.16; N, 4.42.

6.2.2. 1-(3,4-Difluorobenzyl)-6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (2b)

The reaction was similar to the synthesis of **2a**, using **1a** (500 mg, 2.3 mmol) and 3,4-difluorobenzyl bromide (714 mg, 3.4 mmol) to yield 1-(3,4-difluorobenzyl)-6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (**2b**, 702 mg, 89%). Purification on a silica gel column using hexanes–ethyl acetate (9:1) provided the target compound as white crystals, mp 75–77 °C. ^{1}H NMR (DMSO-d_6) δ 1.24 (9H, m), 1.94 (3H, s), 3.82–3.89 (2H, m), 4.39 (2H, s), 5.47 (1H, s), 6.08 (1H, d, J = 8.7 Hz), 6.48 (1H, dd, J = 8.7, 2.9 Hz), 6.61 (1H, d, J = 2.9 Hz), 7.29–7.35 (3H, m); HRESI-MS: $[M+H]^+$ *m/z* 344.1826 (calcd 344.1826). Anal. ($C_{21}H_{23}F_2NO$) Calcd C, 73.45; H, 6.75; N, 4.08. Found: C, 73.32; H, 6.71; N, 4.11.

6.2.3. 1-(3,4-Difluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (3b)

Compound **1b**[8] (420 mg, 2.22 mmol) was combined with 3,4-difluoro benzyl bromide (427 μL, 3.34 mmol), Et_3N (463 μL, 3.33 mmol), and toluene (10 mL) and the mixture was stirred for 24 h under nitrogen. The solvent was evaporated and the residue purified on silica gel using hexanes–dichloromethane (4:1) as the mobile phase to yield 1-(3,4-difluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**3b**, 487 mg, 70%) as a brown oil that

solidified upon standing. ^{1}H NMR (DMSO-d_6) δ 1.30 (6H, s), 2.00 (3H, s), 4.40 (2H, s), 5.49 (1H, s), 6.05 (1H, d, J = 8.7 Hz), 6.37 (1H, dd, J = 8.7, 2.4 Hz), 6.58 (1H, s), 7.29–7.42 (3H, m), 8.52 (1H, s).

6.2.4. 1-(4-Isopropylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (3d)

Compound **1b** (388 mg, 2.05 mmol) was combined with 4-isopropyl benzyl bromide (526 μL, 3.08 mmol), Et_3N (427 μL, 3.08 mmol), and toluene (10 mL). The reaction mixture was stirred for 24 h under nitrogen. The solvent was then evaporated and the residue purified on silica gel using hexanes–dichloromethane (2:1) as the mobile phase to yield 1-(4-isopropylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**3d**, 469 mg, 71%) as a brown oil that solidified upon standing. ^{1}H NMR (DMSO-d_6) δ 1.19 (6H, d, J = 6.9 Hz), 1.28 (6H, s), 1.93 (3H, s), 2.78–2.92 (1H, m), 4.34 (2H, s), 5.45 (1H, s), 6.07 (1H, d, J = 8.7 Hz), 6.33 (1H, d, J = 8.4 Hz), 6.52 (1H, d, J = 2.1 Hz), 7.15–7.26 (4H, m), 8.42 (1H, s).

6.2.5. 1-(3,4-Difluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (4b)

Compound **3b** (491 mg, 1.56 mmol), Et_3N (325 μL, 2.34 mmol), and acetyl chloride (166 μL, 2.34 mmol) were stirred overnight with dichloromethane (10 mL) under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–ethyl acetate (9:1) as the mobile phase to yield 1-(3,4-difluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate **(4b**, 367 mg, 66%) as a yellow oil which solidified on standing. ^{1}H NMR ($CDCl_3$) δ 1.37 (6H, s), 2.02 (3H, s), 2.27 (3H, s), 4.44 (2H, s), 5.41 (1H, s), 6.15 (1H, d, J = 8.7 Hz), 6.64 (1H, dd, J = 8.7, 2.4 Hz), 6.84 (1H, d, J = 2.4 Hz), 7.08–7.20 (3H, m); ^{13}C NMR ($CDCl_3$) δ 18.6, 21.0, 28.3, 47.3, 57.2, 115.1 (d, $^2J_{CF}$ = 24.75 Hz), 116.7, 117.3 (d, $^2J_{CF}$ = 16.5 Hz), 120.8, 121.9 (dd, $^4J_{CF}$ = 3.75 Hz), 123.9, 127.5, 130.3, 136.6 (dd, $^4J_{CF}$ = 3.75 Hz), 141.6 (d, $^2J_{CF}$ = 16.5 Hz), 148.9 (d, $^3J_{CF}$ = 12.75 Hz), 149.0 (d, $^1J_{CF}$ = 244.5 Hz), 149.2 (d, $^1J_{CF}$ = 244.5 Hz), 152.3 (d, $^3J_{CF}$ = 12.75 Hz), 170.1. ESI-MS: $[M+Na]^+$ m/z 380; HRESI-MS: $[M+Na]^+$ m/z 380.1439 (calcd 380.1438). Anal. ($C_{21}H_{21}F_2NO_2$) Calcd C, 70.57; H, 5.92; N, 3.92. Found: C, 70.52; H, 5.96; N, 3.95.

6.2.6. 1-(2-Methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (4c)

Compound **1b** (300 mg, 1.6 mmol) and 2-methoxybenzyl chloride (376 mg, 2.4 mmol) were reacted with Et_3N (335 μL, 2.4 mmol) in toluene (10 mL) to yield, after purification on a silica gel column using hexanes–ethyl acetate (9:1), 1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**3c**, 329 mg, 67%). Compound **3c** (329 mg, 1.06 mmol) was then stirred together with acetyl chloride (500 μL, 7.0 mmol) and Et_3N (1 mL, 7.2 mmol) at room temperature overnight in DCM to yield, after purification on a silica gel column using hexanes–DCM (3:1), **4c** (325 mg, 87%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.37 (6H, s), 2.01 (3H, s), 2.26 (3H, s), 3.93 (3H, s), 4.46 (2H, s), 5.37 (1H, s), 6.11 (1H, d, J = 8.7 Hz), 6.62 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.79 (1H, d, J = 2.4 Hz), 6.87–6.91 (2H, m), 7.22–7.30 (2H, m); ^{13}C NMR ($CDCl_3$) δ 18.7, 21.1, 28.6, 43.0, 55.2, 57.2, 109.5, 111.5, 116.3, 120.6, 120.8, 123.1, 126.7, 127.2, 127.3, 127.4, 130.2, 141.0, 142.1, 156.6, 170.0; ESI-MS: $[M+H]^+$ m/z 352; HRESI-MS: $[M+H]^+$ m/z 352.1912 (calcd 352.1913). Anal. ($C_{22}H_{25}NO_3$) Calcd C, 75.19; H, 7.17; N, 3.99. Found: C, 75.34; H, 7.42; N, 3.77.

6.2.7. 1-(4-Isopropylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (4d)

A solution of **3d** (469 mg, 1.46 mmol), Et_3N (304 μL, 2.19 mmol), acetyl chloride (155 μL, 2.19 mmol), and dichloromethane (10 mL) were stirred overnight under nitrogen. The solvent was evaporated and the residue purified on silica gel using hexanes–dichloromethane (1:1) as the mobile phase to yield 1-(4-isopropylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**4d**, 461 mg, 87%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, d, J = 6.9 Hz), 1.39 (6H, s), 2.03 (3H, s), 2.27 (3H, s), 2.84–2.99 (1H, m), 4.49 (2H, s), 5.39 (1H, s), 6.25 (1H, d, J = 9.0 Hz), 6.65 (1H, dd, J = 2.6, 9 Hz), 6.80 (1H, d, J = 2.6 Hz), 7.19 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 19.1, 21.5, 24.4, 29.0, 34.1, 48.4, 57.5, 112.4, 116.7, 121.1, 123.9, 126.5, 127.0, 127.8, 130.7, 137.1, 141.6, 142.5, 147.6, 170.6; ESI-MS: $[M+Na]^+$ m/z 386; HRESI-MS: $[M+Na]^+$ m/z 386.2094 (calcd 386.2096). Anal. ($C_{24}H_{29}NO_2$) Calcd C, 79.30; H, 8.04; N, 3.85. Found: C, 79.31; H, 8.13; N, 3.81.

6.2.8. 1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride (5a)

Compound **3a**[8] (2.96 g, 10.6 mmol) was dissolved in THF (50 mL) and treated with 1 N HCl (12 mL). The solution was evaporated under reduced pressure, dried overnight under high vacuum, then suspended in dry THF to give **5a** as a white solid (2.77 g, 83%), mp 185–187 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (3H, s), 1.90 (3H, s), 2.11 (3H, d, J = 1.3 Hz), 4.15 (1H, dd, J = 11.4 and 8.2 Hz), 4.59 (1H, dd, J = 11.5 and 3.4 Hz), 5.57 (1H, d, J = 1.0 Hz), 6.56 (2H, d, J = 1.2 Hz), 6.87 (1H, s), 7.33–7.03 (5H, m), 12.76 (1H, br s); EIMS m/z 208.9. Anal. ($C_{19}H_{21}NO{\cdot}HCl$) Calcd C, 72.25; H, 7.02; N, 4.43; Cl, 11.23. Found: C, 72.00; H, 6.96; N, 4.40; Cl, 11.09.

6.2.9. 1-(3,4-Difluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride (5b)

Compound **3b** (186 mg, 0.59 mmol) was mixed with methanolic HCl (7 mL) under nitrogen. After consumption of the starting material, the solvent was evaporated and THF (10 mL) was added. The mixture was kept at 0 °C overnight and the precipitate collected and dried under vacuum to yield **5b** (138 mg, 66%) as an off-white solid, mp 174–175 °C. ^{1}H NMR ($CDCl_3$) δ 1.49 (3H, br s), 1.86 (3H, br s), 2.13 (3H, br s), 4.10 (1H, br s), 4.54 (1H, br s), 5.58 (1H, br s), 7.10–6.60 (6H, m), 8.75 (1H, br s); ^{13}C NMR (methanol-d_4) δ 18.2, 24.4, 54.5, 65.3, 113.6, 115.5, 118.7 (d, $^2J_{CF}$ = 18 Hz), 122.3 (d, $^2J_{CF}$ = 17.3 Hz), 127.1, 129.0, 130.4, 131.1, 133.2, 151.0 (d, $^3J_{CF}$ = 12 Hz), 151.3 (d, $^1J_{CF}$ = 246.75 Hz), 151.4 (d, $^1J_{CF}$ = 246.75 Hz), 154.2 (d, $^3J_{CF}$ = 12 Hz), 160.9; ESI-MS: $[M-Cl]$ m/z 316; HRESI-MS: $[M-Cl]$ m/z 316.1506 (calcd 316.1513). Anal. ($C_{19}H_{20}ClF_2NO$) Calcd C, 64.86; H, 5.73; Cl, 10.08; N, 3.98. Found: C, 64.42; H, 5.99; N, 3.83; Cl, 9.56.

6.2.10. 1-(2-Methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride (5c)

Compound **3c** (332 mg, 1.07 mmol) was mixed with methanolic HCl (10 mL). After consumption of the starting material, the solvent was evaporated and THF (20 mL) was added. The mixture was kept at 0 °C overnight and the precipitate collected and dried under vacuum overnight to yield **5c** as an off-white solid (267 mg, 72%), mp 195–198 °C. ^{1}H NMR (methanol-d_4) δ 1.44 (3H, s), 1.85 (3H, s), 2.19 (3H, s), 3.54 (3H, s), 4.52 (1H, d, J = 11.9 Hz), 4.67 (1H, d, J = 11.9 Hz), 5.84 (1H, s), 6.34 (2H, m), 6.79 (1H, d, J = 8.4 Hz), 6.88 (2H, m), 7.15 (1H, d, J = 7.5 Hz), 7.32 (1H, dd, J = 8.1 Hz); ^{13}C NMR (methanol-d_4) δ 18.3, 24.1, 24.7, 56.1, 65.1, 111.9, 112.9, 114.9, 119.6, 121.8, 124.1, 126.8, 128.6, 131.9, 133.3, 133.6, 134.6, 160.3, 160.7; ESI-MS: $[M-Cl]$ m/z 310; HRESI-MS: $[M-Cl]$ m/z 310.1802 (calcd 310.1807). Anal. ($C_{20}H_{24}ClNO_2$) Calcd C, 69.45; H, 6.99; Cl, 10.25; N, 4.05. Found: C, 68.68; H, 7.03; N, 3.98; Cl, 10.14.

6.2.11. 5,7-Dichloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (7)

A one step Lewis acid catalyzed reaction was used to prepare this compound. A mixture of 4-amino-2,6-dichlorophenol (5.0 g,

28 mmol), excess acetone (20 mL) and $BF_3{\cdot}OEt_2$ (3.5 mL, 28 mmol) in toluene (25 mL) was heated to reflux for 72 h. The reaction was quenched with ice water and extracted with DCM, then the residue was purified on silica gel column using hexanes–ethyl acetate (85:15) to yield 5,7-dichloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**7**, 1.64 g, 23%) as yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.13 (6H, s), 2.18 (3H, s), 4.3 (1H, br s), 5.45 (1H, s), 6.62 (1H, s), 8.75 (1H, br s).

6.2.12. 1-Benzyl-5,7-dichloro-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (8)

A mixture of **7** (500 mg, 1.94 mmol), benzyl bromide (497 mg, 2.91 mmol), and Et_3N (404 μL, 2.90 mmol) were heated to reflux overnight in toluene to yield 1-benzyl-5,7-dichloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol that was further acetylated as described above to yield, after purification on silica gel column using hexanes–DCM (4:1), **8** (570 mg, 75%) as yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$) δ 1.34 (6H, s), 2.35 (3H, s), 2.36 (3H, s), 4.50 (2H, s), 5.54 (1H, s), 6.41 (1H, s), 7.27–7.36 (5H, m); ^{13}C NMR ($CDCl_3$) δ 20.3, 23.2, 27.2, 48.5, 56.0, 112.2, 121.8, 125.5, 126.1, 126.8, 127.1, 128.7, 128.8, 134.5, 135.0, 138.3, 144.8, 168.3; $[M+Na]^+$ *m/z* 412; HRESI-MS: $[M+Na]^+$ *m/z* 412.0830 (calcd 412.0847). Anal. ($C_{21}H_{21}Cl_2NO_2$) Calcd C, 64.62; H, 5.42; Cl, 18.17; N, 3.59. Found: C, 64.66; H, 5.46; Cl, 18.00; N, 3.61.

6.2.13. 1,2-Dihydro-6-methoxy-2,2,4,8-tetramethylquinoline (10a)

4-Methoxy-2-methyl aniline (**9a**, 5 g, 36.4 mmol), acetone (5 mL), and iodine (922 mg, 3.64 mmol) were heated to reflux in toluene (25 mL) for 24 h. The solvent was evaporated and the residue purified on silica gel using hexanes–ethyl acetate (9:1) as the mobile phase to yield **10a** (1 g, 13%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.22 (6H, s), 1.90 (3H, s), 2.06 (3H, s), 3.65 (3H, s), 4.53 (1H, s), 5.35 (1H, s), 6.46–6.53 (2H, m).

6.2.14. 1,2-Dihydro-6,8-dimethoxy-2,2,4-trimethylquinoline (10b)

2,4-Dimethoxy aniline (**9b**) (2.0 g, 13.06 mmol), acetone (5 mL), and iodine (330 mg, 1.31 mmol) were heated to reflux in toluene (25 mL) for 24 h. The solvent was evaporated and the residue purified on silica gel using hexanes–ethyl acetate (10:1) as the mobile phase to yield 1,2-dihydro-6,8-dimethoxy-2,2,4-trimethylquinoline (**10b**, 722 mg, 24%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.20 (6H, s), 1.92 (3H, s), 3.68 (3H, s), 3.76 (3H, s), 4.47 (1H, s), 5.33 (1H, s), 6.28 (1H, d, J = 1.8 Hz), 6.42 (1H, d, J = 1.8 Hz).

6.2.15. 7-Chloro-1,2-dihydro-6-methoxy-2,2,4-trimethylquinoline (10c)

3-Chloro-*p*-anisidine **9c** (5.0 g, 31.7 mmol), acetone (6 mL), and iodine (803 mg, 3.17 mmol) were heated to reflux in toluene (30 mL) for 24 h under nitrogen. The solvent was evaporated and the residue purified on silica gel using hexanes–diethyl ether (9:1) as the mobile phase to yield **10c** (2.33 g, 31%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.17 (6H, s), 1.91 (3H, s), 3.73 (3H, s), 5.32 (1H, s), 5.67 (1H, s), 6.52 (1H, s), 6.72 (1H, s).

6.2.16. 1,2-Dihydro-2,2,4,8-tetramethylquinolin-6-ol (11a)

Compound **10a** (0.966 mg, 4.44 mmol) was heated in 48% HBr (30 mL) under nitrogen to reflux overnight. The mixture was neutralized with aqueous Na_2CO_3 (1 M) then extracted three times with chloroform. The chloroform extract was washed with brine, the solvent removed and the residue purified on silica gel with hexanes–ethyl acetate (10:1) as the mobile phase to yield the demethylated product, 1,2-dihydro-2,2,4,8-tetramethylquinolin-6-ol (**11a**, 481 mg, 53%), as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.18 (6H, s), 1.84 (3H, s), 1.98 (3H, s), 4.31 (1H, s), 5.29 (1H, s), 6.33 (2H, s), 8.25 (1H, s), mp 134–136 °C.

6.2.17. 1,2-Dihydro-8-methoxy-2,2,4-trimethylquinolin-6-ol (11b)

Compound **10b** (1.67 g, 7.16 mmol) was heated in 48% HBr (30 mL) under nitrogen to reflux for 4 h. The mixture was neutralized with 1 M Na_2CO_3 then extracted three times with chloroform. The chloroform extract was washed with brine and the solvent removed. The residue was purified on silica gel with hexanes–ethyl acetate (2:1) as the mobile phase to yield the 6-O-demethylated product, 1-benzyl-1,2-dihydro-8-methoxy-2,2,4-trimethylquinolin-6-ol (**11b**, 700 mg, 45%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 1.15 (6H, s), 1.83 (3H, s), 3.70 (3H, s), 4.27 (1H, s), 5.29 (1H, s), 6.13 (1H, br s), 6.24 (1H, br s), 8.44 (1H, s).

6.2.18. 7-Chloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (11c)

Compound **10c** (2.39 g, 10.05 mmol) was heated in 48% HBr (20 mL) under nitrogen to reflux for 8 h. The mixture was neutralized with Na_2CO_3 (1 M) then extracted three times with ethyl acetate. The ethyl acetate extract was washed with brine and the solvent removed, then the residue was purified on silica gel with hexanes–ethyl acetate (10:1) as the mobile phase to yield the demethylated compound (**11c**, 500 mg, 22%) as a white solid which turned brown over time. ^{1}H NMR (DMSO-d_6) δ 1.15 (6H, s), 1.84 (3H, s), 5.32 (1H, s), 5.46 (1H, s), 6.44 (1H, s), 6.60 (1H, s), 8.87 (1H, s), mp 138–140 °C.

6.2.19. 1-Benzyl-1,2-dihydro-2,2,4,8-tetramethylquinolin-6-yl acetate (12a)

Compound **11a** (932 mg, 4.6 mmol) was added to benzyl bromide (821 μL, 6.9 mmol), Et_3N (960 μL, 6.9 mmol), and toluene (15 mL). The reaction mixture was heated to reflux for 24 h, the solvent was evaporated and the residue purified on silica gel using hexanes–ethyl acetate (9:1) as the mobile phase to yield the *N*1 benzylated product, 1-benzyl-1,2-dihydro-2,2,4,8-tetramethylquinolin-6-ol (950 mg, 70%), as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.06 (6H, s), 1.88 (3H, s), 2.07 (3H, s), 3.90 (2H, s), 5.40, (1H, s), 6.46 (1H, d, J = 2.7 Hz), 6.52 (1H, d, J = 2.7 Hz), 7.10–7.23 (5H, m), 8.94 (1H, s). 1-Benzyl-1,2-dihydro-2,2,4,8-tetramethylquinolin-6-ol (950 mg, 3.2 mmol), acetyl chloride (382 mg, 4.86 mmol), and Et_3N (676 μL, 4.86 mmol) in dichloromethane (25 mL) were stirred overnight at room temperature. The solvent was evaporated and the residue was purified on a silica gel column using hexanes–ethyl acetate (10:1) as the mobile phase to yield 1-benzyl-1,2-dihydro-2,2,4,8-tetramethylquinolin-6-yl acetate (**12a**, 921 mg, 84%) as a colorless oil which solidified on standing. ^{1}H NMR (DMSO-d_6) δ 1.10 (6H, s), 1.90 (3H, s), 2.19 (3H, s), 2.25 (3H, s), 4.00 (2H, s), 5.50, (1H, s), 6.81–6.85 (2H, m), 7.12–7.28 (5H, m); ^{13}C NMR (DMSO-d_6) δ 18.2, 18.5, 21.1, 27.5, 53.5, 55.0, 114.0, 122.9, 126.4, 127.7, 127.9, 128.9, 131.3, 132.9, 134.1, 141.6, 143.4, 146.1, 169.6; ESI-MS: $[M+Na]^+$ *m/z* 358; HRESI-MS: $[M+Na]^+$ *m/z* 358.1783 (calcd 358.1784). Anal. ($C_{22}H_{25}NO_2$) Calcd C, 78.77; H, 7.51; N, 4.18. Found: C, 78.57; H, 7.54; N, 4.22.

6.2.20. 1-Benzyl-1,2-dihydro-8-methoxy-2,2,4-trimethylquinolin-6-yl acetate (12b)

A mixture of **11b** (533 mg, 2.43 mmol), Et_3N (507 μL, 3.45 mmol), benzyl bromide (462 μL, 3.9 mmol), and toluene (15 mL) were heated to reflux overnight. The solvent was evaporated and the residue purified on silica gel using hexanes–ethyl acetate (9:1) as the mobile phase to yield the *N*1 benzylated product, 1-benzyl-1,2-dihydro-8-methoxy-2,2,4-trimethylquinolin-6-ol (496 mg, 66%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 1.06 (6H, s), 1.86 (3H, s), 3.69 (3H, s), 4.05 (2H, s), 5.39 (1H, s), 6.29 (1H, d, J = 2.4 Hz), 6.35 (1H, d, J = 2.4 Hz), 7.23–7.13 (3H,

m), 7.31–7.33 (2H, m), 9.01 (1H, s). 1-Benzyl-1,2-dihydro-8-methoxy-2,2,4-trimethylquinolin-6-ol (409 mg, 1.32 mmol) was combined with Et_3N (276 μL, 1.98 mmol), acetyl chloride (141 μL, 1.98 mmol), and dichloromethane. The reaction mixture was stirred overnight under nitrogen to yield 1-benzyl-1,2-dihydro-8-methoxy-2,2,4-trimethylquinolin-6-yl acetate (**12b**, 410 mg, 88%) as a colorless oil which solidified on standing. ^{1}H NMR (DMSO-d_6) δ 1.33 (6H, s), 2.14 (3H, s), 2.77 (3H, s), 3.95 (3H, s), 4.46 (2H, s), 5.71 (1H, s), 6.87 (1H, d, J = 2.3 Hz), 6.93 (1H, d, J = 2.3 Hz), 7.36–7.49 (3H, m), 7.56–7.59 (2H, m); ^{13}C NMR (DMSO-d_6) δ 18.4, 21.1, 27.5, 52.9, 54.5, 55.9, 105.6, 108.5, 126.2, 127.4, 127.8, 128.4, 130.1, 132.5, 132.9, 142.8, 145.3, 153.0, 169.5; ESI-MS: [M+Na]$^+$ m/z 374; HRESI-MS: [M+Na]$^+$ m/z 374.1731 (calcd 374.1732). Anal. ($C_{22}H_{25}NO_3$) Calcd C, 75.19; H, 7.17; N, 3.99. Found: C, 74.55; H, 7.25; N, 3.90.

6.2.21. 1-Benzyl-7-chloro-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (12c)

A mixture of **11c** (333 mg, 1.49 mmol), Et_3N (310 μL, 2.23 mmol), and benzyl bromide (265 μL, 2.23 mmol) were dissolved in toluene (10 mL) and stirred overnight under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–ethyl acetate (10:1) as the mobile phase to yield the *N*1-benzylated product, 1-benzyl-7-chloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol, as a brown oil (248 mg, 53%). ^{1}H NMR (DMSO-d_6) δ 1.28 (6H, s), 1.91 (3H, s), 4.38 (2H, s), 5.48 (1H, s), 6.09 (1H, s), 6.68 (1H, s), 7.16–7.34, (5H, m), 9.07 (1H, br s). 1-Benzyl-7-chloro-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (248 mg, 0.79 mmol), Et_3N (165 μL, 1.18 mmol), and acetyl chloride (84 μL, 1.18 mmol) were stirred overnight with dichloromethane (15 mL) under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–dichloromethane (2:1) as the mobile phase to yield 1-benzyl-7-chloro-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**12c**, 260 mg, 93%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, s), 1.89 (3H, s), 2.22 (3H, s), 4.38 (2 H, s), 5.29 (1H, s), 6.16 (1H, s), 6.69 (1H, s), 7.10–7.22 (5H, m); ^{13}C NMR ($CDCl_3$) δ 18.8, 20.8, 28.9, 48.4, 57.7, 112.5, 118.2, 122.7, 125.8, 126.3, 127.0, 127.1, 128.9, 130.7, 137.2, 138.8, 143.2, 169.6; ESI-MS: [M+Na]$^+$ m/z 378; HRESI-MS: [M+Na]$^+$ m/z 378.1241 (calcd 378.1237). Anal. ($C_{21}H_{22}ClNO_2$) Calcd C, 70.88; H, 6.23; N, 3.94. Found: C, 70.78; H, 6.16; N, 3.83, mp 147–149 °C.

6.2.22. 7-Bromo-1,2-dihydro-6-methoxy-2,2,4-trimethylquinoline (14)

A solution of 3-bromo-*p*-anisidine (**13**, 2.14 g, 10.6 mmol), acetone (8 mL), and iodine (402 mg, 1.59 mmol) was heated to reflux in toluene (30 mL) for 36 h. The reaction mixture was cooled, washed with brine and the solvent removed. The residue was purified on silica gel using hexanes–dichloromethane (1:1) as the mobile phase to yield **14** (1.43 g, 48%) as a brown oil that solidified on standing. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.91 (3H, s), 3.72 (3H, s), 5.35 (1H, s), 5.68 (1H, s), 6.67 (1H, s), 6.69 (1H, s).

6.2.23. 7-(4-(Trifluoromethyl)phenyl)-1,2-dihydro-6-methoxy-2,2,4-trimethylquinoline (15a)

To a solution of **14** (530 mg, 1.88 mmol) dissolved in toluene (10 mL) were added a solution of 4-(trifluoromethyl)phenyl boronic acid (535 mg, 2.82 mmol) in ethanol (2 mL), a 2 M solution of K_2CO_3 (2 mL), and $Pd(PPh_3)_4$ (108 mg, 5 mol %) at room temperature and the mixture was heated to reflux for 3 h. The solvent was removed and the residue purified on silica gel with hexane–dichloromethane (1:1) as mobile phase to yield **15b** (586 mg, 90%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.21 (6H, s), 1.97 (3H, s), 3.68 (3H, s), 5.38 (1H, s), 5.60 (1H, s), 6.51 (1H, s), 6.77 (1H, s), 7.63–7.77 (4H, m).

6.2.24. 1,2-Dihydro-6-methoxy-2,2,4,7-tetramethylquinoline (15b)

Compound **14** (639 mg, 2.26 mmol), methyl boronic acid (163 mg, 2.72 mmol), $Pd(PPh_3)_4$ (159 mg, 6 mol %), Cs_2CO_3 (2.2 g, 6.79 mmol) and a 10:1 mixture of THF/water (22 mL) were heated to reflux for 24 h. The solvent was removed and the residue purified on silica gel with hexanes–dichloromethane (1:1) as the mobile phase to yield **15c** (292 mg, 59%) as a brown oil that solidified on standing. ^{1}H NMR (DMSO-d_6) δ 1.14 (6H, S), 1.89 (3H, s), 2.02 (3H,s), 3.67 (3H, S), 5.22 (1H, s), 5.29 (1H, s), 6.28 (1H, s), 6.54 (1H, s).

6.2.25. 1-Benzyl-7-(4-(trifluoromethyl)phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (16a)

Compound **15a** (289 mg, 0.83 mmol) was heated in 48% HBr (25 mL) under nitrogen for 7 h. The mixture was cooled, neutralized with 1 M Na_2CO_3 (150 mL) and extracted with ethyl acetate. The solvent was removed and the residue purified on silica gel using dichloromethane as the mobile phase to yield the demethylated product, 7-(4-(trifluoromethyl)phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (63 mg, 23%), a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.18 (6H, s), 1.89 (3H, s), 5.37 (2H, s), 6.48 (1H, s), 6.65 (1H, s), 7.72 (4H, s), 8.69 (1H, s). 7-(4-(Trifluoromethyl)phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (250 mg, 0.75 mmol), Et_3N (156 μL, 1.12 mmol), benzyl bromide (134 μL, 1.12 mmol), and toluene (15 mL) were then heated to reflux overnight under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–dichloromethane (1:1) as the mobile phase to yield the *N*1-benzylated product, 1-benzyl-7-(4-(trifluoromethyl)phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (280 mg, 88%), as a yellow sticky oil. ^{1}H NMR (DMSO-d_6) δ 1.33 (6H, s), 1.95 (3H, s), 4.44 (2H, s), 5.53 (1H, s), 6.19 (1H, s), 6.71 (1H, s), 7.15–7.22 (1H, m), 7.27–7.37 (4H, m), 7.42–7.47 (2H, m), 7.57–7.62 (2H, m), 8.85 (1H, s). 1-Benzyl-7-(4-(trifluoromethyl)phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (280 mg, 0.66 mmol), Et_3N (137 μL, 0.99 mmol), and acetyl chloride (70 μL, 0.99 mmol) were then stirred overnight with dichloromethane (10 mL) under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–dichloromethane (1:1) as the mobile phase to yield 1-benzyl-7-(4-(trifluoromethyl) phenyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**16a**, 280 mg, 91%) as a yellow oil that solidified on standing. ^{1}H NMR ($CDCl_3$) δ 1.32 (6H, s), 1.93 (3H, s), 1.97 (3H, s), 4.42 (2H, s), 5.33 (1H, s), 6.11 (1H, s), 6.72 (1H, s), 7.28–7.10 (7H, m), 7.43 (2H, d, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 18.6, 20.8, 28.7, 48.3, 57.4, 113.3, 117.7, 123.9, 124.2 (q, $^1J_{CF}$ = 270.8 Hz), 125.0 (q, $^3J_{CF}$ = 3.75 Hz), 126.2, 126.8, 127.0, 128.6, 128.9, 129.5 (q, $^2J_{CF}$ = 32.3 Hz), 130.9, 132.4, 138.0, 139.1, 142.1, 142.2, 170.0; ESI-MS: [M+H]$^+$ m/z 466; HRESI-MS: [M+H]$^+$ m/z 466.1977 (calcd 466.1994). Anal. ($C_{28}H_{26}F_3NO_2$) Calcd C, 72.24; H, 5.63; N, 3.01. Found: C, 72.18; H, 5.69; N 3.00.

6.2.26. 1-Benzyl-1,2-dihydro-2,2,4,7-tetramethylquinolin-6-yl acetate (16b)

Compound **15b** (148 mg, 0.68 mmol) was heated in 48% HBr (20 mL) under nitrogen for 2 h. The mixture was cooled, neutralized with 1 M Na_2CO_3 (100 mL) and extracted with ethyl acetate. The solvent was removed and the residue purified on silica gel with hexanes–ethyl acetate (10:1) as the mobile phase to yield the demethylated product, 1,2-dihydro-2,2,4,7-tetramethylquinolin-6-ol (67 mg, 49%), as a white solid that turned brown on standing. ^{1}H NMR (DMSO-d_6) δ 1.13 (6H, s), 1.82 (3H, s), 1.99 (3H, s), 5.06 (1H, s), 5.20 (1H, s), 6.22 (1H, s), 6.44 (1H, s), 8.14 (1H, s). 1,2-Dihydro-2,2,4,7-tetramethylquinolin-6-ol (225 mg, 1.11 mmol), Et_3N

(231 μL, 1.66 mmol), benzyl bromide (197 μL, 1.66 mmol), and toluene (10 mL) were then heated to reflux overnight under nitrogen. The solvent was evaporated and the residue purified on silica gel with hexanes–dichloromethane (1:1) as the mobile phase to yield the *N*1 benzylated product, 1-benzyl-1,2-dihydro-2,2,4,7-tetramethylquinolin-6-ol (243 mg, 75%), as a brown oil. ^{1}H NMR (DMSO-d_6) δ 1.26 (6H, s), 1.87 (3H, s), 1.90 (3H, s), 4.38 (2H, s), 5.37 (1H, s), 6.00 (1H, s), 6.53 (1H, s), 7.14–7.35 (5H, m), 8.28 (1H, s). 1-Benzyl-1,2-dihydro-2,2,4,7-tetramethylquinolin-6-ol (235 mg, 0.80 mmol), Et_3N (167 μL, 1.20 mmol), and acetyl chloride (85 μL, 1.20 mmol) were then stirred overnight with dichloromethane (10 mL) under nitrogen. The solvent was evaporated and the residue purified on silica gel using hexanes–ethyl acetate (10:1) as the mobile phase to yield 1-benzyl-1,2-dihydro-2,2,4,7-tetramethylquinolin-6-yl acetate (**16b**, 168 mg, 63%) as a yellow oil that solidified on standing. ^{1}H NMR (DMSO-d_6) δ 1.31 (6H, s), 1.81 (3H, s), 1.90 (3H, s), 2.21 (3H, s), 4.48 (2H, s), 5.42 (1H, s), 6.08, (1H, s), 6.65 (1H, s), 7.14–7.35 (5H, s); ^{13}C NMR (DMSO-d_6) δ 16.4, 18.4, 20.7, 28.2, 47.5, 57.0, 113.4, 116.9, 121.3, 126.3, 126.6, 128.6, 128.9, 130.1, 139.8, 140.1, 141.8, 169.6; ESI-MS: $[M+H]^+$ *m/z* 336; HRESI-MS: $[M+H]^+$ *m/z* 336.1964 (calcd 336.1964). Anal. ($C_{22}H_{25}NO_2$) Calcd C, 78.77; H, 7.51; N, 4.18. Found: C, 78.04; H, 7.52; N, 4.13.

6.2.27. 4-Methoxy-*N*-(2-methylbut-3-yn-2-yl)benzenamine (19)

A mixture of *p*-anisidine (**17**, 4.95 g, 40.2 mmol), 3-chloro-3-methylbut-1-yne (**18**, 4.59 g, 44.8 mmol), copper(I) chloride (403 mg, 4.08 mmol), and copper powder (316 mg, 4.98 mmol) in triethylamine (80 mL) was stirred overnight. The mixture was filtered through Celite and the Celite pad was washed with ethyl acetate (300 mL). Combined filtrates were washed twice with concentrated NH_4OH solution followed by saturated NaCl solution, dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed on silica eluting with hexanes/ethyl acetate (4:1) to give an orange oil (5.99 g, 79%). ^{1}H NMR (DMSO-d_6) δ 1.45 (6H, s), 3.18 (1H, s), 3.65 (3H, s), 5.06 (1H, s), 6.73 (2H, d, *J* = 8.9 Hz), 6.87 (2H, d, *J* = 8.9 Hz).

6.2.28. 4-Methoxy-*N*-(2-methyl-4-phenylbut-3-yn-2-yl) benzenamine (20)

A mixture of **19** (2.17 g, 10.1 mmol), 4-iodobenzene (2.47 g, 12.1 mmol), bis-(triphenylphosphine)palladium(II) chloride (212 mg, 0.302 mmol), and copper(I) chloride (75.9 mg, 0.399 mmol) in triethylamine (30 mL) was stirred at 60 °C until the reaction was complete by HPLC (about 3 h). The reaction mixture was filtered through silica gel and the silica pad was washed with ethyl acetate. Filtrates were washed with saturated NaCl solution, dried ($MgSO_4$), concentrated, and chromatographed on silica eluting with hexanes–ethyl acetate (4:1) to give an oil (2.15 g, 81%). ^{1}H NMR (DMSO-d_6) δ 1.55 (6H, s), 3.75 (3H, s), 5.17 (1H, br s), 6.76 (2H, d, *J* = 9.0 Hz), 6.94 (2H, d, *J* = 9.0 Hz), 7.34 (5H, s).

6.2.29. 1,2-Dihydro-6-methoxy-2,2-dimethyl-4-phenylquinoline (21)

A mixture of **20** (8.46 g, 32.1 mmol) and copper(I) chloride (3.20 g, 32.3 mmol) in toluene (120 mL) was stirred at reflux overnight. The mixture was filtered through Celite and the Celite pad was washed with ethyl acetate. Combined filtrates were washed with NH_4OH, followed by 10% NaCN, then by brine. The extract was dried ($MgSO_4$), concentrated, and purified on a column of silica eluting with hexanes–ethyl acetate (4:1) to give an oil (5.02 g, 59%). ^{1}H NMR (DMSO-d_6) δ 1.25 (6H, s), 3.52 (3H, s), 5.47 (1H, s), 5.59 (1H, br s), 6.28 (1H, d, J = 2.6 Hz), 6.53 (1H, d, *J* = 8.5 Hz), 6.61 (1H, dd, *J* = 8.6 and 2.7 Hz), 7.27 (2H, m), 7.39 (3H, m).

6.2.30. 1-Benzyl-1,2-dihydro-2,2-dimethyl-4-phenylquinolin-6-yl acetate (22)

A mixture of **21** (5.02 g, 18.9 mmol) in 48% HBr (60 mL) was stirred at reflux for 1 h. The mixture was cautiously poured over a mixture of ice and an excess of $NaHCO_3$. This mixture was extracted with ethyl acetate followed by CH_2Cl_2. The extracts were washed with brine, dried ($MgSO_4$), concentrated, and chromatographed on silica, eluting with hexanes–ethyl acetate (2:1). The crude product was recrystallized from hexanes–ethyl acetate to give 1,2-dihydro-2,2-dimethyl-4-phenylquinolin-6-ol (2.37 g, 49%) as a white solid, mp 179–180 °C. ^{1}H NMR (DMSO-d_6) δ 1.23 (6H, s), 5.36 (1H, br s), 5.42 (1H, d, *J* = 1.3 Hz), 6.21 (1H, d, *J* = 2.2 Hz), 6.42 (2H, m), 7.25 (2H, m), 7.38 (3H, m), 8.29 (1H, s); ESI(+) MS *m/z* 252.8. Anal. ($C_{17}H_{17}NO{\cdot}0.1H_2O$) Calcd C, 80.66; H, 6.80; N, 5.53. Found: C, 80.76; H, 6.80; N, 5.54. A mixture of 1,2-dihydro-2,2-dimethyl-4-phenylquinolin-6-ol (2.30 g, 9.15 mmol), benzyl bromide (2.0 mL, 16.8 mmol), and triethyl amine (2.0 mL, 14.3 mmol) in toluene (90 mL) was then stirred at reflux overnight. The reaction mixture was diluted with water. Layers were separated and the aqueous layer was extracted with ethyl acetate. Combined organic layers were washed with brine, dried ($MgSO_4$), concentrated, and chromatographed on a column of silica, eluting with hexanes–ethyl acetate (4:1) to give 1-benzyl-1,2-dihydro-2,2-dimethyl-4-phenylquinolin-6-ol as an oil that solidified to a glass (3.23 g, 104%). ^{1}H NMR (DMSO-d_6) δ 1.37 (6H, s), 4.43 (2H, s), 5.57 (1H, s), 6.17 (1H, d, *J* = 8.7 Hz), 6.27 (1H, d, *J* = 2.8 Hz), 6.33 (1H, dd, *J* = 8.6 and 2.8 Hz), 7.20 (t, *J* = 7.2 Hz, 1H), 7.32 (m, 4H), 7.42 (m, 5H), 8.41 (s, 1H). Anal. ($C_{24}H_{23}NO{\cdot}0.25H_2O$) Calcd C, 83.32; H, 6.85; N, 4.05. Found: C, 83.07; H, 6.95; N, 3.97. A mixture of 1-benzyl-1,2-dihydro-2,2-dimethyl-4-phenylquinolin-6-ol (2.02 g, 5.92 mmol) and acetyl chloride (1.0 mL, 14.01 mmol) in dichloromethane (50 mL) maintained at 0 °C was then treated with triethylamine (1.5 mL, 10.8 mmol). The mixture was stirred overnight at room temperature, then concentrated and chromatographed on a column of silica, eluting with hexanes–ethyl acetate (10:1) to give **22** as a glass (1.97 g, 86%), mp 57–59 °C. ^{1}H NMR (DMSO-d_6) δ 1.43 (6H, s), 2.10 (3H, s), 4.55 (2H, s), 5.61 (1H, s), 6.28 (1H, d, *J* = 8.9 Hz), 6.42 (1H, d, *J* = 2.8 Hz), 6.64 (1H, dd, *J* = 8.8 and 2.7 Hz), 7.24 (1H, m), 7.38 (9H, m); ESI(+) MS *m/z* 384.6. Anal. ($C_{26}H_{25}NO_2{\cdot}0.2H_2O$) Calcd C, 80.67; H, 6.61; N, 3.62. Found: C, 80.40; H, 6.67; N, 3.51.

6.3. Preparation of compounds for testing

Compounds were dissolved in 100% dimethylsulfoxide (DMSO) and finally diluted in culture medium prior to the in vitro assay. The DMSO concentration never exceeded 1% in such assays. For in vivo experiments, the compounds were dissolved in DMSO and further diluted with distilled H_2O to a final DMSO concentration of 10% prior to each injection into the animals.

6.4. In vitro growth inhibition assay of *T. b. rhodesiense* (STIB900)

IC_{50} values were determined using the Alamar Blue assay and were carried out three times independently and in duplicate. Briefly, the compounds were tested in Minimum Essential Medium with Earle's salts, supplemented as previously described[28] with the following modifications: 2-mercaptoethanol 0.2 mM, sodium pyruvate 1 mM, hypoxanthine 0.5 mM, and 15% heat-inactivated horse serum. Serial drug dilutions were prepared in 96-well microtiter plates and each well inoculated with 2000 bloodstream forms and incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar Blue (12.5 mg resazurin (Sigma) dissolved in 100 mL of phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated

for additional 2–5 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SOFTMAXPRO software.

6.5. In vitro growth inhibition assay of *T. b. brucei* (s427)

The potency of compounds against bloodstream form *T. b. brucei* (s427) was assessed as outlined previously.[29] In brief, 100 μL of late log phase parasites cultured in HMI-9 medium[30] were incubated in the presence or absence of compounds in 96-well plates at an initial density of 10^5 cells/mL at 37 °C in a humidified 5% CO_2 atmosphere for 72 h. Twenty-five microliters of 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was then added to each well, followed by incubation of the plate at 37 °C as described for an additional 2 h. One hundred microliters of 20% SDS lysis buffer in 50% aqueous dimethyl formamide was added to each well and plates were re-incubated as before for 3–6 h. Optical densities were measured at 570 nm using a SpectraMax Plus microplate reader (Amersham Biosciences, Piscataway, NJ). IC_{50} values were determined with the aid of the software program SOFTMAXPRO (Amersham Biosciences, dose–response equation $y = [(a - d)/(1 + (x/c)^b)] + d$, where x = the drug concentration, y = absorbance at 570 nm, a = upper asymptote, b = slope, c = IC_{50}, and d = lower asymptote).

6.6. In vitro cytotoxicity assay (L6 rat myoblast cells)

IC_{50} values were determined using the Alamar Blue assay[31] and were carried out three times independently and in duplicate. Briefly, 4000 L6 cells were seeded in RPMI 1640 medium supplemented with L-glutamine 2 mM, HEPES 5.95 g/L, $NaHCO_3$ 2 g/L, and 10% fetal bovine serum in 96-well microtiter plates. The serial drug dilutions were incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar Blue (12.5 mg resazurin (Sigma) dissolved in 100 mL of phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength 536 nm and an emission wavelength 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SOFTMAXPRO software.

6.7. STIB900 acute mouse model of trypanosomiasis

Experiments were performed as previously reported[5] with minor modifications. Briefly, female NMRI mice were infected intraperitoneally (ip) with 2×10^4 STIB900 bloodstream forms. Experimental groups of four mice were treated ip with test compounds on four consecutive days from day 1 to day 4 post infection. A control group was infected but remained untreated. The tail blood of all mice was checked for parasitemia until 60 days post infection. Day of parasitemia relapse of animals was recorded to calculate the mean relapse time in days. Surviving and aparasitemic mice at day 60 were considered as cured and then euthanized.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.003.

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