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Novel Tetrahydropyran-Based Bacterial Topoisomerase Inhibitors with Potent Anti-Gram Positive Activity and Improved Safety Profile

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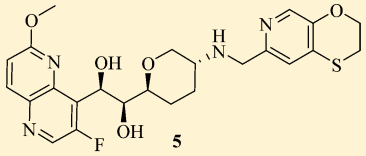
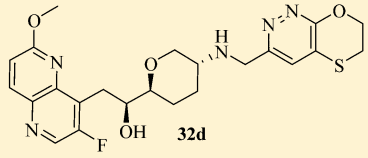
Supporting Information



5

in vivo efficacious against *S. aureus*
but lengthens the QT-interval of the ECG



32d

in vivo efficacious against *S. aureus* and *S. pneumoniae*
No change in cardiovascular parameters (guinea pig model)

ABSTRACT: Novel antibacterial drugs that are effective against infections caused by multidrug resistant pathogens are urgently needed. In a previous report, we have shown that tetrahydropyran-based inhibitors of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) display potent antibacterial activity and exhibit no target-mediated cross-resistance with fluoroquinolones. During the course of our optimization program, lead compound **5** was deprioritized due to adverse findings in cardiovascular safety studies. In the effort of mitigating these findings and optimizing further the pharmacological profile of this class of compounds, we have identified a subseries of tetrahydropyran-based molecules that are potent DNA gyrase and topoisomerase IV inhibitors and display excellent antibacterial activity against Gram positive pathogens, including clinically relevant resistant isolates. One representative of this class, compound **32d**, elicited only weak inhibition of hERG K^+ channels and $hNa_V1.5$ Na^+ channels, and no effects were observed on cardiovascular parameters in anesthetized guinea pigs. In vivo efficacy in animal infection models has been demonstrated against *Staphylococcus aureus* and *Streptococcus pneumoniae* strains.

INTRODUCTION

According to a recent report of the Center for Diseases Control and Prevention,[1] every year in the United States around two million people are falling ill from antibiotic-resistant infections, and 23000 people die because of such infections. Antimicrobial resistance constitutes not only a direct threat, but also put at risk well-established medical procedures from bone and joint replacement surgery to dialysis and organ transplants. All over the world, health authorities are reporting similar threats and are calling for immediate action to pursue discovery and development of novel antibacterial drugs.[2,3] However, the number of potential drugs effective against the "ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species)[4] currently in clinical development remains dramatically low.[5] New drugs must also be devoid of cross-resistance with agents that were formerly of clinical relevance. To overcome cross-resistance, they can either act on an untapped drug target or address a novel binding site of a known drug target. Whereas the identification of novel drug targets remains of prime importance,[6] there are still considerable efforts dedicated to the clinically validated antibacterial drug targets. In this respect, bacterial type II topoisomerases, DNA gyrase and topoisomerase IV (Topo IV), have been looked at intensely.[7] These two homologous and essential enzymes are known to regulate the DNA topology during replication.[8] DNA gyrase introduces negative supercoils into DNA,[9] and Topo IV is mainly responsible of the decatenation of the sister chromatids at the end of the replication process.[10] Topoisomerases are heterotetrameric A_2B_2 complexes made of two GyrA/GyrB and ParC/ParE (GrlA/GrlB in *S. aureus*) subunits for DNA gyrase and Topo IV, respectively. Fluoroquinolone[11] and coumarin antibiotics,[12] two clinically validated classes of antibacterial drugs, block DNA topoisomerases function. Coumarin antibiotics do so through binding to the ATP-binding pocket located within GyrB or ParE subunits.[13] Fluoroquinolones function through binding to the cleavage-ligation site located in GyrA/ParC, stabilizing double-strand breaks in DNA, eventually leading to a rapid bacterial killing.[14] Resistance to fluoroquinolones is nowadays widespread.[15] The main mechanism providing

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resistance to fluoroquinolone results from amino acid changes in the quinolone-resistance determining region (QRDR) of GyrA (Ser84Leu) or GrlA (Ser80Ile).[16−18] A few years ago, GSK[19] and Aventis[20] had discovered chemical entities, related either to **1** (GSK299423)[21] or **2** (NXL-101)[22] (Figure 1), that

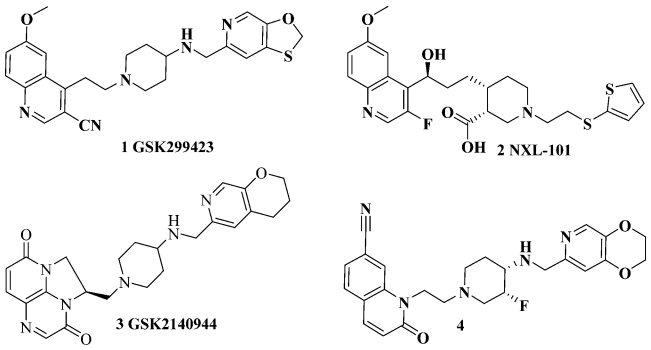

Figure 1. Chemical structures of some NBTIs.

display excellent antibacterial properties and are devoid of cross-resistance with fluoroquinolones while being potent DNA gyrase and/or topoisomerase IV inhibitors. In 2010, the binding mode of compound **1** at the interface between DNA and gyrase of *Staphylococcus aureus* (*S. aureus*) was unambiguously identified. Intriguingly, this compound binds at a site different from the one used by fluoroquinolones.[23] This finding immediately triggered intensive research efforts.[24] Several pharmaceutical companies have reported their research programs. Viquidacin (NXL-101) **2**, developed at Novexel, was the first NBTI to enter clinical development but was discontinued due to QT prolongation.[25] More recently, GSK entered phase 2 dose-ranging study to treat skin infections caused by *S. aureus* with **3** (GSK-2140944).[26] Astra-Zeneca has also reported compounds such as **4**, which may be suitable for the treatment of infections caused by Gram-positive bacteria[27] or for the treatment of tuberculosis.[28]

We have previously reported a series of tetrahydropyran (THP)-based bacterial topoisomerase inhibitors and described how these compounds act as dual DNA gyrase and Topo IV inhibitors.[29] In those series, we demonstrated that NBTI **5** (Figure 2) displayed an excellent antistaphylococcal activity, no cross-resistance with fluoroquinolones, low to moderate resistance frequency, and was efficacious in a murine thigh infection model against *S. aureus*; however, NBTI **5** blocked $hK_V11.1$ ("hERG") channels slightly. In addition, NBTI **5** also blocked $hNa_V1.5$ channels slightly and affected various cardiovascular parameters when administered to anesthetized guinea pigs (data not shown in our earlier disclosure,[29] see thereafter). We therefore based our lead optimization on lowering hERG blockade further, while the inhibition of $hNa_v1.5$ and the effects on cardiovascular parameters in vivo were monitored for selected compounds. Our objective was to discover THP-based NBTIs with a clean cardiovascular safety profile before advancing them into preclinical studies.

Structure−activity relationship studies have established the importance of the two-atom linker −A−B− that unifies the bicyclic aromatic left-hand side (LHS) to the central THP-core in modulating hERG K^+ channel block (Figure 2).[29] To complete our investigations on hydroxylated −A−B− linkers, we turned our attention to NBTIs featuring a $-CH_2-(CHOH)-$ linker. We report herein the synthetic routes that led to the designed hydroxylated linker as well as the fruits of our investigations devoted to the optimization of the cardiac safety profile of THP-based NBTIs.[30] Key properties of our preclinical candidate **32d** are disclosed and illustrate its clinically relevant pharmacological profile.

CHEMISTRY

To rapidly construct the $-CH_2-(CHOH)-$ linker and to examine its influence on biological properties, we first envisioned the synthesis of amine **14b** from ketone **13** obtained by an hydration reaction of alkyne **10b** (R = Bn) (Scheme 1). Alkyne **10a** (R = *t*Bu) was readily built via a Sonogashira-type coupling[31] between known triflate **7a** (FG = OSO_2CF_3) and alkyne **9**, the preparation of which is described in Supporting Information. Exchange of the NH-protecting group (NH-Boc to NH-Cbz) was mandatory to perform the Hg(II)-promoted hydration under harsh acidic conditions.[32] Gratifyingly, when alkyne **10b** was treated with mercury oxide in aqueous sulfuric acid, it afforded a unique product which, after reduction with sodium borohydride and CBz deprotection, yielded **14b**, isolated as a mixture of epimeric alcohols. Amine **14b** was reductively aminated with aldehyde **15a**, leading to compound **16**. Its regioisomer **12** was obtained by reductive amination of amine **11** with aldehyde **15a**.[29] The construction of amine **11**, featuring the $-(CHOH)-CH_2-$ linker, was achieved by trapping lithio derivatives **7b** (FG = Li) with aldehyde **8**.

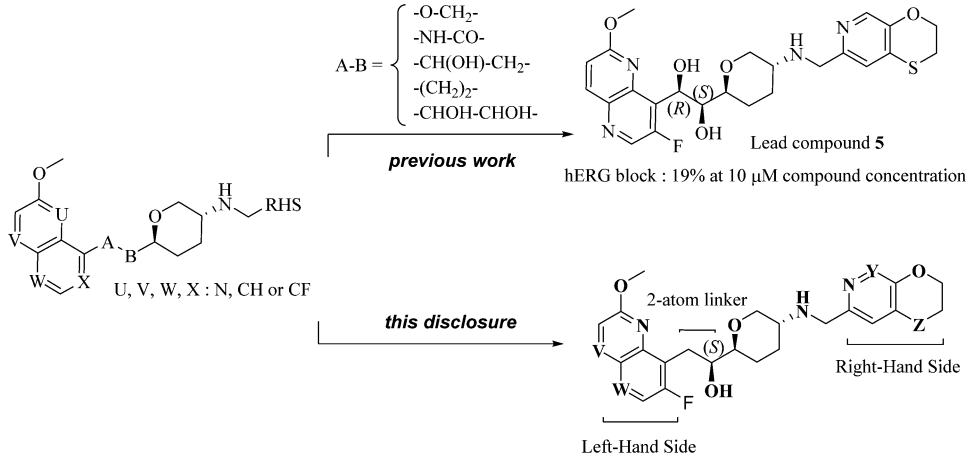

Figure 2. Chemical structures of THP-based NBTIs.

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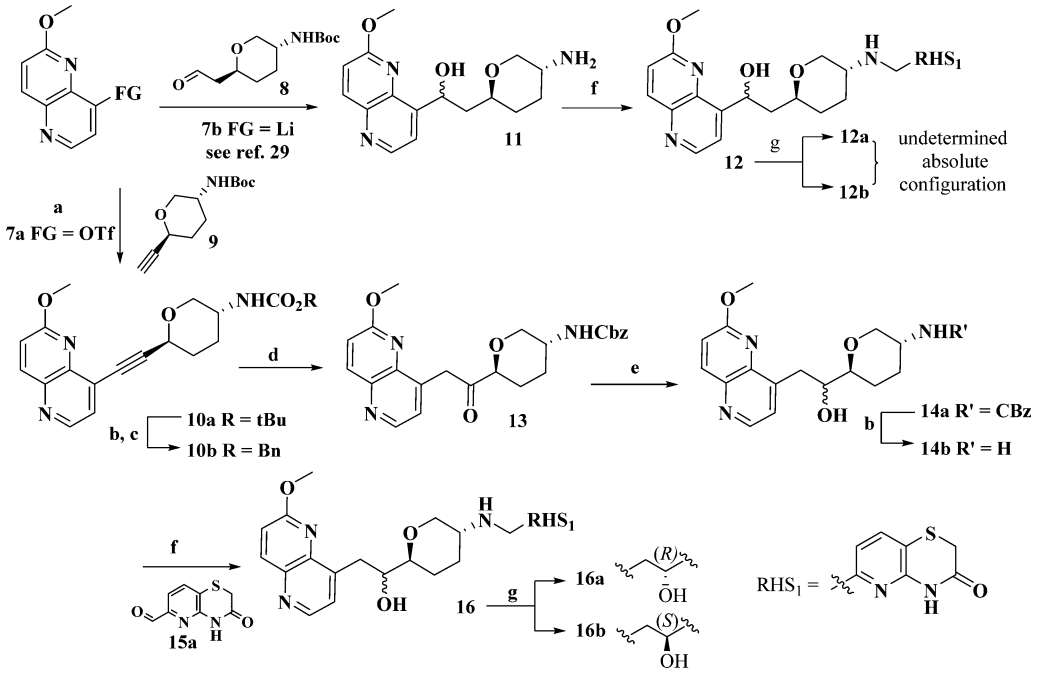
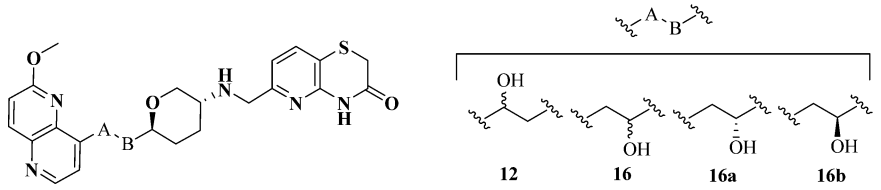
Scheme 1[a]



[a]Reagents: (a) cat. CuI, cat. $PdCl_2(PPh_3)_2$, Et_3N, DMF, rt; (b) TFA; (c) Cbz-Cl, $NaHCO_3$, aq THF; (d) HgO, aq H_2SO_4, 55 °C; (e) $NaBH_4$, MeOH, 0 °C; (f) **15a**, MS 3 Å, DCM–MeOH, 50 °C then $NaBH_4$, 0 °C; (g) chiral HPLC separation.

Table 1. Antibacterial, Topoisomerases, and hERG Activities of NBTIs 5, 12a,b, and 16a,b



	MIC (mg/L)				gyrase SCIA (IC_{50}, μM)				Topo IV RIA (IC_{50}, μM)		
compds	*Sa*[a]	*Sa*^{R}[b]	*Sp*[c]	*Ec*[d]	*Sa*[e]	*Sa*^{Rf}	*Sa*^{QRg}	*Ec*[h]	*Sa*	*Ec*	hERG block (%)[i]
5	≤0.03	2	0.03	8	0.03	32	0.5	8	8	0.125	19
12	≤0.03	1	0.125	4	0.03	>32	0.03	2	>8	0.125	99
12a	0.125	8	0.5	8	0.125	>32	0.03	2	32	0.125	100
12b	≤0.03	1	0.125	4	0.03	>32	<0.03	2	8	0.125	90
16	≤0.03	4	0.25	4	0.5	>32	0.03	8	8	0.125	76
16a	0.25	8	0.5	8	0.5	>32	0.125	32	32	0.125	73
16b	≤0.03	2	0.125	4	0.125	>32	0.03	2	2	0.125	76

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922. [e]Isolated from *Staphylococcus aureus* ATCC 29213. [f]Enzyme containing the D83N mutation isolated from *Sa*^{R} A-234. [g]Quinolone-resistant (QR) enzyme containing a *gyrA* S84L mutation isolated from *Staphylococcus aureus* A-798. [h]Isolated from *Escherichia coli* ATCC 25922. [i]% reduction of hERG K^+ current measured at 10 μM.

Compounds **12** and **16**, isolated both as mixtures of epimers, were separated by chiral HPLC, leading to compounds **12a,b** and **16a,b** respectively. After characterization of their biological properties, compound **16b** was identified as the most promising compound (Table 1). The development of an efficient synthetic access to analogues of NBTI **16b** as well as the assignment of the absolute configuration were prioritized. On the basis of our previous investigations for which the −(CHOH)−(CHOH)− linker was preferably (1*R*,2*S*)-configurated (see compound **5**, Figure 2), we inferred that diastereomer **16b** should be (*S*)-configurated at the level of −−CH_2−(CHOH)− linker.

The stereocontrolled synthesis of **16b** was undertaken from known (1*R*,2*S*)-configurated diol **17** as reported in Scheme 2. After treatment of diol **17** with 1,1′-carbonyl diimidazole (CDI), the reaction was worked up under basic conditions (aqueous $NaHCO_3$), giving rise to cyclic carbonate **18**. Subsequent palladium−catalyzed hydrogenolysis of the benzylic −C−O− bond was readily performed under hydrogen atmosphere in the presence of palladium on charcoal. However, some optimization was necessary to minimize the formation of side products. The use of a mixture of palladium on $CaCO_3$ and palladium on charcoal in the presence of ammonium formate gave rise to a much cleaner reaction and afforded, after

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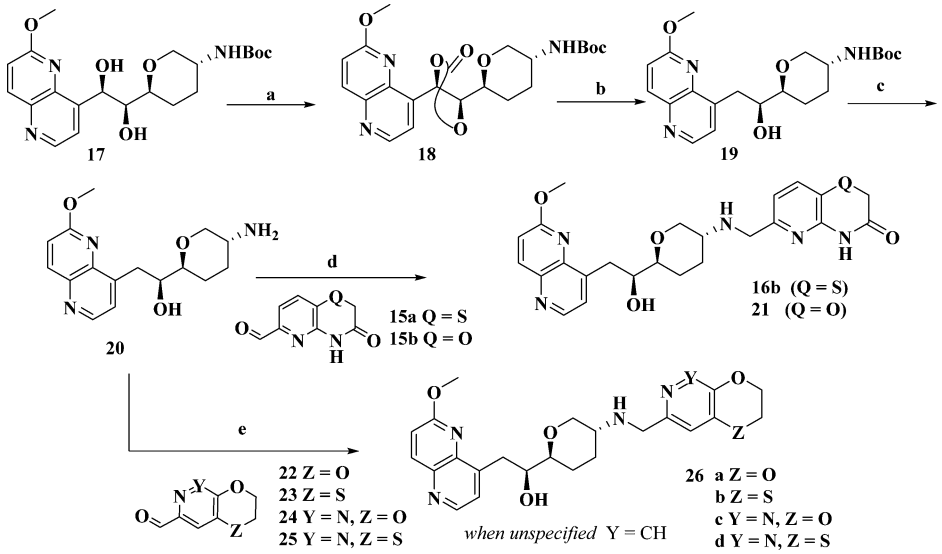
Scheme 2[a]



[a]Reagents: (a) CDI, 2-butanone, 60 °C then aq $NaHCO_3$; (b) 5% Pd on $CaCO_3$, 10% Pd on C, NH_4CO_2H, EtOAc–EtOH, 60 °C; (c) TFA; (d) **15a** or **15b**, MeOH, rt, then $NaBH_4$, 0 °C; (e) **22**, **23**, **24** or **25**, MeOH, 50 °C then $NaBH_4$, 0 °C.

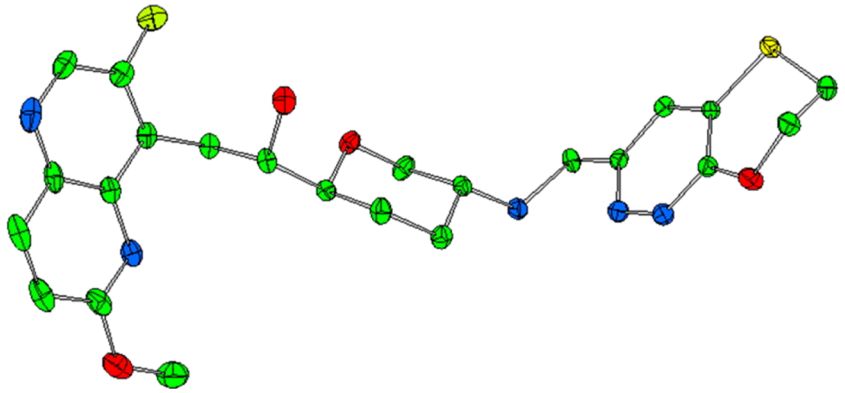


Figure 3. ORTEP representation of the molecular structure of compound **32d** as determined by X-ray diffraction. Hydrogen atoms have been omitted for clarity.[35]

chromatographic purification, benzylic alcohol **19** in 89% yield. Treatment of **19** with TFA provided amine **20**. Using aldehyde **15a** (Q = S), reductive amination of **20** led indeed to derivative **16b**, validating thereby our initial hypothesis and ascertaining the chemical structure. A supplementary confirmation was achieved by a single molecule X-ray structure of compound **32d** (deposited as CCDC 1022935, Figure 3). A series of analogous tetrahydropyranyl derivatives **21** and **26a–d**, were prepared by reductive amination of amine **20** with aldehydes **15b**[33] and **22–25**.

The same synthetic pathway was applied for the preparation of intermediate amines **30a–c** starting from (1*R*,2*S*)-configurated diols **27a–c**, whose the preparation followed the route reported previously.[34] These resulting amines were eventually converted by reductive amination to final derivatives **31a,b** using aldehydes **15a,b** or to derivatives **32–34(a–d)** using aldehydes **22–25**.

(*R*)-epimers of derivatives 1-*epi*-**32a** and 1-*epi*-**32d**, featuring a (*R*)-configurated $-CH_2-(CHOH)-$ two-atom linker, were also prepared from the corresponding (1*S*,2*R*)-configurated diol. These syntheses are described in Supporting Information.

RESULTS AND DISCUSSION

In Vitro Characterization of NBTIs. Enzyme inhibition profiles were assessed for novel chemical entities on a panel of bacterial topoisomerases comprising DNA gyrase and Topo IV isolated from wild-type *S. aureus* and *Escherichia coli* (*E. coli*) strains. Inhibition of DNA gyrase isolated either from a laboratory strain made resistant to **2** (NXL-101) (gyrA:D83N mutant) or a quinolone-resistant strain (gyrA:S84L mutant, dubbed QR mutant) were also measured. The antibacterial activity, expressed as MIC (minimal inhibitory concentration, [mg/L])[36] was measured against a panel of bacterial strains including one wild-type *Streptococcus pneumoniae* (*S. pneumoniae*), one wild-type *E. coli*, and two *S. aureus* (a wild-type and a laboratory mutant carrying gyrA:D83N mutation) strains. Blockade of the hERG K^+ channel was also routinely assessed. As shown in Table 1, compounds **12** and **16**, evaluated as mixture of epimers, showed the same enzymatic profile than our earlier compound **5**, with potent inhibition of wild-type and QR S84L *S. aureus* DNA gyrases and *E. coli* Topo IV (IC_{50}s in the nanomolar range). Inhibitory activity on *E. coli* DNA gyrase and *S. aureus* Topo IV were weaker. No activity was measured on *gyrA* D83N mutated DNA gyrase. Once separated by chiral

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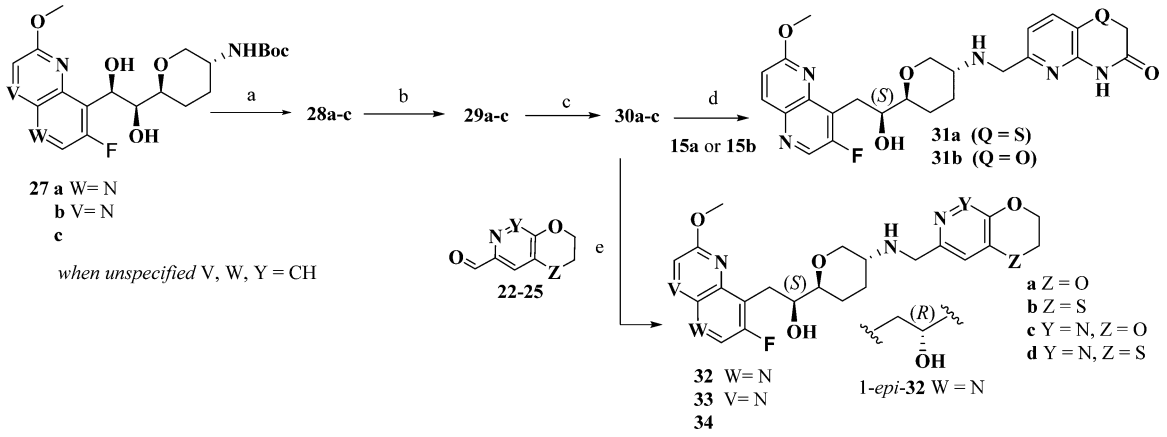
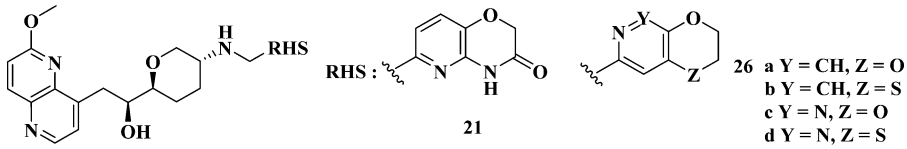
Scheme 3[a]



[a]Reagents: (a) CDI, 2-butanone, 60 °C then aq $NaHCO_3$; (b) 5% Pd on $CaCO_3$, 10% Pd on C, NH_4CO_2H, EtOAc–EtOH, 60 °C; (c) TFA, (d) **15a** or **15b**, MeOH, rt, then $NaBH_4$, 0 °C; (e) **22**, **23**, **24** or **25**, MeOH, 50 °C then $NaBH_4$, 0 °C.

Table 2. Antibacterial, Topoisomerases, and hERG Activities of NBTIs 21 and 26a–d



	MIC (mg/L)				gyrase SCIA (IC_{50}, μM)				Topo IV RIA (IC_{50}, μM)		
compd	*Sa*[a]	*Sa*[Rb]	*Sp*[c]	*Ec*[d]	*Sa*[e]	*Sa*[Rf]	*Sa*[QRg]	*Ec*[h]	*Sa*[e]	*Ec*[h]	hERG block (%)[i]
21	≤0.03	2	0.25	4	0.125	>32	0.03	2	2	0.125	66
26a	≤0.03	4	0.125	8	0.5	>32	0.03	32	32	0.5	22
26b	≤0.03	4	0.125	>8	0.12	8	0.03	32	32	0.125	44
26c	0.25	>8	1	>8	0.5	>32	0.125	>32	>32	8	0
26d	≤0.03	8	0.25	>8	0.5	32	0.5	>32	>32	8	14

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922. [e]Isolated from *Staphylococcus aureus* ATCC 29213. [f]Enzyme containing the D83N mutation isolated from Sa^R A-234. [g]Quinolone-resistant (QR) enzyme containing a *gyrA* S84L mutation isolated from *Staphylococcus aureus* A-798. [h]Isolated from *Escherichia coli* ATCC 25922. [i]% reduction of hERG K^+ current measured at 10 μM.

HPLC, **12b** and **16b** proved to be slightly more potent inhibitors of wild-type *S. aureus* DNA gyrase and topo IV than their respective epimers **12a** and **16a**. The four compounds **12a,b** and **16a,b** showed good antibacterial activity against wild-type *S. aureus* and *S. pneumoniae* and were also as potent against a QR *S. aureus* strain (data not shown). However, we observed that **12b** and **16b** demonstrated more pronounced antibacterial potency against a *gyrA* D83N *S. aureus* strain (with MIC = 1 and 2 mg/L, respectively). These results were in agreement with previous observations for which most potent dual *S. aureus* DNA gyrase–Topo IV inhibitors displayed antibacterial activity against this particular *S. aureus* mutated strain, most probably through their action on Topo IV. The antibacterial activity against *E. coli* was weak with no notable difference among the four compounds. High level of hERG block was observed for these four compounds, with **16a** and **16b** exhibiting lower inhibitory activity (73 and 76% at 10 μM compound concentration) than **12a** and **12b** (90 and 100%, respectively).

The overall profile of compound **16b** emerged as the most favorable one and therefore our focus was set on establishing structure–activity relationships of its analogues rather than on those of **12b**. The absolute stereochemistry of **12a** and **12b** –(CHOH)–CH_2– linker remained undetermined. Analogues of **16b** were produced using to the stereocontrolled syntheses reported in Schemes 2 and 3 that proved to be versatile enough. First, analogues of **16b** featuring various right-hand side (RHS) moieties were evaluated. As shown in Table 2, compound **21**, featuring a pyrido-oxazinone RHS, exhibited the same enzymatic inhibitory and antibacterial activity profile than compound **16b**, with only a slightly lower hERG block (66% versus 77% at 10 μM compound concentration). Compounds **26a,b** containing either a dioxinopyridine or a oxathiinopyridine RHS, retained potent inhibition of wild-type and QR S84L *S. aureus* DNA gyrase and *E. coli* Topo IV. A weaker inhibitory activity on *S. aureus* Topo IV was observed for **26a,b** in comparison to either **16b** or **21**. These enzymatic inhibition data tracked well with the antibacterial activity observed on wild-type Gram-positive strains and the weak activity on the D83N *S. aureus* strain. Analogues **26c,d** featuring a dioxinopyridazine or a oxathiinopyridazine RHS, showed only meaningful inhibitory activity on *S. aureus* DNA gyrase. Weak inhibitory activities on *E. coli* Topo IV and no activity on *E. coli* DNA gyrase or *S. aureus* Topo IV were measured for either of these two compounds. Compound **26c** showed modest antibacterial activity on wild-type *S. aureus* or *S. pneumoniae* strain. Nonetheless, compounds **26c,d** displayed less block of hERG

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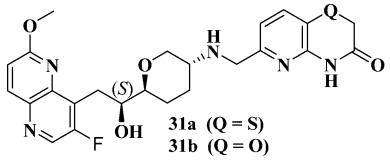
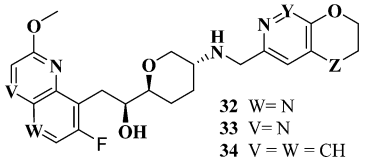
Table 3. Antibacterial, Topoisomerases, and hERG Activities of NBTIs 31,b and 32-34(a−d)





a Y = CH, Z = O
b Y = CH, Z = S
c Y = N, Z = O
d Y = N, Z = S

	MIC (mg/L)				Gyrase SCIA (IC_{50}, μM)				Topo IV RIA (IC_{50}, μM)		
compd	*Sa*[a]	*Sa*^{R}[b]	*Sp*[c]	*Ec*[d]	*Sa*[e]	*Sa*^{Rf}	*Sa*^{QRg}	*Ec*[h]	*Sa*[e]	*Ec*[h]	hERG block (%)[i]
31a	≤0.03	0.06	≤0.03	0.5	0.03	32	<0.03	0.03	0.125	0.03	83
31b	≤0.03	0.25	≤0.03	0.5	0.125	>32	0.03	NT	2	NT	72
32a	≤0.03	1	0.06	8	0.125	>32	0.03	32	8	0.125	33
1-*epi*-**32a**	0.06	>8	0.25	>8	0.125	>32	0.125	32	32	2	37
32b	≤0.03	0.5	≤0.06	4	0.03	2	>0.03	2	22	0.125	59
32c	≤0.03	4	0.25	>8	0.5	>32	0.03	>32	32	2	4
32d	≤0.03	1	0.06	>8	0.125	8	0.03	32	8	0.125	10
1-*epi*-**32d**	0.5	>8	0.5	>8	NT	NT	NT	NT	NT	NT	7
33a	≤0.03	1	0.12	8	0.125	8	0.03	8	8	0.125	49
33b	≤0.03	0.25	≤0.03	4	0.03	0.5	>0.03	2	8	0.125	72
33c	≤0.03	2	0.125	>8	0.125	8	0.125	32	>32	2	17
33d	≤0.03	1	0.06	>8	0.03	2	>0.03	8	2	0.5	14
34a	≤0.03	1	0.06	8	0.125	>32	0.03	8	8	0.5	58
34b	≤0.03	1	0.06	8	0.03	2	>0.03	2	8	0.125	74
34c	≤0.03	2	0.25	>8	0.125	32	0.125	32	32	0.125	10
34d	≤0.03	1	≤0.03	>8	0.125	8	0.03	8	32	0.125	20
CIP	0.5	0.5	1	≤0.03	128	>256	>256	0.5	8	8	

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922. [e]Isolated from *Staphylococcus aureus* ATCC 29213. [f]Enzyme containing the D83N mutation isolated from *Sa*^{R} A-234. [g]Quinolone-resistant (QR) enzyme containing a *gyrA* S84L mutation isolated from *Staphylococcus aureus* A-798. [h]Isolated from *Escherichia coli* ATCC 25922. [i]% reduction of hERG K^+ current measured at 10 μM.

(0 and 14% inhibition at 10 μM compound concentration) than compounds **26a,b** (22 and 44% inhibition, respectively).

Second, the exploration of the structure−activity relationship of the LHS was based on the knowledge previously gathered on the diol-linker series (exemplified with compound **5**), for which it was observed that 3-fluoronaphthyridine and 6-fluoroquinoxaline LHS provided superior antibacterial activity compared to 6-methoxyquinoline. Analogous 2-methoxyquinoline derivatives, featuring the nitrogen atom at the preferred position[29] were also prepared in this series. As shown in Table 3, 3-fluoronaphthyridine derivatives **32a−d** are consistently more potent than their *des*-fluoro analogues **26a−d**. Even if enzymatic inhibitory data collected for **26c** and **32c** on wild-type *S. aureus* DNA gyrase did not show a notable difference, the antibacterial activity of **32c** was significantly improved on *S. aureus* and *S. pneumoniae*, with 4-fold lower MICs than **26c**. There was a trend in favor of improved inhibitory activity on *S. aureus* topoisomerases and *E. coli* Topo IV enzymes for **32a**, **32b**, and **32d** in comparison to their respective *des*-fluoro analogues. In agreement with the findings (Table 1), (*R*)-epimers 1-*epi*-**32a** and 1-*epi*-**32d** elicited less potent antibacterial activity than their respective epimers **32a** and **32d**. The impact of the $-CH_2-(CHOH)-$ linker stereochemistry on the blockade of hERG was marginal. All fluorinated compounds presented in Table 3 displayed to a large extent, excellent antibacterial activity on Gram-positive strains. In comparison to ciprofloxacin (CIP), a fluoroquinolone known to target preferentially Topo IV in *S. aureus*, most of fluorinated compounds such as **32a,b,d** and **33a,b,d** inhibited not only *S. aureus* Topo IV to a similar extent than CIP but also displayed excellent inhibitory activity against DNA gyrase. Compounds **32b** and **33b**, featuring an oxathiinopyridine RHS had good antibacterial activity on D83N *S. aureus* strain (MIC < 1 mg/L) because of either a potent inhibitory activity on *S. aureus* Topo IV for **32b** or inhibitory activity on the mutant D83N *S. aureus* DNA gyrase enzymes for **33b**. However, these favorable antibacterial properties went along with undesired block of hERG. Similarly, compound **34b**, featuring as well an oxathiinopyridine RHS blocked hERG strongly. Their analogues containing a pyridazine-based RHS, compounds **32d** and **33d** exhibited less block of hERG while keeping satisfactory enzymatic inhibitory and antibacterial properties. The beneficial effect of the N-2 nitrogen atom of the RHS on hERG block is a general trend and correlates here with the decreased log *D* (from 2.8 for **32b** to 1.7 for **32d**). Identification of a few compounds, notably **32d** and **33d** that exhibit a better in vitro profile than our former lead NBTI **5** stimulated further efforts to evaluate their in vivo effects.

Cardiovascular Characterization of Selected THP-Based NBTIs. The effects of compounds **31b**, **32a**, **32d**, and **33d** were assessed in more detail on hERG channels as well as on $hNa_V1.5$ channels. These compounds were also examined for their actions on cardiovascular function in anesthetized guinea pigs and compared to the initial compound, NBTI **5**. The four selected compounds exhibited a wide range of hERG blockade (10−72% and 21−92% at 10 and 30 μM, respectively) and $hNa_V1.5$ inhibition (0−23% and 0−59% at 10 and 30 μM, respectively). During the in vivo experiment, three consecutive doses of 3, 10, and 30 mg of compound per kg of bodyweight were administered intravenously over periods

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of 20 min and mean arterial pressure (MAP), heart rate (HR) and ECG parameters (PR, RR, QRS, QT intervals) were recorded continuously. These data, as well as the Bazett-corrected QT interval (QTcBZ)[37] are qualitatively reported in Table 4 as differences from vehicle-treated animals. Plasma drug concentrations were measured at the end of the final infusion period, and are reported as total and free concentrations. In the 6-fluoronaphthyridine series, compound **31b** induced marked decreases of MAP and HR and it increased the PR, QT, and QRS intervals. The effects on QT and QRS can probably be attributed to the blockade of the guinea pig equivalents of hERG and $hNa_V1.5$ channels by compound **31b**, although Bazett-corrected QT remained unchanged. Prolongation of the PR-interval suggests that compound **31b** may also block cardiac $Ca_V1.2$ channels.[38] Compound **32a** showed a very similar profile to compound **31b**, although effects on MAP, HR, PR, and QT were slightly less whereas the effect on QRS was somewhat larger. These effects were consistent with compound **32a** blocking hERG less and $Na_V1.5$ more than compound **31b**. The overall profiles of compounds **31b** and **32a** are similar to the ones found for our earlier lead compound **5** (Table 4).

Table 4. Effects of Selected THP-Based NBTIs on Human Cardiac Ion Channels and on MAP, HR, and ECG in Anesthetized Guinea Pigs

	5	31b	32a	32d	33d
hERG block[a]	19/46	72/92	33/59	10/21	14/29
$hNa_V1.5$ block[a]	<15/22	17/45	23/59	<15	<15
MAP[b]	↔	↓(--)	↓(−)	↔	↓(−)
HR[b]	↓(−)	↓(--)	↓(−)	↔	↑(+)
PR[b]	↔	↑(++)	↑(++)	↔	↔
RR[b]	↑(+)	↑(++)	↑(+)	↔	↓(−)
QRS[b]	↑(+)	↑(++)	↑(++)	↔	↔
QT[b]	↑(+)	↑(+)	↑(+)	↔	↔
QTcBZ[b,c]	↔	↔	↔	↔	↑(+)
[drug] (μM)[d]	67	29	36	39	17
f_u[e]	0.235	0.168	0.11	0.201	0.274
free [drug] (μM)[d]	16	5	4	8	5

[a]% reduction of current measured at 10 and 30 μM compound concentrations. [b]Compared to vehicle treated animals. [c]QTcBZ = $QT/\sqrt{RR}$. [d]At the end of 30 mg/kg/20 min infusion period. (+ or −) magnitude of the difference from vehicle treatment. [e]Unbound fraction measured in guinea pig plasma by rapid equilibrium dialysis.

In the case of compound **32d**, no effects on MAP, HR, and ECG parameters were observed above those recorded for vehicle-treated animals, at the highest drug exposure reached (8 μM free). Compound **32d**, that displayed relatively low inhibition of hERG (21% at 30 μM) and no detectable effect on $hNa_v1.5$; proved to have the cleanest cardiovascular profile in the 6-fluoronaphthyridine series. Its analogue **33d**, featuring a 3-fluoroquinoxaline LHS, showed as well, relatively weak hERG inhibition (29% at 30 μM) and was inactive on $hNa_v1.5$ but, at the highest drug exposure, slight decreases of MAP and RR interval were observed and Bazett-corrected QTc interval was slightly increased. Therefore, from the selected set of compounds we could identify NBTI **32d** with the desired clean cardiovascular profile in vitro and in the anesthetized guinea pig.

The antistaphylococcal activity of compounds **32a**, **32d**, and **33d** was explored in vivo in a neutropenic murine thigh infection model. When administered subcutaneously at a dose of 40 mg/kg, compound **32a** and **33d** (MIC against the *S. aureus* in vivo strain = 0.03 and 0.015 mg/L, respectively) elicited a net 1-log reduction of colony forming units (CFU) in thighs compared to CFU at treatment start when measured 6 h postinfection, i.e., 5 h post treatment. For compound **32d** (MIC against the *S. aureus* in vivo strain = 0.03 mg/L) a net 2-log reduction of CFU was obtained in similar conditions. In vivo pharmacodynamic activity of compound **32d** was studied in more depth and it was found efficacious against various *S. pneumoniae* and *S. aureus* strains (including isolates with demonstrated resistance to beta lactams and quinolones) in thigh and lung infection models.[39] As summarized in Table 5, pharmacokinetic properties of compounds **32a,d** and **33d** were determined in rodents and dog. In mouse, compound **32d** showed low clearance (Cl = 25.4 mL/min/kg) and a longer initial half-life (~1h) when compared to compounds **32a** and **33d** (0.66 and 0.25 h respectively). The better in vivo efficacy observed for **32d** could be attributed to its superior PK profile in mice. All compounds showed low volumes of distribution in rat and dog. Clearance for all compounds was low to medium in these two species. Oral bioavailability for compound **33d** was rather low in rat (*F* 14%) but higher in dog (*F* 43%). The best overall profile was obtained for compound **32d** that showed good bioavailability in both species (*F* 47% and 79% respectively).

Table 5. Mouse, Rat, and Dog Pharmacokinetic Parameters of Selected THP-Based NBTIs

	32a	32d	33d
Mouse[a]			
AUC_n[b] (μg·h/(mL.dose))	0.35	0.65	0.18
Cl/F (mL/min/kg)	47.6	25.4	90.1
β $t_{1/2}$ (h)	0.66	0.95	0.25
Rat[c]			
AUC_n[b] (μg·h/(mL.dose))	0.73	1.28	1.27
Cl (mL/min/kg)	23	13	13
Vd_{ss} (L/kg)	1.1	0.76	0.49
$t_{1/2}$ (h)	0.5	5.0	3.1
%*F*	33	47	14
Dog[d]			
AUC_n[b] (μg·h/(mL.dose))	2.56	4.14	4.4
Cl (mL/min/kg)	6.7	4.0	3.8
Vd_{ss}(L/kg)	1.7	0.92	0.69
$t_{1/2}$ (h)	3.9	3.0	2.2
%*F*	31	79	43

[a]Mouse dosed sc at 25 mg/kg (compound **32a**) or 40 mg/kg (compounds **32d** and **33d**). [b]Dose-normalized AUC obtained after sc or iv dosing. [c]Rat dosed iv at 1 mg/kg (compound **32a**) or 0.5 mg/kg (compounds **32d** and **33d**) and orally at 5 mg/kg (compound **32a**) or 1 mg/kg (compounds **32d** and **33d**). [d]Beagle dog dosed iv at 0.5 mg/kg (compounds **32a** and **32d**) or 1 mg/kg (compound **33d**) and orally at 5 mg/kg (all compounds).

No inhibition of the major cytochrome P450 enzymes (IC_{50}s > 50 μM) and no activation of the pregnane X receptor were found for compound **32d**, suggesting a low potential for drug–drug interaction.

Microbiological Profile of Compound 32d. Compound **32d** was tested against sets of recent clinical isolates of selected pathogens (Table 6). Compound **32d** was highly active against all *S. aureus* isolates tested, including MRSA and fluoroquino-

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Table 6. Antibacterial Activity of NBTI 32d against Selected Pathogens[a]

	Nb of strains	MIC_{50}	MIC_{90}	range
Staphylococcus aureus	48	0.016	0.03	≤0.008−0.06
MRSA subset	27	0.03	0.03	≤0.008−0.06
QR subset	20	0.03	0.03	≤0.008−0.03
Staphylococcus epidermidis	24	0.016	0.03	≤0.016−0.125
Streptococcus agalactiae	13	0.25	0.5	0.125−0.5
Streptococcus pyogenes	14	0.06	0.06	≤0.016−0.06
Streptococcus pneumoniae	31	0.125	0.25	0.03−0.25
Enterococcus faecalis	20	0.5	1	0.06−1
Enterococcus faecium	21	0.25	1	0.016−2
Moraxella catarrhalis	11	0.125	0.125	0.03−0.25
Haemophilus influenzae	11	2	8	0.5−8
Escherichia coli	13	16	32	8−32
Acinetobacter baumannii	21	4	>8	1−8

[a]MIC_{90} (MIC_{50}) is the MIC in mg/L at which at least 90% (50%) of the strains were inhibited.

lone-resistant strains ($MIC_{90} \leq 0.03$ mg/L). It also showed good activity against a variety of other clinically relevant Gram-positive bacteria, including *S. pneumoniae* and *Enterococcus faecium* (*E. faecium*), including vancomycin-resistant strains, with $MIC_{90} \leq 1$ mg/L. While significant activity was observed against *Moraxella catarrhalis*, activities against *Haemophilus influenza* as well as other Gram-negative pathogens such as *E. coli* or *Acinetobacter baumannii* were weaker with $MIC_{90} \geq 8$ mg/L. In summary, compound **32d** has an antibacterial spectrum well suited for the treatment of Gram-positive infections.

Spontaneous resistance frequencies of compound **32d**, measured in various representative Gram-positive strains are summarized in Table 7. In the *S. pneumoniae* and the *E. faecium* strains, resistance frequencies of **32d** were very low ($<10^{-9}$ at 4-fold the MIC), while in *S. aureus* somewhat higher frequencies were observed (10^{-8} at 4-fold the MIC). Nevertheless, very low frequencies were observed at 16-fold the MIC for *S. aureus*. Resistance frequencies of quinolone-resistant strains were not significantly higher than those of quinolone-susceptible strains. *S. aureus* clones spontaneously resistant to **32d** were isolated. All of them showed single mutations in QRDRs of *gyrA* or *gyrB*, the most often encountered mutation occurring at D83 (to N) of *gyrA*. Second-step mutants were subsequently generated with **32d** and exhibited mutations in the QRDR of *grlA*, preferentially at D79. Together with the enzymatic data showed in Table 3, these data suggest that **32d** is a dual gyrase and topoisomerase IV inhibitor, with gyrase being the preferred target in *S. aureus*.

Table 7. Spontaneous Resistance Frequencies Measured with 32d on Selected Pathogens

	resistance frequency	
bacterial strains	at 4× the MIC	at 16× the MIC
S. aureus ATCC 29213[a]	3.0×10^{-8}	6.1×10^{-10}
S. aureus BA-1730 (MRSA, QR)	6.3×10^{-8}	9.3×10^{-10}
S. pneumoniae A-70 (QR)	$<2.9 \times 10^{-9}$	
E. faecium A-949 (VRE, QR)	$<2.7 \times 10^{-9}$	

[a]For CIP: 8.8×10^{-8} (4× the MIC) and $<4.6 \times 10^{-10}$ (16× the MIC)

To assess the bactericidal effect of **32d**, the minimal bactericidal concentration (MBC) of the compound against a panel of eight strains was determined and compared to the respective MIC. As shown in Table 8, compound **32d** acts as bactericidal drug against all Gram-positive strains tested. MBCs were generally around 2-fold higher than the MIC (including MRSA and VRE). Only for one *S. aureus* strain the MBC was >16-fold higher, up from ≤0.016 to 0.25. In comparison, Moxifloxacin (MXF, a fluoroquinolone) displayed a bactericidal activity against quinolone-susceptible strains, while no MBCs could be recorded for Linezolid (LZD) that is known to exhibit a bacteriostatic activity against Gram-positive bacteria (with the notable exception of *S. pneumoniae*).

CONCLUSION

We have reported the synthesis of compound **32d**, a novel dual DNA gyrase and Topo IV inhibitor featuring a tetrahydropyran core and an unprecedented homobenzylic alcohol moiety.[40] Our efforts to optimize antibacterial activity while decreasing the strong blockade of the hERG K^+ channel initially observed for **16b**, led to the discovery of a series of promising derivatives. In general, these derivatives display strong antibacterial activity against clinically relevant Gram-positive pathogens and the required lower hERG inhibition. In comparison to earlier NBTIs that were identified in the course of our research program, compound **32d** displayed a relatively low inhibition of the hERG K^+ channel with an $IC_{20} \approx 30$ μM and no liabilities in a cardiovascular safety experiment conducted in guinea pig. The in vitro properties translated to a useful pharmacological profile. Indeed, compound **32d** was efficacious in vivo in a murine thigh infection models against *S. aureus* or *S. pneumoniae*, and it showed good oral bioavailability in rat and dog. Owing to this profile, a preclinical development program for **32d** was initiated. A more efficient synthetic route to this family of THP-based NBTIs, enabling the preclinical studies was discovered[41] and details will be reported in due course.

EXPERIMENTAL SECTION

MIC and MBC Testing. Minimal inhibitory concentrations (MICs) were determined by the broth microdilution method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI).[36] Stock solutions of compounds were prepared in DMSO and serially diluted in cation-adjusted Mueller-Hinton broth (final DMSO concentration, 2.5% (v/v). Reference antibiotics (ciprofloxacin, linezolid) were tested in parallel and MICs against quality control strains were within the accepted CLSI ranges. For determination of the minimal bactericidal concentration (MBC) MICs were determined as described above. After reading the MICs, the wells were carefully mixed by pipetting and 10 μL from each well was spread on Columbia Blood Agar or Mueller-Hinton Agar (for *Staphylococcus aureus* strains). Colonies were counted after incubation for 24 h at 37 °C. The MBC was defined as the lowest concentration that reduces CFU counts by >99.9% compared to the CFU counts of the inoculum at the beginning of the experiment.

Spontaneous Resistance Frequencies. Large numbers of bacteria (10^9−10^{10}) were plated on Mueller−Hinton agar plates containing the antibiotic compound at 4- and 16-fold the MIC concentration. Resistance frequencies were calculated by dividing the numbers of colonies growing after 48 h of incubation at 37 °C through the total numbers of viable bacteria plated (as determined by colony count on drug-free agar).

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Table 8. Minimal Bactericidal Concentration (MBC) of 32d, LZD, and MXF[a]

strains	resistance phenotype	compounds		
		32d	LZD	MXF
S. aureus ATCC 29213		≤0.016/0.03	2/>32	0.06/0.125
S. aureus A-798	MRSA, QR	≤0.016/0.03	2/>32	8/>8
S. aureus ATCC 43300	MRSA	0.03/0.06	2/>32	0.06/0.125
S. aureus H7935 (A-1093)	MRSA, QR	0.03/0.06	2/>32	8/ND
S. aureus HT203 (A-1160)	caMRSA	≤0.016/0.25	2/>32	0.125/0.125
E. faecalis ATCC 29212		0.5/1	0.5/>32	0.25/0.5
E. faecium A-949	VRE, QR	0.25/0.25	2/>32	>8/ND
S. pneumoniae ATCC 49619		0.06/0.06	0.125/2	0.125/0.25

[a]QR, quinolone-resistant; caMRSA, community acquired MRSA; QS, quinolone-sensitive; LZD, linezolid; MXF, moxifloxacin. Values are MIC/MBC in mg/L.

DNA Gyrase and Topo IV Inhibition Assays. DNA gyrase supercoiling inhibition assay was performed with relaxed pBR322 (purchased from Inspiralis, UK) as a substrate. For *E. coli*, the reaction mixture (20 μL) contained 25 mM Tris HCl pH 7.5, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1 mM ATP, 1.6 mM spermidine, 6.5% glycerol, 50 μg/mL Bovine Serum Albumin (BSA) and 0.1 μg relaxed plasmid. For *S. aureus* the reaction mixture (20 μL) contained 50 mM Tris HCl pH 7.5, 20 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 3 mM ATP, 700 mM potassium glutamate, 50 μg/mL BSA and 0.1 μg relaxed plasmid. The reactions were carried out at 37 °C for 1 h. DNA Topo IV relaxation assay was performed with supercoiled pBR322 as a substrate. For *E. coli*, the reaction mixture (20 μL) contained 50 mM Tris HCl pH 7.8, 20 mM KCl, 6 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 1 mM spermidine, 100 μg/mL BSA and 0.1 μg supercoiled pBR322. For *S. aureus*, the reaction mixture (20 μL) contained 50 mM Tris HCl pH 7.5, Twenty mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 1.5 mM ATP, 5 mM spermidine, 100 mM potassium glutamate, 5 μg/mL BSA, and 0.1 μg supercoiled pBR322. The reaction was carried out at 37 °C for 1 h. Both supercoiling and relaxation reactions were stopped by adding a mixture of EDTA, bromophenol blue, and glycerol. Ten μL of each reaction mixture were loaded on a 1% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0) and run at 1.0 V/cm for 14 to 16 h. Gels were stained in water with ethidium bromide and visualized under ultraviolet light. For supercoiling inhibition assays (SCIA), 50% inhibitory concentration (IC_{50}) was determined visually as being the compound concentration at which the supercoiled band was reduced by 50%. For relaxation inhibition assays (RIA), IC_{50} was defined as the concentration of inhibitor necessary to inhibit the formation of 50% relaxed DNA from supercoiled. The IC_{50} was taken as an average of two inhibitory concentrations if inhibition did not occur exactly within a particular concentration range (for representative gels, see Supporting Information). All gels were also subject to analysis using AlphaEase Stand Alone Software (Alpha Innotech).

$hK_V1.1$ ("hERG") and $hNa_V1.5$ Assay.[42] Compounds were evaluated for block of $hK_V1.1$ and $hNa_V1.5$ channels using CHO cells stably expressing the hERG or the SCN5A genes (bSys, Witterswil, Switzerland) and the QPatch platform (Sophion, Ballerup, Denmark). For $hK_V1.1$, K^+ tail currents were measured at −40 mV following a 500 ms depolarization to +20 mV from a holding voltage of −80 mV. For $hNa_V1.5$, Na^+ peak currents were measured at −20 mV following a 60-s-long, 5-Hz train of 150 ms depolarizations to −20 mV from a holding voltage of −120 mV. The external solution contained 150 mM Na^+, 4 mM K^+, 1 mM Mg^{2+}, and 1.2 mM Ca^{2+}. Compound effects were quantified 3 min after application to the cells.

Guinea Pig Protocol. Normotensive male Dunkin−Hartley guinea pigs were delivered from Harlan Laboratories (Horst, Netherlands) and group-housed during the acclimatization period (>1 week). All animals were housed under identical conditions in climate-controlled rooms with a 12-h light/dark cycle and free access to normal pelleted food, in accordance with the guidelines of the Basellandschaft Cantonal Veterinary Office (license no. 170). The experimental design was selected in accordance with the principles of the 3Rs and the number of animals was kept to a strict minimum to allow statistical significance.

On the day of the study, the guinea pigs (400−1000 g of bodyweight) were anesthetized with a mixture of 60 mg/kg ketamine-HCl (Ketanarkon, Streuli Pharma AG, Uznach) and 8 mg/kg xylazine (Xylazin Streuli, Streuli Pharma AG, Uznach) given intramuscularly, and the animals were placed on a thermostatically controlled heating table to maintain body temperature at 36−38 °C. A catheter was inserted into the left jugular vein for infusion of the compound or vehicle and a derivation lead II was positioned for the ECG recording. A second catheter was inserted in the right carotid artery to measure MAP and HR. After the hemodynamic and ECG parameters had stabilized, they were continuously recorded on a PowerLab data acquisition system using IOX software (Emka Technologies, Paris, France). Data were exported to a Dell Optiplex GX620 computer for analysis (Datanalyst version 2.3.4.6 and ECG-Auto version 2.5.1.18 software, both from Emka Technologies).

The QT interval corrected for HR, QTc Bazett (QTcBZ), was calculated using the following formula: $QTcB\ Z = QT/\sqrt{RR}$. At each time point, QTcBZ was calculated for 30−60 individual ECG waveforms and averaged. After baseline measurements, each guinea pig was infused intravenously with either tested compound or its vehicle during three consecutive 20 min periods using a Precidor pump (Infors AG, Bottmingen, Switzerland). The infusion rate was 0.3 mL/kg/20 min for the first 20 min period, 1 mL/kg/20 min for the second and 3 mL/kg/20 min for the third period. The corresponding doses of tested compound were 3, 10, and 30 mg/kg.

At the end of the last infusion period, a 250 μL blood sample was collected in 5% EDTA (K2E EDTA, BD Vacutainer ref 368856) and centrifuged at 10000*g*/4 °C. The plasma samples were put in a 96-well PCR plate and stored at −20 °C until they were analyzed for tested compounds concentrations using liquid chromatography coupled to mass spectrometry (LC-MS/MS).

Mice in Life Procedures.[43] All animal housing and experiments were performed in agreement with the Swiss Federal Ordinance for animal protection, the animal welfare guide lines from the Cantonal Vetenary Office Basellandschaft, and the Actelion Pharmaceuticals Ltd. internal animal welfare guidelines. Eight to 12 week-old, specific pathogen-free, female NMRI mice weighing 27−30 g were used for all studies (Harlan, The Netherlands). Mice were rendered neutropenic (neutrophils <100/mm^3) by injecting cyclophosphamide (Sigma) intraperitoneally 4 days (150 mg/kg) and 1 day (100 mg/kg) before thigh infection. Previous studies have shown that this regimen produces neutropenia in this model for 5 days. Broth cultures of freshly plated *S. aureus* (laboratory descendent of DSM 11823) were grown to logarithmic phase overnight to an absorbance of 0.3−0.9 at 580 nm. Thigh infections with *S. aureus* were performed by injection of 0.05 mL of inoculum ($10^{7.0-8.0}$ CFU/ml) intra muscularly into both thighs of mice 2 h before administration of test compound (formulated in 5% mannitol in water, administered at 10 mL/kg). Post-mortem, thigh samples were homogenized (Ultra-Turrax tube drive, IKA), serially diluted and plated (spiral platter EddyJet (IUL)). Results were expressed as colony forming units (CFU; CFU-counter Flash & Grow,

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IUL), correlating to remaining bacterial load in the thigh at the end of the study.

Pharmacokinetics in the Mouse. The pharmacokinetic profile was determined in female NMRI mice (n = 18). For this purpose, each compound was formulated as aqueous isotonic 5% D-mannitol solution for subcutaneous administration at a dose of 25, 40, and 40 mg/kg for **32a**, **32d**, and **33d**, respectively. Blood samples (two per mouse) were taken at regular time intervals to cover a 24 h period, and plasma was generated by centrifugation at 4000 rpm at 4 °C. Plasma concentrations were determined using a specific and sensitive LCMS/MS method with a limit of quantification of 1.5 ng/mL.

Pharmacokinetics in the Rat. The pharmacokinetic profile was determined in the male Wistar rat (n = 3). For this purpose, compound **32a** was formulated as an aqueous solution at pH 4.5 for intravenous administration at a dose of 1 mg/kg and as aqueous suspension pH 5.6 for oral dosing at 5 mg/kg; compound **32d** was formulated as a solution in 5% DMSO in pure water for intravenous administration at a dose of 0.5 mg/kg, and as a microsuspension in 7.5% aqueous modified gelatin for oral dosing at 1 mg/kg; compound **33d** was formulated as an aqueous solution at pH 4.74 for intravenous administration at a dose of 0.5 mg/kg and as as a microsuspension in 7.5% aqueous modified gelatin for oral dosing at 1 mg/kg. Blood samples were taken at regular time intervals over a period of 24 h from a preimplanted catheter, and plasma was generated by centrifugation at 4000 rpm at 4 °C. Plasma concentrations were determined using a specific and sensitive LCMS/MS method with a limit of quantification of 1.5 ng/mL.

Pharmacokinetics in the Dog. The pharmacokinetic profile was determined in male Beagle dogs (n = 3). For this purpose, compounds **32a**, **32d**, and **33d** were formulated as aqueous isotonic 5% D-mannitol solutions intravenous administration at a dose of 0.5, 0.5, and 1 mg/kg respectively and as dispersion in gelatin 7.5% via 5% DMSO aqueous suspension for oral dosing at 5 mg/kg. Blood samples were taken at regular time intervals over a period of 24 h from a preimplanted catheter, and plasma was generated by centrifugation at 4000 rpm at 4 °C. Plasma concentrations were determined using a specific and sensitive LCMS/MS method with a limit of quantification of 1.5 ng/mL.

General Chemical Methods. Starting materials, reagents, and solvents were obtained from commercial sources and used as received. All reactions were carried out with continuous stirring under atmosphere of dry nitrogen. The resonance frequency for ^{1}H (^{13}C) on a Varian Gemini 300 is 300 MHz (75 MHz) or on a Bruker Ascend 500 is 500 MHz (125 MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50 ppm), coupling constants (J) are in hertz (Hz). Peak multiplicities are expressed as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), and broad singlet (br s). Melting points were recorded with a Büchi 520 apparatus and are uncorrected. Purification of intermediates and final products was carried out on normal phase using an ISCO CombiFlash system and prepacked SiO_2 cartridges eluted with optimized gradients of either heptane−ethyl acetate mixture or dichloromethane−methanol (doped with 1% v/v of aq NH_4OH for basic compounds). Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F^{254} on glass plates) or by LC-MS (methods and equipment are described in Supporting Information). All target compounds had purity >95%, established on a Waters Atlantis T3, 5 μm, 4.6 mm × 30 mm, eluting with a gradient of 5−95% of acetonitrile in water containing 0.04% of trifluoroacetic acid or on a Waters XBridge C18, OBD, 5 μm, 4.6 mm × 50 mm (Waters, Switzerland), eluting with a gradient of 5−95% of acetonitrile in water containing 13 mM of NH_4OH UV at 230 and 254 nm. Purity and identity were further confirmed by NMR spectroscopy. Retention times (t_R) are expressed in minutes.

General Procedure A: Cyclic Carbonates Formation. To a mixture of diol (1 equiv) in 2-butanone (4 mL/mmol), heated to 60 °C, was added CDI (1.5 equiv). The mixture was stirred 1 h and Na_2CO_3 (5% aqueous, 1.5 mL/mmol) was added. The reaction proceeded at 60 °C overnight. The reaction mixture was cooled. The two layers were decanted, and the aqueous layer was extracted with EtOAc (2 mL/mmol). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by chromatography (heptane−EtOAc) to afford the corresponding cyclic carbonate.

General Procedure B: Hydrogenolysis of Cyclic Carbonates. To a suspension of cyclic carbonate (1 equiv) in EtOH (7 mL/mmol) and EtOAc (0.5 mL/mmol) under nitrogen atmosphere was added ammonium formate (0.35 g/mmol). The mixture was heated to 70 °C. At this point, 5% Pd on $CaCO_3$ (0.04 g/mmol) then 10% Pd/C (0.01 g/mmol) were added. The reaction proceeded at 40 °C for 1 h. Water (3 mL/mmol) and EtOAc (3 mL/mmol): The mixture was filtered and the solids were washed with water (10 mL/mmol) and EtOAc (30 mL/mmol). The two layers were separated, and the aqueous layer was extracted with EtOAc (10 mL/mmol). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by chromatography (heptane−EtOAc) to afford the corresponding alcohol.

General Procedure C: Boc Deprotection. A solution of the Boc-protected amine (1 equiv) in TFA (2 mL/mmol) was stirred at rt for 1 h. The solvent was evaporated in vacuo, and the residue was partitioned between DCM−MeOH mixture (9:1, 10 mL/mmol) and saturated $NaHCO_3$ solution (5 mL/mmol). The pH was adjusted to 10 by adding 32% aqueous NaOH. The two layers were separated, and the aqueous layer was extracted three times with the same mixture (3 × 10 mL/mmol) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by chromatography (DCM−MeOH 19:1, containing 1% aq NH_4OH) to afford the corresponding amine.

General Procedure D: Reductive Amination Using Aldehydes 15a,b. To a solution of amine (1 equiv) in DCE (10 mL/mmol) and MeOH (4 mL/mmol) were added 3 Å molecular sieves (3 g/mmol) and **15a** or **15b** (1.1 equiv). The reaction was heated at 50 °C overnight. After cooling to 0 °C, $NaBH_4$ (400 mol %) was added and the reaction was stirred for 2 h. The reaction mixture was filtered, and the filtrate was diluted with DCM−MeOH (9:1, 10 mL/mmol) and saturated $NaHCO_3$ solution (5 mL/mmol). The two layers were separated, and the organic layer was dried over dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by chromatography (DCM−MeOH containing 1% NH_4OH) to afford the alkylated product.

General Procedure E: Reductive Amination Reaction Using Aldehydes 22−25. To a solution of amine (1equiv) in MeOH (5 mL/mmol) was added the appropriate aldehyde (1 equiv). The reaction mixture was stirred at rt overnight. The reaction mixture was cooled to 0 °C, and $NaBH_4$ (400 mol %) was added. After stirring for 40 min, DCM−MeOH (9:1, 6 mL/mmol) and saturated $NaHCO_3$ (2 mL/mmol) were added. The two layers were decanted, and the aqueous layer was extracted with a DCM−MeOH mixture (9:1, 4 × 5 mL/mmol). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by chromatography (DCM−MeOH containing 1% aq NH_4OH) to afford the corresponding alkylated product.

***tert*-Butyl ((3*R*,6*S*)-6-((6-Methoxy-1,5-naphthyridin-4-yl)-ethynyl)tetrahydro-2*H*-pyran-3-yl)carbamate (10a).** CuI (0.2 g, 0.9 mmol), $PdCl_2(PPh_3)_2$ (0.32 g, 0.45 mmol), **9** (1 g, 4.4 mmol), and **7a** (1.37 g, 4.4 mmol), DMF (5 mL) and Et_3N (3 mL, 21.6 mmol) were successively introduced in a flask. The reaction proceeded 6 h at rt. After the solvent was evaporated, the residue was purified by chromatography (heptane−EtOAc) to afford **10a** (1.07 g, 63% yield). Orange solid; mp = 125 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.74 (d, J = 4.5 Hz, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.63 (d, J = 4.5 Hz, 1 H), 7.18 (d, J = 9.0 Hz, 1 H), 4.99 (m, 1 H), 4.84 (m, 1 H), 4.39 (m, 1 H), 3.76 (m, 1 H), 3.53 (m, 1 H), 2.30 (m, 1 H), 2.22 (m, 1 H), 1.90 (m, 1 H), 1.76 (m, 1 H), 1.48 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 155.2, 147.2, 142.1, 142.0, 140.4, 128.5, 126.7, 117.2, 82.0, 79.6, 67.8, 66.4, 53.9, 45.4, 28.4 (3C), 27.8, 25.9. ESI-MS $(M + H)^+$ m/z 384.0. HR ESI-MS $(M + H)^+$ m/z = 384.1927 (calcd for $C_{21}H_{26}N_3O_4$: 384.1923); t_R =1.28.

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(3*R*,6*S*)-[6-(6-Methoxy-[1,5]naphthyridin-4-ylethynyl)-tetrahydro-pyran-3-yl]-carbamic Acid Benzyl Ester (10b). A solution of **10a** (0.75 g, 1.94 mmol) in TFA (12 mL) was stirred at rt for 10 min. After evaporation to dryness, the residue was partitioned between aqueous NaOH (1M, 7 mL) and a DCM−MeOH mixture (9:1, 50 mL). The aqueous layer was extracted with the same mixture (2 × 50 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was taken up in EtOAc (9 mL) and water (9 mL). $NaHCO_3$ (0.5 g) and benzyl chloroformate (0.32 mL, 2.2 mmol) were added. The reaction proceeded for 30 min. The two layers were decanted, and the aqueous layer was extracted once with EtOAc. The combined organic layers were concentrated to dryness, and the residue was purified by chromatography (heptane−EtOAc) to afford **10b** (R = Bn) (0.24g, 30% yield). White solid; mp = 133 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.74 (d, J = 4.5 Hz, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.63 (d, J = 4.5 Hz, 1 H), 7.33−7.41 (m, 5 H), 7.18 (d, J = 9.0 Hz, 1 H), 5.23 (d, J = 7.6 Hz, 1 H), 5.14 (s, 2 H), 4.87 (br s, 1 H), 4.44 (dd, J = 2.0, 11.7 Hz, 1 H), 4.14 (s, 3 H), 3.84 (m, 1 H), 3.57 (dd, J = 3.7, 11.5 Hz, 1 H), 2.32 (m, 1 H), 2.22 (m, 1 H), 1.90 (m, 1 H), 1.81 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 155.7, 147.2, 142.1, 141.8, 140.4, 136.4, 128.6 (2C), 128.4, 128.3 (2C), 128.2, 126.7, 117.2, 96.3, 82.2, 67.5, 66.8, 66.4, 53.9, 46.0, 27.6, 25.8. ESI-MS (M + H)$^+$ m/z 418.0. HR ESI-MS (M + H)$^+$ m/z = 418.1772 (calcd for $C_{24}H_{24}N_3O_4$: 418.1767); t_R = 1.28.

(3*R*,6*S*)-(6-[2-(6-Methoxy-[1,5]naphthyridin-4-yl)-acetyl]-tetrahydro-pyran-3-yl)-carbamic Acid Benzyl Ester (13). To a solution of **10b** (0.24 g, 0.57 mmol) in MeOH (2 mL) and THF (2 mL) was added a solution of mercury oxide (5.1 mL, prepared from 0.075 g of mercury oxide in 12 mL of a 4% (wt/wt) H_2SO_4 solution in water). The reaction mixture was heated at 55 °C for 4 h. After cooling to rt, $NaHCO_3$ was added until pH 8 was reached. The aqueous layer was extracted with EtOAc (2 × 100 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography (DCM−MeOH 19:1) to afford **13** (0.17 g, 67% yield). White solid; mp = 173 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.72 (d, J = 4.4 Hz, 1 H), 8.26 (d, J = 9.0 Hz, 1 H), 7.56 (d, J = 4.4 Hz, 1 H), 7.30−7.41 (m, 6 H), 7.25 (d, J = 9.0 Hz, 1 H), 5.00−5.06 (m, 2 H), 4.35−4.47 (m, 2 H), 4.02 (m, 1 H), 3.95 (s, 3 H), 3.50 (m, 1 H), 3.15 (m, 1 H), 2.54 (overlaid m, 1 H), 1.96−2.03 (m, 2 H), 1.47−1.64 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 206.1, 161.6, 155.6, 147.5, 141.5, 140.8, 140.3, 136.2, 128.6 (2C), 128.3 (2C), 128.2, 128.1, 125.7, 166.6, 81.8, 70.8, 66.9, 53.7, 46.6, 40.3, 29.7, 27.0. ESI-MS (M + H)$^+$ m/z 436.4. HR ESI-MS (M + H)$^+$ m/z = 436.1873 (calcd for $C_{24}H_{26}N_3O_5$: 436.1872); t_R = 1.15.

(3*R*,6*S*)-(6-((1*RS*)-1-Hydroxy-2-(6-methoxy-[1,5]-naphthyridin-4-yl)-ethyl)-tetrahydro-pyran-3-yl)-carbamic Acid Benzyl Ester (14a). To a solution of **13** (0.17 g, 0.39 mmol) in THF (2 mL) and MeOH (2 mL) was added $NaBH_4$ (0.077 g). The reaction was stirred 30 min, and water (5 mL) was added. The volatiles were removed under reduced pressure, and the residue was extracted twice with EtOAc (2 × 25 mL). The combined extracts were concentrated to dryness, and the residue was purified chromatography (DCM−MeOH 19:1) to afford **14a** (0.045 g, 27% yield). White solid. ^{1}H NMR (500 MHz, $CDCl_3$) mixture of diastereomers δ: 8.69−8.75 (two overlaid d, 2 × 0.5 H), 8.27 (d, J = 9.0 Hz, 0.5 H), 8.24 (d, J = 9.0 Hz, 0.5 H), 7.46−7.50 (m, 1 H), 7.33−7.41 (m, 5 H), 7.16 (d, J = 6.8 Hz, 0.5 H), 7.15 (d, J = 6.8 Hz, 0.5 H), 5.02−5.21 (m, 2 H), 4.46−4.55 (m, 1 H), 4.18−4.29 (m, 1 H), 4.10−4.12 (two overlaid s, 3 H), 4.02 (m, 0.5 H), 3.92 (m, 0.5 H), 3.65−3.83 (m, 1.5 H), 3.57 (m, 0.5 H), 3.21−3.43 (m, 3 H), 2.95−3.11 (m, 2 H), 2.10−2.24 (m, 1 H), 2.03 (m, 0.5 H), 1.87 (m, 0.5 H), 1.72 (m, 0.5 H), 1.56 (m, 0.5 H). ESI-MS (M + H)$^+$ 438.4. HR ESI-MS (M + H)$^+$ m/z = 438.2032 (calcd for $C_{24}H_{28}N_3O_5$: 438.2028); t_R = 1.04.

(1*RS*)-1-((2*S*,5*R*)-5-Amino-tetrahydro-pyran-2-yl)-2-(6-methoxy[1,5]naphthyridin-4 yl)-ethanol (14b). The compound **14b** (0.029 g) was prepared in 92% yield starting from **14a** (0.045 g, 0.10 mmol) according to general procedure C. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) mixture of diastereomers δ: 8.72 (d, J = 4.4 Hz, 0.5 H), 8.70 (d, J = 4.4 Hz, 0.5 H), 8.26 (d, J = 9.1 Hz, 0.5 H), 8.23 (d, J = 9.1 Hz, 0.5 H), 7.49 (d, J = 4.4 Hz, 0.5 H), 7.47 (d, J = 4.4 Hz, 0.5 H), 7.15 (d, J = 9.1 Hz, 0.5 H), 7.14 (d, J = 9.1 Hz, 0.5 H), 5.00 (br s, 1 H), 4.09 (s, 3 × 0.5 H), 4.08 (s, 3 × 0.5 H), 3.99−4.04 (m, 2 × 0.5 H), 3.89−3.94 (m, 2 × 0.5 H), 3.57 (dd, J = 2.8, 13.7 Hz, 0.5 H), 3.43 (dd, J = 3.5, 13.4 Hz, 0.5 H), 3.33 (dd, J = 6.8, 7.2 Hz, 0.5 H), 3.30 (dd, J = 6.8, 7.2 Hz, 0.5 H), 3.25 (ddd, J = 2.1, 5.0, 11.3 Hz, 0.5 H), 3.03 (t, J = 10.7 Hz, 0.5 H), 3.03 (overlaid m, 0.5 H), 2.97 (t, J = 10.7 Hz, 0.5 H), 2.81−2.88 (m, 1 H), 2.05−2.13 (m, 1 H), 1.99 (m, 0.5 H), 1.83 (m, 0.5 H), 1.66 (m, 0.5 H), 1.50 (m, 0.5 H), 1.37 (br s, 2 H), 1.26 (m, 0.5 H), 1.17 (m, 0.5 H). ESI-MS (M + H)$^+$ 304.4.

6-((((3*R*,6*S*)-6-((*R* and *S*)-1-/hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (16a). Starting from of **14b** (0.029 g, 0.095 mmol) and **15** (0.018g, 0.095 mmol), **16** (0.021 g, 47% yield) was prepared using the general procedure D. The diastereomeric mixture was separated on a Chiracel OZ-H column (250 mm × 20 mm, 5 μm) with EtOH−heptane (9:1), containing 0.1% diethylamine. Isomer **16a** eluted first (t_R = 10.0 min), followed by isomer **16b** (t_R = 13.5 min). For isomer **16a**: yellowish solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.88 (s, 1 H), 8.66 (d, J = 4.4 Hz, 1 H), 8.24 (d, J = 9.0 Hz, 1 H), 7.74 (d, J = 7.8 Hz, 1 H), 7.54 (d, J = 4.5 Hz, 1 H), 7.24 (d, J = 9.0 Hz, 1 H), 7.09 (d, J = 7.9 Hz, 1 H), 4.71 (d, J = 6.4 Hz, 1 H), 4.01 (s, 3 H), 3.96 (m, 1 H), 3.84 (m, 1 H), 3.69−3.77 (m, 2 H), 3.60 (dd, J = 3.4, 13.2 Hz, 1 H), 3.53 (s, 2 H), 3.12 (ddd, J = 1.6, 5.1, 11.9 Hz, 1 H); 2.95 (t, J = 10.5 Hz, 1 H), 2.88 (dd, J = 9.2, 13.3 Hz, 1 H), 2.50 (overlaid m, 1 H), 2.05 (m, 1 H), 1.86 (m, 1 H), 1.58 (m, 1 H), 1.24 (br s, 1 H), 1.19 (m, 1 H). ESI-MS (M + H)$^+$ 482.4. HR ESI-MS (M + H)$^+$ m/z = 482.1879 (calcd for $C_{24}H_{28}N_5O_4S$: 482.1862); t_R = 0.59. For isomer **16b**: off-white solid; mp = 172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.93 (s, 1 H), 8.67 (d, J = 4.4 Hz, 1 H), 8.25 (d, J = 9.0 Hz, 1 H), 7.97 (d, J = 7.8 Hz, 1 H), 7.93 (d, J = 8.5 Hz, 1 H), 7.60 (d, J = 7.8 Hz, 1 H), 7.55 (d, J = 4.4 Hz, 1 H), 7.25 (d, J = 9.0 Hz, 1 H), 4.69 (d, J = 6.4 Hz, 1 H), 4.10 (s, 3 H), 3.84−4.1 (overlaid m, 3 H), 3.64 (s, 2 H), 3.50 (dd, J = 3.7, 12.6 Hz, 1 H), 3.21 (m, 1 H), 3.13 (t, J = 10.4 Hz, 1 H), 3.03 (dd, J = 8.9, 12.8 Hz, 1 H), 2.03 (m, 1 H), 1.79−1.50 (m, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.6, 161.2, 158.3, 149.2, 148.0, 145.8, 141.4, 141.1, 141.0, 136.4, 126.2, 117.1, 116.4, 113.2, 80.6, 72.4, 72.1, 53.9, 53.6, 51.6, 34.8, 30.8, 29.2. ESI-MS (M + H)$^+$ m/z 482.2. HR ESI-MS (M + H)$^+$ m/z = 482.1859 (calcd for $C_{24}H_{28}N_5O_4S$: 482.1862); t_R = 0.58.

***tert*-Butyl ((3*R*,6*S*)-6-((4*R*,5*R*)-5-(6-Methoxy-1,5-naphthyridin-4-yl)-2-oxo-1,3-dioxolan-4-yl)tetrahydro-2*H*-pyran-3-yl)-carbamate (18).** The compound **18** was prepared in 68% yield from the diol **17**[29] according to general procedure A. White solid; mp = 206 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.84 (d, J = 4.5 Hz, 1 H), 8.29 (d, J = 9.1 Hz, 1 H), 7.61 (d, J = 4.5 Hz, 1 H), 7.22 (d, J = 9.1 Hz, 1 H), 7.19 (d, J = 0.8 Hz, 1 H), 6.22 (s, 1 H), 4.71 (d, J = 1.9 Hz, 1 H), 4.29 (ddd, J = 2.0, 4.7, 10.7 Hz, 1 H), 4.09 (s, 3 H), 3.80 (dt, J = 2.2, 11.7, 1 H), 3.69 (m, 1 H), 3.13 (m, 1 H), 2.24 (m, 1 H), 1.91 (m, 1 H), 1.72 (m, 1 H), 1.48 (s, 9 H), 1.35 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.2, 154.8, 147.9, 142.1, 141.1, 140.7, 138.8, 135.0, 121.9, 117.5, 82.7, 79.7, 76.5, 76.0, 71.6, 54.2, 46.1, 30.0, 28.6 (3C), 25.7. ESI-MS (M + H)$^+$ m/z 446.2. HR ESI-MS (M + H)$^+$ m/z = 446.1924 (calcd for $C_{22}H_{28}N_3O_7$: 446.1927); t_R = 1.2.

***tert*-Butyl ((3*R*,6*S*)-6-((*S*)-1-Hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (19).** The compound **19** was prepared in 89% yield from carbonate **18** according to general procedure B. White solid; mp = 168 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.71 (d, J = 4.4 Hz, 1 H), 8.25 (d, J = 9.0 Hz, 1 H), 7.50 (d, J = 4.5 Hz, 1 H), 7.15 (d, J = 9.0 Hz, 1 H), 4.28 (m, 1 H), 4.18 (ddd, J = 2.1, 4.8, 10.6 Hz, 1 H), 4.09 (s, 3 H), 4.02 (m, 1 H), 3.62−3.74 (m, 2 H), 3.41 (dd, J = 3.5, 13.3 Hz, 1 H), 3.32 (dd, J = 8.7, 13.3 Hz, 1 H), 3.25 (ddd, J = 2.1, 5.0, 11.3 Hz, 1 H), 3.04 (t, J = 10.7 Hz, 1 H), 2.17 (m, 1 H), 1.87 (m, 1 H), 1.71 (m, 1 H), 1.47 (s, 9 H), 1.31 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7, 155.1, 147.8, 145.1, 141.5, 141.1, 140.7, 125.6, 116.5, 79.6, 79.5, 73.6, 71.4, 53.9, 46.6, 35.2, 30.3, 28.4 (3C), 26.3. ESI-MS (M + H)$^+$ m/z 404.0. HR ESI-MS (M + H)$^+$ m/z = 404.2191 (calcd for $C_{21}H_{30}N_3O_5$: 404.2185); t_R = 1.01.

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(*S*)-1-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (20). The compound **20** was prepared in 98% yield from **19** according to general procedure B. White solid; mp = 103 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.71 (d, J = 4.4 Hz, 1 H), 8.24 (d, J = 9.0 Hz, 1 H), 7.50 (d, J = 4.5 Hz, 1 H), 7.15 (d, J = 9.0 Hz, 1 H), 4.09 (s, 3 H), 3.98−4.05 (m, 2 H), 3.43 (dd, J = 3.5, 13.4 Hz, 1 H), 3.32 (dd, J = 8.7, 13.4 Hz, 1 H), 3.25 (ddd, J = 2.2, 5.0, 11.3 Hz, 1 H), 3.03 (t, J = 10.6 Hz, 1 H), 2.86 (m, 1 H), 2.11 (m, 1 H), 1.83 (m, 1 H), 1.66 (m, 1 H), 1.54 (br s, 3 H), 1.26 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7, 147.8, 145.1, 141.6, 141.1, 140.8, 125.6, 116.5, 79.6, 75.0, 73.8, 53.9, 47.5, 35.5, 33.4, 26.6. ESI-MS (M + H)$^+$ m/z 304.0. HR ESI-MS (M + H)$^+$ m/z = 304.1657 (calcd for $C_{16}H_{21}N_3O_3$: 304.1661); t_R = 0.46.

6-((((3*R*,6*S*)-6-((*S*)-1-Hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (16b). Starting from **20** (0.59 g, 1.97 mmol) and **15a** (0.42 g, 2.1 mmol), **16b** (0.45 g, 47% yield) was prepared using the general procedure D. Analytical data recorded for this sample matched those recorded previously (vide infra).

6-((((3*R*,6*S*)-6-((*S*)-1-Hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (21). Starting from **20** (0.709 g, 2.34 mmol) and **15b** (0.42 g, 2.34 mmol), **21** (0.19 g, 18% yield) was prepared according to the general procedure D. Off-white solid; mp = 188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.14 (s, 1 H), 8.63 (d, J = 4.4 Hz, 1 H), 8.21 (d, J = 9.1 Hz, 1 H), 7.50 (d, J = 4.5 Hz, 1 H), 7.28 (d, J = 8.1 Hz, 1 H), 7.21 (d, J = 9.1 Hz, 1 H), 6.99 (d, J = 8.1 Hz, 1 H), 4.59 (s, 2 H), 4.53 (d, J = 6.4 Hz, 1 H), 3.98 (s, 3 H), 3.98 (overlaid m, 1 H), 3.85 (m, 1 H), 3.62−3.73 (m, 2 H), 3.42 (dd, J = 3.8, 12.8 Hz, 1 H), 3.10 (m, 1 H), 2.88−3.00 (m, 2 H), 1.95−2.07 (m, 2 H), 1.63 (m, 1 H), 1.48 (m, 1 H), 1.19 (m, 1 H). ESI-MS (M + H)$^+$ m/z 466.3.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (26a). Starting from **20** (0.08 g, 0.26 mmol) and **22** (0.044 g, 0.26 mmol), **26a** (0.044 g, 47% yield) was prepared according to the general procedure E. Off-white solid; mp = 116 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (d, J = 4.4 Hz, 1 H), 8.23 (d, J = 9.0 Hz, 1 H), 8.13 (s, 1 H), 7.49 (d, J = 4.4 Hz, 1 H), 7.14 (d, J = 9.0 Hz, 1 H), 6.82 (s, 1 H), 4.33−4.36 (m, 2 H), 4.28−4.31 (m, 2 H), 4.16 (m, 1 H), 4.08 (s, 3 H), 3.99 (m, 1 H), 3.78−3.85 (m, 2 H), 3.70 (m, 1 H), 3.42 (dd, J = 3.4, 13.4 Hz, 1 H), 3.25−3.32 (m, 2 H), 3.15 (t, J = 10.6 Hz, 1 H), 2.71 (m, 1 H), 2.17 (m, 1 H), 1.83 (m, 1 H), 1.59−1.71 (m, 2 H), 1.34 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7, 153.4, 150.2, 147.9, 145.1, 141.7, 141.1, 140.8, 140.1, 138.9, 125.6, 116.4, 110.7, 80.0, 73.8, 72.8, 65.0, 64.1, 53.9, 53.3, 52.1, 35.5, 30.8, 26.5. ESI-MS (M + H)$^+$ m/z 453.0. HR ESI-MS (M + H)$^+$ m/z = 453.2142 (calcd for $C_{24}H_{29}N_4O_5$: 453.2137); t_R = 0.58.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (26b). Starting from **20** (0.1 g, 0.33 mmol) and **23** (0.06 g, 0.33 mmol), **26b** (0.14 g, 90% yield) was prepared according to the general procedure E. White solid; mp = 50 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.71 (d, J = 4.4 Hz, 1 H), 8.24 (d, J = 9.0 Hz, 1 H), 8.05 (s, 1 H), 7.49 (d, J = 4.5 Hz, 1 H), 7.14 (d, J = 9.0 Hz, 1 H), 7.00 (s, 1 H), 4.41−4.45 (m, 2 H), 4.17 (m, 1 H), 4.08 (s, 3 H), 3.99 (m, 1 H), 3.78−3.84 (m, 2 H), 3.42 (dd, J = 3.4, 13.4 Hz, 1 H), 3.26−3.33 (m, 2 H), 3.18−3.31 (m, 2 H), 3.15 (t, J = 10.5 Hz, 1 H), 2.72 (m, 1 H), 2.58 (br s, 2 H), 2.17 (m, 1 H), 1.84 (m, 1 H), 1.65 (m, 1 H), 1.36 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7, 151.3, 147.8, 147.5, 145.2, 141.6, 141.1, 140.7, 139.1, 129.4, 125.6, 120.0, 116.5, 80.0, 73.8, 72.6, 64.8, 54.0, 53.3, 51.7, 35.6, 30.7, 26.5, 25.7. ESI-MS (M + H)$^+$ m/z 469.0. HR ESI-MS (M + H)$^+$ m/z = 469.191 (calcd for $C_{24}H_{29}N_4O_4S$: 469.1909); t_R = 0.61.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]dioxino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (26c). Starting from **18d** (0.08 g, 0.26 mmol) and **24** (0.046 g, 0.26 mmol), **26c** (0.082 g, 67% yield) was prepared according to the general procedure E. Off-white solid; mp = 45 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.70 (d, J = 4.4 Hz, 1 H), 8.23 (d, J = 9.0 Hz, 1 H), 7.49 (d, J = 4.5 Hz, 1 H), 7.14 (d, J = 9.0 Hz, 1 H), 7.02 (s, 1 H), 4.52−4.55 (m, 2 H), 4.37−4.41 (m, 2 H), 4.16 (ddd, J = 2.2, 4.4, 10.8 Hz, 1 H), 4.08 (s, 3 H), 4.03 (apparent d, J = 5.4 Hz, 2 H), 3.99 (m, 1 H), 3.72 (br s, 1 H), 3.42 (dd, J = 3.5, 13.4 Hz, 1 H), 3.26−3.33 (m, 2 H), 3.13 (t, J = 10.6 Hz, 1 H), 2.73 (m, 1 H), 2.20 (m, 1 H), 1.84 (m, 1 H), 1.59−1.72 (m, 2 H), 1.32 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.8, 159.3, 155.5, 147.8, 145.1, 144.3, 141.6, 141.1, 140.8, 125.6, 116.5, 113.4, 80.0, 73.8, 72.6, 64.8 (2C), 54.0, 53.4, 50.4, 35.5, 30.8, 26.5. ESI-MS (M + H)$^+$ m/z 454.1. HR ESI-MS (M + H)$^+$ m/z = 454.2091 (calcd for $C_{23}H_{28}N_5O_5$: 454.2090); t_R = 0.52.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]oxathiino[2,3-*c*]-pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (26d). Starting from **18d** (0.072 g, 0.23 mmol) and **25** (0.046 g, 0.25 mmol), **26d** (0.070 g, 63% yield) was prepared according to the general procedure E. Off-white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.63 (d, J = 4.4 Hz, 1 H), 8.21 (d, J = 9.0 Hz, 1 H), 7.53 (s, 1 H), 7.50 (d, J = 4.5 Hz, 1 H), 7.21 (d, J = 9.0 Hz, 1 H), 4.54−4.58 (m, 2 H), 4.52 (d, J = 6.4 Hz, 1 H), 3.98 (s, 3 H), 3.95 (m, 1 H), 3.80−3.89 (m, 3 H), 3.42 (dd, J = 3.9, 12.9 Hz, 1 H), 3.35−3.30 (m, 2 H), 3.09 (m, 1 H), 2.95 (dd, J = 9.2, 11.8 Hz, 1 H), 2.90 (t, J = 10.7 Hz, 1 H), 2.43 (overlaid m, 1 H), 2.21 (m, 1 H), 2.01 (m, 1 H), 1.62 (m, 1 H); 1.47 (m, 1 H), 1.16 (m, 1 H). ESI-MS (M + H)$^+$ m/z 470.2; .HR ESI-MS (M + H)$^+$ m/z = 470.1861 (calcd for $C_{23}H_{28}N_5O_4S$: 470.1862); t_R = 0.54.

***tert*-Butyl ((3*R*,6*S*)-6-((4*R*,5*R*)-5-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-oxo-1,3-dioxolan-4-yl)tetrahydro-2*H*-pyran-3-yl)carbamate (28a).** The compound **28a** was prepared in 74% yield from diol **27a**[29] using the general procedure A. White solid; mp = 135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1 H), 8.24 (d, J = 9.1 Hz, 1 H), 7.15 (d, J = 9.1 Hz, 1 H), 6.34 (d, J = 5.8 Hz, 1 H), 4.86 (t, J = 5.5 Hz, 1 H), 4.29 (m, 1 H), 4.18 (m, 1 H), 4.11 (s, 3 H), 3.59−3.69 (m, 2 H), 3.11 (t, J = 10.8 Hz, 1 H), 2.17 (m, 1 H), 1.82 (m, 1 H), 1.45 (s, 9 H), 1.43 (overlaid m, 1 H), 1.39 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.3, 156.2 (d, J = 263 Hz), 155.0, 154.9, 140.6, 140.3 (d, J = 4 Hz), 139.4 (d, J = 3 Hz), 138.1 (d, J = 28 Hz), 123.8 (d, J = 8 Hz), 116.6 (d, J = 2 Hz), 81.6, 79.9, 76.3, 71.1, 70.5 (d, J = 6 Hz), 54.6, 46.3, 29.6, 28.4 (3C), 26.6. ESI-MS (M + H)$^+$ m/z 464.3. HR ESI-MS (M + H)$^+$ m/z = 464.1833 (calcd for $C_{22}H_{27}N_3O_7F$: 464.1833); t_R = 1.27.

***tert*-Butyl ((3*R*,6*S*)-6-((4*R*,5*R*)-5-(6-Fluoro-3-methoxyquinoxalin-5-yl)-2-oxo-1,3-dioxolan-4-yl)tetrahydro-2*H*-pyran-3-yl)-carbamate (28b).** The compound **28b** was prepared in 66% yield from the diol **27b**[29] according to general procedure A. White solid; mp = 183.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.65 (s, 1 H), 8.17 (dd, J = 5.9, 9.2 Hz, 1 H), 7.62 (t, J = 9.5 Hz, 1 H), 6.78 (d, J = 7.7 Hz, 1 H), 6.28 (d, J = 6.0 Hz, 1 H), 5.01 (dd, J = 3.6, 5.9 Hz, 1 H), 4.03 (s, 3 H), 3.92 (m, 1 H), 3.51 (m, 1 H), 3.34 (m, 1 H), 3.07 (t, J = 10.5 Hz, 1 H), 1.89 (m, 1 H), 1.64 (m, 1 H), 1.33−1.50 (m, 2 H), 1.36 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.3 (d, J = 255 Hz), 157.8, 155.2, 155.0 (d, J = 5 Hz), 140.0 (d, J = 7 Hz), 139.6 (d, J = 3 Hz), 136.0, 132.9 (d, J = 11 Hz), 116.5 (d, J = 12 Hz), 115.7 (d, J = 26 Hz), 81.8, 79.8, 75.8, 71.4, 70.7 (d, J = 7 Hz), 54.6, 46.1, 28.3 (3C), 25.3. ESI-MS (M + H)$^+$ m/z 464.6. HR ESI-MS (M + H)$^+$ m/z = 464.1826 (calcd for $C_{22}H_{27}N_3O_7F$: 464.1833); t_R = 1.26.

***tert*-Butyl ((3*R*,6*S*)-6-((4*R*,5*R*)-5-(7-Fluoro-2-methoxyquinolin-8-yl)-2-oxo-1,3-dioxolan-4-yl)tetrahydro-2*H*-pyran-3-yl)-carbamate (28c).** The compound **28c** was prepared in 63% yield from the diol **27c**[29] according to general procedure A. White solid; mp = 93 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.00 (d, J = 8.9 Hz, 1 H), 7.79 (dd, J = 6.1, 8.9 Hz, 1 H), 7.19 (t, J = 9.3 Hz, 1 H), 6.94 (d, J = 8.9 Hz, 1 H), 6.33 (m, 1 H), 5.05 (m, 1 H), 4.29 (m, 1 H), 4.23 (m, 1 H), 4.10 (s, 3 H) 3.67 (m, 1 H), 3.49 (m, 1 H), 3.12 (t, J = 10.7 Hz, 1 H), 2.19 (m, 1 H); 1.65−1.78 (m, 2 H), 1.46 (s, 9H), 1.34 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.3, 161.8 (d, J = 252 Hz), 155.4, 155.0, 145.9 (d, J = 7 Hz), 139.1, 131.4 (d, J = 12 Hz), 122.4, 116.0 (d, J = 10 Hz), 113.3 (d, J = 9 Hz), 113.2 (J = 14 Hz), 81.7, 79.7, 76.0, 71.4, 71.1 (d, J = 7 Hz), 54.1, 46.1; 29.9, 28.3 (3C), 26.6. ESI-MS (M + H)$^+$ m/z 463.0. HR ESI-MS (M + H)$^+$ m/z = 463.1888 (calcd for $C_{23}H_{28}N_2O_7F$: 463.1880) ; t_R = 1.33.

***tert*-Butyl ((3*R*,6*S*)-6-((*S*)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)tetrahydro-2*H*-pyran-3-yl)-**

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carbamate (29a). The compound **29a** was prepared in 78% yield from carbonate **28a** according to general procedure B. White solid; mp = 170 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.67 (s, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.10 (d, J = 9.0 Hz, 1 H), 4.28 (m, 1 H), 4.18 (ddd, J = 2.0, 4.7, 10.7 Hz, 1 H), 4.09 (s, 3 H), 4.00 (m, 1 H), 3.63 (m, 1 H), 3.52 (m, 1 H), 3.38−3.46 (m, 2 H), 3.28 (ddd, J = 2.5, 4.4, 11.0 Hz, 1 H), 3.06 (t, J = 10.7 Hz, 1 H), 2.18 (d, J = 12.2 Hz, 1 H), 1.71−1.87 (m, 2 H), 1.47 (s, 9 H), 1.33 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 157.6 (d, J = 256 Hz), 155.1, 141.9 (d, J = 6 Hz), 140.6, 138.6 (d, J = 2 Hz), 138.2 (d, J = 29 Hz), 129.1 (d, J = 13 Hz), 115.4 (d, J = 3 Hz), 79.6, 79.5, 73.3, 71.4, 54.1, 46.5, 30.3, 28.4 (3C), 27.8, 26.6. ESI-MS (M + H)$^+$ m/z 422.2. HR ESI-MS (M + H)$^+$ m/z = 422.2094 (calcd for $C_{21}H_{29}N_3O_5F$: 422.2091); t_R = 1.21.

***tert*-Butyl ((3*R*,6*S*)-6-((*S*)-2-(6-Fluoro-3-methoxyquinoxalin-5-yl)-1-hydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29b).** The compound **29b** was prepared in 73% yield from carbonate **28b** according to general procedure B. Colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.54 (s, 1 H), 7.88 (dd, J = 5.9, 9.1 Hz, 1 H), 7.47 (t, J = 9.2 Hz, 1 H), 4.38 (d, J = 6.1 Hz, 1 H), 4.04 (s, 4 H), 3.74−3.84 (m, 2 H), 3.24−3.33 (m, 2 H), 3.00−3.13 (m, 2 H), 2.79 (t, J = 10.5 Hz, 1 H), 2.53 (overlaid m, 1 H), 1.90 (m, 1 H), 1.44−1.65 (m, 2 H), 1.27 (br s, 2 H), 1.10 (m, 1 H). ESI-MS (M + H)$^+$ m/z 422.5. HR ESI-MS (M + H)$^+$ m/z = 422.2094 (calcd for $C_{21}H_{29}N_3O_5F$: 422.2091); t_R = 1.21.

***tert*-Butyl ((3*R*,6*S*)-6-((*S*)-2-(7-Fluoro-2-methoxyquinolin-8-yl)-1-hydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29c).** The compound **29c** was prepared in 70% yield from carbonate **28c** according to general procedure B. White solid; mp = 67 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, J = 8.8 Hz, 1 H), 7.61 (dd, J = 6.1, 8.8 Hz, 1 H), 7.19 (t, J = 9.0 Hz, 1 H), 6.89 (d, J = 8.8 Hz, 1 H), 4.27 (br s, 1 H), 4.19 (ddd, J = 2.1, 4.8, 10.7 Hz, 1 H), 4.08 (s, 3 H), 4.02 (br s, 1 H), 3.97 (m, 1 H), 3.67 (br s, 1 H), 3.45 (ddd, J = 0.8, 2.1, 11.0 Hz, 1 H), 3.38 (ddd, J = 1.8, 8.4, 11.0 Hz, 1 H), 3.29 (ddd, J = 2.1, 4.7, 11.2 Hz, 1 H), 3.06 (t, J = 10.7 Hz, 1 H) 2.17 (m, 1 H), 1.86 (m, 1 H), 1.73 (m, 1 H), 1.47 (m, 9 H), 1.32 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.8, 161.7 (d, J = 247 Hz), 155.1, 146.5 (d, J = 9 Hz), 139.4, 127.2 (d, J = 11 Hz), 121.9, 120.6 (d, J = 16 Hz), 113.8 (d, J = 27 Hz), 111.9 (d, J = 2 Hz), 79.8, 79.5, 74.2, 71.5, 53.7, 46.6, 30.5, 28.4 (3C), 27.7, 26.4. ESI-MS (M + H)$^+$ m/z 421.0. HR ESI-MS (M + H)$^+$ m/z = 421.2139 (calcd for $C_{22}H_{30}N_2O_5F$: 421.2138); t_R =1.35.

(*S*)-1-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (30a). The compound **30a** was prepared in 90% yield from **29a** according to general procedure B. White solid; mp = 118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ:. 8.67 (s, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.10 (d, J = 9.0 Hz, 1 H), 4.10 (s, 3 H), 3.97−4.04 (m, 2 H), 3.56 (br s, 1 H), 3.46 (ddd, J = 1.5, 4.0, 13.0 Hz, 1 H), 3.40 (ddd, J = 1.4, 8.4, 13.0 Hz, 1 H), 3.29 (ddd, J = 2.3, 4.5, 11.2 Hz, 1 H), 3.05 (t, J = 10.6 Hz, 1 H), 2.86 (m, 1 H), 2.12 (m, 1 H), 1.79 (m, 1 H), 1.70 (m, 1 H), 1.28 (m, 1 H), 1.10 (br s, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 157.6 (d, J = 256 Hz), 141.9 (d, J = 6 Hz), 138.6, 138.5, 138.2 (d, J = 29 Hz), 129.2 (d, J = 13 Hz), 115.4 (d, J = 3 Hz), 79.6, 75.0, 73.4, 54.0, 47.4, 33.5, 28.0, 26.9. ESI-MS (M + H)$^+$ m/z 322.2. HR ESI-MS (M + H)$^+$ m/z = 322.1567 (calcd for $C_{16}H_{21}N_3O_3F$: 322.1567); t_R = 0.57.

(*S*)-1-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethan-1-ol (30b). The compound **30b** was prepared in quantitative yield from **29b** according to general procedure B. White solid; mp = 139−140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.46 (s, 1 H), 7.92 (dd, J = 5.8, 9.1 Hz, 1 H), 7.36 (t, J = 9.1 Hz, 1 H), 4.12 (s, 3 H), 4.04 (ddd, J = 2.2, 4.5, 10.7 Hz, 1 H), 3.93 (m, 1 H), 3.42 (ddd, J = 1.5, 4.0, 13.0 Hz, 1 H), 3.36 (ddd, J = 1.4, 8.4, 13.0 Hz, 1 H), 3.26 (ddd, J = 2.3, 4.5, 11.1 Hz, 1 H), 3.06 (t, J = 10.6 Hz, 1 H), 2.86 (m, 1 H), 2.11 (m, 1 H), 1.77 (m, 1 H), 1.69 (m, 1 H), 1.29 (m, 1 H), 1.25−2.20 (br s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7 (d, J = 248 Hz), 157.4, 140.3 (d, J = 10 Hz), 138.2 (d, J = 3 Hz), 136.0, 128.7 (d, J = 11 Hz), 120.7 (d, J = 17 Hz), 116.1 (d, J = 27 Hz), 79.5, 74.9, 73.8, 53.9, 47.4, 33.4, 28.0, 27.0. ESI-MS (M + H)$^+$ m/z 322.0. HR ESI-MS (M + H)$^+$ m/z = 322.1567 (calcd for $C_{16}H_{21}N_3O_3F$: 322.1567); t_R = 0.59.

(*S*)-1-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (30c). The compound **30c** was prepared in quantitative yield from **29c** according to general procedure B. White solid; mp = 65 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, J = 8.9 Hz, 1 H), 7.61 (dd, J = 6.0, 8.8 Hz, 1 H), 7.19 (t, J = 9.0 Hz, 1 H), 6.89 (d, J = 8.8 Hz, 1 H), 4.08 (s, 3 H), 4.08 (overlaid1 m, 1 H), 3.95 (m, 1 H), 3.45 (ddd, J = 1.6, 3.8, 13.4 Hz, 1 H), 3.38 (ddd, J = 1.8, 8.1, 13.4 Hz, 1 H), 3.30 (ddd, J = 2.0, 5.0, 11.2 Hz, 1 H), 3.12 (t, J = 10.7 Hz, 1 H), 2.94 (m, 1 H), 2.82 (br s, 3 H), 2.13 (m, 1 H), 1.85 (m, 1 H), 1.68 (m, 1 H), 1.35 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.8, 161.7 (d, J = 246 Hz), 146.5 (d, J = 9 Hz), 139.5, 127.3 (d, J = 11 Hz), 121.9, 120.4 (d, J = 16 Hz), 113.8 (d, J = 27 Hz), 112.0 (d, J = 2 Hz), 79.9, 74.3, 73.6, 53.8, 47.4, 32.3, 27.8, 26.5. ESI-MS (M + H)$^+$ m/z 321.0. HR ESI-MS (M + H)$^+$ m/z = 321.1615 (calcd for $C_{17}H_{22}N_2O_3F$: 321.1614); t_R = 0.67.

6-((((3*R*,6*S*)-6-((*S*)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)tetrahydro-2*H*-pyran-3-yl)amino)-methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (31a). Starting from **30a** (0.092 g, 0.28 mmol) and **15a** (0.055 g, 0.28 mmol), **31a** (0.113 g, 79% yield) was prepared according to the general procedure D. Yellowish solid; mp = 93 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.67 (s, 1 H), 8.29 (br s, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.60 (d, J = 7.8 Hz, 1 H), 7.10 (d, J = 9.0 Hz, 1 H), 6.98 (d, J = 7.8 Hz, 1 H), 4.17 (ddd, J = 2.1, 4.3, 10.7 Hz, 1 H), 4.09 (s, 3 H), 3.99 (m, 1 H), 3.83−3.90 (m, 2 H), 3.50 (s, 2 H), 3.46 (ddd, J = 1.2, 4.0, 13.4 Hz, 1 H), 3.40 (ddd, J = 1.2, 8.5, 13.4 Hz, 1 H), 3.33 (ddd, J = 2.2, 4.3, 11.2 Hz, 1 H), 3.18 (t, J = 10.6 Hz, 1 H), 2.73 (m, 1 H), 2.18 (m, 1 H), 1.80 (m, 1 H), 1.74 (br s, 2H), 1 0.69 (m, 1 H), 1.40 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.6, 162.7, 157.5 (d, J = 256 Hz), 156.8, 148.2, 141.8 (d, J = 6 Hz), 140.6, 138.6 (d, J = 2 Hz), 138.1 (d, J = 9 Hz), 136.2, 129.2 (d, J = 13 Hz), 117.9, 115.4 (d, J = 3 Hz), 113.6, 80.1, 73.4, 72.5, 54.1, 53.3, 51.5, 30.7, 29.7, 28.0, 26.7. ESI-MS (M + H)$^+$ m/z 499.9.

6-((((3*R*,6*S*)-6-((*S*)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)tetrahydro-2*H*-pyran-3-yl)amino)-methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (31b). Starting from **30a** (0.092 g, 0.28 mmol) and **15b** (0.055 g, 0.28 mmol), **31b** (0.119 g, 79% yield) was prepared according to the general procedure D. Yellowish solid; mp = 187−188 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.67 (s, 1 H), 8.52 (br s, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 7.23 (d, J = 8.0 Hz, 1 H), 7.10 (d, J = 9.0 Hz, 1 H), 6.94 (d, J = 8.0 Hz, 1 H), 4.67 (s, 2 H), 4.17 (m, 1 H), 4.09 (s, 3 H), 3.99 (m, 1 H), 3.80−3.87 (m, 2 H), 3.45 (ddd, J = 1.2, 4.0, 13.4 Hz, 1 H), 3.40 (ddd, J = 1.2, 8.5, 13.4 Hz, 1 H), 3.33 (ddd, J = 2.2, 4.3, 11.2 Hz, 1 H), 3.16 (t, J = 10.6 Hz, 1 H), 2.72 (m, 1 H), 2.16 (m, 1 H), 1.80 (m, 1 H), 1.59−1.73 (m, 3 H), 1.38 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.3, 162.7, 157.4 (d, J = 256 Hz), 151.9, 141.8 (d, J = 6 Hz), 140.6, 140.0, 138.6 (d, J = 2 Hz), 138.3, 138.1 (d, J = 9 Hz), 129.2 (d, J = 13 Hz), 124.1, 118.1, 115.4 (d, J = 2 Hz), 80.1, 73.4, 72.7, 67.3, 54.1, 53.3, 51.4, 30.8, 28.1, 26.7. ESI-MS (M + H)$^+$ m/z 484.3. HR ESI-MS (M + H)$^+$ m/z = 484.1996 (calcd for $C_{24}H_{27}N_5O_5F$: 484.1996); t_R = 0.64.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (32a). Starting from **30a** (1.47 g, 4.6 mmol) and **22** (0.758 g, 4.6 mmol), **32a** (1.31 g, 61% yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.72 (s, 1 H), 8.24 (d, J = 9.0 Hz, 1 H), 7.99 (s, 1 H), 7.19 (d, J = 9.0 Hz, 1 H), 6.91 (s, 1 H), 4.53 (d, J = 6.1 Hz, 1 H), 4.30−4.37 (m, 2 H), 4.23−4.27 (m, 2 H), 4.00 (s, 3 H), 3.85−3.96 (m, 2 H), 3.59−3.70 (m, 2 H), 3.31 (m, 1 H), 3.07−3.10 (m, 2 H), 2.90 (t, J = 10.5 Hz, 1 H), 2.41 (m, 1 H), 2.09 (m, 1 H), 2.00 (m, 1 H), 1.64 (m, 1 H), 1.47 (m, 1 H), 1.19 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 157.6 (d, J = 256 Hz), 153.3, 150.2, 141.9 (d, J = 6 Hz), 140.6, 140.1, 138.9, 138.5 (d, J = 2 Hz), 138.2 (d, J = 28 Hz), 129.2 (d, J = 13 Hz), 115.3 (d, J = 2 Hz), 110.7, 80.0, 73.4, 72.7, 65.0, 64.1, 54.0, 53.2, 52.0, 30.7, 28.0, 26.7. ESI-MS (M + H)$^+$ m/z 471.2. HR ESI-MS (M + H)$^+$ m/z = 471.2054 (calcd for $C_{24}H_{28}N_4O_5F$: 471.2043); t_R = 0.67.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (32b). Starting from **30a** (5.0 g, 15.7 mmol) and **23** (2.87 g, 15.7 mmol), **32b** (5.47 g, 71%

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yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.72 (d, J = 0.6 Hz, 1 H), 8.25 (d, J = 9.0 Hz, 1 H), 7.92 (s, 1 H), 7.19 (d, J = 9.0 Hz, 1 H), 7.13 (s, 1 H), 4.53 (d, J = 6.1 Hz, 1 H) 4.33−4.37 (m, 2 H), 4.00 (s, 3 H), 3.85−3.96 (m, 2 H), 3.59−3.70 (m, 2 H), 3.31 (m, 1 H), 3.21−3.25 (m, 2 H), 3.06−3.16 (m, 2 H), 2.90 (t, J = 10.5 Hz, 1 H), 2.41 (m, 1 H), 1.95−2.05 (m, 2 H), 1.65 (m, 1 H), 1.48 (m, 1 H), 1.17 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.6, 157.5 (d, J = 256 Hz), 151.5, 147.4, 141.8 (d, J = 6 Hz), 140.5, 139.2, 138.5 (d, J = 2 Hz), 138.2 (d, J = 29 Hz), 129.3, 129.2 (d, J = 13 Hz), 120.0, 115.3 (d, J = 2 Hz), 80.0, 73.4, 72.7, 64.8, 54.1, 53.2, 51.8, 30.7, 28.0, 26.7, 25.7. ESI-MS (M + H)$^+$ m/z 487.3. HR ESI-MS (M + H)$^+$ m/z = 487.1821 (calcd for $C_{24}H_{28}N_4O_4FS$: 487.1815); t_R = 0.71.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]dioxino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (32c). Starting from **30a** (0.053 g, 0.165 mmol) and **24** (0.027 g, 0.17 mmol), **32c** (0.02 g, 25% yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.73 (s, 1 H), 8.25 (d, J = 9.0 Hz, 1 H), 7.20 (d, J = 9.1 Hz, 1 H), 7.17 (s, 1 H), 4.56 (d, J = 6.0 Hz, 1 H) 4.47−4.51 (m, 2 H), 4.36−4.40 (m, 2 H),), 4.00 (s, 3 H), 3.85−3.96 (m, 2 H), 3.81−3.84 (m, 2 H), 3.31 (m, 1 H), 3.07−3.17 (m, 2 H), 2.90 (t, J = 10.5 Hz, 1 H), 2.41 (m, 1 H), 2.26 (m, 1 H), 2.00 (m, 1 H), 1.64 (m, 1 H), 1.49 (m, 1 H), 1.17 (m, 1 H). ESI-MS (M + H)$^+$ m/z 472.2. HR ESI-MS (M + H)$^+$ m/z = 472.1993 (calcd for $C_{23}H_{27}N_5O_5F$: 472.1996); t_R = 0.61.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]oxathiino[2,3-*c*]-pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (32d). Starting from **30a** (2.0 g, 6.27 mmol) and **25** (1.34 g, 6.27 mmol), **32d** (2.06 g, 67% yield) was prepared according to the general procedure E. Off-white solid; mp = 129 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.72 (s, 1 H), 8.24 (d, J = 9.0 Hz, 1 H), 7.53 (s, 1 H), 7.19 (d, J = 9.0 Hz, 1 H), 4.50−4.61 (m, 3 H), 3.98 (s, 3 H), 3.85−3.95 (m, 2 H), 3.77−3.83 (m, 2 H), 3.31 (overlaid m, 1 H), 3.25−3.30 (m, 2 H), 3.05−3.15 (m, 2 H), 2.90 (t, J = 10.7 Hz, 1 H), 2.41 (m, 1 H), 2.20 (m, 1 H), 2.02 (m, 1 H), 1.65 (m, 1 H); 1.50 (m, 1 H), 1.17 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 159.9, 157.5 (d, J = 256 Hz), 156.7, 141.9 (d, J = 6 Hz), 140.6, 138.6 (d, J = 2 Hz), 138.2 (d, J = 29 Hz), 129.2 (d, J = 13 Hz), 125.8, 125.2, 115.4 (d, J = 3 Hz), 80.0, 73.4, 72.6, 66.2, 54.1, 53.4, 50.2, 30.8, 28.0, 26.7, 25.6. ESI-MS (M + H)$^+$ m/z 488.3. HR ESI-MS (M + H)$^+$ m/z = 488.1771 (calcd for $C_{23}H_{27}N_5O_4FS$: 488.1767); t_R = 0.63.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethan-1-ol (33a). Starting from **30b** (0.344 g, 1.0 mmol) and **22** (0.177 g, 1.0 mmol), **33a** (0.334 g, 66% yield) was prepared according to the general procedure E. Off-white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.53 (s, 1 H), 7.99 (s, 1 H), 7.88 (dd, J = 5.8, 9.1 Hz, 1 H), 7.46 (t, J = 9.2 Hz, 1 H), 6.91 (s, 1 H), 4.38 (d, J = 6.1 Hz, 1 H), 4.29−4.34 (m, 2 H), 4.23−4.28 (m, 2 H), 4.02 (s, 3 H), 3.93 (m, 1 H), 3.79 (m, 1 H), 3.60−3.70 (m, 2 H), 3.28 (m, 1 H), 3.03−3.12 (m, 2 H), 2.88 (t, J = 10.5 Hz, 1 H), 2.43 (overlaid m, 1 H), 2.06 (br s, 1 H), 1.98 (m, 1 H), 1.61 (m, 1 H), 1.47 (m, 1 H), 1.17 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.6 (d, J = 249 Hz), 157.4, 153.4, 150.2, 140.3 (d, J = 10 Hz), 140.1, 138.9, 138.2 (d, J = 3 Hz), 136.0, 128.7 (d, J = 11 Hz), 120.7 (d, J = 17 Hz), 116.1 (d, J = 27 Hz), 110.7, 79.9, 73.8, 72.7, 65.0, 64.1, 53.9, 53.2, 52.1, 30.8, 28.0, 26.9. ESI-MS (M + H)$^+$ m/z 471.2. HR ESI-MS (M + H)$^+$ m/z = 471.2040 (calcd for $C_{24}H_{28}N_4O_5F$: 471.2043); t_R = 0.69.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethan-1-ol (33b). Starting from **30b** (0.057 g, 0.177 mmol) and **23** (0.031 g, 0.18 mmol), **33b** (0.027 g, 31% yield) was prepared according to the general procedure E. White foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.54 (s, 1 H), 7.92 (s, 1 H), 7.89 (dd, J = 5.8, 9.1 Hz, 1 H), 4.32−4.39 (m, 3 H), 4.03 (s, 3 H), 3.93 (m, 1 H), 3.79 (m, 1 H), 3.59−3.70 (m, 2 H), 3.30 (m, 1 H), 3.21−3.26 (m, 2 H), 3.03−3.12 (m, 2 H), 2.89 (t, J = 10.4 Hz, 1 H), 2.43 (overlaid m, 1 H), 1.94−2.07 (m, 2 H), 1.62 (m, 1 H), 1.48 (m, 1 H), 1.18 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.7 (d, J = 249 Hz), 157.4, 151.4, 147.4, 140.3 (d, J = 10 Hz), 139.2, 138.2 (d, J = 3 Hz), 136.0, 129.3, 128.7 (d, J = 11 Hz), 120.7 (d, J = 17 Hz), 120.0, 116.1 (d, J = 27 Hz), 79.9, 73.8, 72.6, 64.8, 53.9, 53.3, 51.8, 30.7, 28.0, 26.9, 25.7. ESI-MS (M + H)$^+$ m/z 487.2.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]dioxino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethan-1-ol (33c). Starting from **30b** (0.297 g, 0.92 mmol) and **24** (0.153 g, 0.92 mmol), **33c** (0.124 g, 28% yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.53 (s, 1 H), 7.88 (dd, J = 5.9, 9.1 Hz, 1 H), 7.46 (t, J = 9.2 Hz, 1 H), 7.16 (s, 1 H), 4.45−4.51 (m, 2 H), 4.33−4.40 (m, 3 H), 4.03 (s, 3 H), 3.92 (m, 1 H), 3.75−3.84 (m, 3 H), 3.30 (m, 1 H), 3.03−3.12 (m, 2 H), 2.88 (t, J = 10.5 Hz, 1 H), 2.43 (overlaid m, 1 H), 2.25 (br s, 1 H), 1.99 (m, 1 H), 1.61 (m, 1 H), 1.47 (m, 1 H), 1.16 (m, 1 H). ESI-MS (M + H)$^+$ m/z 472.2. HR ESI-MS (M + H)$^+$ m/z = 472.1990 (calcd for $C_{23}H_{27}N_5O_5F$: 472.1996); t_R = 0.64.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]oxathiino[2,3-*c*]-pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethan-1-ol (33d). Starting from **30b** (1.8 g, 5.6 mmol) and **25** (1.0 g, 5.6 mmol), **33d** (1.99 g, 73% yield) was prepared using the general procedure E. White solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.53 (s, 1 H), 7.88 (dd, J = 5.9, 9.1 Hz, 1 H), 7.53 (s, 1 H), 7.47 (t, J = 9.2 Hz, 1 H), 4.54−4.59 (m, 2 H), 4.39 (d, J = 6.1 Hz, 1 H), 4.02 (s, 4 H), 3.92 (m, 1 H), 3.74−3.83 (m, 3 H), 3.24−3.32 (m, 3 H), 3.02−3.13 (m, 2 H), 2.88 (t, J = 10.4 Hz, 1 H), 2.42 (overlaid m, 1 H), 2.18 (m, 1 H), 1.62 (m, 1 H), 1.48 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.6 (d, J = 249 Hz), 159.9, 157.4, 156.8, 140.3 (d, J = 10 Hz), 138.3 (d, J = 3 Hz), 136.0, 128.7 (d, J = 11 Hz), 125.7, 125.2, 120.6 (d, J = 17 Hz), 116.1 (d, J = 27 Hz), 79.9, 73.8, 72.6, 66.2, 53.9, 53.4, 50.2, 30.8, 28.0, 26.8, 25.6. ESI-MS (M + H)$^+$ m/z 488.5. HR ESI-MS (M + H)$^+$ m/z = 488.1776 (calcd for $C_{23}H_{27}N_5O_4FS$: 488.1767); t_R = 0.66.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (34a). Starting from **30c** (0.73 g, 5.6 mmol) and **22** (0.38 g, 2.3 mmol), **34a** (0.275 g, 26% yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.20 (d, J = 8.9 Hz, 1 H), 7.99 (s, 1 H), 7.76 (dd, J = 6.3, 8.9 Hz, 1 H), 7.25 (t, J = 9.1 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 6.91 (s, 1 H), 4.29−4.34 (m, 3 H), 4.23−4.27 (m, 2 H), 3.96 (s, 3 H), 3.95 (overlaid m, 1 H), 3.82 (m, 1 H), 3.60−3.70 (m, 2 H), 3.31 (overlaid m, 1 H), 3.03−3.12 (m, 2 H), 2.90 (t, J = 10.5 Hz, 1 H), 2.43 (overlaid m, 1 H), 1.93−2.07 (m, 2 H), 1.61 (m, 1 H), 1.47 (m, 1 H), 1.16 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 161.6 (d, J = 246 Hz), 153.5, 150.2, 146.5 (d, J = 9 Hz), 140.1, 139.4, 138.9, 127.2 (d, J = 11 Hz), 121.9, 120.7 (d, J = 16 Hz), 113.8 (d, J = 27 Hz), 111.9 (d, J = 3 Hz), 110.7, 80.3, 74.4, 72.8, 65.0, 64.1, 53.8, 53.3, 52.1, 30.9, 28.0 (d, J = 2 Hz), 26.6. ESI-MS (M + H)$^+$ m/z 470.1. HR ESI-MS (M + H)$^+$ m/z = 470.2093 (calcd for $C_{25}H_{29}N_3O_5F$: 470.2091); t_R = 0.76.

(*S*)-1-((2*S*,5*R*)-5-(((2,3-Dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (34b). Starting from **30c** (0.072 g, 0.23 mmol) and **23** (0.046 g, 0.25 mmol), **34b** (0.082 g, 67% yield) was prepared according to the general procedure E. Off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.20 (d, J = 8.9 Hz, 1 H), 7.92 (s, 1 H), 7.76 (dd, J = 6.2, 8.9 Hz, 1 H), 7.25 (t, J = 9.2 Hz, 1 H), 7.13 (s, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 4.29−4.32 (m, 3 H), 3.97 (s, 3 H), 3.95 (overlaid m, 1 H), 3.84 (m, 1 H), 3.59−3.70 (m, 2 H), 3.31 (overlaid m, 1 H), 3.20−3.25 (m, 2 H), 3.02−3.11 (m, 2 H), 2.89 (t, J = 10.5 Hz, 1 H), 2.44 (overlaid m, 1 H), 1.94−2.08 (m, 2 H), 1.61 (m, 1 H), 1.49 (m, 1 H), 1.17 (m, 1 H). ESI-MS (M + H)$^+$ m/z 486.1. HR ESI-MS (M + H)$^+$ m/z = 486.1863 (calcd for $C_{25}H_{29}N_3O_4FS$: 486.1862); t_R = 0.79.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]dioxino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (34c). Starting from **30c** (0.083 g, 0.26 mmol) and **25** (0.043 g, 0.26 mmol), **34c** (0.061 g, 50% yield) was prepared according to the general procedure E. Off-white solid; mp = 161 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, J = 8.8 Hz, 1

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H), 7.61 (dd, J = 6.1, 8.8 Hz, 1 H), 7.19 (t, J = 9.0 Hz, 1 H), 6.88 (d, J = 8.8 Hz, 1 H), 4.51−4.55 (m, 2 H), 4.37−4.40 (m, 2 H),), 4.17 (ddd, J = 2.2, 4.4, 10.8 Hz, 1 H), 4.08 (s, 3 H), 4.00−4.06 (m, 2 H), 3.95 (m, 2 H), 3.45 (ddd, J = 1.7, 3.7, 14.4 Hz, 1 H), 3.37 (ddd, J = 1.9, 8.5, 14.4 Hz, 1 H), 3.32 (ddd, J = 2.1, 4.8, 11.5 Hz, 1 H), 3.15 (t, J = 10.5 Hz, 1 H), 2.74 (tt, J = 4.1, 11.1 Hz, 1 H), 2.20 (m, 1 H), 1.84 (m, 1 H), 1.74 br s, 2 H), 1.67 (m, 1 H), 1.34 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 161.6 (d, J = 246 Hz), 159.4, 155.5, 146.5 (d, J = 9 Hz), 144.2, 139.4, 127.2 (d, J = 11 Hz), 121.9, 120.7 (d, J = 16 Hz), 113.8 (d, J = 27 Hz), 113.4, 111.9 (d, J = 3 Hz), 80.3, 74.3, 72.7, 64.7, 64.6, 53.8, 53.4, 50.4, 30.9, 27.9 (d, J = 3 Hz), 26.6. ESI-MS $(M + H)^+$ m/z 471.0. HR ESI-MS $(M + H)^+$ m/z = 471.2046 (calcd for $C_{24}H_{28}N_4O_5F$: 471.2043); t_R = 0.71.

(*S*)-1-((2*S*,5*R*)-5-(((6,7-Dihydro-[1,4]oxathiino[2,3-*c*]-pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (34d). Starting from **30c** (0.053 g, 0.16 mmol) and **25** (0.030 g, 0.16 mmol), **34d** (0.03 g, 37% yield) was prepared according to the general procedure E. Off-white solid; mp = 165 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, J = 8.8 Hz, 1 H), 7.61 (dd, J = 6.1, 8.9 Hz, 1 H), 7.33 (s, 1 H), 7.19 (t, J = 9.0 Hz, 1 H), 6.88 (d, J = 8.8 Hz, 1 H), 4.64−4.69 (m, 2 H), 4.17 (ddd, J = 2.2, 4.4, 10.9 Hz, 1 H), 4.08 (s, 3 H), 3.98−4.05 (m, 2 H), 3.46 (ddd, J = 1.6, 3.6, 13.8 Hz, 1 H), 3.38 (ddd, J = 1.8, 8.5, 13.8 Hz, 1 H), 3.32 (ddd, J = 2.0, 4.8, 11.4 Hz, 1 H), 3.21−3.25 (m, 2 H), 3.15 (t, J = 10.5 Hz, 1 H), 2.74 (tt, J = 4.1, 11.1 Hz, 1 H), 2.20 (m, 1 H), 1.84 (m, 1 H), 1.74 br s, 2 H), 1.67 (m, 1 H), 1.34 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.7, 161.7 (d, J = 246 Hz), 159.9, 156.8, 146.5 (d, J = 9 Hz), 139.4, 127.2 (d, J = 11 Hz), 125.7, 125.2, 121.9, 120.7 (d, J = 16 Hz), 113.8 (d, J = 27 Hz), 111.9 (d, J = 3 Hz), 80.3, 74.3, 72.6, 66.2, 53.8, 53.5, 50.2, 30.9, 27.9 (d, J = 2 Hz), 26.6, 25.6. ESI-MS $(M + H)^+$ m/z 487.0. HR ESI-MS $(M + H)^+$ m/z = 487.1815 (calcd for $C_{24}H_{28}N_4O_4FS$: 487.1815); t_R = 0.73.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details on synthesis and characterization of compounds **9**, **12a** and **12b**, **27c**, 1-*epi*-**32a**, and 1-*epi*-**32d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

[+]W.K. and C.H. retired on December 31, 2012.

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■ ABBREVIATIONS USED

NBTI, novel (nonfluoroquinolone) bacterial type II topoisomerase inhibitor; QR, quinolone-resistant; QS, quinolone-sensitive; QTcBZ, Bazett-corrected QT interval (= QT/$\sqrt{RR}$); CIP, ciprofloxacin; LZD, linezolid; MXF, moxifloxacin; LHS, bicyclic aromatic left-hand side; RHS, aromatic right-hand side (as positioned in Figure 2); CDI, 1,1′-carbonyl diimidazole; CFU, colony forming unit; RIA, relaxation inhibitory activity; SCIA, supercoiling inhibitory activity; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate

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