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Antitrypanosomal Activity of 1,2-Dihydroquinolin-6-ols and Their Ester Derivatives

Jean Fotie,[†,⊥] Marcel Kaiser,[‡] Dawn A. Delfín,[†] Joshua Manley,[†] Carolyn S. Reid,[†] Jean-Marc Paris,[§] Tanja Wenzler,[‡] Louis Maes,[∥] Kiran V. Mahasenan,[†] Chenglong Li,[†] and Karl A. Werbovetz*[,†]

[†]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210,* [‡]*Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, CH-4002 Basel, Switzerland,* [§]*Laboratoire de Biochimie, École Nationale Supérieure de Chimie de Paris, UMR CNRS 7573, 11 Rue Pierre et Marie Curie, 75005 Paris, France, and* [∥]*Faculty of Pharmaceutical, Biomedical, and Veterinary Sciences, University of Antwerp, Antwerp, Belgium.* [⊥]*Present address: Department of Chemistry and Physics, 217 Pursley Hall, Southeastern Louisiana University, Hammond, Louisiana 70402.*

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The current chemotherapy for second stage human African trypanosomiasis is unsatisfactory. A synthetic optimization study based on the lead antitrypanosomal compound 1,2-dihydro-2,2,4-trimethylquinolin-6-yl 3,5-dimethoxybenzoate (TDR20364, **1a**) was undertaken in an attempt to discover new trypanocides with potent in vivo activity. While 6-ether derivatives were less active than the lead compound, several *N*1-substituted derivatives displayed nanomolar IC_{50} values against *T. b. rhodesiense* STIB900 in vitro, with selectivity indexes up to >18000. 1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**10a**) displayed an IC_{50} value of 0.014 μM against these parasites and a selectivity index of 1700. Intraperitoneal administration of **10a** at 50 (mg/kg)/day for 4 days caused a promising prolongation of lifespan in *T. b. brucei* STIB795-infected mice (>14 days vs 7.75 days for untreated controls). Reactive oxygen species were produced when *T. b. brucei* were exposed to **10a** in vitro, implicating oxidative stress in the trypanocidal mode of action of these 1,2-dihydroquinoline derivatives.

Introduction

Human African trypanosomiasis (HAT[a]) or sleeping sickness is caused by *Trypanosoma brucei* subspecies, parasites that are transmitted by the tsetse fly. Early symptoms of HAT include malaise and irregular fevers as well as enlarged lymph glands and spleen. Parasites are later found in the central nervous system (CNS), where they cause progressive neurological disorders ending in death in the absence of chemotherapy.[1] Approximately 17 500 cases of sleeping sickness were reported for the year 2004, although the disease incidence is probably 3−4 times higher[2] (for a review on the current epidemiological situation, see Simarro et al.[3]). Pentamidine and suramin are used to treat the first phase but do not cross the blood−brain barrier efficiently and thus are not used to treat second stage disease. Melarsoprol, an organoarsenical, is used against second stage disease but causes reactive encephalopathy in 5−10% of cases, half of which are fatal.[4,5] Failure rates with melarsoprol have risen to as high as 30% in some parts of Africa.[4] Eflornithine is an alternative to melarsoprol treatment for second stage *T. b. gambiense* infection but is ineffective against *T. b. rhodesiense* and must be administered by slow intravenous infusion in high doses over a long course.[4,6] Recently, eflornithine failure in sub-Saharan Africa has been observed (Jean Jannin, personal communication). Eflornithine is currently being studied in combination with the anti-Chagas disease drug nifurtimox against second stage infections with *T. b. gambiense* in the hopes of extending the usefulness of the former drug.[7] While several other classes of molecules have displayed activity against African trypanosomes, no other drugs are approved for use against second stage disease. A safe, effective, and inexpensive replacement for melarsoprol and eflornithine against second stage HAT is desperately needed.

As part of the continuing search for novel entities with better efficacy and lower toxicity for the treatment of second stage HAT, a screening program sponsored by the World Health Organization's Special Programme for Research and Training in Tropical Diseases (WHO/TDR) was carried out at Tibotec Belgium and the Swiss Tropical Institute (STI), using compounds sourced from Specs (www.specs.net). This program identified a series of dihydroquinolines with promising antitrypanosomal activity, of which 1,2-dihydro-2,2,4-trimethylquinolin-6-yl 3,5-dimethoxybenzoate (TDR20364, **1a**) was studied in the greatest detail. This agent displayed potent and selective antitrypanosomal activity against the *T. b. rhodesiense* strain STIB900 (IC_{50} = 0.054 μM, SI = 300). However, no cures were achieved and no prolongation of survival was observed in *T. b. brucei* (STIB795) infected NMRI mice treated with **1a** at a daily dose of 50 mg/kg/day

*To whom correspondence should be addressed. Phone: (614) 292-5499. Fax: (614) 292-2435. E-mail: werbovetz.1@osu.edu.

[a] Abbreviations: CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester; CNS, central nervous system; COSY, correlation spectroscopy; CYP450, cytochrome P450; DCM, dichloromethane; ESI-MS, electrospray ionization mass spectrometry; Et_3N, triethylamine; FDA, Food and Drug Administration; HAT, human African trypanosomiasis; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum coherence; HRESI-MS, high resolution electrospray ionization mass spectrometry; MLSP, melarsoprol; PPT, podophyllotoxin; ROS, reactive oxygen species; SI, selectivity index; STI, Swiss Tropical Institute; WHO/TDR, World Health Organization's Special Programme for Research and Training in Tropical Diseases.

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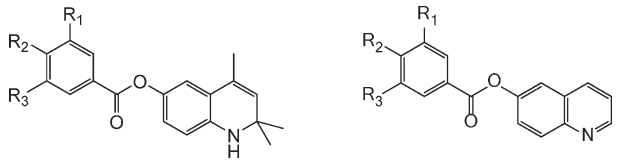


1a $R_1 = R_3 = OMe$, $R_2 = H$
1b $R_1 = H$, $R_2, R_3 = O\text{-}CH_2\text{-}O$

2a $R_1 = R_3 = OMe$, $R_2 = H$
2b $R_1 = H$, $R_2, R_3 = O\text{-}CH_2\text{-}O$

Figure 1. Structures of **1a**, **1b**, **2a**, and **2b**.

ip for 4 days. Nonetheless, the outstanding in vitro antitrypanosomal activity of **1a** suggested that this compound could serve as a hit for the identification of new antitrypanosomal drug candidates. The compound series was assigned to The Ohio State University by WHO/TDR for medicinal chemistry optimization with parasitology support from STI.

We report here initial lead identification studies based on **1a** that resulted in the preparation, characterization, and evaluation of a series of 1,2-dihydroquinoline derivatives for their antitrypanosomal activity. The in vivo evaluation of some of these compounds as well as a preliminary investigation of their trypanocidal mechanism of action is also described.

Chemistry

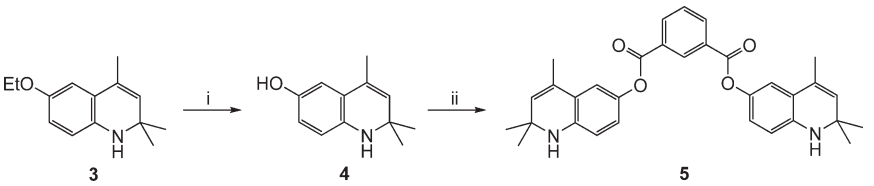
Quinolin-6-yl benzoate derivatives **2a** and **2b** were obtained by esterification of commercially available 6-hydroxyquinoline with 3,5-dimethoxybenzoyl chloride (readily prepared by heating 3,5-dimethoxybenzoic acid to reflux in thionyl chloride[8]) and with commercially available piperonyloyl chloride, respectively (see Figure 1 for structures of **1a**, **1b**, **2a**, and **2b**). 1,2-Dihydro-2,2,4-trimethylquinolin-6-ol (**4**) was obtained by heating commercially available ethoxyquin (**3**) to reflux in 48% HBr.[9] Compound **4** was esterified with 3,5-dimethoxybenzoyl chloride, piperonyloyl chloride, or isophthaloyl dichloride (see Scheme 1) in dichloromethane (DCM), using triethylamine (Et_3N) as base, to afford compounds **1a**, **1b**, or **5**, respectively.

Compound **4** also served as a versatile precursor for the synthesis of 1,2-dihydro-2,2,4-trimethylquinoline derivatives possessing an ether linkage at the 6-position (**6a**−**g**, **7**) and *N*1-benzylated 1,2-dihydro-2,2,4-trimethylquinolin-6-ol derivatives (**9a**−**o**). Selective modification of **4** occurs either at the 6-*O* atom by using an appropriate electrophile in the presence of NaH/DMF to yield 6-*O*-benzylated derivatives (Scheme 2)[10] or at *N*1 with an appropriate electrophile in the presence of Et_3N/toluene to yield the 6-hydroxy-*N*1-substituted derivatives (Scheme 3). This latter reaction was incomplete when acetone or THF were used as solvent. Employing more strongly basic conditions (NaH/DMF) affords not only the 6-*O*-substituted derivatives (70−85%) as major products but also the corresponding *N*1,O-disubstituted compounds (**8a**−**g**) as side products (see Scheme 2). Three of these side products (**8e**−**g**) were isolated, characterized, and tested for their biological activity. Furthermore, the *N*1-benzylated compounds were very susceptible to auto-oxidation, as they turned to a dark, sticky gum within a few hours after preparation regardless of the synthetic and storage conditions. Despite the fact that a rapid color change was observed with **9a** and that a satisfactory elemental analysis could not be obtained for this compound, no changes in the ^1H NMR spectrum of **9a** were observed when the pure material was stored at room temperature in an open air environment for 6 days. Only **11a** and **11b**, which were prepared as described for compounds **9a**−**o** and obtained as brown needles, were stable as free alcohols, while the other compounds required protection by either acetylation or benzoylation to avoid a color change and to permit satisfactory elemental analysis. Accordingly, the *N*1-benzylated derivatives (**9a**−**o**), acetyl chloride, and Et_3N were allowed to stir at room temperature overnight in THF or DCM under nitrogen to yield **10a**−**o**. Reaction of 1,2-dihydro-2,2,4-trimethylquinolin-6-ols **9a**, **9e**, and **9f** with 3,5-dimethoxybenzoyl chloride yielded **12a**−**c**, while reaction of **9a** with benzoyl chloride, 2-phenylacetyl chloride, trimethylacetyl chloride, octanoyl chloride, and cyclohexane carboxylic acid chloride yielded **13a**−**e**, respectively. The 6-*O*,*N*1-diacetylated derivative (**15a**) and the *N*1-methylated 6-*O*-acetyl analogue (**15b**) were also prepared. Compound **15a** was obtained by heating a mixture of **4**, acetyl chloride, and NaH in THF to reflux to achieve diacetylation, while **15b** was obtained by heating 1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (**14**) with iodomethane in refluxing toluene in the presence of Et_3N as base (the structures for **11a**, **11b**, **12a**−**c**, **13a**−**e**, **15a**, and **15b** are in Figure 2).

6-*O*-Benzylated and *N*1-benzylated derivatives can be easily differentiated by their ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum of 6-*O*-benzylated derivatives, a singlet of one proton around δ 5.40−5.60 ppm (NH) and another singlet of two protons around δ 4.90−4.98 ppm (CH_2−O) can be observed in addition to the aromatic protons, but no signal around δ 8.30−8.50 ppm attributable to a phenolic hydroxyl group is present. However, the ^1H NMR spectrum of *N*1-benzylated derivatives displays a singlet around δ 4.40−4.60 ppm (CH_2−N) and a hydroxyl resonance (around δ 8.30−8.50 ppm) but no NH signal. These observations were confirmed by 2D NMR spectroscopy comparing compound **6a** as a representative 6-*O*-benzylated derivative and **11a** as a representative *N*1-modified derivative. The heteronuclear multiple bond correlation (HMBC) spectrum of **11a** displayed key correlations between the protons at δ 4.41 ppm (benzylic protons) and the quaternary carbon (C-2) at δ 56.8 ppm and between the same protons and the aromatic quaternary carbon (C-8a) at δ 137.2 ppm (see Figure 3). No correlation was observed between these benzylic protons and the phenolic carbon (C-6) at δ 148.8 ppm. These data demonstrate that the methylpyridyl side chain is bound to the *N*1-atom. Such correlations between the benzylic protons and C-2 and C-8a were absent in the HMBC spectrum of **6a**. Instead, a correlation between the benzylic protons and the phenolic carbon at δ 148.5 ppm was observed (see Figure 3), confirming that the benzyl side chain is linked at the 6-*O* atom.

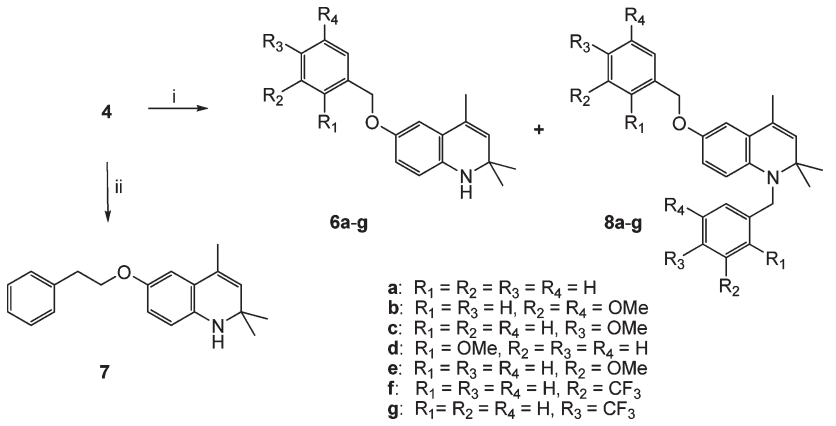
Essential precursors 1,2-dihydro-2,2,4-trimethylquinoline (**16a**) and 1,2-dihydro-2,2,4-trimethylquinolin-7-ol (**16b**) were not commercially available, so these compounds were prepared in our laboratory. Dihydroquinolines have been synthesized via a variety of methods.[11−14] We chose to prepare **16a** and **16b** through a modified Skraup cyclization, which involves reaction of the appropriate aniline derivative and a ketone in the presence of catalytic iodine.[15−18] As an adaptation of this method, a mixture of the appropriate aniline derivative and acetone in toluene was heated to reflux for 24 h in the presence of a catalytic quantity of iodine to provide the corresponding dihydroquinoline. The methyl group on the 7-*O* atom (if present) was removed using BBr_3 in DCM.[19,20] This was achieved by allowing the reaction mixture initially maintained at −78 °C to warm to room temperature. *N*-Substitution and acetylation were achieved as reported above to provide **17a** and **17b** (Scheme 4).

Scheme 1[a]



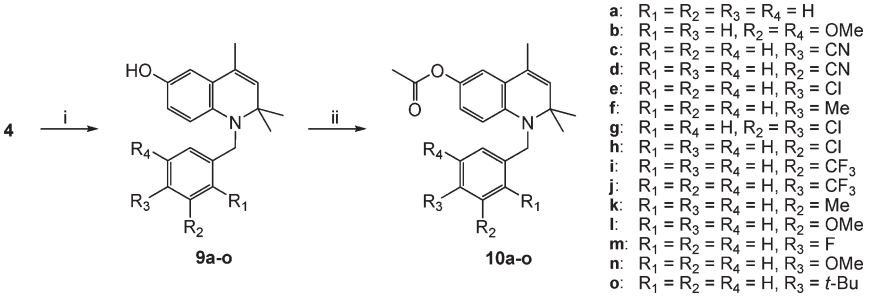
[a] Reagents and conditions: (i) HBr, reflux, 16 h; (ii) isophthaloyl dichloride, DCM, Et_3N.

Scheme 2[a]



[a] Reagents and conditions: (i) benzyl bromide derivative, NaH, DMF, room temp, 48 h; (ii) 2-bromoethylbenzene, NaH, DMF, room temp, 48 h.

Scheme 3[a]



[a] Reagents and conditions: (i) benzyl bromide derivative, Et_3N, toluene, reflux, overnight; (ii) CH_3COCl, Et_3N, DCM, room temp, overnight.

The 1,2-dihydro-2,2-dimethylquinoline core was prepared by reaction between the appropriate aniline derivative and 3-chloro-3-methyl-1-butyne in Et_3N in the presence of copper metal and copper chloride to yield the corresponding *N*-(2-methylbut-3-yn-2-yl)arylamine (**18**).[18] Ring closure was performed in toluene in the presence of copper chloride to yield the desired 1,2-dihydro-2,2-dimethylquinoline.[18,21,22] *O*-Dealkylation, *N*-substitution, and *O*-acetylation (if applicable) were achieved using the same conditions as reported above (Scheme 5).

Antitrypanosomal Activity

A total of 53 compounds (**1a**−**20b**) were evaluated in vitro for their antitrypanosomal activity and cytotoxicity. The results of the testing against *T. b. rhodesiense* bloodstream form trypomastigotes (STIB900) as well as the assessment of the cytotoxicity of compounds **1a**−**20b** against rat myoblast cells (L6) are summarized in Tables 1−4, with melarsoprol and podophyllotoxin used as the reference agents. Compounds **1a**−**20b** displayed different levels of antitrypanosomal activity against *T. b. rhodesiense* (STIB900), with IC_{50} values ranging from 7 nM to 89 μM. The two 6-hydroxyquinoline ester derivatives (**2a** and **2b**) were the least active of the series, with IC_{50} values of 89 and 80 μM, respectively (Table 1). Among the 1,2-dihydroquinolines, the 6-ether (**6a**−**g**, **7**) and the *N*1-benzylated 6-ether (**8e**−**g**) derivatives were far less active than the 6-esters or their *N*1-benzylated counterparts. The 6-ether derivatives displayed IC_{50} values ranging from 3.7 μM (for **7**) to 13 μM (for **6b**), while the *N*1-benzylated 6-ether derivatives (**8e**−**g**) were much less active (IC_{50} values of 86 and 63 μM for **8g** and **8e**, respectively) except for **8f** which displayed an IC_{50} value of 3.6 μM (Table 2). However, all the 1,2-dihydroquinoline ester derivatives (**1a**−**b**, **5**; see Table 1), the *N*1-benzylated 6-hydroxy compounds (**9a**−**b**, **e**−**f**, **n**, **11a**,**b**, **20a**), and their ester derivatives (**10a**−**o**, **12a**−**c**, **13a**−**e** and **20b**) were among the most active agents (Tables 3 and 4). Compound **5** (IC_{50} < 0.008 μM) was the most potent of the ester derivatives, while **9a** (IC_{50} = 0.007 μM) was the most potent of the *N*1-benzylated congeners. The 6-*O*-acetyl and 6-*O*-benzoyl derivatives of **9a**, compounds **10a** (IC_{50} = 0.014 μM) and **13a** (IC_{50} = 0.013 μM), were the most potent of the 6-*O*-acyl *N*1-benzylated derivatives. Interestingly, any substitution on the

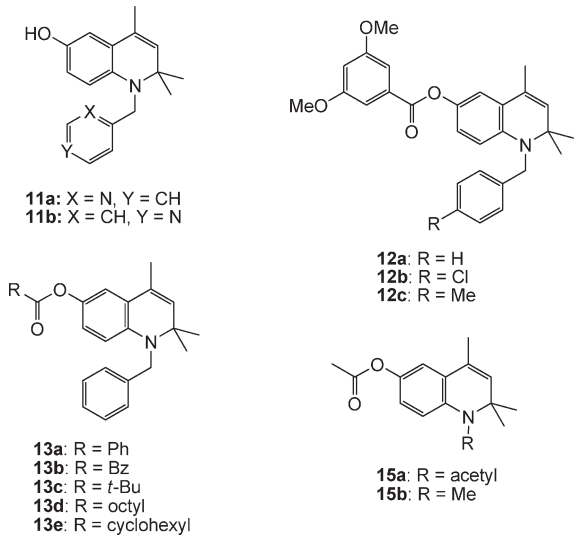


Figure 2. Structures of **11a**, **11b**, **12a**−**c**, **13a**−**e**, **15a**, and **15b**.

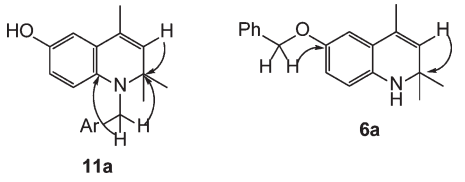


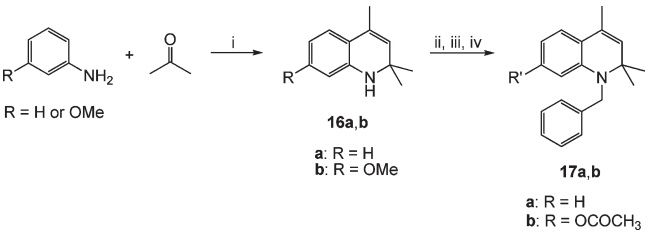
Figure 3. Key HMBC correlations for **11a** and **6a**.

phenyl ring of the *N*1-benzyl group resulted in a loss of activity (compare **9a** with **9b**, **9e**, **9f**, **9n** and compare **10a** with **10b-o**), albeit this was a minor loss in some cases. The identity of the ester group at the 6-*O* atom had little effect on activity, as there was only a 2-fold difference in potency among *N*1-benzylated esters **10a**, **12a**, and **13a-e**. The 6-*O*-acetyl *N*1-acetyl analogue (**15a**, IC_{50} = 5.3 μM) and the 6-*O*-acetyl-*N*1-methyl derivative (**15b**, IC_{50} = 1.4 μM) exhibited reduced activity compared to all of the *N*1-benzylated derivatives possessing an OH group at position 6 or their esterified counterparts. The nor-hydroxy derivative 1-benzyl-1,2-dihydro-2,2,4-trimethylquinoline (**17a**, IC_{50} = 57 μM) displayed weak activity. 1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-7-yl acetate (**17b**, IC_{50} 4.4 μM), the 7-*O*-acetyl derivative, was less potent than the 6-*O*-acetyl compounds (although it was more active than nor-hydroxy congener **17a**).

Cytotoxicity and Selectivity

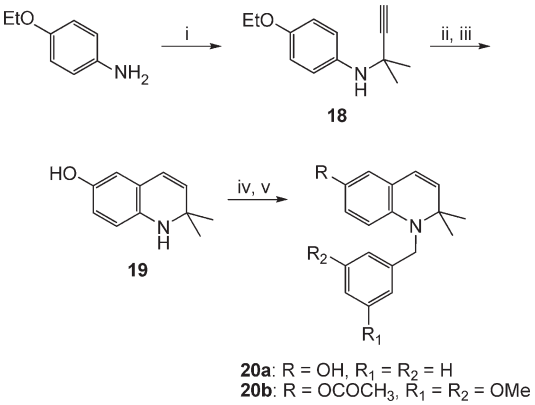
Tables 1−4 also summarize the in vitro toxicities of the compounds to L6 rat myoblasts and their in vitro antitrypanosomal selectivity indexes (SI) relative to L6 cells, expressed as the ratio IC_{50}(L6)/IC_{50}(*T. b. rhodesiense*). The 6-*O*-benzylated dihydroquinolines (compounds **6a**−**g**, IC_{50} = 45−77 μM) and their *N*1,*O*-disubstituted congeners (compounds **8e**−**g**, $IC_{50} \geq 180$ μM) were less toxic to L6 myoblasts than either the *N*1-benzylated 6-hydroxydihydroquinolines (compounds **9a**,**b**, **9e**,**f**, and **9n**, IC_{50} = 6.2−11 μM) or the 6-*O*-acetyl-*N*1-benzyl derivatives (compounds **10a-o**, IC_{50} = 9−31 μM). Acetylation of **9a**,**b**, **9e**,**f**, and **9n** to give **10a**,**b**, **10e**,**f**, and **10n** resulted in at least a 2-fold decrease in cytotoxicity. With the exception of **12a** (IC_{50} = 24 μM), replacement of the methyl ester present in compounds **10a**−**n** with a larger ester substituent resulted in a decrease in cytotoxicity (compounds **12b**,**c** and **13a**−**e**, $IC_{50} \geq 160$ μM). Most notably,

Scheme 4[a]



[a] Reagents and conditions: (i) I_2, toluene, reflux, 24 h; (ii) BBr_3, DCM (−78 to 25 °C), if applicable; (iii) benzyl bromide, Et_3N, reflux, overnight; (iv) CH_3COCl, Et_3N, DCM, room temp, overnight, if applicable.

Scheme 5[a]



[a] Reagents and conditions: (i) 3-chloro-3-methyl-1-butyne, Cu, CuCl (10%/10% w/v), Et_3N, room temp; (ii) CuCl (10%), toluene, reflux; (iii) HBr, reflux, 16 h; (iv) $ArCH_2Br$, Et_3N, reflux, overnight; (v) CH_3COCl, Et_3N, DCM, if applicable.

many of these compounds displayed SI values exceeding 1000 (**5**, SI > 7800; **10a**, SI = 1700; **12b**, SI > 3900; **12c**, SI > 6000; **13a**, SI > 18 000; **13b**, SI = 10 000; **13d**, SI = 13 000; **13e**, SI > 8200).

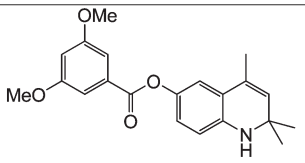
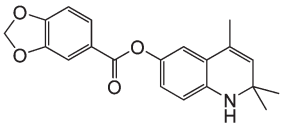
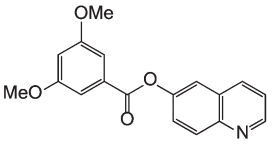
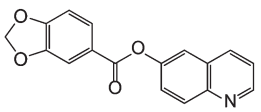
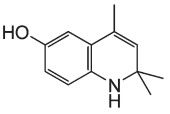
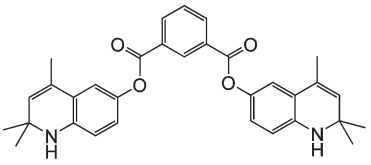
In Vivo Antitrypanosomal Activity

Based on the potent in vitro antitrypanosomal activity and selectivity of the *N*1-benzyl dihydroquinoline 6-esters against *T. b. rhodesiense*, compounds **10a**, **10d**, and **10m** were selected for evaluation in a murine model of African trypanosomiasis. These agents were administered to *T. b. brucei* (STIB795) infected mice by intraperitoneal injection at a daily dose of 50 mg/kg for 4 days. The results of this in vivo evaluation, reported in Table 5, revealed that **10a** was the most active compound. While all animals in the other groups had died because of infection by day 14, each of the mice receiving compound **10a** was still alive at this point. Nonetheless, mice in this group relapsed by day 10 (were positive for parasites) and were euthanized on day 14 because of animal welfare regulations. Compounds **10d** and **10m** were substantially less active than **10a**, and the survival times of these groups were not statistically different from that of the control group. No overt signs of toxicity related to the compounds were noted in any of the mice.

Mode of Action

Since we suspected reactive oxygen species (ROS) to be involved in the mechanism of action of these agents, **10a**, its 7-*O*-acetyl counterpart **17b**, and the nor-hydroxy compound

Table 1. In Vitro Antrypanosomal Activity and Cytotoxicity of Compounds **1a**−**5**

Compound	Structure	*T. b. rhodesiense*[c] (IC_{50}, μM)	Cytotoxicity[d] (IC_{50}, μM)	SI[e]	ClogP[f]
1a		0.054	16	300	3.69
1b		0.30	29	99	3.72
2a		89	200	2	3.35
2b		80	40	0.5	3.38
4		0.78	18	23	2.07
5		<0.008	63	>7800	5.85
MLSP[a]		0.008	7.9	1000	-
PPT[b]		-	0.012	-	-

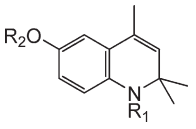
[a] MLSP, melarsoprol. [b] PPT, podophyllotoxin. [c] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[23] [d] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[24] [e] Selectivity index expressed as the ratio IC_{50}(L6)/IC_{50}(*T. b. rhodesiense*). [f] Calculated using ChemDraw Pro 11.0.1 (CambridgeSoft).

17a were evaluated for their ability to generate ROS within the parasite. Our assay to detect ROS in *T. b. brucei* in vitro employed 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA), which has been used previously to assess cellular ROS levels.[25,26] CM-H_2DCFDA diffuses through membranes during cell loading. Then intracellular esterases produce the corresponding carboxy derivative, which oxidizes to a highly fluorescent derivative in the presence of reactive oxygen species. Figure 4 shows the results obtained from the evaluation of **10a**, **17a**, and **17b** compared to H_2O_2 and 1% DMSO used as positive and negative controls, respectively. Compound **10a**, which bears a 6-acetoxy group, caused a concentration-dependent increase in fluorescence in trypanosome-containing samples incubated with this compound for 24 h. A slight increase in fluorescence was observed when parasites were exposed to 0.1 μM **10a**, while fluorescence levels were almost 4-fold higher than negative control samples when trypanosomes were incubated with 10 μM **10a**. *T. b. brucei* exposed to 250 μM H_2O_2 showed only a 3.3-fold increase in fluorescence compared to the negative control. Compound **17a**, which lacks a hydroxyl group, and **17b**, which possesses a 7-acetoxy group, cause no increase in fluorescence when trypanosomes were exposed to 0.1, 1, and 10 μM concentrations of these agents.

Discussion

As part of a continuing search for novel entities with better efficacy and lower toxicity for the treatment of late stage HAT, a screening program supported by WHO/TDR identified a promising series of 1,2-dihydroquinolines with antitrypanosomal activity. While in vitro testing of **1a** against *T. b. rhodesiense* (STIB900) revealed the potent activity of this compound (IC_{50} = 0.054 μM), it was inactive in a murine *T. b. brucei* model. Subsequent studies described here have identified dihydroquinolines with potent and selective in vitro antitrypanosomal activity, and **10a** displays promising in vivo activity in an animal model of African trypanosomiasis.

Table 2. In Vitro Antrypanosomal Activity and Cytotoxicity of Ethers **6a**−**8g**



compd	R_1	R_2	IC_{50}, μM			ClogP[f]
			T. b. rhodesiense[c]	cytotoxicity[d]	SI[e]	
6a	H	benzyl	4.1	56	14	4.06
6b	H	3,5-dimethoxybenzyl	13	68	5	3.81
6c	H	4-methoxybenzyl	5.2	77	15	3.94
6d	H	2-methoxybenzyl	5.3	48	9	3.94
6e	H	3-methoxybenzyl	11	61	5	3.94
6f	H	3-trifluoromethylbenzyl	8.8	45	5	4.98
6g	H	4-trifluoromethylbenzyl	4.5	48	11	4.98
7	H	phenethyl	3.7	83	22	4.34
8e	3-methoxybenzyl	3-methoxybenzyl	63	>210	>3	6.08
8f	3-trifluoromethylbenzyl	3-trifluoromethylbenzyl	3.6	>180	>50	8.18
8g	4-trifluoromethylbenzyl	4-trifluoromethylbenzyl	86	>180	>2	8.18
MLSP[a]			0.008	7.9	1000	−
PPT[b]			−	0.012	−	−

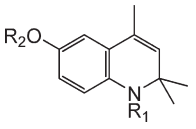
[a] MLSP, melarsoprol. [b] PPT, podophyllotoxin. [c] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[23] [d] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[24] [e] Selectivity index expressed as the ratio IC_{50}(L6)/IC_{50}(*T. b. rhodesiense*). [f] Calculated using ChemDraw Pro 11.0.1 (CambridgeSoft).

Dihydroquinolines are mainly known for their antioxidant activity,[27−31] although they have also been reported to possess anti-inflammatory,[32,33] fungicidal,[34] antiatherosclerotic,[35] and hormone receptor modulator[36,37] properties. However, compound **3**, also known as ethoxyquin, remains the most well-known member of this class of molecules. Compound **3**, a Food and Drug Administration (FDA) approved antioxidant commonly used as a preservative in the food processing industry,[38,39] is a fat stabilizer and preservative found primarily in premium pet foods containing high fat levels. It has been used for over 30 years to prevent oxidation and rancidity in fats in order to maintain the wholesomeness and the quality in pet foods. The FDA has also approved its use as food additive in the production of paprika, chili powder, and ground chili. Studies have shown that **3** displays a cancer chemoprotective effect by inducing detoxification enzymes such as glutathione *S*-transferase[40] and aldehyde reductase.[41,42] Since **3** is an FDA-approved food additive and the related compounds studied here possess high selectivity indexes, the further investigation of these agents as antitrypanosomal drug candidates is warranted.

The fact that esterified 6-hydroxyquinoline compounds **2a** and **2b** were inactive (IC_{50} of 89 and 80 μM, respectively) against *T. b. rhodesiense* in vitro while the 1,2-dihydroquinoline derivatives (**1a** and **1b**) bearing the same ester side chains, respectively, displayed good activities (IC_{50} of 0.054 and 0.30 μM, respectively) suggests that the dihydroquinoline core is required for antitrypanosomal activity. This hypothesis was further supported by the observation that **4**, the hydroxy derivative of the initial lead compound **1a**, displayed significant antitrypanosomal activity (IC_{50} = 0.78 μM), while its isophthalate dimer derivative (**5**) exhibited outstanding potency (IC_{50} < 0.008 μM). It is also important to note that **4** was less active than any of the ester derivatives, possibly because of its higher hydrophilicity that may restrict passive diffusion of the compound into the parasite. The calculated log *P* (ClogP) values for **4**, **15a**, and **15b** are lower than the corresponding values for other dihydroquinoline-6-esters examined in this study (see Tables 1, 3, and 4), providing further support for this hypothesis. This could also explain why the pyridylmethyl derivatives **11a** and **11b** were generally less active than the *N*1-benzyl derivatives **10a**−**o**, **12a**−**c**, and **13a**−**e**. A scatter plot of IC_{50} values vs ClogP for all monomeric dihydroquinolines containing a 6-hydroxyl group or a 6-*O*-acylated function (compounds **1a**,**b**, **9a**,**b**, **9e**,**f**, **9n**, **10a**−**o**, **11a**,**b**, **12a**−**c**, **13a**−**e**, **15a**,**b**, and **20a**,**b**; see Supporting Information Figure S1) indicates that such agents with ClogP values less than 4 tend to possess weaker in vitro antitrypanosomal potency than those with ClogP values of 4 or greater. The hydrophilicity of **4** might also explain why the parent compound (**1a**) was inactive in the murine trypanosomiasis model. In vivo, rapid conversion of **1a** to **4** is likely to occur through the action of host esterases. This latter compound may be less likely to enter the parasite or could have been excreted rapidly from the animal (perhaps aided by phase 2 metabolism). Thus, although compound **5** exhibited potent in vitro antitrypanosomal activity, we did not pursue the in vivo evaluation of this compound because of our belief that **4** would be rapidly generated in the animal model.

A series of 1,2-dihydroquinolines possessing side chains that would be less susceptible to rapid host metabolism was also prepared. This was achieved by attaching benzyl side chains at the 6-*O*-position, at the *N*1-atom, or at both positions to generate a series of 6-*O*-benzylated ether (**6a**−**g**), *N*1-benzylated (**9a**−**o**, **11a**,**b**), or 6-*O*,*N*1-dibenzylated (**8e**−**g**) derivatives. A benzyl side chain was chosen both to mimic the side chain present in the parent compound and to introduce a group where several analogues could be prepared to aid in further developing a structure−activity relationship. In the in vitro assay against *T. b. rhodesiense*, all the 6-ether compounds (**6a**−**g**, **7**) displayed reduced activity (IC_{50} = 3.7−13 μM) compared to the lead compound **1a** (IC_{50} = 0.054 μM), while all the 6-*O*,*N*1-dibenzylated derivatives (**8e**−**g**) were very weak except for **8f** (3.6 μM), a compound that exhibited

Table 3. In Vitro Antrypanosomal Activity and Cytotoxicity of Compounds **9a**−**13e**



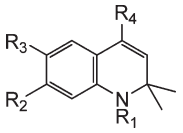
			IC50, μM			
compd	R_1	R_2	*T. b. rhodesiense*[c]	cytotoxicity[d]	SI[e]	ClogP[f]
9a	benzyl	H	0.007	6.8	970	4.34
9b	3,5-dimethoxybenzyl	H	0.14	11	83	4.09
9e	4-chlorobenzyl	H	0.061	7.9	130	4.90
9f	4-methylbenzyl	H	0.058	8.1	140	4.83
9n	4-methoxybenzyl	H	0.17	6.2	37	4.21
10a	benzyl	acetyl	0.014	24	1700	4.32
10b	3,5-dimethoxybenzyl	acetyl	0.10	25	240	4.06
10c	4-cyanobenzyl	acetyl	0.26	29	110	4.35
10d	3-cyanobenzyl	acetyl	0.18	31	170	4.35
10e	4-chlorobenzyl	acetyl	0.18	22	120	4.87
10f	4-methylbenzyl	acetyl	0.39	21	54	4.8
10g	3,4-dichlorobenzyl	acetyl	0.053	28	530	5.43
10h	3-chlorobenzyl	acetyl	0.039	29	740	4.87
10i	3-trifluoromethyl-benzyl	acetyl	0.041	27	660	5.24
10j	4-trifluoromethylbenzyl	acetyl	0.36	14	39	5.24
10k	3-methylbenzyl	acetyl	0.34	28	82	4.8
10l	3-methoxybenzyl	acetyl	0.32	29	91	4.19
10m	4-fluorobenzyl	acetyl	0.041	29	710	4.47
10n	4-methoxybenzyl	acetyl	0.048	15	310	4.19
10o	4-*tert*-butylbenzyl	acetyl	0.67	9	13	6.02
11a	2-pyridylmethyl	H	1.10	18	17	3.40
11b	4-pyridylmethyl	H	0.34	13	39	2.98
12a	benzyl	3,5-dimethoxybenzoyl	0.023	24	1000	5.96
12b	4-chlorobenzyl	3,5-dimethoxybenzoyl	0.048	> 190	> 3900	6.52
12c	4-methylbenzyl	3,5-dimethoxybenzoyl	0.033	> 200	> 6000	6.45
13a	benzyl	benzoyl	0.013	> 240	> 18000	6.21
13b	benzyl	phenylacetyl	0.015	160	11000	6.16
13c	benzyl	trimethylacetyl	0.028	170	6000	6.24
13d	benzyl	octanoyl	0.017	220	13000	7.06
13e	benzyl	cyclohexanecarbonyl	0.028	> 230	> 8200	6.29
MLSP[a]			0.008	7.9	1000	−
PPT[b]			−	0.012	−	−

[a] MLSP, melarsoprol. [b] PPT, podophyllotoxin. [c] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[23] [d] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[24] [e] Selectivity index expressed as the ratio IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*). [f] Calculated using ChemDraw Pro 11.0.1 (CambridgeSoft).

unexplained but nonetheless modest potency. On the other hand, the 6-hydroxy-*N*1-benzylated compounds (**9a**,**b**, **9e**,**f**, **9n**) possessed in vitro antitrypanosomal activities (IC_{50} = 0.007−0.17 μM) that were equally potent or superior to that of the parent compound **1a**. Compound **9a** was the most potent of the series, with an IC_{50} of 0.007 μM. However, as mentioned previously, these *N*1-benzylated compounds were susceptible to auto-oxidation except for **11a**,**b** and **20a**. This type of behavior has been reported previously for 5-hydroxydiclofenac, a *p*-hydroxyaniline containing metabolite of the anti-inflammatory drug diclofenac. 5-Hydroxydiclofenac is believed to undergo auto-oxidation because of its ability to form glutathione conjugates in the absence of enzymatic activation.[43,44] Thus, the unstable alcohols obtained in our studies were protected through esterification to generate prodrugs (**10a**−**o**, **12a**−**c**, **13a**−**e**). These compounds were prepared because we hypothesized that the ester function would be hydrolyzed both in vitro and in vivo to generate the active species. This strategy was successful in that all the 6-*O*-acylated, *N*1-benzylated congeners displayed excellent antitrypanosomal activity (IC_{50} = 0.013−0.67 μM). Substitution of the phenyl ring present in the *N*1-benzyl group resulted in a decrease in antitrypanosomal activity, as compounds **10b**−**o** were 3.3- to 56-fold less active than **10a**. There is no clear antitrypanosomal structure−activity relationship for these phenyl substitutions, as some meta- and para-substituted compounds show potent activity (**10g**−**i**, **10m**, **10n**, with IC_{50} values from 0.039 to 0.051 μM) while others do not (such as **10e** and **10l**, with IC_{50} values of 0.18 and 0.32 μM, respectively). Nonetheless, all of these compounds possessed submicromolar IC_{50} values and were substantially more active than the 6-*O*-benzylated ethers and 6-*O*,*N*1-dibenzylated (**8e**−**g**) derivatives.

Furthermore, **15a** and **15b**, two 6-acetoxy derivatives carrying acetyl and methyl groups at the *N*1 position, respectively, displayed only marginal activity (IC_{50} of 5.3 and 1.4 μM, respectively), suggesting an important role for the *N*1-benzyl side chain on the antitrypanosomal activity of these compounds. Removing the methyl group at the 4-position of the dihydroquinoline ring (**20a** and **20b**, IC_{50} of 0.13 and 0.20 μM, respectively) decreased the activity of these compounds compared to their 4-methyl counterparts (**9a** and **10b**, IC_{50} of 0.007

Table 4. In Vitro Antrypanosomal Activity and Cytotoxicity of Compounds **15a**−**20b**



					IC_{50}, μM			
compd	R_1	R_2	R_3	R_4	*T. b. rhodesiense*[c]	cytotoxicity[d]	SI[e]	ClogP[f]
15a	acetyl	H	acetoxy	Me	5.3	160	29	1.69
15b	Me	H	acetoxy	Me	1.4	41	29	2.83
17a	benzyl	H	H	Me	57	110	2	4.73
17b	benzyl	acetoxy	H	Me	4.4	71	16	4.32
20a	benzyl	H	OH	H	0.13	5.5	42	4.16
20b	3,5-dimethoxy-benzyl	H	acetoxy	H	0.20	10	50	3.89
MLSP[a]					0.008	7.9	1000	−
PPT[b]					−	0.012	−	−

[a] MLSP, melarsoprol. [b] PPT, podophyllotoxin. [c] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[23] [d] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[24] [e] Selectivity index expressed as the ratio IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*). [f] Calculated using ChemDraw Pro 11.0.1 (CambridgeSoft).

Table 5. Activity of **10a**, **10d**, and **10m** against *T. b. brucei* STIB795 in Mice[a]

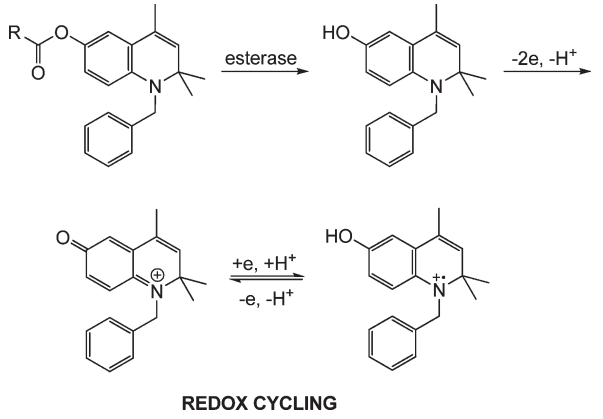
compd	dose ((mg/kg)/day), route	formulation	mean survival time ± standard deviation (day)
control		10% DMSO	7.75 ± 3.50
10a	4 × 50, ip	10% DMSO	>14[b]
10d	4 × 50, ip	10% DMSO	9.25 ± 2.22
10m	4 × 50, ip	10% DMSO	8.75 ± 3.78

[a] The veterinary reference drug diminazene typically cures animals in this assay (no parasitemia detected 30 days after infection) when given at 4 × 10 (mg/kg)/day ip. [b] Mice relapsed by day 10 and were euthanized on day 14 after infection; $p > 0.05$ compared to the control group.

and 0.014 μM, respectively). The *N*1-benzylated derivatives carrying the same ester side chain as the parent compound (**12a**−**c**) also displayed very good in vitro potency (IC_{50} of 0.023, 0.048, and 0.033 μM, respectively). Compounds carrying aliphatic (**13c**−**e**, IC_{50} of 0.028, 0.017, and 0.028 μM, respectively) or a 2-phenylacetyl (**13b**, IC_{50} of 0.015 μM) ester side chains were as potent and selective as their benzoylated or acetylated counterparts, with no major differences in activity among molecules possessing the various side chains.

Perhaps the most striking observation from this structure−activity relationship investigation is the fact that compounds lacking the 6-oxygen atom altogether (**17a**) or bearing an oxygen atom at the 7-position rather than the 6-position (**17b**) were far less potent than those containing an alcohol or acyloxy group at the 6-position. These key observations highlight the importance of a 6-oxygen atom for antitrypanosomal activity in this series of compounds and serve as the foundation for our mechanistic hypothesis. On the basis of the in vitro antitrypanosomal structure−activity relationship and previous literature concerning ethoxyquin cited below, we hypothesize that the antitrypanosomal 1,2-dihydroquinolines increase the oxidative stress on the parasite, with a free hydroxyl group at the 6-position being critical for activity. We propose that compounds esterified at the 6-oxygen atom are hydrolyzed rapidly by trypanosomal esterases, followed by two-electron oxidation to a quinone imine species.[28,45] This quinone imine could then undergo a single electron reduction to form a semiquinone that may enhance the rate of formation of reactive oxygen species such as superoxide and/or other free radicals.[46,47] The added strain on the parasite's antioxidant machinery could lead to lethal consequences for the organism (see Scheme 6). However, **17a** and **17b**, molecules that cannot form a quinone imine intermediate and cause little or no production of reactive oxygen species in the parasite, are far less potent against trypanosomes in vitro. Quinones are known to be substrates for trypanothione reductase,[46,48] and this enzyme is a potential target of these 1,2-dihydroquinoline derivatives. This hypothesis merits further investigation.

Scheme 6[a]



[a] Proposed antitrypanosomal mechanism of 2,2,4-trimethyl-1,2-dihydroquinolines.

When comparing the antioxidant effect of ethoxyquin with that of some of its derivatives, De Koning and Milkovitch found that 1,2-dihydroquinolines that lack a hydroxyl or alkoxyl group at the 6-position of their core (even with such a group at the 8 position) displayed little or no antioxidant properties.[49] Thorisson et al. found that a quinone imine derivative of ethoxyquin displayed significant antioxidant

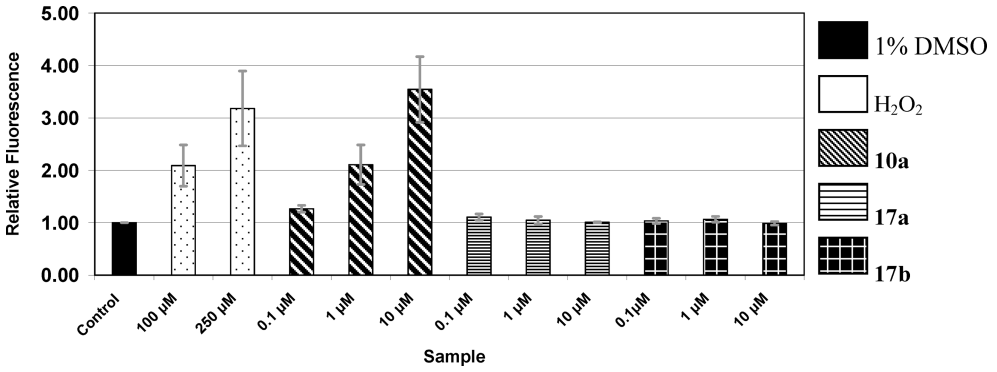


Figure 4. Evaluation of the capacity of compounds **10a**, **17a**, and **17b** to generate reactive oxygen species in *T. b. brucei* using a CM-H_2DCFDA fluorescence assay. Parasites loaded with CM-H_2DCFDA were incubated at 37 °C for 24 h with or without test compounds at the concentrations indicated, and fluorescence intensity was measured by flow cytometry as indicated in the Experimental Section. The results shown represent the mean ± standard error obtained in three independent experiments.

activity.[28] These authors hypothesized that this activity could be due to the ability of the quinone imine species to act as a radical acceptor. Hayes et al. later postulated that the anticancer chemopreventive effect of ethoxyquin stems from the fact that ethoxyquin is metabolized in vivo to dihydroquinolin-6-ol **4** (see Figure 1) by CYP450 isoenzymes, and this latter compound displayed a chemopreventive effect through its oxidation to a quinone imine.[50] At first glance, the antioxidant properties of the quinone imine observed in earlier chemoprevention studies seem to contradict our hypothesis that these trypanocidal molecules act by increasing oxidative stress in the parasite. However, given that trypanothione reductase carries out the single electron reduction of quinones and naphthoquinones,[46,48] the quinone imine may be handled similarly by the parasite enzyme and could be the source of significant amounts of reactive oxygen species in the trypanosome. To examine whether the quinone imine form of **9a** was stabilized compared to other compounds of lesser trypanocidal potency, quantum chemical calculations were performed for **9a** along with dihydroquinolin-6-ol **4**, ether **6a**, and the dihydroquinolin-6-ols corresponding to **15a** and **15b** (see Supporting Information Table S1). Compounds **6a** and the dihydroquinolin-6-ol corresponding to **15a** clearly show the least potential to be oxidized to the quinone imine since (1) their HOMO orbitals gain the least stability energy during oxidation and (2) their overall solution phase energies show the greatest increase during oxidation. These electronic data help rationalize the poor antitrypanosomal activities of **6a** and **15a**. However, these calculations do not allow us to distinguish between **4**, **9a**, and the dihydroquinolin-6-ol corresponding to **15b**. Since the ClogP values of **4** and **15b** are significantly lower than that of **10a** and its corresponding dihydroquinolin-6-ol **9a** as mentioned earlier, lipophilicity is likely to play an important role in trypanocidal potency.

While concentrations of **10a** above the IC_{50} value of this compound are required to detect elevated levels of reactive oxygen species in trypanosomes (Figure 4), redox cycling may still be responsible for the trypanotoxic effect of **10a**. Since quinones can act as subversive substrates for trypanosomatid trypanothione reductase in vitro and result in oxygen consumption,[46] redox cycling in live trypanosomes with the quinone imine formed from **10a** would generate superoxide that could be reduced by trypanosome superoxide dismutase,[51] thus preventing the detection of ROS at low concentrations of **10a**. Such levels of **10a** may still be toxic, however, because of the consumption of reducing equivalents that would occur during futile redox cycling of the quinone imine. At higher concentrations of **10a**, the trypanosome superoxide dismutase may be overwhelmed, resulting in the detection of ROS. Another possible explanation for the relatively low levels of ROS observed at 100 nM **10a** could be that the ROS determination was made after 24 h of exposure to **10a**, while the IC_{50} value shown in Table 3 was measured after a 70 h incubation of trypanosomes with **10a**. The observed structure−activity relationship is also consistent with attack on the quinone imine intermediate by a cellular nucleophile, resulting in the death of the parasite through interference with a critical process within the trypanosome or the formation of lethal adducts. However, given the importance of trypanothione reductase to trypanosomatids, the ability of related quinones and naphthoquinones to act as subversive substrates for this enzyme,[46,48] and the high selectivity of these dihydroquinolines for *T. brucei* compared to L6 cells (Table 1), we feel that the quinone imine intermediate shown in Scheme 6 is unlikely to exert trypanocidal effects by acting as an electrophile.

Quinone imine metabolites of drugs such as acetaminophen and diclofenac are nonetheless believed to cause hepatotoxicity due to their reactivity with cellular nucleophiles.[52−54] A potential strategy to decrease the risk of hepatic injury due to administration of these dihydroquinoline compounds for the treatment of HAT is to develop ester-containing derivatives that are hydrolyzed specifically by trypanosomal esterases, thus restricting the generation of the quinone imine species to the site of infection. Also, acetaminophen-induced liver injury is commonly treated by the administration of *N*-acetylcysteine,[55] which reverses depletion of glutathione levels.[56] Considering the severity of second stage HAT, the lack of appropriate treatments for this disease, and the potent in vitro activity of these simple dihydroquinolines, investigation of this class of antitrypanosomal molecules should continue while carefully monitoring the potential toxicity of a quinone imine intermediate.

Conclusion

HAT is still a huge problem in sub-Saharan Africa. The disease causes much physical and economic hardship, particularly in rural communities that are affected by the tsetse vector and that do not have easy access to modern medical facilities. The current drugs used to treat second stage HAT are losing their effectiveness, most likely because of the emergence of drug-resistant parasites. We report here the

synthesis of a series of 1,2-dihydroquinolines with nanomolar in vitro antitrypanosomal potency. Prodrug **10a** extended the lifespan of mice infected with *T. b. brucei*, indicating that further investigation of this class of molecules as potential candidates against HAT is essential. Future studies will focus on preparing potent, metabolically stable analogues possessing properties that will allow the compounds to cross the blood−brain barrier and thus display efficacy against second stage HAT. Efforts will also be undertaken to further elucidate the metabolism, pharmacokinetics, and the antitrypanosomal mechanism of action of this novel and promising class of compounds.

Experimental Section

General Experimental. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and HMBC spectra were recorded on a Bruker 300 UltraShield spectrometer. Electrospray ionization mass spectrometry (ESI-MS) and high resolution ESI-MS (HRESI-MS) were performed by The Ohio State University Mass Spectrometry and Proteomics Facility. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Anhydrous DCM and toluene were distilled over P_2O_5 or $CaCl_2$ and stored under molecular sieves (type 4A, $^1/_{16}$ in. pellets). THF was distilled over Na metal and used immediately after collection. Reaction mixtures were monitored using TLC silica gel 60 F_{254} plates from EMD Chemicals Inc. Gravity and flash column chromatography were performed using type 60A silica gel (60−230 mesh) from Fisher Scientific. All the compounds were further purified using silica gel GF preparative 1000 μm UV_{254} plates from Analtech. As described in the text, *N*1-benzyl-2,2,4-trimethyldihydroquinolin-6-ols were susceptible to oxidation and were converted to their stable ester derivatives. All other target compounds displayed a purity of ≥95% (elemental analyses within 0.4% of theoretical values) unless otherwise noted. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

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.

Preparation and Characterization of Compounds. Preparation of 6-*O*-Acyl Derivatives. Ester compounds were prepared by allowing a mixture of either 6-hydroxyquinoline or 1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**4**) and the appropriated acyl chloride in DCM or THF to stir overnight in the presence of Et_3N. While 6-hydroxyquinoline is commercially available, 1,2-dihydro-2,2,4-trimethylquinolin-6-ol was obtained by heating the commercially available ethoxyquin to reflux in 48% HBr for 16 h.[9] Many of the acyl chlorides used were commercially available. Those that were not were prepared by heating the corresponding carboxylic acid to reflux in thionyl chloride.[8]

Quinolin-6-yl 3,5-Dimethoxybenzoate (2a). A mixture of 6-hydroxyquinoline (300 mg, 2.07 mmol) and 3,5-dimethoxybenzoyl chloride (414 mg, 2.07 mmol) in Et_3N (10 mL) and THF (15 mL) was allowed to stir overnight at room temperature. Excess Et_3N and solvent were evaporated under reduced pressure, and the residue was further purified on a silica gel column using hexanes−ethyl acetate (6:4) to yield **2a** (383 mg, 60%) as a white powder, mp 102−104 °C. ^{1}H NMR ($CDCl_3$): δ 3.88 (6H, s), 6.75 (1H, s), 7.38 (2H, 2s), 7.43 (1H, m), 7.59 (1H, dd, J_1 = 9.2 and J_2 = 2.2 Hz), 7.69 (1H, d, J = 2.2 Hz), 8.17 (2H, m), 8.93 (1H, d, J = 3.3 Hz). ^{13}C NMR ($CDCl_3$): δ 55.7, 106.6, 107.8, 118.6, 121.6, 124.8, 128.6, 131.1, 131.1, 135.8, 146.4, 148.8, 150.3, 160.9, 164.9. ESI-MS: $[M + H]^+$ m/z 310, $[M + Na]^+$ m/z 332. HRESI-MS: $[M + H]^+$ m/z 310.1079 (calcd 310.1079). Anal. ($C_{18}H_{15}NO_4$) C, H,N.

Quinolin-6-yl Benzo[*d*][1,3]dioxole-5-carboxylate (2b). The reaction was similar to the one above using 6-hydroxyquinoline (300 mg, 2.07 mmol) and piperonyloyl chloride (382 mg, 2.07 mmol) to yield **2b** (326 mg, 54%). Purification was carried out on a silica gel column using hexanes−ethyl acetate (3:7) to provide the desired material as a white powder, mp 153−155 °C. ^{1}H NMR ($CDCl_3$): δ 6.09 (2H, s), 6.93 (1H, d, J = 8.3 Hz), 7.42 (1H, m), 7.57 (1H, dd, J_1 = 9.3 and J_2 = 2.1 Hz), 7.65 (1H, s), 7.68 (1H, d, J = 2.1 Hz), 7.87 (1H, d, J = 8.3 Hz), 8.15 (2H, m), 8.92 (1H, d, J = 3.6 Hz). ^{13}C NMR ($CDCl_3$): δ 102.1, 108.2, 110.0, 118.6, 121.6, 123.1, 124.9, 126.4, 128.6, 131.1, 135.8, 146.4, 148.0, 148.8, 150.2, 152.4, 164.5. ESI-MS: $[M + H]^+$ m/z 294, $[M + Na]^+$ m/z 316. HRESI-MS: $[M + H]^+$ m/z 294.0770 (calcd 294.0776). Anal. ($C_{17}H_{11}NO_4$) C, H, N.

1,2-Dihydro-2,2,4-trimethylquinolin-6-ol (4). Ethoxyquin was suspended in 48% HBr and heated to reflux for 16 h. The mixture was cooled to room temperature and partitioned between chloroform and aqueous Na_2CO_3 (1 M).[9] The organic layer was further washed with water, concentrated under reduced pressure, and purified on a silica gel column using hexanes−ethyl acetate (7:3) to yield **4** (47%) as a yellow powder, mp 178−179 °C (lit.[44] 180−181 °C). ^{1}H NMR (DMSO-d_6): δ 1.33 (6H, s), 1.99 (3H, s), 5.78 (1H, s), 6.82 (1H, d, J = 8.3 Hz), 6.86 (1H, s), 7.38 (1H, d, J = 8.3 Hz), 9.96 (1H, brs, NH), 10.87 (1H, brs, OH). Anal. ($C_{12}H_{15}NO$) C, H, N.

1,2-Dihydro-2,2,4-trimethylquinolin-6-yl 3,5-Dimethoxybenzoate (1a). The reaction was similar to the preparation of **2a** using compound **4** (600 mg, 3.2 mmol) and 3,5-dimethoxybenzoyl chloride (642 mg, 3.2 mmol) to yield, after purification on a silica gel column using hexanes−ethyl acetate (8:2), **1a** (669 mg, 59%) as a yellow powder. This compound was further recrystallized from hexanes−ethyl acetate−dichloromethane (2:2:1) to yield yellow needles, mp 130−132 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (6H, s), 1.97 (3H, s), 3.86 (6H, s), 5.37 (1H, s), 6.46 (1H, d, J = 8.4 Hz), 6.71 (1H, s), 6.83 (1H, dd, J_1 = 8.4 and J_2 = 2.4 Hz), 6.89 (1H, d, J = 2.4 Hz), 7.34 (1H, s), 7.35 (1H, s). ^{13}C NMR ($CDCl_3$): δ 18.7, 31.1, 52.2, 55.8, 106.4, 107.8, 113.2, 116.8, 121.2, 122.5, 128.3, 129.5, 132.0, 141.4, 142.3, 160.9, 165.8. ESI-MS: $[M + H]^+$ m/z 354, $[M + Na]^+$ m/z 376. HRESI-MS: m/z 354.1711 (calcd 354.1705). Anal. ($C_{21}H_{23}NO_4$) C, H, N.

1,2-Dihydro-2,2,4-trimethylquinolin-6-yl Benzo[*d*][1,3]dioxole-5-carboxylate (1b). The reaction was similar to the preparation of **2b** using compound **4** (300 mg, 1.6 mmol) and piperonyloyl chloride (292 mg, 1.6 mmol) to yield, after purification on a silica gel column using hexanes−ethyl acetate (4:1), **1b** (326 mg, 65%) as yellow crystals, mp 134−136 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (6H, s), 1.97 (3H, s), 3.72 (1H, brs, NH), 5.36 (1H, s), 6.07 (2H, s), 6.45 (1H, d, J = 8.4 Hz), 6.82 (1H, d, J = 8.4 Hz), 6.90 (2H, d, J = 8.4 Hz), 7.62 (1H, s), 7.82 (1H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 18.5, 31.0, 52.0, 101.9, 108.1, 109.9, 113.0, 116.7, 121.1, 122.2, 123.9, 126.0, 128.1, 129.3, 141.1, 142.2, 147.8, 152.0, 165.2. ESI-MS: $[M + H]^+$ m/z 338, $[M + Na]^+$ m/z 360. HRESI-MS: $[M + Na]^+$ m/z 360.1215 (calcd 360.1212). Anal. ($C_{20}H_{19}NO_4$) C, H, N.

Bis(1,2-dihydro-2,2,4-trimethylquinolin-6-yl) Isophthalate (5). The bis(1,2-dihydro-2,2,4-trimethylquinolin-6-yl) isophthalate is a dimer of **4** and was prepared under similar conditions to those employed to synthesize **1a**,**b** using compound **4** (300 mg, 1.6 mmol) and isophthaloyl dichloride (161 mg, 0.8 mmol) to yield, after purification on a silica gel column using hexanes−ethyl acetate (4:1), **5** (282 mg, 70%) as yellow crystals, mp 213−215 °C. ^{1}H NMR ($CDCl_3$): δ 1.31 (12H, s), 1.98 (6H, s), 3.70 (2H, brs, NH), 5.38 (2H, s), 6.50 (2H, d, J = 8.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 6.93 (2H, s), 7.66−7.68 (1H, m), 8.44 (2H, d, J = 7.5 Hz), 9.00 (1H, s). ^{13}C NMR ($CDCl_3$): δ 18.5, 31.0, 52.1, 113.1, 116.6, 121.0, 122.4, 128.1, 128.9, 129.3, 130.6, 131.6, 134.7, 141.1, 142.1, 165.0. ESI-MS: $[M + H]^+$ m/z 509, $[M + Na]^+$ m/z 531. HRESI-MS: $[M + H]^+$ m/z 509.2454 (calcd 509.2440). Anal. ($C_{32}H_{32}N_2O_4$) calcd: C, 75.57; H, 6.34; N, 5.51. Found: C, 74.97; H, 6.31; N, 5.40.

Preparation of 6-*O*-Ether Derivatives. These compounds were prepared by allowing **4**, an appropriate benzyl halide (1.5 equiv), and NaH (1.5 equiv) in DMF to stir for 48 h at room temperature under a nitrogen atmosphere.[10]

6-(Benzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6a). Compound **4** (300 mg, 1.6 mmol) and benzyl bromide (271 mg, 1.6 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6a** (257 mg, 58%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 4.96 (2H, s), 5.31 (1H, s), 5.43 (1H, brs, NH) (based on HSQC data), 6.41 (1H, d, J = 8.1 Hz), 6.62−6.64 (2H, m), 7.30−7.44 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.2, 30.2, 51.0, 70.0, 110.5, 112.9, 115.0, 121.3, 127.5, 127.6, 128.3, 129.5, 137.8, 138.6, 149.5. ESI-MS: $[M + H]^+$ m/z 280. HRESI-MS: $[M + H]^+$ m/z 280.1715 (calcd 280.1701). Anal. ($C_{19}H_{21}NO$) C, H, N.

6-(3,5-Dimethoxybenzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6b). Compound **4** (300 mg, 1.6 mmol) and 3,5-dimethoxybenzyl bromide (366 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6b** (307 mg, 57%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 3.72 (6H, s), 4.90 (2H, s), 5.30 (1H, s), 5.42 (1H, brs, NH), 6.39−6.42 (2H, m), 6.58− 6.63 (4H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.7, 51.5, 55.6, 70.3, 99.7, 105.7, 111.0, 113.4, 115.5, 121.7, 128.0, 130.0, 139.1, 140.8, 149.9, 160.9. ESI-MS: $[M + H]^+$ m/z 340. HRESI-MS: $[M + H]^+$ m/z 340.1898 (calcd 340.1912). Anal. ($C_{21}H_{25}NO_3$) C, H, N.

6-(4-Methoxybenzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6c). Compound **4** (300 mg, 1.6 mmol) and 4-methoxybenzyl bromide (319 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6c** (245 mg, 50%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 3.74 (3H, s), 4.87 (2H, s), 5.30 (1H, s), 5.40 (1H, brs, NH), 6.41 (1H, d, J = 8.1 Hz), 6.60−6.63 (2H, m), 6.92 (2H, d, J = 8.5 Hz), 7.34 (2H, d, J = 8.5 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.7, 51.5, 55.5, 70.2, 111.0, 113.4, 114.2, 115.5, 121.8, 128.0, 129.8, 130.0, 130.2, 139.1, 150.0, 159.3. ESI-MS: $[M + H]^+$ m/z 310. HRESI-MS: $[M + H]^+$ m/z 310.1812 (calcd 310.1807). Anal. ($C_{20}H_{23}NO_2$) C, H, N.

6-(2-Methoxybenzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6d). Compound **4** (300 mg, 1.6 mmol) and 2-methoxybenzyl chloride (248 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6d** (281.1 mg, 57%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 3.81 (3H, s), 4.93 (2H, s), 5.30 (1H, s), 5.42 (1H, brs, NH), 6.41 (1H, d, J = 8.1 Hz), 6.60−6.63 (2H, m), 6.95−6.97 (1H, m), 7.02 (1H, d, J = 8.1 Hz), 7.30−7.35 (1H, m), 7.39 (1H, d, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.7, 51.5, 55.8, 65.5, 110.8, 111.2, 113.4, 115.3, 120.7, 121.8, 126.0, 128.0, 129.4, 129.5, 130.0, 139.1, 150.2, 157.2. ESI-MS: $[M + H]^+$ m/z 310. HRESI-MS: $[M + H]^+$ m/z 310.1803 (calcd 310.1807). Anal. ($C_{20}H_{23}NO_2$) C, H, N.

6-(3-Methoxybenzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6e). Compound **4** (300 mg, 1.6 mmol) and 3-methoxybenzyl bromide (319 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6e** (267.8 mg, 55%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.17 (6H, s), 1.88 (3H, s), 3.76 (3H, s), 4.94 (2H, s), 5.31 (1H, s), 5.42 (1H, brs, NH), 6.41 (1H, d, J = 8.1 Hz), 6.62−6.65 (2H, m), 6.88 (1H, d, J = 8.4 Hz), 6.99−7.01 (2H, m), 7.26−7.32 (1H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.7, 51.5, 55.5, 70.3, 111.0, 113.4, 113.5, 113.6, 115.5, 120.1, 121,8, 128.0, 129.9, 130.0, 139.2, 140.0, 150.0, 159.8. ESI-MS: $[M + H]^+$ m/z 310. HRESI-MS: $[M + H]^+$ m/z 310.1805 (calcd 310.1807). Anal. ($C_{20}H_{23}NO_2$) C, H, N.

6-(3-(Trifluoromethyl)benzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6f). Compound **4** (300 mg, 1.6 mmol) and 3-(trifluoromethyl)benzyl bromide (379 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6f** (259 mg, 47%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 5.07 (2H, s), 5.31 (1H, s), 5.44 (1H, brs, NH), 6.41 (1H, d, J = 8.1 Hz), 6.64−6.67 (2H, m), 7.61−7.66 (2H, m), 7.72−7.75 (2H, m). ^{13}C NMR (DMSO-d_6): δ 18.6, 30.8, 51.5, 69.7, 111.2, 113.4, 115.7, 121.8, 124.3 (q, $^3J_{CF}$ = 3.8 Hz), 124.7 (q, $^3J_{CF}$ = 3.8 Hz), 124.8 (q, $^1J_{CF}$ = 270.8 Hz), 127.9, 129.7 (q, $^2J_{CF}$ = 32.3 Hz), 130.0, 132.0, 139.4, 139.9, 149.7. ESI-MS: $[M + H]^+$ m/z 348. HRESI-MS: $[M + H]^+$ m/z 348.1581 (calcd 348.1575). Anal. ($C_{20}H_{20}F_3NO$) C, F, H, N.

6-(4-(Trifluoromethyl)benzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (6g). Compound **4** (300 mg, 1.6 mmol) and 4-(trifluoromethyl)benzyl bromide (379 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **6g** (286 mg, 52%) as a brown sticky oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 5.08 (2H, s), 5.31 (1H, s), 5.44 (1H, brs, NH), 6.40 (2H, d, J = 8.1 Hz), 6.62−6.65 (2H, m), 7.64 (2H, d, J = 7.5 Hz), 7.74 (2H, d, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.8, 51.5, 69.6, 111.0, 113.3, 115.5, 121.8, 124.7 (q, $^1J_{CF}$ = 270.75 Hz), 125.7 (q, $^3J_{CF}$ = 3.75 Hz), 127.9, 128.4 (q, $^2J_{CF}$ = 31.50 Hz), 130.0, 139.3, 143.3, 149.6. ESI-MS: $[M + H]^+$ m/z 348. HRESI-MS: $[M + H]^+$ m/z 348.1573 (calcd 348.1575). Anal. ($C_{20}H_{20}F_3NO$) C, F, H, N.

1,2-Dihydro-2,2,4-trimethyl-6-(phenethyloxy)quinoline (7). Compound **4** (300 mg, 1.6 mmol) and (2-bromoethyl)benzene (293 mg, 1.6 mmol) were used under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **7** (93 mg, 20%) as a brown sticky oil. ^{1}H NMR (DMSO-d_6): δ 1.16 (6H, s), 1.87 (3H, s), 2.97 (2H, t, J = 6.9 Hz), 4.05 (2H, t, J = 6.9 Hz), 5.30 (1H, s), 5.39 (1H, brs, NH), 6.40 (1H, d, J=8.1 Hz), 6.54−6.57 (2H, m), 7.20−7.26 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 30.7, 35.8, 51.4, 69.3, 110.6, 113.4, 115.2, 121.8, 126.6, 128.0, 128.7, 129.4, 130.0, 139.0, 139.1, 150.0. ESI-MS: $[M + H]^+$ m/z 294. HRESI-MS: $[M + H]^+$ m/z 294.1859 (calcd 294.1858). Anal. ($C_{20}H_{23}NO$) C, H, N.

1-(3-Methoxybenzyl)-6-(3-methoxybenzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (8e). 8e, a side product from the preparation of **6e**, was obtained as a yellow sticky oil (157 mg, 23%). ^{1}H NMR (DMSO-d_6): δ 1.28 (6H, s), 1.94 (3H, s), 3.70 (3H, s), 3.73 (3H, s), 4.37 (2H, s), 4.91 (2H, s), 5.46 (1H, s), 6.11 (1H, d, J = 8.7 Hz), 6.55 (1H, dd, J_1 = 8.7 and J_2 = 2.7 Hz), 6.70 (1H, d, J = 2.7 Hz), 6.75 (1H, d, J = 8.4 Hz), 6.85−6.97 (5H, m), 7.17−7.27 (2H, m). ^{13}C NMR (DMSO-d_6): δ 18.8, 28.0, 47.9, 55.3, 55.5, 56.9, 70.0, 111.5, 111.7, 112.6, 112.7, 113.5, 113.6, 114.4, 118.7, 120.1, 124.1, 127.4, 129.9, 130.0, 131.4, 138.6, 139.8, 142.4, 150.1, 159.8, 159.9. ESI-MS: $[M + H]^+$ m/z 430, $[M + Na]^+$ m/z 452. HRESI-MS: $[M + Na]^+$ m/z 452.2204 (calcd 452.2202). Anal. ($C_{28}H_{31}NO_3$) C, H, N.

1-(3-(Trifluoromethyl)benzyl)-6-(3-(trifluoromethyl)benzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (8f). 8f, a side product from the preparation of **6f**, was obtained as yellow sticky oil that solidified upon standing (280 mg, 35%). ^{1}H NMR (DMSO-d_6): δ 1.30 (6H, s), 1.94 (3H, s), 4.52 (2H, s), 5.04 (2H, s), 5.48 (1H, s), 6.10 (1H, d, J=8.7 Hz), 6.58 (1H, dd, J_1=8.7 and J_2=2.1 Hz), 6.75 (1H, d, J = 2.1 Hz), 7.50−7.69 (8H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 27.8, 47.5, 57.0, 69.3, 111.8, 112.5, 114.5, 123.0 (q, $^3J_{CF}$=3.75 Hz), 123.6 (q, $^3J_{CF}$=3.75 Hz), 123.7 (q, $^3J_{CF}$=3.75 Hz), 124.3 (q, $^3J_{CF}$=3.75 Hz), 124.8 (q, $^1J_{CF}$ = 271.2 Hz), 125.8 (q, $^1J_{CF}$ = 272.0 Hz), 127.4, 129.6 (q, $^2J_{CF}$ = 36.0 Hz), 131.6 (q, $^2J_{CF}$=27.75 Hz), 138.5, 139.7, 142.4, 150.1. ESI-MS: $[M + H]^+$ m/z 506, $[M + Na]^+$ m/z 528. HRESI-MS: $[M + H]^+$ m/z 506.1904 (calcd 506.1918). Anal. ($C_{28}H_{25}F_6NO$) C, F, H, N.

1-(4-(Trifluoromethyl)benzyl)-6-(4-(trifluoromethyl)benzyloxy)-1,2-dihydro-2,2,4-trimethylquinoline (8g). 8g, a side product from the preparation of **6g**, was obtained as a yellow sticky oil (257 mg, 32%). ^{1}H NMR (DMSO-d_6): δ 1.29 (6H, s), 1.95 (3H, s), 4.51

(2H, s), 5.05 (2H, s), 5.49 (1H, s), 6.06 (1H, d, J = 8.7 Hz), 6.56 (1H, d, J = 8.7 Hz), 6.74 (1H, s), 7.52 (2H, d, J = 8.1 Hz), 7.61 (2H, d, J = 9.0 Hz), 7.64 (2H, d, J = 9.0 Hz), 7.71 (2H, d, J = 8.1 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 27.9, 47.5, 57.0, 69.2, 111.8, 112.5, 114.4, 124.4, 124.7 (q, $^1J_{CF}$ = 270.75 Hz), 124.8 (q, $^1J_{CF}$ = 270.75 Hz), 125.6 (q, $^3J_{CF}$ = 3.75 Hz), 127.4 (q, $^2J_{CF}$ = 30.75 Hz), 128.9 (q, $^2J_{CF}$ = 31.5 Hz), 131.6, 138.4, 143.1, 145.8, 150.0. ESI-MS: $[M+H]^+$ m/z 506, $[M + Na]^+$ m/z 528. HRESI-MS: $[M + H]^+$ m/z 506.1908 (calcd 506.1918). Anal. ($C_{28}H_{25}F_6NO$) C, F, H, N.

Preparation of *N*-Substituted Compounds. All of the *N*-substituted compounds were prepared by heating a mixture of **4** and the appropriate benzyl halide (1.5 equiv) in refluxing toluene, using Et_3N (1.5 equiv) as base. Since the *N*-substituted compounds appeared to be very susceptible to auto-oxidation, they were further protected by either acetylation or benzoylation except for **11a**, **11b**, and **20a**, which were obtained as needles and thus were stable as the free hydroxy compounds.

1,2-Dihydro-2,2,4-trimethyl-1-((pyridin-2-yl)methyl)quinolin-6-ol (11a). Compound **4** (300 mg, 1.6 mmol) and 2-(bromomethyl)pyridine hydrobromide (401 mg, 1.6 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (7:3), **11a** as brown needles (132 mg, 23%), mp 193−195 °C. ^{1}H NMR (DMSO-d_6): δ 1.29 (6H, s), 1.91 (3H, s), 4.41 (2H, s), 5.45 (1H, s), 6.00 (1H, d, J = 8.7 Hz), 6.30 (1H, dd, J_1 = 8.7 and J_2 = 2.7 Hz), 6.52 (1H, d, J = 2.7 Hz), 7.18−7.22 (1H, m), 7.27 (1H, d, J = 7.8 Hz), 7.62−7.68 (1H, m), 8.50 (1H, brs, OH), 8.52 (1H, d, J=4.5 Hz). ^{13}C NMR (DMSO): δ 18.8, 27.7, 50.5, 56.8, 111.3, 112.7, 114.9, 120.8, 122.3, 124.4, 127.5, 131.4, 137.0, 137.2, 148.8, 149.4, 161.0. The HSQC and HMBC data enabled us to find the key correlation between the protons at δ 4.41 ppm (benzylic protons) and the quaternary carbon (C-2) at δ 56.8 ppm. The H-3 proton (5.45 ppm) also displayed a correlation with the carbon at δ 56.8 ppm, confirming the identity of this carbon as C-2. These data demonstrate that the methylpyridinyl side chain is bound to the *N*1-atom. ESI-MS: $[M + H]^+$ m/z 281. HRESI-MS: $[M + H]^+$ m/z 281.1659 (calcd 281.1654). Anal. ($C_{18}H_{20}N_2O$) C, F, H, N.

1,2-Dihydro-2,2,4-trimethyl-1-((pyridin-4-yl)methyl)quinolin-6-ol (11b). Compound **4** (300 mg, 1.6 mmol) and 4-(bromomethyl)pyridine hydrobromide (401 mg, 1.6 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (7:3), **11b** as brown needles (162 mg, 36%), mp 187−188 °C. ^{1}H NMR (DMSO-d_6): δ 1.26 (6H, s), 1.92 (3H, s), 4.40 (2H, s), 5.45 (1H, s), 5.95 (1H, d, J = 8.7 Hz), 6.31 (1H, dd, J_1 = 8.7 and J_2 = 2.4 Hz), 6.53 (1H, d, J = 2.4 Hz), 7.30 (2H, d, J = 5.4 Hz), 8.45 (2H, d, J = 5.4 Hz), 8.51 (1H, brs, OH). ^{13}C NMR (DMSO-d_6): δ 18.8, 27.6, 47.1, 56.8, 111.4, 112.7, 114.9, 122.2, 124.3, 127.5, 131.3, 136.7, 148.9, 150.0, 150.6. ESI-MS: $[M + H]^+$ m/z 281. HRESI-MS: $[M + H]^+$ m/z 281.1654 (calcd 281.1654). Anal. ($C_{18}H_{20}N_2O$) calcd: C, 77.11; H, 7.19; N, 9.99. Found: C, 76.63; H, 7.20; N, 9.83.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10a). Compound **4** (300 mg, 1.6 mmol) and benzyl bromide (410 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on silica gel column using hexanes−ethyl acetate (9:1), 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9a**) (398 mg, 89%). **9a** (300 mg, 1.07 mmol) was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (1:1), **10a** (328 mg, 95%) as yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.33 (6H, s), 1.92 (3H, s), 2.17 (3H, s), 4.89 (2H, s), 5.49 (1H, s), 6.14 (1H, d, J = 8.7 Hz), 6.58 (1H, dd, J_1 = 8.7 and J_2 = 2.7 Hz), 6.73 (1H, d, J = 2.7 Hz), 7.18−7.20 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 21.2, 28.5, 47.8, 57.3, 112.0, 116.9, 121.4, 123.4, 126.5, 127.0, 128.9, 131.2, 140.1, 141.3, 141.9, 170.1. ESI-MS: $[M + Na]^+$ m/z 344. HRESI-MS: $[M + Na]^+$ m/z 344.1624 (calcd 344.1626). Anal. ($C_{21}H_{23}NO_2$) C, H, N.

1-(3,5-Dimethoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10b). Compound **4** (500 mg, 2.64 mmol) and 3,5-dimethoxybenzyl bromide (916 mg, 3.96 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−DCM (1:1), 1-(3,5-dimethoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9b**) (762 mg, 85%) as a brown oil. **9b** (300 mg, 0.88 mmol) was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (1:1), **10b** (310 mg, 92%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.31 (6H, s), 1.92 (3H, s), 2.18 (3H, s), 3.69 (6H, s), 4.40 (2H, s), 5.47 (1H, s), 6.15 (1H, d, J = 8.7 Hz), 6.34 (1H, d, J = 2.5 Hz), 6.46 (2H, m), 6.62 (1H, dd, J_1=8.7 and J_2 = 2.7 Hz), 6.72 (1H, d, J = 2.7 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 21.2, 28.5, 48.0, 57.3, 60.2, 98.1, 104.6, 112.0, 116.9, 121.4, 123.3, 126.9, 131.1, 141.4, 141.9, 142.8, 161.1, 170.1. ESI-MS: $[M + Na]^+$ m/z 404. HRESI-MS: $[M + Na]^+$ m/z 404.1832 (calcd 404.1838). Anal. ($C_{23}H_{27}NO_4$) C, H, N.

1-(4-Cyanobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10c). Compound **4** (500 mg, 2.6 mmol) and 4-(bromomethyl)benzonitrile (784 mg, 4.0 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (8:2), 4-((6-hydroxy-2,2,4-trimethylquinolin-1(2*H*)-yl)methyl)benzonitrile (**9c**) (684 mg, 86%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **10c** (739 mg, 94.9%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.31 (6H, s), 1.92 (3H, s), 2.18 (3H, s), 4.59 (2H, s), 5.50 (1H, s), 6.07 (1H, d, J=8.7 Hz), 6.53 (1H, dd, J_1=8.7 Hz and J_2=2.4 Hz), 6.74 (1H, d, J = 2.4 Hz), 7.50 (2H, d, J=8.1 Hz), 7.77 (2H, d, J = 8.1 Hz). ^{13}C NMR (DMSO d_6): δ 18.7, 21.2, 28.4, 47.6, 57.4, 109.8, 111.8, 117.2, 119.4, 121.5, 123.6, 126.9, 127.6, 131.2, 132.9, 141.5, 141.6, 146.5, 170.1. ESI-MS: $[M + H]^+$ m/z 347. HRESI-MS: $[M + H]^+$ m/z 347.1752 (calcd 347.1760). Anal. ($C_{22}H_{22}N_2O_2$) C, H, N.

1-(3-Cyanobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10d). Compound **4** (300 mg, 1.6 mmol) and 3-(bromomethyl)benzonitrile (470 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (8:2), 3-((6-hydroxy-2,2,4-trimethylquinolin-1(2*H*)-yl)methyl)benzonitrile (**9d**) (386 mg, 79.3%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **10d** (382 mg, 87.0%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.35 (6H, s), 2.01 (3H, s), 2.25 (3H, s), 4.48 (2H, s), 5.40 (1H, s), 6.05 (1H, d, J=8.7 Hz), 6.62 (1H, dd, J_1 = 8.7 Hz and J_2=2.7 Hz), 6.81 (1H, d, J = 2.7 Hz), 7.40 (1H, m), 7.50−7.62 (3H, m). ^{13}C NMR ($CDCl_3$): δ 18.9, 21.3, 28.5, 47.8, 57.5, 112.0, 113.0, 117.1, 119.2, 121.0, 124.3, 127.8, 129.7, 130.1, 130.5, 130.8, 131.0, 141.5, 141.6, 142.0, 170.3. ESI-MS: $[M + H]^+$ m/z 347. HRESI-MS: $[M + H]^+$ m/z 347.1758 (calcd 347.1760). Anal. ($C_{22}H_{22}N_2O_2$) C, H, N.

1-(4-Chlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10e). Compound **4** (300 mg, 1.6 mmol) and 4-chlorobenzyl bromide (493 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (19:1), **9e** (457 mg, 91%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10e** (477 mg, 92%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.31 (6H, s), 1.92 (3H, s), 2.18 (3H, s), 4.48 (2H, s), 5.48 (1H, s), 6.11 (1H, d, J=8.7 Hz), 6.60 (1H, dd, J_1=8.7 Hz and J_2 = 2.3 Hz), 6.73 (1H, d, J=2.3 Hz), 7.31−7.38 (4H, m). ^{13}C NMR (DMSO d_6): δ 18.7, 21.2, 28.4, 47.2, 57.4, 111.9, 117.0, 121.5, 123.5, 126.9, 128.4, 128.9, 131.2, 131.3, 139.2, 141.5, 141.6, 170.1. ESI-MS: $[M + H]^+$ m/z 356. HRESI-MS: $[M + H]^+$ m/z 356.1421 (calcd 356.1417). Anal. ($C_{21}H_{22}ClNO_2$) C, Cl, H, N.

1-(4-Methylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10f). Compound **4** (300 mg, 1.6 mmol) and 4-methylbenzyl bromide (444 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1),

1-(4-methylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9f**) (400 mg, 85%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10f** (434 mg, 95%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.32 (6H, s), 1.91 (3H, s), 2.19 (3H, s), 2.22 (3H, s), 4.43 (2H, s), 5.47 (1H, s), 6.13 (1H, d, J = 8.7 Hz), 6.58 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.72 (1H, d, J = 2.7 Hz), 7.01 (2H, d, J = 7.8 Hz), 7.20 (2H, d, J = 7.8 Hz). ^{13}C NMR (DMSO d_6): δ 18.7, 21.1, 21.2, 28.5, 47.6, 57.3, 112.0, 116.9, 121.4, 123.3, 126.4, 126.9, 129.5, 131.2, 135.9, 136.9, 141.3, 141.9, 170.1. ESI-MS: [M + H]$^+$ m/z 336. HRESI-MS: [M + H]$^+$ m/z 336.1966 (calcd 336.1964). Anal. ($C_{22}H_{25}NO_2$) C, H, N.

1-(3,4-Dichlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10g). Compound **4** (300 mg, 1.6 mmol) and 3,4-dichlorobenzyl chloride (469 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(3,4-dichlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9g**) (318 mg, 57%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10g** (346 mg, 97%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.30 (6H, s), 1.92 (3H, s), 2.18 (3H, s), 4.50 (2H, s), 5.49 (1H, s), 6.12 (1H, d, J = 8.7 Hz), 6.62 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.75 (1H, d, J = 2.4 Hz), 7.28 (1H, d, J = 8.1 Hz), 7.52−7.57 (2H, m). ^{13}C NMR (DMSO d_6): δ 18.7, 21.2, 28.3, 46.8, 57.4, 111.9, 117.2, 121.5, 123.6, 126.9, 128.5, 129.3, 131.1, 131.3, 131.5, 141.4, 141.7, 141.8, 170.1. ESI-MS: [M + H]$^+$ m/z 390. HRESI-MS: [M + H]$^+$ m/z 390.1031 (calcd 390.1028). Anal. ($C_{21}H_{21}Cl_2NO_2$) C, Cl, H, N.

1-(3-Chlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10h). Compound **4** (300 mg, 1.6 mmol) and 3-chlorobenzyl bromide (493 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(3-chlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9h**) (467 mg, 93%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10h** (466 mg, 88%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.31 (6H, s), 1.92 (3H, s), 2.18 (3H, s), 4.51 (2H, s), 5.49 (1H, s), 6.14 (1H, d, J = 8.7 Hz), 6.62 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.75 (1H, d, J = 2.4 Hz), 7.25−7.34 (4H, m). ^{13}C NMR (DMSO d_6): δ 18.7, 21.2, 28.4, 47.3, 57.4, 111.9, 117.1, 121.5, 123.5, 125.2, 126.3, 126.9, 127.0, 130.8, 131.2, 133.6, 141.6, 141.7, 143.1, 170.1. ESI-MS: [M + H]$^+$ m/z 356. HRESI-MS: [M + H]$^+$ m/z 356.1420 (calcd 356.1417). Anal. ($C_{21}H_{22}ClNO_2$) C, Cl, H, N.

1-(3-(Trifluoromethyl)benzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10i). Compound **4** (300 mg, 1.6 mmol) and 3-(trifluoromethyl)benzyl bromide (574 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(3-(trifluoromethyl)benzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9i**) (457 mg, 82%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10i** (497 mg, 97%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.37 (6H, s), 2.02 (3H, s), 2.26 (3H, s), 4.53 (2H, s), 5.40 (1H, s), 6.12 (1H, d, J = 8.7 Hz), 6.63 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.81 (1H, d, J = 2.4 Hz), 7.41−7.60 (4H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.4, 47.9, 57.3, 111.7, 116.7, 120.8, 122.9 (q, $^3J_{CF}$ = 3.5 Hz), 123.7 (q, $^3J_{CF}$ = 3.5 Hz), 123.8, 126.0 (q, $^1J_{CF}$ = 271.8 Hz), 127.5, 129.1 (q, $^2J_{CF}$ = 32.3 Hz), 132.0, 140.7, 141.5, 141.6, 170.2. ESI-MS: [M + H]$^+$ m/z 390. HRESI-MS: [M + H]$^+$ m/z 390.1682 (calcd 390.1681). Anal. ($C_{22}H_{22}F_3NO_2$) C, F, H, N.

1-(4-(Trifluoromethyl)benzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10j). Compound **4** (300 mg, 1.6 mmol) and 4-(trifluoromethyl)benzyl bromide (574 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(4-(trifluoromethyl)benzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9j**) (463 mg, 84%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (7:3), **10j** (363 mg, 70%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.37 (6H, s), 2.02 (3H, s), 2.26 (3H, s), 4.54 (2H, s), 5.40 (1H, s), 6.10 (1H, d, J = 8.7 Hz), 6.63 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.81 (1H, d, J = 2.7 Hz), 7.46 (2H, d, J = 8.1 Hz), 7.57 (2H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.5, 47.9, 57.3, 111.7, 116.7, 120.8, 123.8, 124.8 (q, $^1J_{CF}$ = 270.75 Hz), 125.7 (q, $^3J_{CF}$ = 3.75 Hz), 126.5, 127.4, 129.1 (q, $^2J_{CF}$ = 32.25 Hz), 130.3, 141.5, 143.8, 170.2. ESI-MS: [M + H]$^+$ m/z 390. HRESI-MS: [M + H]$^+$ m/z 390.1682 (calcd 390.1681). Anal. ($C_{22}H_{22}F_3NO_2$) C, F, H, N.

1-(3-Methylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10k). Compound **4** (300 mg, 1.6 mmol) and 3-methylbenzyl bromide (444 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(3-methylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9k**) (415 mg, 88%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (7:3), **10k** (425 mg, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.38 (6H, s), 2.02 (3H, s), 2.27 (3H, s), 2.37 (3H, s), 4.47 (2H, s), 5.39 (1H, s), 6.22 (1H, d, J = 8.7 Hz), 6.63 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.79 (1H, d, J = 2.4 Hz), 7.05 (1H, m), 7.17−7.21 (3H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 21.5, 28.6, 48.3, 57.2, 112.0, 116.4, 120.8, 123.2, 123.5, 126.7, 127.4, 127.5, 128.5, 130.3, 138.2, 139.4, 141.2, 142.1, 170.3. ESI-MS: [M + H]$^+$ m/z 336. HRESI-MS: [M + H]$^+$ m/z 336.1964 (calcd 336.1964). Anal. ($C_{22}H_{25}NO_2$) C, H, N.

1-(3-Methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10l). Compound **4** (300 mg, 1.6 mmol) and 3-methoxybenzyl bromide (483 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(3-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9l**) (432 mg, 88%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (7:3), **10l** (383 mg, 78%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.38 (6H, s), 2.00 (3H, s), 2.26 (3H, s), 3.79 (3H, s), 4.46 (2H, s), 5.37 (1H, s), 6.21 (1H, d, J = 8.7 Hz), 6.62 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.77 (1H, d, J = 2.7 Hz), 6.92−6.97 (2H, m), 7.21−7.28 (2H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.5, 48.2, 55.2, 57.2, 111.6, 111.9, 112.1, 116.4, 118.5, 120.7, 123.6, 127.4, 129.6, 130.2, 141.2, 141.4, 142.0, 159.9, 170.3. ESI-MS: [M + H]$^+$ m/z 352. HRESI-MS: [M + H]$^+$ m/z 352.1913 (calcd 352.1913). Anal. ($C_{22}H_{25}NO_3$) C, H, N.

1-(4-Fluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10m). Compound **4** (300 mg, 1.6 mmol) and 4-fluorobenzyl bromide (454 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(4-fluorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9m**) (400 mg, 85%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10m** (429 mg, 94%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.36 (6H, s), 2.00 (3H, s), 2.26 (3H, s), 4.45 (2H, s), 5.38 (1H, s), 6.15 (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), 6.97−7.02 (2H, m), 7.28−7.32 (2H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.5, 47.5, 57.2, 111.8, 115.4 ($^2J_{CF}$ = 21.75 Hz), 116.5, 120.7, 123.7, 127.4, 127.6 ($^3J_{CF}$ = 8.25 Hz), 130.3, 134.9, 141.3, 141.7, 161.7 ($^1J_{CF}$ = 242.25 Hz), 170.2. ESI-MS: [M + H]$^+$ m/z 340. HRESI-MS: [M + H]$^+$ m/z 340.1714 (calcd 340.1713). Anal. ($C_{21}H_{22}FNO_2$) C, F, H, N.

1-(4-Methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10n). Compound **4** (300 mg, 1.6 mmol) and 4-methoxybenzyl bromide (483 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(4-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9n**) (441 mg, 90%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM

(3:1), **10n** (366 mg, 73%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.37 (6H, s), 2.00 (3H, s), 2.26 (3H, s), 3.80 (3H, s), 4.44 (2H, s), 5.37 (1H, s), 6.21 (1H, d, J = 8.7 Hz), 6.63 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.78 (1H, d, J = 2.4 Hz), 6.86 (2H, d, J = 8.4 Hz), 7.26 (2H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.6, 47.6, 55.3, 57.2, 111.9, 114.0, 116.4, 120.7, 123.5, 127.2, 127.3, 130.3, 131.2, 141.1, 142.0, 158.4, 170.3. ESI-MS: $[M + H]^+$ m/z 352. HRESI-MS: $[M + H]^+$ m/z 352.1913 (calcd 352.1913). Anal. ($C_{22}H_{25}NO_3$) C, H, N.

1-(4-*tert*-Butylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (10o). Compound **4** (300 mg, 1.6 mmol) and 4-(*tert*-butyl)benzyl bromide (545 mg, 2.4 mmol) were reacted under the conditions described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), 1-(4-*tert*-butylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol (**9o**) (425 mg, 80%). This latter compound was further acetylated to yield, after purification on a silica gel column using hexanes−DCM (3:1), **10o** (383 mg, 80%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.33 (9H, s), 1.38 (6H, s), 2.01 (3H, s), 2.26 (3H, s), 4.47 (2H, s), 5.38 (1H, s), 6.23 (1H, d, J = 8.7 Hz), 6.63 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.78 (1H, d, J = 2.4 Hz), 7.27 (2H, d, J = 8.1 Hz), 7.34 (2H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 18.7, 21.1, 28.6, 31.4, 34.4, 47.9, 57.1, 112.0, 116.3, 120.7, 123.5, 125.4, 125.8, 127.4, 130.3, 136.3, 141.1, 142.1, 149.4, 170.3. ESI-MS: $[M + H]^+$ m/z 378. HRESI-MS: $[M + H]^+$ m/z 378.2432 (calcd 378.2433). Anal. ($C_{25}H_{31}NO_2$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl-3,5-dimethoxybenzoate (12a). 9a (700 mg, 2.5 mmol) and 3,5-dimethoxybenzoyl chloride (762 mg, 3.8 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (8:2), **12a** (862 mg, 78%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.35 (6H, s), 1.94 (3H, s), 3.81 (6H, s), 4.52 (2H, s), 5.51 (1H, s), 6.20 (1H, d, J = 8.7 Hz), 6.74 (1H, d, J = 8.7 Hz), 6.82−6.88 (2H, m), 7.07−7.22 (2H, m), 7.28−7.33 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 28.5, 47.8, 56.0, 57.4, 106.2, 107.7, 112.1, 116.9, 121.4, 123.5, 126.5, 126.9, 127.0, 128.9, 131.3, 131.7, 140.0, 141.4, 142.1, 161.0, 165.2. ESI-MS: $[M + Na]^+$ m/z 466. HRESI-MS: $[M + Na]^+$ m/z 466.1952 (calcd 466.1994). Anal. ($C_{28}H_{29}NO_4$) C, H, N.

1-(4-Chlorobenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl-3,5-dimethoxybenzoate (12b). 9e (300 mg, 0.96 mmol) and 3,5-dimethoxybenzoyl chloride (289 mg, 1.44 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (3:7), **12b** (397 mg, 87%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.38 (6H, s), 2.03 (3H, s), 3.87 (6H, s), 4.48 (2H, s), 5.41 (1H, s), 6.19 (1H, d, J = 9.0 Hz), 6.72−6.77 (2H, m), 6.93 (1H, d, J = 2.4 Hz), 7.29 (4H, m), 7.34 (2H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 28.5, 47.6, 55.6, 57.2, 106.2, 107.6, 111.9, 116.7, 120.8, 123.8, 127.5, 127.6, 128.7, 130.3, 131.8, 132.3, 137.9, 141.6, 141.6, 160.7, 165.5. ESI-MS: $[M+Na]^+$ m/z 500. HRESI-MS: $[M+H]^+$ m/z 500.1602 (calcd 500.1599). Anal. ($C_{28}H_{28}ClNO_4$) C, Cl, H, N.

1-(4-Methylbenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl-3,5-dimethoxybenzoate (12c). 9f (300 mg, 1.02 mmol) and 3,5-dimethoxybenzoyl chloride (307 mg, 1.53 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (3:7), **12c** (393 mg, 84%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.37 (6H, s), 2.00 (3H, s), 2.31 (3H, s), 3.84 (6H, s) 4.47 (2H, s), 5.37 (1H, s), 6.24 (1H, d, J = 9.0 Hz), 6.69−6.73 (2H, m), 6.89 (1H, d, J = 2.8 Hz), 7.10 (2H, d, J = 7.5 Hz), 7.22 (2H, d, J = 7.5 Hz), 7.31 (2H, m). ^{13}C NMR ($CDCl_3$): δ 18.9, 21.2, 28.8, 48.1, 55.8, 57.4, 106.4, 107.8, 112.2, 116.6, 121.0, 123.8, 126.3, 127.6, 129.5, 130.5, 132.1, 136.3, 136.5, 141.6, 142.3, 160.9, 165.7. ESI-MS: $[M + Na]^+$ m/z 480. HRESI-MS: $[M + Na]^+$ m/z 480.2152 (calcd 480.2151). Anal. ($C_{29}H_{31}NO_4$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Benzoate (13a). 9a (500 mg, 1.8 mmol) and benzoyl chloride (379 mg, 2.7 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (8:2), **13a** (570 mg, 82.6%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.42 (6H, s), 2.05 (3H, s), 4.56 (2H, s), 5.43 (1H, s), 6.29 (1H, d, J = 8.7 Hz), 6.79 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.96 (1H, d, J = 2.4 Hz), 7.26−7.30 (2H, m), 7.32−7.41 (3H, m), 7.50−7.55 (2H, m), 7.62−7.64 (1H, m), 8.22 (2H, d, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 18.8, 28.6, 48.2, 57.3, 112.1, 116.6, 120.9, 123.7, 126.2, 126.7, 127.5, 128.5, 128.6, 128.8, 130.1, 130.3, 133.3, 139.4, 141.4, 142.1, 165.8. ESI-MS: $[M + Na]^+$ m/z 406. HRESI-MS: $[M + Na]^+$ m/z 406.1750 (calcd 406.1783). Anal. ($C_{26}H_{25}NO_2$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl-2-phenylacetate (13b). 9a (700 mg, 2.5 mmol) and 2-phenylacetyl chloride (587 mg, 3.8 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (85:15), **13b** (847 mg, 85.2%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.32 (6H, s), 1.92 (3H, s), 3.87 (2H, s), 4.49 (2H, s), 5.48 (1H, s), 6.15 (1H, d, J = 8.7 Hz), 6.58 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.74 (1H, d, J = 2.7 Hz), 7.18−7.35 (10H, m). ^{13}C NMR ($CDCl_3$): δ 18.7, 28.5, 41.4, 48.1, 57.2, 111.9, 116.3, 120.6, 123.5, 126.2, 126.6, 127.0, 127.4, 128.6, 128.7, 129.3, 130.3, 133.8, 139.4, 141.3, 142.0, 170.7. ESI-MS: $[M + Na]^+$ m/z 420. HRESI-MS: $[M + Na]^+$ m/z 420.1925 (calcd 420.1939). Anal. ($C_{27}H_{27}NO_2$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Pivalate (13c). 9a (400 mg, 1.4 mmol) and trimethylacetyl chloride (253 mg, 2.1 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (8:2), **13c** (464 mg, 91.2%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.24 (9H, s), 1.32 (6H, s), 1.93 (3H, s), 4.49 (2H, s), 5.48 (1H, s), 6.15 (1H, J = 8.7 Hz), 6.54 (1H, dd, J_1 = 8.7 and J_2 = 2.4 Hz), 6.68 (1H, d, J = 2.4 Hz), 7.19−7.30 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 27.3, 28.4, 40.9, 47.8, 57.3, 112.1, 116.7, 121.2, 123.5, 126.5, 126.9, 127.0, 128.9, 131.2, 140.1, 141.6, 141.8, 177.2. ESI-MS: $[M + Na]^+$ m/z 386. HRESI-MS: $[M + Na]^+$ m/z 386.2065 (calcd 386.2096). Anal. ($C_{24}H_{29}NO_2$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Octanoate (13d). 9a (1 g, 3.6 mmol) and octanoyl chloride (878 mg, 5.4 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (85:15), **13d** (1.23 g, 84.2%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, J = 6.9 Hz), 1.26−1.28 (8H, m), 1.33 (6H, s), 1.58 (2H, dd, J_1 = J_2 = 7.2 Hz), 1.92 (3H, s), 2.47 (2H, t, J = 7.2 Hz), 4.49 (2H, s), 5.49 (1H, s), 6.14 (1H, d, J = 8.7 Hz), 6.56 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.70 (1H, d, J = 2.7 Hz), 7.20−7.31 (5H, m). ^{13}C NMR (DMSO-d_6): δ 14.4, 18.7, 22.5, 24.9, 28.5, 28.8, 28.9, 31.6, 33.9, 47.8, 57.3, 112.0, 116.8, 121.4, 123.4, 126.5, 126.9, 128.7, 128.9, 131.2, 140.1, 141.3, 141.9, 172.7. ESI-MS: $[M + Na]^+$ m/z 428. HRESI-MS: $[M + Na]^+$ m/z 428.2535 (calcd 428.2565). Anal. ($C_{27}H_{35}NO_2$) C, H, N.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ylcyclohexanecarboxylate (13e). 9a (2 g, 7.2 mmol) and cyclohexane carboxylic acid chloride (1.57 g, 10.7 mmol) were allowed to react under conditions similar to those used in the preparation of **1a**,**b** to yield, after purification on a silica gel column using hexanes−DCM (8:2), **13e** (2.5 g, 89.1%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.22−1.45 (6H, m), 1.33 (6H, s), 1.54−1.61 (2H, m), 1.67−1.74 (3H, m), 1.92 (3H, s), 4.49 (2H, s), 5.48 (1H, s), 6.14 (1H, d, J = 8.7 Hz), 6.55 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz), 6.69 (1H, d, J = 2.7 Hz), 7.24−7.31 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.7, 22.1, 25.1, 25.8, 29.0, 42.5, 47.8, 57.3, 112.0, 116.8, 121.3, 123.4, 126.5, 126.9, 128.7, 128.9, 131.2, 140.1, 141.4, 141.8, 174.7. ESI-MS: $[M + Na]^+$ m/z

412. HRESI-MS: $[M + Na]^+$ m/z 412.2235 (calcd 412.2252). Anal. ($C_{26}H_{31}NO_2$) C, H, N.

1-Acetyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl Acetate (15a). Compound **4** (500 mg, 2.64 mmol), excess acetyl chloride (5 equiv), and NaH (3 equiv) in THF were heated to reflux overnight under nitrogen. The solvent was then evaporated and the residue partitioned between water and DCM, and purified on a silica gel column using hexanes−DCM (1:1) to yield **14a** (560 mg, 77.6%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.44 (6H, s), 1.96 (3H, s), 2.08 (3H, s), 2.26 (3H, s), 5.66 (1H, s), 6.93 (1H, d, J = 8.6 Hz), 6.95−6.96 (2H, m). ^{13}C NMR (DMSO-d_6): δ 17.8, 21.3, 26.1, 26.7, 57.8, 116.7, 120.7, 124.4, 127.2, 129.7, 134.4, 136.9, 147.5, 169.7, 171.9. ESI-MS: $[M + Na]^+$ m/z 296. HRESI-MS: $[M + Na]^+$ m/z 296.1258 (calcd 296.1263). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

1,2-Dihydro-1,2,2,4-tetramethylquinolin-6-yl Acetate (15b). 1,2-Dihydro-2,2,4-trimethylquinolin-6-yl acetate (**14**) was obtained by acetylation of **4**. ^{1}H NMR (DMSO-d_6): δ 1.19 (6H, s), 1.84 (3H, s), 2.18 (3H, s), 5.32 (1H, s), 5.84 (1H, brs, NH), 6.40 (1H, d, J = 7.8 Hz), 6.61−6.64 (2H, m). Compound **14** (562 mg, 2.4 mmol), CH_3I (756 μL, 12.1 mmol), and Et_3N (507 μL, 3.6 mmol) in toluene (25 mL) were heated to reflux overnight. The solvent was then evaporated and the residue was purified on a silica gel column using hexanes−ethyl acetate (9:1) to yield 1,2-dihydro-1,2,2,4-tetramethylquinolin-6-yl acetate (**15b**) (495 mg, 84%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.32 (6H, s), 1.97 (3H, s), 2.29 (3H, s), 2.80 (3H, s), 5.35 (1H, s), 6.48 (1H, d, J = 8.7 Hz), 6.79 (1H, d, J = 2.7 Hz), 6.84 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.7 Hz). ^{13}C NMR ($CDCl_3$): δ 18.5, 21.1, 27.1, 30.8, 56.3, 110.8, 116.3, 120.9, 124.0, 127.6, 131.1, 141.4, 143.1, 170.2. ESI-MS: $[M + H]^+$ m/z 268. HRESI-MS: $[M + H]^+$ m/z 268.1302 (calcd 268.1313). Anal. ($C_{15}H_{19}NO_2$) C, H, N.

1,2-Dihydro-2,2,4-trimethylquinoline (16a). This compound was prepared by heating a mixture of aniline (1 g, 10.7 mmol) and an excess of acetone (5 mL) in toluene (25 mL) to reflux in the presence of iodine (272 mg, 1.07 mmol) for 24 h.[14−16] The solvent was then evaporated and the residue purified on a silica gel column using hexanes−DCM (9:1) to yield 1,2-dihydro-2,2,4-trimethylquinoline (**16a**) (538 mg, 29%). ^{1}H NMR ($CDCl_3$): δ 1.41 (6H, s), 2.19 (3H, s), 3.74 (1H, brs, NH), 5.46 (1H, s), 6.57 (1H, d, J=7.8 Hz), 6.81 (1H, dd, J_1=J_2 = 7.8 Hz), 7.15 (1H, dd, J_1=J_2=7.8 Hz), 7.23 (1H, d, J=7.8 Hz). ^{13}C NMR ($CDCl_3$): δ 18.7, 31.2, 51.9, 113.1, 117.2, 121.6, 123.8, 128.5, 128.6, 143.5.

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinoline (17a). 1,2-Dihydro-2,2,4-trimethylquinoline (**16a**) (300 mg, 1.7 mmol) was benzylated at the N1 position as described above with benzyl bromide (444 mg, 2.6 mmol) to yield, after purification on a silica gel column using hexanes−DCM (7:3), **17a** (409 mg, 91.3%) as a yellow oil that solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 1.42 (6H, s), 2.09 (3H, s), 4.56 (2H, s), 5.38 (1H, s), 6.30 (1H, d, J = 8.4 Hz), 6.64−6.66 (1H, m), 6.93−6.96 (1H, m), 7.13 (1H, d, J = 7.5 Hz), 7.25−7.41 (5H, m). ^{13}C NMR ($CDCl_3$): δ 18.8, 28.7, 48.0, 57.1, 111.8, 116.0, 122.7, 123.5, 126.2, 126.5, 127.9, 128.6, 129.3, 139.7, 144.1. ESI-MS: $[M + Na]^+$ m/z 286. HRESI-MS: $[M + Na]^+$ m/z 286.1583 (calcd 286.1572). Anal. ($C_{19}H_{21}N$) C, H, N.

1,2-Dihydro-7-methoxy-2,2,4-trimethylquinoline (16b). This compound was prepared by heating to reflux a mixture of m-anisidine (1 g, 8.1 mmol) and an excess of acetone (5 mL) in toluene (25 mL) in the presence of iodine (206 mg, 0.81 mmol) for 24 h to yield **16b** (660 mg, 40%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.18 (6H, s), 1.85 (3H, s), 3.65 (3H, s), 5.11 (1H, s), 5.79 (1H, brs, NH), 6.01−6.05 (2H, m), 6.83 (1H, d, J = 7.8 Hz). ^{13}C NMR (DMSO-d_6): δ 18.7, 31.4, 51.6, 55.0, 98.0, 101.4, 114.5, 124.6, 126.2, 127.7, 145.9, 160.3. Molecular formula: $C_{13}H_{17}NO$. The chemical shifts of protons and carbons of this compound were attributed using a HSQC spectrum, and the structure of the compound was further confirmed by key correlations observed in the HMBC spectrum. Correlations between H-5 (δ 6.83) and C-4 (127.7) and between C-6 (δ 101.4) and C-7 (δ 160.3) were observed but not with C-8 (δ 98.0), proving that C-8 and H-5 are separated by more than three bonds. H-8 displayed correlations with C-6 (δ 101.4), C-7 (δ160.3), and C-8a (δ 145.9) but not with C-5 (δ 124.6). This provides evidence that the methoxy group is attached to carbon C-7. This methoxy group was converted to a 7-hydroxy group using BBr_3 (3 equiv) in DCM (25 mL), with the temperature being allowed to rise from −78 C° (1 h) to 25 C° (18 h)[19,20] to yield 1,2-dihydro-2,2,4-trimethylquinolin-7-ol (276 mg, 45%).

1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-7-yl Acetate (17b). 1,2-Dihydro-2,2,4-trimethylquinolin-7-ol (300 mg, 1.6 mmol) was benzylated at the N1 position with benzyl bromide (410 mg, 2.4 mmol), and the product was further acetylated as described above to yield **17b** (230 mg, 45%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.31 (6H, s), 1.95 (3H, s), 2.11 (3H, s), 4.49 (2H, s), 5.40 (1H, s), 5.90 (1H, d, J = 2.7 Hz), 6.26 (1H, dd, J_1 = 8.1 Hz and J_2 = 2.7 Hz), 6.99 (1H, d, J = 8.1 Hz), 7.15−7.28 (5H, m). ^{13}C NMR (DMSO-d_6): δ 18.8, 21.2, 28.8, 47.5, 57.5, 104.9, 109.0, 120.2, 124.3, 126.4, 126.9, 128.6, 128.8, 129.5, 139.7, 145.5, 151.5, 169.3. ESI-MS: $[M + H]^+$ m/z 344. HRESI-MS: $[M+H]^+$ m/z 344.4033 (calcd 344.4026). Anal. ($C_{21}H_{23}NO_2$) C, H, N.

1,2-Dihydro-2,2-dimethylquinolin-6-ol (19). A mixture of p-phenetidine (4.0 g, 29.2 mmol), 3-chloro-3-methyl-1-butyne (3.6 g, 35.1 mmol), CuCl (287 mg, 2.9 mmol), and Cu (184 mg, 2.9 mmol) in Et_3N (75 mL) was allowed to stir at room temperature, and the reaction was monitored by TLC until completion (4−5 h).[18] The reaction mixture was dissolved in CH_2Cl_2 and washed twice with 200 mL of a saturated solution of NH_4Cl. The solvent was then evaporated and the residue purified on a silica gel column using hexanes−EtOAc (95:5) to yield 4-ethoxy-N-(2-methylbut-3-yn-2-yl)benzenamine (**18**) (3.26 g, 55%) as a yellow oil. **18** (3.26 g, 16.0 mmol) and CuCl (159 mg, 1.6 mmol) in toluene was heated to reflux overnight.[18,21,22] The solvent was then evaporated and the residue purified on a silica gel column using hexanes−ethyl acetate (9:1) to yield 6-ethoxy-1,2-dihydro-2,2-dimethylquinoline (2.10 g, 65%) that was subsequently heated to reflux in HBr for 16 h to yield 1,2-dihydro-2,2-dimethylquinolin-6-ol (**19**) (1.6 g, 88.4%), mp 155−157 °C. ^{1}H NMR (DMSO-d_6): δ 1.17 (6H, s), 5.15 (1H, brs, NH), 5.43 (1H, d, J = 9.6 Hz), 6.11 (1H, d, J = 9.6 Hz), 6.29 (1H, d, J = 9.3 Hz), 6.31−6.38 (2H, m), 8.30 (1H, brs, OH). ^{13}C NMR (DMSO-d_6): δ 30.5, 51.7, 113.2, 113.7, 115.7, 120.6, 123.9, 132.4, 137.3, 148.4. ESI-MS: $[M + Na]^+$ m/z 198.

1-Benzyl-1,2-dihydro-2,2-dimethylquinolin-6-ol (20a). 19 (300 mg, 1.7 mmol) was benzylated at the N1 position with benzyl bromide (445 mg, 2.6 mmol) as described above to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **20a** (341 mg, 76%) as purple crystals, mp 162−165 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (6H, s), 4.38 (2H, s), 5.56 (1H, d, J = 9.6 Hz), 6.01 (1H, d, J = 8.7 Hz), 6.22 (1H, d, J = 9.6 Hz), 6.28 (1H, dd, J_1 = 8.7 Hz and J_2 = 2.4 Hz), 6.37 (1H, d, J_2 = 2.4 Hz), 7.17−7.31 (5H, m), 8.44 (1H, brs, OH). ^{13}C NMR (DMSO-d_6): δ 27.7, 47.8, 57.1, 112.9, 114.0, 115.2, 122.8, 123.7, 126.6, 126.8, 128.8, 133.5, 137.1, 141.0, 148.7. ESI-MS: $[M + Na]^+$ m/z 288. HRESI-MS: $[M + H]^+$ m/z 288.1354 (calcd 288.1364). Anal. ($C_{18}H_{19}NO$) C, H, N.

1-(3,5-Dimethoxybenzyl)-1,2-dihydro-2,2-dimethylquinolin-6-yl Acetate (20b). 19 (300 mg, 1.7 mmol) was benzylated at the N1 position with 3,5-dimethoxybenzyl bromide (601 mg, 2.6 mmol) as described above to yield a brown oil (446 mg, 81%) that was further acetylated to yield, after purification on a silica gel column using hexanes−ethyl acetate (9:1), **20b** (453 mg, 90%) as a yellow oil that solidified upon standing. ^{1}H NMR (DMSO-d_6): δ 1.33 (6H, s), 2.17 (3H, s), 3.69 (6H, s), 4.41 (2H, s), 5.60 (1H, d, J = 9.6 Hz), 6.13 (1H, d, J = 8.4 Hz), 6.28 (1H, d, J = 9.6 Hz), 6.30−6.34 (1H, m), 6.45−6.47 (2H, m), 6.57 (1H, d, J = 8.4 Hz), 6.43−6.45 (1H, m). ^{13}C NMR (DMSO-d_6): δ 21.2, 28.5, 47.9, 55.5, 57.6, 98.2, 104.6, 112.0, 119.8, 121.5, 121.9, 122.9, 133.4, 141.4, 141.8, 142.8, 161.1, 170.0. ESI-MS: $[M + Na]^+$ m/z 390. HRESI-MS: $[M + Na]^+$ m/z 390.1665 (calcd 390.1681). Anal. ($C_{22}H_{25}NO_4$) C, H, N.

Quantum Chemical Calculations. Quantum chemical optimization calculations were performed using Jaguar, version 7.6, installed on an AMD Opteron 64 bit dual core workstation with 4GB RAM running the openSuse 10.1 operating system. Density functional theory with B3LYP hybrid functionals was used to perform the calculation with a 6-31+G** basis set. Jaguar's standard Poisson−Boltzmann continuum solvation model (PBF) was implemented in the calculation with water as solvent. Initial compound coordinates were built on Maestro, version 9.0 (Schrodinger Suite 2009), modeling suite environment. Molecular mechanics conjugate gradient method was applied to minimize the geometries utilizing OPLS2005 force field (maximum iteration 500, gradient 0.05). Jaguar calculations were performed on conjugated and open shell models of each compound.

In Vitro Susceptibility Testing of Trypanosomes and L6 Myoblasts. The in vitro antitrypanosomal activity and cytotoxicity of compounds of interest were determined following established protocols.[23,24,57]

In Vivo Assay. Compounds were evaluated in mice infected with *Trypanosoma b. brucei* (STIB 795) following an acute in vivo model developed at STI. In brief, the tested compounds were first dissolved in 100% DMSO followed by dilution in water to a final concentration of 10% DMSO. Female NMRI mice (20−25 g) were kept in standard Macrolon cages at a constant temperature of 21 °C and 60−70% relative humidity and were provided commercial pellets and water ad libitum. On day 0 for each group (control as well as experimental), four mice were infected intraperitoneally with 1×10^4 bloodstream form *T. b. brucei* from a stock of cryopreserved stabilates containing 10% glycerol. The stabilate was suspended in phosphate/saline/glucose buffer (9.0 g/L $Na_2HPO_4 \cdot 2H_2O$, 0.47 g/L $NaH_2PO_4 \cdot 2H_2O$, 2.55 g/L NaCl, 1% w/v glucose, pH 8.0) to obtain a trypanosome cell density of 4×10^4/mL. Each mouse was injected with 0.25 mL. On days 3−6, animals were treated on 4 consecutive days with the compound of interest by the intraperitoneal route, with a tolerated dose that was determined earlier. The concentration of test compound is such that 0.1 mL/10 g can be injected. On day 7, parasitemia of all mice is checked by tail blood examination and recorded as well as on day 10. After day 10, parasitemia is monitored twice per week until day 30. For dead mice, the day of death is recorded. On day 30, surviving and aparasitaemic mice are considered cured, and they are later euthanized.

CM-H_2DCFDA Fluorescence Assay. The ability of compounds **10a**, **17a**, and **17b** to generate reactive oxygen species in *T. b. brucei* was assessed using a fluorescence assay employing the dye CM-H_2DCFDA. *Trypanosoma b. brucei* (MITat1,2, variant 221) were maintained in HMI-9 medium.[58] Parasites were collected from late log phase cell culture by centrifugation at 1500*g* for 15 min at room temperature. The parasites were then suspended at a cell density of 1×10^6 cells/mL in HMI-9 medium lacking FBS containing 10 μg/mL CM-H_2DCFDA and were incubated for 60 min at 37 °C to accumulate the dye. The parasites were then centrifuged as before and resuspended in 10 mL of HMI-9 medium at a cell density of 1×10^6 cells/mL. Then 500 μL of this cell suspension was placed in each well of a 24-well plate. Test compounds were then added to the wells, and the cells were incubated as before for 24 h (the maximum amount of DMSO in these cultures was 1%). The parasites were then transferred to microcentrifuge tubes and centrifuged at 300*g* for 15 min. The supernatant was removed, and the cells were resuspended in 1 mL of phosphate-buffered saline. Fluorescence intensity in 10 000 individual cells was measured by flow cytometry, using a Becton Dickinson FACSCalibur instrument (Rutherford, NJ). The data were then analyzed using FlowJo software.

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Supporting Information Available: Scatter plot of IC_{50} values vs ClogP for monomeric dihydroquinolines containing a 6-hydroxyl group or a 6-*O*-acylated function (Figure S1) and quantum chemical calculations for **4**, **6a**, **9a**, and the dihydroquinolin-6-ols corresponding to **15a** and **15b** (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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