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Article

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A Cinchona Alkaloid Antibiotic that Appears to Target ATP Synthase in *Streptococcus pneumoniae*

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Abstract

Optochin, a cinchona alkaloid derivative discovered over 100 years ago, possesses highly selective antibacterial activity towards *Streptococcus pneumoniae.* Pneumococcal disease remains the leading source of bacterial pneumonia and meningitis worldwide. The structure activity relationships of optochin were examined through modification to both the quinoline and quinuclidine subunits, which led to the identification of analogue **48** with substantially improved activity. Resistance and molecular modeling studies indicate **48** likely binds to the c-ring of ATP synthase near the conserved glutamate 52 ion binding site while mechanistic studies demonstrated **48** causes cytoplasmic acidification. Initial pharmacokinetic and drug metabolism analysis of optochin and **48** revealed limitations of these quinine analogues, which were rapidly cleared resulting in poor in vivo exposure through hydroxylation pendant to the quinuclidine and *O*-dealklyation of the quinoline. Collectively, the results provide a foundation to advance **48** and highlight ATP synthase as a promising target for antibiotic development.

Keywords: *Streptococcus pneumoniae*, pneumococcal disease, antibiotic, cinchona alkaloid, quinine, optochin, ATP synthase.

Introduction

Pneumococcal disease caused by the gram-positive bacterium *Streptococcus pneumoniae* remains the leading source of bacterial pneumonia and meningitis worldwide. Additionally, *S. pneumoniae* is responsible for bacteremia and many common upper respiratory tract infections including sinusitis and otitis media.[1,2] The pneumococcal conjugate vaccine PCV13 provides immunity to thirteen of the most prevalent *S. pneumoniae* serotypes and has significantly reduced the global burden of pneumococcal disease. The wide deployment of PCV13 and the heptavalent predecessor PCV7 has led to a corresponding increase in drug-resistant *S. pneumoniae* (DRSP), thus complicating therapy for individuals that are either unvaccinated or infected with any of the more than eighty serotypes not covered by these vaccines.[3-5] Narrow spectrum agents effective against DRSP are therefore desirable and could complement existing vaccination programs to decolonize asymptomatic carriers. The concept of narrow spectrum antibacterial agents, defined as active against a single or related species, is gaining traction due to advances in rapid diagnostics in clinical microbiology labs coupled with the importance of the human microbiome in maintaining health and preventing disease.[6] Moreover, narrow spectrum agents are expected to have a smaller impact on human and environmental resistome, which serve as a reservoir for transmissible resistance elements.[7]

The antibiotic optochin **1**[8] (Figure 1A), a cinchona alkaloid derivative, possesses highly selective pneumococcal activity and was also shown to effectively treat septicemia in animals and humans.[9-11] However, development of optochin was discontinued because of toxicity observed in humans with incidence of optical disturbances in up to 5% of patients given ~25 mg/kg/day and moderate activity in treating lobar pneumonia, the most prevalent infection caused by *S. pneumoniae*.[10] Clinical experience indicated optochin appeared to be effective in the early course of pneumonia infections, but that *S. pneumoniae* became "optochin-fast" or resistant during treatment, likely owing to sub-optimal exposure

due to lack of pharmacokinetic (PK) and pharmacodynamic (PD) information and inabilty to administer higher doses to compensate for the presumptive low lung exposure.[10] It should be highlighted that all of this aforementioned work was done over a century ago or more than three decades before penicillin revolutionized the treatment of bacterial infections. The analytical tools available to perform proper PK/PD studies were simply not available, the word "antibiotic" did not yet exist in the scientific lexicon, and the concept of drug resistance was inchoate. Optochin, thus remains largely forgotten in the history of antibiotics, but could be considered the very first antibiotic discovered if one defines an antibiotic as a natural product or derivative thereof with antibacterial activity that is not a general cytotoxin.[12,13]

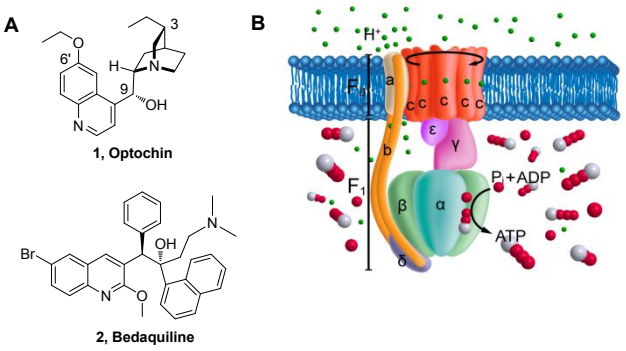


Figure 1. A) Structures of optochin (**1**) and bedaquiline (**2**). The numbering of optochin is based on the convention used for quinine. B) ATP synthase is a nanoscale rotary motor composed of two multimeric subunits (the membrane bound F_o subunit and F_1 subunit), which transforms proton motive force into rotational energy that is used to phosphorylate ADP. The F_o subunit is a cylinder with a central pore (the c-ring) comprised of multiple c-subunits (10-14 subunits in *S. pneumoniae*).

Given optochins remarkable selective antibacterial activity, it has been used in clinical microbiology laboratories as an important diagnostic reagent for distinguishing pneumococcal strains from other alpha-hemolytic streptococci for more than seventy years.[14] The mechanism of action (MOA) of optochin has been inferred through genetic studies that show optochin-resistance maps to *atpE*, which encodes for the c-ring of ATP synthase (Figure 1B). ATP synthase is a multimeric complex and plays a central role in energy metabolism by synthesizing a majority of the ATP in most bacteria.[15] ATP synthase is essential in *S. pneumoniae*[16] and has been chemically validated as a new antibacterial target

with the recent FDA approval of bedaquiline **2**, a diarylquinoline drug that selectively blocks ATP production in *Mycobacterium tuberculosis* (*Mtb*) by coincidentally also binding to the c-ring of ATP synthase.[17] Bedaquiline achieves an extraordinary 10,000-fold selectivity for the mycobacterial ATP synthase over human mitochondrial ATP synthase and other bacterial homologues demonstrating it is possible to achieve high species selectivity with this new molecular target. Interestingly, ATP synthase was recognized as the likely target of optochin nearly 10 years before the first report of bedaquiline, yet none of the published work on bedaquiline or any derivative has mentioned this prior precedent.[18]

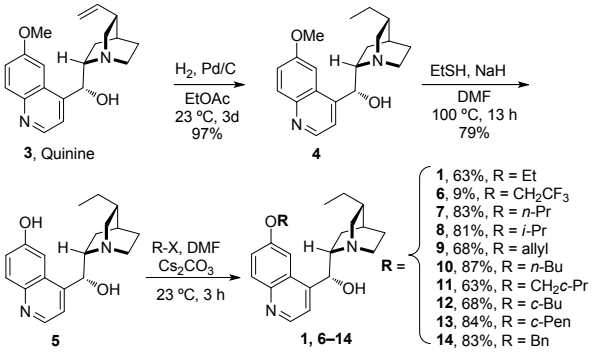
Herein we have examined the structure–activity relationships (SAR) of optochin that govern anti-pneumococcal activity and toxicity through the synthesis of a systematic series of analogues. Based on the recent revelations into optochin's MOA, we hypothesized the observed adverse effects in animals and humans may have been caused by mechanism-based toxicity due to inhibition of human mitochondrial ATP synthase. The high biochemical selectivity attained with bedaquiline suggests the feasibility of separating potency and toxicity of ATP synthase inhibitors. Moreover, the 4-[(quinuclidin-2-yl)hydroxymethyl]quinoline scaffold of optochin has superior physicochemical properties (clogP 3.1, MW 340) compared to bedquiline (clogP 7.3, MW 556) and thus represents a more attractive molecular scaffold for development of new ATP synthase inhibitors.[17]

RESULTS

Chemistry. We began by exploring the SAR at C-6′ of optochin (see Figure 1 for numbering of optochin). Morgenroth and Levy reported in 1911 that an ethyl substituent was optimal and indicated many homologues were less active; although information on many of the actual analogues prepared as well as chemical and microbiological characterization by contemporary standards were not provided.[8,19,20] We therefore synthesized a systematic series of analogues at the C-6′ position as shown

in Scheme 1. Catalytic hydrogenation of quinine **3** afforded dihydroquinine **4** in 97% yield.[21] Demethylation was accomplished employing sodium thioethoxide in DMF at 100 °C to furnish phenol **5**.[22, 23] This nucleophilic cleavage condition was preferred over more traditional methods using Lewis acids or potent electrophiles because of the nucleophilicity of the quinuclidine nitrogen atom. The desired compounds were prepared by phenol alkylation of **5** with a variety of primary and secondary alkyl bromides and iodides using cesium carbonate in DMF to afford optochin **1** and **6–14** in yields ranging from 9–87%.[24]

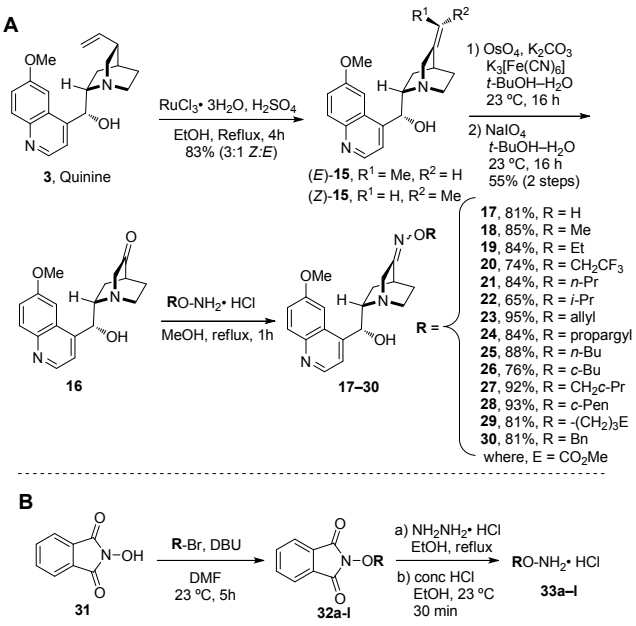
Scheme 1. Synthesis of C-6′ analogues.



The vinyl group at C-3 of quinine provides another convenient synthetic handle to introduce diversity, thus we next explored modification at this position. Ruthenium-catalyzed isomerization of the vinyl moiety of quinine **3** provided exocyclic olefin **15** as an inseparable 3:1 mixture of geometric isomers (Scheme 2A).[25] Oxidation of the exocyclic olefin in **15** was accomplished by a two-step procedure employing osmium tetroxide catalyzed dihydroxylation followed by oxidative cleavage of the resultant diol to furnish ketone **16** in 56% overall yield.[26, 27] Condensation of **16** with hydroxylamine in refluxing methanol provided oxime **17**.[28] A wide variety of other oxime derivatives, **18–30** were prepared analogously by condensation with the appropriate alkoxyamines. Interestingly, *n*-propyl **21**, *i*-propyl **22**, cyclopropylmethyl **27**, and cyclopentyl **28** were isolated as single undefined geometric

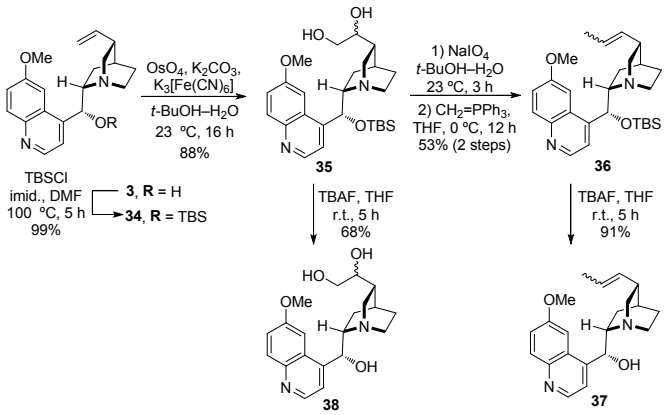
isomers, whereas all of the other analogues were obtained as approximately 2:1 mixtures of geometric isomers. The synthesis of the corresponding alkoxyamines was conveniently performed by alkylation of *N*-hydroxyphthalimide **31** with various alkylbromides using DBU in DMF to afford **32a–l** (Scheme 2B).[29] Hydrazinolysis of **32a–l** liberated the alkoxyamines, which were precipitated to provide the desired alkyoxyamine building blocks **33a–l** as the HCl salts.[30]

Scheme 2. Synthesis of C-3 oxime analogues.



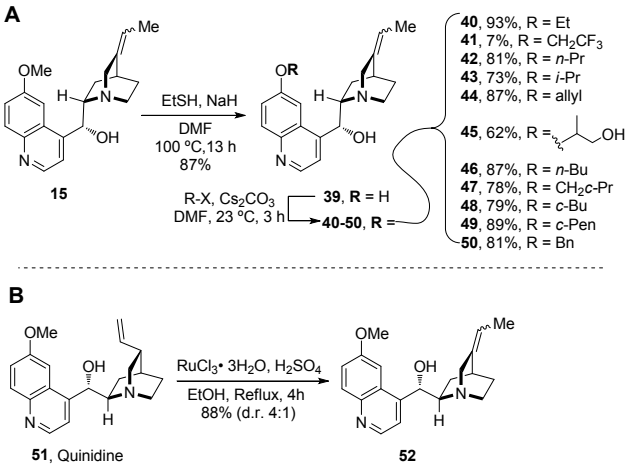
For the next set of analogues the secondary alcohol of quinine **3** was TBS protected using standard conditions to afford **34** (Scheme 3).[31] Lemieux-Johnson oxidation by sequential dihydroxylation to **35** and sodium periodate oxidation furnished an intermediate aldehyde.[27] Interestingly, competitive oxidation of the quinuclidine nitrogen was observed when **35** was not purified. Wittig olefination proceeded to afford **36** smoothly at 0 °C without significant epimerization of the aldehyde at C-3 and subsequent TBAF deprotection yielded **37**.[32] Subjection of the intermediate diol **35** to TBAF similarly provided **38**.[27,31]

Scheme 3. Modification of the vinyl moiety at C-3.



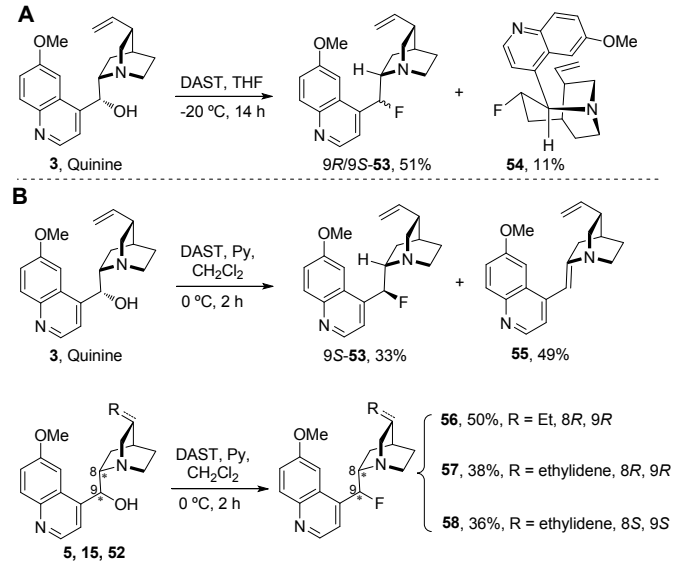
As a result of the enhanced activity and reduced toxicity observed with compound **15** containing an exocyclic olefin at C-3 (*vide infra*) this scaffold was prioritized for further SAR studies. Compound **15** contains a methyl ether at C-6′ of the quinoline and our SAR studies with the optochin series of compounds indicates this position can further modulate potency, thus a systematic series of analogues was synthesized as shown in Scheme 4. Demethylation of **15** employing sodium thioethoxide smoothly afforded apo-cupreine **39**,[23] which was reacted with a series of alkyl bromides and alkyl iodides to furnish **40–50** (Scheme 4A).[24] To investigatie the importance of the stereochemistry at C-8 and C-9 of **15**, quinidine **51** was converted to the diastereomeric analogue **52** (Scheme 4B).[25]

Scheme 4. apo-Cupreine analogues.



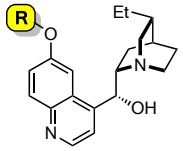
As a last series of analogues, fluorination of the C-9 alcohol of quinine **3** was explored as a model substrate. In the absence of base, quinine **3** reacted with diethylaminosulfur trifluoride (DAST) to give a mixture of C-9 fluorinated diastereomers **53** and the rearranged fluorinated product **54** (**Scheme 5A**). The identity of the rearranged product **54** was previously established by X-ray crystallography.[33] In contrast, in the presence of pyridine, a single C-9 fluorinated diastereomer **53** and eliminated product **55** were obtained.[34] Using this method, we then synthesized the fluorinated products **56**, **57**, and **58** respectively from **5**, **15** and quinidine derivative **52** with yield ranging from 36% to 50% (**Scheme 5B**).

Scheme 5. Fluorinated derivatives.



Micobiology. All optochin analogues were initially evaluated against *S. pneumoniae* strain D39 according to CLSI guidelines as described in the Experimental Section to determine the minimum inhibitory concentrations (MIC) that resulted in complete inhibition of observable growth and for mammalian toxicity against the HepG2 cell line to determine the concentration which resulted in 50% decrease of cell viability (CC_{50}). Optochin and quinine were included as controls and the relative potencies of the compounds discussed below are with respect to optochin. The SAR at C-6 shows an ethyl substituent is preferred as the MIC is 2–4 μg/mL for optochin **1** whereas the methyl analogue **4** loses 16-fold in potency and the unsubstituted phenol is 32-fold less active. The 2,2,2-trifluoroethyl analogue **6**, *n*-propyl **7**, *iso*-propyl **8** and allyl **9** are equipotent with MICs of 4 μg/mL demonstrating slightly larger groups are also tolerated. However, substituents beyond three carbons begin to show loses in potency with *n*-butyl **10** and cyclopropylmethyl **11** displaying 2-fold loses in potency and benzyl **14** a more pronounced 8–16 decrease in potency. We observed that more compact cycloalkyl substituents including cyclobutyl **12** and cyclopentyl **13** were equipotent to optochin. The cytotoxicity loosely tracked with antibacterial activity wherein weakly active compounds possessing MICs greater than 32 μg/mL exhibited no apparent toxicity at 100 μM, the highest concentration evaluated. On the other hand compounds, whose MICs were between 2–16 μg/mL displayed cytotoxicities (CC_{50}) from 41 to 86 μM. Cyclopentyl **13** and optochin **1** deviated from this trend and lacked observable cytotoxicity despite their potent antibacterial activities.

Table 1. SAR at C-6′.



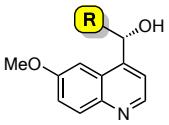
Compound	R	MIC μg/mL (μM)	CC_{50} μM[b]
Optochin (**1**)	Et	2–4 (6–12)	>100
Quinine (**3**)	*n.a.*[a]	128 (395)	>100
4	Me	32 (98)	>100
5	H	64 (204)	>100
6		4 (10)	72
7	*n*-Pr	4 (11)	57
8	*i*-Pr	4 (11)	86
9	allyl	4–8 (11–22)	78
10	*n*-Bu	8 (22)	41
11		8 (22)	87
12		2 (5)	51
13		2 (5)	>100
14	benzyl	16–32	76

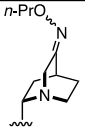
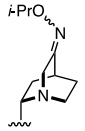
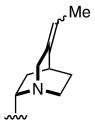
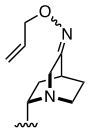
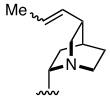
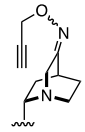
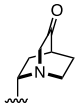
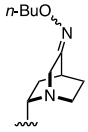
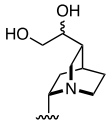
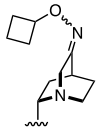
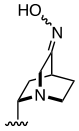
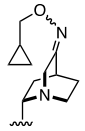
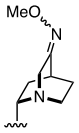
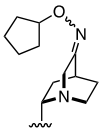
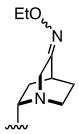
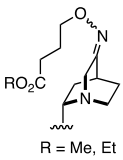
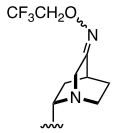
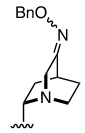
[a]not applicable, see structure in Scheme 1. [b]Standard deviation was less than 10% of the mean value for all compounds.

The SAR of the quinuclidine moiety at C-3′ was performed with the C-6′ position fixed as a methyl ether (Table 2). The MIC of quinine **3** containing a vinyl moiety at C-3 was 128 μg/mL. Saturation of the vinyl moiety in analogue **4** provided a 4-fold increase in potency while isomerization of the double bond to exocylic olefin **15** or addition of a methyl in propenyl **37** resulted in a striking 32-fold increase in activity relative to quinine. The ketone **16** arising from oxidative cleavage of **15** and diol **38** derived from dihydroxylation of vinyl group of **3** were both devoid of activity suggesting polar functional groups at C-3 are poorly tolerated. The exocyclic olefin in **15** orients the side chain at a completely different vector, which may explain its enhanced activity. Unfortunately, access to diverse alkylidene analogues at this position were synthetically challenging, thus isosteric oxime derivatives that are also

sp^2 hybridized at C-3 were explored. The unsubstitued oxime **17** was inactive; however, introduction of *O*-alkyl groups on the oxime led to enhancements in potency providing MICs of 16–32 μg/mL with methyl **18**, *n*-propyl **21**, *iso*-propyl **22**, cyclopropylmethyl **27**, cyclobutyl **26**, cyclopentyl **28**, and benzyl **30** oxime analogues. The SAR is relatively flat in this series of compounds, but some outliers were observed including ethyl **19** and 2,2,2-trifluoroethyl **20** oximes, whose MICs were ≥ 128 μg/mL while the MICs of allyl **23** and propargyl **24** oximes were 64 μg/mL. The *n*-butyl oxime derivative **25** exhibited the most potent activity of all oximes with an MIC of 8–16 μg/mL or 8-16-fold greater than the parent compound quinine **3**. The butyryl oxime **29** containing a terminal methyl ester in the carbon chain abrogated the benefits found oxime butyl ether **25** shifting the MIC to 128 μg/mL. Overall, compounds **15** containing an exocyclic olefin and **37** emerged as the most potent analouge from this series with an MIC of 4 μg/mL followed by oxime butyl ether **25** with an MIC of 8–16 μg/mL. None of the compounds except allyl **23** displayed any cytotoxicity at 100 μM, the highest concentration evaluated.

Table 2. SAR at C-3.



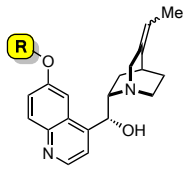
Compound	R	MIC μg/mL (μM)	CC_{50} (μM)	Compound	R	MIC μg/mL (μM)	CC_{50} (μM)
Quinine (**3**)		128 (395)	>100	**21**		32 (86)	>100
4		32 (98)	>100	**22**		16–32 (43–86)	>100
15		4 (12)	>100	**23**		64 (174)	51[a]
37		4 (12)	>100	**24**		64 (175)	>100
16		>128 (>410)	>100	**25**		8–16 (21~42)	>100
38		>128 (>357)	>100	**26**		32 (84)	>100
17		>128 (>391)	>100	**27**		16–32 (42–84)	>100
18		16–32 (375–750)	>100	**28**		16–32 (40–81)	>100
19		128 (360)	>100	**29**	 R = Me, Et	128 (299)	>100
20		>128 (>313)	>100	**30**		32 (77)	>100

[a]Standard deviation was less than 10% of the mean value.

The promising activity of **15** combined with the ease of synthesis prompted optimization of the substituent at C-6′ because our SAR studies on the optochin scaffold indicated this position allows further tuning of potency. The simple unsubstituted compound **39** containing a free phenol lost 4-fold in potency relative to **15** while the ethyl analogue **40** realized a substantial 8-fold increase in activity providing an MIC of 0.5 μM (Table 3). The *iso*-propyl analogue **43** was equipotent to **40**, but most other small substituents led to a slight loss of activity with MICs ranging from 2–4 μg/mL as observed with 2,2,2-trifluoroethyl **41**, *n*-propyl **42**, allyl **44**, 1-hydroxypropan-2-yl **45**, *n*-butyl **46**, cyclopropylmethyl **47**, and cyclopentyl **49**. However, cyclobutyl **48** broke this trend and yielded the most potent optochin analogue yet described with an MIC of 0.25 μg/mL. Given the potency of **48**, we sought to determine if activity resided in one of the geometric isomers and thus separated the mixture by preparative reverse-phase HPLC and assigned each isomer by NOE studies. Thus, irradiation of the C-2 methylene of the major isomer led to an enhancement of the C-11 methyl group while irradiation of the C-10 vinyl proton led to enhancement of the C-4 methine group, securing the structural assignment as *Z* (note: the methyl group is in the plane with N-1, C-2, C-4 and directed towards the C-2 methylene) The minor isomer was assigned as *E* in a similar manner. The *E*-isomer conferred optimal activity and was 2-fold more potent than (*Z*)-**48**. Analogues with larger substituents progressively lost activity as illustrated by benzyl **50**, whose MIC increased to 16 μg/mL. The overall trends for this compounds series paralleled the SAR of our initial optochin analogues (Table 1) with small alkyl substitutents between 2–5 carbons being preferred with significant reductions in potency as the chain length decreased or increased beyond this narrow range. Interestingly, the cytotoxicity did not track with antibacterial activity and several compounds including

39–41, **45**, and **47** displayed no cytotoxicity while the remaining analogues exhibited modest cytotoxicity with CC_{50} values ranging from 27 to 65 μM.

Table 3. SAR at C-6′ of apo-cupreine analogues.



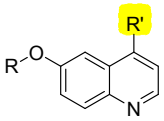
Compound	R	MIC μg/mL (μM)	CC_{50}[a] (μM)
39	H	16 (52)	>100
15	Me	4 (12)	>100
40	Et	0.5 (1)	>100
41		2 (5)	>100
42	*n*-Pr	1–2 (3–6)	51
43	*i*-Pr	0.5 (1)	54
44	allyl	2 (4)	54
45		2 (5)	>100
46	*n*-Bu	4 (11)	27
47		2 (5)	>100
48		0.25 (0.7)	56
49		2 (5)	48
50	benzyl	16 (40)	47
(*E*)-**48**		0.25 (0.7)	65
(*Z*)-**48**		0.5 (1.4)	59

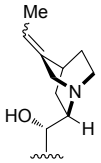
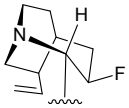
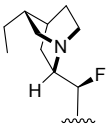
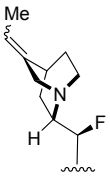
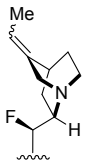
[b]Standard deviation was less than 10% of the mean value for all compounds.

Our last series of analogues explored the importance of the stereochemical requirements of C-8 and C-9 along with the role of the alcohol at C-9 (Table 4). Epimerization of the stereocenters at C-8 and C-9 in **52** obliterated activity demonstratng a strict requirement for the native 8*S*, 9*R*-stereochemistry. Bioisosteric replacement of the 9-hydroxy with a fluorine atom in the 9*S*-configuration with dihydroquinine or apo-cupreine derivatives **57** and **58** ablated activity illustrating the importance of the alcohol at this position. Finally, we obtained a small amount of a rearranged scaffold azabicyclo[3.2.2]nonane **54** during our attempts to fluorinate the C-9 position of the model substrate quinine. Intiguingly, this compound was equipotent to quinine suggesting **54** could be a viable scaffold

for future medicinal chemistry efforts through appropriate modification at C-6′ and reduction of the olefin. We also tested **56** obtained during our studies, but this was inactive consistent with the SAR trends observed in the previously described series of compounds. Taken together, our results indicate the 8*S*, 9*R*-stereochemistry of **48** is required for activity, the C-9 alcohol is also essential, and fluorination at C-9 is poorly tolerated. None of these derivatives displayed any cytotoxicity at the highest concentration evaluated (CC_{50} > 100 μM).

Table 4. SAR at C-8 and C-9 positions.



Compound	R	R'	MIC μg/mL (μM)	CC_{50} (μM)
52	Me		>128 (>395)	>100
54	Me		128 (392)	>100
56	Me		>128 (>390)	>100
57	Me		128 (392)	>100
58	Me		>128 (>392)	>100

The minimum bactericidal concentration (MBC) of selected compounds was evaluated against *S. pneumoniae* D37 is shown in Table 5. Compound **48** demonstrated potent bactericidal activity with an

MBC of 1-2 µg/mL followed closely by optochin **1**, whose MBC was 4 µg/mL. The kinetics of bacterial killing were assessed *in vitro* using time-kill assays by incubating **48** with *S. pneumoniae* at 1×, 5× and 50× the MIC. However, a significant decrease in the number of bacteria as measured by OD_{620} or plating to determine the residual colony forming units (CFU) These experiments revealed **48** was bacteriostatic during the first 18 hours (Figure 2). Experiments with higher concentrations of antibiotic (up to 1000× the MIC) as well as with a lower initial inoculum (10^7 CFU) yielded the same outcome. Vancomycin was used as a positive control and displayed rapid bactericidal activity reducing the initial inoculum by about 1,000-fold units within 3 hours at 5× the MIC. Attempts to extend the duration of the experiments were confounded by well known autolytic behavior of *S. pneumoniae* that occurs in vitro as observed by the decreased CFU of the untreated control after 5 hours (data not shown).

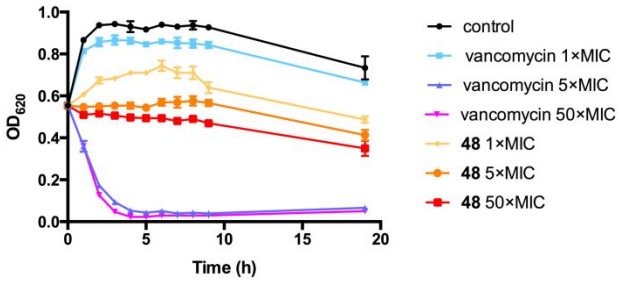


Figure 2. In vitro time-kill analysis of *S. pneumoniae*. Bacterial killing was monitored by measuring the optical density at 620 nm (OD_{620}) up to 18 hours. Four independent time-kill experiments were performed, and one representative experiment is shown. The CFU at each time point was independently determined by plating and the results paralleled the OD_{620} measurements. Compound 48 was evaluated as a *E/Z* mixture.

Table 5. MBC of **1**, **39**, and **48** (*E*/*Z* mixture).

Compound	MIC (µg/mL)	MBC (µg/mL)
Optochin (**1**)	2–4	4
apo-Cupreine (**39**)	16	16
48	0.25–0.5	1–2

S. pnuemoniae mutants that were resistant to **48** were isolated with a frequency of ~1 in 10^6 CFU when **48** was present at a concentration of 4× MIC (Figure 3). This frequency decreased to ~2 in 10^7 CFU at 8× MIC and to ~1 in 10^8 CFU at 16× MIC. Mutants resistant to optochin (**1**) were recovered at a similar frequency. For rifampicin used as positive control, we observed a frequency of resistance of about 2 in 10^7 at 4× MIC, which decreased to 4 in 10^8 at 8× and 16× MIC.

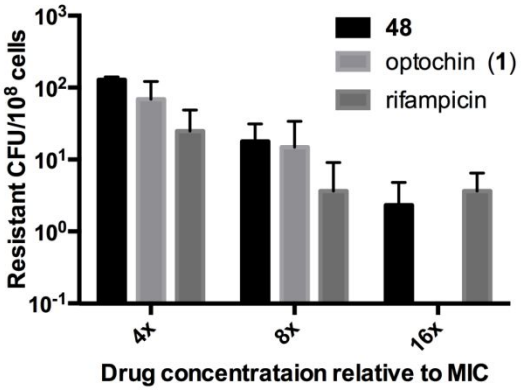


Figure 3. Frequency of the emergence of spontaneous resistance in *S. pneumoniae* D39 on standard solid medium in the presence of **48** (*E*:*Z* mixture) at concentrations 4×, 8×, and 16× the MIC (1, 2, and 4 μg/mL). Optochin **1** (8, 16, and 32 μg/mL) and rifampicin (0.125, 0.25, and 0.5 μg/mL) were used as controls. Data were obtained twice independently with two to three replicates (except for the data for optochin at 16× MIC, which was a single experiment with no recoverable colonies). The mean value is given for platting 10^8 CFU and the error bars represent the standard error of the mean (SEM). Error bars for compound **48** at 4× the MIC are not shown because only two data points were obtained (120 and 138 colonies were recovered). The individual data points are provided in Table S1 in the Supporting Information.

We next evaluated **48** along with apo-cupreine **39** and optochin **1** against a panel of other *S. pneumoniae* strains including drug-sensitive, multidrug resistant, and optochin-resistant strains (Table 6). Compound **48** maintained fairly uniform activity against four *S. pneumoniae* multidrug resistant strains including serotypes 6B, 19A, and 19F (Table 6, entries 3–6) with MICs ranging from 0.25–1 μg/mL. Interestinly, apo-cupreine **39** that also contains the C-3′ exocyclic olefin found in **48** lost considerable activity against several of drug-resistant strains (Table 6, entries 4–5). We also generated two optochin-resistant strains through point mutations (G142C and G145A) of *atpE* in *S. pneumoniae* strain R6 using

an efficient PCR-based approach based on previously described resistant studies (Table 6, entries 7–8).[18,35] The MIC values of optochin **1** and **48** were each shifted 16–32-fold confirming AtpE as a likely molecular target of these compounds.

Table 6. Inhibitory activity against resistant *S. pneumoniae.*

Entry	Strain	Resistance[a]	MIC, µg/mL		
			Optochin (1)	**apo-Cupreine (39)**	**48** (*E:Z* mixture)
1	D39	DS	2–4	8–16	0.25–0.5
2	R6	DS	2	n.d.	0.5
3	TH2784 (19F*)	APCET	2	4	0.5
4	TH2582 (19A)	APChEMC	2	64	0.25–0.5
5	TH2889 (6B)	APEErMoC	2	128	0.5
6	TH2863 (19F)	PEMT	4–8	n.d.	0.5–1
7	R6-*atpE*$_{G142C}$	Optochin	64	n.d.	8–16
8	R6-*atpE*$_{G145A}$	Optochin	16	n.d.	4

[a]Abbreviation: n.d.: not determined, DS: Drug Sensitive, A: Amoxicillin, P: Penicillin, C: Cefotaxime, E: Erythromycin, T: Tetracycline, Ch: Chloramphenicol, M: Meropenem, Er: Ertapenem, Mo: Moxifloxacin.

To further validate *atpE* as the target, we sequenced the *atpE* gene of 29 mutants from the resistance tests discussed in Figure 2 isolated at 4, 8, and 16× MIC of **48**. Indeed all of the mutants contained missense mutations in *atpE* as shown in Table 7. Interestingly, at 4× the MIC, all mutations (10/10) mapped to G18S within the N-terminal helix of AtpE while at 8× the MIC, the mutations were found at different positions (A31V and A49S). We also sequenced five mutants from multiple experiments recovered at 16× the MIC of **48** and mutations mapped to F50L and F45C within the C-terminal helix of AtpE. For comparison, 15 mutants resistant to optochin (**1**) were sequenced and mutations mapped to similar positions (Table 7).

Table 7. Mutational substitutions in *S. pneumoniae atpE*.

Compound[a]	DNA sequence	Amino acid sequence	# of clones
48 (4× MIC)	G52A	G18S	10
48 (8× MIC)	C92T	A31V	9
	C145T	A49S	6
48 (16× MIC)	T148C	F50L	4
	T134G	F45C	1
Optochin (**1**), (4× MIC)	G52A	G18S	7
	G145T	A49S	1
Optochin (**1**), (8× MIC)	G49A	G14S	5
	T72A	N24K	2

[a]The value in parentheses, i.e. (4× MIC), refers to the concentration of compound used to select the isolated mutant. Compound 48 was used as an *E:Z* mixture.

We hypothesized ATP synthase in *S. pneumoniae* is involved in pH homeostatsis as observed in other fermentive microorganisms by operating in the reverse direction to regulate the intracellular pH (pH_{IB}) by consuming ATP.[36-38] To explore the physiological consequences of inhibition of *S. pneumoniae* ATP synthase, we determined the pH_{IB} of strain D39 and two isogenic optochin-resistant mutants (D39-*atpE*$_{G142C}$ and D39-*atpE*$_{G145A}$) using a pH-sensitive ratiometric GFP biosensor on a plasmid that allowed non-invasive measurements of pH_{IB}.[39,40] The bacterial cells in medium at pH 7.2 were treated with **48**, DMSO only (negative control), or the proton uncoupler carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP) (positive control) and the pH was measured at 5 minute intervals (Figure 4).

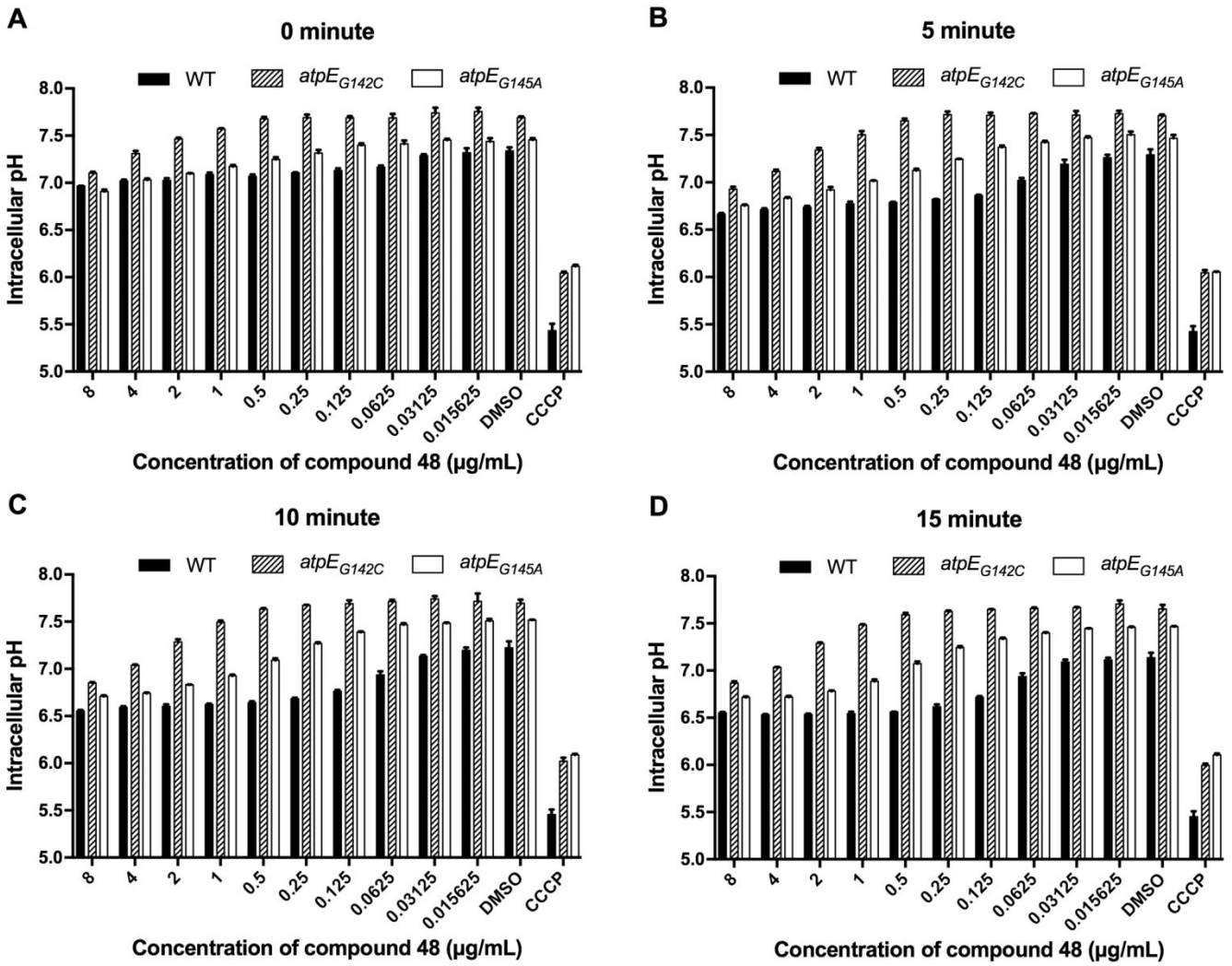


Figure 4. The impact of compound 48 treatment on intracellular pH of *S. pneumoniae*. D39 WT, D39 *atpE*$_{G142C}$, D39 *atpE*$_{G145A}$ were treated with vehicle control DMSO (0.5% v/v), compound 48, or CCCP (10 μM) in PBS pH7.2 buffer supplemented with 10 mM glucose. pH measurements immediately and at 5-min intervals after treatment were shown in A, B, C and D, respectively. Experiments were performed two times independently in triplicate. Representative data from one independent experiment is shown with mean ± standard deviation.

The basal pH_{IB} of wild-type *S. pneumoniae* treated with DMSO was 7.34 ± 0.04. Treatment with **48** resulted in a time and concentration-dependent decrease in pH_{IB} (Figure 4). For example, at 5 minutes, the pH_{IB} decreased to 7.02 ± 0.03 with 0.0625 μg/mL **48** (0.25×MIC) and to 6.82 ± 0.003 with 0.25 μg/mL **48** (1×MIC), but then quickly began to level off reaching 6.66 ± 0.01 at 8 μg/mL **48** (32×MIC) (Figure 4B). The impact of **48** on pH_{IB} was rapid, thus addition of **48** to wild-type *S. pneumoniae* at 0.25

μg/mL (1×MIC) immediately lowered the pH to 7.11 ± 0.003 (ΔpH = -0.23 compared to the DMSO only control at the same time point), then to 6.82 ± 0.003 (ΔpH = -0.47) at 5 minutes, followed by a more gradual decline to 6.68 ± 0.01 (ΔpH = -0.54) at 10 minutes that remained essentially unchanged at 15 minutes (ΔpH = -0.52) (Figures 4A-D). Addition of DMSO only (negative control) did not impact pH_{IB} whereas addition of 10 μM of the proton uncoupler CCCP (positive control) caused an immediate reduction of the intracellular pH to 5.43 ± 0.07 (ΔpH = -1.9) that persisted for the duration of the experiment. To provide support for the selectivity of this process, we also evaluated two isogenic *S. pneumoniae* mutant strains D39-*atpE*$_{G145A}$ and D39-*atpE*$_{G142C}$ whose MICs of 2 and 8 μg/mL are shifted 8 and 32-fold, respectively. Treatment of the more resistant strain D39-*atpE*$_{G142C}$ with 0.25 μg/mL **48**, which is the MIC of the wild-type strain, had essentially no impact on pH_{IB} at any time point (ΔpH $\leqslant$ 0.03). The mutant strain D39-*atpE*$_{G142C}$ did undergo cytoplasmic acidification with **48**, but only at substantially higher concentrations than required for the wild-type *S. pneumoniae* D39. The isogenic *S. pneumoniae* mutant D39-*atpE*$_{G145A}$ that exhibits low-level resistance (MIC = 2 μg/ml) showed a comparable time-dependent decrease in intracellular pH as the wild-type; but only at concentration of **48** equal to or greater than 2 μg/mL. Taken together these data show **48** results in mild cytoplasmic acidification that is dependent on AtpE.

To confirm the microbiological selectivity, we evaluated **48** against a panel of gram positive and negative organisms as well as a couple of other streptococcal species. As shown in Table 8, compound **48** was weakly active towards *Enterococcus faecium, Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter clocae* displaying MICs of ≥128 μg/mL. Compound **48** retained moderate activity against other pathogenic streptococci with MICs of 32 μg/mL and 16 μg/mL against *Streptococcus pyogenes* and *Streptococcus mitis*, respectively.

Table 8. Susceptibility of gram positive and negative bacteria to **48** (*E*:*Z* mixture).

Species	Strain	MIC (μg/mL)
Staphylococcus aureus	TH4114	128
Streptococcus pyogenes	ST157	32
Streptococcus mitis	ST138	16
Enterococcus faecium	TH6064	64–128
Enterobacter clocaae	TH11715	>128
Escherichia coli	TH4746	>128
Klebsiella pneumoniae	ATCC13883	128
Acinetobacter baumannii	TH9781	128
Pseudomonas aeruginosa	TH4090	>128
Mycobacterium tuberculosis	H37Rv	>256

The effect of four antibiotics (vancomycin, penicillin, levofloxacin and rifampicin) used to clinically treat pneumococci was next examined in combination with **48** by checkboard assay with *S. pneumoniae* D39. The fractional inhibition concentration indices (FICI) were calculated to assess drug interaction between these compounds. The minimum $FICI_m$ values for each combination ranged from 0.63 to 1. indicating these antibiotics exhibit no interaction (FICI >1 to ≤ 4). Representative data from a single experiment are shown in Figure 5.

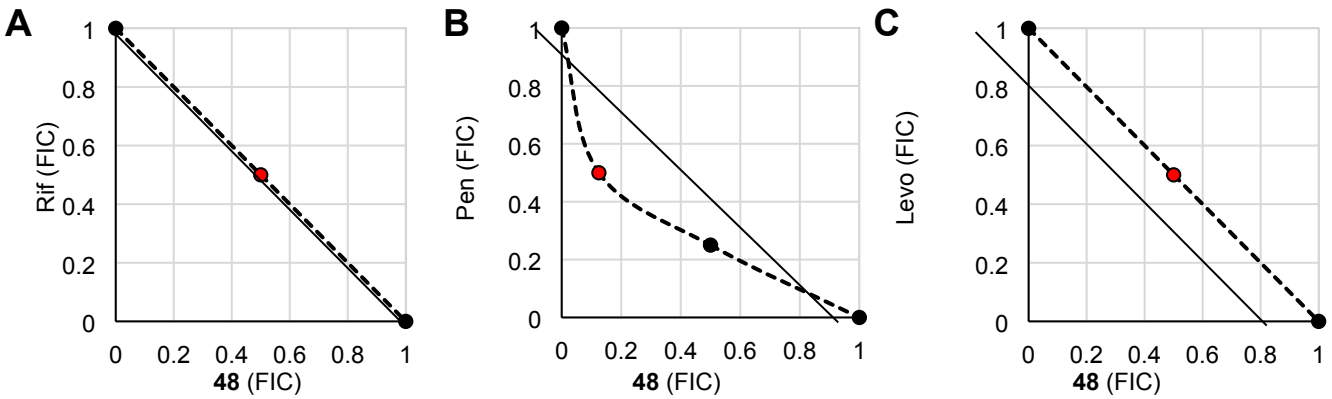


Figure 5. Graphical representation of checkerboard assays used to determine drug interactions with **48** (*E*/*Z* mixture). Representative data is presented for **48** in combination with rifampicin (panel A), penicillin (panel B), and levofloxacin (panel C). $FICI_m$, minimum value of FICI in the tested combinations is 1.0 (panel A), 0.63 (panel B), and 1.0 (panel C). Representative data from at least

two independent experiments are shown. $FICI_m$, minimum value of FICI in the tested combinations is shown. Synergy ($FICI_m \leq 0.5$). No interaction ($FICI_m > 0.5–4$). Three to four independent checkerboard assays were performed, and one representative experiment is shown.

To assess the potential for mitochondrial toxicity of **48** caused by inhibition of ATP synthase, we performed cell viability assays employing glucose or galactose as the primary carbon sources following the protocol described by Will and co-workers.[41] Highly proliferative HepG2 cells have adapted their metabolism under standard cell culture conditions to obtain ATP primarily from glycolysis and thus are insensitive to inhibitors of ATP synthase. By contrast, metabolism of galactose does not generate ATP by glycolysis; consequently, cells grown on galactose supplemented medium are highly sensitive to inhibition of mitochondrial function because they are forced to rely on the mitochondrial oxidative phosphorylation pathway to generate a proton motive force that in turn drives ATP synthase in the mitochondrial membrane. The impact of optochin **1**, apo-cupreine **39**, and **48** on cell viability of HepG2 cells did not depend on carbon source (glucose or galactose) confirming these quinine derivatives do not disrupt mitochondrial function (see Table 9 and a representative dose-response curve for **48** in Figure 6A). By contrast, the positive control, oligomycin, a potent inhibitor of mammalian ATP synthase inhibited cell viability more than 90% (CC_{90}) at ≤ 0.3 μM in galactose medium. The CC_{90} value was shifted by four orders of magnitude to 300 μM when grown in glucose-supplemented medium (Figure 6B). The negative control tamoxifen, which does act on ATP synthase, displayed equipotent cytotoxicity in both media (Figure 6C). Taken together, our results demonstrate that optochin **1** and derivatives **39** and **48** do not pertub mitochondrial function indicating these molecules likely do not inhibit mammalian ATP synthase due to their intrinsic biochemical selectivity for the *S. pneumoniae* homologue.

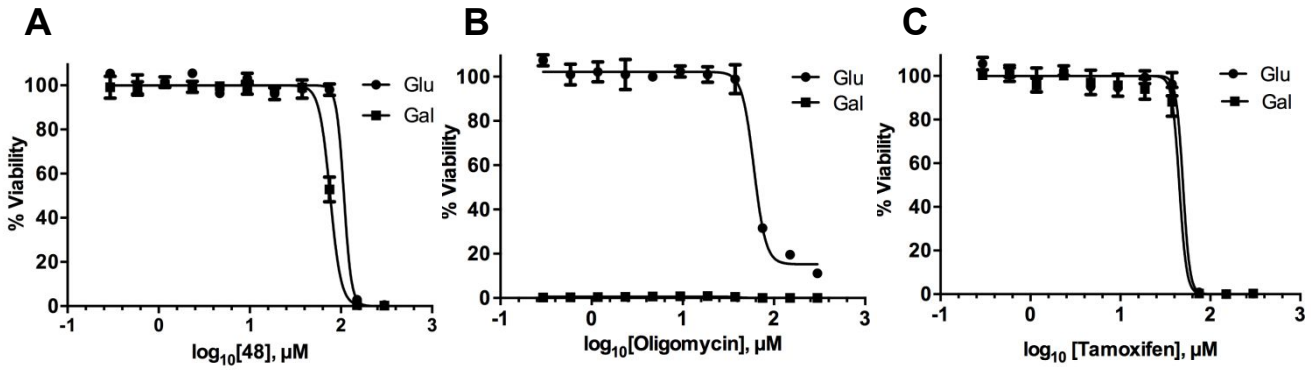


Figure 6. Dose-response curves for cell viabililty of HepG2 cells treated with compound **48** as an *E:Z* mixture (panel A), oligomycin (panel B) and tamoxifen (panel C) for 24 hours using the CellTiter-Glo® luminescent cell viability assay. Cells were grown in DMEM medium either supplemented with 10 mM glucose (solid circles) or 10 mM galactose (solid squares).

Table 9. Mitochondrial Toxicity of HepG2 cells.[a]

Compound	CC_{90}, +Glu (μM)	CC_{90}, +Gal (μM)
Tamoxifen	75	75
Oligomycin	300	<0.3
Optochin (**1**)	300	300
Apo-Cupreine (**39**)	>300	300
48 (*E/Z* mixture)	150	150

[a]Inhibition of 90% cell viability (IC_{90}) measured against HepG2 cells grown in DMEM medium supplemented either with 10 mM glucose (+Glu) or 10 mM galactaose (+Gal)

Pharmacokinetics and Metabolism Studies. Each of the pure diasteromers *Z*-**48** and *E*-**48** along with optochin **1** and apo-cupreine **39** were evaluated in single dose pharmacokinetic experiments using male ICR mice administered as a bolus orally (*p.o.*) at 25 mg/kg and intravenously (*i.v.*) at 2.5 mg/kg. The serum concentration-versus-time curves shown in Figure 7 were used to determine the pharmacokinetic parameters (Table 10) by noncompartmental analysis. After intravenous (*i.v.*) administration (2.5 mg/kg), all compounds exhibited biexponential kinetics with a rapid distribution phase followed by a slow terminal elimination phase resulting in an extremely large steady state volumes of distribution (VD_{ss}) ranging from 8.8 L/kg for *E*-**48** to 20 L/kg for apo-cupreine **39** with optochin possessing an intermediate value of 12.7 L/kg. Each of the compounds also exhibited extremely high clearances (Cl) ranging from 172–274 $mL \cdot min^{-1} \cdot kg^{-1}$, which indicates extra-hepatic

metabolism since the clearance vastly exceeds mouse hepatic blood flow (90 mL·min^{-1}·kg^{-1}) suggesting tissue oxidases may play a significant role in metabolism of these quinine derivatives. The high clearance overrides the extensive tissue distribution resulting in short half-lives ($t_{1/2\beta}$) from 0.42 to 0.53 hours for *Z*-**48**, *E*-**48**, and optochin **1**. After oral administration of optochin **1**, apo-cupreine **39**, *Z*-**48** and *E*-**48**, the compounds reached peak plasma concentrations (C_{max}) of 639, 619, 207 and 331 ng/mL. The oral bioavailabilities (F) of optochin **1**, apo-cupreine **39**, *Z*-**48** and *E*-**48** calculated from the area-under-the-curve (AUC) of the concentration-time plots shown in Figure 7 were 109%, 23%, 14% and 21%, respectively. Based on the C_{max} values, only *E*-**48** exceeded its MIC when dosed orally. Even with its impressive bioavailability optochin **1** was unable to approach its MIC. These preliminary PK studies suggest the poor in vivo performance of optochin was likely caused by poor oral exposure as a result of rapid clearance. While *E*-**48** provides improved oral exposure as measured by the MIC/AUC ratio, it also suffers from rapid clearance and low bioavailability.

Table 10. Pharmacokinetic parameters of **1**, **39**, *Z*-**48**, and *E*-**48** after a single oral or *i.v.* administration to male ICR mice.

Parameters	Units	Optochin (**1**)		apo-Cupreine (**39**)		Z-**48**		*E*-**48**	
		*p.o.*25 mg/kg	*i.v.*2.5 mg/kg	*p.o.*25 mg/kg	*i.v.*2.5 mg/kg	*p.o.*25 mg/kg	*i.v.*2.5 mg/kg	*p.o.*25 mg/kg	*i.v.*2.5 mg/kg
$t_{1/2\beta}$	h	1.00	0.53	0.91	1.12	0.69	0.42	0.70	0.44
T_{max}	h	0.5	0.033	0.083	0.033	0.25	0.033	0.083	0.033
C_{max}	ng/mL	639	375	619	511	207	521	331	399
$AUC_{(0-t)}$	h×ng/mL	1569	144	442	190	319	235	368	175
VD_{ss}	L/kg	-	12.7	-	20.0	-	6.30	-	8.8
Cl	mL/min/kg	-	274	-	207	-	172	-	230
F%		109	-	23.3	-	13.6	-	21.0	-

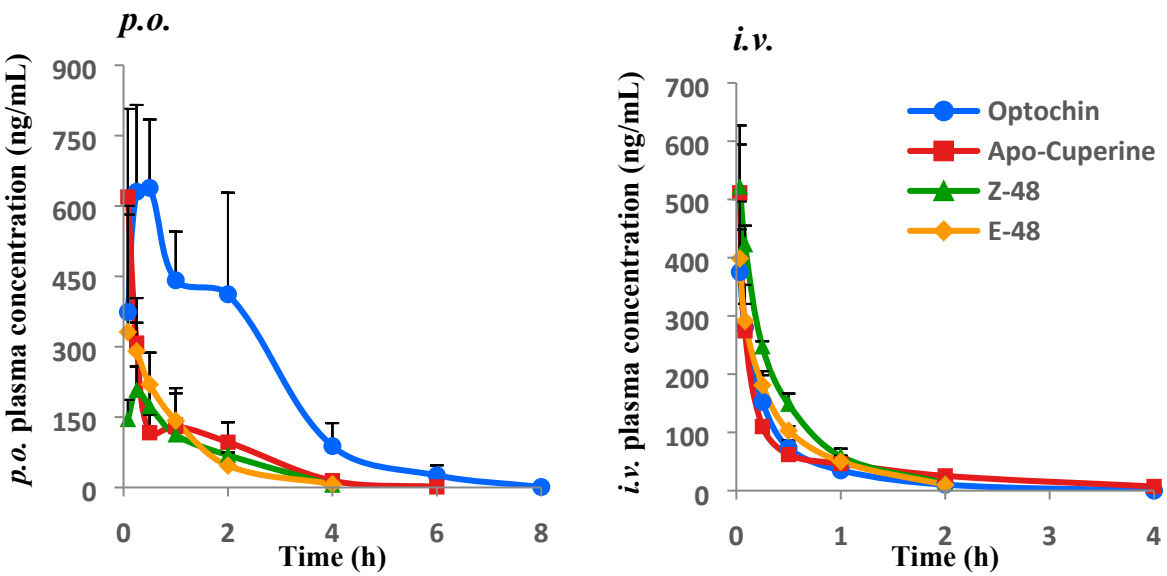


Figure 7. Mean plasma concentration versus time profile after single *p.o.* (25 mg/kg) and *i.v.* (2.5 mg/kg) bolus administration of **1**, **39**, *Z*-**48**, and *E*-**48** to male ICR mice. Error bars represent standard deviation of the mean (n = 3).

Based on the excellent pharmacokinetic behavior of quinine,[42] we were surprised that the related derivatives optochin (**1**), apo-cupreine **39**, and **48**, were so rapidly cleared. Given quinine primarily undergoes hepatic metabolism, we incubated **1**, **39**, and ***Z*-48** with ICR mouse liver microsomes (MLM) and observed 87%, 89%, and 99.8% metabolism, respectively after 60 minutes. Further characterization of the metabolites of ***Z*-48** generated by MLMs using LC-MS/MS revealed the time-dependent formation of seven metabolites (M1-M7) ranked in order of their relative abundance with M1-M3 accounting for 80% of the total abundance based on ion counts. The primary metabolite M1 was formed by hydroxylation (+16 Da) pendant to the quinuclidine ring while M2 was formed by *O*-dealkylation of the cyclobutyl group (-54 Da) and M3 resulted from hydroxylation of M2 on or pendant to the quinuclidine ring (-54 Da + 16 Da). Further hydroxylation of M1 on the quinoline (+32 Da) provided M4 and oxidation of M1 to a putative keto derivative (+16 Da – 2Da) yielded M7. Metabolism of M2 via glucuronidation afforded M5 (-54 Da + 176 Da) while oxidation of M2 furnished ketone M6 (-54 Da – 2 Da). Putative structural assignments of M1-M7 are shown in Figure 8 based on accurate mass

measurements of the MS^1 and MS^2 fragment ions. The structures of metabolites M2 (i.e. apo-cupreine) was confirmed by chemical synthesis. We also performed a similar anlaysis on optochin (**1**) and apo-cupreine (**39**) and the results are summarized in the supporting information. Taken together, this analysis demonstrates hydroxylation of the quinucline is the major pathway for all compounds followed by *O*-dealkylation at C-6′.

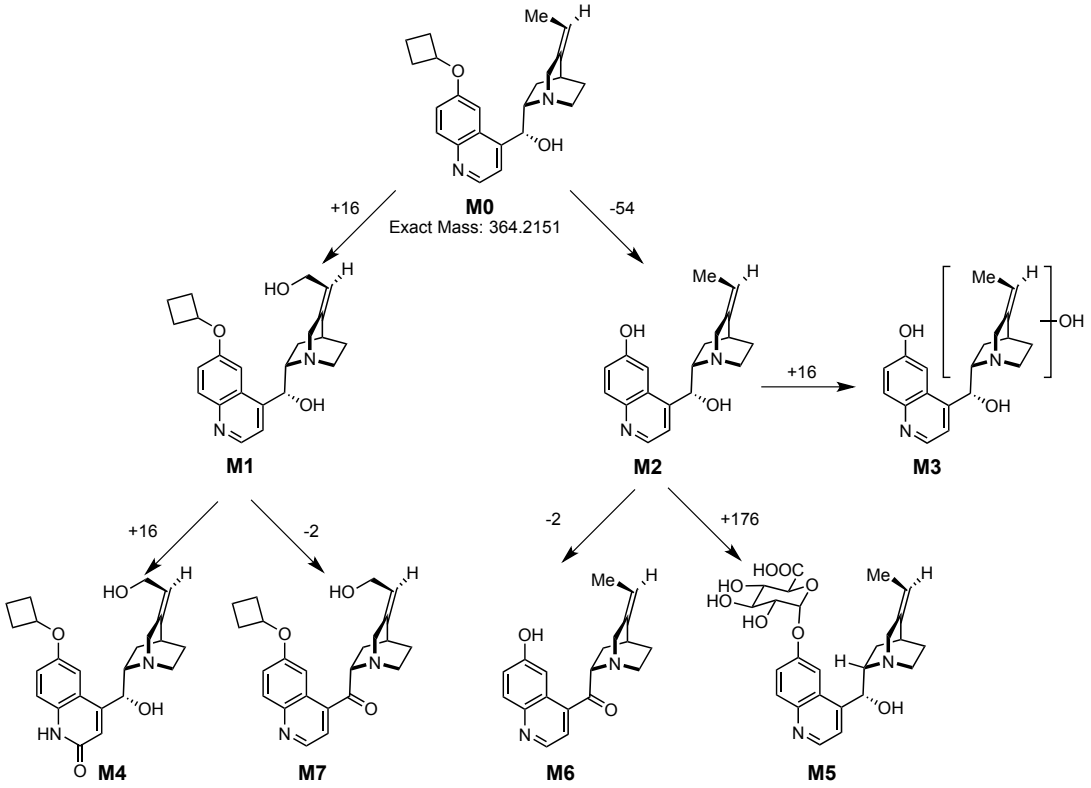


Figure 8. Proposed metabolic scheme for ***Z*-48** in MLM.

DISCUSSION

Given the paucity of new antibiotics and growing antimicrobial resistance epidemic, re-examination of old antibiotics like optochin represents a promising strategy to replenish the antibiotic pipeline. The putative molecular target of optochin is the multimeric ATP synthase; however, a cell-free biochemical assay was not initially available for this challenging target. Thus optimization was solely guided by whole-cell antibacterial activity, which can be confounded by differential bacterial accumulation and off-target activity across a compound series. Despite, these limitations, clear SAR trends were observed as discussed below. The SAR of optochin was interrogated at each of the most synthetically accessible positions of the 4-[(quinuclidin-2-yl)hydroxymethyl]quinoline scaffold. Morgenroth and Levy had reported that an ethyl group at the quinoline C-6′ position of optochin was optimal, while the methyl, isopropyl, isobutyl, and isopentyl homologues were respectively, 20-, 4,- 150-, and 20-fold less potent.[10] We observed slightly different relative trends in activity. While our methyl analogue **7** was 16-fold less active than optochin and the isopropyl analogue **10** was 2-fold less potent, consistent with the reported values, we found the SAR to be considerably more flat for homologues **8–13**, which contained between two and four carbons (e.g. 2,2,2-trifluoethyl, *n*-propyl, iso-propyl, allyl, *n*-butyl, and cyclopropylmethyl). Each of these compounds was only 2-4-fold less potent than optochin. Further exploration led to cyclobutyl **14** and cyclopentyl **15** with equivalent potencies to optochin, which deviates from the anticipated trends of the historical data. Our findings indicate slightly more flexibility at the quinoline C-6′ position than anticipated. Comparison of the quinoline SAR at C-6′ of different quinuclidine congeners (Tables 1 and 3) reveals changing the quinuclidine moiety also subtly impacts the relative SAR trends observed at C-6′. Thus for the 3-ethyl substitued quinuclidine analogues (e.g. optochin analogues, Table 1), the optimal C-6′ substituent is an ethyl. However, the 3-ethylidene substitued

quinuclidine analogues (Table 3) display a slightly different trend wherein the cyclobutyl substituent is preferred.

We explored the SAR at C-3 of the quinuclidine through the synthesis of a systematic series of oxime derivatives. This was accomplished by isomerization of vinyl group of quinine **3** to the C-3 ethylidene isomer **15**, which in turn could be efficiently oxidatively cleaved to furnish the C-3 ketone derivative **16**. We successfully synthesized 14 oxime derivatives **17–30** from **16** and identified several compounds (**18**, **21**, **22**, **26**, **28**, and **30**) that were equipotent to the parent dihydroquinine **7** whose MIC was 32 μg/mL (see Table 2). However, clear SAR trends did not emerge as highlighted by the uneven potency trends of **18–21** where the unsubstituted and ethyl oxime derivatives **17** and **19** (MIC > 128 ug/mL) were inactive while the methyl oxime **18** and *n*-propyl oxime **21** were active (MIC = 32 μg/mL). The intermediate **15** containing the exocyclic olefin exhibited an unexpected and dramatic 8-fold increase in potency (MIC = 4 μg/mL) relative to dihydroquinine **7**. Butler and Cretcher had described the isolation of a quinine derivative in 1935 termed apo-cupreine containing an ethylidene at C-3 of the quinuclidine, but a phenol C-6′ of the quinoline (a molecule, identical in structure to **39** that could be isolated as mixture of likely *E* and *Z* isomers).[43] The apo-cupreines displayed no toxicity when dosed in mice (30/30 mice survived a 5 mg dose ~ 250 mg/kg) whereas only 5/30 mice survived an equivalent dose of optochin.[43] In 2014, Sullivan and co-workers re-examined a related analogue termed hydroxyethyl-apo-cupreine for anti-malarial activity and confirmed it much less toxic than quinine.[44] These data suggested to us that toxicity could potentially be minimized by incorporation of an ethylidene substituentat C-3 rather than the ethyl group found in optochin. We hypothesized the sp^2 hybridized ethylidene substituent, which orients the ethylidene group in a different vector, decreases undesirable off-target activity resulting in the improved safety profile. We therefore set out to further optimize compound **15** by modification of the C-6′ substituent and identified derivative **48** with an

impressive MIC of 0.25 μg/mL that is 8–16 more potent than optochin. Attempts to further improve upon the potency of **48** by alteration of the stereochemistry at C-8/9 or modification of the C-9 hydroxyl proved futile. Thus bioisosteric replacement of the C-9 hydroxyl with a fluoro group abolished activity. Overall, these initial SAR studies identified **48**, which is an order of magnitude more potent than optochin.

We initially hypothesized the adverse effects observed with optochin in humans were due to mechanism-based toxicity; however, the lack of mitochondrial toxicity suggests optochin and analogue **48** do not inhibit mammalian ATP synthase or distrupt mitochondrial function. Pharmacokinetic analysis demonstrates both optochin and analogue **48** are rapidly cleared resulting in low exposure, thus it seems plausible that the parent compounds do not contribute to toxicity. Rather we speculate toxicity may be caused by one of the metabolites as has been suggested for quinine, which undergoes similar metabolism.[45] Further work will be required to evaluate this new hypothesis and future efforts will be directed at blocking the metabolic labile positions of **48** as well as toxicological evaluation of the major metabolites.

Kunin and Ellis reported that mefloquine which bears some structural resemblance to optochin exhibited antibacterial activity against gram-positive organisms including *S. pneumoniae (*MIC 0.4–0.8 μg/mL), *Staphylococcus aureus* (MIC = 16 μg/mL), *Staphylococcus epidermidis* (MIC = 16 μg/mL), and *Enterococcus faecalis* (MIC = 16 μg/mL) with substantially reduced activity agasint gram-negative bacteria (MICs ≥ 32 μg/mL).[46] Like optochin, they observed mutations to mefloquine mapped to the F_o subunit of ATP synthase in *S. pneumoniae*. These mutational studies were biochemically validated employing a reconstituted membrane assay that measured the ATPase activity of ATP synthase isolated from a panel of susceptible and laboratory-generated resistant strains.[47] Strong correlation (r^2 = 0.91) was observed between the biochemical IC_{50} values for inhibition of ATPase activity and the MIC values

against *S. pneumoniae* providing strong evidence for the primary mechanism of action. These co-workers also synthesized several mefloquine analogues with improved pneumococcal activity and significantly expanded spectrum of activity.[46] For example, their mefloquine analogue OSU-99 (Figure 9) showed excellent activity against *S. aureus*, *S. epidermidis*, *S. pyogenes*, *E. faecalis*, *E. faecium* with MICs of 1.5–6.3 μg/mL and MIC ranging from 0.08–1.25 against 21 clinical *S. pneumoniae* isolates. Mefloquine is an approved drug for malaria; however, its primary liabilities are its poor drug disposition properties including an exceedingly high plasma protein binding (>98%), large volume of distribution (V_d ~ 20 L/kg), and low clearance, which result in very low plasma concentrations (C_{max} ~50 ng/mL), and an exceptional long terminal elimination half-life ($t_{1/2\beta}$ ~20 days).[48] Additionally, mefloquine is well known for numerous toxicities. These combined issues have presumably contributed to the lack of antibacterial drug development of mefloquine and the reported analogues, which are even more lipophilic than mefloquine.

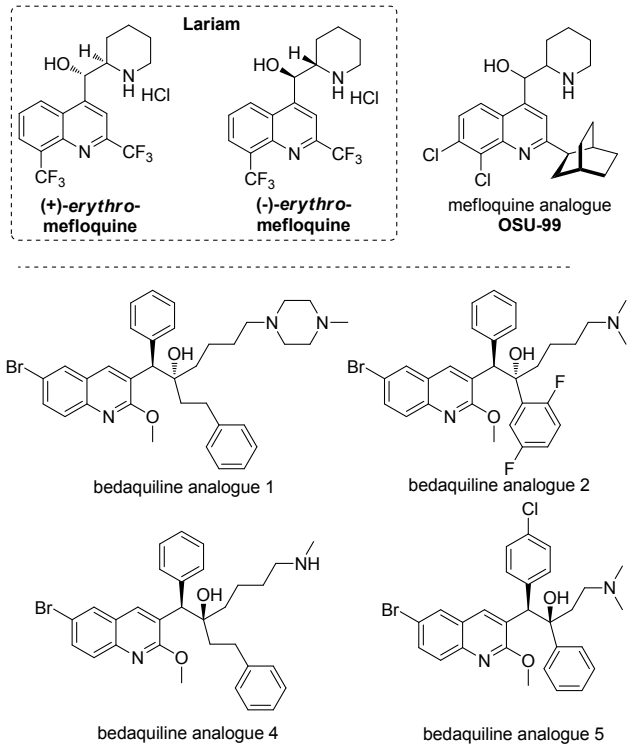


Figure 9. Structrues of mefloquine and bedaquiline analogues.

In 2012, Koul, Bald and co-workers disclosed their efforts to expand the spectrum of activity of bedaquiline beyond mycobacteria.[49] Over 700 analogues of bedaquiline were evaluated and a few compounds were identified with activity towards gram positive bacteria including *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *E. faecalis*, and *B. subtilis*. The most potent compound (bedaquiline analogue 1) had MICs ranging from 0.25–4 μg/mL against the aforementioned pathogens. Interestingly, one of the derivatives (bedaquiline analogue 5, Figure 9) was selective towards *S. pneumoniae* with an MIC of 1 μg/mL. Further microbiological studies were performed with *S. aureus* where bedaquiline analogues 1 and 2 were shown to be rapidly bactericidal depleting an initial inoculum by greater than 10,000-fold CFU/mL within 24 hours at 10× their MIC values.[49] Resistance to these compounds against *S. pneumonaie*, the most susceptible pathogen, occurred with a mutation frequency of 8×10^{-7} at 10× their MIC and mapped to *atpE* with mutations at V48I and V60A, results that are almost identical to our observations with optochin analogue **48**.[49] The bedaquiline analogues were shown to inhibit ATP synthase from *S. aureus* (*S. pneumoniae* ATP synthase was not examined) with IC_{50} values ranging from 1.4–2.7 μg/mL.[49] However, the compounds were also quite cytotoxic and active against human mitochondrial ATP synthase with IC_{50}'s ranging from 23–29 μg/mL providing selectivity indexes (SI) of only 9–21, whereas bedaquiline exhibited a SI greater than 10,000.[49] Taken together with the aforementioned mefloquine analogues, these studies further corroborate ATP synthase as a vulnerable and chemically validated target in *S. pneumoniae* and highlight the potential to expand the spectrum of activity of optochin. Additionally, several other non-quinoline ATP synthase inhibitors have recently been reported for other bacteria.[50,51]

Genetic disruption studies have confirmed ATP synthase is essential in *S. pneumoniae*.[16] However, analysis of the genome sequence of the reference strain *S. pneumoniae* R6 shows it lacks genes necessary for an electron transport chain (ETC) and a complete tricarboxylic acid (TCA) cycle,

indicating *S. pneumoniae* primarily relies on glycolysis for energy metabolism.[52] ATP synthase in *S. pneumoniae* is therefore unlikely to be responsible for production of ATP due to the lack of a functional ETC to generate an electrochemical gradient. Rather, it seems plausible that *S. pneumoniae* ATP synthase is involved in pH homeostatsis as observed in other fermentive microorganisms by operating in the reverse direction to regulate the intracellular pH by consuming ATP.[36-38] Consequently, we hypothesized that optochin and **48** inhibits ATP synthase, which disrupts pH homeostasis lowering intrabacterial pH, leading to the observed growth inhibition. The catatlytic proficiency of many glycolytic enzymes is substantially reduced at lower pH, so we expect acidification may also perturb glycolysis and indirectly inhibit energy metabolism. In support of this mechanism of action we demonstrated **48** leads to cytoplasmic acidification that is dependent on AtpE. This is fundamentally different from bedaquiline that collapses the pH gradient and depletes ATP levels in mycobacteria through direction inhibition of ATP synthase and/or acting as H^+/K^+ ionophore.[53-58]

The putative molecular target of optochin and our lead compound **48** based on genetic resistance mapping is the c-ring of ATP synthase encoded by *atpE*. ATP synthase is a complex molecular machine composed of two multimeric subunits (the membrane bound F_o subunit and F_1 subunit) responsible for synthesizing ATP from the transmembrane proton gradient.[15,59,60] The F_o subunit is a cylinder with a central pore (the c-ring) comprised of multiple c-subunits (10-14 subunits in *S. pneumoniae* and 9 in *M. tuberculosis*). Resistance to **48** mapped to amino acids 18 and 31 in the N-terminal helix and 45, 49, and 50 in the C-terminal helix of the *S. pneumoniae* AtpE ortholog. Interestingly, Phe^{50} at the putative binding site in *S. pneumoniae* and *S. mitis* (the only other bacteria with an MIC of $\leqslant$ 16 ug/mL) is not conserved in subunit c of ATP synthase for the other bacteria examined (Figure 10), which employ a more bulky leucine at this position. Resistance to bedaquiline maps to similar positions in the C-terminal helix of the corresponding mycobacterial c-subunit as shown in the alignment in Figure 10.[17,61,62]

Bedaquiline was recently co-crystallized with the c-subunit from *Mycobacterium phlei* and binding overlapped with the residues identified from the resistance studies serving to validate the observed mutants in *M. tuberculosis*.[63] Based on the structural similarity of **48** to bedaquiline (i.e. both are 6-substituted quinolines containing an amino alcohol side chain at the 3- or 4-positions) we hypothesize **48** binds in a similar location on helix 2 of the *S. pneumoniae* ATP synthase adjacent to the critical glutamate 52 ion-binding site, which is to responsible for proton binding, shuttling, and release.

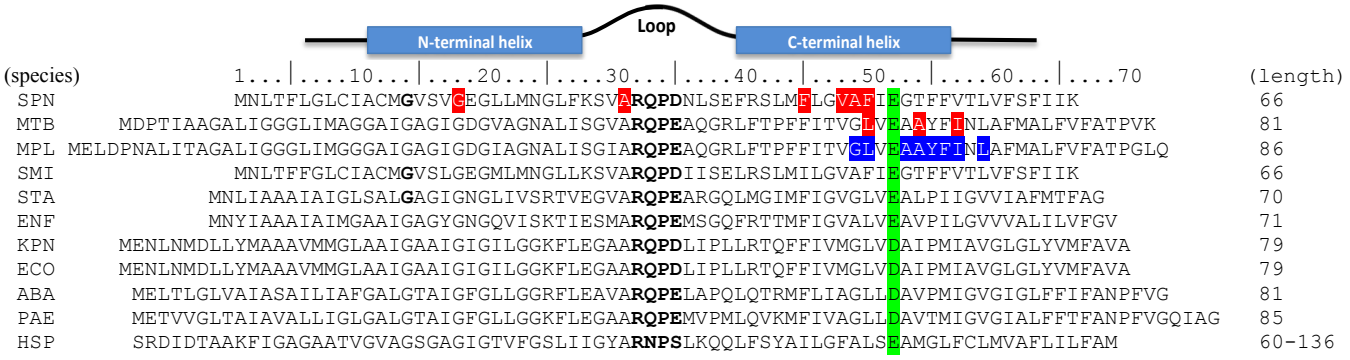


Figure 10. Amino acid alignment of *S. pneumoniae* and other ATP synthase c-subunits. The location of the N- and C-terminal helices and the loop region are indicated (top). Amino acid numbering (top) is according to *S. pneumoniae*. Amino acids shown to cause respective resistance to optochin in *S. pneumoniae* and bedaquiline in *M. tuberculosis* are indicated in red. Residues involved in drug coordination of bedaquilline based on the x-ray structure are shown in blue (PDB 4v1f). The ion-binding glutamate/aspartate is indicated in green. Species used: SPN = *Streptococcus pneumoniae* D39 (Genebank Accession number ABJ53659); MTB = *Mycobacterium tuberculosis*, H37Rv (Genebank Accession number: CCP44062.1); MPL = *Mycobacterium phlei*, CCUG 28060 (Genebank Accession number: SMH63861.1); SMI = *Streptococcus mitis,* SK137 (Genebank Accession number: KJQ73325.1); STA = *Staphylococcus aureus* MRSA USA 300 (Genebank Accession number: ABD22522.1); ENF = *Enterococcus faecium* Aus0004 (Genebank Accession number: AFC64146.1); KPN = *Klebsiella pneumoniae*, ATCC 13883 (Genebank Accession number: KFJ74351); ECO = *Escherichia coli*, K12 (Genebank Accession number: AIZ93459.1); ABA = *Acinetobacter baumannii*, strain AYE (Genebank Accession number: CAM88485.1); PAE = *Pseudomonas aeruginosa*, PA01-VE2 (Genebank Accession number: AGY64756.1); HSP = *Homo sapiens* (residues 60–136, Genebank Accession number: NP_001002027.1).

To provide further insight into the observed resistance studies, we constructed a homology model of the c-ring of *S. pneumoniae* using the bedaquiline co-crystal structure as a template.[63] As shown in Figure 11, the mutations cluster approximately 1-2 helical turns away from the bedaqualine binding site

in the c-ring of *S. pneumoniae.* This suggests that **48** adopts a different binding mode when compared to bedaqualine which may in part, explain the differences in selectivity noted. Further support for this hypothesis can be found in an analysis of the bedaqualine binding site structure that indicates the secondary amine projects into a cleft containing the key glutamate anchor. An alignment of the tertiary amino group of the quinuclidine bicyclic ring structure superposed to the seconday amine of bedaqualine is shown in Figure 11B. The result indicates **48** is sterically occluded from the binding site pocket, preventing salt link formation with the glutamate of the *M. phlei* c-ring. While the model explains the failure of **48** to bind *M. phlei*, further work is requied to characterize the c-ring structure of ATP synthase in *S. pneumoniae* to construct a detailed binding site model that explains selectivity. In particular, it will be critical to determine the number of monomeric units that comprise the c subunit to arrive at meaningful homology models given the limited sequence identity shared across species (<35%).

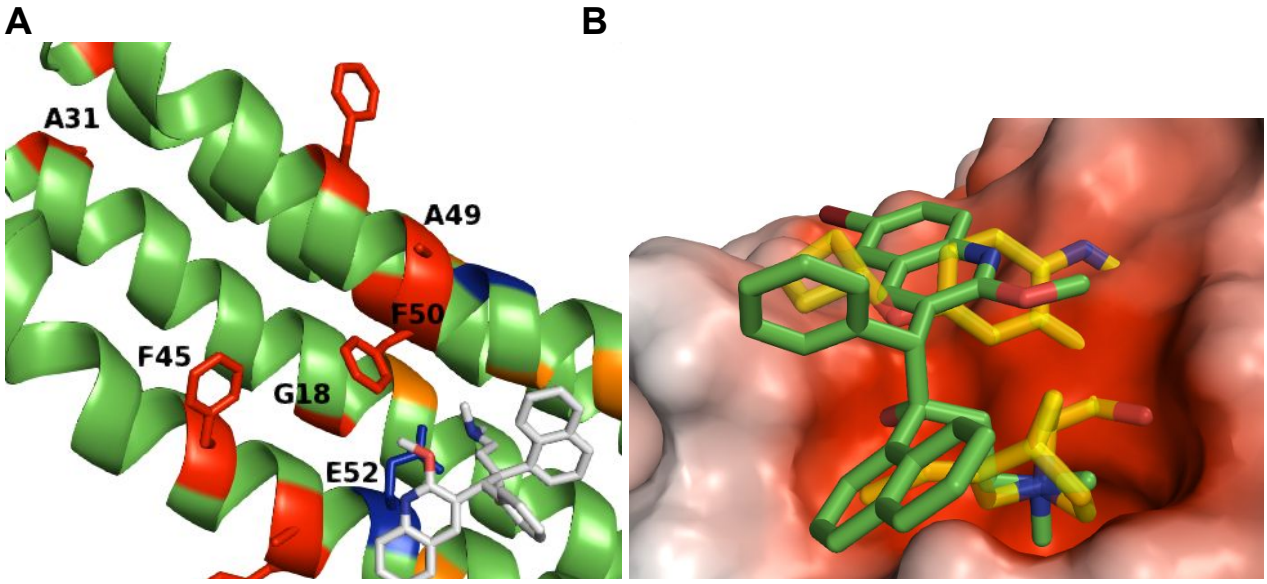


Figure 11. A) Homology model of the *S. pneumoniae* c-ring monomeric units showing location of the resistance mutations for bedaqualine and **48**. The structure was generated using the *M. phlei* crystal structure (4v1f). Bedaquiline induced mutations in *Mtb* are colored orange. Residues that mutate to form resistance to **48** in *S. pneumoniae* are colored red. Key GLU residue is shown in blue. B) Superposition of amino groups of bedaqualine (green) and **48** (yellow) showing the binding site cleft containing the glutamate anchor. Electrostatic potential surface showing negative polarity of pocket is shown for reference.

CONCLUSION

We have reexamined in much greater detail the structure-activity relationships of the antibiotic optochin, a semisynthetic derivative of quinine through the synthesis of more than 50 analogues. Initial SAR focused on the synthetically accessible positions at C-6′ of the quinoline and C-3 of the quinuclidine. These efforts led to the identification of analogue **48**, whose MIC for *Streptococcus pneumoniae* was 0.25 μg/mL or 8–16 times lower than optochin. The SAR showed that small substituents between 2-4 carbons were optimal at C-6′ while the C-3 was very sensitive to modification, but small alkyl and alkylidene substituents were preferred. Like optochin, compound **48** demonstrated remarkable selectivity and was weakly active or inactive towards each of the ESKAPE pathogens as well as *Escherichia coli* and *Mycobacterium tuberculosis* (MIC ≥128 μg/mL). Resistance and molecular modeling studies indicate **48** likely binds to the c-ring of near the conserved glutamate 52 ion binding site. Preliminary mechanism of action studies demonstrated **48** leads to cytoplasmic acidification that may be due to inhibition of ATP synthase. We demonstrated that **48** does not disrupt mammalian mitochondrial function and thus does not target human ATP synthase. Initial pharmacokinetic and drug metabolism analysis of optochin and **48** revealed these quinine analogues were rapidly cleared resulting in poor in vivo exposure through hydroxylation of the quinuclidine and *O*-dealklyation of the quinoline. Overall, these results provide a foundation for future studies aimed at further improving the activity and pharmacokinetic behavior of optochin as well as understanding optochin's mechanism of action, which we speculate is due to disruption of pH homeostasis.

EXPERIMENTAL SECTION

General materials and methods. Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AVANCE III 400 (400 MHz), JEOL ECZ 400S (400 MHz), Zhongke-Niujin WNMR-I 500 (500 MHz) or Agilent VNS-600 (600 MHz) spectrometers. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton ($CDCl_3$ δ 7.26, $(CD_3)_2SO$ δ 2.50, CD_3OD δ 3.31), carbon ($CDCl_3$ δ 77.16, $(CD_3)_2SO$ δ 39.52, CD_3OD δ 49.00) fluorine ($CFCl_3$ δ 0.00) was used as a reference. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), ABq (AB quartet), quint (quintet), m (multiplet), dd (doublet of doublet), bs (broad singlet). For clarity, some ^{13}C NMR chemical shifts are given only for the major diastereomer. Coupling constants were reported in Hertz (Hz). Optical rotations are quoted in 10^3 deg·cm^{-3} at concentrations (*c*) in g·100 mL^{-1}. Melting points were recorded on a Kofler hot block and are uncorrected. DSC measurements were carried out on the Mettler Toledo TGA/DSC 3^+ equipment. All high resolution mass spectra were obtained on a Thermofisher Exactive Plus mass spectrometer. For thin layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F254) were used, and compounds were visualized with a UV light at 254 nm. Flash chromatography separations were performed on Merck 60 (0.040–0.063 mm) mesh silica gel. Preparative reversed-phase HPLC purification was performed on an Ultimate XB 10 μm C18 250 × 50 mm column at room temperature operating at 100 mL/min with detection at 210 nm employing a linear gradient from 70 to 90% MeCN (mobile phase B) in 0.05% formic acid at pH 2.7 (mobile phase A) for 40 min (Method A). Analytical reversed-phase HPLC was performed on a Kromasil 5 μm C18 250 × 4.6 mm column at 40 °C operating at 1 mL/min with detection at 254 nm employing a linear gradient from 30% to 70% MeOH (mobile phase B) in 0.15% ammonium acetate at pH 5.0 (mobile phase A) for 40 min (Method B). All compounds were evaluated for purity by analytical HPLC monitoring at 254 nm and shown to be >95% pure. For compounds obtained as a mixture of diastereomers, the purity

refers to the sum of the purities of the individual diastereomers. Purities along with retention times and k' values are given for each final compound in the individual experimentals. Additionally, HPLC traces of every final compound are provided in the Supporting Information.

General Procedure for the C-6 Demethylation of Cinchona Alkaloids. NaH (60% in mineral oil, 2.0 equiv) was suspended in DMF (0.4 M for sodium ethanthiolate). To this suspension at 0 °C was added ethanethiol (3.0 equiv) dropwise over 3 min. The reaction mixture was stirred at room temperature for 10 min before the addition of cinchona alkaloid (1.0 equiv) in DMF (0.4 M for limiting reagent) and futher stirred for 13 h at 100 °C. The reaction mixture was cooled to 23 °C and neutralized with aqueous 1 N HCl. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine and dried with $MgSO_4$. The drying agent was filtered and resulting filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (5:1 CH_2Cl_2–MeOH) to give the title compound.

General Procedure for the C-6 Alkylation Reaction of 5 or apo-Cupreine 39. Cesium carbonate (2.0 equiv) was added to a stirred solution of compound **5** or apo-cupreine **39** (1.0 equiv) in dry DMF (0.1 M for limiting reagent) and stirred at 25 °C for 10 min. The bromoalkane or iodoalkane (1.2 equiv) was added, and the reaction mixture was stirred for 3 h at 25 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine and dried with $MgSO_4$. The drying agent was filtered and resulting filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (30:1~5:1 CH_2Cl_2–MeOH) to give the title compound.

General Procedure for the Olefin Migration of Quinine or Quinindine. To a solution of cinchona alkaloid (1.0 equiv) in EtOH (0.1 M for limiting reagent) was added concentrated H_2SO_4 (2.0 equiv) and the mixture was stirred for 10 min at room temperature. $RhCl_3 \cdot 3H_2O$ (0.03 equiv) was added

and the mixture was refluxed for 2 h. After evaporation of the EtOH, the reaction mixture was diluted with CH_2Cl_2, basified with 10% K_2CO_3, washed with brine and dried with $MgSO_4$. The drying agent was filtered and resulting filtrate was concentrated. Purification of the residue by column chromatography on silica gel (25:1 CH_2Cl_2–MeOH) gave the title compound as a *Z/E*-mixture.

General Procedure for the Oximation Reaction of Compound 18. A mixture of the ketone **18** (1.0 equiv) and hydroxylamine hydrochloride or *O*-alkylhydroxylamine hydrochloride (1.0 equiv) in methanol (0.1 M for limiting reagent) was refluxed 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine and dried with $MgSO_4$. The drying agent was filtered and resulting filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (15:1~5:1 CH_2Cl_2–MeOH) to give the title compound.

General Procedure for the Deprotection of TBS at C-9 Position of Cinchona Alkaloids. The silyl ether (1.0 equiv), TBAF (1M in THF, 1.0 equiv), and THF (0.1 M for limiting reagent) were stirred 6 hours. The THF was then removed under vacuum, and the resulting oil was dissolved in dichloromethane and washed with aq $NaHCO_3$. Upon removal of the dichloromethane the remaining oil was purified by flash column chromatography (10:1 ~ 3:1 CH_2Cl_2–MeOH) to give the title compound.

General Procedure for the Fluorination Reaction of Cinchona Alkaloids. To a solution of cinchona alkaloid (1.0 equiv) in CH_2Cl_2 (0.1 M for limiting reagent) and pyridine (2 equiv) was added DAST (1.5 equiv) at 0 °C, and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash chromatography (50:1~10:1 CH_2Cl_2–MeOH) afforded the desired flourinated product along with eliminated byproduct.

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (1). Optochin **1** was prepared following the general procedure for C-6 phenol alkylation employing **5** (177 mg, 0.567 mmol) and bromoethane (50 μL, 0.670 mmol) to afford (123 mg, 63%) as a white solid: HPLC purity 95.0%, t_R = 18.3 min; mp of optochin hydrochloride salt 250–252 °C, lit. mp 252–254 °C[64]; R_f = 0.48 (20:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 3328, 2957, 2562, 1458, 1240, 1046, 860, 825; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 4.5 Hz, 1H), 7.66 (d, *J* = 4.5 Hz, 1H), 7.63 (d, *J* = 9.2 Hz, 1H), 6.87 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.72 (d, *J* = 2.5 Hz, 1H), 6.33 (s, 1H), 6.21 (s, 1H), 4.55–4.35 (m, 1H), 3.80–3.70 (m, 1H), 3.60–3.48 (m, 1H), 3.38 (dd, *J* = 13.1, 10.7 Hz, 1H), 3.28–3.19 (m, 1H), 3.05 (td, *J* = 11.8, 5.5 Hz, 1H), 2.68–2.58 (m, 1H), 2.33–2.18 (m, 1H), 2.15–2.10 (m, 1H), 2.03–1.94 (m, 1H), 1.91–1.62 (m, 2H), 1.25 (t, *J* = 6.9 Hz, 3H), 1.25–1.10 (m, 2H), 0.77 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 146.7, 144.0, 143.6, 131.2, 125.2, 122.5, 118.7, 99.8, 66.1, 64.9, 60.1, 57.0, 44.2, 35.6, 27.0, 24.8, 24.7, 17.8, 14.5, 11.4; HRMS (ESI+) calcd for $C_{21}H_{29}N_2O_2$ $[M+H]^+$ 341.2224, found 341.2210 (error 3.9 ppm); $[\alpha]_D^{23}$ = +1.1 (*c* 0.500, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (4). To a solution of quinine (3.24 g, 10 mmol, 1.00 equiv) in MeOH (10 mL) at 25 °C, was added 20% *w*/*w* Pd/C (800 mg). The reaction mixture was stirred for 3 d at the same temperature. The suspension was filtered through Celite and washed with EtOAc (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (20:1 CH_2Cl_2–MeOH) to afford the title compound (3.20 g, 97%) as a white solid: HPLC purity 98.9%, t_R = 28.3 min; mp 176–178 °C, lit. mp 171–172 °C[21]; R_f = 0.46 (10:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} (cm^{-1}) 3174, 2931, 2870, 1509, 1241, 1031, 828; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 4.5 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.49 (d, *J* = 4.5 Hz, 1H), 7.29 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.22 (d, *J* = 2.6 Hz, 1H), 5.56 (d, *J* = 3.7 Hz, 1H), 4.13 (br s, 1H), 3.86 (s, 3H), 3.57–3.34 (m, 1H),

3.21–2.93 (m, 2H), 2.68–2.54 (m, 1H), 2.36 (d, J = 13.5 Hz, 1H), 1.80–1.65 (m, 3H), 1.49–1.31 (m, 3H), 1.31–1.10 (m, 2H), 0.79 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 147.8, 147.6, 144.2, 131.5, 126.6, 121.5, 118.5, 101.4, 71.8, 59.8, 58.6, 55.8, 43.4, 37.5, 28.2, 27.7, 25.4, 21.3, 12.1; HRMS (ESI+) calcd for $C_{20}H_{27}N_2O_2$ $[M+H]^+$327.2067, found 327.2054 (error 4.0 ppm); $[\alpha]_D^{23}$ = –92.8 (*c* 0.800, $CHCl_3$), $[\alpha]_D^{22}$ = –141 (*c* 0.2, $CHCl_3$)[21]. All spectroscopic data were in agreement with the literature values.[21]

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-hydroxyquinoline (5). Compound **5** was prepared following the general procedure for C-6 phenol demethylation employing **4** (650 mg, 2.00 mmol) to afford the title compound (493 mg, 79%) as a white solid: HPLC purity 95.0%, t_R = 14.4 min; mp 223–224 °C, lit. mp 230 °C[64]; R_f = 0.42 (5:1 CH_2Cl_2/MeOH); IR (KBr) γ_{max} 3165, 2931, 2871, 1618, 1467, 1241, 857. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 4.4 Hz, 1H), 7.85 (d, J = 9.1 Hz, 1H), 7.45 (d, J = 2.5 Hz, 1H), 7.41 (d, J = 4.4 Hz, 1H), 7.26 (dd, J = 9.1, 2.5 Hz, 1H), 5.75 (br s, 1H), 5.12 (d, J = 6.3 Hz, 1H), 3.28–3.11 (m, 1H), 3.02 (q, J = 7.9 Hz, 1H), 2.83 (dd, J = 13.2, 9.6 Hz, 1H), 2.51 (br s, 1H), 2.47–2.38 (m, 1H), 2.15–2.12 (m, 1H), 1.79–1.59 (m, 4H), 1.31–1.24 (m, 4H), 0.78 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.7, 148.6, 147.1, 143.7, 131.6, 127.6, 121.6, 119.4, 105.4, 72.1, 60.5, 58.2, 42.4, 37.5, 28.6, 27.6, 25.7, 23.9, 12.5; HRMS (ESI+) calcd for $C_{19}H_{25}N_2O_2$ $[M+H]^+$ 313.1911, found 313.1897 (error 4.3 ppm); $[\alpha]_D^{23}$ = –231 (*c* 0.700, $CHCl_3$) , lit $[\alpha]_D^{27}$= –149.4 (*c* 1.23, EtOH)[65]. All spectroscopic data were in agreement with the literature values.[65]

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-(2,2,2-trifluoroethoxy)quinoline (6). Compound **6** was prepared following the general procedure for C-6 phenol alkylation employing **5** (100 mg, 0.321 mmol) and 1,1,1-trifluoro-2-iodoethane (41 μL, 0.39 mmol) to afford the title compound (12 mg, 9%) as a white solid: HPLC purity 99.2%, t_R = 27.7 min; mp 190–192 °C; R_f = 0.45 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, J = 4.5 Hz,

1H), 7.67 (d, J = 4.5 Hz, 1H), 7.62 (d, J = 9.2 Hz, 1H), 6.95 (dd, J = 9.2, 2.6 Hz, 1H), 6.90 (d, J = 2.6 Hz, 1H), 6.39 (d, J = 2.4 Hz, 1H), 6.16 (d, J = 4.7 Hz, 1H), 4.67–4.44 (m, 2H), 4.28–4.20 (m, 1H), 3.39 (dd, J = 13.0, 10.9 Hz, 1H), 3.23–3.12 (m, 1H), 3.07 (dt, J = 13.0, 6.6 Hz, 1H), 2.70–2.60 (m, 1H), 2.30–2.19 (m, 1H), 2.17–2.04 (m, 1H), 2.03–2.00 (m, 1H), 1.88–1.66 (m, 3H), 1.25–1.10 (m, 2H), 0.78 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 147.8, 144.5, 144.0, 131.8, 124.6, 123.6 (q, J = 279 Hz), 122.0, 118.8, 100.4, 66.2 (q, J = 35.0 Hz), 65.8, 60.2, 56.6, 44.2, 35.5, 26.9, 24.7, 24.5, 17.7, 11.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ –73.01 (t, J = 8.2 Hz); HRMS (ESI+) calcd for $C_{21}H_{26}N_2O_2F_3$ $[M+H]^+$ 395.1941, found 395.1910 (error 7.9 ppm); $[\alpha]_D^{23}$ = +19.2 (*c* 0.300, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-propoxyquinoline (7). Compound **7** was prepared following the general procedure for C-6 phenol alkylation employing **5** (108 mg, 0.348 mmol) and 1-bromopropane (38 μL, 0.42 mmol) to afford the title compound (102 mg, 83%) as a white solid: HPLC purity 95.4%, t_R = 20.7 min; mp 189–191 °C; R_f = 0.44 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 4.5 Hz, 1H), 7.64 (d, J = 4.5 Hz, 1H), 7.63 (d, J = 9.2 Hz, 1H), 6.93 (dd, J = 9.2, 2.5 Hz, 1H), 6.78 (d, J = 2.5 Hz, 1H), 6.36 (s, 1H), 5.93 (br s, 1H), 4.69–4.43 (m, 1H), 3.82–3.70 (m, 1H), 3.66–3.51 (m, 1H), 3.45–3.32 (m, 1H), 3.32–3.18 (m, 1H), 3.06 (td, J = 11.8, 5.6 Hz, 1H), 2.68–2.60 (m, 1H), 2.33–2.17 (m, 1H), 2.20–2.07 (m, 1H), 1.99 (d, J = 2.7 Hz, 1H), 1.82–1.79 (m, 1H), 1.79–1.61 (m, 4H), 1.27–1.16 (m, 2H), 1.05 (t, J = 7.8 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 146.7, 143.9, 143.6, 131.2, 125.2, 122.5, 118.6, 99.8, 70.6, 66.1, 60.1, 57.0, 44.1, 35.6, 27.0, 24.8, 24.7, 22.4, 17.8, 11.5, 10.7; HRMS (ESI+) calcd for $C_{22}H_{31}N_2O_2$ $[M+H]^+$ 355.2380, found 355.2376 (error 1.2 ppm); $[\alpha]_D^{23}$ = –0.7 (*c* 0.3, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-isopropoxyquinoline (8). Compound **8** was prepared following the general procedure for C-6 phenol alkylation employing **5** (101 mg, 0.324 mmol) and 2-bromopropane (37 μL, 0.39 mmol) to afford the

title compound (93 mg, 81%) as a light yellow solid: HPLC purity 98.9%, t_R = 28.3 min; mp 181–182 °C; R_f = 0.48 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 4.5 Hz, 1H), 7.64 (d, *J* = 9.3 Hz, 1H), 7.62 (d, *J* = 4.5 Hz, 1H), 6.95 (dd, *J* = 9.3, 2.5 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.40 (s, 1H), 5.68 (br s, 1H), 4.89–4.72 (m, 1H), 4.54 (t, *J* = 8.9 Hz, 1H), 3.41 (dd, *J* = 12.7, 10.5 Hz, 1H), 3.35 (q, *J* = 7.5 Hz, 1H), 3.19–3.05 (m, 1H), 2.81–2.57 (m, 1H), 2.33–2.17 (m, 1H), 2.18–2.05 (m, 1H), 2.03–2.00 (m, 1H), 1.93–1.61 (m, 4H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.22 (d, *J* = 5.8 Hz, 3H), 1.21–1.11 (m, 1H), 0.77 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 146.7, 143.6, 143.3, 131.4, 125.0, 123.3, 118.4, 101.0, 69.5, 66.3, 60.2, 57.0, 44.1, 35.6, 27.0, 24.8, 24.5, 22.2, 20.8, 17.8, 11.4; HRMS (ESI+) calcd for $C_{22}H_{31}N_2O_2$ $[M+H]^+$ 355.2372, found 355.2380 (error 2.2 ppm); $[\alpha]_D^{23}$= +17.6 (*c* 0.300, $CHCl_3$).

6-Alloxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (9). Compound **9** was prepared following the general procedure for C-6 phenol alkylation employing **5** (131 mg, 0.420 mmol) and allyl bromide (43 μL, 0.50 mmol) to afford the title compound (101 mg, 68%) as a white solid: HPLC purity 95.3%, t_R = 19.1 min; mp 183–184 °C; R_f = 0.52 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (d, *J* = 4.4 Hz, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.64 (d, *J* = 4.4 Hz, 1H), 6.99 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.77 (d, *J* = 2.5 Hz, 1H), 6.42 (s, 1H), 6.07–5.99 (m, 1H), 5.60 (br s, 1H), 5.39 (d, *J* = 17.3 Hz, 1H), 5.18 (d, *J* = 10.6 Hz, 1H), 4.66–4.61 (m, 1H), 4.43 (d, *J* = 14.1 Hz, 1H), 4.31 (d, *J* = 14.1 Hz, 1H), 3.40 (dd, *J* = 13.2, 10.6 Hz, 1H), 3.27 (t, *J* = 9.1 Hz, 1H), 3.11–3.05 (m, 1H), 2.71–2.66 (m, 1H), 2.32–2.15 (m, 1H), 2.10 (dd, *J* = 13.2, 7.3 Hz, 1H), 2.02–1.94 (m, 1H), 1.86–1.73 (m, 3H), 1.20–1.14 (m, 2H), 0.78 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 147.0, 143.72, 143.67, 133.3, 131.4, 125.0, 122.5, 118.8, 116.0, 100.4, 69.4, 66.1, 60.1, 57.1, 44.4, 27.0, 25.5, 24.7, 24.6, 17.9, 11.5; HRMS (ESI+) calcd for $C_{22}H_{29}N_2O_2$ $[M+H]^+$ 353.2224, found 353.2218 (error 1.5 ppm); $[\alpha]_D^{23}$ = +23.1 (*c* 0.3, $CHCl_3$).

6-Butoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (10). Compound **10** was prepared following the general procedure for C-6 phenol alkylation employing **5** (107 mg, 0.343 mmol) and 1-bromobutane (44 μL, 0.41 mmol) to afford the title compound (110 mg, 87%) as a white solid: HPLC purity 98.7%, t_R = 24.5 min; mp 191–192 °C; R_f = 0.46 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 4.5 Hz, 1H), 7.62 (d, *J* = 4.5 Hz, 1H), 7.62 (d, *J* = 9.2 Hz, 1H), 6.90 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.75 (d, *J* = 2.5 Hz, 1H), 6.35 (s, 1H), 6.01 (br s, 1H), 4.54 (t, *J* = 16.2 Hz, 1H), 3.84–3.71 (m, 1H), 3.73–3.60 (m, 1H), 3.45–3.32 (m, 1H), 3.31–3.17 (m, 1H), 3.05 (td, *J* = 11.9, 5.5 Hz, 1H), 2.63 (dd, *J* = 17.1, 4.0 Hz, 1H), 2.34–2.15 (m, 1H), 2.17–2.05 (m, 1H), 2.03–1.98 (m, 1H), 1.89–1.80 (m, 1H), 1.77–1.59 (m, 3H), 1.56–1.40 (m, 2H), 1.25–1.18 (m, 3H), 1.00 (t, *J* = 7.3 Hz, 3H), 0.78 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 146.7, 143.9, 143.5, 131.2, 125.2, 122.5, 118.6, 99.8, 69.2, 66.1, 60.1, 57.0, 44.1, 35.6, 31.4, 27.0, 24.8, 24.7, 19.5, 17.8, 14.3, 11.5; HRMS (ESI+) calcd for $C_{23}H_{33}N_2O_2$ $[M+H]^+$ 369.2537, found 369.2535 (error 0.5 ppm); $[\alpha]_D^{23}$ = +20.4 (*c* 0.5, $CHCl_3$).

6-Cyclopropylmethoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (11). Compound **11** was prepared following the general procedure for C-6 phenol alkylation employing **5** (101 mg, 0.324 mmol) and cyclopropylmethyl bromide (37 μL, 0.39 mmol) to afford the title compound (78 mg, 63%) as a white solid: HPLC purity 96.0%, t_R = 20.2 min; mp 195–197 °C; R_f = 0.52 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.4 Hz, 1H), 7.75 (d, *J* = 9.3 Hz, 1H), 7.62 (d, *J* = 4.4 Hz, 1H), 7.09 (d, *J* = 9.3 Hz, 1H), 6.89 (d, *J* = 2.6 Hz, 1H), 6.41 (s, 1H), 4.53–4.46 (m, 1H), 3.83 (dd, *J* = 10.4, 7.0 Hz, 1H), 3.55 (dd, *J* = 10.4, 7.0 Hz, 1H), 3.41 (t, *J* = 11.9 Hz, 1H), 3.26 (t, *J* = 9.1 Hz, 1H), 3.12–3.05 (m, 1H), 2.67 (d, *J* = 12.6 Hz, 1H), 2.38–2.07 (m, 2H), 2.03 (d, *J* = 9.1 Hz, 1H), 1.80–1.40 (m, 1H), 1.20–1.04 (m, 5H), 0.80 (t, *J* = 7.3 Hz, 3H), 0.62–0.58 (m, 2H), 0.48–0.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 146.9, 143.81, 143.77, 131.4, 125.3,

122.8, 118.7, 100.1, 74.1, 66.2, 60.1, 57.1, 44.2, 35.6, 27.0, 24.8, 24.6, 17.94, 11.5, 10.1, 3.3, 3.2; HRMS (ESI+) calcd for $C_{23}H_{31}N_2O_2$ $[M+H]^+$ 367.2380, found 367.2377 (error 0.8 ppm); $[\alpha]_D^{23} = -8.8$ (*c* 0.60, $CHCl_3$).

6-Cyclobutoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (12). Compound **12** was prepared following the general procedure for C-6 phenol alkylation employing **5** (100 mg, 0.321 mmol) and bromocyclobutane (39 μL, 0.39 mmol) to afford the title compound (85 mg, 68%) as a white solid: HPLC purity 97.8%, t_R = 21.7 min. mp 188–190 °C; R_f = 0.55 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 4.5 Hz, 1H), 7.96 (d, *J* = 9.3 Hz, 1H), 7.54 (d, *J* = 4.5 Hz, 1H), 7.25 (dd, *J* = 9.3, 2.5 Hz, 1H), 7.07 (d, *J* = 2.5 Hz, 1H), 5.69 (br s, 1H), 4.74–4.67 (m, 1H), 3.64 (br s, 1H), 3.29–3.11 (m, 2H), 2.73 (t, *J* = 13.7 Hz, 1H), 2.62–2.33 (m, 3H), 2.24–1.95 (m, 3H), 1.90–1.60 (m, 4H), 1.60–1.35 (m, 3H), 1.24 (d, *J* = 8.9 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 147.5, 146.6, 144.3, 131.8, 126.5, 122.2, 118.5, 102.6, 71.7, 59.7, 58.5, 43.5, 37.3, 30.5, 30.2, 29.7, 27.8, 27.6, 25.3, 21.1, 13.4, 12.0; HRMS (ESI+) calcd for $C_{23}H_{31}N_2O_2$ $[M+H]^+$ 367.2380, found 367.2367 (error 3.5 ppm); $[\alpha]_D^{23} = -57.9$ (*c* 0.500, $CHCl_3$).

6-Cyclopentoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (13). Compound **13** was prepared following the general procedure for C-6 phenol alkylation employing **5** (65 mg, 0.21 mmol) and bromocyclopentane (27 μL, 0.25 mmol) to afford the title compound (67 mg, 84%) as a white solid: HPLC purity 97.3%, t_R = 24.6 min; mp 205–207 °C; R_f = 0.50 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 4.5 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.64 (d, *J* = 4.5 Hz, 1H), 6.94 (dd, *J* = 9.2, 2.0 Hz, 1H), 6.75 (s, 1H), 6.26 (s, 1H), 4.74 (t, *J* = 5.2 Hz, 1H), 4.41 (br s, 1H), 3.47–3.13 (m, 2H), 3.02 (t, *J* = 14.3 Hz, 1H), 2.63 (dd, *J* = 15.1, 3.8 Hz, 1H), 2.11 (t, *J* = 12.7 Hz, 1H), 2.07–1.85 (m, 4H), 1.80–1.63 (m, 5H), 1.63–1.46 (m, 4H), 1.18–1.09

(m, 3H), 0.76 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 146.9, 144.2, 143.5, 131.3, 125.3, 123.2, 118.6, 101.2, 79.1, 67.0, 60.0, 57.2, 44.0, 35.9, 32.8, 31.8, 27.1, 25.3, 24.7, 24.1, 24.0, 18.4, 11.5; HRMS (ESI+) calcd for $C_{24}H_{33}N_2O_2$ $[M+H]^+$ 381.2537, found 381.2530 (error 1.7 ppm); $[\alpha]_D^{23}$ = +24.9 (*c* 0.200, $CHCl_3$).

6-Benzyloxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (14). Compound **14** was prepared following the general procedure for C-6 phenol alkylation employing **5** (65 mg, 0.21 mmol) and benzyl bromide (30 μL, 0.25 mmol) to afford the title compound (70 mg, 83%) as a white solid: HPLC purity 96.3%, t_R = 22.9 min; mp 200–202°C; R_f = 0.45 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 4.5 Hz, 1H), 7.66 (d, J = 9.2 Hz, 1H), 7.59 (d, J = 4.5 Hz, 1H), 7.54 (s, 1H), 7.52 (s, 1H), 7.36–7.29 (m, 2H), 7.28 (d, J = 4.5 Hz, 1H), 7.03 (dd, J = 9.2, 2.5 Hz, 1H), 6.90–6.83 (m, 1H), 6.40 (s, 1H), 5.00 (ABq, $\Delta\delta_{AB}$ = 0.21, J_{AB} = 13.2 Hz, 2H), 4.55 (t, J = 12.0 Hz, 1H), 3.34 (dd, J = 13.1, 10.7 Hz, 1H), 3.25–3.09 (m, 1H), 3.05 (dt, J = 12.0, 6.0 Hz, 1H), 2.43 (dd, J = 12.0, 4.4 Hz, 1H), 2.31–2.13 (m, 1H), 2.13–2.01 (m, 1H), 2.00–1.91 (m, 1H), 1.87–1.61 (m, 3H), 1.22–0.99 (m, 3H), 0.75 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 147.1, 144.1, 143.6, 137.3, 131.4, 128.2, 127.2, 126.9, 125.0, 122.5, 118.7, 100.3, 69.9, 66.0, 60.2, 57.0, 44.2, 35.5, 26.9, 24.8, 24.5, 17.7, 11.2; HRMS (ESI+) calcd for $C_{26}H_{31}N_2O_2$ $[M+H]^+$ 403.2374, found 403.2380 (error 1.6 ppm); $[\alpha]_D^{23}$ = +32.3 (*c* 0.250, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (15). Compound **15** was prepared following the general procedure for olefin migration employing quinine **3** (970 mg, 2.99 mmol) to afford (806 mg, 83%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 98.7% (two isomers, 22.5% and 76.1%), t_R = 18.7 and 19.3 min; mp 172–173 °C; R_f = 0.60 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.72 (d, J = 4.6 Hz, 1H), 8.00 (d, J = 9.2 Hz, 1H), 7.52

(d, J = 4.6 Hz, 1H), 7.35 (dd, J = 9.2, 2.7 Hz, 1H), 7.23 (d, J = 2.7 Hz, 1H), 5.63 (d, J = 4.7 Hz, 1H), 5.24 (q, J = 6.6 Hz, 0.75H, major), 5.12 (q, J = 6.6 Hz, 0.25H, minor), 3.92 (s, 2.2H, major), 3.90 (s, 0.8H, minor), 3.55–3.35 (m, 3H), 3.16 (t, J = 5.4 Hz, 1H), 2.95–2.61 (m, 2H), 2.35 (s, 1H), 1.93 (t, J = 10.4 Hz, 1H), 1.87–1.66 (m, 2H), 1.61–1.55 (m, 1H), 1.53 (d, J = 6.9 Hz, 0.8H, minor), 1.45 (d, J = 6.9 Hz, 2.2H, major); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 147.9, 147.5, 144.1, 140.9, 131.4, 126.6, 121.4, 118.4, 114.6, 101.4, 71.9, 60.1, 56.7, 55.7, 44.0, 33.4, 28.1, 27.9, 12.3; HRMS (ESI+) calcd for $C_{20}H_{25}N_2O_2$ [M+H] $^+$ 325.1911, found 325.1897 (error 4.3 ppm); $[\alpha]_D^{23}$ = –160 (*c* 1.20, $CHCl_3$). All spectroscopic data were in agreement with the literature values.[25]

6-Methoxy-(*R*)-4-[(1*S*,2*S*,4*S*)-5-oxo-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (16). Compound **15** (100 mg, 0.31 mmol, 1.0 equiv) was added to a vigorously stirred two-phase system of K_2CO_3 (129 mg, 0.93 mmol, 3.0 equiv) and $K_3[Fe(CN)_6]$ (306 mg, 0.93 mmol, 3.0 equiv) in *tert*-butyl alcohol/H_2O (1:1, 3.1 mL). After 45 min, osmium(VIII)oxide solution (0.14 mL, 2.5% in water) was added in small portions, and the reaction mixture was stirred for 15 h at room temperature, then the reaction mixture was diluted with CH_2Cl_2 (20 mL), followed by washing with saturated aqueous $NaHCO_3$ (5 mL) and 10% aqueous $NaHSO_3$ (5mL). The combined organic layer was dried over $MgSO_4$, evaporated to obtain the crude diol as a white solid.

To a solution of the crude diol prepared above in *tert*-butanol (2 mL), was added a saturated solution of $NaIO_4$ (88 mg, 0.41 mmol, 1.3 equiv) in H_2O (2 mL). The mixture was stirred vigorously for 2 h at at room temperature, treated with aqueous $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3×20 mL). The extracts were dried over $MgSO_4$, concentrated, and the crude product was purified by column chromatography (5:1 CH_2Cl_2–MeOH) to yield the desired ketone **16** (53 mg, 55%) a white solid: HPLC purity 96.8%, t_R = 13.0 min; mp 205–206 °C; R_f = 0.42 (10:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 3074, 1728, 1512, 1258, 851, 833; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (d, J = 4.3 Hz, 1H), 7.93 (d, J = 9.2 Hz,

1H), 7.60 – 7.44 (m, 2H), 7.39 (d, *J* = 9.2 Hz, 1H), 5.85–5.77 (m, 1H), 5.40 (t, *J* = 5.2 Hz, 1H), 3.92 (s, 3H), 3.48–3.40 (m, 1H), 3.24 (q, *J* = 7.7 Hz, 1H), 3.17–3.11 (m, 2H), 2.62 (t, *J* = 5.8 Hz, 1H), 2.33 (s, 1H), 2.19 (dd, *J* = 13.2, 7.7 Hz, 1H), 2.11–2.01 (m, 1H), 1.97–1.91 (m, 1H), 1.79 (t, *J* = 5.5 Hz,1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 219.8, 156.9, 148.7, 147.5, 143.9, 131.1, 126.9, 121.1, 119.1, 102.4, 70.6, 64.2, 60.6, 55.5, 54.9, 41.1, 28.0, 24.8; HRMS (ESI+) calcd for $C_{18}H_{21}N_2O_3$ $[M+H]^+$ 313.1547, found 313.1533 (error 4.4 ppm);$[\alpha]_D^{23}$ = –208 (*c* 0.700, MeOH).

(*R*)-4-((1*S*,2*S*,4*S*)-5-Hydroxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (17). Compound **17** was prepared following the general procedure for C-3 oximation employing **16** (150 mg, 0.480 mmol) and hydroxylamine hydrochloride (33 mg, 0.48 mmol) to afford the title compound (126 mg, 81%, ^{1}H NMR indicates 5:1 diastereomeric ratio through the integral value of =N-O**H** protons) as a white solid: HPLC purity 97.4% (two isomers, 82.6% and 14.8%), t_R = 10.5 and 10.9 min; mp 212–213 °C; R_f = 0.30 (5:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 3250, 2597, 1617, 1430, 1228, 939, 857; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.42 (br s, 0.17H, minor), 12.32 (br s, 0.83H, major), 10.95 (br s, 0.17H, minor), 10.90 (br s, 0.83H, major), 8.71 (d, *J* = 4.5 Hz, 1H), 7.92 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.61 (d, *J* = 4.5 Hz, 1H), 7.57 (d, *J* = 2.3 Hz, 1H), 7.38 (dd, *J* = 9.2, 2.3 Hz, 1H), 6.55 (d, *J* = 3.4 Hz, 1H), 6.33 (br s, 0.17H, minor), 6.29 (br s, 0.83H, major), 4.35–4.03 (m, 3H), 4.00 (s, 3H), 3.78 (t, *J* = 8.3 Hz, 1H), 2.75 (s, 1H), 2.35–2.19 (m, 1H), 2.12–2.05 (m, 1H), 1.85 (t, *J* = 10.5 Hz,1H), 1.44–1.25 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.8, 152.0, 147.3, 145.1, 143.6, 131.1, 125.5, 121.9, 118.9, 102.0, 65.5, 59.5, 56.7, 54.9, 43.3, 27.8, 22.8, 21.6; HRMS (ESI+) calcd for $C_{18}H_{22}N_3O_3$ $[M+H]^+$ 328.1656, found 328.1641(error 4.5 ppm); $[\alpha]_D^{23}$ = –18.9 (*c* 0.89, MeOH).

6-Methoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-methoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (18). Compound **18** was prepared following the general procedure for C-3 oximation employing **16** (200 mg, 0.640 mmol) and *O*-methylhydroxylamine hydrochloride (54 mg,

0.64 mmol) to afford the title compond (185 mg, 85%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =N-OC**H$_3$** protons) as a white solid: HPLC purity 98.9% (two isomers), t_R = 17.2 min; mp 195–197 °C; R_f = 0.31 (10:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 3255, 2938, 2871, 2480, 1620, 1510, 1241, 1044; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.5 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 0.25H, minor), 7.97 (d, *J* = 9.1 Hz, 0.75H, major), 7.50 (d, *J* = 4.5 Hz, 0.75H, major), 7.49 (d, *J* = 4.5 Hz, 0.25H, minor), 7.33 (dd, *J* = 9.1, 2.8 Hz, 0.25H, minor), 7.32 (dd, *J* = 9.1, 2.8 Hz, 0.75H, major), 7.18 (d, *J* = 2.8 Hz, 0.25H, minor), 7.17 (d, *J* = 2.8 Hz, 0.75H, major), 5.69 (br s, 0.75H, major), 5.66 (br s, 0.25H, minor), 3.89 (s, 3H), 3.78 (s, 2.2H, major), 3.75 (s, 0.8H, minor), 3.69–3.48 (m, 3H), 3.43 (d, *J* = 8.8 Hz, 1H), 3.23–3.05 (m, 1H), 2.77 (t, *J* = 14.9 Hz, 1H), 2.63 (s, 1H), 2.19–2.09 (m, 1H), 2.03–1.88 (m, 1H), 1.77 (d, *J* = 14.9 Hz, 1H), 1.55 (t, *J* = 11.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 and 164.1, 157.94 and 157.91, 147.6 and 146.8, 144.3, 131.8 (2C), 126.4 and 126.2, 121.7, 118.2 and 118.1, 101.0 and 100.9, 71.8, 61.43 and 61.35, 60.6 and 60.4, 56.6 and 55.8, 55.7 and 55.3, 43.4, 29.8 and 29.7, 26.0 and 25.8, 24.4 and 24.1; HRMS (ESI+) calcd for $C_{19}H_{24}N_3O_3$ $[M+H]^+$ 342.1812, found 342.1797 (error 4.5 ppm); $[\alpha]_D^{23}$ = –124 (*c* 1.30, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (19). Compound **19** was prepared following the general procedure for C-3 oximation employing **16** (100 mg, 0.320 mmol) and *O*-ethylhydroxylamine hydrochloride (36 mg, 0.32 mmol) to afford the title compound (95 mg, 84%, ^{1}H NMR indicates 2:1 diastereomeric ratio through the integral value of -N=OCH$_2$C**H$_3$** protons) as a white solid: HPLC purity 97.7% (two isomers), t_R = 19.1 min; mp 194–195 °C; R_f = 0.33 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 4.5 Hz, 1H), 7.99 (d, *J* = 9.2 Hz, 0.4H, minor), 7.98 (d, *J* = 9.2 Hz, 0.6H, major), 7.52 (d, *J* = 4.5 Hz, 0.6H, major), 7.51 (d, *J* = 4.5 Hz, 0.4H, minor), 7.41–7.30 (m, 1H), 7.22–7.16 (m, 1H), 5.71 (br s, 0.6H, major), 5.67 (br s, 0.4H, minor), 4.12–3.93 (m, 2H), 3.90 (s, 3H), 3.76–3.49 (m, 3H), 3.46 (d, *J* = 3.5 Hz, 1H), 3.19 (t,

J = 8.1 Hz, 1H), 2.91–2.72 (m, 1H), 2.64 (s, 1H), 2.21–2.09 (m, 1H), 2.03 (t, *J* = 11.2 Hz, 1H), 1.94 (t, *J* = 7.9 Hz, 1H), 1.88–1.68 (m, 1H), 1.20 (t, *J* = 7.5 Hz, 1.2H), 1.18 (t, *J* = 7.5 Hz, 1.8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0 and 161.8, 157.93 and 157.89, 147.5, 147.0 and 146.9, 144.18 and 144.15, 131.6, 126.4 and 126.2, 121.7, 118.3 and 118.2, 101.08 and 101.04, 71.6 and 71.5, 69.2 and 69.0, 60.6 and 60.4, 56.6 and 55.8, 55.7 and 55.4, 43.5, 29.8 and 29.7, 25.9 and 25.7, 24.4 and 24.1, 14.58 and 14.55; HRMS (ESI+) calcd for $C_{20}H_{26}N_3O_3$ $[M+H]^+$ 356.1969, found 356.1959 (error 2.8 ppm); $[\alpha]_D^{23}$ = –200 (*c* 0.300, $CHCl_3$).

6-Methoxy-(*R*)-4-{[(1*S*,2*S*,4*S*,5*Z/E*)-5-(2,2,2-trifluoroethoxy)imino-1-azabicyclo[2.2.2]octan-2-yl]hydroxymethyl}quinoline (20). Compound **20** was prepared following the general procedure for C-3 oximation employing **16** (62 mg, 0.20 mmol) and *O*-1,1,1-trifluoroethylhydroxylamine hydrochloride (30 mg, 0.20 mmol) to afford of the title compound (60 mg, 74%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 98.1% (two isomers), t_R = 20.8 min; mp 185–187 °C; R_f = 0.37 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (d, *J* = 4.5 Hz, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.47 (d, *J* = 4.5 Hz, 1H), 7.34 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.17 (d, *J* = 2.6 Hz, 1H), 5.61 (br s, 0.67H, major), 5.58 (br s, 0.33H, minor), 4.36–4.26 (m, 2H), 3.91 (s, 2H, major), 3.90 (s, 1H, minor), 3.68–3.51 (m, 3H), 3.51–3.35 (m, 1H), 3.13 (d, *J* = 9.3 Hz, 1H), 2.79–2.74 (m, 1H), 2.64 (s, 1H), 2.17 (dd, *J* = 13.3, 8.4 Hz, 0.67H, major), 2.07 (dd, *J* = 13.3, 8.4 Hz, 0.33H, minor), 1.95 (t, *J* = 11.0 Hz, 0.66H, major), 1.84 (t, *J* = 11.0 Hz, 0.34H, minor), 1.81–1.64 (m, 1H), 1.59–1.55 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 and 164.7, 157.94 and 157.89, 147.6 and 147.1, 144.21 and 144.18, 131.7, 130.40 and 130.34, 126.39 and 126.30, 123.76 and 123.71 (q, *J* = 278.0 Hz), 121.7, 118.2 and 118.1, 101.01 and 100.95, 71.90, 70.5 and 70.4 (q, *J* = 34.0 Hz), 60.5 and 60.3, 56.2 and 55.7, 55.6 and 55.1, 43.2, 25.9 and 25.7, 25.1 and 24.9, 23.9 and 22.7; ^{19}F NMR (376 MHz, $CDCl_3$)

δ –73.98 (t, *J* = 8.3 Hz), –73.85 (t, *J* = 8.3 Hz); HRMS (ESI+) calcd for $C_{20}H_{23}N_3O_3F_3$ $[M+H]^+$ 410.1675, found 410.1667 (error 1.9 ppm); $[\alpha]_D^{23}$= –153 (*c* 0.300, $CHCl_3$).

6-Methoxy-(*R*)-4-[(1*S*,2*S*,4*S*)-5-propoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (21). Compound **21** was prepared following the general procedure for C-3 oximation employing **16** (100 mg, 0.320 mmol) and *O*-*n*-propylhydroxylamine hydrochloride (48 mg, 0.32 mmol) to afford the title compound (99 mg, 84%, sole product) as a white solid: HPLC purity 99.1%, t_R = 21.9 min; mp 196–197 °C; R_f = 0.35 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.4 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.50 (d, *J* = 4.4 Hz, 1H), 7.32 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.17 (d, *J* = 2.6 Hz, 1H), 5.70 (s, 1H), 3.92 (dd, *J* = 6.9, 3.3 Hz, 2H), 3.89 (s, 3H), 3.71–3.49 (m, 3H), 3.27–3.08 (m, 1H), 2.91–2.69 (m, 1H), 2.64 (s, 1H), 2.15 (dd, *J* = 13.0, 8.1 Hz, 1H), 1.94 (d, *J* = 11.4 Hz, 1H), 1.77 (d, *J* = 12.5 Hz, 1H), 1.59 (qd, *J* = 13.9, 8.1 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 157.9, 147.6, 146.9, 144.2, 131.7, 126.3, 121.7, 118.2, 101.0, 75.3, 71.6, 60.6, 55.8, 55.4, 43.5, 29.8, 26.1, 25.9, 22.4, 10.4; HRMS (ESI+) calcd for $C_{21}H_{28}N_3O_3$ $[M+H]^+$ 370.2125, found 370.2118 (error 1.9 ppm); $[\alpha]_D^{23}$ = –80.2 (*c* 0.300, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*)-5-Isopropoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (22). Compound **22** was prepared following the general procedure for C-3 oximation employing **16** (100 mg, 0.320 mmol) and *O*-*i*-propylhydroxylamine hydrochloride (48 mg, 0.32 mmol) to afford the title compound (77 mg, 65%, sole product) as a white solid: HPLC purity 99.2%, t_R = 21.6 min; mp 190–192 °C; R_f = 0.40 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (d, *J* = 4.4 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 4.4 Hz, 1H), 7.28 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.15 (d, *J* = 2.6 Hz, 1H), 5.74 (s, 1H), 4.19 (quint, *J* = 6.1 Hz, 1H), 3.87 (s, 3H), 3.73 (d, *J* = 15.0 Hz, 1H), 3.68–3.50 (m, 2H), 3.15 (d, *J* = 9.6 Hz, 1H), 2.87–2.72 (m, 1H), 2.65 (s, 1H), 2.16 (dd, *J* = 12.6, 8.1 Hz, 1H), 1.97 (t, *J* = 11.6 Hz, 1H), 1.78 (d, *J* = 12.6 Hz, 1H), 1.53 (td, *J* = 12.6, 3.7 Hz, 1H), 1.18 (d, *J* = 6.1 Hz, 3H),

1.16 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 157.9, 147.5, 146.9, 144.1, 131.6, 126.2, 121.7, 118.2, 101.0, 75.1, 71.2, 60.7, 55.9, 55.5, 43.6, 29.8, 26.0, 25.6, 21.7 (2C); HRMS (ESI+) calcd for $C_{21}H_{28}N_3O_3$ $[M+H]^+$ 370.2123, found 370.2118 (error 0.6 ppm); $[\alpha]_D^{23}$ = –65.3 (*c* 0.300, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Allyloxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (23). Compound **23** was prepared following the general procedure for C-3 oximation employing **16** (62 mg, 0.20 mmol) and *O*-allylhydroxylamine hydrochloride (22 mg, 0.20 mmol) to afford the title compound (70 mg, 95%, ^{1}H NMR indicates 2:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 95.5% (two isomers), t_R = 19.8 min; mp 185–186 °C; R_f = 0.36 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 4.5 Hz, 1H), 8.00 (d, J = 9.2 Hz, 1H), 7.64–7.46 (m, 1H), 7.36–7.33 (m, 1H), 7.18 (d, J = 2.6 Hz, 1H), 6.02–5.83 (m, 1H), 5.76–5.59 (m, 1H), 5.28–5.09 (m, 2H), 4.52–4.36 (m, 2H), 3.91 (s, 2H, major), 3.90 (s, 1H, minor), 3.73–3.50 (m, 2H), 3.52–3.40 (m, 1H), 3.21–3.06 (m, 1H), 2.77 (t, J = 14.6 Hz, 1H), 2.68–2.60 (m, 1H), 2.21–2.07 (m, 1H), 2.06–2.00 (m, 1H), 1.97–1.93 (m, 1H), 1.85–1.79 (m, 1H), 1.57–1.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 157.95 and 157.92, 147.7, 146.8, 144.37 and 144.34, 134.5 and 134.3, 131.8, 126.4 and 126.3, 121.7, 118.2 and 118.1, 117.5 and 117.3, 101.03 and 100.99, 74.6 and 74.4, 72.1, 60.6 and 60.4, 56.6 and 55.8, 55.7 and 55.5, 43.4, 26.1 and 25.9, 25.4 and 25.2, 24.6 and 24.2; HRMS (ESI+) calcd for $C_{21}H_{26}N_3O_3$ $[M+H]^+$ 368.1969, found 368.1944 (error 6.6 ppm); $[\alpha]_D^{23}$ = –171 (*c* 0.500, $CHCl_3$).

6-Methoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-propargyloxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (24). Compound **24** was prepared following the general procedure for C-3 oximation employing **16** (66 mg, 0.21 mmol) and *O*-(prop-2-yn-1-yl) hydroxylamine hydrochloride (23 mg, 0.21 mmol) to afford the title compound (65 mg, 84%, ^{1}H NMR indicates 2:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 95.8% (two isomers),

t_R = 17.5 min; mp 199–200 °C; R_f = 0.30 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 4.5 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 4.5 Hz, 1H), 7.35 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.19 (d, *J* = 2.7 Hz, 1H), 5.65 (br s, 0.66H, major), 5.62 (br s, 0.34H, minor), 4.57 (d, *J* = 2.4 Hz, 1.3H, major) , 4.55 (d, *J* = 2.4 Hz, 0.7H, minor), 3.92 (s, 2H, major), 3.91 (s, 1H, minor), 3.75–3.50 (m, 2H), 3.50–3.42 (m, 1H), 3.15 (t, *J* = 9.6 Hz, 1H), 2.87–2.69 (m, 1H), 2.69 (t, *J* = 3.4 Hz, 1H), 2.41, (tt, *J* = 8.0, 1.7 Hz, 0.66H, major), 2.39 (tt, *J* = 8.0, 1.7 Hz, 0.34H, minor), 2.23–2.09 (m, 1H), 2.05 (t, *J* = 11.1 Hz, 1H), 1.98–1.89 (m, 1H), 1.86–1.72 (m, 1H), 1.58 (t, *J* = 11.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 157.95 and 157.92, 147.7, 146.85 and 146.79, 144.35 and 144.33, 131.8, 126.4 and 126.3, 121.7, 118.2 and 118.1, 101.06 and 101.00, 80.0 and 79.8, 74.3 and 74.2, 72.1, 61.0 and 60.9, 60.5 and 60.4, 56.5 and 55.73, 55.67 and 55.4, 43.3 and 43.2, 29.9 and 29.7, 26.0 and 25.9, 24.8 and 24.1; HRMS (ESI+) calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$ 366.1812, found 366.1801 (error 2.8 ppm); $[\alpha]_D^{23}$ = –154 (*c* 0.200, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Butoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (25). Compound **25** was prepared following the general procedure for C-3 oximation employing **16** (100 mg, 0.320 mmol) and *O*-*n*-butylhydroxylamine hydrochloride (45 mg, 0.32 mmol) to afford the title compound (108 mg, 88%, ^{1}H NMR indicates 2:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 96.2% (two isomers, 61.2% and 35.0%), t_R = 26.1 and 26.4 min; mp 192–193 °C; R_f = 0.38 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 4.5 Hz, 1H), 8.01 (d, *J* = 9.2, 0.34H, minor), 8.00 (d, *J* = 9.2 Hz, 0.66H, major), 7.53 (d, *J* = 4.2 Hz, 0.66H, major), 7.52 (d, *J* = 4.2 Hz, 0.34H, minor), 7.36 (dd, *J* = 9.2, 3.1 Hz, 0.34H, minor), 7.35 (dd, *J* = 9.3, 3.1 Hz, 0.66H, major), 7.23–7.17 (m, 1H), 5.76 (br s, 0.66H, major), 5.71 (br s, 0.34H, minor), 4.00–3.95 (m, 2H), 3.94 (s, 2H, major), 3.93 (s, 1H, minor), 3.60 (q, *J* = 18.4 Hz, 2H), 3.50–3.93 (m, 1H), 3.20 (td, *J* = 8.6, 3.9 Hz, 1H), 2.91–2.72 (m, 1H), 2.68–2.62 (m, 1H), 2.20–2.15 (m, 1H), 2.07–2.03 (m, 1H), 1.97–1.97 (m, 1H), 1.85–1.80 (m, 1H), 1.64–1.46 (m, 3H), 1.43–1.27 (m, 2H), 0.90 (t, *J* =

7.4 Hz, 2H, major), 0.88 (t, *J* = 7.4 Hz, 1H, minor); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 158.01 and 157.96, 147.7, 146.54 and 146.47, 144.40 and 144.37, 131.89, 126.3 and 126.2, 121.76 and 121.74, 118.17 and 118.10, 100.97 and 100.92, 73.7 and 73.5, 60.6 and 60.4, 56.6 and 55.8, 55.7 and 55.4, 43.5, 31.17 and 31.13, 29.8 and 29.7, 26.0 and 25.8, 25.2 and 25.0, 24.4 and 24.1, 19.20 and 19.18, 14.0; HRMS (ESI+) calcd for $C_{22}H_{30}N_3O_3$ $[M+H]^+$ 384.2282, found 384.2271 (error 2.6 ppm); $[\alpha]_D^{23} = -144$ (*c* 0.400, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Cyclobutoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (26). Compound **26** was prepared following the general procedure for C-3 oximation employing **16** (62 mg, 0.20 mmol) and *O*-cyclobutylhydroxylamine hydrochloride (25 mg, 0.20 mmol) to afford the title compound (58 mg, 76%, ^{1}H NMR indicates 2:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as a white solid: HPLC purity 99.8% (two isomers, 77.2% and 22.6%), t_R = 25.6 and 26.7 min; mp 196–197 °C; R_f = 0.37 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 4.5 Hz, 1H), 8.02 (d, *J* = 9.2 Hz, 0.34H, minor), 8.00 (d, *J* = 9.2 Hz, 0.66H, major), 7.54 (d, *J* = 4.5 Hz, 0.66H, major), 7.53 (d, *J* = 4.5 Hz, 0.34H, minor), 7.36 (dd, *J* = 9.2, 2.6 Hz, 0.34H, minor), 7.35 (dd, *J* = 9.2, 2.6 Hz, 0.66H, major), 7.20 (d, *J* = 2.6 Hz, 1H), 5.74 (br s, 0.66H, major), 5.69 (br s, 0.34H, minor), 4.52 (sext, *J* = 7.6 Hz, 1H), 3.92 (s, 2H, major), 3.91 (s, 1H, minor), 3.80–3.52 (m, 3H), 3.47 (t, *J* = 5.2 Hz, 1H), 3.22–3.09 (m, 1H), 2.91–2.69 (m, 1H), 2.65 (s, 1H), 2.33–2.08 (m, 3H), 2.07–1.90 (m, 3H), 1.87–1.63 (m, 2H), 1.60–1.47 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 157.99 and 157.94, 147.7, 146.5, 144.40 and 144.37, 131.9, 126.4 and 126.2, 121.7, 118.13 and 118.06, 100.99 and 100.95, 76.2 and 76.0, 60.6 and 60.4, 56.7 and 55.8, 55.7 and 55.5, 43.5, 29.7 and 29.4, 26.0 and 25.8, 24.5 and 24.2, 22.7, 18.5, 14.1, 12.7; HRMS (ESI+) calcd for $C_{22}H_{28}N_3O_3$ $[M+H]^+$ 382.2116, found 382.2125 (error 2.4 ppm); $[\alpha]_D^{23} = -231$ (*c* 0.100, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*)-5-Cyclopropylmethoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (27). Compound **27** was prepared following the general procedure for C-3 oximation employing **18** (62 mg, 0.20 mmol) and *O*-(cyclopropylmethyl) hydroxylamine hydrochloride (25 mg, 0.20 mmol) to afford the title compound (70 mg, 92%, sole product) as a white solid: HPLC purity 98.0%, t_R = 21.7 min; mp 194-195 °C; R_f = 0.34 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.5 Hz, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 4.5 Hz, 1H), 7.32 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.18 (d, *J* = 2.6 Hz, 1H), 5.70 (br s, 1H), 3.90 (s, 3H), 3.77 (dd, *J* = 7.0, 2.0 Hz, 2H), 3.73–3.49 (m, 3H), 3.17 (t, *J* = 10.3 Hz, 1H), 2.78 (t, *J* = 14.3 Hz, 1H), 2.63 (s, 1H), 2.20–2.09 (m, 1H), 2.03–1.87 (m, 1H), 1.87–1.72 (m, 1H), 1.63–1.46 (m, 1H), 1.12–0.97 (m, 1H), 0.48 (q, *J* = 4.7 Hz, 2H), 0.21 (q, *J* = 4.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 157.9, 147.6, 146.8, 144.3, 131.8, 126.3, 121.7, 118.1, 101.0, 78.4, 71.7, 60.6, 55.8, 55.5, 43.5, 29.9, 29.7, 26.0, 25.8, 10.1 (2C); HRMS (ESI+) calcd for $C_{22}H_{28}N_3O_3$ $[M+H]^+$ 382.2107, found 382.2125 (error 4.9 ppm); $[\alpha]_D^{23}$ = –151 (*c* 0.200, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*)-5-Cyclopentoxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (28). Compound **28** was prepared following the general procedure for C-3 oximation employing **18** (62 mg, 0.20 mmol) and *O*-cyclopentylhydroxylamine hydrochloride (57 mg, 0.20 mmol) to afford the title compound (71 mg, 93%, sole product) as a white solid: HPLC purity 98.4%, t_R = 26.8 min; mp 196–197 °C; R_f = 0.35 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 4.5 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.52 (d, *J* = 4.5 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.16 (d, *J* = 2.5 Hz, 1H), 5.73 (br s, 1H), 4.67–4.45 (m, 1H), 3.89 (s, 3H), 3.72 (d, *J* = 7.0 Hz, 1H), 3.57 (q, *J* = 18.7 Hz, 2H), 3.21–3.09 (m, 1H), 2.83–2.71 (m, 1H), 2.65 (s, 1H), 2.20–2.09 (m, 1H), 2.04–1.89 (m, 1H), 1.81–1.41 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 157.9, 147.6, 146.6, 144.3, 131.8, 126.2, 121.7, 118.2, 101.0, 84.7, 71.5, 60.6, 55.8, 55.5, 43.5, 32.10, 32.02, 29.9, 26.0 (2C), 23.9 (2C); HRMS

(ESI+) calcd for $C_{23}H_{30}N_3O_3$ $[M+H]^+$396.2282, found 396.2250 (error 8.0 ppm); $[\alpha]_D^{23} = -138$ (*c* 0.200, $CHCl_3$).

6-Methoxy-(*R*)-4-{[(1*S*,2*S*,4*S*,5*Z/E*)-5-(4-methoxy-4-oxo)butoxyimino-1-azabicyclo[2.2.2]octan-2-yl]hydroxymethyl}quinoline and 6-Methoxy-(*R*)-4-{[(1*S*,2*S*,4*S*,5*Z/E*)-5-(4-ethoxy-4-oxo)butoxyimino-1-azabicyclo[2.2.2]octan-2-yl]hydroxymethyl}quinoline (29). Compound **29** was prepared following the general procedure for C-3 oximation employing **16** (62 mg, 0.20 mmol) and a mixture of ethyl 4-(aminooxy)butanoate hydrochloride and methyl 4-(aminooxy)butanoate hydrochloride (34 mg, 0.20 mmol) [in Scheme 2B, compound **31** is used as the methyl ester, but in the reaction from **32** to **33**, the transesterification reaction was carried out using ethanol as a solvent, resulting in the compound **33** being a mixture of methyl ester and ethyl esters. Therefore, the result of this reaction was a mixture of methyl ester and ethyl ester] to afford the title compound (69 mg, 81%, ^{1}H NMR indicates 3:2 ester ratio through the integral value of –COOC**H$_3$** protons and –COOC**H$_2$**C**H$_3$** protons) as a white solid: HPLC purity 95.2% (two esters, 49.3% and 45.9%), t_R = 18.2 and 19.8 min; mp 190–192 °C; R_f = 0.39 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 4.5 Hz, 1H), 8.01 (d, *J* = 9.2 Hz, 0.4H, minor), 7.99 (d, *J* = 9.2 Hz, 0.6H, major), 7.52 (d, *J* = 4.5 Hz, 1H), 7.33 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.19 (d, *J* = 2.5 Hz, 1H), 5.74 (br s, 0.6H, major), 5.69 ((br s, 0.4H, minor), 4.09 (q, *J* = 7.1 Hz, 0.8H (COO**Et**), minor), 4.04–3.93 (m, 2H), 3.92 (s, 1.8H, major), 3.91 (s, 1.2H, minor), 3.70–3.65 (m, 1H), 3.63 (s, 1.8H (COO**Me**), major), 3.56 (s, 1H), 3.52 (s, 1H), 3.44 (s, 1H), 3.24–3.09 (m, 1H), 2.90–2.71 (m, 1H), 2.64 (s, 1H), 2.34 (q, *J* = 7.4 Hz, 2H), 2.21–2.09 (m, 1H), 1.98–1.80 (m, 3H), 1.79 (d, *J* = 10.2 Hz, 1H), 1.56 (t, *J* = 10.2 Hz, 1H), 1.22 (t, *J* = 7.2 Hz, 1.2H (COO**Et**), minor); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7 and 173.3, 162.51 and 162.45, 157.99 and 157.95, 147.6, 146.61 and 146.59, 144.35 and 144.31, 131.8, 126.3 and 126.2, 121.8 and 121.7, 118.18 and 118.12, 100.98 and 100.92, 72.63 and 72.57, 71.7 and 71.5, 60.6 (**Et**), 60.42 and 60.37, 56.6 and 55.8, 55.7 and

55.3, 51.6 (**Me**), 43.5, 32.0 and 31.0, 30.9 and 30.7, 29.8 and 29.4, 25.9 and 25.7, 24.5 and 24.1, 14.2 (**Et**); HRMS (ESI+) calcd for $C_{23}H_{30}N_3O_5$ and $C_{24}H_{32}N_3O_5$ $[M+H]^+$ 428.2180 and 442.2336, found 428.2150 and 442.2303 (error 7.6 and 7.1 ppm); $[\alpha]_D^{23} = -128.0$ (*c* 0.4, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Benzyloxyimino-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (30). Compound **30** was prepared following the general procedure for C-3 oximation employing **16** (66 mg, 0.21 mmol) and *O*-benzylhydroxylamine hydrochloride (34 mg, 0.21 mmol) to afford the title compound (75 mg, 81%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of quinoline H-2′ protons) as a white solid: HPLC purity 95.6% (two isomers), t_R = 24.1 min; mp 202–203 °C; R_f = 0.33 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 4.5 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 0.34H, minor), 7.99 (d, *J* = 9.2 Hz, 0.66H, major), 7.50 (d, *J* = 4.5 Hz, 0.66H, major), 7.49 (d, *J* = 4.5 Hz, 0.34H, minor), 7.35 (dd, *J* = 9.2, 2.5 Hz, 0.34H, minor), 7.34 (dd, *J* = 9.2, 2.5 Hz, 0.66H, major), 7.33–7.29 (m, 2H), 7.28–7.26 (m, 3H), 7.17 (d, *J* = 2.5 Hz, 1H), 5.74 (br s, 0.66H, major), 5.70 (br s, 0.34H, minor), 5.09–4.86 (m, 2H), 3.90 (s, 3H), 3.74–3.50 (m, 3H), 3.43 (s, 1H), 3.22–3.05 (m, 1H), 2.74 (t, *J* = 12.7 Hz, 1H), 2.65 (s, 1H), 2.20–2.07 (m, 1H), 2.01–1.87 (m, 1H), 1.76 (d, *J* = 13.9 Hz, 1H), 1.55 (t, *J* = 12.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 157.94 and 157.92, 147.7, 146.85 and 146.82, 144.35 and 144.32, 138.0 and 137.8, 131.8, 128.4 and 128.3, 128.1, 127.9 and 127.8, 127.7, 126.39 and 126.31, 121.7, 118.2 and 118.1, 101.03 and 101.00, 75.7 and 75.5, 72.0 and 71.8, 60.6 and 60.4, 56.6 and 55.8, 55.7 and 55.6, 43.43 and 43.40, 29.9, 29.7, 26.1 and 25.2, 24.7 and 24.1; HRMS (ESI+) calcd for $C_{25}H_{28}N_3O_3$ $[M+H]^+$418.2125, found 418.2108 (error 2.8 ppm); $[\alpha]_D^{23} = -189$ (*c* 0.300, $CHCl_3$).

(*R*)-6-Methoxy-4-[(1*S*,2*S*,4*S*,5*R*)-5-vinyl-1-azabicyclo[2.2.2]octan-2-yl)*tert*-butyldimethylsilyloxymethyl]quinoline (34). To a solution of quinine (6.28 g, 20.0 mmol, 1.0 equiv) in DMF (50 mL) was added imidazole (5.44 g, 80.0 mmol, 4.0 equiv) and TBDMSCl (9.10 g, 60.0

mmol, 3.0 equiv). The solution was stirred 5 h at 100 °C and worked up by adding toluene (50 mL) and washing with water (50 mL). The toluene was removed under reduced pressure and the crude oil was purified by flash chromatography (50:1 CH_2Cl_2–MeOH) to afford the title compund (9.11 g, 99%) as a white solid: mp 177–179 °C; R_f = 0.60 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, *J* = 4.5 Hz, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.77 (s, 1H), 7.49 (d, *J* = 4.5 Hz, 1H), 7.40 (dd, *J* = 9.3, 2.5 Hz, 1H), 6.79 (s, 1H), 5.56 (ddd, *J* = 17.2, 10.4, 6.8 Hz, 1H), 5.13–4.79 (m, 2H), 4.18 (s, 3H), 4.08 (s, 1H), 3.39 (t, *J* = 12.2 Hz, 1H), 3.26 (t, *J* = 9.6 Hz, 1H), 3.16 (d, *J* = 13.9 Hz, 2H), 2.70 (s, 1H), 2.26 (t, *J* = 11.0 Hz, 1H), 2.15–2.00 (m, 2H), 1.99–1.80 (m, 1H), 1.49 (t, *J* = 12.0 Hz, 1H), 1.01 (s, 9H), 0.39 (s, 3H), –0.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 146.6, 144.6, 144.1, 137.4, 131.8, 125.8, 123.5, 118.9, 117.5, 100.7, 67.7, 60.4, 58.1, 54.5, 42.9, 37.2, 26.9, 26.0, 24.7, 18.5, 18.0, –4.3, –4.5; HRMS (ESI+) calcd for $C_{26}H_{39}N_2O_2Si$ $[M+H]^+$ 439.2775, found 439.2778 (error 0.5 ppm); $[\alpha]_D^{23}$= –106 (*c* 0.600, $CHCl_3$).

(*R*)-4-[(1*S*,2*S*,4*S*,5*R*)-5-(1,2-Dihydroxyethyl)-1-azabicyclo[2.2.2]octan-2-yl]*tert*-butyldimethylsilyloxymethyl-6-methoxyquinoline (35). Compound **34** (3.15 g, 7.18 mmol, 1.0 equiv) was added to a vigorously stirred two-phase system of K_2CO_3 (2.98 g, 21.5 mmol, 3.0 equiv) and $K_3[Fe(CN)_6]$ (7.09 g, 21.5 mmol, 3.0 equiv) in *tert*-butyl alcohol/H_2O (1:1, 60 mL). After 45 min, an aqueous solution of osmium (VIII) oxide (0.30 mL, 2.5% in water) was added in small portions, and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed successively with saturated aqueous $NaHCO_3$ (100 mL) and 10% aqueous $NaHSO_3$ (100 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. Purification by flash chromatography (10:1 CH_2Cl_2–MeOH) afforded the title compound (2.98 g, 88%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of quinioline H-2′) as a white solid: mp 225–226 °C; R_f = 0.45 (5:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz,

$CDCl_3$) δ 8.65 (s, 0.75H, major) , 8.57 (s, 0.25H, minor), 8.13–7.69 (m, 1H), 7.56–7.39 (m, 1H), 7.33 (d, *J* = 9.6 Hz, 1H), 7.24 (s, 1H), 5.61 (s, 1H), 3.92 (s, 2.2H, major), 3.88 (s, 0.8H, minor), 3.72–3.60 (m, 1H), 3.56 (s, 1H), 3.36 (d, *J* = 8.3 Hz, 1H), 3.28 (d, *J* = 9.8 Hz, 1H), 3.01 (s, 1H), 2.92 (d, *J* = 7.3 Hz, 1H), 2.85–2.65 (m, 1H), 2.60–2.55 (m, 1H), 2.37–2.00 (m, 2H), 1.92–1.82 (m, 1H), 1.72 (s, 1H), 1.63 (d, *J* = 11.2 Hz, 1H), 1.55 (s, 1H), 1.40 (d, *J* = 11.1 Hz, 1H), 1.20 (d, *J* = 7.0 Hz, 1H), 0.96 (s, 6.8H, major), 0.82 (s, 2.2H, minor), 0.10 (s, 2.2H, major), –0.01 (s, 0.8H, minor), –0.41 (s, 2.2H, major), –0.41 (s, 0.8H, minor); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1 and 156.7, 148.34 and 148.25, 147.2 and 147.1, 145.2 and 144.1, 131.6 and 131.2, 127.1 and 126.2, 121.6 and 121.5, 118.8 and 118.6, 100.9 and 100.8, 74.5 and 73.7, 72.6 and 72.3, 65.4 and 64.7, 61.6 and 60.9, 55.9 and 55.1, 43.4 and 43.3, 38.8 and 38.7, 28.6 and 28.1, 27.7 and 27.5, 26.0 and 25.8, 24.1 and 23.9, 22.5 and 21.0, 18.2 and 18.1, –4.2 and –4.6, –5.16 and –5.23; HRMS (ESI+) calcd for $C_{26}H_{41}N_2O_4Si$ $[M+H]^+$ 473.2830, found 473.2831 (error 0.2 ppm); $[\alpha]_D^{23} = -102$ (*c* 1.60, MeOH).

(*R*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-(*Z*/*E*)-prop-1-en-1-yl)-1-azabicyclo[2.2.2]octan-2-yl)*tert*-butyldimethylsilyloxymethyl]-6-methoxyquinoline (36). To a solution of **35** (823 mg, 1.74 mmol, 1.0 equiv) in *tert*-butanol (9 mL) was added a saturated solution of $NaIO_4$ (484 mg, 2.26 mmol, 1.3 equiv) in H_2O (3 mL) dropwise. The mixture was stirred vigorously for 16 h at at room temperature, treated with aqueous $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (3×20 mL), dried over $MgSO_4$, filtered, and the filtrate concentrated under redued pressure. Purification by flash chromatography (40:1 CH_2Cl_2–MeOH) afforded the intermediate aldehyde (587 mg, 79%) as an approximately 2:1 mixture of isomers (presumably epimers at C-3) as a white solid: R_f = 0.58 (10:1 CH_2Cl_2–MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 9.72 (s, 0.6H, major), 9.62 (s, 0.4H, minor), 8.75–8.61 (m, 1H), 8.04–7.98 (m, 1H), 7.89–7.73 (m, 0.6H, major), 7.52–7.49 (m, 0.4H, minor), 7.45–7.33 (m, 1H), 7.15–7.10 (m, 1H), 5.73–5.47 (m, 1H), 3.96 (s, 1.8H, major), 3.94 (s, 1.2H, minor), 3.50–3.41 (m, 2H), 3.24 (dd, *J* = 13.6, 7.3 Hz, 1H),

3.12–2.99 (m, 1H), 2.95–2.85 (m, 1H), 2.77–2.57 (m, 1H), 2.45–2.25 (m, 1H), 2.04 (dd, J = 12.4, 8.6 Hz, 1H), 1.93–1.73 (m, 1H), 1.66–1.55 (m, 1H), 1.51–1.31 (m, 1H), 0.98 (s, 5.4H, major), 0.96 (s, 3.6H, minor), 0.18 (s, 1.8H, major), 0.15 (s, 1.2H, minor), –0.35 (s, 1.8H, major), –0.38 (s, 1.2H, minor). The resulting unstable aldehyde was immediately used in the next reaction.

To a stirred suspension of ethyltriphenylphosphonium bromide (2.05 g, 5.52 mmol, 4.0 equiv) in THF (30 mL) was added a solution of *n*-BuLi (2.5 M in Hexane, 1.93 mL, 4.82 mmol, 3.5 equiv) at 0 °C, and the resulting orange solution was stirred at 0 °C for 10 min. To the reaction mixture was added dropwise a solution of the aldehyde prepared above (587 mg, 1.38 mmol, 1.0 equiv) in THF (20 mL) via a double-tipped stainless steel needle at 0 °C. After stirring the reaction mixture for 12 h, the reaction was quenched with aqueous $NaHCO_3$ (20 mL), the aqueous mixture was extracted with EtOAc (3×30 mL), and the combined organic extracts were dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. Purification by flash chromatography (50:1 CH_2Cl_2–MeOH) afforded the intermediate TBS protected olefin (418 mg, 67%, ^{1}H NMR indicates 1.1:1 diastereomeric ratio through the integral value of =CH-**C**$\mathbf{H_3}$) as a white solid: mp 185–187 °C; R_f = 0.55 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, J = 4.5 Hz, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.61 (s, 1H), 7.50 (d, J = 4.5 Hz, 1H), 7.38 (dd, J = 9.1, 2.6 Hz, 1H), 5.58–5.27 (m, 1H), 5.11 (t, J = 10.0 Hz, 1H), 4.12 (s, 3H), 4.05–3.78 (m, 2H), 3.50–3.38 (m, 1H), 3.28–3.01 (m, 2H), 2.88 (br s, 2H), 2.25–2.10 (m, 2H), 2.05–1.70 (m, 3H), 1.55 (d, J = 8.4 Hz, 1.4H, minor), 1.55 (d, J = 8.4 Hz, 1.6H, major), 0.99 (s, 9H), 0.35 (s, 3H), –0.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4 and 159.2, 146.7 and 146.6, 144.6, 131.8, 130.8 and 130.6, 128.6, 127.8, 125.8, 123.22 and 123.21, 119.01 and 118.95, 100.63 and 100.58, 68.2, 60.8, 58.0, 57.3, 42.9, 31.7, 29.7, 26.7, 26.0, 25.8, 25.2, 19.1, 18.0, 13.2, –4.3, –4.4; HRMS (ESI+) calcd for $C_{27}H_{41}N_2O_2Si$ $[M+H]^+$ 453.2932, found 453.2932 (error 0.0 ppm); $[\alpha]_D^{23}$= –103 (*c* 0.600, $CHCl_3$).

6-Methoxy-(*R*)-4-{((1*S*,2*S*,4*S*,5*S*)-5-[((*Z/E*)-prop-1-en-1-yl)-1-azabicyclo[2.2.2]octan-2-yl]hydroxymethyl}quinoline (37). The intermediate TBS protected olefin product **36** prepared above (500 mg, 1.10 mmol) was submitted to the general procedure for TBS deprotection employing TBAF (1.11 mL, 1.11 mmol) to afford the title compound (340 mg, 91%, ^{1}H NMR indicates 1.1:1 diastereomeric ratio through the integral value of -C**H**OH) as a white solid: HPLC purity 99.8% (two isomers, 52.2% and 47.7%), t_R = 19.3 and 19.5 min; mp 188–189 °C; R_f = 0.45 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70–8.64 (m, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.53 (d, *J* = 4.5 Hz, 0.45H, minor), 7.51 (d, *J* = 4.5 Hz, 0.55H, major), 7.32 (d, *J* = 9.2 Hz, 1H), 7.23 (s, 0.55H, major), 7.22 (s, 0.45H, minor), 5.63 (br s, 0.45H, minor), 5.56 (br s, 0.55H, major), 5.52–5.42 (m, 0.9H, minor), 5.42–5.29 (m, 1.1H, major), 3.88 (s, 3H), 3.47 (br s, 1H), 3.22–3.07 (m, 2H), 2.75–2.69 (m, 1H), 2.54 (d, *J* = 12.9 Hz, 2H), 2.41 (q, *J* = 7.7 Hz, 1H), 1.87 (t, *J* = 10.9 Hz, 1H), 1.77–1.62 (m, 2H), 1.60–1.49 (m, 3H), 1.42 (dd, *J* = 16.4, 8.5 Hz, 1H), 1.27 (d, *J* = 15.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.77 and 157.75, 147.66 and 147.64, 147.50 and 147.46, 144.32 and 144.28, 134.3 and 132.9, 131.69 and 131.67, 126.66 and 126.62, 125.0 and 124.6, 121.5, 118.5, 101.3, 72.0 and 71.9, 60.1 and 59.5, 58.8 and 57.8, 55.71 and 55.68, 44.1 and 43.2, 33.6 and 32.8, 28.3 and 27.7, 27.5 and 27.1, 22.0 and 21.4, 13.2 and 13.1; HRMS (ESI+) calcd for $C_{21}H_{27}N_2O_2$ $[M+H]^+$ 339.2067, found 339.2072 (error 4.4 ppm); $[\alpha]_D^{23}$ = –97.5 (*c* 0.300, $CHCl_3$).

(*R*)-4-[(1*S*,2*S*,4*S*,5*R*)-5-(1,2-Dihydroxyethyl)-1-azabicyclo[2.2.2]octan-2-yl]hydroxymethyl-6-methoxyquinoline (38). Compound **38** was prepared following the general procedure for TBS deprotection employing **35** (670 mg, 1.42 mmol) and TBAF (1.43 mL, 1.43 mmol) to afford the title compound (346 mg, 68%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of -C**H**OH) as a white solid: HPLC purity 98.1% (two isomers), t_R = 5.5 min; mp 240–241 °C, lit. mp 221 °C[66]; R_f = 0.23 (5:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 3351, 2944, 1621, 1511, 1096, 831, 622; ^{1}H NMR

(400 MHz, CD_3OD) δ 8.63 (d, *J* = 4.6 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 0.25H, minor), 7.89 (d, *J* = 9.2 Hz, 0.75H, major), 7.70 (d, *J* = 4.6 Hz, 0.25H, minor), 7.69 (d, *J* = 4.6 Hz, 0.75H, major), 7.46 (d, *J* = 2.6 Hz, 1H), 7.37 (dd, *J* = 9.2, 2.6 Hz, 0.25H, major), 7.36 (dd, *J* = 9.2, 2.6 Hz, 0.75H, major), 5.88 (br s, 0.75H, major), 5.81 (br s, 0.25H, minor), 3.99 (s, 2H, major), 3.98 (s, 1H, minor), 3.62–3.45 (m, 1H), 3.52–3.36 (m, 1H), 3.32–3.08 (m, 5H), 3.08–2.75 (m, 1H), 2.10–1.80 (m, 4H), 1.69–1.60 (m, 2H), 1.40 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.23–1.03 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.49 and 158.47, 148.2 and 147.9, 146.6, 143.2, 129.9, 126.5 and 126.4, 122.14 and 122.11, 118.8 and 118.7, 101.08 and 101.01, 73.4 and 72.9, 69.2 and 69.0, 64.6 and 64.1, 60.0 and 59.7, 55.50 and 55.46, 53.7 and 53.2, 43.3 and 43.2, 37.1 and 36.9, 26.2 and 26.0, 24.6 and 23.3, 19.8 and 19.6; HRMS (ESI+) calcd for $C_{20}H_{27}N_2O_4$ $[M+H]^+$ 359.1965, found 359.1949 (error 4.7 ppm); $[\alpha]_D^{23}$= –136 (*c* 0.600, $CHCl_3$), lit $[\alpha]_D^{23}$= –140[66]. All spectroscopic data were in agreement with the literature values.[67]

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-hydroxyquinoline (39, Apo-cupreine). Compound **39** was prepared following the general procedure for C-6 phenol demethylation alkaloids employing **15** (200 mg, 0.616 mmol), NaH (50 mg, 1.3 mmol) and EtSH (0.14 mL, 1.9 mmol) to afford the title compound (168 mg, 87%, ^1H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a light yellow solid: HPLC purity 96.7% (two isomers, 7.0% and 89.7%), t_R = 16.0 and 16.7 min; mp 211–212 °C, lit. mp 205 °C[66]; R_f = 0.44 (5:1 CH_2Cl_2/MeOH); IR (KBr) γ_{max} 3230, 2943, 1621, 1469, 1224, 859, 644; ^1H NMR (500 MHz, DMSO-d_6) δ 10.17 (br s, 0.25H), 10.16 (br s, 0.75H), 8.67 (d, *J* = 4.3 Hz, 1H), 7.90 (d, *J* = 9.1 Hz, 1H), 7.55–7.49 (m, 2H), 7.39 (d, *J* = 9.1 Hz, 1H), 6.47 (br s, 1H), 5.99 (br s, 1H), 5.41 (d, *J* = 7.5 Hz, 0.75H, major), 5.34 (d, *J* = 7.5 Hz, 0.25H, minor), 4.29–3.83 (m, 3H), 3.56–3.46 (m, 1H), 3.40–3.25 (m, 1H), 2.60–2.58 (m, 1H), 2.20 (t, *J* = 10.6 Hz, 1H), 2.04–2.00 (m, 1H), 1.77–1.73 (m, 1H), 1.56 (d, *J* = 6.8 Hz, 0.8H, minor), 1.49 (d, *J* = 6.8 Hz, 2.2H, major), 1.28–1.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.4,

147.1, 143.4, 131.8, 131.7, 126.5, 122.0, 119.3, 118.3, 105.3, 105.1, 66.7, 60.5, 60.3, 54.6, 44.3, 31.3, 24.6, 12.8; HRMS (ESI+) calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$ 311.1754, found 311.1739 (error 4.8 ppm); $[\alpha]_D^{23}$ = –163 (*c* 0.800, MeOH) , lit $[\alpha]_D^{23}$= –215 (*c* 1.0, EtOH)[43].

6-Ethoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (40). Compound **40** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and bromoethane (29 μL, 0.39 mmol) to afford the title compound (101 mg, 93%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a white solid: HPLC purity 97.9% (two isomers, 14.6% and 83.3%), t_R = 21.3 and 22.2 min; mp 190–191 °C; R_f = 0.49 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.5 Hz, 0.75H, major), 8.64 (d, *J* = 4.5 Hz, 0.25H, minor), 7.67 (d, *J* = 9.2 Hz, 1H), 7.64 (d, *J* = 4.5 Hz, 1H), 6.94 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.82 (d, *J* = 2.5 Hz, 1H), 6.48 (s, 1H), 5.38 (q, *J* = 7.0 Hz, 0.75H, major), 5.30 (q, *J* = 7.0 Hz, 0.25H, minor), 4.73–4.50 (m, 1H), 3.92–3.81 (m, 1H), 3.82–3.73 (m, 2H), 3.71–3.54 (m, 1H), 3.43–3.26 (m, 1H), 3.16 (td, *J* = 12.5, 5.1 Hz, 1H), 2.55 (s, 1H), 2.41–2.10 (m, 2H), 1.91–1.75 (m, 1H), 1.53 (d, J = 6.9 Hz, 0.8 Hz, minor), 1.45 (d, *J* = 6.9 Hz, 2.2H, major), 1.33 (t, *J* = 6.9 Hz, 3H), 1.25–1.20 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 146.8, 143.8, 143.7, 131.3, 125.2, 122.5, 120.2, 118.5, 118.4, 99.9, 66.2, 64.9, 61.1, 55.9, 45.0, 31.5, 24.6, 23.7, 14.6, 12.8; HRMS (ESI+) calcd for $C_{21}H_{27}N_2O_2$ $[M+H]^+$ 339.2067, found 339.2058 (error 2.6 ppm); $[\alpha]_D^{23}$ = –64.0 (*c* 0.300, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-(2,2,2-trifluoroethoxy)quinoline (41). Compound **41** was prepared following the general procedure for C-6 phenol alkylation employing **39** (85 mg, 0.27 mmol, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) and 1,1,1-trifluoro-2-iodoethane (32 μL, 0.33 mmol) to afford the title compound (8 mg, 7%) as a white solid: HPLC purity 97.2% (two isomers), t_R = 17.8 min; mp 174–

175 °C; R_f = 0.50 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 4.5 Hz, 0.25H, minor), 8.69 (d, *J* = 4.5 Hz, 0.75H, major), 7.66 (d, *J* = 4.5 Hz, 1H), 7.63 (d, *J* = 9.1 Hz, 1H), 7.00–6.86 (m, 2H), 6.53 (br s, 0.25H, minor), 6.51 (br s, 0.75H, major), 6.20 (br s, 1H), 5.51–5.20 (m, 1H), 4.65 (td, *J* = 12.7, 7.0 Hz, 1H), 4.57–4.41 (m, 1H), 4.39–4.18 (m, 1H), 3.87–3.72 (m, 2H), 3.37–3.10 (m, 2H), 2.57 (s, 1H), 2.40–2.14 (m, 2H), 2.05–2.00 (m, 1H), 1.94–1.77 (m, 1H), 1.53 (d, *J* = 6.9 Hz, 0.8H, minor), 1.46 (d, *J* = 6.9 Hz, 2.2H, major); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5 and 155.3, 148.1 and 147.9, 144.4 and 143.9, 132.1 and 131.81, 131.78 and 130.0, 129.2, 125.0, 124.5, 122.2 (q, *J* = 277.7 Hz), 120.7 and 120.6, 118.63 and 118.55, 100.3, 66.0 (q, *J* = 34.5 Hz), 60.9 and 60.7, 57.5 and 55.9, 45.2 and 44.9, 31.9 and 31.3, 29.3 and 27.2, 25.5 and 24.52, 24.45 and 23.5, 22.8 and 22.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ –73.08 (t, *J* = 8.2 Hz) and –73.09 (t, *J* = 8.2 Hz); HRMS (ESI+) calcd for $C_{21}H_{24}N_2O_2F_3$ $[M+H]^+$ 393.1784, found 393.1774 (error 2.7 ppm); $[\alpha]_D^{23}$ = –196 (*c* 0.800, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-propoxyquinoline (42). Compound **42** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and 1-bromopropane (36 μL, 0.39 mmol) to afford the title compound (92 mg, 81%, ^{1}H NMR indicates 5:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a white solid: HPLC purity 99.0% (two isomers, 14.9% and 84.2%), t_R = 25.8 and 26.8 min; mp 185–187 °C; R_f = 0.54 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.5 Hz, 1H), 7.77 (d, *J* = 9.2 Hz, 1H), 7.59 (d, *J* = 4.5 Hz, 1H), 7.09 (d, *J* = 9.2 Hz, 1H), 6.97 (br s, 1H), 6.19 (br s, 1H), 5.30 (q, *J* = 6.6 Hz, 0.83H, major), 5.23 (q, *J* = 6.6 Hz, 0.17H, minor), 3.94–3.53 (m, 4H), 3.28 (t, *J* = 8.5 Hz, 1H), 3.11–2.96 (m, 1H), 2.48 (s, 1H), 2.23–1.95 (m, 2H), 1.85–1.63 (m, 3H), 1.53 (d, *J* = 6.8 Hz, 0.5H), 1.46 (d, *J* = 6.8 Hz, 2.5H), 1.35–1.23 (m, 2H), 1.06 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 147.12, 147.06, 143.8, 131.4, 125.7, 122.3, 122.2, 118.43, 118.37,

100.6, 70.3, 61.0, 60.6, 56.2, 44.7, 32.2, 29.7, 25.7, 22.5, 12.7, 10.7; HRMS (ESI+) calcd for $C_{22}H_{29}N_2O_2$ $[M+H]^+$ 353.2224, found 353.2217 (error 1.7 ppm); $[\alpha]_D^{23} = -104$ (*c* 0.500, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-isopropoxyquinoline (43). Compound **43** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and 2-bromopropane (38 μL, 0.39 mmol) to afford the title compound (83 mg, 73%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a white solid: HPLC purity 97.6% (two isomers, 17.6% and 80.0%), t_R = 23.5 and 24.4 min; mp 180–181 °C; R_f = 0.56 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 4.5 Hz, 1H), 7.67 (d, *J* = 9.0 Hz, 1H), 7.61 (d, *J* = 4.5 Hz, 1H), 7.02–6.88 (m, 2H), 6.52 (br s, 1H), 5.36 (q, *J* = 6.4 Hz, 0.75H, major), 5.30 (q, *J* = 6.4 Hz, 0.25H, minor), 4.94–4.74 (m, 1H), 4.58 (t, *J* = 13.2 Hz, 1H), 3.89–3.62 (m, 2H), 3.36 (t, *J* = 9.3 Hz, 1H), 3.27–3.08 (m, 1H), 2.64–2.53 (m, 1H), 2.34–2.11 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 0.8H, minor), 1.44 (d, *J* = 6.9 Hz, 2.2H, major), 1.36 (d, *J* = 6.0 Hz, 3H), 1.27 (d, *J* = 5.9 Hz, 3H), 1.26–1.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 146.8, 143.6, 143.4, 131.4, 125.1, 123.3, 120.3, 118.30, 118.26, 101.1, 69.6, 66.4, 61.4, 55.9, 44.9, 31.5, 24.6, 23.7, 22.3, 21.0, 12.7; HRMS (ESI+) calcd for $C_{22}H_{29}N_2O_2$ $[M+H]^+$ 353.2224, found 353.2220 (error 1.1 ppm); $[\alpha]_D^{23} = -46.1$ (*c* 0.300, $CHCl_3$).

6-Allyloxy-(*R*)-4-[(1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (44). Compound **44** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and allyl bromide (33 μL, 0.387 mmol) to afford the title compound (98 mg, 87%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a white solid: HPLC purity 96.8% (two isomers, 17.6% and 79.2%), t_R = 22.5 and 23.5 min; mp 177–179 °C; R_f = 0.50 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.4 Hz, 1H), 7.71 (d, *J* = 9.2 Hz, 1H), 7.63 (d, *J* = 4.4 Hz, 1H), 7.04 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.87 (d, *J* =

2.5 Hz, 1H), 6.56 (s, 1H), 6.10–6.03 (m, 1H), 5.45 (d, J = 17.2 Hz, 1H), 5.42 (q, J = 6.2 Hz, 0.75H, major), 5.34 (q, J = 6.2 Hz, 0.25H, minor), 5.22 (d, J = 10.7 Hz, 1H), 4.65–4.60 (m, 1H), 4.45 (q, J = 17.5 Hz, 2H), 3.82 (q, J = 16.2 Hz, 2H), 3.35 (t, J = 9.1 Hz, 1H), 3.26–3.10 (m, 1H), 2.56 (s, 1H), 2.36–2.13 (m, 2H), 1.90–1.82 (m, 1H), 1.53 (d, J = 6.8 Hz, 0.8H, minor), 1.46 (d, J = 6.8 Hz, 2.2H, major), 1.25–1.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 147.1, 143.8, 143.6, 133.3, 131.5, 130.2, 125.1, 122.5, 120.5, 118.6, 116.3, 100.5, 69.6, 66.2, 61.2, 56.0, 45.1, 31.5, 24.5, 23.8, 12.8; HRMS (ESI+) calcd for $C_{22}H_{27}N_2O_2$ $[M+H]^+$ 351.2067, found 351.2660 (error 2.0 ppm); $[\alpha]_D^{23}$ = – 7.8 (*c* 0.30, $CHCl_3$).

(*R*)-4-((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-[(2*R*/*S*)-2-hydroxy-1-(methyl)ethoxy]quinoline (45). Compound **45** was prepared following the general procedure for C-6 phenol alkylation employing **39** (82 mg, 0.26 mmol) and 2-iodopropan-1-ol (93 mg, 0.32 mmol) to afford the title compound (60 mg, 62%, HPLC peak area indicates 0.2:1:0.4:1 diastereomeric ratio) as a white solid: HPLC purity (four isomers, 7.2%, 36.6%, 16.0% and 39.5%; total 99.3%), t_R = 17.2, 17.7, 18.7 and 19.2 min; mp 197–199 °C; R_f = 0.34 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 4.5 Hz, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.58–7.53 (m, 1H), 7.33–7.26 (m, 3H), 5.93 (br s, 0.4H, minor), 5.83 (br s, 0.6H, major), 5.23–4.92 (m, 1H), 4.65 (q, J = 5.8 Hz, 1H), 3.96–3.80 (m, 1H), 3.74–3.60 (m, 1H), 3.56–3.30 (m, 1H), 3.32–3.26 (m, 1H), 3.23–2.92 (m, 1H), 2.89–2.64 (m, 1H), 2.34 (d, J = 7.1 Hz, 1H), 2.17–1.92 (m, 1H), 1.93–1.73 (m, 1H), 1.65–1.50 (m, 1H), 1.53–1.29 (m, 6H), 1.25–1.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 and 156.2, 147.69 and 147.65, 147.5 and 147.1, 144.1 and 144.0, 131.8 and 131.7, 126.1, 123.0 and 122.5, 118.27 and 118.22, 118.18, 116.1 and 115.7, 103.4 and 103.1, 75.4 and 75.3, 70.8 and 70.3, 65.6 and 65.3, 60.63 and 60.59, 60.2 and 58.8, 56.5 and 56.3, 44.3 and 44.2, 33.0 and 32.8, 27.1 and 27.0, 26.2 and 25.9, 12.4 and 12.3;

HRMS (ESI+) calcd for $C_{22}H_{29}N_2O_3$ $[M+H]^+$ 369.2173, found 369.2160 (error 3.4 ppm); $[\alpha]_D^{23} = -159$ (*c* 0.400, $CHCl_3$).

6-Butoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (46). Compound **46** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and 1-bromobutane (42 μL, 0.39 mmol) to afford the title compound (103 mg, 87%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =C**H**-**C**H_3) as a white solid: HPLC purity 96.9% (two isomers, 30.2% and 66.7%), t_R = 18.8 and 19.3 min; mp 180–181 °C; R_f = 0.53 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 4.5 Hz, 1H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.60 (d, *J* = 4.5 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.87 (s, 1H), 6.32 (br s, 1H), 5.34 (q, *J* = 6.9 Hz, 0.75H, major), 5.30 (q, *J* = 6.9 Hz, 0.25H, minor), 4.55–4.41 (m, 1H), 3.97–3.58 (m, 4H), 3.30 (t, *J* = 8.8 Hz, 1H), 3.15–3.00 (m, 1H), 2.51 (s, 1H), 2.31–2.04 (m, 2H), 1.87–1.67 (m, 3H), 1.58–1.49 (m, 2H), 1.45 (d, *J* = 6.8 Hz, 3H), 1.30–1.19 (m, 2H), 1.00 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 146.9, 144.5, 143.7, 131.3, 125.4, 122.3, 119.2, 118.43, 118.37, 100.2, 68.9, 61.0, 60.7, 56.1, 44.8, 31.9, 31.3, 29.7, 24.9, 19.5, 14.2, 12.7; HRMS (ESI+) calcd for $C_{23}H_{31}N_2O_2$ $[M+H]^+$ 367.2380, found 367.2375 (error 1.3 ppm); $[\alpha]_D^{23} = -76.2$ (*c* 0.300, $CHCl_3$).

6-Cyclopropylmethoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (47). Compound **47** was prepared following the general procedure for C-6 phenol alkylation employing **39** (85 mg, 0.27 mmol) and cyclopropylmethyl bromide (32 μL, 0.33 mmol) to afford the title compound (78 mg, 78%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =C**H**-**C**H_3) as a white solid: HPLC purity 96.1% (two isomers, 23.1% and 73.0%), t_R = 20.5 and 21.5 min; mp 183–185 °C; R_f = 0.50 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.4 Hz, 0.75H, major), 8.65 (d, *J* = 4.4 Hz, 0.25H, minor), 7.92 (d, *J* = 9.2, 0.75H, major), 7.91 (d, *J* = 9.2 Hz, 0.25H, minor), 7.52 (d, *J* = 4.4 Hz, 1H), 7.29 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.14 (d, *J* =

2.5 Hz, 0.75H, major), 7.10 (d, *J* = 2.5 Hz, 0.25H, minor), 5.78 (s, 1H), 5.22 (q, *J* = 6.7 Hz, 0.75H, major), 5.13 (q, *J* = 6.7 Hz, 0.25H, minor), 3.94–3.80 (m, 1H), 3.77–3.69 (m, 2H), 3.78–3.70 (m, 2H), 3.21–3.05 (m, 1H), 2.88–2.77 (m, 1H), 2.38 (s, 1H), 2.10–1.92 (m, 1H), 1.83 (t, *J* = 10.7 Hz, 1H), 1.69–1.57 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 0.8H), 1.44 (d, *J* = 6.8 Hz, 2.2H), 1.43–1.23 (m, 2H), 0.64 (q, *J* = 8.0 Hz, 2H), 0.38 (q, *J* = 5.0, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 147.5, 146.6, 144.1, 131.6, 126.3, 122.1, 118.49, 118.45, 115.8, 101.7 and 101.6, 73.3 and 73.2, 70.9, 60.8 and 60.4, 58.8 and 56.6, 44.2, 33.0, 27.2, 25.7, 12.7 and 12.5, 10.2, 3.32, 3.26; HRMS (ESI+) calcd for $C_{23}H_{29}N_2O_2$ $[M+H]^+$ 365.2224, found 365.2211 (error 3.4 ppm); $[\alpha]_D^{23} = -129$ (*c* 0.500, $CHCl_3$).

6-Cyclobutoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (48). Compound **48** was prepared following the general procedure for C-6 phenol alkylation employing **39** (85 mg, 0.27 mmol) and bromocyclobutane (31 μL, 0.33 mmol) to afford the title compound (79 mg, 79%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of =CH-C**H$_3$**) as a white solid: HPLC purity 98.2% (two isomers, 33.1% and 65.1%), t_R = 22.3 and 23.2 min; mp 179–183 °C; R_f = 0.52 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72–8.65 (m, 1H), 7.92 (d, *J* = 9.2 Hz, 0.75H, major), 7.91 (d, *J* = 9.2 Hz, 0.25H, minor), 7.56 (d, *J* = 4.5 Hz, 1H), 7.22–7.16 (m, 1H), 7.01 (s, 0.75H, major), 6.97 (s, 0.25H, minor), 6.10–5.79 (m, 1H), 5.24 (q, *J* = 6.8 Hz, 0.75H, major), 5.18 (q, *J* = 6.8 Hz, 0.25H, minor), 4.70–4.57 (m, 1H), 3.93 (br s, 1H), 3.54 (q, *J* = 17.2 Hz, 2H), 3.18 (t, *J* = 9.9 Hz, 1H), 2.97–2.76 (m, 1H), 2.59–2.38 (m, 3H), 2.15–2.06 (m, 3H), 1.95–1.85 (m, 1H), 1.82–1.62 (m, 3H), 1.52 (d, *J* = 7.6 Hz, 0.8H, minor), 1.44 (d, *J* = 7.6 Hz, 2.2H, major), 1.40–1.30 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 147.4, 146.1, 144.07 and 144.04, 131.7, 126.2, 122.2, 118.52, 118.49, 116.6, 102.1 and 102.0, 71.5, 70.2, 60.9 and 60.5, 58.7, 56.5, 44.4, 32.8, 30.5 and 30.1, 26.8, 25.6, 13.4, 12.7 and 12.5; HRMS (ESI+) calcd for $C_{23}H_{29}N_2O_2$ $[M+H]^+$ 365.2224, found 365.2218 (error 1.5 ppm); $[\alpha]_D^{23} = -85.0$ (*c* 0.400, $CHCl_3$).

6-Cyclobutoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (*Z*-48) and 6-Cyclobutoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (*E*-48). The mixture of diastereomeric ***Z*-48** and ***E*-48** (500 mg) was separated by preparative reverse-phase on an Ultimate XB 10 μm C18 250 × 50 mm column employing Method A to afford ***Z*-48** (122 mg) and ***E*-48** (51 mg).

***Z*-48:** HPLC purity 98.4%, t_R = 22.3 min; mp 181–183 °C; R_f = 0.52 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 4.5 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.48 (d, *J* = 4.5 Hz, 1H), 7.39–7.13 (m, 1H), 7.08 (d, *J* = 2.7 Hz, 1H), 5.60 (d, *J* = 4.0 Hz, 1H), 5.15 (q, *J* = 6.6 Hz, 1H), 4.65 (quint, *J* = 7.1 Hz, 1H), 3.60–3.50 (m, 1H), 3.49–3.29 (m, 2H), 3.07 (td, *J* = 9.1, 3.9 Hz, 1H), 2.83–2.60 (m, 1H), 2.59–2.35 (m, 3H), 2.32 (q, *J* = 2.4 Hz, 1H), 2.25–2.02 (m, 2H), 1.98–1.78 (m, 2H), 1.79–1.62 (m, 2H), 1.62–1.51 (m, 1H), 1.42 (dd, *J* = 6.7, 1.6 Hz, 3H), 1.38–1.32 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 147.5, 147.4, 144.1, 140.9, 131.6, 126.5, 121.9, 118.5, 114.7, 102.8, 72.2, 71.7, 60.7, 56.8, 44.1, 33.4, 30.6, 30.2, 28.1, 27.9, 13.4, 12.4; NOE ^{1}H NMR (400 MHz, $CDCl_3$) δ irradiate 5.15 (enhancements at 2.32, 1.42); irradiate 3.35 (enhancements at 3.07, 2.71, 1.42); irradiate 1.42 (enhancements at 5.15, 3.35); HRMS (ESI+) calcd for $C_{23}H_{29}N_2O_2$ $[M+H]^+$ 365.2224, found 365.2215 (error 2.3 ppm); $[\alpha]_D^{23}$ = – 178 (*c* 0.900, $CHCl_3$).

***E*-48:** HPLC purity 98.9%, t_R = 23.2 min; mp 185–186 °C; R_f = 0.52 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.5 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 4.5 Hz, 1H), 7.27 (d, *J* = 6.8 Hz, 1H), 7.07 (d, *J* = 2.7 Hz, 1H), 5.63 (d, *J* = 4.1 Hz, 1H), 5.10 (q, *J* = 6.6 Hz, 1H), 4.66 (quint, *J* = 7.1 Hz, 1H), 3.75–3.45 (m, 1H), 3.46–3.24 (m, 2H), 3.09 (td, *J* = 9.1, 4.0 Hz, 1H), 2.87–2.64 (m, 2H), 2.50–2.40 (m, 2H), 2.29–2.06 (m, 2H), 1.95–1.80 (m, 2H), 1.79–1.57 (m, 2H), 1.57–1.52 (m, 1H), 1.51 (dd, *J* = 6.9, 2.0 Hz, 3H), 1.34 (td, *J* = 9.6, 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 147.6, 147.2, 144.1, 139.9, 131.7, 126.5, 122.0, 118.4, 114.4, 102.7, 72.2, 71.8, 60.3, 59.3, 44.2, 30.6,

30.2, 27.2, 26.5, 26.0, 13.4, 12.7; NOE ^{1}H NMR (400 MHz, $CDCl_3$) δ irradiate 5.10 (enhancements at 3.35, 1.51); irradiate 3.35 (enhancements at 5.10, 3.09, 2.76); irradiate 1.51 (enhancements at 5.10, 2.76, 1.55); HRMS (ESI+) calcd for $C_{23}H_{29}N_2O_2$ $[M+H]^+$ 365.2224, found 365.2217 (error 1.9 ppm); $[\alpha]_D^{23}$ = – 202 (*c* 0.300, $CHCl_3$).

6-Cyclopentoxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (49). Compound **49** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and bromocyclopentane (41 μL, 0.39 mmol) to afford the title compound (108 mg, 89%, ^{1}H NMR indicates 4:1 diastereomeric ratio through the integral value of =C**H**-CH_3) as a white solid: HPLC purity 98.5% (two isomers, 21.0% and 77.5%), t_R = 25.8 and 27.1 min; mp 186–187 °C; R_f = 0.53 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 4.4 Hz, 1H), 7.94 (d, *J* = 9.1 Hz, 1H), 7.54 (d, *J* = 4.4 Hz, 1H), 7.28–7.21 (m, 1H), 7.15 (s, 0.8H, major), 7.12 (s, 0.2H, minor), 5.79 (s, 1H), 5.20 (d, *J* = 7.0 Hz, 0.8H, major), 5.14 (d, *J* = 7.0 Hz, 0.2H, minor), 4.78 (d, *J* = 7.4 Hz, 1H), 3.78–3.72 (m, 1H), 3.59–3.38 (m, 2H), 3.17 (t, *J* = 5.2 Hz, 1H), 2.88–2.80 (m, 1H), 2.37 (s, 1H), 2.08–1.68 (m, 8H), 1.64–1.55 (m, 3H), 1.52 (d, *J* = 6.8 Hz, 0.6H), 1.44 (d, *J* = 6.8 Hz, 2.4H), 1.36 (t, *J* = 11.6 Hz, 1H), 1.28–1.20 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 147.4, 146.6, 144.0, 131.6, 126.3, 122.7, 118.4, 115.7, 102.9, 102.8, 79.5, 71.3, 60.8, 56.7, 44.2, 33.1, 32.9, 32.5, 29.7, 27.5, 27.3, 24.1, 12.4; HRMS (ESI+) calcd for $C_{24}H_{31}N_2O_2$ $[M+H]^+$ 379.2380, found 379.2369 (error 2.8 ppm); $[\alpha]_D^{23}$ = –126 (*c* 0.700, $CHCl_3$).

6-Benzyloxy-(*R*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl]quinoline (50). Compound **50** was prepared following the general procedure for C-6 phenol alkylation employing **39** (100 mg, 0.322 mmol) and benzyl bromide (46 μL, 0.39 mmol) to afford the title compound (105 mg, 81%, ^{1}H NMR indicates 3:1 diastereomeric ratio through the integral value of quinioline H-5′) as a white solid: HPLC purity 98.6% (two isomers, 11.2% and 87.4%), t_R =

24.6 and 24.9 min; mp 197–199 °C; R_f = 0.49 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 4.4 Hz, 1H), 7.74 (d, *J* = 9.2 Hz, 1H), 7.64–7.52 (m, 3H), 7.43–7.35 (m, 2H), 7.30 (d, *J* = 4.4 Hz, 1H), 7.12 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.03 (s, 0.8H, major), 7.00 (s, 0.2H, minor), 6.62 (s, 1H), 5.33 (q, *J* = 6.9 Hz, 0.8H, major), 5.26 (q, *J* = 6.9 Hz, 0.2H, minor), 5.16 (d, *J* = 13.0 Hz, 1H), 4.96 (d, *J* = 13.0 Hz, 1H), 4.63 (t, *J* = 7.5 Hz, 1H), 3.72 (q, *J* = 16.1 Hz, 2H), 3.31 (t, *J* = 9.3 Hz, 1H), 3.22–3.14 (m, 1H), 2.54 (s, 1H), 2.37–2.08 (m, 2H), 1.90–1.81 (m, 1H), 1.51 (d, *J* = 6.8 Hz, 0.6H, minor), 1.45 (d, *J* = 6.8 Hz, 2.4H, major), 1.25–1.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 147.2, 143.9, 143.7, 137.2, 131.6, 128.3, 127.4, 127.04, 126.98, 125.1, 122.7, 120.4, 118.6, 100.5, 70.6, 66.1, 61.2, 56.0, 45.2, 31.5, 24.6, 23.8, 12.9; HRMS (ESI+) calcd for $C_{26}H_{29}N_2O_2$ $[M+H]^+$ 401.2224, found 401.2216 (error 1.9 ppm); $[\alpha]_D^{23}$ = –182 (*c* 0.200, $CHCl_3$).

(*S*)-4-((1*S*,2*R*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)hydroxymethyl-6-methoxyquinoline (52). Compound **52** was prepared following the general procedure for olefin migration employing quinidine **51** (232 mg, 0.715 mmol) to afford the title compound (205 mg, 88%, ^{1}H NMR indicates 4:1 diastereomeric ratio through the integral value of =C**H**-**C**H_3) as a white solid: HPLC purity 99.4% (two isomers, 21.6% and 77.8%), t_R = 17.5 and 18.0 min; mp 199–200 °C, lit. mp 178–181 °C[68]; R_f = 0.50 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 4.5 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.48 (d, *J* = 4.5 Hz, 1H), 7.28 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.20 (d, *J* = 2.7 Hz, 1H), 5.66 (t, *J* = 5.3 Hz, 1H), 5.26–5.19 (m, 0.8H, major), 5.16–5.11 (m, 0.2H, minor), 4.13 (d, *J* = 17.2 Hz, 1H), 3.84 (s, 3H), 3.31 (d, *J* = 17.2 Hz, 1H), 3.20 (td, *J* = 8.9, 4.0 Hz, 1H), 2.91 (ddt, *J* = 12.7, 5.8, 3.0 Hz, 1H), 2.76 (ddd, *J* = 13.2, 9.9, 7.5 Hz, 2H), 2.42–2.24 (m, 1H), 2.06–1.83 (m, 1H), 1.58 (ddd, *J* = 10.5, 5.8, 2.3 Hz, 2H), 1.50 (d, *J* = 6.8 Hz, 3H), 1.42 (td, *J* = 9.3, 4.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 147.6, 147.5, 144.2, 141.2, 131.6, 126.5, 121.6, 118.5, 113.6, 101.2, 71.8, 59.6, 55.3, 52.4 and 51.3, 51.0 and 50.1, 33.4, 27.5, 27.2 and 26.2, 12.8 and 12.4; HRMS (ESI+) calcd for $C_{20}H_{25}N_2O_2$

$[M+H]^+$ 325.1911, found 325.1896 (error 4.6 ppm); $[\alpha]_D^{23}$ = +15.1 (*c* 0.850, $CHCl_3$) lit $[\alpha]_D^{25}$= +178.25 (*c* 0.40, EtOH)[68]. All NMR data were in agreement with the literature values.[68]

4-((2*R*,3*S*,5*R*,6*R*)-3-Fluoro-6-vinyl-1-azabicyclo[3.2.2]nonan-2-yl)-6-methoxyquinoline (54). DAST (367 μL, 3.0 mmol, 1.5 equiv) was added dropwise to a suspension of quinine **3** (648 mg, 2.0 mmol, 1.0 equiv) in THF (4 mL) at –20 °C under an atmosphere of argon. The mixture was stirred 14 h at –20 °C, then quenched with saturated aqueous $NaHCO_3$ solution (15 mL) and extracted with CH_2Cl_2 (3×15 mL). The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (10:1 CH_2Cl_2–MeOH) afforded the fluorinated diastereomers **53** (332 mg, 51%) and compound **54** (71 mg, 11%) as white solids. Data for **54**: HPLC purity 97.8%, t_R = 25.7 min; mp 182–183 °C; R_f = 0.55 (10:1 CH_2Cl_2–MeOH); IR (KBr) γ_{max} 2939, 1620, 1506, 1225, 1022, 839; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 4.6 Hz, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 7.47–7.31 (m, 2H), 7.31–7.23 (m, 1H), 5.94 (ddd, *J* = 17.0, 10.5, 6.3 Hz, 1H), 5.55–5.29 (m, 1H), 5.21–4.98 (m, 2H), 4.47 (t, *J* = 9.6 Hz, 1H), 3.96 (s, 3H), 3.43 (dd, *J* = 8.7, 4.8 Hz, 1H), 3.24–3.08 (m, 1H), 2.93 (dd, *J* = 15.0, 10.1 Hz, 1H), 2.87–2.63 (m, 2H), 2.46 (d, *J* = 5.7 Hz, 1H), 2.20 (ddt, *J* = 7.5, 5.6, 3.9 Hz, 1H), 2.13–1.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 147.5, 144.9 (d, *J* = 38.3 Hz), 139.9, 131.8, 128.7, 121.2, 117.5, 115.2, 102.5, 88.4 (d, *J* = 179.2 Hz), 77.3, 65.4 (d, *J* = 21.3 Hz), 55.5, 49.1, 47.4, 40.7, 37.1 (d, *J* = 18.6 Hz), 30.8, 29.0 (d, *J* = 10.6 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ –176.3 (dt, *J* = 47.5, 8.4 Hz); HRMS (ESI+) calcd for $C_{20}H_{24}N_2OF$ $[M+H]^+$ 327.1867, found 327.1856 (error 3.3 ppm); $[\alpha]_D^{23}$ = –110 (*c* 0.400, $CHCl_3$).

6-Methoxy-(*S*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-vinyl-1-azabicyclo[2.2.2]octan-2-yl)fluoromethyl]quinoline (53) and 6-Methoxy-(*Z/E*)-4-[((1*S*,4*S*,5*R*)-5-vinyl-1-azabicyclo[2.2.2]octan-2-ylidene)methyl]quinoline (55). Compounds **53** and **55** were prepared following the general procedure for C-9 fluorination employing quinine **3** (108 mg, 0.333 mmol), DAST (64 μL, 0.50 mmol), and

pyridine (54 μL, 0.67 mmol) to afford **53** (53 mg, 49%) and **55** (33 mg, 33%, ^{1}H NMR indicates 9:1 diastereomeric ratio through the integral value of –OC**H$_3$** protons) as light yellow solids.

Data for 53: HPLC purity 97.1%, t_R = 20.6 min; mp 189–191 °C; R_f = 0.39 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 4.4 Hz, 1H), 8.06 (d, *J* = 9.6 Hz, 1H), 7.45–7.34 (m, 3H), 5.86 (dd, *J* = 48.6, 9.4 Hz, 1H), 5.77 (ddd, *J* = 17.4, 10.4, 7.3 Hz, 1H), 5.03–4.96 (m, 2H), 3.95 (s, 3H), 3.63–3.42 (m, 1H), 3.38–3.19 (m, 2H), 2.90 (dt, *J* = 15.1, 7.8 Hz, 1H), 2.83–2.73 (m, 1H), 2.41–2.19 (m, 1H), 1.73 (dt, *J* = 6.1, 3.0 Hz, 1H), 1.62 (td, *J* = 7.8, 2.9 Hz, 2H), 1.41 (t, *J* = 9.8 Hz, 1H), 1.01–0.86 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 147.4, 144.9, 141.4, 140.4 (d, *J* = 18.5 Hz), 132.0, 127.0 (d, *J* = 2.7 Hz), 121.9, 120.2 (d, *J* = 6.7 Hz), 114.7, 101.8 (d, *J* = 3.1 Hz), 91.4 (d, *J* = 179.7 Hz), 59.2 (d, *J* = 19.7 Hz), 55.9, 55.7, 41.5, 39.5, 27.9, 27.2, 24.4 (d, *J* = 4.6 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ –176.3 (dd, *J* = 48.6, 13.4 Hz); HRMS (ESI+) calcd for $C_{20}H_{24}N_2OF$ $[M+H]^+$ 327.1867, found 327.1865 (error 0.6 ppm); $[\alpha]_D^{23}$ = +78 (*c* 0.100, $CHCl_3$)

Data for 55: HPLC purity 97.0% (two isomers, 10.9% and 86.0%), t_R = 24.6 and 24.8 min; mp 175–176 °C; R_f = 0.65 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 4.6 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.81 (d, *J* = 4.6 Hz, 1H), 7.33 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.27–7.20 (m, 1H), 6.42 (s, 1H), 6.05–5.80 (m, 1H), 5.18–4.93 (m, 2H), 3.95 (s, 2.7H, major), 3.94 (s, 0.3H, minor), 3.28 (dd, *J* = 13.8, 9.2 Hz, 1H), 3.11–2.88 (m, 2H), 2.80–2.64 (m, 2H), 2.47–2.32 (m, 2H), 2.07–1.91 (m, 1H), 1.82–1.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 154.4, 147.9, 144.7, 140.9, 139.8, 131.5, 127.7, 121.6, 120.9, 114.8, 114.5, 102.2, 55.6, 53.8, 47.9, 39.7, 30.6, 29.7, 27.3; HRMS (ESI+) calcd for $C_{20}H_{23}N_2O$ $[M+H]^+$ 307.1805, found 307.1794 (error 3.5 ppm); $[\alpha]_D^{23}$ = –128 (*c* 0.100, $CHCl_3$).

(*S*)-4-[((1*S*,2*S*,4*S*,5*R*)-5-Ethyl-1-azabicyclo[2.2.2]octan-2-yl)fluoromethyl]-6-methoxyquinoline (56). Compound **56** was prepared following the general procedure for C-9 fluorination employing compound **4** (163 mg, 0.522 mmol), DAST (102 μL, 0.792 mmol) and pyridine (85 μL, 1.1 mmol) to

afford the title compound (83 mg, 50%) as a light yellow solid: HPLC purity 98.8%, t_R = 23.1 min; mp 192–194 °C; R_f = 0.38 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 4.3 Hz, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 7.44–7.39 (m, 3H), 5.97 (dd, *J* = 48.4, 9.4 Hz, 1H), 3.97 (s, 3H), 3.74–3.53 (m, 1H), 3.53–3.33 (m, 2H), 3.04 (t, *J* = 7.8 Hz, 1H), 2.62 (dd, *J* = 13.4, 6.0 Hz, 1H), 1.78–1.46 (m, 4H), 1.40 (q, *J* = 7.2 Hz, 1H), 1.38–1.22 (m, 5H), 1.02–0.93 (m, 1H), 0.83 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 147.3, 145.0, 138.6 (d, *J* = 17.5 Hz), 132.0, 127.0, 122.4, 120.6 (d, *J* = 6.5 Hz), 101.7 (d, *J* = 3.2 Hz), 90.6 (d, *J* = 181.0 Hz), 59.5 (d, *J* = 20.1 Hz), 56.8, 56.1, 41.7, 36.1, 29.7, 27.0, 24.5, 23.1, 11.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ –175.9 (d, *J* = 48.4 Hz); HRMS (ESI+) calcd for $C_{20}H_{26}N_2OF$ $[M+H]^+$ 329.2024, found 329.2028 (error 1.9 ppm); $[\alpha]_D^{23}$ = +21.9 (*c* 0.700, $CHCl_3$).

(*S*)-4-[((1*S*,2*S*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)fluoromethyl]-6-methoxyquinoline (57). Compound **57** was prepared following the general procedure for C-9 fluorination employing compound **15** (195 mg, 0.601 mmol), DAST (0.12 mL, 0.90 mmol) and pyridine (97 μL, 1.2 mmol) to afford the title compound (75 mg, 38%, ^{19}F NMR indicates 3:1 diastereomeric ratio through the integral value of CH-**F**) as a light yellow solid: HPLC purity 98.9% (two isomers, 59.1% and 39.8%), t_R = 27.5 and 28.1 min; mp 190–191 °C; R_f = 0.39 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77–8.74 (m, 1H), 8.06–8.00 (m, 1H), 7.44–7.35 (m, 3H), 5.89 (dd, *J* = 48.3, 9.1 Hz, 1H), 5.20–5.12 (m, 1H), 3.93 (s, 3H), 3.58–3.50 (m, 3H), 3.38–3.30 (m, 1H), 2.93 (dt, *J* = 14.9, 7.2 Hz, 1H), 2.24 (s, 1H), 1.67 (t, *J* = 6.9 Hz, 2H), 1.48 (d, *J* = 5.0 Hz, 3H), 1.37–1.09 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 147.3, 144.9, 140.64 and 140.58, 140.45 and 140.39, 132.0, 126.9 (d, *J* = 2.2 Hz), 121.82 and 121.80, 120.1 (d, *J* = 6.7 Hz), 115.2 and 114.7, 102.0 (d, *J* = 3.1 Hz), 91.4 (d, *J* = 179.7 Hz), 60.1 (d, *J* = 19.5 Hz) and 59.9 (d, *J* = 19.5 Hz), 55.9 and 55.6, 42.1, 32.8 and 31.9, 31.01 and 30.97, 30.16 and 30.12, 28.3, 12.7 and 12.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ –176.2 and –176.4 (dd, *J* = 48.3,

13.4 Hz); HRMS (ESI+) calcd for $C_{20}H_{24}N_2OF$ $[M+H]^+$ 327.1867, found 327.1855 (error 3.7 ppm); $[\alpha]_D^{23}$ = +5.1 (*c* 0.30, $CHCl_3$).

(*S*)-4-[((1*S*,2*R*,4*S*,5*Z/E*)-5-Ethylidene-1-azabicyclo[2.2.2]octan-2-yl)fluoromethyl]-6-methoxyquinoline (58). Compound **58** was prepared following the general procedure for C-9 fluorination employing quinidine **51** (181 mg, 0.558 mmol), DAST (0.11 mL, 0.84 mmol), and pyridine (90 μL, 1.1 mmol) to afford the title compound (66 mg, 36%, ^{1}H NMR indicates 5:1 diastereomeric ratio through the integral value of C**H**-F protons) as a light yellow solid: HPLC purity 97.6% (two isomers, 18.5% and 79.1%), t_R = 19.4 and 20.1 min; mp 192–193 °C; R_f = 0.35 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75–8.72 (m, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 7.42–7.30 (m, 3H), 5.91 (dd, *J* = 48.4, 9.6 Hz, 0.17H, minor), 5.90 (dd, *J* = 48.4, 9.6 Hz, 0.83H), 5.26 (q, *J* = 3.5 Hz, 1H), 4.03 (d, *J* = 17.2 Hz, 1H), 3.91 (s, 3H), 3.73 (d, *J* = 9.6 Hz, 1H), 3.57 (d, *J* = 17.2 Hz, 1H), 3.32–2.93 (m, 2H), 2.28 (q, *J* = 2.6 Hz, 1H), 1.66 (ddt, *J* = 9.6, 7.0, 3.1 Hz, 2H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.42–1.35 (m, 1H), 1.30–1.19 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 147.2, 145.0, 136.9, 132.1, 126.8, 122.8, 122.6, 120.7 (d, *J* = 6.1 Hz), 119.6, 101.3, 89.2 (d, *J* = 184 Hz), 59.6 (d, *J* = 20.6 Hz), 56.3, 49.7, 47.8, 31.1, 28.4, 24.3, 12.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ –174.7 (d, *J* = 48.4 Hz); HRMS (ESI+) calcd for $C_{20}H_{24}N_2OF$ $[M+H]^+$ 327.1867, found 327.1855 (error 3.8 ppm); $[\alpha]_D^{23}$ = +36.1 (*c* 0.400, $CHCl_3$).

General procedure for bacterial cultivation. Before being used for antibacterial activities of optochin and its derivatives, *S. pneumoniae*, *S. mitis*, *S. pyogenes, E. faecium* were grown to mid-log phase in Todd-Hewitt broth (BD) supplemented with 5% yeast extract (THY) at 37 °C with 5% CO_2; *A. baumannii, E. cloacae*, *E. coli, P. aeruginosa, S. aureus,* and *K. pneumoniae* in Luria broth; *M. tuberculosis* in 30-mL square bottles (Nalgene) containing 7H9 medium

supplemented with tyloxapol (0.05% vol/vol), oleate-albumin-dextrose-catalase (OADC; Becton Dickinson 10% vol/vol), and glycerol (0.2% vol/vol).

MIC. The minimum inhibitory concentration (MIC) for each compound was experimentally measured through a microdilution method according to guidelines described by the Clinical and Laboratory Standards Institute (CLSI). Compounds were dissolved in DMSO at 20 mg/mL and serially diluted with MHB in 96 well plates affording a final concentation of 0.5% DMSO in all wells in a total volume of 100 μL. The bacterial culture prepared above (100 μL) was subsequently added to the plates, which were incubated for 20 h at 37 °C and growth of the bacterial culture was visually detected by evaluating the culture turbidity. The MIC was defined as the lowest concentration of each compound that completely inhibit bacterial growth. All the MIC measurements were performed twice with three replicates per experiment. Antimycobacterial activity was assessed as described.[69] Briefly, cells were inoculated into each well containing 7H9 medium (100 μL/well) to an initial OD_{600} of 0.001. Plates were incubated at 37 °C, and growth was monitored at 7 and 10 days. The MIC was determined as the minimum concentration of compound required to inhibit 90% of growth by visual inspection.

MBC. Minimum bactericidal concentration (MBC) was measured by spreading each well of the bacterial cultures at or above MIC on TSA blood plates. The plates were incubated at 37 °C with 5% CO_2 for 24 h. The MBC was defined as the lowest concentration of each compound that prevented growth of bacteria on the agar plate.

Generation of optochin-resistant mutants. Optochin resistant strains were constructed using *S. pneumoniae* R6 as decribed.[18] Point mutations in *atpE* were prepared by substituting G for C at position 142 or substituting G for A at position 145. These two mutations conferred optochin resistance, which has already been reported.[18] For constucting $R6_{G142C}$ mutant, the up- and down-stream sequences of *atpE* were amplified using R6 genomic DNA and primer pairs Pr13331/Pr13332 and Pr13336/Pr13338,

respectively. The two products were then fused by overlap PCR before being used to transform R6 as described.[70] The transformants were selected on TSA plates containing 12.5 μg/mL optochin. The $R6_{G145A}$ mutant was generated with the same procedure except using primer pairs Pr13331/Pr13333 and Pr13335/Pr13338. Sequences for primer pairs: Pr13331:ttcttcagaacgacctagcttagca; Pr13332: agtttgatgtttttaggtCttgcctttattgaaggaactttctttgtaact; Pr13333: agtttgatgtttttaggtgttAcctttattgaaggaactttctttgtaact; Pr13335: aagttccttcaataaaggTaacacctaaaaacatcaaactacgaaactcag; Pr13336: aagttccttcaataaaggcaaGacctaaaaacatcaaactacgaaactcag; Pr13338: tacaatcgtttgaatcgtgatatgc.

Frequency of Resistance Determination. The frequency of resistance (FOR) was measured as described.[1871] Briefly, the mid-log phase cultures of *S. pneumoniae* D39 (OD_{620} 0.4-0.5) were spread to MHA blood plates containing various concentrations of target compounds. The plates were incubated at 37°C for 24 h before the colony forming units (CFU) were counted. The FOR was obtained by dividing CFU values of compound-containing samples with those of the samples lacking the same compounds. The *atpE* mutations of representative mutants were characterized by cultivating individual colonies from the agar plates for FOR in THY broth that was supplemented with the same concentrations of target compounds as the original agar plates, and used to purified genomic DNA. The *atpE* gene of each clone was amplified by polymerase chain reaction (PCR) with primers Pr13358 and Pr13631, and sequenced with the same primers. Specific *atpE* mutations were identified by comparing the sequence of each mutant with that of the wild type D39 (NC_008533.2). The sequence of the two primers is as followed: Pr13358: 5'-aatgttaggttctgtaaatccaataacaaa-3'; Pr13631: 5'-ttgaaatttagcaaaatggaaataatttt-3'.

Intrabacterial pH Determination. The pH-sensitive green fluorescent protein gene (pH-GFP) was amplified from the pUV15-pHGFP plasmid kindly provided by Dr. Carl Nathan[40] using primers Pr14798 5'-GAGAAAGCTTGATATCACCTTATTTGTATAGTTCA-3' and Pr14799 5'-

GAGACTCGAGATGAGTAAAGGAGAAGAACTTTTCACTG-3'. The amplicon was digested with HindIII and XhoI, and ligated to the HindIII/XhoI-digested pIB166 plasmid[72] and transformed into *S. pneumoniae* D39 and its optochin-resistant derivatives. The optochin-resistant mutants of D39 (D39-*atpE*$_{G142C}$ and D39-*atpE*$_{G145A}$) were generated as described for constrution of R6-*atpE*$_{G142C}$ and R6-*atpE*$_{G145A}$ mutants. Compound **48** was serially diluted 2-fold in a black 96-well plate from 8 μg/mL down 0.0078 μg/mL). CCCP and DMSO were used as positive and negative controls, respectively. All compounds were dissolved in Resuspending buffer (PBS pH 7.2 supplemented with 10 mM glucose and 0.5% DMSO). The pellet of mid-log phase cells was resuspended in Resuspending buffer, then were energized at 37 ˚C and added to the compound dilutions (the final cell density was OD_{620} of 0.4) in a total volume of 100 μL. The GFP fluorescence was measured immediatedly (0 min) and at 5-min intervals. The fluorescence values were converted to the pH values in reference to the calibration curve (Supporting Information Figure S1). Two independent biological experiments were performed in triplicate.

Checkerboard assay. Synergy was evaluated using a checkerboard assay as described.[73] The optochin analog **48** was serially diluted in MHB to a final volume of 100 μL in 96-well plates in a lateral manner, with the highest (2 × MIC) and lowest (no compound) concentrations in the first and last columns, respectively. The second drugs were then diluted in a vertical fashion with the highest (2 × MIC) and lowest (no compound) concentrations in the first and last row, respectively. Pneumococcal cells were prepared as described above and added into the plate (10^6 CFU in 100 μL), incubated at 37°C with 5% CO_2 for 20 h. FICI was calculated as followed: FICI = FIC drug A + FIC drug B; FIC drug A =

[MIC of drug A in the presence of drug B]/[MIC of drug A]; FIC drug B = [MIC drug B in presence of Drug A]/[MIC of drug B].

Killing curve. Bacterial killing kinetics of compound **48** was determined essentially as described.[74] Compounds were mixed with the mid-log phase cutures of D39, which were incubated at 37 °C with 5% CO_2. At selected time points, the optical density (OD_{620}) of each culture was measured before an aliquot (40 uL) taken to determine viable bacteria (CFU) on TSA blood agar plates. The experiment was done at least three times with three replicates for each sample each time.

Cell Cytotoxicity Assay. Human liver cells (HepG2, ATCC HB-8065) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. All reactions were performed in duplicate. All the final compounds were prepared as 100 mM stock solutions in DMSO. For the highest test concentration,1 μL of the stock solution, 99 μL of DMEM, and the 100 μL of HepG2 cells in DMEM [(2.5-5.0) × 10^3 cells per well] were plated in 96-well plates, yielding a final volume of 100 μL and compound concentration of 100 μL. A serial dilution to provide final compound concentration of 50, 25, 12.5 and 6.25 μM were also prepared. Control wells contained 1% DMSO (negative control). The plate was incubated for 48 h at 37 °C in a 5% CO_2/95% air humidified atomosphere. The DMEM medium was aspirated, and Almamar Blue Kit (Fanbo BioChemcals) was added to each well, and the plate was incubated as described above for 2-4 h. The plate was read on a Multimode Plate Reader (Enspire^R, PerkinElmer) at 570 nm for formazan and 600 nm for background subtraction. Cell viability was estimated as the percentage absorbance of sample relative to the DMSO control. Percent cell viability was plotted against test concentration to determine CC_{50}.

Mitochondrial toxicity assay. This was performed following the described protocol using both high-glucose (25 mM) Dulbecco's Modified Eagle Medium (DMEM) and galactose media employing

DMEM deprived of glucose, but supplemented with 10 mM galactose.[41] Cell viability was evaluated after 24 h of culture by adding 10 μL of the Cell Titer Glo Reagent (Promega) to each well at room temperature and the cellular ATP concentrations were measured as per the manufacturers instructions.

Pharmacokinetics Study. Animal Care and Welfare Committee of Institute of Materia Medica, Chinese Academy of Medical Sciences approved all animal protocols (1 Xian nong tan Street, Xicheng District, Beijing, China; protocol #SYXK 2014-0023). All animal programs are in compliance with the Guide for the Care and Use of Laboratory Animals issued by Beijing Association on Laboratory Animal Care (BALAC). The single dose pharmacokinetic studies were performed with male ICR mice by monitoring plasma time course of optochin **1**, apo-cupreine **39**, ***Z*-48** and ***E*-48** after administration via oral (*p.o.*) and intravenous (*i.v.*) routes. Optochin **1** and apo-cupreine **39** were respectively prepared as 2.5 mg/mL suspension with pure water (containing Tween). ***Z*-48** and ***E*-48** were suspend with 0.5% CMC to make a 2.5 mg/mL suspension for oral use. Optochin **1** and apo-cupreine **39** were respectively formulated as 0.25 mg/mL solution with 1.25% methanol in saline. ***Z*-48** and ***E*-48** were respectively formulated as 0.25 mg/mL suspension for intravenous injection. Sixty-four mice were divided into 8 groups, 10 in each oral group and 6 in each intravenous group. After fasting 12 h with free access to water, mice were given a single *p.o.* or *i.v.* dose of 25 or 2.5 mg/kg by oral gavage or injection respectively. Blood samples (50 μL) were obtained from the orbital vein at 5, 15, 30 min, 1, 2, 5, 6, 8 and 12 h after oral administration and 2, 5, 15, 30 min, 1, 2, 5, 6, 8, 12, 24 h after injection into the intravenous injection. 20 μL of plasma was separated and 40 μL of acetonitrile (200 ng/mL) containing internal standard (propranolol, 200 ng/mL) was added. After mixing, the mixture was centrifuged (14,000 rpm × 5 min, twice) and 1 μL of supernatant was taken for LC-MS/MS analysis.

Reverse-phase LC was performed on a Shiseido C18 column (100 mm × 2.1 mm, 2.7 μm particle size). Mobile phase A was 0.1% aqueous formic acid while mobile phase B was 0.1% formic acid in

methanol. Initial conditions were 10% B from 0 to 0.7 min, after which the %B was increased to 95% from 0.7 to 1.2 min. The column was washed in 95% B for 3 min, returned to 10% over 0.3 min, and allowed to re-equilibrate for 2 min in 10% B to provide a total run time of 6.5 min. The flow rate was 0.2 mL/min and the column oven was maintained at 37 °C. The injection volume was 5 μL. All analytes were analyzed by mass spectrometry in positive ionization mode by Multiple Reaction Monitoring (MRM): *m/z* 341→174 (optochin, **1**), *m/z* 311→184 (apo-cupreine, **39**) *m/z* 365→146 (***Z*-48**), *m/z* 365→184 (***E*-48**), *m/z* 263→180 (internal standard: propranolol).

Analyte and internal standard peak areas were calculated (Xcalibur, version 2.0.7). Analyte peak areas were normalized to the corresponding internal standard peak areas and the analyte concentrations were determined using an appropriate standard curve for each compound. The standard solutions were prepared as follows: optochin **1**, apo-cupreine **39** were dissolved in methanol (20 mg/mL), ***Z*-48** and ***E*-48** were dissolved in DMSO (20 mg/mL) the stock solutions were diluted by acetonitrile containing 200 ng/mL of the internal standard (propranolol, 200 ng/mL) to a concentration of 5, 10, 20, 50, 100, 200, 500, 800, 1000 ng/mL test solution. A solution of propranolol (200 ng/mL) in acetonitrile was prepared as the internal standard. To 20 μL of mouse plasma blank were added 20 μL of compound working solution of different concentrations and 20 μL of acetonitrile containing 200 ng/mL of the internal standard (propranolol, 200 ng/mL) respectively. After mixing, the solutions were centrifuged (14000*g* × 5 min, twice) and the supernatants (1 μL) were analyzed for LC–MS/MS analysis. PK parameters were calculated from concentration-time profiles by non-compartamental analysis (Phoenix WinNonLin, version 6.3, Pharsight Corporation).

Microsomal Stability and Metabolite Identification. Microsomal studies were performed using ICR mouse liver microsomes prepared as described using differential centrifugation.[75] The liver microsome incubation system includes mouse liver microsomal protein (1 mg/mL), test compounds (10

μM), NADPH generation system 20 μL, alamethincin (50 μg/mg protein), UDPGA (5 mM), Tris-HCl buffer (50 mM, pH = 7.4) in a total volume of 200 μL. The compound-containing liver microsomes were incubated with NADPH for 10 min, and then incubated with UDPGA for 30, 60, and 90 min. 50 μL was taken and 100 μL of acetonitrile was added to terminate the reaction. After vortexing, centrifugation (14,000*g* × 5 min) was performed twice, and 3 μL of the supernatant was taken for analysis. LC/MS/MS was performed using a Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer. All experiments were performed in duplicate.

Reverse-phase LC was performed on a Zobax C18 (100 mm×2.1 mm， 3.5 μm). Mobile phase A was 0.1% aqueous formic acid while mobile phase B was 0.1% formic acid in methanol. The mobile phase was delivered with a gradient elution profile as follows: 0 min, 10% B; 15 min, 40% B; 17-19 min, 100% B; 20-23 min, 10% B. The total runtime for each injection was 23 min. The flow rate was 0.3 mL/min and the column oven was maintained at 30 °C.

The analysis was performed in full MS/dd-MS2 mode. For a full MS scan, the selected scan range was from *m/z* 100 to 500 and the resolution was 70,000, while the automatic gain control (AGC) target was set to 1.0e6 with a maximum injection time (IT) of 100 ms. For the dd-MS2 scan, the fragmentation mass spectra were recorded at a mass resolving power of 17,500 FWHM with a quadrupole isolation window of 1.5 *m/z* for precursor ions. AGC target and maximum IT for the dd-MS2 scan was 2.0e5 and 50 ms.

XCalibur 4.1 software (Thermo Fisher Scientific, San Jose, CA, USA) was used for instrument control and data processing. Compound Discover 2.0 software was used for metabolite identification.

ASSOCIATED CONTENT

Supporting Information: Copies of ^{1}H NMR and ^{13}C NMR spectra of all new compounds, HPLC chromatograms of all final compounds, and molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

[α], specific rotation; ATP, adenosine triphosphate; AUC, area under the curve; CC_{50}, 50% cytotoxicity concentration; CFU, colony forming unit; Cl, clearance; CLSI, clinical & laboratory standard institute; C_{max}, maximum serum concentration following an oral dose; CMC, carboxymethyl cellulose; DAST, diethylaminosulfurtrifluoride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMEM, Dulbecco's modified eale medium; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; DRSP, drug-resistant *Streptococcus pneumoniae*; ETC, electron transport chain; F, bioavailability; FBS, fetal bovine serum; FDA, Food and Drug Administration; FICI, fractional inhibition concentration indices; FOR, frequency of resistance; HCl, hydrogen chloride; HPLC, high-pressure liquid chromatography; HRMS, high-

resolution mass spectrometry; ICR, institute of cancer research; IR, infrared; *i.v.*, intravenous; LC-MS, liquid chromatography-mass spectrometry; MBC, minimum bactericidal concentration; MHB, Muller-Hinton broth; MIC, minimum inhibitory concentration; MLM, mouse liver microsomes; MOA, mechanism of action; *Mtb*, *Mycobacterium tuberculosis*; NOE, nuclear Overhauser effect; OD, optical density; PCV 7, 7-valent pneumococcal conjugate vaccine; PCV 13, 13-valent pneumococcal conjugate vaccine; PD, pharmacodynamic; PK, pharmacokinetic; *p.o.*, oral; R_f, retention factor (in chromatography); SAR, structure-activity relationships; SI, selectivity indexes; $t_{1/2}$, terminal elimination half-life following an oral dose; TBAF, tetra-*n*-butylammonium bromide; TBS, *tert*-butyldimethylsilyl; THF, tetrahydrofuran; THY, Todd-Hewitt broth plus 0.5% yeast extract; TAC, tricarboxylic acid cycle; VD_{ss}, steady state volumes of distribution.

Statement

Authors will release the atomic coordinates and experimental data upon article publication.

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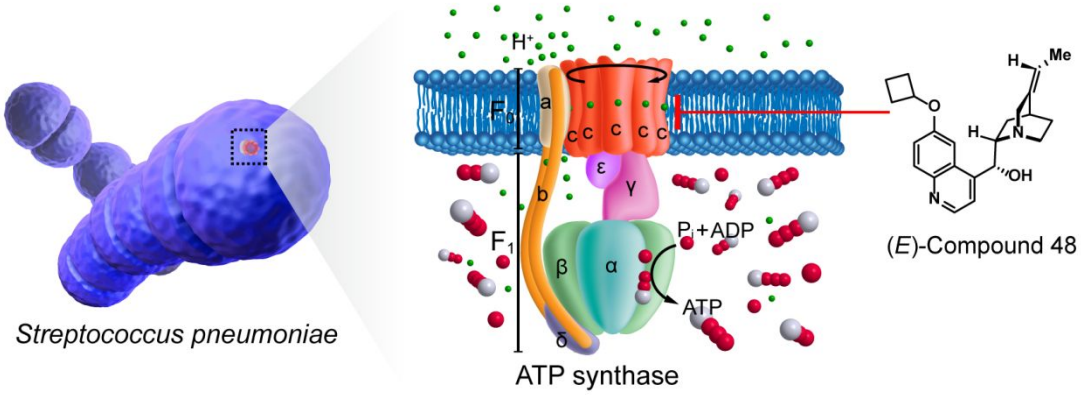
TOC Graphic


Streptococcus pneumoniae
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