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Novel N-Linked Aminopiperidine Inhibitors of Bacterial Topoisomerase Type II with Reduced pK_a: Antibacterial Agents with an Improved Safety Profile

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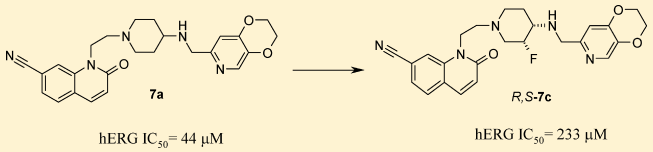
ABSTRACT: Novel non-fluoroquinolone inhibitors of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) are of interest for the development of new antibacterial agents that are not impacted by target-mediated cross-resistance with fluoroquinolones. N-Linked amino piperidines, such as **7a**, generally show potent antibacterial activity, including against quinolone-resistant isolates, but suffer from hERG inhibition (IC_{50} = 44 μM for **7a**) and QT prolongation in vivo. We now disclose the finding that new analogues of **7a** with reduced pK_a due to substitution with an electron-withdrawing substituent in the piperidine moiety, such as *R,S*-**7c**, retained the Gram-positive activity of **7a** but showed significantly less hERG inhibition (IC_{50} = 233 μM for *R,S*-**7c**). This compound exhibited moderate clearance in dog, promising efficacy against a MRSA strain in a mouse infection model, and an improved in vivo QT profile as measured in a guinea pig in vivo model. As a result of its promising activity, *R,S*-**7c** was advanced into phase I clinical studies.



INTRODUCTION

The evolution of drug resistance in pathogenic bacteria is of global concern,[1] and research into the development of new antibacterial agents that lack cross-resistance to commercially available agents is needed. Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) remain challenging pathogens capable of producing devastating disease, such as necrotizing fasciitis, osteomyelitis, and rapidly progessive pneumonia in the hospital setting.[2] Current levels of resistance in MRSA against the newer agents daptomycin and linezolid are low, but recent outbreaks of infections due to *cfr*-mediated ribosomal methylation that lead to linezolid-resistant MRSA are concerning because they confer resistance by a single gene on a transferable plasmid[3] and could potentially spread rapidly. Toxicity concerns associated with the prolonged use of linezolid often limits its use in longer duration dosing, for example, in the treatment of osteomyelitis. Inhibition of daptomycin activity by pulmonary surfactants precludes its use in the treatment of pneumonia.[4] Therefore, there is an urgent need to develop novel anti-MRSA drugs that are safe and effective to use, especially in a pneumonia setting, and that are not affected by cross-resistance with the current agents.

Bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) are clinically proven antibacterial targets, with many important agents of the fluoroquinolone class of drugs targeting the GyrA and ParC subunits of DNA gyrase and topoisomerase IV, respectively. We recently reported on novel N-linked aminopiperidines[5] with an unsubstituted piperidine moiety. These compounds are members of the NBTIs (novel (non-fluoroquinolone) bacterial type II topoisomerase inhibitors)[6−8] and are not impacted by target mutations that cause resistance to fluoroquinolones. Members of the NBTI class of inhibitors form a ternary complex with the topoisomerase and uncleaved DNA. Structural studies have revealed that the left-hand side (LHS) of the inhibitors interacts with the DNA substrate, whereas the right-hand side (RHS) binds to the topoisomerase protein. Exploration of left-hand side (LHS) and right-hand side (RHS) modifications led us to focus on two LHS cores, 7-cyano-2-quinolone and 7-methoxy-2-oxoquinoxalinone, and two RHS cores, pyrido dioxino and pyrido oxazinone, as exemplified by **7a** and **8b** (Figure 1).

N-Linked aminopiperidines such as **7a** and **8b** exhibit ∼10-fold less hERG inhibition (44 and 35 μM) compared to earlier C-linked analogues (hERG IC_{50} values of <10 μM), likely as a result of reduced log *D*.[5] However, in our experience, compounds like **7a** and **8b** still demonstrated QT prolongation in vivo in preclinical models at less than 20 μM free concentration. For example, **7a** caused >10% $MAPD_{90}$ prolongation in the guinea pig at 16 μM free concentration (Figure 2). Our estimates for effective therapeutic plasma concentrations ($ETPC_{unbound}$) for these compounds were typically in the range of 2 to 10 μM free concentration (data not shown). The resulting ratios for hERG $IC_{50}/ETPC_{unbound}$ of <22 reflected a high risk for QT

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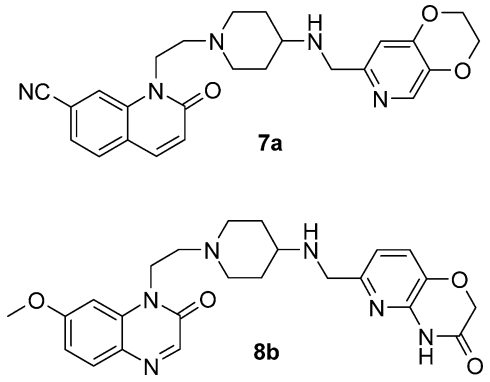


Figure 1. Unsubstituted N-linked aminopiperidines.

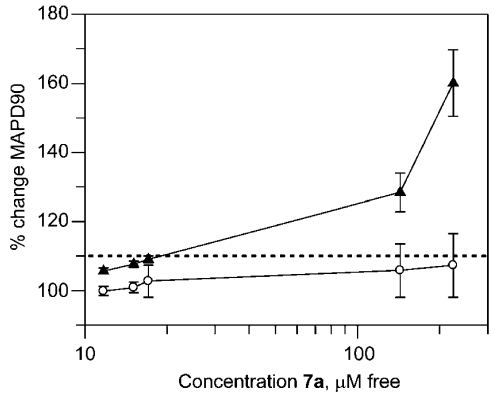


Figure 2. Effects on cardiac repolarization potential ($MAPD_{90}$) of the guinea pig for compound **7a**. Following infusion of **7a** (closed triangles), a maximum increase on $MAPD_{90}$ of 60 ± 10% was observed with 224 μM free plasma exposure for **7a**. The calculated EC_{10} (dashed line) for **7a** was 16 μM free concentration. Vehicle controls (open circles) were performed in separate cohorts of animals and are plotted in alignment with the compound dosing. Error bars shown are ± SEM, n = 6.

prolongation signals in man. A recent analysis of human thorough QT studies at Pfizer[9] demonstrated that a hERG IC_{50} within 60-fold of the $ETPC_{unbound}$ results in an 82% chance of causing a significant signal in a human thorough QT study (hTQTS).

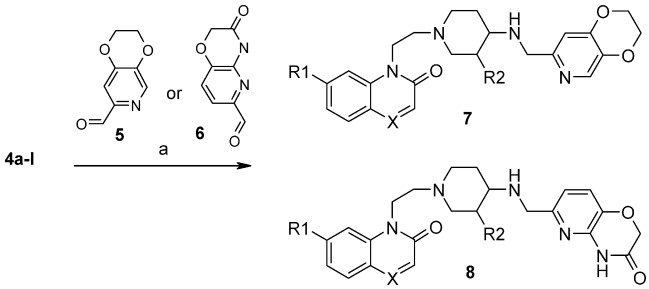
We therefore aimed to further reduce hERG inhibition within the N-linked aminopiperidines. To reduce the risk of a positive signal in the hTQTS, we aimed for a >100 ratio of hERG IC_{50}/$ETPC_{unbound}$, which translates approximately into a hERG IC_{50} target of >200 μM. Since we had previously optimized antibacterial hERG activities by manipulation of log D,[5] our strategy for this second effort was to reduce pK_a.

CHEMISTRY

Compounds were assembled through reductive aminations of amines **4a–l** with aldehydes **5**[10] or **6**[11] (Scheme 1).[12]

For the synthesis of the amines **4**, LHSs **1a**, **1b**,[5] and **1c** were alkylated with mesylates **2a–g** and **39** (Schemes 2 and 7). The mesylates were unstable under storage and were freshly prepared for immediate use from the corresponding alcohols (Schemes 4 and 7–10). Alkylations with **2a–g** generally afforded the desired N-alkylated products together with smaller amounts of O-alkylation products. O-Alkylated products generally had higher R_f value by TLC than N-alkylated product and were efficiently removed by chromatography. N-Alkylation in **3** was confirmed by NMR studies, with carbon chemical shifts for the CH_2N

Scheme 1[a]



a: X= C; R_1= CN; R_2= H
b: X= N; R_1= OMe; R_2= H
c: X= C; R_1= CN; R_2= F, cis
d: X= N; R_1 = OMe; R_2= F, cis
e: X= C; R_1= CN; R_2= F, trans
f: X= N; R_1 = OMe; R_2= F, trans
g: X= C; R_1= CN; R_2= OH, cis
h: X= N; R_1 = OMe; R_2= OH, cis
i: X= N; R_1 = OMe; R_2= OH, trans
j: X= C; R_1= CN; R_2= OMe, cis
k: X= N; R_1= OMe; R_2= OMe, cis
l: X= C; R_1= CN; R_2= OMe, trans
m: X= N; R_1= OMe; R_2= OMe, trans

[a]Reagents: (a) Molecular sieves 3 Å, $CHCl_3$/MeOH, 70 °C, then $NaBH(OAc)_3$, 0 °C to room temp.

moiety observed in the characteristic range of 40–45 versus 60–70 ppm for CH_2O in the case of O-alkylation.

The *cis*- and *trans*-fluoropiperidine mesylates **2b–d** were synthesized from the known fluoropiperidine derivatives **11–13**[13–15] by protection group manipulation and alkylation with TBDMS-protected bromoethanol as the key synthetic steps (Scheme 4). For the synthesis of the *cis*- and *trans*-hydroxy and methoxy piperidines, we followed a similar strategy, employing unprotected bromoethanol for alkylation of the piperidines (Schemes 7–10).

4b containing a 7-cyano-2-quinolone LHS was initially synthesized by alkylation of the commercially available 7-bromoquinolone **1a** to give **3a**, which was converted to the 7-cyano derivative by palladium-catalyzed coupling with in situ generated Bu_3SnCN. Subsequently, we developed several improvements for the synthesis of the compounds that are worth highlighting. A new efficient synthesis of the 7-cyano-substituted LHS **1c** eliminated the need for a cyanation step later in the synthesis (Scheme 3). A further improvement to the synthesis was affected by alkylation of the LHS with 2-bromo-1,1-diethoxyethane, followed by deprotection of the acetal with HCl, as described for the aldehyde **34** (Scheme 6), which could be alkylated reductively with piperidine derivatives such as **31** and **51** (Schemes 5 and 11). This sequence was broadly applicable to LHS moieties, eliminated the need for the unstable mesylates, and led to shorter routes for analogues with piperidine modifications.

RESULTS AND DISCUSSION

We explored reduction of pK_a through substitution at the 3-position of the piperidine moiety with electron-withdrawing substituents, specifically both *cis*- and *trans*-substitution with hydroxy, methoxy, and fluoro groups (Table 1). In order to discern small differences in pK_a values, these values were determined by pH metric titration. We discuss the pK_a of the basic secondary amine only because the tertiary piperidine is a weak base, with pK_a values ranging from 3.12 to 5.55 in

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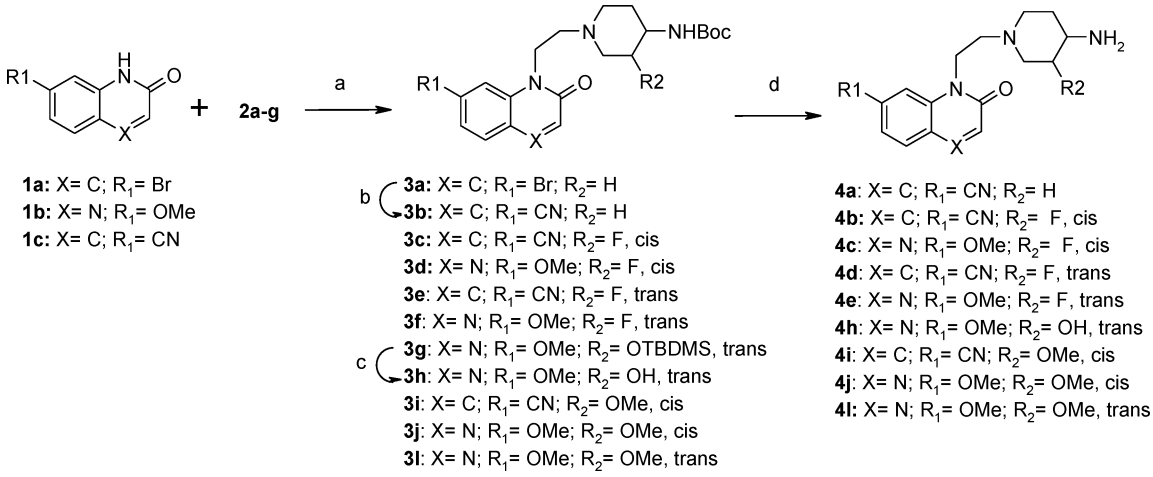
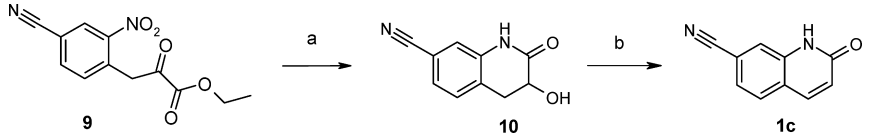
Scheme 2[a]



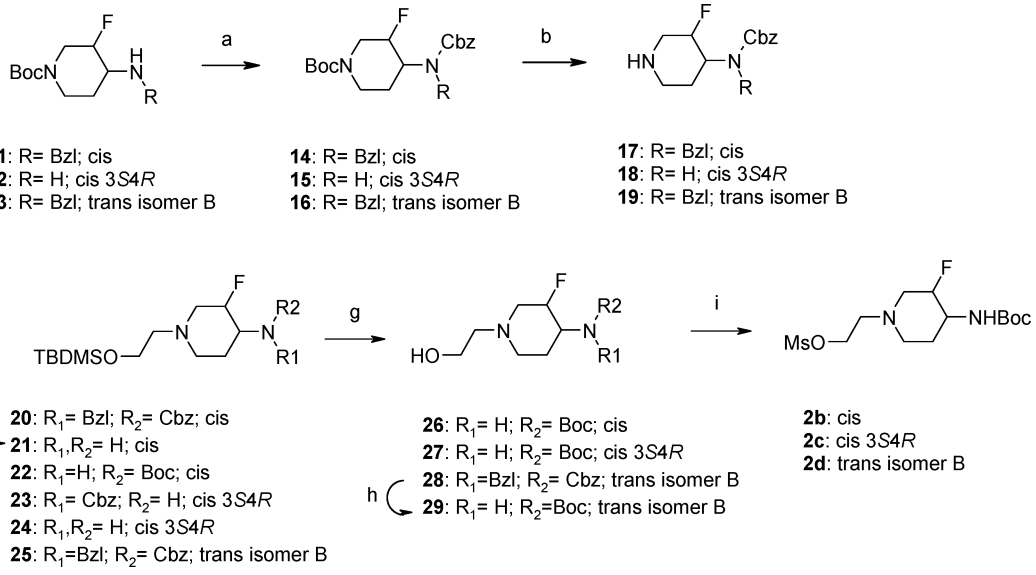
[a]Reagents: (a) NaH, DMF, 0 °C to room temp; (b) KCN, $Sn(Bu)_3Cl$, $Pd_2(dba)_3$, Xantphos, CH_3CN, 85 °C, 76%; (c) TBAF, THF, 0 °C to room temp, 79%; (d) TFA/DCM, 0 °C.

Scheme 3[a]



[a]Reagents: (a) $NaBH_4$, CH_3CN, room temp, then AcOH and Fe, 65 °C, 66%; (b) DBU, CH_3CN, 75 °C, 68%.

Scheme 4[a]



[a]Reagents: (a) BzlOCCl, Na_2CO_3, dioxane, 0 °C to room temp; (b) HCl, dioxane, 0 °C; (c) $TBDMSO(CH_2)_2Br$, Cs_2CO_3, CH_3CN, 60 °C; (d) $Pd(OH)_2/C$, H_2, MeOH, room temp, 98%; (e) Boc_2O, THF, room temp, 82%; (f) Pd/C, H_2, EtOH, room temp, 93%; (g) TBAF, THF, 0 °C; (h) $Pd(OH)_2/C$, H_2, MeOH, room temp, 76%; (i) MsCl, NEt_3, CH_2Cl_2, 0 °C.

substituted piperidines. Compounds **8b−m** with a pyrido oxazinone RHS showed a third pK_a of ∼10.4, which is due to the weakly acidic nature of the oxazinone RHS NH moiety.

In the fluoro series, *cis*-fluoro analogues of **7a**, *R,S*-**7c** and *S,R*-**7c**, led to a reduction of pK_a for the secondary amine functionality from 8.27 in **7a** to 7.03 in **7c**. *R,S*-**7c** and *S,R*-**7c** inhibited hERG with IC_{50} values of 233 and 199 μM, respectively, representing an approximately 5-fold improvement over **7a** (hERG IC_{50} = 44 μM). The pK_a was further reduced in the *trans*-fluoro analogue **7e** (pK_a = 6.66). The same trend was previously observed for related *cis*- and *trans*-3-fluoropiperidines but with larger shifts in pK_a.[13] However, **7e** was a more potent inhibitor of hERG with an IC_{50} of 122 μM. This apparent disconnect in the SAR can be explained by comparing the log *D* values of the parent **7a** (log *D* = 0.68), the *cis*-fluoro analogue **7c** (0.96), and *trans*-fluoro analogue **7e** (1.53). It is well-established that both

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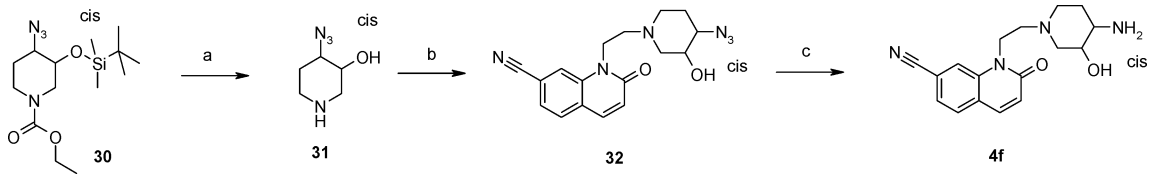
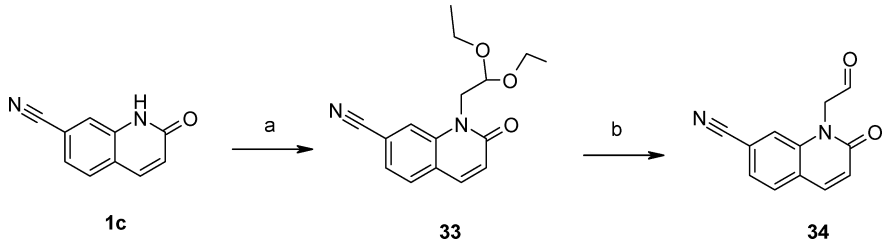
Scheme 5[a]



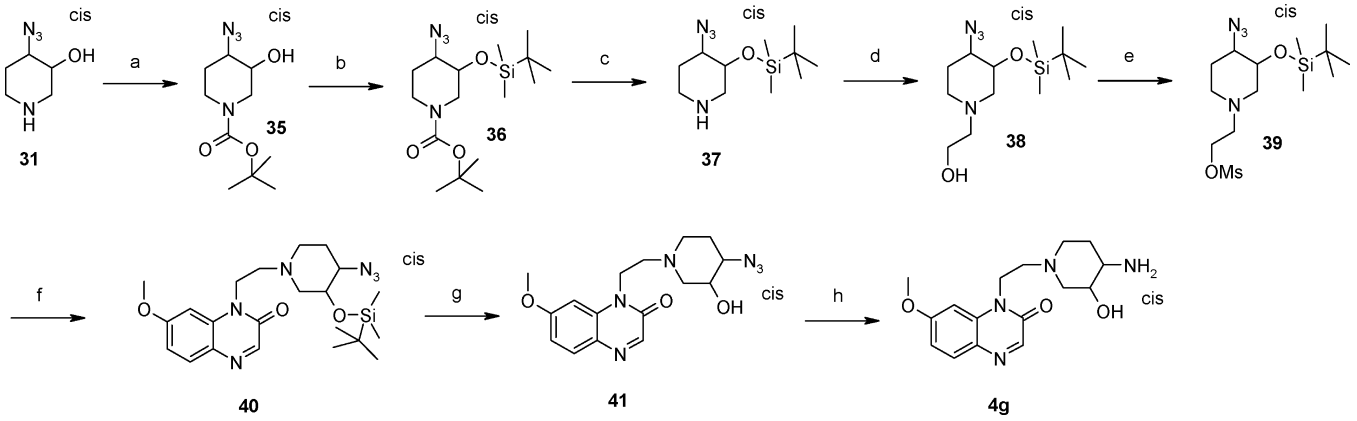
[a]Reagents: (a) KOH, EtOH, 90 °C, 43%; (b) **34**, THF, 75 °C then $NaB(OAc)_3H$, room temp, 49%; (c) $P(Ph)_3$, CH_3CN/H_2O, room temp, 90%.

Scheme 6[a]



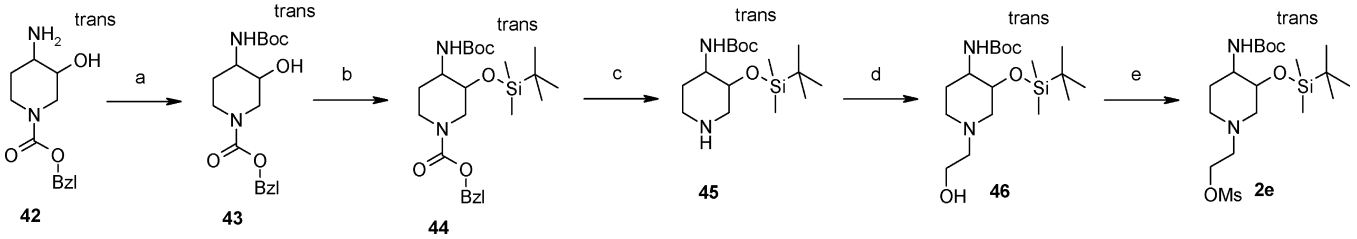
[a]Reagents: (a) $BrCH_2CH(OEt)_2$, Cs_2CO_3, NMP, 70 °C, 60%; (b) concd HCl, CH_3CN, room temp, 100%.

Scheme 7[a]



[a]Reagents: (a) Boc_2O, KOH, *i*PrOH/CH_2Cl_2, 0 °C to room temp, 35%; (b) TBDMSiCl, imidazole, DMF, 0 °C to room temp, 89%; (c) TFA/CH_2Cl_2, 0 °C, 98%; (d) $Br(CH_2)_2OH$, $NEt(iPr)_2$, CH_3CN, 70 °C, 95%; (e) MsCl, NEt_3, CH_2Cl_2, 0 °C; (f) **1b**, NaH, DMF then **39**, 0 °C to room temp, 52%; (g) TBAF, THF, room temp, 94%; (h) $P(Ph)_3$, CH_3CN/H_2O, room temp, 90%.

Scheme 8[a]



[a]Reagents: (a) Boc_2O, $NaHCO_3$, $EtOAc/H_2O$, room temp, quant; (b) TBDMSiCl, imidazole, DMF, room temp, 69%; (c) Pd/C, H_2, MeOH, room temp, quant; (d) $Br(CH_2)_2OH$, $NEt(iPr)_2$, CH_3CN, 70 °C, 67%; (e) MsCl, NEt_3, CH_2Cl_2, 0 °C.

pK_a and log *D* will have an impact on hERG inhibition.[16] Therefore, in the optimization against hERG by lowering pK_a, the concomitant increase of log *D* needs to be considered. In this case, a balance was obtained with the *cis*-fluoro analogue **7c**.

Reduction of pK_a is expected to potentially reduce hERG inhibition through reduction of binding affinity. We investigated binding to the hERG channel with the [^{3}H]-astemizole binding assay[17] on a limited number of compounds, and indeed, both isomers of **7c** showed higher astemizole binding IC_{50} values (>200 μM, compared to 39 μM for the parent **7a**). Reduced binding by **7c** relative to **7a** seems to be a result of the relative large reduction of pK_a for **7c** (by 1.24 log units) since the difference in log *D* is relatively small (0.28 log units). The *trans*-isomer **7e** had an astemizole binding IC_{50} of 57 μM, which is

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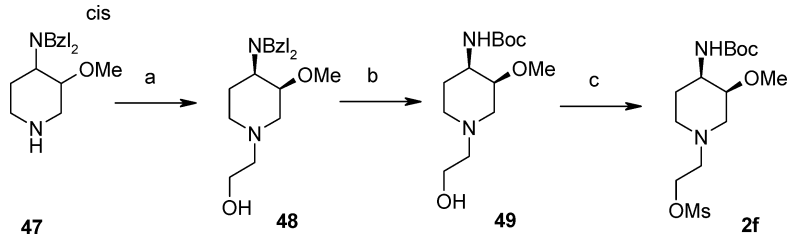
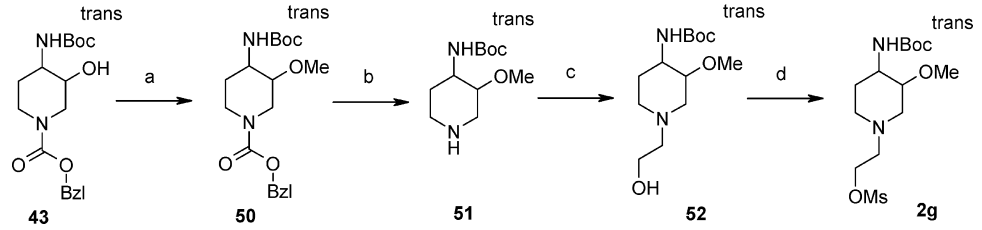
Scheme 9[a]



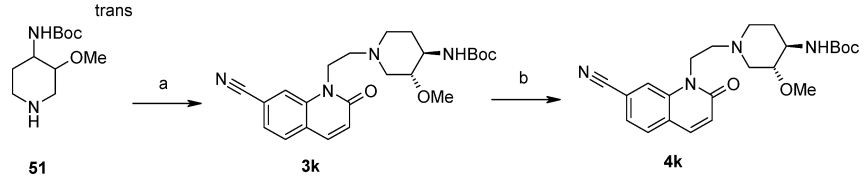
[a]Reagents: (a) $Br(CH_2)_2OH$, $NEt(iPr)_2$, CH_3CN, 70 °C, 68%; (b) Boc_2O, $Pd(OH)_2/C$, H_2, MeOH, room temp, 74%; (c) MsCl, NEt_3, CH_2Cl_2, 0 °C.

Scheme 10[a]



[a]Reagents: (a) NaOH, $(Me)_2SO_4$, $Bzl(Et)_3N^+Cl^-$, toluene/H_2O, room temp, 78%; (b) Pd/C, H_2, MeOH, room temp, 98%; (c) $Br(CH_2)_2OH$, $NEt(iPr)_2$, CH_3CN, 70 °C, 57%; (d) MsCl, NEt_3, CH_2Cl_2, 0 °C.

Scheme 11[a]



[a]Reagents: (a) **34**, THF, 75 °C then $NaB(OAc)_3H$, room temp; (b) TFA/CH_2Cl_2, 0 °C, quant.

lower than the value for the *cis*-isomers **7c**, despite a further reduction in pK_a for **7e** by 0.37 log units. However, the difference in pK_a for **7e** and **7c** is relatively small and associated with a relatively large difference in log *D* by 0.56 log units, indicating that the higher log *D* of **7e** causes tighter binding to the hERG channel.

The hERG Ionworks is a cellular assay, and the key binding sites for small molecule ligands to the hERG channel are facing the intracellular side.[17] Reductions in pK_a and resultant increases in log *D* are expected to increase functional hERG inhibition in the Ionworks assay by increasing permeability.

An optimal balance between pK_a and log *D* will therefore take into account effects on both binding and permeability.

It is interesting to note that when the same linker modifications were performed on the parent compound **8b**, bearing a quinoxalinone LHS and a pyrido oxazinone RHS, the *cis*-fluoro analogues **8d** had higher hERG IC_{50} values than the *trans*-analogue **8f** but were not improved over the parent **8b** (hERG IC_{50} values of 35, 25/32, and 19 μM for parent, *cis*-fluoro isomers, and *trans*-fluoro isomers, respectively). Fluoro-substituted analogues *S*,*R*-**8c** and **7f** that bear either the quinoxalinone LHS or the pyrido oxazinone RHS are also slightly more potent hERG inhibitors than the corresponding analogues with the cyanoquinolone LHS and pyrido dioxino RHS found in **7a**, indicating that hERG inhibition with N-linked piperidines is more potent with a quinoxalinone LHS or a pyrido oxazinone RHS; however, the lack of improvement for the *cis*-fluoro analogues **8d** compared to parent **8b** is surprising.

All 3-fluoro-substituted piperidines, especially *cis*-substituted analogues (compounds **7c** and **8c**), showed less potency against the target topo IV from *Escherichia coli* than the unsubstituted parents **7a** and **8b**. We saw a correlation between the IC_{50} values of topo IV from *E. coli* and the gyrase from *Staphylococcus aureus* (*S. aureus* data not shown). The reduction in target potency with substituted piperidines was likely due to conformational effects on the piperidine moiety, which will impact the position of the LHS and RHS, as well as the position of the basic amine, which are important for binding. The piperidine moiety itself is positioned in the target binding site in the solvent-exposed space.[6] Compounds were tested for antibacterial activity against Gram-positive and Gram-negative organisms. Despite the drop in target potency with *cis*-fluoro-substituted piperidines, antibacterial activity against Gram-positive organisms was largely maintained (compounds **7c**, **8c**, and **8d**) relative to the unsubstituted parents **7a** and **8b**, as apparent by similar MICs and free fractions (Table 1). This indicated better permeability into Gram-positive bacteria with the reduced pK_a/higher log *D* compounds. The higher log *D* preference for agents targeting Gram-positive organisms has been noted previously for this series of compounds[5] and as a general trend for antibacterials.[18] On the other hand, cellular activity (MICs) against the Gram-negative organisms *Pseudomonas aeruginosa* and *E. coli* was less potent for all fluoro-substituted piperidines compared to

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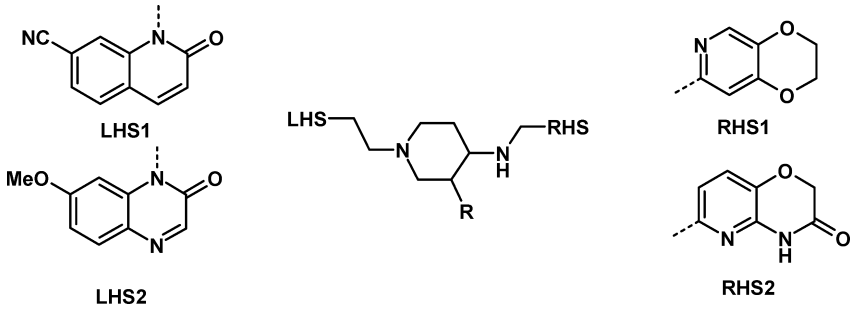
Table 1. SAR of Substituted Piperidines (Minimum Inhibitory Concentration (MIC, μg/mL): Lowest Drug Concentration That Reduced Growth by 80% or More)



compd	LHS	RHS	configuration	*S.a.*[a] MIC (μg/mL)	*S.p.*[b] MIC (μg/mL)	*P.ae.*[c] MIC (μg/mL)	*E. coli*[d] MIC (μg/mL)	fu[e] (%)	*E. coli* topo IV[f] IC_{50} (nM)	log *D*[g]	pK_a[h]	hERG[i] IC_{50} (μM)
7a	1	1	H	0.03	0.08	>8	0.5	28	3.2	0.68	8.27/5.75	44
8b	2	2	H	0.03	0.08	1	0.13	26	2.2	0.60	8.42/5.56/10.46	35
						fluoro-substituted analogues (R = F)						
R,S-**7c**	1	1	*cis*, 3*R*4*S*	0.06	0.13	>8	4	25	48	0.96	7.03/4.47	233
S,R-**7c**	1	1	*cis*, 3*S*4*R*	0.13	0.13	>8	4	20	60	0.97	ND	199
S,R-**8c**	1	2	*cis*, 3*S*4*R*	0.06	0.25	8	1	20	11	0.66	7.28/4.32/10.28	168
R,S-**8d**	2	2	*cis*, 3*R*4*S*	0.03	0.13	8	0.5	ND	ND	ND	ND	25
S,R-**8d**	2	2	*cis*, 3*S*4*R*	0.03	0.13	8	0.5	25	ND	0.83	ND	32
7e	1	1	*trans*, enantiomer B	0.13	0.25	>8	8	9	16	1.53	6.66/4.07	122
7f	2	1	*trans*, enantiomer B	0.06	0.13	>8	8	9	26	1.68	6.46/3.95	75
8f	2	2	*trans*, enantiomer B	<0.01	0.06	4	0.5	4	<9	1.38	6.86/3.12/10.40	19
						hydroxy-substituted analogues (R = OH)						
R,S-**7g**	1	1	*cis*, 3*R*4*S*	0.06	ND	>8	1	19	25	0.46	8.00/5.55	156
S,R-**7g**	1	1	*cis*, 3*S*4*R*	0.06	ND	>8	1	24	41	0.45		117
R,S-**8g**	1	2	*cis*, 3*R*4*S*	0.25	ND	2	0.50	21	17	0.11		305
S,R-**8g**	1	2	*cis*, 3*S*4*R*	0.50	ND	2	0.50	18	15	0.10		>333
R,S-**7h**	2	1	*cis*, 3*R*4*S*	0.03	ND	8	0.50	19	5	0.77		>33
S,R-**7h**	2	1	*cis*, 3*S*4*R*	0.06	ND	>8	1	14	6	0.78	7.97/5.42	15
R,S-**8h**	2	2	*cis*, 3*R*4*S*	0.06	ND	2	0.25	14	17	0.39		172
S,R-**8h**	2	2	*cis*, 3*S*4*R*	0.13	ND	2	0.25	5	23	0.37		152
7i	2	1	*trans*, enantiomer B	0.06	0.06	>8	2	18	3	0.9	7.40/4.33	>100
						methoxy-substituted analogues (R = OMe)						
S,R-**7j**	1	1	*cis*, 3*S*4*R*	0.13	0.13	>8	4	54	55	0.63	7.99/5.15	174
S,R-**8j**	1	2	*cis*, 3*S*4*R*	0.13	0.50	8	1	50	28	0.32	ND	189
S,R-**7k**	2	1	*cis*, 3*S*4*R*	0.06	0.13	>8	4	33	76	0.90	8.01/5.00	75
S,R-**8k**	2	2	*cis*, 3*S*4*R*	0.06	0.25	4	1	28	76	0.51	8.17/5.10/10.39	88
7l	1	1	*trans*, enantiomer 1	0.25	0.50	>8	8	13	96	1.07	7.25/4.34	176
8l	1	2	*trans*, enantiomer 1	0.13	0.25	>8	2	27	35	0.81	7.53/4.22/10.46	124
7m	2	1	*trans*, enantiomer 1	0.25	0.25	>8	8	11	113	1.27	7.00/4.32	93
8m	2	2	*trans*, enantiomer 1	0.03	0.13	8	1	18	45	0.97	7.45/4.34/10.48	27

[a]Methicillin-susceptible *Staphylococcus aureus*. [b]*Streptococcus pneumonia* D39 (penicillin-susceptible). [c]*Pseudomonas aeruginosa* PAO1. [d]*Escherichia coli* W3110. [e]Fraction unbound, human, % free. [f]*Escherichia coli* topo IV IC_{50}. [g]Partion coefficient at pH 7.4. [h]First value listed is for secondary amine. [i]hERG Ionworks IC_{50}.[28] ND: Not determined.

unsubstituted parents **7a** and **8b**, indicating that the more basic **7a** and **8b** may permeate better into Gram-negative bacteria. This could be due to the nature of the outer membrane in Gram-negative organisms as a permeability barrier, where porins are generally more permissive to positively charged compounds.[19]

Hydroxy-substituted piperidines (compounds **7g–i**, **8g–h**) showed a reduction in pK_a compared that of parent **7a** and **8b** that was more pronounced in the *trans* series, but to an overall lesser extent than seen with the more electronegative fluoro-substituted compounds (pK_a of 7.4 and ~8 for the secondary amine in the *trans*- and *cis*-hydroxy piperidines, respectively). Log *D* values for hydroxyl-substituted compounds were lower than for the corresponding fluoro-substituted analogues. hERG inhibition was reduced relative to parent **7a** and **8b** with most of the analogues, especially with *S,R*-**8g**, which had a low log *D* of 0.1. Compound **7h** isomers showed relative potent inhibition of hERG, which is likely due to the higher log *D* of ~0.77 and a relative high pK_a, which is probably similar to **7g** at pK_a = 8.0. The

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trans-isomer **7i** has a slightly higher log *D* of 0.9 but a lower pK_a, which may explain why **7i** shows a relatively lower level of hERG inhibition (IC_{50} >100 μM) compared to **7h**. As with fluoro-substituted analogues, the balance between reduction in pK_a and the level of lipophilicity seems to be important for the level of hERG inhibition. With regard to antibacterial activity, *cis*-hydroxy substitution led to reduced binding to the target, and only the *trans*-analogue **7i** showed similar potency on the target as the parent. Antibacterial activity against the Gram-positive organism *S. aureus* was largely maintained relative to parent with compounds of log *D* > ~0.4. The isomers of **8g**, which had a low log *D* of ~0.1, were less potent.

MICs against the Gram-negative pathogens *P. aeruginosa* and *E. coli* were consistently higher for all hydroxy-substituted analogues relative to parent. Although the MIC shift was modest, it suggests that this substitution, due to its slight reduction in pK_a, may be detrimental to permeability into these Gram-negative organisms.

Methoxy-substituted piperidines (compounds **7j−m**, **8j−m**) showed reductions in pK_a very similar to the hydroxy-substituted analogues for the *cis*-analogues and a slightly lower pK_a for the *trans*-isomer (comparing compounds **7m** and **7i**, pK_a of 7.0 and 7.4, respectively). Log *D* values for methoxy-substituted compounds were slightly higher than for the hydroxy-substituted analogues. hERG IC_{50} values for methoxy-substituted analogues were, in general, improved over the unsubstituted parents and similar to the hydroxy analogues. An interesting difference in hERG inhibition was found between the *trans*-analogues **7l** and **8m**, which showed a marked difference in hERG IC_{50} values (176 vs 27 μM, respectively), with the log *D* of **8m** being only 0.1 units lower but the pK_a by 0.2 units higher than for **7l**. While not conclusive, these results again suggest that relative small differences in pK_a may explain significant differences in hERG inhibition.

The impact on QT interval was investigated with *R,S*-**7c** in vivo in the guinea pig. The compound did not cause a statistically significant change in $MAPD_{90}$ at the highest exposure tested (67 μM total or 30 μM free) (Figure 3). This represents a significant improvement for *R,S*-**7c** over the unsubstituted parent **7a**, which gave an EC_{10} value of 16 μM free concentration in this model. Compound *R,S*-**7c** also demonstrated reduced ancillary pharmacology compared to **7a** when tested in a secondary pharmacology panel consisting of 104 targets at 30 μM (results not shown).

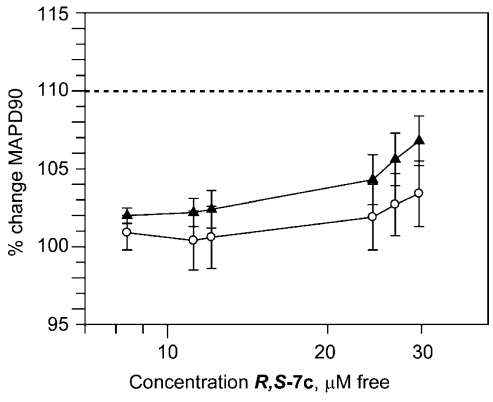

Figure 3. Effects on cardiac repolarization potential ($MAPD_{90}$) of the guinea pig for compound *R,S*-**7c**. Following infusion of *R,S*-**7c** (closed triangle), $MAPD_{90}$ was not significantly different ($P < 0.05$) from vehicle treated animals (open circles) at any plasma concentration achieved (highest concentration tested 30 μM free). As there were no biologically or statistically significant changes, an EC_{10} (dashed line) could not be calculated for *R,S*-**7c**. Error bars shown are ± SEM, $n = 6$.

The optimized *cis*-fluoro analogue *R,S*-**7c** showed excellent activity against hospital- and community-acquired MRSA, vancomycin-resistant *S. aureus* (VRSA), as well as against linezolid-resistant isolates (Table 2), indicating lack of cross-resistance with anti-MRSA agents. Pharmacokinetic properties of selected compounds were determined in the rat and dog (Table 3). The fluoro-substituted compounds *R,S*-**7c** and *S,R*-**8c** showed lower volumes of distribution in both species compared to unsubstituted parent compounds **7a** and **8b**, likely as a result of reduction in pK_a. Clearance for all four compounds was high in rat and low to medium in dog, correlating roughly with in vitro intrinsic clearance in hepatocytes. Compounds *R,S*-**7c** and *S,R*-**8c** were predicted to show low to medium clearance in man, based on intrinsic clearance in human hepatocytes. Bioavailability of compound *R,S*-**7c** was low in rat but higher in dog, correlating with clearance and indicating that first pass metabolism may be a limiting factor for bioavailability in rodents.

Table 2. MICs against Resistant Strains of *Staphylococcus aureus*[a]

phenotype	strain	*R,S*-**7c**	linezolid	vancomycin	levofloxacin
CA-MRSA[b]	ARC3189	0.06	2	1	8
HA-MRSA[c]	ARC1692	0.125	2	2	0.125
VRSA[d]	ARC3186	0.015	1	>64	16
LRSA[e]	ARC3583	0.125	16	ND	>16

[a]Minimum inhibitory concentration (MIC, μg/mL): lowest drug concentration that reduced growth by 80% or more. [b]Community-acquired methicillin-resistant (USA300). [c]Hospital-acquired methicillin-resistant (ATCC33591). [d]Vancomycin-resistant, from NARSA (VRS3). [e]Linezolid-resistant, containing the cfr resistance plasmid.[3] ND: Not determined.

Compound *R,S*-**7c** was highly cleared in mouse (results not shown), and for this reason, it was dosed for efficacy studies in mice together with the cytochrome P_{450} inhibitor azabenzotriazole (ABT).[20] Compound *R,S*-**7c** was active against MRSA ATCC33591 in a neutropenic mouse thigh infection model (Table 4), with a 1.5 log reduction in colony forming units (CFU) observed at a dose of 60 mg/kg/day when co-dosed with ABT. The CFU reduction per dose was similar to that of levofloxacin in this model; however, the AUC requirement was higher for *R,S*-**7c**.

CONCLUSIONS

We report on the reduction of hERG inhibiton in N-linked aminopiperidine NBTIs by substitution in the 3-position of the piperidine moiety with electron-withdrawing substituents. The *cis*-fluoro-substituted analogues **7c** were optimal, resulting in ~5-fold increased hERG IC_{50} values. Reducing pK_a, while minimizing the associated increase in lipophilicity, was important for successful reduction of hERG inhibition. Small differences in pK_a and log *D* may explain marked differences in hERG activity. Compound *R,S*-**7c** was tested in the guinea pig model for QT prolongation to free exposures approximately 2-fold that for **7a** where 10% increased prolongation was observed. We show that NBTIs with reduced pK_a are excellent antibacterial agents against the Gram-positive bacteria *Streptococcus pneumoniae* and *S. aureus*, including resistant isolates. *R,S*-**7c** was evaluated in a neutropenic mouse thigh infection model against an MRSA

Table 3. Pharmacokinetics in Rat and Dog[a]

	compound	Cl int (Hep) (μL/min/10^6)	clearance (mL/min/kg)	volume (L/kg)	half-life (h)	AUC (iv) (μg·h/mL)	bioavailability (%)
rat	**7a**	42	127	21	3.0	0.37	31
	8b	26	252	23	1.9	0.19	
	R,S-**7c**	13	54	3.0	0.9	3.1	18
	S,R-**8c**	14	62	3.0	ND	2.5	
dog	**7a**	1.8	18	8.0	8.4	2.6	72
	8b	<1	8.5	2.0	13	6.8	
	R,S-**7c**	4.5	6.2	1.5	3.4	27	60
	S,R-**8c**	8.6	7.3	1.7	5.4	25	
man	*R,S*-**7c**	5.2					
	S,R-**8c**	2.0					

[a]Pharmacokinetic parameters following administration of 3 mg/kg iv bolus injection (compounds **7a**, **8b**, rat, dog), or 10 mg/kg iv infusion (compounds *R,S*-**7c**, *S,R*-**8c**, rat, dog). Oral bioavailability was assessed following a 10 mg/kg oral dose.

Table 4. Exposure and Efficacy of Compound *R,S*-7c Compared to Levofloxacin in a Mouse Thigh Model[a]

	compound *R,S*-**7c**		levofloxacin	
	AUC∞ (μg/h/mL)	log reduction in CFU/g thigh vs start of treatment	AUC∞ (μg/h/mL)	log reduction in CFU/g thigh vs start of treatment
20 mg/kg/day	18	0.1	ND	ND
40 mg/kg/day	37	1.0	4	0.3
60 mg/kg/day	53	1.5	11[b]	1.4[b]

[a]In vivo activity against *S. aureus* ATCC33591 (ARC1692). [b]80 mg/kg/day dose.

strain and found to be efficacious with a 1.5 log reduction in CFU at a dose of 60 mg/kg/day in the presense of ABT. The pharmacokinetic properties of selected compounds were studied in rat and dog. A good correlation was seen for clearance to in vitro hepatocyte values for intrinsic clearance. Human hepatocyte values predicted low to medium clearance in man for *cis*-fluoro-substituted analogues. The *cis*-fluoro analogue *R,S*-**7c** had the overall best profile of the compounds investigated for this study and was advanced into phase I studies.

EXPERIMENTAL SECTION

Minimum Inhibitory Concentration Testing. Minimum inhibitory concentrations were determined by broth microdilution according to the Clinical and Laboratory Standards Institute guidelines.[21] Inoculants were incubated at 35 °C on blood agar plates (Remel #01202) for 18 to 24 h. Compounds were dissolved in 100% DMSO and diluted to 2% DMSO (v/v) in culture medium to 11 doubling dilutions from 64 to 0.06 μg/mL. Specific culture media: For *S. aureus*, *P. aeruginosa*, and *E. coli*, Mueller Hinton Broth 2 (Difco). For *S. pneumoniae*, Mueller Hinton Broth 2 plus 2.5% (v/v) lysed horse blood (Hema Resource and Supply #15-14-0100-28). Plates were read by spectrophotometry at 620 nm.

Topoisomerase IV Assay. The assay for the ATPase activity *E. coli* topo IV, employing the malachite green phosphate detection reagent,[22] was performed as described previously.[5]

Plasma Protein Binding. Plasma protein binding was determined using the Dianorm equilibrium dialysis chamber. Compound (10 μM concentration) was spiked in the plasma chamber (donor side), and phosphate buffer was placed in the receiver side. The unit was rotated at 37 °C for 16 h. Drug concentration was determined for the plasma sample that represents the bound fraction and the buffer sample that represents the free fraction. LC/MS-MS quantitative sample analysis was achieved using an Ace C18 50 × 4.6 mm column (MacMod, PA) and electrospray ionization MRM detection (PE Sciex API 4000 mass spectrometer, Applied Biosystems CA). Plasma samples (50 μL) were treated with methanol (150 μL) containing an internal standard to precipitate the protein. Concentration determination was based on a standard curve (10 nM to 10 μM), and data were processed by the Analyst version 1.4.1. software.

Log *D* Determination. The partition coefficient (log *D*) was measured by shake flask method, using 10 mM phosphate buffer at pH 7.4 and *n*-octanol. The samples were allowed to reach equilibrium by shaking for 1 h at 1200 rpm, and sample analysis was done by LC/UV, with MS for mass confirmation.

pK_a Determination. Values of pK_a were determined at Sirius Analytical Instruments Ltd. (Forest Row Business Park, Station Road, Forest Row, East Sussex, TH18 5DW) by the Gold Standard pH metric assay on a Sirius T3 automated system in triplicate. The accuracy of the measurement was approximately 0.02 log units.

Animals. Wistar Han rats for pharmacokinetic studies and guinea pigs for cardiac electrophysiology studies were obtained from Charles River Laboratories (Raleigh, NC). CD-1 mice were obtained from Charles River Laboratories (Kingston, NY). All animals were housed and acclimated in AstraZeneca animal facilities before each study. All experimental procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Pharmacokinetic Studies. Pharmacokinetic properties of selected compounds were studied in the rat. Groups of three Wistar Han rats were administered test compound at a dose of 3 or 4 mg/kg, by bolus injection into a cannulated jugular vein. Oral bioavailability was determined following a 10 mg/kg dose given by oral gavage. Serial 200 μL samples of whole blood were taken at time intervals. Concentration of compound in plasma was determined by LC-MS/MS, and pharmacokinetic parameters were estimated using a non-compartmental model in WinNonLin (Pharsight). Exposure in CD-1 mice was determined for analysis of the efficacy studies. At timed intervals, groups of three mice were sacrificed and whole blood samples collected by cardiac puncture. Plasma samples were prepared and analyzed as described above. Similarly, plasma pharmacokinetics were determined from 0 to 24 h in male beagle dogs (n = 3) following 15 min iv infusions at 3 mg/kg or oral administration at 10 mg/kg.

***S. aureus* Neutropenic Thigh Infection Model.** Compound *R,S*-**7c** was studied in a neutropenic mouse thigh infection model as described by Mills et al.[23] Briefly, mice were rendered neutropenic by injecting cyclophosphamide (Sigma-Aldrich, St. Louis, MO) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before experimental infection. Mice were infected with methicillin-resistant *S. aureus* ATCC33591 to achieve a target inoculum of 5×10^5 CFU/thigh. Two hours prior to infection, mice received a single administration of 100 mg/kg aminobenzotriazole (ABT) orally to inhibit cytochrome P_{450} activity.[20] Groups of five animals each received an intraperitoneal injection of *R,S*-**7c** at the doses specified in Table 4, prepared in 5% dextrose with lactic acid pH 5.0, on a qd regime starting 2 h after infection. An additional group of five mice received vehicle alone. Efficacy was determined 24 h after the start of treatment. Thigh tissue was homogenized with an Omni TH homogenizer (Omni International,

Warranton, VA), plated onto tryptic soy agar plates, and incubated at 37 °C overnight for CFU/gram thigh determination.

Effects on Cardiac Repolarization Potential of the Guinea Pig in Vivo. Cardiac electrophysiological characterization of compounds was performed by the method described by Duker et al.[29] Male guinea pigs were anaesthetized with Nembutal and tracheotomized for mechanical ventilation. The carotid artery and jugular vein were cannulated, and to eliminate autonomic influence on the heart, a bilateral vagotomy was performed and propranolol (0.5 mg/kg) given intravenously. Needle electrodes were positioned for recording of lead II electrocardiogram. The chest was opened and a bipolar electrode was clipped to the left atrial appendage for cardiac pacing. A suction electrode for recording of the monophasic action potential (MAPD) was positioned on the left ventricular epicardial wall. Delay of cardiac repolarization is reflected in an electrocardiogram as an increase in the QT interval and can be indirectly measured as a prolongation of the ventricular monophasic action potential (MAP) duration. Compounds were infused intravenously with two 17 min infusion periods, and monophasic action potential was continuously recorded during cardiac pacing. During the first infusion period for **7a** (vehicle was water pH adjusted to 5 with lactic acid), 11.7 mg/kg was administered, while 29.4 mg/kg was delivered during the second infusion period. During first infusion period for *R,S*-**7c** (20% SBECD vehicle), 11.7 mg/kg was administered, while 14.7 mg/kg was delivered during the second infusion period. Blood samples were collected at each dose level for determination of plasma concentration.

General Chemical Methods. All commercially available solvents and reagents were used without further purification. All moisture-sensitive reactions were carried out under a nitrogen atmosphere in commercially available anhydrous solvents. Column chromatography was performed on 230−400 mesh silica gel 60. Aluminum-backed sheets of silica gel 60 F254 (EM Science) were used for TLC. Melting points were obtained with a Mel-TempII melting point apparatus from Laboratory Devices, Inc. and are uncorrected. ^{1}H NMR spectra were recorded at 300 or 400 MHz. Chemical shifts are reported in parts per million (δ) relative to solvent. The purity of tested compounds was assessed by LC-MS. Reverse phase HPLC was carried out using YMC Pack ODS-AQ (100 × 20 mm ID, S-5 μ particle size, 12 nm pore size) on Agilent instruments. Mass spectroscopy was performed using a Micromass Quattro Micro mass spectrometer (for ESP) and an Agilent 1100 MSD instrument (for APCI). All compounds tested possessed a purity of ≥95%.

2-Oxo-1,2-dihydroquinoline-7-carbonitrile (1c). A mixture of **10** (15.51 g, 82.42 mmol) and 1,8-diazabicyclo[5.4.0]undec-7ene (DBU) in acetonitrile (155 mL) was heated at 75 °C for 2.5 h. The reaction mixture was cooled to room temperature, and a precipitate was collected by filtration, washed with water (77 mL) and with methanol (77 mL), and dried under reduced pressure to give the product as an off-white solid, 9.71 g (68%): mp > 250 °C MS (ESP) m/z 171 (MH^+); ^{1}H NMR (DMSO-d_6) δ 6.67 (d, 1H); 7.48−7.68 (m, 2H); 7.85 (d, 1H); 7.98 (d, 1H); 12.01 (s, 1H).

***cis*(±)-2-{4-[(*tert*-Butoxycarbonyl)amino]-3-fluoropiperidin-1-yl}ethyl methanesulfonate (2b).** A mixture of **26** (314 mg, 1.2 mmol) in dry dichloromethane (5 mL) and triethylamine (0.236 mL, 1.7 mmol) was treated at 0 °C with methanesulfonyl chloride (0.111 mL, 1.44 mmol). After 90 min, potassium phosphate buffer (pH 7, 1 M, 5 mL) was added, dichloromethane was removed under reduced pressure, and it was extracted with ice-cold ethyl acetate (20 mL). The aqueous phase was back-extracted once with ethyl acetate (10 mL), and the combined organic phases were dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue was taken up in DMF (2 mL). This crude preparation of the mesylate was used without delay for the next step.

2-((3*S*,4*R*)-4-(*tert*-Butoxycarbonylamino)-3-fluoropiperidin-1-yl)ethylmethanesulfonate (2c). The compound was prepared from **24** using the same sequence of reactions as for the converion of racemic **21** to **2b**: MS (ESP) m/z 341 (MH^+).

2-{4-[(*tert*-Butoxycarbonyl)amino]-3-fluoropiperidin-1-yl}-ethyl methanesulfonate, *trans*-Enantiomer B (2d). **29** (2.0 g, 7.62 mmol), triethylamine (1.5 mL, 10.7 mmol), and methanesulfonyl chloride (0.71 mL, 9.15 mmol) were reacted following the procedure for **2b**. The crude product was used directly in the next step without further purification.

2-(*trans*(±)-4-[(*tert*-Butoxycarbonyl)amino]-3-{[*tert*-butyl-(dimethyl)silyl]oxy}piperidin-1-yl)ethyl methanesulfonate (2e). **46** (1.0 g, 2.7 mmol), triethylamine (0.52 mL, 3.74 mmol), and methanesulfonyl chloride (0.25 mL, 3.21 mmol) were reacted using the general procedure for **2**. The crude product was used directly in the next step without further purification.

2-{(3*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]-3-methoxypiperidin-1-yl}ethyl methanesulfonate (2f). **49** (540 mg, 1.97 mmol), triethylamine (0.38 mL, 2.76 mmol), and methanesulfonyl chloride (0.18 mL, 2.36 mmol) were reacted as described for **2b**. The crude preparation of the mesylate product in DMF was used without delay for the next step.

2-{*trans*(±)-4-[(*tert*-Butoxycarbonyl)amino]-3-methoxypiperidin-1-yl}ethyl methanesulfonate (2g). **52** (0.74 g, 2.7 mmol), triethylamine (0.53 mL, 3.78 mmol), and methanesulfonyl chloride (0.25 mL, 3.24 mmol) were reacted as described for **2b**. The crude preparation of the mesylate product in DMF was used without delay for the next step.

General Procedure for 3 by Alkylation of 1 with 2. A solution of **1** (2 mmol) in dry dimethylformamide (10 mL) was treated at 0 °C under stirring with sodium hydride (60% in oil, 2 mmol). The cooling bath was removed, and the mixture was stirred for 30 min at room temperature. Freshly prepared **2** in a solution with DMF (0.58 mmol/mL, 2 mmol) was added, and the resulting mixture was stirred overnight at room temperature. DMF was removed under reduced pressure, and the residue was taken up in ethyl acetate (100 mL) and saturated aqueous sodium hydrogencarbonate solution (30 mL). The aqueous phase was back-extracted once with ethyl acetate (50 mL). The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Generally, O-alkylation was observed as a minor product and removed by chromatography. Chromatography conditions are given below for individual compounds.

***tert*-Butyl-{1-[2-(7-bromo-2-oxoquinolin-1(2*H*)-yl)ethyl]-piperidin-4-yl}carbamate (3a).** Prepared from commercially available **1a** and **2a**[5] according to the general procedure for **3**. Chromatography on silica gel with hexanes/acetone (5:2) gave 9.87 g (66%) of the product as a colorless solid: mp 155 °C; MS (ESP) m/z 450/452 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.32 (m, 2H); 1.36 (s, 9H); 1.65 (m, 2H); 2.01 (t, 2H); 2.46 (m, 2H); 2.90 (m, 2H); 3.19 (m, 1H); 4.29 (t, 2H); 6.61 (d, 1H); 6.75 (d, 1H); 7.41 (d, 1H); 7.65 (d, 1H); 7.73 (br s, 1H); 7.89 (d, 1H).

***tert*-Butyl-{1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)ethyl]-piperidin-4-yl}carbamate (3b).** A mixture of **3a** (9.85 g, 21.9 mmol) and potassium cyanide (2.14 g, 32.8 mmol) in dry acetonitrile (60 mL) was degassed and flushed with nitrogen three times. Tributyltinchloride (0.059 mmol, 1.13 mL of a 51.6 mM solution in heptane) was added, followed by 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (63 mg, 0.11 mmol) and tris(dibenzylideneacetone)dipalladium(0) (100 mg, 0.11 mmol), and it was degassed and flushed with nitrogen like above. The mixture was stirred for 30 min at room temperature and then degassed and flushed with nitrogen again. It was heated at 85 °C for 20 h. The solvent was removed under reduced pressure and the residue taken up in dichloromethane (500 mL) and washed with water (200 mL). The aqueous phase was back-extracted once with dichloromethane (200 mL), and combined organic phases were dried over sodium sulfate. Solvent was removed under reduced pressure, and the residue was crystallized from acetonitrile (∼60 mL) to give the product as a colorless solid: 6.57 g (76%), mp 202 °C; MS (ESP) m/z 397 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 2H); 1.36 (s, 9H); 1.64 (m, 2H); 2.02 (m, 2H); 2.50 (m, 2H, under solvent peak); 2.90 (m, 2H); 3.15 (m, 1H); 4.34 (t, 2H); 6.74−6.78 (m, 2H); 7.63 (m, 1H); 7.89 (d, 1H); 7.99 (d, 1H); 8.05 (s, 1H).

***cis*(±)-*tert*-Butyl-{1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)-ethyl]-3-fluoropiperidin-4-yl}carbamate (3c).** Prepared from **1c** and **2b** according to the general procedure for **3**. Chromatography on silica gel with hexanes/ethyl acetate (2:3) gave the product as a colorless solid: 397 mg (73%); MS (ESP) m/z 415 (MH^+); ^{1}H NMR (DMSO-

d_6) δ 1.37 (s, 9H); 1.48 (m, 1H); 1.67 (m, 1H); 2.27 (m, 2H); 2.56 (m, 2H); 2.96 (m, 1H); 3.15 (m, 1H); 3.46 (m, 1H); 4.34 (m, 2H); 4.60 (m, 1H); 6.77 (m, 1H); 6.91 (m, 1H); 7.64 (m, 1H); 7.90 (m, 1H); 7.99 (m, 1H); 8.07 (s, 1H).

***tert*-Butyl-(3*S*,4*R*)-1-(2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)-ethyl)-3-fluoropiperidin-4-ylcarbamate (3c, Single Enantiomer).** Prepared from **1c** and **2c** according to the procedure for racemic **3c**: $[\alpha]_D$ = +0.063 (c = 0.2, DMSO).

***cis*(±)-*tert*-Butyl-{3-fluoro-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate (3d).** Prepared from **1b**[5] and **2b** according to the general procedure for **3**. Chromatography on silica gel with hexanes/ethyl acetate (2:3) gave 222 mg (51%) product as a solid: MS (ESP) m/z 421 (MH^+); ^{1}H NMR ($CDCl_3$-d) δ 1.44 (s, 9H); 1.86 (m, 2H); 2.40 (m, 2H); 2.80 (m, 2H); 3.15 (m, 1H); 3.41 (m, 1H); 3.70 (m, 1H); 3.94 (s, 3H); 4.42 (m, 2H); 4.70 (m, 2H); 6.93 (m, 2H); 7.77 (m, 1H); 8.11 (s, 1H).

***tert*-Butyl-{1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)ethyl]-3-fluoropiperidin-4-yl}carbamate, *trans*-Enantiomer B (3e).** Prepared from **1c** (0.5 g, 2.94 mmol) and **2d** (3.82 mmol) according to the general procedure for **3**. Chromatography on silica gel with a gradient of 10−50% acetone in hexanes gave 0.64 g (53%) of the product as an off-white solid: MS (ESP) m/z 415 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.25−1.43 (m, 11H); 1.67−1.78 (m, 1H); 2.04−2.17 (m, 2H); 2.57−2.68 (m, 2H); 2.80−2.89 (m, 1H); 3.25−3.32 (m, 1H); 4.27−4.47 (m, 2H); 4.30 (m, 1H); 6.78 (d, 1H); 6.99 (d, 1H); 7.66 (dd, 1H); 7.91 (d, 1H); 8.01 (d, 1H); 8.09 (s, 1H).

***tert*-Butyl-{3-fluoro-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate, *trans*-Enantiomer B (3f).** Prepared from **1b** (0.52 g, 2.95 mmol) and **2d** (3.82 mmol) according to the procedure for **3e**, to give 0.93 g (78%) of the product as an off-white solid: MS (ESP) m/z 421 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.23−1.45 (m, 11H); 1.64−1.80 (m, 1H); 2.04−2.19 (m, 2H); 2.61−2.71 (m, 2H); 2.84 (d, 1H); 3.25−3.33 (m, 1H); 3.92 (s, 3H); 4.27−4.43 (m, 2H); 4.28 (m, 1H); 16.94−7.05 (m, 3H); 7.75 (d, 1H); 8.04 (s, 1H).

***tert*-Butyl-{*trans*(±)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}-carbamate (3g).** **1b** (430 mg, 2.43 mmol), **2e** in DMF (~0.27 mmol/mL, 2.70 mmol), and sodium hydride (60% in oil, 110 mg, 2.70 mmol) were reacted using the general procedure for **3**. The crude product was purified by chromatography on silica gel, eluting with a gradient of 10−25% acetone in hexanes to give 600 mg (46%) of the product as an off-white solid: MS (ESP) m/z 533 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H); 0.79 (s, 9H); 1.33 (s, 9H); 1.37−1.47 (m, 1H); 1.49−1.59 (m, 1H); 1.87 (t, 1H); 1.95−2.07 (m, 1H); 2.55−2.66 (m, 2H); 2.77−2.89 (m, 1H); 2.92−3.02 (m, 1H); 3.11 (s, 1H); 3.30−3.40 (m, 1H); 3.89 (s, 3H); 4.17−4.41 (m, 2H); 6.57 (d, 1H); 6.91−7.04 (m, 2H); 7.72 (d, 1H); 8.01 (s, 1H).

***tert*-Butyl-{3-hydroxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate *trans*-Enantiomers (3h, Isomers 1 and 2).** A solution of **3g** (0.60 g, 1.13 mmol) in THF (20 mL) was treated at 0 °C with a solution of tetrabutylammonium fluoride in THF (1M, 2.2 mL). The reaction was stirred at room temperature for 2 h, then concentrated to dryness under reduced pressure. The crude residue was taken up in ethyl acetate and washed with water. The aqueous phase was re-extracted three times with ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica gel with 0−5% methanol in dichloromethane gave 0.27 g of the desired product and 0.12 g of an O-acetylated side product. The side product was taken up in methanol and treated with a catalytic amount of potassium carbonate. This was stirred at room temperature for 1 h, resulting in complete conversion to the alcohol. The reaction mixture was concentrated to dryness. The residue was partitioned between aqueous potassium phosphate buffer (pH = 7) and ethyl acetate. The aqueous phase was re-extracted 2× with ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered, and concentrated to dryness giving an additional 100 mg of the desired (79% total) of product: MS (ESP) m/z 419 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.18−1.33 (m, 1H); 1.38 (s, 9H); 1.63−1.77 (m, 1H); 1.86 (t, 1H); 1.97 (t, 1H); 2.54−2.64 (m, 2H); 2.80−2.93 (m, 1H); 2.96−3.09 (m, 2H); 3.23 (dd, 1H); 3.92 (s, 3H); 4.32 (t, 2H); 4.67 (d, 1H); 6.62 (d, 1H); 6.94−7.06 (m, 2H); 7.69−7.79 (m, 1H); 8.04 (s, 1H).

The mixture of enantiomers was separated by supercritical fluid chromatography on a Chiralpak AD-H column (250 × 21 mm, 5 μm) eluting with an isocratic gradient of 25% isopropyl alcohol/0.1% dimethylethylamine at a flow rate of 60 mL/min. This gave 130 mg of **3h**, isomer **1** (first eluting enantiomer) and 130 mg of **3h**, isomer **2** (second eluting enantiomer).

***tert*-Butyl-{(3*S*,4*R*)-1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)-ethyl]-3-methoxypiperidin-4-yl}carbamate (3i).** **1c** (370 mg, 2.20 mmol), **2f** in DMF (~0.24 mmol/mL, 2.40 mmol), and sodium hydride (60% in oil, 110 mg, 2.60 mmol) were reacted following the general procedure for **3**. Chromatography on silica gel with 25−35% acetone in hexanes gave 370 mg (39%) of the product as an off-white solid: MS (ESP) m/z 427 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H); 1.42−1.51 (m, 1H); 1.57−1.71 (m, 1H); 2.18−2.40 (m, 2H); 2.56 (t, 2H); 2.65−2.76 (m, 1H); 2.78−2.90 (m, 1H); 3.18 (s, 3H); 3.27 (s, 1H); 3.58 (s, 1H); 4.30−4.46 (m, 2H); 6.37 (d, 1H); 6.78 (d, 1H); 7.66 (dd, 1H); 7.91 (d, 1H); 8.01 (d, 1H); 8.09 (s, 1H).

***tert*-Butyl-{(3*S*,4*R*)-3-methoxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate (3j).** **1b** (320 mg, 1.79 mmol), **2f** in DMF (~0.20 mmol/mL, 1.97 mmol), and sodium hydride (60% in oil, 86 mg, 2.15 mmol) were reacted following the general procedure for **3**. Chromatography on silica gel with 15−25% acetone in hexanes gave 420 mg (55%) of the product as a colorless solid: MS (ESP) m/z 433 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H); 1.43−1.51 (m, 1H); 1.57−1.72 (m, 1H); 2.20−2.40 (m, 2H); 2.55−2.66 (m, 2H); 2.67−2.78 (m, 1H); 2.80−2.93 (m, 1H); 3.18 (s, 3H); 3.29 (s, 1H); 3.51−3.65 (m, 1H); 3.92 (s, 3H); 4.24−4.43 (m, 2H); 6.40 (d, 1H); 6.96−7.05 (m, 2H); 7.75 (d, 1H); 8.04 (s, 1H).

***tert*-Butyl-{1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)ethyl]-3-methoxypiperidin-4-yl}carbamate, *trans*-Enantiomer 1 (3k).** A mixture of **51** (0.63 g, 2.7 mmol), **34** (0.57 g, 2.7 mmol), and sodium triacetoxyborohydride (1.7 g, 8.1 mmol) were reacted following the procedure for **32** to give 0.74 g (62%) of the racemic mixture of the product: MS (ESP) m/z 427 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.19−1.33 (m, 1H); 1.37 (s, 9H); 1.64−1.73 (m, 1H); 1.77 (m, 1H); 1.99 (m, 1H); 2.59 (m, 2H); 2.79−2.87 (m, 1H); 2.93−3.04 (m, 1H); 3.05−3.15 (m, 1H); 3.23−3.30 (m, 1H); 3.28 (s, 3H); 4.30−4.47 (m, 2H); 6.79 (d, 2H); 7.66 (dd, 1H); 7.91 (d, 1H); 8.01 (d, 1H); 8.09 (s, 1H).

The mixture of enantiomers was separated by HPLC on a Chiralpak AD column (20 × 250 mm, 10 μm) with an isocratic gradient of 80% hexanes, 20% 1:1 ethanol/methanol, 0.1% diethylamine at a flow rate of 20 mL/min. This gave 0.28 g of **3k** (*trans*-enantiomer **1**) (second eluting peak, (+) isomer) and 0.32 g of *tert*-butyl-{1-[2-(7-cyano-2-oxoquinolin-1(2*H*)-yl)ethyl]-3-methoxypiperidin-4-yl}carbamate (first eluting peak, (−) isomer).

***tert*-Butyl-{3-methoxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate, (+) *trans*-Enantiomer 1 (3l).** **1b** (430 mg, 2.45 mmol), **2g** (~0.27 mmol/mL, 2.70 mmol), and sodium hydride (60% in oil, 110 mg, 2.70 mmol) were reacted using a the general procedure for **3**. The crude product was purified by flash chromatography eluting with a gradient of 15−35% acetone in hexanes to give 490 mg (45%) of the racemic mixture of the products as an off-white solid: MS (ESP) m/z 433 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.21−1.33 (m, 1H); 1.37 (s, 9H); 1.63−1.74 (m, 1H); 1.78 (t, 1H); 2.01 (t, 1H); 2.62 (t, 2H); 2.80−2.90 (m, 1H); 2.96−3.06 (m, 1H); 3.07−3.18 (m, 1H); 3.22−3.29 (m, 4H); 3.93 (s, 3H); 4.27−4.43 (m, 2H); 6.78 (d, 1H); 6.96−7.06 (m, 2H); 7.75 (d, 1H); 8.05 (s, 1H).

The mixture of enantiomers was separated by supercritical fluid chromatography on a Chiralpak AD-H column (250 × 21 mm, 5 μm) eluting with an isocratic gradient of 20% isopropyl alcohol/0.1% dimethylethylamine at a flow rate of 60 mL/min. This gave 190 mg of **3l** (second eluting compound, (+) *trans*-enantiomer **1**) and 190 mg of *tert*-butyl-{3-methoxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]-piperidin-4-yl}carbamate (first eluting peak, (−) isomer).

General Procedure for 4 by Deprotection of 3. A solution of **3** (16.6 mmol) in dichloromethane (100 mL) was treated with trifluoroacetic acid (40 mL) at 0 °C for 30 min. The solvent was removed under reduced pressure and the residue co-distilled once with

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dichloromethane, then taken up in dichloromethane (200 mL) and washed with saturated sodium hydrogencarbonate solution (50 mL, pH adjusted to 10 with sodium hydroxide). The aqueous phase was back-extracted with dichloromethane (3 × 100 mL) and dried over sodium sulfate. The combined organic phases were concentrated under reduced pressure to give the products.

1-[2-(4-Aminopiperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4a). Prepared from **3b** (6.57 g, 16.57 mmol) according to the general procedure for **4**, in quantitative yield: off-white solid, mp 138 °C; MS (ESP) m/z 297 (MH^+); ^1H NMR (DMSO-d_6) δ 1.13 (m, 2H); 1.48 (m, 1H); 1.62 (m, 2H); 2.01 (t, 2H); 2.50 (m, 2H, under solvent peak); 2.86 (m, 2H); 4.35 (t, 2H); 6.76 (d, 1H); 7.63 (d, 1H); 7.90 (d, 1H); 7.98 (d, 1H); 8.07 (s, 1H).

***cis*(±)-1-[2-(4-Amino-3-fluoropiperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4b).** Prepared from **3c** (397 mg, 0.95 mmol) according to the general procedure for **4**, except chloroform was used as the solvent and aqueous workup was omitted. **4c** was obtained as the trifluoroacetate salt in quantitative yield: MS (ESP) m/z 315 (MH^+).

1-(2-((3*S*,4*R*)-4-Amino-3-fluoropiperidin-1-yl)ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4b, Single Isomer). Prepared from chiral **3c**, according to the procedure for racemic **4b**: MS (ESP) m/z 315 (MH^+).

***cis*(±)-1-[2-(4-Amino-3-fluoropiperidin-1-yl)ethyl]-7-methoxyquinoxalin-2(1*H*)-one, Trifluoroacetic Acid Salt (4c).** **3d** (222 mg, 0.53 mmol) was reacted following the procedure for **4b**. The title compound was obtained in the form of a bis-trifluoroacetic acid salt in quantitative yield: MS (ESP) m/z 315 (MH^+).

1-{2-[4-Amino-3-fluoropiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile, *trans*-Enantiomer B (4d). Prepared from **3e** (300 mg, 0.72 mmol) according to the general procedure for **4** to give 0.25 g of the crude product as an oil: MS (ESP) m/z 315 (MH^+).

1-{2-[4-Amino-3-fluoropiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Enantiomer B (4e). Prepared from **3f** (330 mg, 0.78 mmol) according to the general procedure for **4** to give 0.27 g of the crude product as an oil: MS (ESP) m/z 321 (MH^+).

1-{2-[(3*R*,4*S*)-4-Amino-3-hydroxypiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4f, Isomer 1) and 1-{2-[(3*S*,4*R*)-4-Amino-3-hydroxypiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4f, Isomer 2). A mixture of **32** (0.545 g, 1.61 mmol) and triphenylphophine (0.507 g, 1.93 mmol) in acetonitrile/water (9:1, 50 mL) was stirred at room temperature for 6 days. The reaction mixture was concentrated to dryness under reduced pressure. Chromatography on silica gel with dichloromethane/methanol (6:1, containing 0.2% ammonium hydroxide) gave the racemic mixture of **4f** as a colorless hard foam: 0.452 g (90%); MS (ESP) m/z 313 (MH^+); ^1H NMR (DMSO-d_6) δ 1.48 (m, 2H); 2.20−2.40 (m, 2H); 2.45−2.61 (m, 4H); 2.72 (m, 1H); 3.44 (m, 1H); 4.35 (dd, 2H); 6.78 (d, 1H); 7.64 (d, 1H); 7.90 (d, 1H); 8.00 (d, 1H); 8.08 (d, 1H) (OH and NH_2 protons were exchanged with methanol).

The racemic mixture was separated on a Chiralpak AD column (250 × 20 mm, 10 μm) with 60% hexanes and 40% ethanol/methanol (1:1), containing 0.1% diethyl amine. Isomer **2** was eluting first, $[\alpha]_D$ = +45.5, followed by isomer **1**, $[\alpha]_D$ = −45.9 (in methanol/chloroform 1:1, c = 1).

1-{2-[(3*R*,4*S*)-4-Amino-3-hydroxypiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one (4g, Isomer 1) and 1-{2-[(3*S*,4*R*)-4-Amino-3-hydroxypiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one (4g, Isomer 2). A mixture of **41** (0.507 g, 1.47 mmol) and triphenylphophine (0.463 g, 1.77 mmol) in acetonitrile/water (9:1, 20 mL) was stirred at room temperature for 5 days. The reaction mixture was concentrated to dryness under reduced pressure. The residue was taken up in dichloromethane (5 mL) and chromatographed on silica gel with dichloromethane/methanol (6:1, containing 0.2% ammonium hydroxide) to give the racemic mixture of **4g** as a colorless hard foam (0.422 g, 90%): MS (ESP) m/z 319 (MH^+); ^1H NMR (DMSO-d_6) δ 1.48 (m, 2H); 1.89 (m, 1H); 2.28 (m, 1H); 2.37 (dd, 1H); 2.54−2.62 (m, 3H); 2.71 (m, 1H); 3.45 (m, 1H); 3.91 (s, 3H); 4.30 (dd, 2H); 6.96−7.00 (m, 2H); 7.73 (d, 1H); 8.03 (s, 1H) (OH and NH_2 protons were exchanged with methanol). The racemic mixture was separated on a Chiralpak AD column (250 × 20 mm, 10 μm) with ethanol/methanol (1:1), containing 0.1% diethyl amine. Isomer **2** was eluting first, $[\alpha]_D$ = +45.5, followed by isomer **1**, $[\alpha]_D$ = −44.7 (in methanol/chloroform 1:1, c = 1).

1-{2-[4-Amino-3-hydroxypiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Isomer 2 (4h). **3h**, isomer **2** (130 mg, 0.31 mmol) was reacted with trifluoroacetic acid following the general procedure for **4** to give 84 mg (85%) of the crude product as an off-white foam: MS (ESP) m/z 319 (MH^+).

1-{2-[(3*S*,4*R*)-4-Amino-3-methoxypiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile (4i). **3i** (370 mg, 0.87 mmol) was reacted with trifluoroacetic acid according to the general procedure for **4** give 300 mg (quant) of the crude product as an oil: MS (ESP) m/z 327 (MH^+).

1-{2-[(3*S*,4*R*)-4-Amino-3-methoxypiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one (4j). **3j** (420 mg, 0.97 mmol) was reacted with trifluoroacetic acid according to the general procedure for **4** to give 310 mg (97%) of the crude product as an oil: MS (ESP) m/z 333 (MH^+).

1-{2-[4-Amino-3-methoxypiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile, *trans*-Enantiomer 1 (4k). **3k** (280 mg, 0.66 mmol) was reacted with trifluoroacetic acid according to the general procedure for **4** to give 240 mg of the crude product as an oil: MS (ESP) m/z 327 (MH^+).

1-{2-[4-Amino-3-methoxypiperidin-1-yl]ethyl}-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Enantiomer 1 (4l). **3l** (190 mg, 0.44 mmol) was reacted with trifluoroacetic acid according to the general procedure for **4** to give 150 mg (quant) of the crude product as an oil: MS (ESP) m/z 333 (MH^+).

General Procedure for 7 and 8 by Reductive Amination of 4. A solution of **4** (0.20 mmol) and 1 equiv of aldehyde, either (2,3-dihydro[1,4]dioxino[2,3-*c*]pyridine-7-carbaldehyde **5**[10] for **7** or 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde **6**[11] for **8**, in dry chloroform/methanol (5 mL, 1:1) was heated over freshly activated 3 Å molecular sieves (pearled) at 70 °C for 3 h. The reaction mixture was cooled to 0 °C, and sodium triacetoxyborohydride (0.6 mmol) was added. The reaction mixture was stirred at room temperature for 30 min, then filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was taken up in dichloromethane (50 mL) and saturated aqueous sodium hydrogencarbonate solution (5 mL). The pH of the aqueous phase was adjusted to a pH of 10 with 1 M aqueous sodium hydroxide solution. The aqueous phase was back-extracted twice with dichloromethane (2 × 20 mL), and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure.

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile (7a). **4a** (70 mg, 0.24 mmol), **5**[10] (40 mg, 0.24 mmol), and sodium triacetoxyborohydride (150 mg, 0.75 mmol) were reacted as described in the general procedure for **7**. Chromatography on silica gel with dichloromethane/methanol (6:1) and crystallization from dichloromethane/ether/hexanes gave the monoacetate salt of the product as a colorless solid: 69 mg (58%), mp 130−135 °C; MS (ESP) m/z 446 (MH^+); ^1H NMR ($CDCl_3$-*d*) δ 1.19 (m, 2H); 1.73 (m, 2H); 1.89 (s, 3H); 2.00 (t, 2H); 2.34 (m, 1H); 2.51 (m, 2H, under solvent peak); 2.88 (m, 2H); 3.65 (s, 2H); 4.24−4.37 (m, 6H); 6.76 (d, 1H); 6.92 (s, 1H); 7.63 (dd, 1H); 7.90 (d, 1H); 7.97−8.00 (m, 2H); 8.07 (br s, 1H).

1-(2-{(3*R*,4*S*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-fluoropiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile (*R*,*S*-7c) and 1-(2-{(3*S*,4*R*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-fluoropiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile (*S*,*R*-7c). **4b** (0.95 mmol), **5**[10] (160 mg, 0.95 mmol), and sodium triacetoxyborohydride (600 mg, 2.8 mmol) were reacted as described in the general procedure for **7**, except *N*,*N*-diisopropylethylamine (15 equiv) was added together with **4b**. Reverse phase chromatography with water/acetonitrile/ammonium acetate afforded the product as a tan foam: 276 mg (62%); MS (ESP) m/z 464 (MH^+); ^1H NMR ($CDCl_3$-*d*) δ 1.86 (m, 2H); 2.34 (m, 3H); 2.71 (m, 2H); 2.82 (m, 1H); 3.03 (m, 1H); 3.30 (m, 1H); 3.93 (m, 2H); 4.30 (m, 4H); 4.42 (m, 2H); 4.90 (m,

1H); 6.80 (d, 1H); 6.90 (s, 1H); 7.45 (d, 1H); 7.65 (m, 2H); 7.78 (s, 1H); 8.09 (s, 1H).

The racemic mixture was separated on a Chiralpak AD, 250 × 20 mm, 10 μ column (50% methanol, 50% ethanol, 0.1% diethylamine). *R,S*-**7c** eluted first, $[\alpha]_D$ = +14.3 (*c* = 0.3, methanol) (89 mg), followed by *S,R*-**7c**, $[\alpha]_D$ = −11.6 (*c* = 0.328, methanol) (80 mg).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]-3-fluoropiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile, *trans*-Enantiomer B, Bis-hydrochloride Salt (7e). **4d**, **5**,[10] and sodium triacetoxyborohydride were reacted as described in the general procedure for **7**. Chromatography on silica gel with a gradient of 0−5% methanol in dichloromethane gave the free base of the title composition. This was taken up in 1:1 dichloromethane/diethyl ether (5 mL) and treated with 1.0 M HCl in ether (∼2 equiv), resulting in a precipitate. This mixture was concentrated to dryness, and the resulting solid was reconstituted in water and lyophilized to give the title compound: MS (ESP) *m/z* 464 (MH^+); ^{1}H NMR (D_2O) δ 1.81−1.94 (m, 1H); 2.30−2.43 (m, 1H); 3.13−3.26 (m, 1H); 3.32−3.42 (m, 1H); 3.42−3.50 (m, 1H); 3.53 (t, 2H); 3.57−3.66 (m, 1H); 3.79−3.93 (m, 1H); 4.11−4.27 (m, 2H); 4.38−4.43 (m, 2H); 4.50−4.56 (m, 2H); 4.62−4.75 (m, 1H); 4.80−4.88 (m, 1H); 4.85 (m, 1H); 6.85 (d, 1H); 7.25 (s, 1H); 7.68 (dd, 1H); 7.89 (d, 1H); 7.98 (s, 1H); 8.03 (d, 1H); 8.20 (s, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]-3-fluoropiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Enantiomer B, Bis-hydrochloride Salt (7f). **4e**, **5**,[10] and sodium triacetoxyborohydride were reacted as described in the procedure for **7e** to give the title compound as the bis-hydrochloride salt: MS (ESP) *m/z* 470 (MH^+); ^{1}H NMR (D_2O) δ 1.81−1.97 (m, 1H); 2.32−2.45 (m, 1H); 3.20−3.32 (m, 1H); 3.35−3.44 (m, 1H); 3.52−3.72 (m, 4H); 3.91 (s, 3H); 3.85−3.95 (m, 1H); 4.13−4.28 (m, 2H); 4.37−4.43 (m, 2H); 4.51−4.56 (m, 2H); 4.57−4.73 (m, 2H); 4.94 (m, 1H); 6.90 (d, 1H); 7.10 (dd, 1H); 7.30 (s, 1H); 7.78 (d, 1H); 8.03 (s, 1H); 8.22 (s, 1H).

1-(2-{(3*R*,4*S*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-hydroxypiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile, Bis-hydrochloride Salt (*R,S*-7g). **4f** (3*R*,4*S* isomer) (74 mg, 0.24 mmol), **5**[10] (39 mg, 0.24 mmol), and sodium triacetoxyborohydride (150 mg, 0.75 mmol) were reacted as described in the general procedure for **7**. Chromatography was done on silica gel with dichloromethane/methanol (8:1 to 4:1). Fractions containing product were pooled and concentrated to dryness. The residue was taken up in dichloromethane/diethyl ether (1:2, 10 mL), and HCl in diethyl ether (2 M, ∼0.15 mL) was added. The mixture was concentrated to dryness under reduced pressure, co-distilled two times with dichloromethane (2 × 15 mL), and triturated from ether to give the title composition as a colorless solid: 91 mg (72%), mp >210 °C; MS (ESP) *m/z* 462 (MH^+); ^{1}H NMR (DMSO-d_6) δ 2.18 (m, 2H); 3.15 (m, 1H); 3.25−3.36 (m, 4H); 3.69 (m, 2H); 4.10−4.49 (m, 7H); 4.61 (dd, 2H); 6.64 (br s, 1H); 6.83 (d, 1H); 7.30 (s, 1H); 7.72 (d, 1H); 7.97 (d, 1H); 8.08 (d, 1H); 8.22 (m, 2H); 9.45 (br s, 2H); 10.00 (br s, 1H).

1-(2-{(3*S*,4*R*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-hydroxypiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile, Bis-hydrochloride Salt (*S,R*-7g). **5**[10] and **4f** (3*S*,4*R* isomer) were reacted using the procedure for *R,S*-**7g**: MS (ESP) *m/z* 462 (MH^+).

1-(2-{(3*R*,4*S*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-hydroxypiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, Bis-hydrochloride Salt (*R,S*-7h). **5**[10] and **4g** (3*R*,4*S* isomer) were reacted following the procedure for **7g**: MS (ESP) *m/z* 468 (MH^+); ^{1}H NMR (D_2O) δ 2.28 (m, 2H); 3.20 (ddd, 1H); 3.31 (m, 1H); 3.45−3.76 (m, 4H); 3.92 (s, 3H); 3.99 (m, 1H); 4.29−4.62 (m, 8H); 4.83 (m, 1H); 6.93 (m, 1H); 7.12 (m, 1H); 7.22 (d, 1H); 7.81 (dd, 1H); 8.06 (d, 1H); 8.19 (d, 1H).

1-(2-{(3*S*,4*R*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-hydroxypiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, Bis-hydrochloride Salt (*S,R*-7h). **5**[10] and **4g** (3*S*,4*R* isomer) were reacted following the procedure for **7g**: MS (ESP) *m/z* 468 (MH^+).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]-3-hydroxypiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Enantiomer B, Bis-hydrochloride Salt (7i). **4h** (42 mg, 0.13 mmol) and **5**[10] (22 mg, 0.13 mmol) were reacted with sodium triacetoxyborohydride (83 mg, 0.39 mmol) as described in the general procedure for **7g** to give 45 mg of the product as a yellow solid: MS (ESP) *m/z* 467 (MH^+); ^{1}H NMR (D_2O) δ 1.82−1.99 (m, 1H); 2.42−2.53 (m, 1H); 3.01 (t, 1H); 3.08−3.20 (m, 1H); 3.32−3.44 (m, 1H); 3.58 (t, 2H); 3.80−3.95 (m, 5H); 3.96−4.09 (m, 1H); 4.32−4.50 (m, 6H); 4.65 (t, 2H); 6.87 (d, 1H); 7.07 (dd, 1H); 7.29 (s, 1H); 7.76 (d, 1H); 8.00 (s, 1H); 8.21 (s, 1H).

1-(2-{(3*S*,4*R*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-methoxypiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile, Bis-hydrochloride Salt (*S,R*-7j). **4i** (93 mg, 0.29 mmol), **5**[10] (47 mg, 0.29 mmol), and sodium triacetoxyborohydride (180 mg, 0.86 mmol) were reacted according to the procedure for **7g** to give 95 mg of the product as a colorless solid: MS (ESP) *m/z* 476 (MH^+); ^{1}H NMR (D_2O) δ 2.14−2.33 (m, 2H); 3.12−3.27 (m, 2H); 3.42−3.49 (m, 3H); 3.54−3.62 (m, 2H); 3.63−3.76 (m, 2H); 4.09 (s, 1H); 4.25 (d, 1H); 4.32−4.39 (m, 4H); 4.43−4.50 (m, 2H); 4.50−4.65 (m, 1H); 4.83 (d, 1H); 6.80 (d, 1H); 7.29 (s, 1H); 7.61 (dd, 1H); 7.82 (d, 1H); 7.91 (s, 1H); 7.97 (d, 1H); 8.22 (s, 1H).

1-(2-{(3*S*,4*R*)-4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)amino]-3-methoxypiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, Bis-hydrochloride Salt (*S,R*-7k). **4j** (160 mg crude, 0.48 mmol), **5**[10] (80 mg, 0.48 mmol), and sodium triacetoxyborohydride (310 mg, 1.44 mmol) were reacted following the general procedure for **7**. Chromatography on silica gel with a gradient of 2−5% methanol in dichloromethane containing 0.25% ammonium hydroxide gave 160 mg (70%) of the free base of the title composition as an oil. This was taken up in dichloromethane/diethyl ether (1:1, 5 mL) and treated with 2.0 M HCl in ether (∼2 equiv). The resulting precipitate was collected by filtration, reconstituted in water, and lyophilized to give 148 mg of the of the bis-hydrochloride salt of the product as a solid: MS (ESP) *m/z* 482 (MH^+); ^{1}H NMR (D_2O) δ 2.04−2.33 (m, 2H); 3.05−3.25 (m, 2H); 3.44 (s, 3H); 3.50−3.71 (m, 3H); 3.87 (s, 3H); 4.04 (s, 1H); 4.21 (d, 3H); 4.26−4.32 (m, 3H); 4.33−4.40 (m, 2H); 4.45−4.58 (m, 1H); 4.74−4.87 (m, 1H); 6.82−6.92 (m, 1H); 7.02−7.12 (m, 2H); 7.75 (d, 1H); 8.01 (s, 1H); 8.09 (s, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]-3-methoxypiperidin-1-yl}ethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile, *trans*-Enantiomer 1, Bis-hydrochloride Salt (7l). **4k** (105 mg crude, 0.32 mmol), **5**[10] (53 mg, 0.32 mmol), and sodium triacetoxyborohydride (205 mg, 0.97 mmol) were reacted following the procedure for *S,R*-**7k** to give 63 mg of the title composition as a solid: MS (ESP) *m/z* 476 (MH^+); ^{1}H NMR (D_2O) δ 1.84−2.00 (m, 1H); 2.37−2.49 (m, 1H); 2.93−3.06 (m, 1H); 3.10−3.23 (m, 1H); 3.33−3.44 (m, 4H); 3.53−3.80 (m, 4H); 4.19 (d, 1H); 4.27−4.40 (m, 4H); 4.40−4.48 (m, 2H); 4.62−4.76 (m, 2H); 6.79 (d, 1H); 7.22 (s, 1H); 7.62 (dd, 1H); 7.83 (d, 1H); 7.91 (s, 1H); 7.97 (d, 1H); 8.14−8.20 (m, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]-3-methoxypiperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one, *trans*-Enantiomer 1, Bis-hydrochloride Salt (7m). **4l** (75 mg, 0.23 mmol), **5**[10] (37 mg, 0.23 mmol), and sodium triacetoxyborohydride (150 mg, 0.69 mmol) were reacted following the procedure for **7g** to give 63 mg of the product as a yellow solid: MS (ESP) *m/z* 482 (MH^+); ^{1}H NMR (D_2O) δ 1.81−2.02 (m, 1H); 2.34−2.52 (m, 1H); 2.92−3.05 (m, 1H); 3.10−3.23 (m, 1H); 3.37−3.49 (m, 4H); 3.54−3.72 (m, 3H); 3.73−3.82 (m, 1H); 3.85−3.90 (m, 3H); 4.22 (d, 1H); 4.29−4.41 (m, 4H); 4.43−4.51 (m, 2H); 4.60−4.80 (m, 2H); 6.83−6.91 (m, 1H); 7.07 (dd, 1H); 7.26−7.31 (m, 1H); 7.74 (d, 1H); 7.97−8.03 (m, 1H); 8.17−8.26 (m, 1H).

6-[({1-[2-(7-Methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]-piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (8b). 1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxyquinoxalin-2(1*H*)-one (60 mg, 0.20 mmol),[5] **6**[11] (36 mg, 0.20 mmol), and sodium triacetoxyborohydride (130 mg, 0.60 mmol) were reacted as described in the general procedure for **8**. Chromatography on silica gel with 5% methanol in dichloromethane containing 0.25% ammonium hydroxide and crystallization from dichloromethane/ethyl acetate gave the free base of the product as a colorless solid: 45 mg (50%); MS (ESP) *m/z* 465 (MH^+); ^{1}H NMR ($CDCl_3$-*d*) δ 1.06−1.39 (m, 2H); 1.66−1.87

(m, 2H); 2.04 (t, 2H); 2.33−2.49 (m, 1H); 2.56 (t, 2H); 2.92 (d, 2H); 3.70 (s, 2H); 3.92 (s, 3H); 4.32 (t, 2H); 4.61 (s, 2H); 6.86−7.09 (m, 3H); 7.30 (d, 1H); 7.75 (d, 1H); 8.04 (s, 1H); 11.18 (s, 1 H).

1-[2-((3*S*,4*R*)-3-Fluoro-4-{[(3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile (8c). 1-{2-[(3*S*,4*R*)-4-Amino-3-fluoropiperidin-1-yl]ethyl}-2-oxo-1,2-dihydroquinoline-7-carbonitrile trifluoroacetate, **4b** (3*S*4*R* isomer) (1.2 mmol), prepared following the procedure for racemic **4b**, except instead of racemic **2b**, the single enantiomer **2c** was used, was suspended in chloroform/methanol (1:2, 30 mL) and neutralized by dropwise addition of *N*,*N*-diisopropylethylamine. To this solution were added **6**[11] (258 mg, 1.45 mmol) and sodium triacetoxyborohydride (512 mg, 2.42 mmol), and the reaction was preformed as described in the general procedure for **8**. Reverse phase chromatography with water/acetonitrile/ammonium acetate afforded the product as an off-white solid after lyophilization. The hydrochloride salt was prepared by dissolving the lyopholization product in dichloromethane (5 mL) and adding 2.2 equiv of 4 N HCl in dioxane, which gave a colorless solid: 154 mg (26%); MS (ESP) *m*/*z* 477 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.61 (m, 2H); 2.20 (m, 2H); 2.52 (m, 4H); 3.04 (m, 2H); 3.72 (s, 2H); 4.34 (m, 2H); 4.59 (s, 2H); 4.82 (m, 1H); 6.67 (d, 1H); 7.01 (d, 1H); 7.28 (d, 1H); 7.64 (d, 1H); 7.89 (d, 1H); 8.00 (d, 1H); 8.09 (s, 1H); 11.19 (s, 1H).

6-[({(3*R*,4*S*)-3-Fluoro-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one, Bis-hydrochloride Salt (*R*,*S*-8d) and 6-[({(3*S*,4*R*)-3-Fluoro-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one, Bis-hydrochloride Salt (*S*,*R*-8d). A solution of **4c** (0.53 mmol) in dichloroethane/methanol (1:1, 20 mL) was neutralized with *N*,*N*-diisopropylethylamine. **6**[11] (113 mg, 0.63 mmol) was added, and the reaction was stirred at reflux over 3 Å molecular sieves overnight. The reaction mixture was cooled to 0 °C, and sodium cyanoborohydride (40 mg, 0.63 mmol) was added. The mixture was stirred at room temperature for 2 h, then filtered through a fritted funnel and concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated sodium bicarbonate followed by saturated sodium chloride. The organic extracts were dried over magnesium sulfate and concentrated under reduced pressure. Reverse phase chromatography with water/methanol/trifluoroacetic acid yielded the product as trifluoroacetic acid salt. The salt was dissolved in water and chloroform and basified with saturated sodium carbonate. The layers were separated, and the aqueous phase was extracted with chloroform. The combined organic phases were dried over magnesium sulfate and concentrated under reduced pressue to give a racemic mixture of the free bases of the title compositions as a solid: 26 mg (10%); MS (ESP) *m*/*z* 483 (MH^+); ^{1}H NMR ($CDCl_3$-*d*) δ 1.70 (m, 4H); 2.35 (m, 2H); 2.64 (m, 1H); 2.73 (m, 2H); 3.04 (m, 1H); 3.31 (m, 1H); 3.86 (s, 2H); 3.92 (s, 3H); 4.35 (m, 2H); 4.63 (s, 2H); 4.83 (m, 1H); 6.86 (m, 1H); 6.92 (m, 1H); 6.98 (m, 1H); 7.21 (m, 1H); 7.77 (m, 1H); 8.11 (s, 1H). The racemic mixture was separated by chiral chromatography (HPLC, Chiralcel OJ, 250 × 20 mm, 10 μ mobile phase: 50% hexane, 25% ethanol, 25% methanol, 0.1% diethylamine). The free base of the 3*S*,4*R* enantiomer eluted first. The hydrochloride salts of both enantiomers were prepared by dissolving the free bases in dichloromethane (2 mL) and adding 2.2 equiv of 4 N HCl in dioxane. The resulting colorless precipitates were collected by filtration and dried to give the HCl salts of the enantiomers as colorless solids.

6-[({3-Fluoro-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one, *trans*-Enantiomer B (8f). **4e** and **6**[11] were reacted as described in the procedure for **8b**: MS (ESP) *m*/*z* 483 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.14−1.29 (m, 1H); 1.82−1.95 (m, 1H); 2.04−2.20 (m, 2H); 2.52−2.61 (m, 1H); 2.60−2.70 (m, 2H); 2.78−2.89 (m, 1H); 3.16−3.27 (m, 1H); 3.67−3.80 (m, 2H); 3.88−3.96 (m, 3H); 4.29−4.43 (m, 2H); 4.30 (m, 1H); 4.61 (s, 2H); 6.96−7.06 (m, 3H); 7.30 (d, 1H); 7.75 (d, 1H); 8.00−8.09 (m, 1H); 11.19 (s, 1H).

1-[2-((3*R*,4*S*)-3-Hydroxy-4-{[(3-oxo-3,4-dihydro-2*H*-pyrido-[3,2-*b*][1,4]oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile, Bis-hydrochloride Salt (*R*,*S*-8g). **6**[11] and **4f** (3*R*,4*S* isomer) were reacted as described for **7g** to give the title compound: MS (ESP) *m*/*z* 475 (MH^+); ^{1}H NMR (D_2O) δ 2.28 (m, 2H); 3.15 (ddd, 1H); 3.26 (m, 1H); 3.40−3.72 (m, 4H); 3.91 (m, 1H); 4.28 (s, 2H); 4.52 (m, 1H); 4.61 (m, 1H); 4.71 (s, 2H); 4.80 (m, 1H); 6.65 (d, 1H); 7.08 (d, 1H); 7.35 (d, 1H); 7.66 (d, 1H); 7.87 (d, 1H); 7.96 (s, 1H); 8.02 (d, 1H).

1-[2-((3*S*,4*R*)-3-Hydroxy-4-{[(3-oxo-3,4-dihydro-2*H*-pyrido-[3,2-*b*][1,4]oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile, Bis-hydrochloride Salt (*S*,*R*-8g). **6**[11] and **4f** (3*S*4*R* isomer) were reacted as described for **7g** to give the title compound: MS (ESP) *m*/*z* 475 (MH^+).

6-[({(3*R*,4*S*)-3-Hydroxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*]-[1,4]oxazin-3(4*H*)-one, Bis-hydrochloride Salt (*R*,*S*-8h). **6**[11] and **4g** (3*R*,4*S* isomer) were reacted as described for **7g** to give the title compound: MS (ESP) *m*/*z* 481 (MH^+); ^{1}H NMR (DMSO-d_6) δ 2.19 (m, 2H); 3.19 (m, 1H); 3.30−3.48 (m, 4H); 3.62−3.83 (m, 2H); 3.96 (s, 3H); 4.18 (m, 2H); 4.54−4.76 (m, 3H); 4.69 (s, 2H); 6.54 (m, 1H); 7.04 (dd, 1H); 7.21 (d, 1H); 7.26 (d, 1H); 7.44 (d, 1H); 7.79 (d, 1H); 8.09 (s, 1H); 9.28 (m, 1H); 9.61 (m, 1H); 10.26 (m, 1H); 11.41 (s, 1H).

6-[({(3*S*,4*R*)-3-Hydroxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*]-[1,4]oxazin-3(4*H*)-one, Bis-hydrochloride Salt (*S*,*R*-8h). **6**[11] and **4g** (3*S*,4*R* isomer) were reacted as described for **7g** to give the title compound: MS (ESP) *m*/*z* 481 (MH^+); ^{1}H NMR (D_2O) δ 2.28 (m, 2H); 3.17 (ddd, 1H); 3.27 (m, 1H); 3.51−3.75 (m, 4H); 3.91 (s, 3H); 3.99 (m, 1H); 4.28 (m, 2H); 4.50−4.62 (m, 2H); 4.71 (s, 2H); 4.80 (m, 1H); 6.90 (d, 1H); 7.08 (d, 1H); 7.11 (dd, 1H); 7.34 (d, 1H); 7.79 (d, 1H); 8.05 (s, 1H).

1-[2-((3*S*,4*R*)-3-Methoxy-4-{[(3-oxo-3,4-dihydro-2*H*-pyrido-[3,2-*b*][1,4]oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile (*S*,*R*-8j). **4i** (93 mg, 0.29 mmol), **6**[11] (51 mg, 0.29 mmol), and sodium triacetoxyborohydride (180 mg, 0.86 mmol) were reacted using the general procedure for **8** to give 80 mg (57%) of the title compound as an off-white solid: MS (ESP) *m*/*z* 489 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.36−1.51 (m, 1H); 1.59−1.75 (m, 1H); 2.23−2.37 (m, 1H); 2.56 (t, 2H); 2.60−2.80 (m, 3H); 3.17 (s, 3H); 3.26−3.32 (m, 2H); 3.68 (q, 2H); 4.25−4.45 (m, 2H); 4.61 (s, 2H); 6.78 (d, 1H); 6.99 (d, 1H); 7.29 (d, 1H); 7.65 (dd, 1H); 7.91 (d, 1H); 8.00 (d, 1H); 8.10 (s, 1H); 11.20 (s, 1H).

6-[({(3*S*,4*R*)-3-Methoxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*]-[1,4]oxazin-3(4*H*)-one (*S*,*R*-8k). **4j** (160 mg, 0.48 mmol), **6**[11] (85 mg, 0.48 mmol), and sodium triacetoxyborohydride (310 mg, 1.44 mmol) were reacted as described in the general procedure for **8** to give 139 mg (58%) of the product: MS (ESP) *m*/*z* 495 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.36−1.53 (m, 1H); 1.59−1.77 (m, 1H); 2.24−2.37 (m, 1H); 2.40−2.46 (m, 1H); 2.60 (t, 2H); 2.65−2.84 (m, 2H); 3.14−3.21 (m, 3H); 3.30−3.34 (m, 2H); 3.67 (q, 2H); 3.92 (s, 3H); 4.22−4.45 (m, 2H); 4.61 (s, 2H); 6.92−7.10 (m, 3H); 7.30 (d, 1H); 7.75 (d, 1H); 8.04 (s, 1H); 11.15−11.27 (m, 1H).

1-[2-(3-Methoxy-4-{[(3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*]-[1,4]oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile, *trans*-Enantiomer 1 (8l). **4k** (105 mg, 0.32 mmol), **6**[11] (57 mg, 0.32 mmol), and sodium triacetoxyborohydride (205 mg, 0.97 mmol) were reacted as described in the general procedure for **8**. Chromatography on silica gel with a gradient of 2−10% methanol in dichloromethane containing 0.25% ammonium hydroxide gave 88 mg (56%) of the title compound as an off-white solid: MS (ESP) *m*/*z* 489 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.00−1.15 (m, 1H); 1.76 (m, 1H); 1.87 (d, 1H); 1.99 (m, 1H); 2.22−2.34 (m, 1H); 2.59 (m, 2H); 2.78−2.96 (m, 2H); 3.25−3.33 (m, 2H); 3.30 (s, 3H); 3.55−3.77 (m, 2H); 4.30−4.50 (m, 2H); 4.61 (s, 2H); 6.79 (d, 1H); 6.97 (d, 1H); 7.28 (d, 1H); 7.66 (dd, 1H); 7.91 (d, 1H); 8.01 (d, 1H); 8.12 (s, 1H); 11.21 (s, 1H).

6-[({3-Methoxy-1-[2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one, *trans*-Enantiomer 1 (8m). **4l** (75 mg, 0.23 mmol), **6**[11] (40 mg, 0.23 mmol), and sodium triacetoxyborohydride (150 mg, 0.69 mmol) were reacted as described in the general procedure for **8** to give 73 mg (66%) of the product: $[\alpha]_D$ = +17.5 (methanol, *c* = 0.56); MS (ESP) *m*/*z* 495 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.04−1.22

(m, 1H); 1.78 (t, 1H); 1.83−1.93 (m, 1H); 2.00 (m, 1H); 2.22−2.35 (m, 1H); 2.62 (t, 2H); 2.81−3.00 (m, 2H); 3.20−3.30 (m, 4H); 3.56−3.78 (m, 2H); 3.92 (s, 3H); 4.26−4.44 (m, 2H); 4.61 (s, 2H); 6.92−7.08 (m, 3H); 7.29 (d, 1H); 7.75 (d, 1H); 8.05 (s, 1H); 11.21 (s, 1H).

3-Hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline-7-carbonitrile (10). Ethyl 3-(4-cyano-2-nitrophenyl)-2-oxopropanoate **9**[24] (6.5 kg, 24.8 mol) and acetonitrile (21 L) were stirred at 22 °C, sodium borohydride (0.30 kg, 7.9 mol) was added in portions, and the mixture was then stirred for 1 h at 24 °C. Acetic acid (65 L) was charged to the solution, and the internal temperature was raised to 65 °C. Iron (3.3 kg) was added to the solution in portions (6 × 0.5 kg) over 1 h. After a further 1 h, the product was isolated by filtration, washed sequentially with water (3 × 25 L) and ethanol (29 L), and dried under reduced pressure to give the product as a beige solid: 3.07 kg (66%); mp >250 °C; MS (ESP) m/z 189 (MH^+); ^{1}H NMR (DMSO-d_6) δ 2.90−3.20 (m, 2H); 4.10−4.20 (m, 1H); 5.65 (d, 1H); 7.15 (s, 1H); 7.35−7.45 (m, 2H); 10.38 (s, 1H).

***cis*(±)-*tert*-Butyl-4-{benzyl[(benzyloxy)carbonyl]amino}-3-fluoropiperidine-1-carboxylate (14).** To a mixture of **11**[13] (1.1 g, 3.6 mmol) in dioxane (20 mL) and saturated sodium carbonate (10 mL) at 0 °C was added dropwise benzyl chloroformate (0.76 mL, 5.4 mmol), and the reaction mixture was stirred at 0 °C for 1 h. Ethyl acetate (20 mL) and brine (20 mL) were added, and the layers were separated. The aqueous phase was extracted once with ethyl acetate, and the combined organic phases were dried over magnesium sulfate and concentrated under reduced pressure to give the product as a colorless solid: 1.4 g (89%); MS (ESP) m/z 343 (MH^+-Boc). ^{1}H NMR ($CDCl_3$-d) δ 1.46 (s, 9H); 1.46 (m, 1H); 2.00 (m, 1H); 2.91 (m, 2H); 4.33 (m, 4H); 4.86 (m, 2H); 5.16 (m, 2H); 7.28 (m, 10H).

(3*S*,4*R*)-*tert*-Butyl-4-(benzyloxycarbonylamino)-3-fluoropiperidine-1-carboxylate (15). To a mixture of (3*S*,4*R*)-*tert*-butyl-4-amino-3-fluoropiperidine-1-carboxylate **12**[14,15] (5.1 g, 23.37 mmol) in dioxane (150 mL) and saturated sodium carbonate (50 mL) at 0 °C was added benzyl chloroformate (5.00 mL, 35.05 mmol). After 15 min, the reaction mixture was diluted with ethyl acetate and saturated sodium chloride. The layers were separated, and the organic extracts were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. Chromatography on silica gel with 0−50% ethyl acetate in hexanes gave the product as an off-white solid: 8 g (97%); MS (ESP) m/z 353 (MH^+); ^{1}H NMR ($CDCl_3$-d) δ 1.44 (m, 9H); 1.73 (m, 2H); 2.80 (m, 2H); 3.60 (m, 1H); 4.30 (m, 2H); 4.65 (m, 1H); 5.06 (m, 1H); 5.09 (s, 2H); 7.34 (m, 5H).

***trans*(±)-*tert*-Butyl-4-{benzyl[(benzyloxy)carbonyl]amino}-3-fluoropiperidine-1-carboxylate (16).** To a solution of trans(±)-*tert*-butyl-(4-benzylamino)-3-fluoropiperidine-1-carboxylate **13**[13] (10.3 g, 33.4 mmol) in 1,4-dioxane (100 mL) and sodium carbonate (5.31 g, 50.1 mmol) in water (20 mL) was added benzyl chloroformate (5.89 mL, 41.8 mmol) dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was then concentrated to near dryness and diluted with ethyl acetate. The organic phase was washed with water and brine, then dried over sodium sulfate. Chromatography on silica with 20% ethyl acetate in hexanes gave the product as a solid (12.5 g, 94%): MS (ESP) m/z 343 (MH^+-Boc); ^{1}H NMR ($CDCl_3$-d) δ 1.45 (s, 9H); 1.67 (d, 2H); 1.84 (m, 1H); 2.59−2.75 (m, 2H); 3.91−4.07 (m, 2H); 4.48 (d, 2H); 4.63 (d, 1H); 5.18 (s, 2H); 7.20−7.34 (m, 10H).

***cis*(±)-Benzylbenzyl[1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-ethyl)-3-fluoropiperidin-4-yl]carbamate (17).** To a solution of **14** (6 g, 13.5 mmol) in dichloromethane (50 mL) at 0 °C was added 4 N HCl in dioxane (6.8 mL). The reaction mixture was stirred at room temperature overnight. The precipitate was collected by filtration to afford the product as a colorless solid: 4.4 g (86%): MS (ESP) m/z 343 (MH^+).

Benzyl-(3*S*,4*R*)-3-fluoropiperidin-4-ylcarbamate, Hydrochloride Salt (18). To solution of **15** (8 g, 22.7 mmol) in dichloromethane (200 mL) at 0 °C was added 4 M hydrogen chloride in dioxane (11.35 mL, 45.4 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. Another equivalent of 4 M hydrogen chloride in dioxane was added, and the reaction was stirred for another 4 h. The resulting colorless precipitate was collected by filtration and dried under reduced pressure to give 5.9 g of the product (90%): MS (ESP) m/z 253 (MH^+).

***trans*-(±)-Benzylbenzyl(3-fluoropiperidin-4-yl)carbamate hydrochloride (19).** To a solution of **16** (12.05 g, 28.2 mmol) in dichloromethane (50 mL) at 0 °C was added hydrogen chloride (1 M in diethyl ether, 56.5 mL, 56.5 mmol). The mixture was stirred at room temperature for 1 h. The solid was isolated by filteration and washed with diethyl ether to give the mono-hydrochloride salt of the product (10.1 g, 95%): MS (ESP) m/z 343 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.68 (m, 1H); 2.00−2.15 (m, 1H); 3.08 (m, 1H); 3.18 (m, 1H); 3.34 (m, 2H); 3.50 (m, 1H); 4.34−4.49 (m, 2H); 4.65 (m, 1H); 5.02 (s, 1H); 5.14 (d, J = 19.40 Hz, 2H); 7.15−7.30 (m, 8H); 7.32 (m, 2H).

***cis*(±)-Benzylbenzyl[1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-ethyl)-3-fluoropiperidin-4-yl]carbamate (20).** A mixture of **17** (4.3 g, 6.1 mmol), (2-bromoethoxy)-*tert*-butyldimethylsilane (9.8 mL, 45.7 mmol), and cesium carbonate (9.9 g, 30.4 mmol) in acetonitrile (150 mL) was heated at 60 °C overnight. The reaction mixture was filtered and concentrated under reduced pressure. Chromatography on silica gel with hexanes/ethyl acetate (3:2) afforded the product as a colorless oil: 5.2 g (91%); MS (ESP) m/z 501 (MH^+).

***cis*(±)-1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-3-fluoropiperidin-4-amine (21). 20** (5.2 g, 10.4 mmol) was hydrogenated in anhydrous methanol (15 mL) on palladium hydroxide 20 wt % on carbon (31 mg) for 24 h, then filtered through Celite and concentrated under reduced pressure to give the product as a colorless oil: 2.8 g (98%); MS (ESP) m/z 277 (MH^+).

***cis*(±)-*tert*-Butyl-[1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-3-fluoropiperidin-4-yl]carbamate (22). 21** (2.8 g, 10.4 mmol) and di-*tert*-butyldicarbonate (3.4 g, 15.6 mmol) were combined in tetrahydrofuran (50 mL) at room temperature. After 90 min, the reaction mixture was concentrated under reduced pressure. Chromatography on silica gel with hexanes/ethyl acetate (3:2) afforded the product as a colorless oil: 3.2 g (82%); ^{1}H NMR ($CDCl_3$-d) δ 0.03 (s, 6H); 0.86 (s, 9H); 1.43 (s, 9H); 1.77 (m, 2H); 2.25 (m, 1H); 2.37 (m, 1H); 2.58 (m, 2H); 2.95 (m, 1H); 3.26 (m, 1H); 3.62 (m, 1H); 3.74 (m, 2H); 4.65 (m, 1H); 4.83 (m, 1H).

Benzyl (3*S*,4*R*)-1-(2-*tert*-Butyldimethylsilyloxy)ethyl)-3-fluoropiperidin-4-ylcarbamate (23). To a stirred mixture of **18** (5.9 g, 20.43 mmol) and cesium carbonate (33.3 g, 102.17 mmol) in acetonitrile (300 mL) at room temp was added (2-bromoethoxy)(*tert*-butyl)dimethylsilane (21.92 mL, 102.17 mmol). The reaction was stirred at 60 °C overnight, then filtered through a fritted funnel and concentrated. Chromatography on silica gel with 0−50% ethyl acetate in hexanes afforded the title compound as a yellow oil: 8 g (95%); MS (ESP) m/z 411 (MH^+).

(3*S*,4*R*)-1-(2-*tert*-Butyldimethylsilyloxy)ethyl)-3-fluoropiperidin-4-amine (24). A solution of **23** (8 g, 19.48 mmol) in ethanol (100 mL) was hydrogenated on palladium on carbon (10%, activated, 1.037 g) under normal pressure at room temp overnight. The reaction mixture was filtered through a 0.45 μm membrane, and solvent was evaporated under reduced pressure to give the product as an oil (5 g, 93%): MS (ESP) m/z 277 (MH^+); ^{1}H NMR ($CDCl_3$-d) δ 0.04 (s, 6H); 0.87 (s, 9H); 1.75 (m, 4H); 2.35 (m, 2H); 2.56 (m, 2H); 2.81 (m, 2H); 3.15 (m, 1H); 3.74 (m, 2H); 4.57 (m, 1H).

***trans*(±)-Benzylbenzyl-1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-ethyl)-3-fluoropiperidin-4-yl]carbamate (25).** A mixture of **19** (7.98 g, 21.1 mmol), (2-bromoethoxy)-*tert*-butyldimethylsilane (6.85 g, 27.5 mmol) and cesium carbonate (17.9 g, 55.0 mmol) in acetonitrile (60 mL) was heated to 60 °C for 12 h. The reaction mixture cooled to room temperature and concentrated under reduced pressure to dryness. The residue was diluted with ethyl acetate and washed with water and brine. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica with 10% acetone in hexanes gave the product as oil (8.9 g, 84%): MS (ESP) m/z 501 (MH^+); ^{1}H NMR ($CDCl_3$-d) δ 0.04−0.07 (s, 6H); 0.77−0.88 (s, 9H); 1.58−1.74 (m, 2H); 2.05−2.20 (m, 2H); 2.44−2.58 (m, 2H); 2.69−2.84 (m, 1H); 3.24 (m, 1H); 3.65 (s, 2H); 4.44−4.59 (m, 3H); 5.11 (s, 2H); 7.13−7.28 (m, 9H); 7.34 (m, 2H).

***cis*(±)-*tert*-Butyl-[3-fluoro-1-(2-hydroxyethyl)piperidin-4-yl]-carbamate (26).** To a solution of **22** (530 mg, 1.4 mmol) in tetrahydrofuran (10 mL) at 0 °C was added tetrabutylammonium

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fluoride (1 M in THF, 2.8 mL). After 30 min, the reaction was quenched with saturated sodium bicarbonate and extracted twice with ethyl acetate, dried over magnesium sulfate, and concentrated. Silica gel chromatography with 2.5% methanol in ethyl acetate afforded the product as a colorless solid: 314 mg (85%); ^{1}H NMR ($CDCl_3$-*d*) δ 1.43 (s, 9H); 1.81 (m, 2H); 2.30 (m, 1H); 2.36 (m, 1H); 2.59 (m, 2H); 2.75 (m, 1H); 2.95 (m, 1H); 3.24 (m, 1H); 3.61 (m, 2H); 3.71 (m, 1H); 4.68 (m, 1H); 4.85 (m, 1H).

(3*S*,4*R*)-1-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-3-fluoropiperidin-4-amine (27). The compound was prepared from **24** using the procedure for the synthesis of racemic **26** from **21**.

Benzylbenzyl[3-fluoro-1-(2-hydroxyethyl)piperidin-4-yl]carbamate, *trans*-Enantiomer B (28). A solution of tetrabutylammonium fluoride in tetrahydrofuran (1 M, 21.3 mL, 21.3 mmol) was added to **25** (8.9 g, 17.8 mmol) in tetrahydrofuran (20 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 1 h. The mixture was then cooled to 0 °C and quenched with water. The mixture was extracted with ethyl acetate and washed with brine. The combined organic phase was dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica with 40% acetone in hexanes gave the racemic product as an oil (5.1 g, 74%): MS (ESP) *m*/*z* 387 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 1H); 1.67 (m, 1H); 2.02 (m, 2H); 2.40 (m, 2H); 2.74 (m, 1H); 3.14−3.28 (m, 2H); 3.43 (m, 2H); 3.93 (m, 1H); 4.40 (t, 2H); 4.50 (m, 1H); 5.06 (m, 2H); 7.15 (m, 1H); 7.20−7.31 (m, 8H); 7.36 (m, 1H). The racemic mixture was separated on a Chiralpak AD column (500 × 20 mm, 20 μm) with ethanol/methanol (1:1), containing 0.1% diethyl amine. *trans*-Enantiomer B (**27**) was the second eluting enatiomer. The chiral purity (using an analytical method equivalent to the preparative method described above) was determined to be >98% ee.

***tert*-Butyl-[3-fluoro-1-(2-hydroxyethyl)piperidin-4-yl]carbamate, *trans*-Enantiomer B (29).** A mixture of **28** (5.6 g, 14.4 mmol) and palladium hydroxide on carbon (20%, 0.5 g) in methanol was stirred under an atmosphere of hydrogen overnight at normal pressure. Hydrogen was removed by purging with nitrogen, di-*tert*-butyldicarbonate (3.5 g, 15.8 mmol) was added, and the mixture was stirred under nitrogen for 1 h. The reaction mixture was filtered through Celite and the filtrate concentrated to dryness under reduced pressure. Chromatography on silica gel with 10% methanol (containing 0.1% ammonium hydroxide) in ethyl acetate gave 2.9 g (76%) of product as an oil: ^{1}H NMR ($CDCl_3$-*d*) δ 1.36−1.55 (m, 10H); 2.02−2.31 (m, 3H); 2.52−2.64 (m, 2H); 2.72−2.82 (m, 2H); 3.09−3.20 (m, 1H); 3.60 (t, 3H); 4.31 (m, 1H); 4.80 (d, 1H).

4-Azidopiperidin-3-ol (31).[25] To a solution of ethyl 4-azido-3-(*tert*-butyldimethylsilyloxy)piperidine-1-carboxylate **30**[25] (22.3 g, 67.89 mmol) in ethanol (300 mL) was added potassium hydroxide (38.1 g, 678.9 mmol) in water (75 mL). The mixture was heated at 90 °C for 6 h. Most of the solvent was removed under reduced pressure, and the residue was diluted with brine (30 mL). The mixture was extracted with dichloromethane (3 × 200 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was dried under high vacuum to remove most of the silanol. The residue was taken up in ether (∼100 mL), hexanes were added (∼150 mL), and most of the ether was removed under reduced pressure. The precipitate was collected by filtration and washed with hexanes to give the product as a slightly pink solid: 4.18 g (43%); ^{1}H NMR (DMSO-d_6) δ 5.00 (d, 1H); 3.63 (m, 1H); 3.50 (m, 1H); 2.75−2.60 (m, 2H); 2.60−2.50 (m, 1H); 2.50−2.35 (m, 1H); 1.85 (m, 1H); 1.67 (m, 1H); 1.51 (m, 1H).

1-[2-*cis*(±)-(4-Azido-3-hydroxypiperidin-1-yl)ethyl]-2-oxo-1,2-dihydroquinoline-7-carbonitrile (32). A mixture of **34** (4.44 g, 20.91 mmol) and **31** (3.27 g, 23 mmol) in THF (200 mL) was heated under stirring at 75 °C for 3 h. The mixture was cooled to room temperature and sodium triacetoxyborohydride (13.30 g, 62.73 mmol) was added. It was stirred for 2 h at room temperature, then quenched by addition of methanol (100 mL). The reaction mixture was stirred overnight, filtered through a 0.45 μm membrane, and the solid residue was washed with MeOH/dichloromethane (1:3, 2 × 20 mL). The filtrate and wash were combined and concentrated to dryness under reduced pressure. Chromatography was done on silica gel with hexanes/acetone (1:1 to 1:2). Fractions containing product were pooled and concentrated under reduced pressure to give the product as a colorless solid: 3.43 g (49%); MS (ESP) *m*/*z* 339 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.56 (m, 1H); 1.71 (m, 1H); 2.25−2.63 (m, 6H); 3.67 (m, 2H); 4.35 (dd, 2H); 5.06 (m, 1H); 6.78 (d, 1H); 7.64 (dd, 1H); 7.90 (d, 1H); 8.00 (d, 1H); 8.08 (br s, 1H).

1-(2,2-Diethoxyethyl)-2-oxo-1,2-dihydroquinoline-7-carbonitrile (33). A mixture of **1c** (35.0 g, 201 mmol), 2-bromo-1,1-diethoxyethane (44.1 mL, 281 mmol), and cesium carbonate (78.5 g, 241 mmol) in dry NMP (200 mL) was stirred at 70 °C overnight. The reaction mixture was diluted with water (350 mL) and extracted with butyl acetate (2 × 350 mL). The combined organic phases were filtered through Celite and washed with water (1 × 175 mL). The butyl acetate solution was concentrated to 140 mL and diluted with *iso*-hexane (525 mL). The precipitate was isolated by filtration and washed with *iso*-hexane (70 mL). This gave 34 g (60%) of the product as a colorless solid after drying, which was used without further purification: MS (ESP) *m*/*z* 309 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.96 (t, 6H); 3.34−3.47 (m, 2H); 3.56−3.73 (m, 2H); 4.39 (d, 2H); 4.72 (t, 1H); 6.80 (d, 1H); 7.62 (d, 1H); 7.89 (d, 1H); 8.02 (d, 1H); 8.13−8.22 (m, 1H).

2-Oxo-1-(2-oxoethyl)-1,2-dihydroquinoline-7-carbonitrile (34). To a solution of **33** (21.5 g, 75.1 mmol) in acetonitrile (230 mL) was added concentrated hydrochloric acid (2 equiv, 12.5 mL) at room temp. After 1 h, the resulting precipitate was collected by filtration. This gave 16 g (100%) of the product as a colorless solid after drying, which was used without further purification: MS (ESP) *m*/*z* 213 (MH^+); ^{1}H NMR (DMSO-d_6) δ 5.25−5.38 (m, 2H); 6.82 (d, 1H); 7.67 (d, 1H); 7.95 (d, 1H); 8.02−8.14 (m, 2H); 9.64−9.74 (m, 1H).

***tert*-Butyl-(*cis*(±))-4-azido-3-hydroxypiperidine-1-carboxylate (35).** To a mixture of **31** (2.1 g, 14.8 mmol) and potassium hydroxide (2.5 g, 44 mmol) in isopropyl alcohol (20 mL) and dichloromethane (25 mL) was added at 0 °C a solution of di-*tert*-butyldicarbonate (3.9 g, 17.7 mmol) in dichloromethane (10 mL). The cooling was removed, and it was stirred 2 h at room temperature. It was quenched with water (50 mL), and isopropyl alcohol and dichloromethane were removed under reduced pressure. It was neutralized with potassium phosphate buffer (1 M, pH 7, 100 mL), extracted with ethyl acetate twice (2 × 300 mL), and the combined organic phases were dried over sodium sulfate. Solvent was removed under reduced pressure, and the residue was titurated from hexanes (∼20 mL) to give 0.966 g of product as a colorless solid. Chromatography of the mother liquors with hexanes/ethyl acetate (5:1) afforded an additional 0.353 g of product (35%): MS (ESP) *m*/*z* 265 (MNa^+); ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H); 1.58 (m, 1H); 1.74 (m, 1H); 3.20−3.40 (m, 4H); 3.69 (m, 2H); 5.40 (d, 1H).

***tert*-Butyl-(*cis*(±))-4-azido-3-{[*tert*-butyl(dimethyl)silyl]oxy}-piperidine-1-carboxylate (36).** A mixture of **35** (1.76 g, 7.25 mmol) and imidazole (0.74 g, 10.9 mmol) in DMF (7 mL) at 0 °C was treated with *tert*-butyldimethylsilyl chloride (1.3 g, 8.7 mmol). Cooling was removed, and the mixture was stirred overnight at room temperature. It was cooled to 0 °C and quenched with phosphate buffer (1M, pH 7, 20 mL). After 15 min, the mixture was diluted with ethyl acetate (100 mL), the organic phase was washed with water (2 × 50 mL) and dried over sodium sulfate. Chromatography on silica gel with hexanes/ethyl acetate (9:1) gave the product as a colorless oil: 2.3 g (89%); ^{1}H NMR (DMSO-d_6) δ 0.10 (s, 6H); 0.87 (s, 9H); 1.37 (s, 9H); 1.56−1.80 (m, 2H); 3.09−3.30 (m, 2H); 3.46 (m, 2H); 3.62 (m, 1H); 3.88 (m, 1H).

(*cis*(±))-4-Azido-3-{[*tert*-butyl(dimethyl)silyl]oxy}piperidine (37). A solution of **36** (2.3 g, 6.45 mmol) in dichloromethane (50 mL) was treated at 0 °C with trifluoroacetic acid (5 mL). After 3 h, the mixture was concentrated under reduced pressure and the residue was co-distilled twice with dichloromethane. The residue was taken up in dichloromethane (100 mL) and washed with saturated aqueous sodium hydrogencarbonate solution (30 mL). The aqueous phase was back-extracted once with dichloromethane (100 mL), and the combined organic phases were dried over sodium sulfate to give the product as a slightly yellow oil: 1.625 g (98%); ^{1}H NMR (DMSO-d_6) δ 0.07 and 0.09 (2 × s, 6H); 0.88 (s, 9H); 1.49−1.73 (m, 2H); 2.45 (m, 1H); 2.56−2.69 (m, 3H); 3.65 (m, 1H); 3.79 (m, 1H).

2-(*cis*(±))-(4-Azido-3-{[*tert*-butyl(dimethyl)silyl]oxy}-piperidin-1-yl)ethanol (38). A mixture of **37** (1.625 g, 6.34 mmol),

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N,*N*-diisopropylethylamine (1.65 mL, 9.5 mmol), and 2-bromoethanol (0.584 mL, 8.25 mmol) in dry acetonitrile (17 mL) was heated in the microwave oven at 70 °C for 2 h. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate (∼150 mL) and washed with saturated aqueous sodium hydrogencarbonate solution (∼25 mL). The aqueous phase was back-extracted once with ethyl acetate (100 mL), and the combined organic phases were dried over sodium sulfate. Chromatography on silica gel with dichloromethane/methanol (20:1) gave 1.80 g (95%) of product as a colorless oil: MS (ESP) m/z 301 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.08 (s, 6H); 0.87 (s, 9H); 1.65 (m, 2H); 2.18 (m, 1H); 2.25−2.60 (m, 5H); 3.44 (m, 2H); 3.73 (m, 1H); 3.91 (m, 1H); 4.35 (m, 1H).

2-(*cis*(±))-(4-Azido-3-{[*tert*-butyl(dimethyl)silyl]oxy}-piperidin-1-yl)ethyl methanesulfonate (39). **38** (1.8 g, 6 mmol) was reacted with triethylamine (1.18 mL, 8.4 mmol) and methanesulfonyl chloride (0.556 mL, 7.2 mmol) as described for **2b**. The crude preparation of the mesylate was used without delay for the next step: MS (ESP) m/z 379 (MH^+).

1-[2-(*cis*(±))-4-Azido-3-{[*tert*-butyl(dimethyl)silyl]oxy}-piperidin-1-yl)ethyl]-7-methoxyquinoxalin-2(1*H*)-one (40). **1b** (0.528 g, 3.0 mmol), **39** (3 mmol), and sodium hydride (in oil, 60%, 132 mg) were reacted following the general procedure for **3** to give the product as a hard foam: 0.721 g (52%); MS (ESP) m/z 459 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.03 and 0.05 (2 × s, 6H); 0.82 (s, 9H); 1.65 (m, 2H); 2.25−2.70 (m, 6H); 3.70 (m, 1H); 3.90 (s, 3H); 3.83 (m, 1H); 4.24 (m, 1H); 4.39 (m, 1H); 6.96−7.00 (m, 2H); 7.73 (m, 1H); 8.02 (s, 1H).

1-[2-{(*cis*(±))-(4-Azido-3-hydroxypiperidin-1-yl)}ethyl]-7-methoxyquinoxalin-2(1*H*)-one (41). A solution of **40** (0.721 g, 1.57 mmol) in THF (5 mL) was treated dropwise at room temperature with a solution of tetrabutylammonium fluoride in THF (1M, 2.2 mL). After 1 h, saturated aqueous sodium hydrogencarbonate solution (10 mL) was added and THF was removed under reduced pressure. It was extracted with dichloromethane/ether (1:1, ∼200 mL). The aqueous phase was back-extracted with dichloromethane (100 mL), and the combined organic phases were dried over sodium sulfate. Chromatography on silica gel with hexanes/acetone (1:1) gave the product as a colorless hard foam: 0.507 g (94%); MS (ESP) m/z 345 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.58 (m, 1H); 1.70 (m, 1H); 2.25−2.65 (m, 6H); 3.67 (m, 2H); 3.90 (s, 3H); 4.31 (dd, 2H); 5.11 (m, 1H); 6.97−7.00 (m, 2H); 7.73 (d, 1H); 8.03 (s, 1H).

Benzyl-*trans*(±)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxypiperidine-1-carboxylate (43).[26] A mixture of benzyl trans(±)-4-amino-3-hydroxypiperidine-1-carboxylate **42**[25] (3.0 g, 12.0 mmol), di-*tert*-butyldicarbonate (2.9 g, 13.2 mmol), and sodium bicarbonate (3.0 g, 36.0 mmol) in ethyl acetate/water (1:1, 100 mL) was stirred vigorously overnight. The biphasic mixture was separated. The aqueous phase was re-extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure to give 4.2 g (quant) of the product as a colorless solid. This material was used without further purification: ^{1}H NMR (DMSO-d_6) δ 1.15−1.32 (m, 1H); 1.35−1.42 (m, 9H); 1.71−1.83 (m, 1H); 2.60−2.79 (m, 1H); 2.82−2.98 (m, 1H); 3.15−3.29 (m, 2H); 3.74−3.86 (m, 1H); 3.88−3.98 (m, 1H); 5.00 (d, 1H); 5.04−5.08 (m, 2H); 6.73 (d, 1H); 7.25−7.42 (m, 5H).

Benzyl-*trans*(±)-4-[(*tert*-butoxycarbonyl)amino]-3-{[*tert*-butyl(dimethyl)silyl]oxy} piperidine-1-carboxylate (44). A mixture of **43**[26] (2.0 g, 5.7 mmol), imidazole (0.58 g, 8.6 mmol), and *tert*-butyl(chloro)dimethylsilane (1.0 g, 6.9 mmol) in DMF (15 mL) was stirred at room temperature under nitrogen overnight. Water (50 mL) was added to the reaction, and the mixture was extracted 2× with ether. The combined organic phases were dried over sodium sulfate and concentrated to dryness. Chromatography on silica gel with 10−25% acetone in hexanes giving 1.8 g (69%) of the product as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H); 0.80 (s, 9H); 1.27−1.41 (m, 10H); 1.61−1.72 (m, 1H); 2.59−3.05 (m, 2H); 3.30−3.40 (m, 2H); 3.69−3.95 (m, 2H); 4.92−5.14 (m, 2H); 6.68 (d, 1H); 7.24−7.40 (m, 5H).

***tert*-Butyl-(*trans*(±)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-piperidin-4-yl)carbamate (45).** **44** (1.8 g, 3.9 mmol) was hydrogenated in methanol (50 mL) over 10% palladium on carbon (∼400 mg) at normal pressure for 1 h. The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to give 1.3 g (quant) of the product as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H); 0.80 (s, 9H); 1.20−1.30 (m, 1H); 1.33 (s, 9H); 1.53 (d, 1H); 2.15 (dd, 1H); 2.23−2.39 (m, 1H); 2.74 (d, 1H); 2.88 (dd, 1H); 3.20−3.30 (m, 2H); 4.08 (s, 1H); 6.58 (d, 1H).

***tert*-Butyl-[*trans*(±)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-1-(2-hydroxyethyl)piperidin-4-yl]carbamate (46).** **45** (1.3 g, 3.9 mmol), 2-bromoethanol (0.36 mL, 5.1 mmol), and ethyl(diisopropyl)amine (1.0 mL, 5.9 mmol) were reacted using the procedure described for **38** to give 1.0 g (67%) of the desired product: ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H); 0.79 (s, 9H); 1.33 (s, 9H); 1.39 (dd, 1H); 1.46−1.58 (m, 1H); 1.71−1.82 (m, 1H); 1.82−1.93 (m, 1H); 2.33 (t, 2H); 2.72 (d, 1H); 2.80−2.90 (m, 1H); 2.99−3.16 (m, 1H); 3.32−3.47 (m, 3H); 4.36 (t, 1H); 6.56 (d, 1H).

2-[(3*S*,4*R*)-4-(Dibenzylamino)-3-methoxypiperidin-1-yl]-ethanol (48). A mixture of *cis*(±)*N*,*N*-dibenzyl-3-methoxypiperidin-4-amine **47**[27] (1.7 g, 5.5 mmol), bromoethanol (0.5 mL, 7.1 mmol), and *N*,*N*-diisopropylethylamine (1.4 mL, 8.3 mmol) were reacted like described for **38**, heating for 1 h at 70 °C. Chromatography on silica gel with 5% methanol in dichloromethane containing 0.25% ammonium hydroxide gave 1.3 g (68%) of the *cis*-racemic product as a colorless solid: MS (ESP) m/z 355 (MH^+); ^{1}H NMR (DMSO-d_6) δ 1.44−1.58 (m, 1H); 1.64 (d, 1H); 1.79−2.08 (m, 2H); 2.32 (t, 2H); 2.36−2.45 (m, 1H); 2.88 (d, 1H); 3.13 (d, 1H); 3.30 (s, 3H); 3.40−3.49 (m, 2H); 3.56 (s, 1H); 3.59−3.87 (m, 4H); 4.34 (s, 1H); 7.11−7.24 (m, 2H); 7.24−7.40 (m, 8 H).

The enantiomers were separated by chiral chromatography on a chiral cell OJ column (250 × 20 mm, 10 μm) eluting with 1:1 methanol/ethanol and 0.1% diethylamine at 10 mL/min flow rate. The (−) isomer (3*R*,4*S*) eluted first followed by the (+) isomer (3*S*,4*R*) (**48**).

***tert*-Butyl-[(3*S*,4*R*)-1-(2-hydroxyethyl)-3-methoxypiperidin-4-yl]carbamate (49).** A solution of **48** (3*S*,4*R* isomer) (940 mg, 2.66 mmol) and di-*tert*-butyldicarbonate (0.67 mL, 2.92 mmol) in methanol (100 mL) was hydrogenated over 20% palladium hydroxide on carbon (240 mg) overnight. The reaction mixture was filtered through Celite and concentrated to dryness under reduced pressure. Chromatography on silica gel with 2−10% methanol in chloroform gave 540 mg (74%) of the product as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H); 1.43−1.50 (m, 1H); 1.58−1.73 (m, 1H); 2.15 (d, 2H); 2.37 (t, 2H); 2.54−2.64 (m, 1H); 2.75−2.88 (m, 1H); 3.22 (s, 3H); 3.27−3.32 (m, 1H); 3.41−3.59 (m, 3H); 4.37 (t, 1H); 6.35 (d, 1H).

Benzyl-*trans*(±)-4-[(*tert*-butoxycarbonyl)amino]-3-methoxypiperidine-1-carboxylate (50). **43** (1.0 g, 2.86 mmol) was suspended in 10 mL of toluene and treated with a 50 wt % solution of aqueous sodium hydroxide (6 mL) followed by dimethylsulfate (0.33 mL, 3.43 mmol) and benzyl triethylammonium chloride (catalytic amount). The reaction was stirred vigorously for 1 h. The reaction was quenched with ice. The phases were separated. The aqueous phase was re-extracted with ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica gel with 25−50% acetone in hexanes gave 0.78 g (78%) of the product as a colorless oil: MS (ESP) m/z 365 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.55−0.68 (m, 10H); 1.04−1.19 (m, 1H); 2.17−2.46 (m, 2H); 2.55−2.64 (m, 2H); 2.67−2.80 (m, 1H); 2.85−3.07 (m, 1H); 3.10−3.31 (m, 1H); 4.09 (s, 3H); 4.32 (s, 2H); 6.45−6.61 (m, 5H).

***tert*-Butyl-[*trans*(±)-3-methoxypiperidin-4-yl]carbamate (51).**[14] **50** (0.98 g, 2.69 mmol) was hydrogenated in methanol (50 mL) over 10% Pd/C (400 mg) at normal pressure. After 1 h, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to give 0.61 g (98%) of the product as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 1.14−1.29 (m, 1H); 1.34−1.42 (m, 9H); 1.68 (d, 1H); 2.11 (dd, 1H); 2.26−2.38 (m, 1H); 2.71−2.82 (m, 1H); 2.86−2.98 (m, 1H); 3.14−3.21 (m, 3H); 3.26 (s, 3H); 6.75−6.86 (m, 1H).

***tert*-Butyl-[*trans*(±)-1-(2-hydroxyethyl)-3-methoxypiperidin-4-yl]carbamate (52).** **51** (1.1 g, 4.8 mmol), 2-bromoethanol (0.44 mL, 6.2 mmol), and ethyl(diisopropyl)amine (1.25 mL, 7.2 mmol) were as described for **37** to give 0.74 g (57%) of the product as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 1.24−1.34 (m, 1H); 1.38 (s, 9H); 1.62−1.77 (m,

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2H); 1.82−1.97 (m, 1H); 2.38 (t, 2H); 2.73 (d, 1H); 2.98−3.18 (m, 3H); 3.27 (s, 3H); 3.46 (q, 2H); 4.39 (t, 1H); 6.78 (d, 1H).

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Notes

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ABBREVIATIONS USED

NBTI, novel (non-fluoroquinolone) bacterial type II topoisomerase inhibitor; LHS, bicyclic aromatic left-hand side; RHS, aromatic right-hand side (as positioned in Figure 1); MIC, minimal inhibitory concentration; CFU, colony forming units; ND, not determined; hERG, human ether-a-go-go-related gene; $MAPD_{90}$, monophasic action potential duration at 90%

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