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Novel N-Linked Aminopiperidine Inhibitors of Bacterial Topoisomerase Type II: Broad-Spectrum Antibacterial Agents with Reduced hERG Activity

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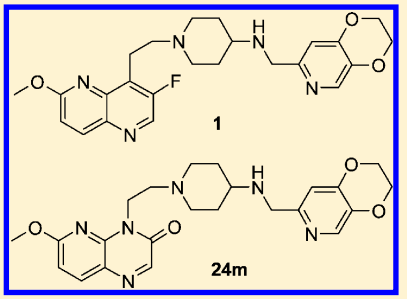
ABSTRACT: Novel non-fluoroquinolone inhibitors of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) are of interest for the development of new antibacterial agents that are not impacted by target-mediated cross-resistance with fluoroquinolones. Aminopiperidines that have a bicyclic aromatic moiety linked through a carbon to an ethyl bridge, such as **1**, generally show potent broad-spectrum antibacterial activity, including quinolone-resistant isolates, but suffer from potent hERG inhibition (IC_{50}= 3 μM for **1**). We now disclose the finding that new analogues of **1** with an N-linked cyclic amide moiety attached to the ethyl bridge, such as **24m**, retain the broad-spectrum antibacterial activity of **1** but show significantly less hERG inhibition (IC_{50}= 31 μM for **24m**) and higher free fraction than **1**. One optimized analogue, compound **24l**, showed moderate clearance in the dog and promising efficacy against *Staphylococcus aureus* in a mouse thigh infection model.



INTRODUCTION

The prevalence of drug-resistant pathogenic bacteria is increasing globally[1] and is necessitating research into development of new antibacterial agents that lack cross-resistance mediated by mutations in the bacterial targets. 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. Novel (non-fluoroquinolone) bacterial type II topoisomerase inhibitors (NBTIs) have been described in the literature[2−4] and are not impacted by target mutations that cause resistance to fluoroquinolones. The binding site of NBTIs in the DNA-bound gyrase complex of *Staphylococcus aureus* has been determined by crystallography[2] and does not overlap with the two fluoroquinolone binding sites. NBTIs are thus of particular interest for the development of new antibacterial agents because they act on a clinically proven antibacterial target through a novel mechanism of inhibition and retain activity against fluoroquinolone-resistant isolates.

NBTIs were first discovered in the laboratories of GlaxoSmithKline and Aventis Pharma AG, and several pharmaceutical companies are now filing patent applications or publishing in this area.[5−10] Viquidacin (NXL-101), an NBTI discovered by Aventis and developed by Novexel, underwent phase I trials but was discontinued as a result of QTc prolongation observed in phase I trials,[11] emphasizing the intrinsic challenges for achieving an acceptable cardiovascular safety profile with this class of compounds. The chemical structures of NBTIs are quite diverse but have an overall similar topology: A bicyclic aromatic left-hand side (LHS) that stacks between two base pairs of the DNA in the GyrA/DNA complex, an aromatic right-hand side (RHS) that interacts with the protein, and a linker between the LHS and RHS that is mostly solvent exposed but does pick up an interaction with Asp83 that has been shown to be important for binding.[2]

We were interested in the aminopiperidine subclass of NBTIs exemplified by compounds **1** and **2** (Figure 1),

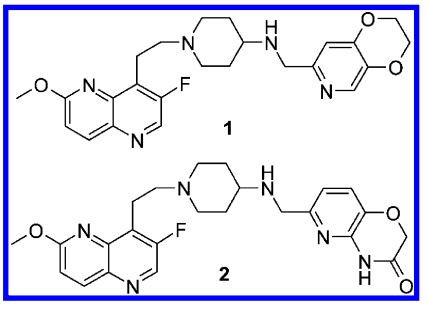


Figure 1. C-Linked aminopiperidine leads from the patent literature (GSK).[6]

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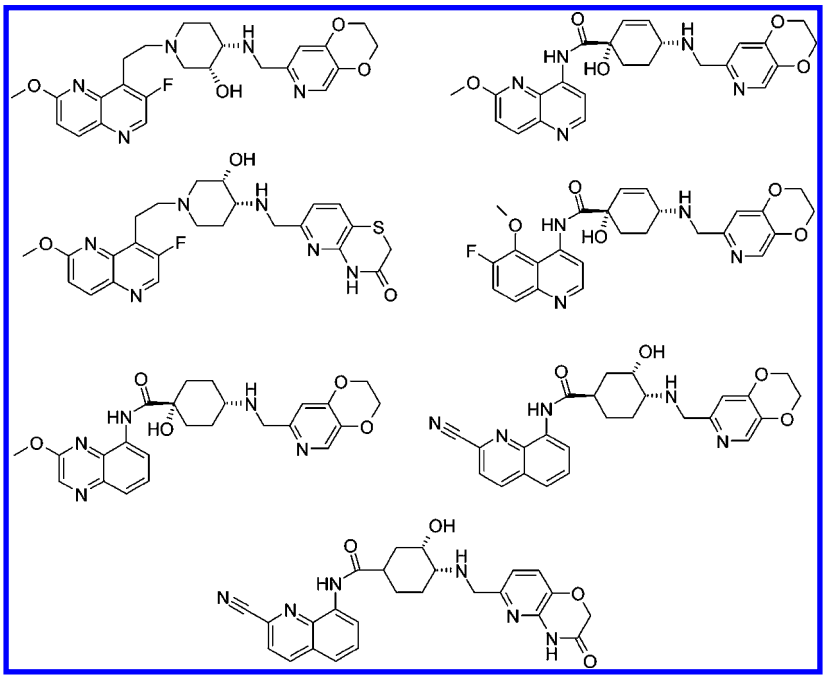


Figure 2. Compounds from the literature[6,38,39] used to build the pharmacophore model.

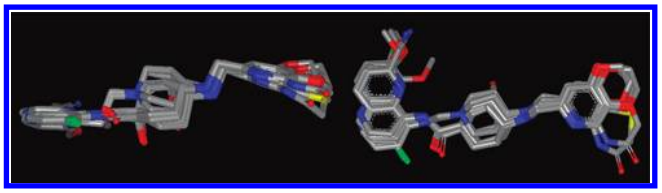


Figure 3. Pharmacophore-based overlay of seven NBTI reference compounds.

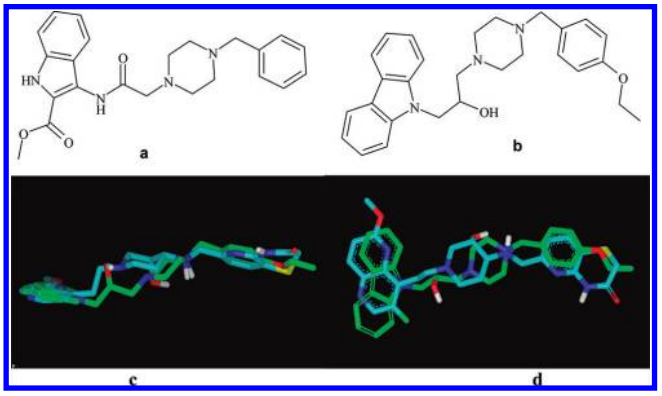


Figure 4. (a) Active hit from the pharmacophore and shape virtual screen. (b) N-Linked hit from the virtual screen. (c) Overlay of a N-linked LHS hit (green carbons) onto the query molecule (cyan carbons) used to define the pharmacophore search. (d) Orthogonal view of (c).

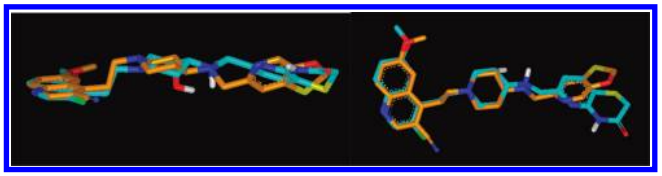
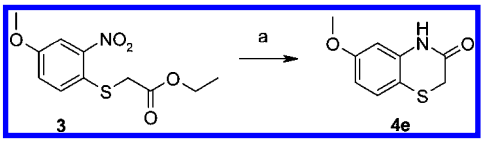


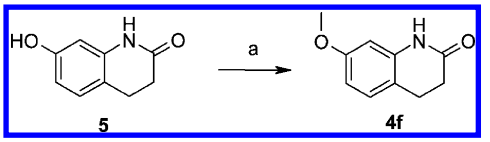
Figure 5. Overlay of a NBTI inhibitor from pharmacophore modeling (cyan carbons) versus NBTI inhibitor conformation (gold carbons) observed bound to GyrA from the reported crystal structure.[2]

Scheme 1[a]



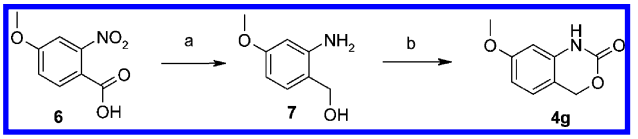
[a]Reagents: (a) Fe, AcOH, 90 °C, 93%.

Scheme 2[a]



[a]Reagents: (a) NEt_3, $(CH_3)_3SiCHN_2$, CH_2Cl_2/MeOH/CH_3CN, room temp, 67%.

Scheme 3[a]



[a]Reagents: (a) *N*-methylmorpholine, isobutyl chloroformate, THF, room temp, then $NaBH_4$, water, then Pd on C/H_2, THF, 24%; (b) NEt_3, triphosgene, toluene, room temp, 36%.

identified by researchers at GSK,[6] because **1** and **2** displayed potent broad-spectrum antibacterial activity combined with good physicochemical properties, such as sufficiently high solubility for iv formulation. It soon became apparent to us that **1** and **2** carried considerable cardiovascular safety risk arising from potent hERG inhibition, with IC_{50} values of 3 and 4 μM, respectively. We embarked on a lead optimization

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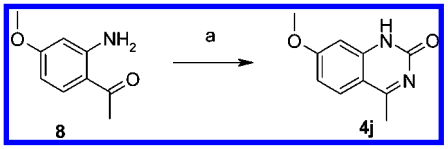
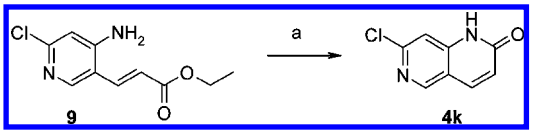
Scheme 4[a]



[a]Reagents: (a) KOCN, AcOH, room temp, 64%.

Scheme 5[a]



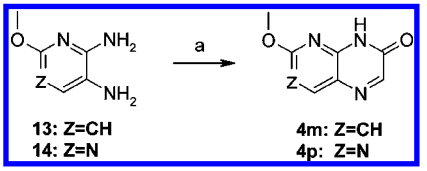
[a]Reagents: (a) DBU, N(^{i}Pr)$_2$Et, 100−120 °C, 70%.

program with the goal of identifying novel analogues that retain the potent antibacterial activity and good physicochemical properties of the leads but show significantly reduced hERG liability.

MOLECULAR MODELING

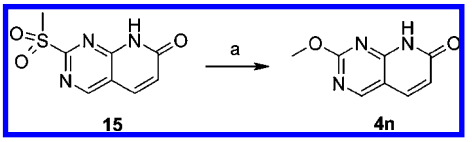
One well-established strategy for identifying new leads is virtual screening of molecular databases for compounds matching a pharmacophore model. Since the NBTI X-ray structure with GyrA was not available at the outset, we employed a ligand-based approach to building an NBTI pharmacophore model. The known NBTI literature was examined. Compounds with reported antibacterial activity were identified, and a diverse, conformationally constrained subset of seven compounds was selected (Figure 2). The TFIT pharmacophore modeling software[12] was used to identify low-energy conformations that maximized the overlap of the LHS aromatic rings, the basic center of the amino group, and the RHS aromatic rings of the seven reference NBTIs. The array of tentative "bioactive" conformations identified using this approach is shown in Figure 3. A pharmacophore and shape search of a multiconformer version of our corporate database yielded several interesting hits that matched the LHS, RHS, and basic center pharmacophore features of NBTIs. Some of the virtual screening hits were selected for IC_{50} testing in a supercoiling assay.[13] While the virtual screening hit shown in Figure 4a was active in the supercoiling assay (IC_{50} = 1.5uM), the most useful aspect of the pharmacophore search output was in the inspiration for new compound design. For example, our focus quickly turned to considering the use of N-linked LHS chemistry after seeing the overlays for an N-linked LHS hit (Figure 4b−d). The recent publication of the X-ray structure of an NBTI bound to a *Staphylococcus aureus* gyrase/DNA complex provided experimental evidence for the bioactive conformation of the aminopiperidine class of NBTI antibacterials.[2] The NBTI conformation observed in the X-ray structure compared to the bioactive conformation from our ligand-based approach (Figure 5) showed a high degree of similarity, with only a slight rotation of the RHS in the ligand-based model. This result is not surprising given that the seven compounds used to build the pharmacophore model contained more conformational constraints in the LHS and linker and little information to constrain the conformation of the RHS. Overall, the ligand-based pharmacophore model has proven to be a powerful tool in our search for improved NBTIs and has helped us design new and potent NBTI chemotypes.

Scheme 7[a]



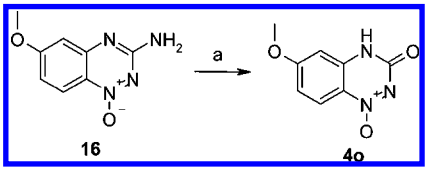
[a]Reagents: (a) ethyl glyoxylate, MeOH, room temp, 13% for **4m**, 95% for **4p**.

Scheme 8[a]



[a]Reagents: (a) NaOMe, MeOH, 70 °C, 71%.

Scheme 9[a]



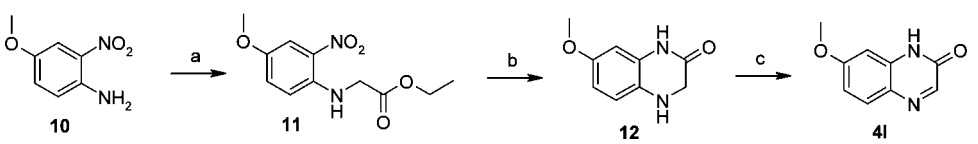
[a]Reagents: (a) H_2SO_4, room temp, then $NaNO_2$, 90%.

CHEMISTRY

Compounds were assembled through N-alkylation of heterocycles **4**, followed by reductive aminations with RHS aldehydes as the key synthetic steps[5] (Scheme 11).

The syntheses of LHS heterocycles **4** are outlined in Schemes 1−9. Alkylations of **4** with mesylate **19** gave aminopiperidine derivatives **20** (Scheme 11). Mesylate **19** was freshly prepared for immediate use from the alcohol **18** (Scheme 10). **19** is unstable under storage. Alkylations with **19**

Scheme 6[a]



[a]Reagents: (a) ethyl bromoacetate, K_2CO_3, 150 °C; (b) H_2, Pd/C, MeOH/AcOH, room temp, 64% (combined for steps a and b); (c) H_2O_2, water, 80 °C, then AcOH, room temp, 91%.

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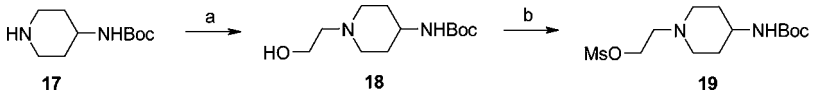
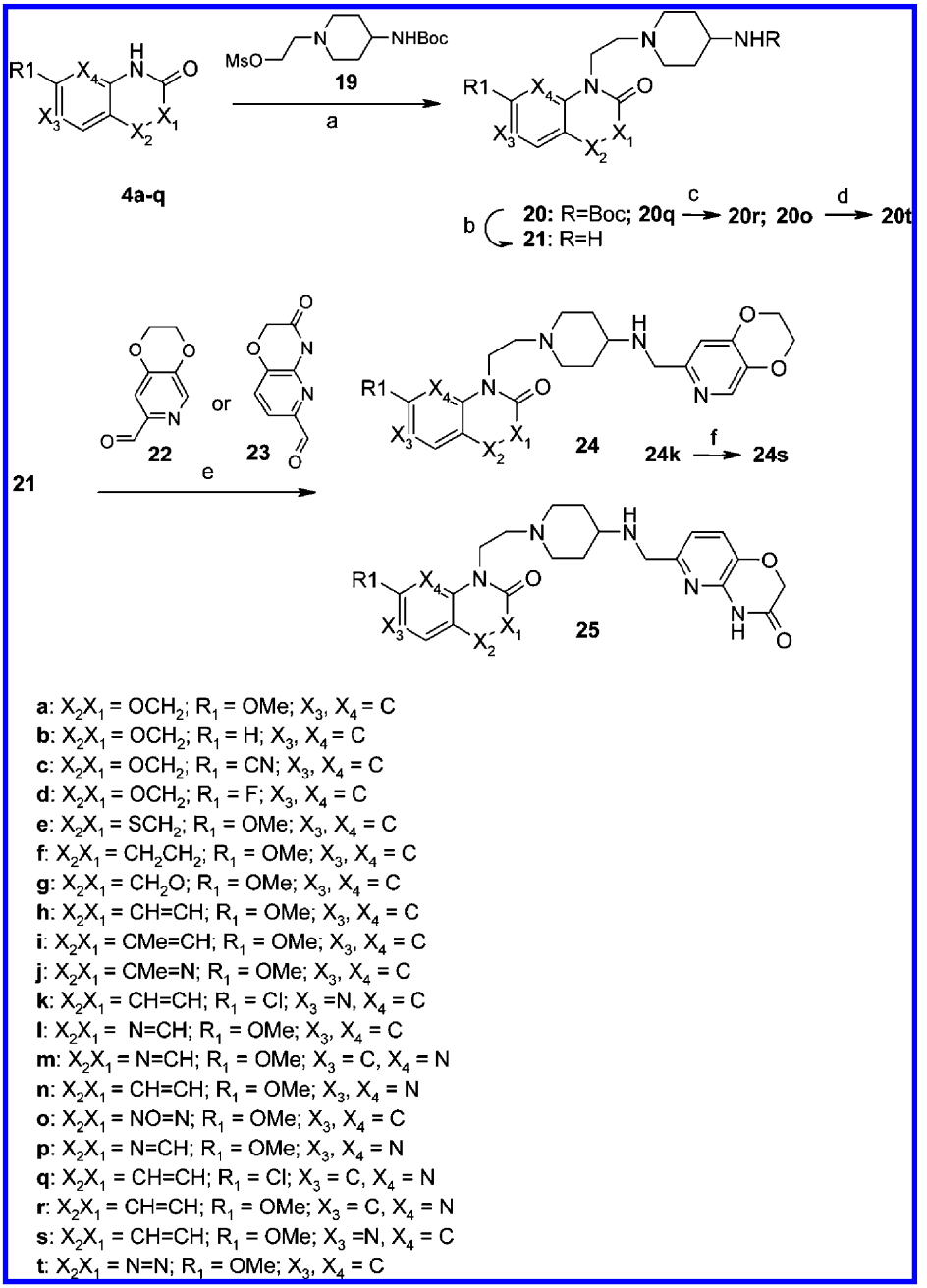
Scheme 10[a]



[a]Reagents: (a) 2-bromoethanol, NEt_3, CH_3CN, 50 °C, 66%; (b) MsCl, NEt_3, CH_2Cl_2, 0 °C.

Scheme 11[a]



[a]Reagents: (a) NaH, DMF, 0 °C to room temp; (b) TFA, CH_2Cl_2, 0 °C; (c) NaOMe, MeOH, 140 °C, 61%; (d) sodium dithionite, DMF/water, 100 °C, 27%; (e) 3 Å molecular sieves, $CHCl_3$/MeOH, 70 °C, then $NaBH(OAc)_3$, 0 °C to room temp; (f) NaOMe/MeOH, 78 °C, 38%.

generally afforded **20** together with smaller amounts of O-alkylation products. O-Alkylated product generally had higher R_f by TLC than N-alkylated product and was efficiently removed by chromatography. N-Alkylation of **20** was confirmed by NMR studies, with carbon chemical shifts for the CH_2N moiety observed in the characteristic range of 40−45 ppm vs 60−70 ppm for CH_2O in the case of O-alkylation. Further transformations on the LHS were carried out in some cases on the Boc-protected intermediates (**20q**, **20o**), rather than unprotected interemediates **4**, because of the higher

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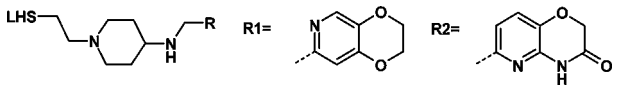
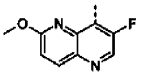
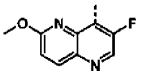
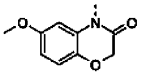
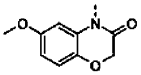
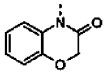
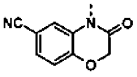
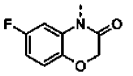
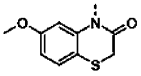
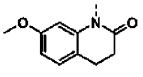
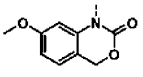
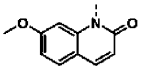
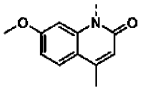
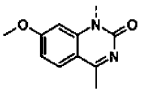
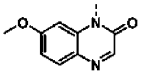
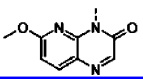
solubility of **20** in most organic solvents. Cleavage of the Boc group in **20** gave free amines **21**, generally in quantitative yields. Reductive aminations of aldehydes **22**[14] and **23**[15] with amines **21** gave the best results when performed with the free

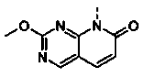
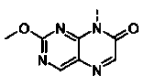
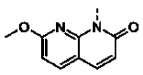
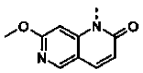
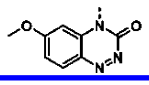
Table 1. SAR of Left-Hand Side (LHS)[i]



Compd	LHS	R	*S.a.*[a] MIC	*S.p.*[b] MIC	*P.ae.*[c] MIC	*E.coli*[d] MIC	*E. coli* TopoIV[e] IC50 *(nM)*	logD[f]	fu[g] (%)	hERG[h] *IC50 (μM)*
1		R1	<0.01	<0.07	4	0.13	1.3	1.46	17	3
2		R2	0.02	0.03	1	0.06	0.6	1.78	6	4
24a		R1	0.13	0.25	>8	1	ND	1.24	18	13
25a		R2	0.13	0.5	4	0.25	5.0	0.87	11	13
24b		R1	2	4	>8	8	100	1.04	26	20
25c		R2	0.13	0.5	2	0.25	3.2	0.24	23	41
25d		R2	0.25	0.5	3	0.25	ND	0.90	17	9
24e		R1	8	>8	>8	>8	ND	1.78	9	6
25f		R2	>8	>8	>8	>8	780	0.66	4	29
25g		R2	1	0.5	4	0.5	4.4	0.50	26	42
24h		R1	0.03	0.06	>8	1.5	22	1.32	31	34
24i		R1	0.02	0.03	>8	0.5	ND	1.72	11	23
24j		R1	2	1	>8	>8	82	0.29	40	>33
24l		R1	0.02	0.06	4	0.5	5.0	0.93	57	19
24m		R1	0.02	0.03	4	0.25	1.5	0.83	47	31

dx.doi.org/10.1021/jm2008826|*J. Med. Chem.* 2011, 54, 7834–7847

Table 1. continued

Compd	LHS	R	*S.a.*[a] MIC	*S.p.*[b] MIC	*P.ae.*[c] MIC	*E.coli*[d] MIC	*E. coli* TopoIV[e] IC50 *(nM)*	logD[f]	fu[g] (%)	hERG[h] *IC50 (μM)*
24n		R1	0.13	0.25	>8	2	13	0.20	27	206
24p		R1	0.25	0.25	>8	2	28	-0.14	96	81
24r		R1	0.02	0.05	>8	0.5	2.0	1.16	31	40
24s		R1	1	1	>8	8	28	0.68	17	>33
25t		R2	>8	8	8	2	26	-0.65	44	>33

[a]Methicillin-susceptible *Staphylococcus aureus*. [b]*Streptococcus pneumonia* D39 (penicillin-susceptible). [c]*Pseudomonas aeruginosa* PAO1. [d]*Escherichia coli* W3110. [e]*Escherichia coli* TopoIV IC_{50}. [f]Partion coefficient at pH 7.4. [g]Fraction unbound, human, % free. [h]hERG Ionworks IC_{50}.[19] [i]Minimum inhibitory concentration (MIC, μg/mL): lowest drug concentration that reduced growth by 80% or more.

bases of **21a−t** in the presence of freshly activated molecular sieves. The mixtures were initially heated to drive imine formation to completion, then cooled for the reduction with sodium triacetoxyborohydride. When the reduction was carried out at elevated temperatures or with more reactive reducing agents, such as sodium borohydride, then over-reduction was observed with quinoxalinones, for example, with **21l**.

RESULTS AND DISCUSSION

A prototype for the N-linked aminopiperidine series was identified as a hit in a virtual screen against the pharmacophore model. We realized that linking the LHS through the nitrogen of a cyclic amide moiety such as in **24m** would enable us to explore a novel molecular framework with the potential for lower lipophilicity compared to earlier compounds from this class. We rationalized that the carbonyl group of the LHS may give good target potency by restraining conformational rotation in the ethyl bridge, similar to the fluoro substituent in **1** and **2**,[6] albeit potentially with a lower log *D*. Reduction of log *D* was seen as desirable to improve the safety profile of the compounds, especially to reduce hERG inhibition.[16] A series of new compounds was prepared,[5] combining novel bicyclic N-linked LHSs with RHSs that were optimized for the corresponding C-linked series.[6] N-Linked aminopiperidines **24** and **25** and C-linked reference compounds **1** and **2** were evaluated for inhibition of bacterial topoisomerase IV, antibacterial activity, and inhibition of the hERG ion channel (Table 1).

N-Linked aminopiperidines showed potent binding to bacterial topoisomerase IV from *E. coli* with IC_{50} in the low nanomolar range for the best compounds (**25c**, **25g**, **24m**, **24l**). The benzoxazinone RHS moiety gave slightly higher target potency than the dioxinopyridine RHS (cf. **1** to **2**). The presence of a substituent in the LHS was important for potent binding, which was evident by the 20-fold difference in IC_{50} between the unsubstituted **24b** and the methoxy derivative **25a**. The methoxy substituent was replaced by a fluoro or a cyano substituent to give compounds with similar potencies (**25c,d**). 4-Methyl substitution was tolerated (**24i**). A partially saturated moiety in the LHS was also tolerated, as was apparent from the potent IC_{50} values for benzoxazinones **24a,c,d** and the cyclic carbamate **25g**. However, the thiazinone **24e** and the dihydroquinoline **25f** lacked potent target affinity, which indicated that the degree of puckering as a function of bond length may be an important factor for recognition of the LHS. Taking the 2-quinolone derivative **24h** as a starting point, the introduction of additional nitrogen atoms in the LHS was tolerated. Derivatives containing 4- or 8-aza substituions (**24l**, **24m**, and **24r**) showed an increase in target potency, whereas the introduction of a nitrogen in the 3- or 6-position resulted in a reduction in potency (**24j**, **24n**, **24p**, **24s**, and **25t**).

The antibacterial activity (MIC) exhibited by N-linked aminopiperidines was similar to that of the C-linked reference compounds **1** and **2** and inhibited the growth of Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) (Table 1). Plasma protein binding for some N-linked aminopiperidines (**24h**, **24l**, **24m**) was significantly reduced compared to **1** and **2**, likely due to lower log *D* values. Aminopiperidines tended to be more potent against Gram-positive organisms than against Gram-negatives. The situation was reversed though for **25t**, which had a relatively low log *D* of −0.65 and retained Gram-negative activity but lacked potent activity against Gram-positive organisms. This finding is in agreement with the general observation that log *D* for agents targeting Gram-positive organisms tend to be higher than for agents that target Gram-negative organisms.[17] This observation is likely due to the structure of the outer membrane in Gram-negatives, with hydrophilic porins presenting an important access route.[18]

Aminopiperidines were evaluated for inhibition of the hERG channel in a functional ionworks assay.[19] IC_{50} correlated

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Table 2. Pharmacokinetics in Rat and Dog[a]

	compd	clearance (mL min^{-1} kg^{-1})	volume (L/kg)	half-life (h)	AUC (iv) μg·h/mL	bioavailability (%)
rat	**25c**	66	23	5.8	0.64	
	24l	338	32	2.8	0.19	
dog	**25c**	7.8	61	166	3.09	64
	24l	21	11	8.3	2.3	

[a]Pharmacokinetic parameters following administration of 4 mg/kg (compound **24l**, rat) or 3 mg/kg (compounds **25c** and **24l**, dog), iv bolus injection. Oral bioavailability was assessed following a 10 mg/kg dose.

Table 3. Exposure and Efficacy of Compound 24l Compared to Levofloxacin in a Mouse Pneumonia Model [a]

	compound **24l**		levofloxacin	
	AUC_∞ (μg h^{-1} mL^{-1})	log reduction in cfu/lung vs vehicle-treated control	AUC_∞ (μg h^{-1} mL^{-1})	log reduction in cfu/lung vs vehicle-treated control
12 (mg kg^{-1} day^{-1})	0.47	0.05	ND	ND
24 (mg kg^{-1} day^{-1})	0.76	1.17	2.50[b]	1.46[b]
36 (mg kg^{-1} day^{-1})	1.17	1.50	ND	ND
48 (mg kg^{-1} day^{-1})	1.79	2.79	ND	ND

[a]In vivo activity against *Staphylococcus aureus* 516 (ARC516). [b]20 mg kg^{-1} day^{-1} dose.

roughly with log *D*,[16] with the more polar N-linked compounds like **24m** showing a significantly improved hERG profile over the C-linked reference compound **1** (31 μM vs 3 μM). The structural feature of an N-linked LHS thus represents an important advance for the design of NBTIs with reduced QT liability. Still, further improvements of the hERG profile are likely needed to eliminate QT liability in this series completely.

The pharmacokinetics of selected compounds were determined in the rat and dog (Table 2). N-Linked aminopiperidines showed high volumes of distribution in both species, with high clearance in the rat and moderate clearance in the dog. Compound **25c** showed good bioavailability in the dog (64%).

Compound **24l** was active against *Staphylococcus aureus* in a neutropenic mouse thigh infection model (Table 3), with a 1 log reduction in colony forming units (cfu) observed at a dose of 24 mg kg^{-1} day^{-1}. The response per dose was similar to that of the comparator, levofloxacin, in this model.

CONCLUSIONS

We report the use of a rationally conceived pharmacophore model and a virtual screening exercise for idea generation in our bacterial topoisomerase inhibitor program. This led to the identification of novel aminopiperidine inhibitors with an N-linked cyclic amide containing LHS instead of the usual C-linked quinoline and naphthyridine LHS found in the patent literature. We report on the synthesis and biological evaluation of N-linked aminopiperidines and show that these analogues display excellent broad-spectrum antibacterial activity with a significantly improved hERG profile. Pharmacokinetic parameters for two optimized analogues (**25c** and **24l**) were determined in the rat and dog, showing high clearance in the rat but moderate clearance in the dog. Compound **24l** was evaluated in a neutropenic mouse thigh infection model against *Staphylococcus aureus* and found to be efficacious with a 1 log reduction in cfu at a dose of 24 mg kg^{-1} day^{-1}. N-Linked aminopiperidines thus show promise for the development of novel antibacterial agents.

EXPERIMENTAL SECTION

Minimum Inhibitory Concentration Testing. Minimum inhibitory concentrations were determined by broth microdilution according to the Clinical and Laboratory Standards Institute guidelines.[20] 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 medium was as follows: For *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*: Mueller Hinton broth, Difco no. 275730. For *Streptococcus pneumoniae*: 2.2% (w/v) Mueller Hinton broth, 0.65% (v/v) lysed horse blood (Hema Resource and Supply no. 15-14-0100-28). Inoculants were incubated at 35 °C on blood agar plates (Remel no. 01202) for 18−24 h. Plates were read by spectrophotometry at 620 nm.

Topoisomerase IV Assay. The assay utilizes the ATPase activity of the ParE subunit of reconstituted *Escherichia coli* ParC/ParE tetramer protein. Inhibition of ATPase activity was monitored by reduced production of inorganic phosphate, a product of the ATPase reaction. Inorganic phosphate was quantified using the ammonium molybdate/malachite green-based detection system. For determination of IC_{50} values, assays were performed in 384-well microtiter plates. Each well contained a dilution range of the compound dissolved in DMSO. In addition, each well contained 20 mM Tris, pH 8.0, 50 mM ammonium acetate, 0.16 mM ATP, 0.005% Brij-35, 8.0 mM magnesium chloride, 0.5 mM EDTA, 2.5% v/v glycerol, 5 mM dithiothreitol, 0.005 mg/mL sheared salmon sperm DNA, 0.5 nM *E. coli* ParC protein, and 0.5 nM *E. coli* ParE protein. Final volume of assays was 30 μL. Mixtures were incubated for 24 h at room temperature, and reactions were quenched with the addition of 30 μL of malachite green reagent[21] via a bulk reagent dispenser. Plates were incubated 3−5 min at room temperature, and then absorbance at 650 nM was measured using a Spectramax 384 plate reader. Values reported are the mean from three replicate experiments.

Plasma Protein Binding. Plasma protein binding was determined using the Dianorm equilibrium dialysis chamber. Compound (10 μM) was spiked in the plasma chamber (donor side); 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 drug concentration was determined for the buffer sample that represents the free fraction. LC/MS−MS quantitative sample analysis was achieved using an Ace C18 50 mm × 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). Data were processed by the Analyst, version 1.4.1, software.

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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.

Animals. Wistar Han rats for pharmacokinetic 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 the animal facility on site 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 mg/kg 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 noncompartmental 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.

***Staphylococcus aureus* Neutropenic Thigh Infection Model.** Compound **24l** was studied in a neutropenic mouse thigh infection model as described in the paper by Mills et al.[22] Briefly, mice were rendered neutropenic by injecting cyclophosphamide (Sigma-Aldrich, St. Louis, MO) intraperitoneally for 4 days (150 mg/kg body weight) and 1 day (100 mg/kg) before experimental infection. Mice were infected with Methicillin-susceptible *Staphylococcus aureus* (ARC516, AstraZeneca culture collection) to achieve a target inoculum of 5×10^5 cfu/thigh. Groups of five animals each received an intraperitoneal injection of **24l** at the doses specified in Table 3, prepared in 5% dextrose with lactic acid, pH 5.0, on a q.d. 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, Warrenton, VA), plated onto tryptic soy agar plates, and incubated at 37 °C overnight for cfu determination.

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-Temp II 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 ppm (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 mm × 20 mm i.d., S-5μ particle size, 12 nm pore size) on Agilent instruments. Mass spectrometry 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 at least 95%.

6-Methoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (4e). To a solution of ethyl [(4-methoxy-2-nitrophenyl)thio]acetate[23] (3 g, 11 mmol) in acetic acid (30 mL) was added iron powder (1.7 g, 31 mmol). The reaction mixture was heated at 90 °C for 3 h. It was cooled to room temperature, diluted with ethyl acetate, filtered through Celite, and concentrated to dryness under reduced pressure. Silica gel chromatography with hexanes/ethyl acetate (3:2) gave 2 g (93%) of the product as a colorless solid. MS (ESP) m/z 196 (MH^+). ^{1}H NMR ($CDCl_3$-*d*) δ: 3.39 (s, 2H); 3.78 (s, 3H); 6.44 (d, 1H); 6.59 (dd, 1H); 7.20 (d, 1H); 8.63 (brs, 1H).

7-Methoxy-3,4-dihydroquinolin-2(1*H*)-one (4f). A mixture of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one **5**[24] (3 g, 17 mmol) and triethylamine (3.14 mL, 22 mmol) in dichloromethane/methanol/acetonitrile (10:1:10, 168 mL) was treated with (trimethylsilyl)-diazomethane (2 M solution in hexanes, 10.25 mL, 20.5 mmol). The mixture was stirred overnight at room temperature, and the solvent was removed under reduced pressure. Chromatography on silica gel with hexanes/acetone (1:1) gave 2.2 g (67%) of the product as a colorless solid. MS (ESP) m/z 178.16 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 2.38 (t, 2H); 2.81 (t, 2H); 3.68 (s, 3H); 6.68−6.78 (m, 3H); 9.90 (brs, 1H).

7-Methoxy-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (4g). A solution of (2-amino-4-methoxyphenyl)methanol 7 (744 mg, 4.85 mmol) in toluene (25 mL) was treated with triethylamine (1.36 mL, 9.7 mmol) and triphosgene (1.58 g, 5.34 mL). The reaction mixture was stirred at room temperature for 1.5 h and then was heated to 110 °C for an additional 2 h. The reaction mixture was cooled to room temperature, diluted with water (25 mL), and filtered. The aqueous layer was extracted with toluene (3 × 30 mL) and ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Chromatography on silica gel with hexanes/ethyl acetate (3:2) gave 314 mg (36%) of the product as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 3.78 (s, 3H); 5.26 (s, 2H); 6.38 (d, 1H); 6.59 (d, 1H); 6.98 (d, 1H); 8.36 (brs, 1H).

7-Methoxy-4-methylquinazolin-2(1*H*)-one (4j). To a solution of commercially available 2-amino-4-methoxyacetophenone **8** (1.07 g) in acetic acid (20 mL) was added potassium cyanate (1.19 g) at room temperature. The reaction mixture was stirred overnight, then poured into water and neutralized with solid sodium hydrogen carbonate. The mixture was extracted with ethyl acetate and then with chloroform. The combined organic phases were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Chromatography on silica gel with 0−10% methanol in dichloromethane gave 0.79 g (64%) of the product as a colorless solid. MS (ESP) m/z 191 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 2.59 (s, 3H); 3.85 (s, 3H); 6.70 (d, 1H); 6.82 (dd, 1H); 7.87 (d, 1H).

7-Chloro-1,6-naphthyridin-2(1*H*)-one (4k). A solution of (*E*)-ethyl 3-(4-amino-6-chloropyridin-3-yl)acrylate **9**[25] (0.617 g, 2.73 mmol) in diisopropylethylamine (5 mL) was treated with DBU (0.7 mL, 4.5 mmol). The reaction mixture was heated to 100 °C for 18 h. The temperature was increased to 120 °C for an additional 24 h. The solvent was removed at reduced pressure. The residue was purified by chromatography on silica gel, eluting with 0−6% methanol in dichloromethane to give the product as a yellow solid (0.346 g, 70%). ^{1}H NMR (methanol-d_4) δ: 6.68 (d, $J = 9.6$ Hz, 1H); 7.30 (s, 1H); 8.03 (d, $J = 9.6$ Hz, 1H); 8.67 (s, 1H).

7-Methoxyquinoxalin-2(1*H*)-one (4l).[26,27] To a solution of 8% aqueous sodium hydroxide (1.32 L) was added **12** (100 g) followed by a solution of 30 wt % hydrogen peroxide in water (1.17 L). The reaction mixture was slowly heated to 80 °C and maintained at this temperature for 4 h. The heating source was removed, and acetic acid (150 mL) was added dropwise. The suspension was stirred overnight at room temperature and the precipitated solid was collected by filtration to afford the product as a tan solid (90 g, 91%). MS (ESP) m/z 177 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 3H); 6.76 (d, 1H); 6.90 (dd, 1H); 7.67 (d, 1H); 7.97 (s, 1H); 12.32 (brs, 1H).

6-Methoxypyrido[2,3-*b*]pyrazin-3(4*H*)-one (4m). To a solution of commercially available 3,4-diamino-6-methoxypyridine **13** (1.11 g) in methanol (20 mL) was added ethyl glyoxalate (3.5 mL). The reaction mixture was stirred overnight at room temperature, then filtered and washed with methanol (the precipitate contained the undesired regioisomer 6-methoxypyrido[2,3-*b*]pyrazin-2(1*H*)-one). The filtrate was concentrated and suspended in diethyl ether to give 0.18 g product (13%) as a colorless solid. MS (ESI) m/z 178 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 6.77 (d, 1H); 8.01 (s, 1H); 8.07 (d, 1H); 12.83 (s, 1H).

2-Methoxy-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (4n).[28] A mixture of 2-methanesulfinyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one **15**[29] (740 mg, 3.5 mmol) in NaOMe solution (0.5 M in methanol,

70 mL, 15 mmol) was heated at 70 °C for 2 h, then cooled to room temperature. The mixture was quenched with glacial acetic acid, filtered through Celite and the residue rinsed with methanol. The combined filtrate and wash were concentrated, and the residue was partitioned between water and dichloromethane. The layers were separated, and the aqueous phase was back-extracted with chloroform/isopropyl alcohol (3:1, 5 × 50 mL). The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica gel with dichloromethane/methanol (3:1) gave the product as a colorless solid in 71% yield. MS (ESP) m/z 178 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 4.02 (s, 3H); 6.50 (d, J = 9.4 Hz, 1H); 7.97 (d, J = 9.4 Hz, 1H); 8.93 (s, 1H).

6-Methoxy-3-oxo-3,4-dihydrobenzo[*e*][1,2,4]triazine 1-Oxide (4o).[30] To a suspension of 3-amino-6-methoxybenzo[*e*]-[1,2,4]triazine 1-oxide **16**[31] (1.17 g, 6.08 mmol) in water (15 mL) was added concentrated sulfuric acid (3.5 mL), followed by sodium nitrite (1.6 g, 23 mmol) in small portions. After 15 min the reaction mixture was filtered and the residue was washed with water and dried under reduced pressure at 105 °C to give the product as a colorless solid in 90% yield, mp 238−240 °C. MS (ESP) m/z 194 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 3.88 (s, 3H); 6.69 (s, 1H); 6.92 (d, J = 9.3 Hz, 1H); 8.03 (d, J = 9.3 Hz, 1H); 12.48 (s, 1H).

2-Methoxypteridin-7(8*H*)-one (4p). To a solution of commercially available 2-methoxy-4,5-pyrimidine diamine **14** (1.83 g) in methanol (25 mL) was added ethyl glyoxalate (5 mL). The mixture was stirred overnight at room temperature, then heated to reflux for 2 h. The reaction mixture was cooled with an ice bath and the resulting precipitate was collected by filtration and washed with methanol to yield the product as an off-white solid (2.20 g, 95%). MS (ESP) m/z 179 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 3.98 (s, 3H); 8.06 (s, 1H); 8.95 (s, 1H); 13.08 (s, 1H).

(2-Amino-4-methoxyphenyl)methanol (7).[32] To a solution of commercially available 4-methoxy-2-nitrobenzoic acid **6** (4 g, 20.66 mmol) in tetrahydrofuran (20 mL) at −15 °C under nitrogen was added *N*-methylmorpholine (2.2 mL, 20 mmol) followed by isobutyl chloroformate (2.6 mL, 20 mmol) dropwise. After 5 min, the reaction mixture was filtered and the solid was rinsed with tetrahydrofuran (20 mL). The filtrate was cooled to −15 °C, and a solution of sodium borohydride in water (3 M, 10 mL) was added. The mixture was stirred for 5 min, then partitioned between water and dichloromethane. The aqueous layer was extracted with dichloromethane (3 × 100 mL), and the combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in tetrahydrofuran (35 mL). Palladium (10% on carbon, 400 mg) was added, and the mixture was stirred under hydrogen gas for 18 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. Chromatography on silica gel with hexanes/ethyl acetate (3:2) gave 744 mg (24%) of the product as a yellow solid. ^{1}H NMR (DMSO-d_6) δ: 3.63 (s, 3H); 4.30 (s, 2H); 4.84−4.96 (m, 2H); 6.08 (dd, 1H); 6.20 (d, 1H); 6.90 (d, 1H).

Ethyl *N*-(4-Methoxy-2-nitrophenyl)glycinate (11). A mixture of 4-methoxy-2-nitroaniline (25.0 g, 0.15 mol), ethyl bromoacetate (200 mL, 1.8 mol), and potassium carbonate (31.1 g, 0.23 mol) was heated at 150 °C for 4.5 h. The mixture was cooled to room temperature, and aqueous sodium hydroxide solution (1M, 600 mL) was added. This mixture was extracted with ethyl acetate (2 × 500 mL). The combined organic phases were dried over magnesium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with 25−50% acetone in hexanes to give 22.1 g of the crude product as a red solid. ^{1}H NMR revealed the presence of ∼20% dialkylated product. This material was used without further purification for the next step. ^{1}H NMR (DMSO-d_6) δ: 1.18−1.23 (t, 3H); 3.74 (s, 3H); 4.12−4.18 (q, 2H); 4.23−4.25 (d, 2H); 6.90 − 6.93 (d, 1H); 7.25−7.29 (dd, 1H); 7.51−7.52 (d, 1H); 8.23−8.27 (t, 1H).

7-Methoxy-3,4-dihydroquinoxalin-2(1*H*)-one (12).[26,27] **11** (15.8 g crude) was taken up in 200 mL of 1:1 methanol/acetic acid, treated with 10% palladium on carbon (2 g), and stirred in an atmosphere of hydrogen overnight. The reaction mixture was filtered through Celite and the filtrate was concentrated to dryness to give 10.6 g of the crude product as a tan solid. This material was used without further purification. MS (ESP) m/z 179 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 3.61 (m, 5H); 5.57 (m, 1H); 6.35−6.40 (m, 2H); 6.60 (m, 1H); 10.13 (brs, 1H).

***tert*-Butyl [1-(2-Hydroxyethyl)piperidin-4-yl]carbamate (18).** A mixture of commercially available *tert*-butyl piperidin-4-ylcarbamate **17** (5 g, 25 mmol), 2-bromoethanol (1.77 mL, 25 mmol), and triethylamine (3.86 mL, 27.5 mmol) in acetonitrile (20 mL) was heated in a sealed tube at 50 °C for 16 h. The solvent was removed under reduced pressure, and the residue was taken up in ethyl acetate (300 mL) and washed with saturated aqueous sodium hydrogen carbonate solution (100 mL). The aqueous phase was back-extracted once with ethyl acetate (100 mL), and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography on silica gel with dichloromethane/methanol (4:1) gave 4.04 g (66%) of the product as a colorless solid, mp 66 °C. MS (ESP) m/z 245 (MH^+). ^{1}H NMR (DMSO-d_6) δ: 1.33 (m, 2H); 1.36 (s, 9H); 1.62 (m, 2H); 1.92 (t, 2H); 2.32 (t, 2H); 2.77 (m, 2H); 3.17 (m, 1H); 3.43 (m, 2H); 4.34 (t, 1H); 6.73 (d, 1H).

2-{4-[(*tert*-Butoxycarbonyl)amino]piperidin-1-yl}ethyl Methanesulfonate (19). A mixture of **18** (1.7 g, 7 mmol) in dry dichloromethane (20 mL) and triethylamine (1.4 mL, 9.8 mmol) was treated at 0 °C with methanesulfonyl chloride (0.65 mL, 8.4 mmol). After 45 min the reaction was complete by TLC (chloroform/methanol 6:1, R_f = 0.54). Potassium phosphate buffer (pH 7, 1 M, 50 mL) was added. Dichloromethane was removed under reduced pressure, and the residue was extracted with ice-cold ethyl acetate (2 × 100 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure, and the crude preparation of the mesylate was used without delay for the next step. MS (ESP) m/z 323.18 (MH^+).

General Procedure for 20 by Alkylation of 4 with 19. A solution of **4** (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 **19** 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 hydrogen carbonate 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-(6-Methoxy-3-oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)ethyl]piperidine-4-yl}carbamate (20a). 20a** was prepared from **4a**[33] and **19** according to the general procedure for **20**. Chromatography on silica gel with hexanes/ethyl acetate (1:1) afforded the product in 60% yield. MS (ESP) m/z 406 (MH^+). ^{1}H NMR ($CDCl_3$-*d*) δ: 1.34 (m, 2H); 1.36 (m, 2H); 1.62 (m, 2H); 1.98 (m, 2H); 2.43 (m, 2H); 2.84 (m, 2H); 3.20 (m, 1H); 3.73 (s, 3H); 3.96 (m, 2H); 4.53 (s, 2H); 6.56 (m, 1H); 6.76 (m, 1H); 6.92 (m, 1H).

***tert*-Butyl {1-[2-(3-Oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)-ethyl]piperidin-4-yl}carbamate (20b). 20b** was prepared from commercially available **4b** and **19** according to the general procedure for **20**. Chromatography on silica gel with dichloromethane/methanol (20:1) gave the product in 63% yield as a yellow gum. MS (ESP) m/z 376 (MH^+).

***tert*-Butyl {1-[2-(6-Cyano-3-oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)ethyl]piperidin-4-yl}carbamate (20c). 20c** was prepared from **4c**[34] and **19** according to the general procedure for **20**. Chromatography on silica gel with hexanes/ethyl acetate (1:3) afforded the product in 76% yield. MS (ESP) m/z 401 (MH^+). ^{1}H NMR ($CDCl_3$-*d*) δ: 1.41 (m, 2H); 1.42 (s, 9H); 1.93 (m, 2H); 2.21 (m, 2H); 2.58 (m, 2H); 2.87 (m, 2H); 3.45 (m, 1H); 4.02 (m, 2H); 4.43 (m, 1H); 4.67 (s, 2H); 7.03 (d, 1H); 7.30 (m, 1H); 7.47 (s, 1H).

***tert*-Butyl {1-[2-(6-Fluoro-3-oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)ethyl]piperidin-4-yl}carbamate (20d).** **20d** was prepared from **4d**[35] and **19** according to the general procedure for **20**. Chromatography on silica gel with hexanes/ethyl acetate (1:1) gave the product in 67% yield. MS (ESP) m/z 394.26 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.39 (m, 2H); 1.43 (s, 9H); 1.92 (m, 2H); 2.20 (m, 2H); 2.58 (t, 2H); 2.88 (m, 2H); 3.45 (m, 1H); 3.98 (t, 2H); 4.41 (m, 1H); 4.55 (s, 2H); 6.68 (m, 1H); 6.85 (m, 1H); 6.91 (m, 1H).

***tert*-Butyl {1-[2-(6-Methoxy-3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)ethyl]piperidin-4-yl}carbamate (20e).** **20e** was prepared from **4e** and **19** according to the general procedure for **20**. Chromatography on silica gel with hexanes/ethyl acetate (1:3) afforded the product in 85% yield. MS (ESP) m/z 422.24 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.40 (m, 2H); 1.45 (s, 9H); 1.92 (m, 2H); 2.22 (m, 2H); 2.62 (t, 2H); 2.88 (m, 2H); 3.35 (s, 2H); 3.49 (m, 1H); 3.82 (s, 3H); 4.08 (t, 2H); 4.43 (m, 1H); 6.61 (dd, 1H); 6.86 (d, 1H); 7.28 (s, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate (20f).** **20f** was prepared from **4f** and **19** according to the general procedure for **20**. Chromatography on silica gel, eluting with ethyl acetate and then acetone/dichloromethane (4:1), gave the product as a colorless oil, (82%). MS (ESP) m/z 404 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.32 (m, 2H); 1.36 (s, 9H); 1.64 (m, 2H); 1.93−2.00 (m, 4H); 2.37 (m, 2H); 2.47 (m, 2H); 2.78 (m, 2H); 3.16 (m, 1H); 3.71 (s, 3H); 3.91(t, 2H); 6.72−6.82 (m, 3H); 7.05 (d, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-2-oxo-2*H*-3,1-benzoxazin-1(4*H*)-yl)ethyl]piperidin-4-yl}carbamate (20g).** A solution of **4g** (310 mg, 1.8 mmol) in dry *N*,*N*-dimethylformamide (5 mL) was treated at 0 °C under stirring with lithium bis(trimethylsilyl)amide (1 M in tetrahydrofuran, 1.9 mL, 1.9 mmol). The mixture was stirred for 30 min at room temperature and treated with **19** (2.03 mmol) as described in the general procedure for **20**. Chromatography on silica gel with dichloromethane/methanol (95:5) gave 333 mg (47%) of the product as a colorless solid. MS (ESP) m/z 406 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.34−1.46 (m, 11H); 1.86 (d, 2H); 2.12−2.25 (m, 2H); 2.60−2.64 (m, 2H); 2.60−2.64 (m, 2H); 2.81−2.90 (m, 2H); 3.38−3.45 (m, 1H); 3.76 (s, 3H); 3.97−3.91 (m, 2H); 4.32−4.42 (m, 1H); 5.05 (s, 2H); 6.49−6.56 (m, 2H); 6.94 (d, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-2-oxoquinolin-1(2*H*)-yl)ethyl]-piperidin-4-yl}carbamate (20h).** **20h** was prepared from **4h**[24] and **19** according to the general procedure for **20**. Chromatography on silica gel with hexanes/acetone (1:1) gave the product as a colorless hard foam, 20% yield. MS (ESP) m/z 402 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.30−1.42 (m, 2H); 1.36 (s, 9H); 1.66 (m, 2H); 2.04 (m, 2H); 2.45−2.53 (m, 2H); 2.92 (m, 2H); 3.15 (m, 1H); 3.88 (s, 3H); 4.31 (t, 2H); 6.39 (m, 1H); 6.76 (m, 1H); 6.88 (m, 1H); 6.93 (m, 1H); 7.62 (d, 1H); 7.80 (d, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate (20i).** **20i** was prepared from **4i**[36] and **19** according to the general procedure for **20**. Chromatography on silica gel, eluting with a gradient of 5−7% methanol in methylene chloride, gave the product as a light brown oil, 74% yield. MS (ESP) m/z 411 (MH^+).

***tert*-Butyl {1-[2-(7-Methoxy-4-methyl-2-oxoquinazolin-1(2*H*)-yl)ethyl]piperidin-4-yl}carbamate (20j).** **20j** was prepared from **4j** and **19** according to the general procedure for **20**. Reverse phase HPLC chromatography on a 19 mm × 100 mm ODO AQ C18 column, eluting with a gradient of 0.1% TFA/water to 0.1% TFA/acetonitrile, gave the product in 35% yield. MS (ESP) m/z 417 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.36−1.43 (m, 2H); 1.37−1.47 (m, 9H); 1.89−1.98 (m, 2H); 2.21−2.31 (m, 2H); 2.66−2.70 (m, 2H); 2.71 (s, 2H); 2.87−2.97 (m, 2H); 3.47 (s, 1H); 3.94 (s, 3H); 4.28−4.37 (m, 2H); 4.43 (s, 1H); 6.78 (d, 1H); 6.82 (dd, 1H); 7.80 (d, 1H).

***tert*-Butyl 1-(2-(7-Chloro-2-oxo-1,6-naphthyridin-1(2*H*)-yl)-ethyl)piperidin-4-ylcarbamate (20k).** A suspension of **4k** (0.348 g, 1.933 mmol) in DMF (7 mL) was treated with lithium bis-(trimethylsilyl)amide (1 M, 2.0 mL). The mixture was stirred at room temperature for 10 min, then cooled to 0 °C and reacted with **19** according to the general procedure for **20**. Chromatography on silica gel with 0−5% methanol in dichloromethane gave the crude product as a mixture with **4k**. This mixture was carried on to the next step without further purification. ^1H NMR (methanol-d_4) δ: 1.33 (s, 9H); 1.75 (d, J = 13.0 Hz, 2H); 1.99−2.22 (m, 2H); 2.47−2.68 (m, 2H); 2.71−2.98 (m, 2H); 3.48−3.61 (m, 1H); 4.30 (t, J = 7.1 Hz, 1H); 6.60 (dd, J = 18.3, 9.6 Hz, 1H); 7.57 (s, 1H); 7.90 (t, J = 9.8 Hz, 1H); 8.56 (d, J = 2.8 Hz, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl]-piperidin-4-yl}carbamate (20l).** **20l** was prepared from **4l** and **19** according to the general procedure for **20**. Chromatography on silica gel with 25% acetone in hexanes gave the product as a colorless solid, 29% yield. MS (ESP) m/z 403 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.26−1.40 (m, 11H); 1.57−1.72 (m, 2H); 1.97−2.11 (m, 2H); 2.51−2.61 (m, 2H); 2.85−2.98 (m, 2H); 3.19 (s, 1H); 3.92 (s, 3H); 4.32 (t, 2H); 6.76 (d, 1H); 6.95−7.04 (m, 2H); 7.70−7.78 (m, 1H); 8.04 (s, 1H).

***tert*-Butyl {1-[2-(6-Methoxy-3-oxopyrido[2,3-*b*]pyrazin-4(3*H*)-yl)ethyl]piperidin-4-yl}carbamate (20m).** **20m** was prepared from **4m** and **19** according to the general procedure for **20**. Chromatography on silica gel with 0−25% acetone in dichloromethane gave the product in 67% yield. MS (ESI) m/z 404 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.31−1.40 (m, 2H); 1.40−1.46 (m, 9H); 1.87−1.95 (m, 2H); 2.15−2.27 (m, 2H); 2.69−2.75 (m, 2H); 2.93−3.02 (m, 2H); 3.40−3.51 (m, 1H); 4.02 (s, 3H); 4.35−4.46 (m, 1H); 4.51−4.60 (m, 2H); 6.73 (d, 1H); 8.02 (d, 1H); 8.15 (s, 1H).

***tert*-Butyl 1-(2-(2-Methoxy-7-oxopyrido[2,3-*d*]pyrimidin-8(7*H*)-yl)ethyl)piperidin-4-ylcarbamate (20n).** **20n** was prepared from **4n** and **19** according to the general procedure for **20**. Chromatography on silica gel with 10% methanol in dichloromethane gave the product in 59% yield. MS (ESP) m/z 404 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.28 (m, 2H); 1.38 (s, 9H); 1.65 (m, 2H); 1.90−2.10 (m, 2H); 2.56 (m, 1H); 2.92 (m, 2H); 3.18 (m, 1H); 4.01 (s, 3H); 4.38 (t, J = 6.9 Hz, 2H); 6.50−6.59 (m, 1H); 6.76 (m, J = 7.5 Hz, 1H); 7.89−8.01 (m, 1H); 8.83−8.98 (m, 1H).

4-(2-(4-(*tert*-Butoxycarbonylamino)piperidin-1-yl)ethyl)-6-methoxy-3-oxo-3,4-dihydrobenzo[*e*][1,2,4]triazine 1-Oxide (20o). **20o** was prepared from **4o** and **19** according to the general procedure for **20**. The residue obtained after aqueous workup was taken up in ether, and the precipitate was collected by filtration and dried under reduced pressure to give the product in 39% yield. MS (ESP) m/z 442 (MNa^+). ^1H NMR ($CDCl_3$) δ: 1.22 (s, 1H); 1.26 (s, 1H); 1.30 (s, 1H); 1.35 (s, 9H); 1.64 (d, J = 10.93 Hz, 2H); 2.03 (t, J = 10.83 Hz, 2H); 2.58 (t, J = 6.50 Hz, 2H); 2.85−2.95 (m, 2H); 3.17 (s, 1H); 3.98 (s, 3H); 4.28 (t, J = 6.5 Hz, 2H); 6.75 (d, J = 7.72 Hz, 1H); 6.99 (dd, J = 9.42, 2.26 Hz, 1H); 7.04 (d, J = 2.07 Hz, 1H); 8.14 (d, J = 9.42 Hz, 1H).

***tert*-Butyl {1-[2-(2-Methoxy-7-oxopteridin-8(7*H*)-yl)ethyl]-piperidin-4-yl}carbamate (20p).** **20p** was prepared from **4p** and **19** according to the general procedure for **20**. Chromatography on silica gel with a gradient of 0−5% methanol in dichloromethane gave the product as a colorless hard foam in 83% yield. MS (ESP) m/z 405 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.24−1.35 (m, 2H); 1.43 (s, 9H); 1.84−1.95 (m, 2H); 2.14−2.24 (m, 2H); 2.71 (t, 2H); 2.91−2.96 (m, 2H); 3.38−3.49 (m, 1H); 4.06−4.12 (m, 3H); 4.33−4.43 (m, 1H); 4.46 (t, 2H); 8.13 (s, 1H); 8.93 (s,1H).

***tert*-Butyl-{1-[2-(7-chloro-2-oxo-1,8-naphthyridin-1(2*H*)-yl)-ethyl]piperidin-4-yl}carbamate (20q).** **20q** was prepared from 7-chloro-1,8-naphthyridin-2(1*H*)-one **4q**[37] and **19** according to the general procedure for **20**. Chromatography on silica gel with 20% acetone in hexanes gave the product as a colorless solid in 67% yield. MS (ESP) m/z 407 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.20 (s, 2H); 1.35 (s, 9H); 1.56−1.67 (m, 2H); 1.92−2.05 (m, 2H); 2.45−2.51 (m, J = 3.20 Hz, 2H); 2.52 (s, 1H); 2.85−2.97 (m, 2H); 3.09−3.23 (m, 1H); 4.34−4.43 (m, 2H); 6.70 (d, J = 9.4 Hz, 1H); 7.39 (d, J = 8.1 Hz, 1H); 7.97 (d, J = 9.6 Hz, 1H); 8.21 (d, J = 8.1 Hz, 1H).

***tert*-Butyl {1-[2-(7-Methoxy-2-oxo-1,8-naphthyridin-1(2*H*)-yl)ethyl]piperidine-4-yl}carbamate (20r).** A solution of **20q** (0.68 g, 1.67 mmol) and sodium methoxide (2.55 mmol) in methanol (5.0 mL) was heated in a sealed tube at 140 °C for 30 min using microwave irradiation. The reaction mixture was concentrated under reduced pressure and then quenched with water. The product was extracted with ethyl acetate, and the organic phase was washed with

brine and then dried over sodium sulfate. Chromatography on silica gel with 10% methanol in dichloromethane gave the product as a colorless solid in 61% yield. MS (ESP) m/z 403 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.28 (s, 1H); 1.35 (s, 9H); 1.63−1.71 (m, 2H); 2.06 (t, 2H); 2.52 (m, 1H); 2.87−2.94 (m, 2H); 2.96 (m, 2H); 3.13−3.20 (m, 1H); 3.20 − 3.29 (m, 2H); 3.97 (s, 3H); 4.41−4.49 (m, 1H); 6.48 (d, J = 9.5 Hz, 1H); 6.70 (d, J = 8.1 Hz, 1H); 7.07 (d, J = 9.5 Hz, 1H); 8.04 (d, J = 8.1 Hz, 1H).

***tert*-Butyl 1-(2-(6-Methoxy-3-oxobenzo[*e*][1,2,4]triazin-4(3*H*)-yl)ethyl)piperidin-4-ylcarbamate (20t).** To a solution of **20o** (0.440 g, 1.05 mmol) in DMF (7 mL) were added water (7 mL) and sodium dithionite (900 mg). The mixture was heated in a microwave reactor at 100 °C for 2 h. The reaction mixture was washed with brine and extracted with ethyl acetate (40 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with 0−10% methanol in methylene chloride to give the product in 27% yield. MS (ESP) m/z 404 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.17−1.33 (m, 2H); 1.37 (s, 9H); 1.64 (m, 2H); 1.90−2.14 (m, 2H); 2.61 (m, 2H); 3.17 (m, 1H); 4.01 (s, 3H); 4.27 (t, J = 6.3 Hz, 2H); 6.76 (d, 1H); 7.01 (m, 1H); 7.11 (d, 1H); 7.95 (m, 2H); 8.27 (d, 1H).

General Procedure for 21 by Deprotection of 20. A solution of **20** (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 codistilled once with dichloromethane, then taken up in dichloromethane (200 mL) and washed with saturated sodium hydrogen carbonate 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.

4-[2-(4-Aminopiperidin-1-yl)ethyl]-6-methoxy-2*H*-1,4-benzoxazin 3(4*H*)-one (21a). **21a** was prepared from **20a** according to the general procedure for **21**, but aqueous workup was omitted, to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 306 (MH^+).

4-[2-(4-Aminopiperidin-1-yl)ethyl]-2*H*-1,4-benzoxazin-3(4*H*)-one (21b). **21b** was prepared from **20b** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 276 (MH^+).

4-[2-(4-Aminopiperidin-1-yl)ethyl]-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carbonitrile (21c). **21c** was prepared from **20c** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 301 (MH^+).

4-[2-(4-Aminopiperidin-1-yl)ethyl]-6-fluoro-2*H*-1,4-benzoxazin-3(4*H*)-one (21d). **21d** was prepared from **20d** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 294 (MH^+).

4-[2-(4-Aminopiperidin-1-yl)ethyl]-6-methoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (21e). **21e** was prepared from **20e** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 322 (MH^+).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxy-3,4-dihydroquinolin-2(1*H*)-one (21f). **21f** was prepared from **20f** according to the general procedure for **21**. The product was obtained in 82% yield as a colorless oil. MS (ESP) m/z 304 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.19 (m, 2H); 1.62 (m, 2H); 1.96 (t, 2H); 2.36 (t, 2H); 2.46 (m, 2H); 2.73−2.80 (m, 5H); 3.71 (s, 3H); 3.91 (m, 2H); 6.77−6.84 (m, 2H); 7.04 (m, 1H).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxy-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (21g). **21g** was prepared from **20g** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield as a brown oil. MS (ESP) m/z 302 (MH^+).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxyquinolin-2(1*H*)-one (21h). **21h** was prepared from **20h** according to the general procedure for **21**. The product was obtained in quantitative yield as a colorless oil. MS (ESP) m/z 302 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.21 (m, 2H); 1.65 (m, 2H); 2.04 (t, 2H); 2.40−2.52 (m, 2H); 2.89 (m, 2H); 3.69 (m, 1H); 3.88 (s, 3H); 4.31 (t, 2H); 6.40 (m, 1H); 6.88 (m, 1H); 6.94 (m, 1H); 7.63 (m, 1H); 7.80 (m, 1H).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxy-4-methylquinolin-2(1*H*)-one (21i). **21i** was prepared from **20i** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 316 (MH^+).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxy-4-methylquinazolin-2(1*H*)-one (21j). **21j** was prepared from **20j** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield. MS (ESP) m/z 317 (MH^+);. ^1H NMR ($CDCl_3$) δ: 1.36−1.46 (m, 2H); 1.80−1.88 (m, 2H); 2.21 (td, 2H); 2.67−2.76 (m, 6H); 2.98 (d, 2H); 3.95 (s, 3H); 4.30−4.38 (m, 2H); 6.80−6.85 (m, 2H); 7.77−7.83 (m, 1H).

1-(2-(4-Aminopiperidin-1-yl)ethyl)-7-chloro-1,6-naphthyridin-2(1*H*)-one (21k). A solution of **20k** (0.449 g, 1.106 mmol) in dichloromethane was treated with a solution of hydrochloric acid in dioxane (4M, 8 mmol) The mixture was stirred at room temperature overnight. The precipitate was collected by filtration to give the bis-hydrochloride salt of the product as a colorless solid in quantitative yield. MS (ESP) m/z 307 (MH^+).

1-[2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxyquinoxalin-2(1*H*)-one (21l). **21l** was prepared from **20l** according to the general procedure for **21** to give the crude product in 77% yield as an oil. MS (ESP) m/z 303 (MH^+).

4-[2-(4-Aminopiperidin-1-yl)ethyl]-6-methoxypyrido[2,3-*b*]-pyrazin-3(4*H*)-one (21m). **21m** was prepared from **20m** according to the general procedure for **21** to give the crude product in 94% yield. MS (ESP) m/z 304 (MH^+).

8-(2-(4-Aminopiperidin-1-yl)ethyl)-2-methoxypyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (21n). **21n** was prepared from **20n** according to the procedure for **21a** to give the trifluoroacetate salt of the product in quantitative yield as a brown hard foam. MS (ESP) m/z 304 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.73 (m, 2H); 1.94−2.24 (m, 2H); 3.14 (m, 2H); 3.30 (brs, 1H); 3.83 (m, 2H); 4.04 (s, 3H); 4.61 (m, 2H); 6.62 (m, 1H); 8.01 (d, J = 9.6 Hz, 1H); 8.15 (m, 3H); 8.97 (s, 1H); 9.66 (brs, 1H).

8-[2-(4-Aminopiperidin-1-yl)ethyl]-2-methoxypteridin-7(8*H*)-one (21p). **21p** was prepared from **20p** according to the general procedure for **21** to give the crude product in quantitative yield. MS (ESP) m/z 305 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.21−1.33 (m, 2H); 1.72−1.82 (m, 2H); 2.13 (td, 2H); 2.64 (s, 1H); 2.71 (t, 2H); 2.92−3.01 (m, 2H); 4.11 (s, 3H); 4.48 (t, 2H); 8.13 (s, 1H); 8.92 (s, 1H).

1-{2-(4-Aminopiperidin-1-yl)ethyl]-7-methoxy-1,8-naphthyridin-2(1*H*)-one (21r). **21r** was prepared from **20r** according to the general procedure for **21**, except after evaporation of the reaction mixture, the residue was diluted with water and the aqueous layer washed with ethyl acetate. The aqueous layer was concentrated under vacuum using acetonitrile as an azeotrope to afford the trifluoroacetic acid salt of the product in 78% yield as an off-white solid. MS (ESP) m/z 303 (MH^+).

4-(2-(4-Aminopiperidin-1-yl)ethyl)-6-methoxybenzo[*e*]-[1,2,4]triazin-3(4*H*)-one (21t). **21t** was prepared from **20t** according to the general procedure for **21** to give the product in 58% yield. MS (ESP) m/z 304 (MH^+).

General Procedure for 24 and 25 by Reductive Amination of 21. A solution of **21** (0.20 mmol) and 1 equiv of aldehyde, either (2,3-dihydro[1,4]dioxino[2,3-*c*]pyridine-7-carbaldehyde **22**[14] for **24** or 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde **23**[15]for **25**, 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.

4-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-6-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (24a). **24a** was prepared from **21a**, *N*,*N*-diisopropylethylamine (15 equiv), and **22**[14] according to the general procedure for **24** except as reducing agent, sodium cyanoborohydride (0.3 mmol) was

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used. Reverse phase chromatography with water/acetonitrile and TFA as modifier gave the trifluoroacetic acid salt of the product. The salt was partitioned between chloroform and sodium hydrogen carbonate solution. The aqueous phase was back-extracted with chloroform and the combined organic phases were dried over magnesium sulfate and concentrated under reduced pressure to give the free base of the product in 32% yield as a gum. MS (ESP) m/z 455 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.46 (m, 2H); 1.88 (m, 2H); 2.14 (m, 2H); 2.54 (m, 1H); 2.58 (t, J = 7.4 Hz, 2H); 2.95 (m, 2H); 3.78 (s, 3H); 3.79 (s, 2H); 4.01 (t, J = 7.4 Hz, 2H); 4.29 (m, 4H); 4.52 (s, 2H); 6.50 (dd, J = 6.2, 2.7 Hz, 1H); 6.67 (d, J = 2.7 Hz, 1H); 6.81 (s, 1H); 6.89 (d, J = 8.7 Hz, 1H); 8.08 (s, 1H).

6-[({1-[2-(6-Methoxy-3-oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one (25a). **25a** was prepared from **21a**, *N*,*N*-diisopropylethylamine (15 equiv) and **23**[15] according to the procedure for **24a** (17% yield). MS (ESP) m/z 468 (MH^+).

4-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethy)-2*H*-1,4-benzoxazin-3(4*H*)-one (24b). **24b** was prepared from **21b**, *N*,*N*-diisopropylethylamine (15 equiv), and **22**[14] according to the procedure for **24a** (16% yield). MS (ESP) m/z 425 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.2–3.8 (m, 14H); 3.87 (s, 1H); 4.42 (m, 4H); 4.75 (s, 2H); 7.11 (m, 3H); 7.40–7.60 (m, 2H); 8.29 (s, 1H); 10.01 (s, 2H); 10.01 (s, 2H); 11.35 (brs, 1H).

3-Oxo-4-[2-(4-{[(3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]-oxazin-6-yl)methyl]amino}piperidin-1-yl)ethyl]-3,4-dihydro-2*H*-1,4-benzoxazine-6-carbonitrile (25c). **25c** was prepared from **21c**, *N*,*N*-diisopropylethylamine (15 equiv), and **23**[15] according to the procedure for **24a** (19% yield). MS (ESP) m/z 463 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.49 (m, 2H); 1.94 (m, 2H); 2.14 (m, 2H); 2.56 (m, 1H); 2.59 (t, J = 6.7 Hz, 2H); 2.94 (m, 2H); 3.84 (s, 2H); 4.03 (t, J = 6.7 Hz, 2H); 4.62 (s, 2H); 4.67 (s, 2H); 6.94 (d, J = 8.1 Hz, 1H); 7.03 (d, J = 8.5 Hz, 1H); 7.19 (d, J = 7.9 Hz, 1H); 7.30 (dd, J = 6.6, 1.7 Hz, 1H); 7.46 (d, J = 1.5 Hz, 1H).

6-[({1-[2-(6-Fluoro-3-oxo-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one (25d). **25d** was prepared from **21d**, *N*,*N*-diisopropylethylamine (15 equiv), and **23**[15] according to the procedure for **24a** (16% yield). MS (ESP) m/z 456 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.52 (m, 2H); 1.94 (m, 2H); 2.15 (m, 2H); 2.57 (m, 1H); 2.59 (t, J = align="left"7.1 Hz, 2H); 2.97 (m, 2H); 3.84 (s, 2H); 4.01 (t, J = 7.1 Hz, 2H); 4.54 (s, 2H); 4.62 (s, 2H); 6.68 (m, 1H); 6.86 (m, 1H); 6.89 (m, 1H); 6.95 (d, J = 8.1 Hz, 1H); 7.20 (d, J = 8.1 Hz, 1H).

4-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-6-methoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (24e). **24e** was prepared from **21e**, *N*,*N*-diisopropylethylamine (15 equiv), and **22**[14] according to the procedure for **24a** (13% yield). MS (ESP) m/z 471 (MH^+). ^1H NMR ($CDCl_3$-d) δ: 1.47 (m, 2H); 1.91 (m, 2H); 2.15 (m, 2H); 2.54 (m, 1H); 2.60 (t, J = 6.4 Hz, 2H); 2.94 (m, 2H); 3.33 (s, 2H); 3.47 (s, 2H); 3.80 (s, 5H); 4.08 (t, J = 6.5 Hz, 2H); 4.29 (m, 4H); 6.58 (dd, J = 6.0, 2.6 Hz, 1H); 6.80 (s, 1H); 6.88 (d, J = 2.4 Hz, 1H); 7.23 (d, J = 8.4 Hz, 1H); 8.08 (s, 1H).

6-[({1-[2-(7-Methoxy-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)-ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one (25f). **25f** was prepared from **21f** and **23**[15] according to the general procedure, but the aqueous workup was omitted. Chromatography on a Phenomenex Synergy Polar-RP4um column, eluting with 30–60% acetonitrile, containing 10 mM ammonium acetate at pH 8, followed by chromatography on silica gel with dichloromethane/methanol (7:1) gave the free base of the product. The free base was dissolved in dichloromethane/ether (10 mL, 1:1), and HCl in ether (1 M, 3 mol equiv) was added under vigorous stirring. The mixture was evaporated to dryness under reduced pressure, and the residue was taken up as a suspension in dichloromethane/hexanes (10 mL, 1:1). The solid was collected by filtration and dried under reduced pressure to give the bis HCl salt of the product as a colorless solid in 24% yield, mp >285 °C (dec). MS (ESP) m/z 466 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.10 (m, 2H); 2.36 (m, 2H); 2.53 (t, 2H); 2.86 (t, 2H); 3.10 (m, 2H); 3.16 (m, 2H); 3.36 (m, 1H); 3.70 (m, 2H); 3.73 (s, 3H); 4.16 (m, 2H); 4.28 (m, 2H); 4.70 (s, 2H); 6.80 (m, 1H); 6.86 (s, 1H); 7.23–7.28 (m, 2H); 7.45 (d, 1H); 9.70 (brs, 2H); 11.07 (brs, 1H); 11.37 (s, 1H).

6-[({1-[2-(7-Methoxy-2-oxo-2*H*-3,1-benzoxazin-1(4*H*)-yl)-ethyl]piperidin-4-yl}amino)methyl]-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one (25g). **25g** was prepared from **21g**, *N*,*N*-diisopropylethylamine (15 equiv), and **23**[15] according to the general procedure for **25**. Chromatography on silica gel with dichloromethane/methanol (9:1) gave the free base of the product as a colorless foam. The free base was taken up in 1,4-dioxane (2 mL), and HCl in 1,4-dioxane (4M, 2 mol equiv) was added dropwise under rapid stirring. The precipitate was collected by filtration to give the bis-hydrochloride salt of the product in 48% yield as a colorless solid. MS (ESP) m/z 468 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.09 (m, 2H); 2.31–2.44 (m, 2H); 3.00–3.15 (m, 2H); 3.66–3.78 (m, 2H); 3.82 (s, 3H); 4.13–4.22 (m, 2H); 4.22–4.35 (m, 2H); 4.69 (s, 2H); 5.25 (s, 2H); 6.70 (d, 1H); 6.85 (s, 1H); 7.20 (dd, 2H); 7.45 (d, 1H); 9.56–9.71 (m, 2H); 11.05–11.19 (m, 1H); 11.37 (s, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-7-methoxyquinolin-2(1*H*)-one (24h). **24h** was prepared from **21h** and **22**[14] according to the general procedure for **24**. Chromatography on silica gel with dichloromethane/methanol (8:1 to 4:1) gave the free base of the title compound as a colorless oil. The HCl salt was prepared according to the protocol for **25f** to give the bis-HCl salt of the product in 48% yield as a colorless solid, mp 243 °C. MS (ESP) m/z 451 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.00–3.80 (m, 11H); 3.96 (s, 3H); 4.20–4.45 (m, 6H); 4.68 (m, 2H); 6.45 (d, 1H); 6.93 (d, 1H); 7.18 (s, 1H); 7.30 (s, 1H); 7.68 (d, 1H); 7.88 (d, 1H); 8.25 (s, 1H); 9.74 (brs, 2H); 11.18 (brs, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-7-methoxy-4-methylquinolin-2(1*H*)-one (24i). **24i** was prepared from **21i**, *N*,*N*-diisopropylethylamine (15 equiv), and **22**[14] according to the procedure for **24a**, except the product was obtained as a TFA salt after reverse phase chromatography (6% yield). MS (ESP) m/z 465 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.35 (s, 3H); 3.79 (s, 3H); 6.20 (s, 1H); 6.80 (m, 2H); 7.58 (d, 1H); 11.44 (brs, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-7-methoxy-4-methylquinazolin-2(1*H*)-one (24j). **24j** was prepared from **21j** and **22**[14] according to the general procedure for **24**. Purification of the crude product was performed by reverse phase HPLC on a 19 mm × 100 mm ODO AQ C18 column, eluting with a gradient of 0.1% TFA/water to 0.1% TFA/acetonitrile. Fractions containing product were concentrated under reduced pressure. The residue was taken up in methanol (10 mL), and 1 M HCl in ether (2 mol equiv) was added. The precipitate was collected by filtration to give the bis-HCl salt of the product as a colorless solid, 52% yield. MS (ESP) m/z 466 (MH^+). ^1H NMR (DMSO-d_6) δ: 2.03–2.14 (m, 2H); 2.31–2.42 (m, 2H); 2.82 (s, 3H); 3.07–3.14 (m, 2H); 3.32–3.44 (m, 2H); 3.77–3.86 (m, 2H); 4.07–4.15 (m, 3H); 4.22–4.33 (m, 2H); 4.34–4.40 (m, 2H); 4.41–4.47 (m, 2H); 4.67–4.77 (m, 2H); 7.11 (d, 1H); 7.22 (s, 1H); 7.37 (s, 1H); 8.19 (d, 1H); 8.29 (s, 1H); 9.81 (s, 1H); 11.28 (s, 1H).

7-Chloro-1-(2-(4-((2,3-dihydro[1,4]dioxino[2,3-c]pyridin-7-yl)methylamino)piperidin-1-yl)ethyl)-1,6-naphthyridin-2(1*H*)-one (24k). **24k** was prepared from **21k**, *N*,*N*-diisopropylethylamine (2.2 equiv), and **22**[14] according to the procedure for **24**. The residue obtained after aqueous workup was purified by silica gel chromatography, eluting with 0–12% methanol in dichloromethane to give the product as a colorless solid in 41% yield. ^1H NMR ($CDCl_3$-d) δ: 1.56–1.71 (m, 2H); 2.01 (d, J = 11.3 Hz, 2H); 2.19–2.36 (m, 1H); 2.69 (t, J = 7.1 Hz, 2H); 3.08 (t, J = 7.6 Hz, 2H); 3.60–3.76 (m, 2H); 3.90 (s, 2H); 4.23–4.42 (m, 5H); 6.72 (d, J = 9.6 Hz, 1H); 6.85 (s, 1H); 7.43 (s, 1H); 7.71 (d, J = 9.6 Hz, 1H); 8.11 (s, 1H); 8.55 (s, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-7-methoxyquinoxalin-2(1*H*)-one (24l). **24l** was prepared from **21l** and **22**[14] according to the general procedure for **24**. Chromatography on silica gel with 5% methanol in dichloromethane containing 0.25% ammonium hydroxide gave the free base of the product as an oil. The free base was taken up in isopropanol and treated with HCl in dioxane (4 M, 3 equiv). Solvent

was removed under reduced pressure to give the bis-hydrochloride salt of the product in 27% yield as an off-white solid. MS (ESP) m/z 452 (MH^+). ^1H NMR (D_2O) δ: 1.86−2.02 (m, 2H); 2.36−2.49 (m, 2H); 3.09−3.23 (m, 2H); 3.52 (t, 2H); 3.56−3.68 (m, 1H); 3.82−3.95 (m, 5H); 4.32−4.40 (m, 4H); 4.43−4.50 (m, 2H); 4.61 (t, 2H); 6.82 (d, 1H); 7.03 (dd, 1H); 7.29 (s, 1H); 7.71 (d, 1H); 7.97 (s, 1H); 8.22 (s, 1H).

4-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-6-methoxypyrido[2,3-*b*]pyrazin-3(4*H*)-one (24m). **24m** was prepared from **21m** and **22**[14] according to the general procedure for **24**. Chromatography on silica gel with 0−2% methanol in dichloromethane gave the product in 64% yield. MS (ESP) m/z 453 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.18 (q, 2H); 1.74 (d, 2H); 2.02 (t, 2H); 2.30−2.40 (m, 1H); 2.60 (t, 2H); 2.89 (d, 2H); 3.65 (s, 2H); 3.98 (s, 3H); 4.26 (dd, 2H); 4.29−4.34 (m, 2H); 4.40 (t, 2H); 6.83 (d, 1H); 6.92 (s, 1H); 7.98 (s, 1H); 8.10 (s, 1H); 8.12 (d, 1H).

8-(2-(4-((2,3-Dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)-methylamino)piperidin-1-yl)ethyl)-2-methoxypyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (24n). **24n** was prepared from **21n**, *N*,*N*-diisopropylethylamine (5 equiv), and **22**[14] according to the procedure for **24a**. Chromatography on silica gel with 15% methanol in dichloromethane gave the product as a colorless solid in 44% yield. MS (ESP) m/z 453 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.23 (m, 2H); 1.80 (m, 2H); 2.07 (t, J = 10.8 Hz, 2H); 2.39 (brs, 2H); 2.95 (m, 2H); 3.71 (m, 2H); 4.06 (s, 3H); 4.34 (m, 2H); 4.36−4.48 (m, 4H); 6.61 (d, J = 9.5 Hz, 1H); 6.99 (s, 1H); 8.00 (d, J = 9.5 Hz, 1H); 8.06 (s, 1H); 8.97 (s, 1H).

8-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidin-1-yl}ethyl)-2-methoxypteridin-7(8*H*)-one (24p). **24p** was prepared from **21p** and **22**[14] according to the general procedure for **24**. Chromatography on silica gel with 0−20% methanol in dichloromethane gave the monoacetic acid salt of the product as a colorless solid in 43% yield. MS (ESP) m/z 454 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.18−1.28 (m, 2H); 1.79 (d, 2H); 1.90 (s, 2H); 2.00 (t, 2H); 2.58 (t, 2H); 2.90 (d, 2H); 3.78 (s, 2H); 4.03 (s, 3H); 4.26−4.35 (m, 6H); 6.96 (s, 1H); 8.04 (s, 1H); 8.15 (s, 1H); 8.99 (s, 1H).

1-(2-{4-[(2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-ylmethyl)-amino]piperidine-1-yl}ethyl)-7-methoxy-1,8-naphthyridin-2(1*H*)-one (24r). **24r** was prepared from **21r**, *N*,*N*-diisopropylethylamine (2 equiv), and **22**[14] according to the general procedure for **24**. Chromatography on silica gel with 5% methanol in dichloromethane, containing 0.5% ammonium hydroxide, gave the product as an oil in 45% yield. MS (ESP) m/z 452 (MH^+). ^1H NMR (DMSO-d_6) δ: 0.78−0.92 (m, 1H); 1.15−1.29 (m, 2H); 1.74 (dd, 2H); 2.02 (m, 2H); 2.26−2.40 (m, 1H); 2.49 (m, 2H); 2.51−2.61 (m, 2H); 2.91 (m, 2H); 3.66 (m, 2H); 3.96 (s, 3H); 4.23−4.29 (m, 2H); 4.29−4.35 (m, 2H); 4.38−4.50 (m, 2H); 6.48 (d, J = 9.4 Hz, 1H); 6.71 (d, J = 8.3 Hz, 1H); 6.92 (s, 1H); 7.84 (d, J = 9.4 Hz, 1H); 7.97−8.06 (m, 2H).

1-(2-(4-((2,3-Dihydro[1,4]dioxino[2,3-*c*]pyridin-7-yl)-methylamino)piperidin-1-yl)ethyl)-7-methoxy-1,6-naphthyridin-2(1*H*)-one (24s). To a solution of sodium methoxide in methanol (0.5 M, 1.4 mL) was added **24k** (0.103 g, 0.23 mmol). The mixture was heated at 68 °C for 3 h. More sodium methoxide solution (0.5 M, 1.4 mL) was added, and heating was continued for 18 h at 78 °C. The mixture was cooled to room temperature and diluted with ethyl acetate and water. The pH of the aqueous layer was adjusted to pH 5 with 1 N HCl. The layers were separated, and the aqueous layer was washed with ethyl acetate (2 × 15 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give the product as a tan solid in 38% yield. ^1H NMR (DMSO-d_6) δ: 1.47 (m, 2H); 2.03 (m, 2H); 2.77 (brs, 1H); 3.00 (m, 2H); 3.88−4.06 (m, 5H); 4.17−4.47 (m, 7H); 6.47 (d, J = 9.6 Hz, 1H); 6.77 (s, 1H); 7.08 (s, 1H); 7.92 (d, J = 9.6 Hz, 1H); 8.12 (s, 1H); 8.56 (s, 1H); 9.13−10.02 (m, 2H).

6-((1-(2-(6-Methoxy-3-oxobenzo[*e*][1,2,4]triazin-4(3*H*)-yl)-ethyl)piperidin-4-ylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]-oxazin-3(4*H*)-one (25t). **25t** was prepared from **21t**, *N*,*N*-diisopropylethylamine (15 equiv), and **23**[15] according to the general procedure for **25**. Chromatography on silica gel with 0−20% methanol in dichloromethane gave the product in 30% yield. MS (ESP) m/z 466 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.08−1.27 (m, 2H); 1.75 (m, 2H); 1.91 (m, 2H); 2.03 (m, 2H); 2.30−2.44 (m, 1 H); 2.61 (m, 2H); 2.89 (m, 2H); 3.94−4.05 (m, 3H); 4.28 (t, J = 6.5 Hz, 2H); 4.61 (s, 2H); 6.93−7.04 (m, 2H); 7.11 (dd, J = 9.0, 2.2 Hz, 1H); 7.29 (d, J = 8.1 Hz, 1H); 8.27 (d, J = 9.0 Hz, 1H); 11.16 (brs, 1H).

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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; MIC, minimal inhibitory concentration; cfu, colony forming units; ESP, electrospray

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