Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01831 • Publication Date (Web): 13 Apr 2017

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Synthesis and Characterization of Tetrahydropyran-Based Bacterial Topoisomerase Inhibitors with Antibacterial Activity Against Gram-Negative Bacteria

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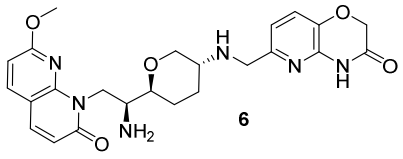
ABSTRACT

There is an urgent unmet medical need for novel antibiotics that are effective against a broad range of bacterial species, especially multi-drug resistant ones. Tetrahydropyran-based inhibitors of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) display potent activity against Gram-positive pathogens and no target-mediated cross-resistance with fluoroquinolones. We report our research efforts aimed at expanding the antibacterial spectrum of this class of molecules towards difficult-to-treat Gram-negative pathogens. Physicochemical properties (polarity and basicity) were considered to guide the design process. Dibasic tetrahydropyran-based compounds such as **6** and **21,** are potent inhibitors of both DNA gyrase and topoisomerase IV, displaying antibacterial activities against Gram-positive and Gram-negative pathogens (*Staphylococcus aureus*, *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Compounds **6** and **21** are efficacious in clinically relevant murine infection models.



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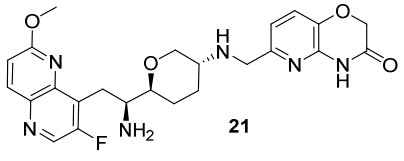

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INTRODUCTION

Antimicrobial resistance has been recognized as a major threat to human health on a worldwide scale.[1] Of particular concern are multi-drug resistant (MDR) Gram-negative bacteria (GNB) that cause an increasing number of infections no longer treatable with available chemotherapies.[2] Despite financial incentives to promote the discovery and development of new antibiotics,[3] the number of antimicrobials that were FDA-approved or that are in advanced clinical development (phase 2 or 3) for the treatment of Gram-negative infections has remained dramatically low.[4] With rare exceptions,[5] all of these anti-GNB drugs act only on a handful of well characterized targets[6] and belong to old classes discovered more than fifty years ago (β-lactams, tetracyclines, fluoroquinolones and aminoglycosides). Therefore, cross-resistance issues that limit their bacterial coverage and clinical utility, are widespread. To minimize these challenges and address the growing medical need, truly novel antibacterial drugs that act via a novel mechanism of action, inhibiting either an unexploited target or addressing a novel site on a validated one,[7] must be discovered.

As many of the targeted cellular pathways components are located in the bacterial cytoplasm, antibacterial drug discoverers have to manage to pair target potency with sufficient cytoplasmic drug accumulation. While broad on-target activity can be assessed at an early phase of the discovery process considering target site conservation across bacterial species, predicting accumulation of a drug in bacteria and especially in GNB remains elusive. Indeed, the Gram-negative cell envelope differs largely from the one of Gram-positive bacteria (GPB), limiting drastically the accessibility of the cell interior to chemicals.[8] Additionally, Gram-negatives have a higher propensity to actively expel antibiotics than GPB, as a result of a wider array of efflux pumps. These two mechanisms that preclude drug accumulation and decrease susceptibility of GNB,[9] are intimately related to the physicochemical properties of antibiotics.[10,11] Careful monitoring and tuning of these physicochemical properties is required to increase accumulation in GNB and enable broad antibacterial coverage.

Among the few biological targets amenable to broad antibacterial activity are DNA gyrase and topoisomerase IV (Topo IV). These two type II bacterial DNA topoisomerases are heterotetramers of

$GyrA_2GyrB_2$ (DNA gyrase) and $ParC_2ParE_2$ (Topo IV) in *Escherichia coli* (*E. coli*), which maintain DNA in a proper topological state during DNA replication and transcription.[12,13] DNA topoisomerase function is blocked by fluoroquinolones (FQs) that have been in clinical use since the 1960's for the treatment of a broad range of infections, including those caused by GNB (e.g urinary tract infections due to *E. coli*). Several structurally unrelated classes of DNA topoisomerase inhibitors acting at binding sites different from the one of FQs have been reported,[14] but only few of them display broad antibacterial activity. Among those, pyrazole derivative **1** is a potent and dual DNA topoisomerase inhibitor, with antibacterial activity against both GPB and GNB, while displaying no cross-resistance with FQs.[15,16]

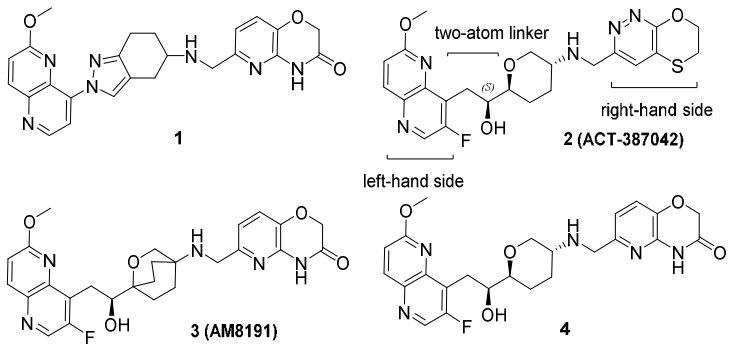


Figure 1: Chemical structures of some representative non-fluoroquinolone bacterial topoisomerase inhibitors.

In previous disclosures, we have detailed the discovery and optimization work conducted on novel (non-fluoroquinolone) bacterial topoisomerase inhibitors (NBTIs) centered around a tetrahydropyran core (henceforth dubbed THP-based NBTIs).[17] This work led to the selection of compound **2** (ACT-387042) as a preclinical development candidate for the treatment of infections caused by Gram-positive pathogens.[18,19] Subsequently, related oxabicyclooctane-linked NBTIs, such as **3** (AM8191) have also been described as broad spectrum antibacterial agents.[20] Here, we report our efforts aimed at the discovery of THP-based NBTIs with an expanded antibacterial spectrum covering some of the most problematic GNB in the clinic, *i.e* carbapenem-resistant *Enterobacteriaceae* (CRE), MDR *Pseudomonas aeruginosa* (*P. aeruginosa*) and MDR *Acinetobacter baumannii* (*A. baumannii*), while keeping an acceptable pharmacological and safety profile.[21,22]

To gain some insights into the relationship that exists between biochemical potency on DNA topoisomerases and antibacterial activity against GNB, we determined the inhibitory profile of our preclinical candidate **2** and its analogs **4**[17] against DNA gyrase and Topo IV enzymes isolated from wild-type *E. coli, A. baumannii* and *P. aeruginosa*. Antibacterial activity against antibiotic-susceptible *E. coli* and *P. aeruginosa* strains and an FQ-resistant *A. baumannii* strain (due to mutations in the quinolone-resistance determining regions of GyrA and Par C) were determined. Ciprofloxacin (CIP), a FQ with potent anti-GNB activity was used as a reference although its mode of action (poisoning of DNA topoisomerases) differs from the one of NBTIs (catalytic inhibitors).[23]

Table 1: Antibacterial and topoisomerases activities of CIP, NBTIs **2** and **4**

	MIC (μg/mL)			Gyrase SCIA (IC_{50}, μM)			TopoIV RIA (IC_{50}, μM)		
	Ec[a]	*Ab*[b]	*Pa*[c]	*Ec*[d]	*Ab*[e]	*Pa*[f]	*Ec*[d]	*Ab*[e]	*Pa*[f]
2	>8	2	>8	8	2	0.5	0.125	0.125	0.125
4	0.5	0.125	4	0.125	0.125	0.125	0.03	0.125	0.125
CIP	≤0.03	>8	0.25	0.5	2	0.5	8	32	8

[a] *Escherichia coli* ATCC 25922. [b] *Acinetobacter baumannii* T6474 containing a Ser83-Leu mutation in GyrA as well as a Ser80-Leu mutation in the ParC. [c] *Pseudomonas aeruginosa* ATCC 27853. [d] wild-type (wt) *Escherichia coli*. [e] wt *Acinetobacter baumannii*. [f] wt *Pseudomonas aeruginosa*.

As indicated in Table 1, DNA gyrases of GNB were ~16-fold more sensitive to CIP than Topo IV enzymes. With IC_{50}s = 0.5 μM against *E. coli* and *P. aeruginosa* DNA gyrases, CIP exhibits a potent antibacterial activity against wild-type *E. coli* and *P. aeruginosa* strains. The wild-type *A. baumannii* DNA gyrase enzyme was also inhibited by CIP (IC_{50} = 2 μM). As expected, the *A. baumannii* T6474 strain was resistant to CIP (MIC > 8 μg/mL). NBTI **2** featuring an oxathiino-pyridazine based right-hand side (RHS) displayed potent inhibitory activity against all Gram-negatives Topo IV enzymes (0.125 μM), whereas weaker inhibitory potencies against DNA gyrase enzymes were measured, ranging from 0.5 μM (*P. aeruginosa*) to 8 μM (*E. coli*). In contrast to CIP, the enzymatic inhibition profile of **2** did not result in antibacterial activity in vitro, except in case of *A. baumannii* that was susceptible to **2** (MIC = 2 μg/mL). On the other hand, pyrido-oxazinone-based compound **4** displayed potent, dual and balanced inhibition of all DNA topoisomerases isolated from GNBs, that tracked well with good MICs measured against Gram-negative *E. coli* and *A. baumannii* (MICs = 0.5 and

0.125 μg/mL respectively). Despite its better inhibitory potency on isolated *P. aeruginosa* DNA topoisomerases than CIP, NBTI **4** exhibited a weaker whole-cell activity against *P. aeruginosa* (MIC = 4 μg/mL). The noticeable difference in susceptibility observed for **4** against *P. aeruginosa* in comparison to other GNB tested, was attributed to insufficient cytoplasmic accumulation in this particular organism. Because of its broad, potent inhibitory and antibacterial activities as well as its lack of cross-resistance with FQs, compound **4** was selected as a starting point.

DESIGN HYPOTHESIS

Our working hypothesis to optimize anti-GNB activity of proprietary THP-based NBTIs was based on observations made by O'Shea and Moser that anti Gram-negative agents are generally more polar (lower *c*log$D_{7.4}$ values) and more positively charged at pH 7.4 than agents active against Gram-positive pathogens only.[10] Therefore, we sought analogs of NBTI **4** with increased polarity hoping that drug accumulation and, consecutively susceptibility of GNB (and in particular *P. aeruginosa*) to THP-based NBTIs will improve. The pyrido-oxazinone RHS, resulting in dual and potent DNA topoisomerase inhibition was prioritized throughout the course of our study. Putting aside oral absorption considerations,[24] we first envisioned to increase the polarity of THP-based NBTIs by designing molecules that would be more protonated at physiological pH. As indicated in Figure 2, with a calculated pK_a (*c*pK_a) of 7.6 for the secondary amine, about 50% of NBTI **4** should be protonated at pH 7.4. Taking into account the constraints set by the specific binding mode of NBTIs (see thereafter, Figure 3), an option to render **4** more polar was to swap the hydroxyl group present on the two-atom linker (that connects the THP-core to the left-hand side (LHS), Figure 1) for an amino group, creating molecules with a dibasic character. This change would likely have little influence on the binding affinity of the inhibitor but derivatives **11** and **16** with a -$C_\alpha H(NH_2)$-CH_2- or a -CH_2-$C_\beta H(NH_2)$- linker respectively, are seemingly more polar with a *c*log$D_{7.4}$ around -1.5 (compared to a *c*log$D_{7.4}$ =0.24 for **4)** (Figure 2). As the *c*pK_a for the primary amine group present in compounds **11** and **16** reached 9.3, a charged species was expected to be their major component at pH ~7.4.

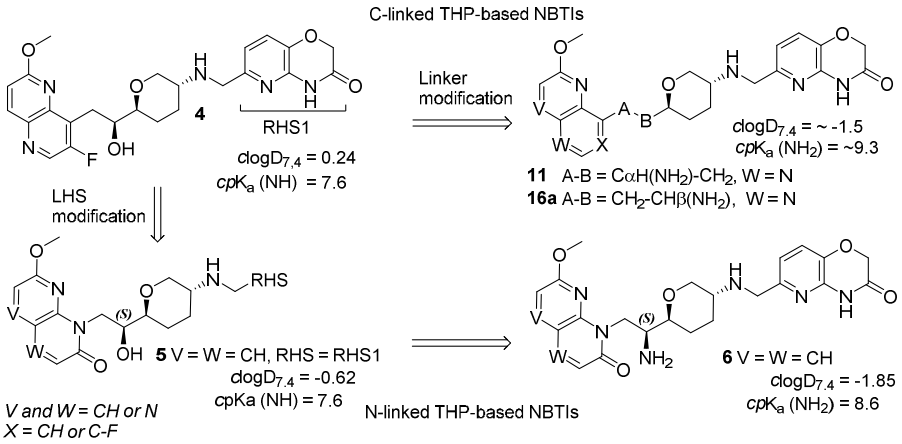


Figure 2: Envisioned structural modifications of NBTI **4** resulting in NBTIs with increased polarity

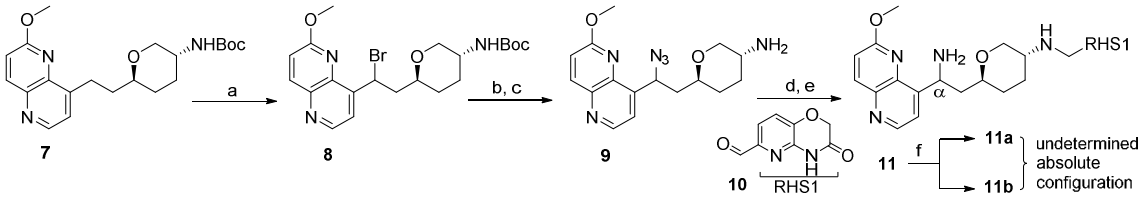
We also envisioned to increase polarity of our series by utilizing naphthyridinones or analogous LHS, attached to the THP-core from a N-atom through a -CH_2-CH(OH)- linker. Even though the polarity of the resulting structures increased in a limited extent (decreased of *c*log$D_{7.4}$ from 0.24 for **4** to -0.62 for **5**) and basicity remained constant (*cp*K_a = 7.6), the construction of such novel molecules via epoxide opening was an attractive prospect. It indeed provided a rapid entry point to novel entities and an opportunity to subsequently reach dibasic entities featuring as well a -CH_2-C_βH(NH_2)- type linker. Thus, dibasic derivatives such as derivative **6** had a marked increase in polarity (*c*log$D_{7.4}$ = -1.85), being mostly present as charged species at pH 7.4 (with a *cp*K_a = 8.6 for the primary amine). NBTI **11** featuring a -C_αH(NH_2)-CH_2- linker was synthesized from known compound **7**[17] following Scheme 1.

CHEMISTRY

Bromination of compound **7** at benzylic position was performed in refluxing carbon tetrachloride using *N*-bromosuccinimide and azobisisobutyronitrile as a radical initiator, leading to bromide **8** as a mixture of epimers. Treatment with sodium azide (NaN_3) in hot dimethylformamide (DMF) and subsequent removal of the *tert*-butyloxycarbonyl (Boc) group using trifluoroacetic acid (TFA) afforded free amine **9**. The latter, upon reaction with aldehyde **10** in MeOH, led to an imine that was further reduced with sodium borohydride. The resulting azide derivative was finally reduced using polymer-bound triphenylphosphine (PPh_3) in aqueous tetrahydrofuran (THF) to afford NBTI **11**, isolated as an equimolar mixture of C_α-epimers. Chiral HPLC separation was performed, leading to

both **11a** and **11b** (for details see Supporting Information). The absolute stereochemistry of the C_α (carbon bearing the primary amine) of both epimers was not assigned.

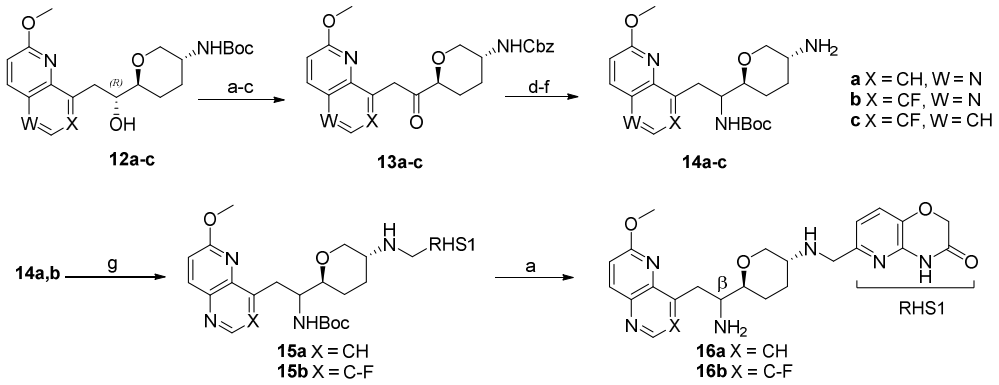
Scheme 1[a]



[a]Reagents: (a) NBS, AIBN, CCl_4, 80°C (b) NaN_3, DMF, 80°C (c) TFA, rt (d) **10**, MeOH, rt then $NaBH_4$, 0°C (e) polymer-bound PPh_3, THF-H_2O, 60°C (f) chiral HPLC separation.

THP-based NBTIs **16a,b** and **20-23** featuring a -CH_2-C_βHNH_2- linker were obtained as depicted in Schemes 2 and 3, from compounds **12a-c**.[18] N-Boc groups were cleaved using TFA, and resulting free amines were treated with benzyl chloroformate (Cbz-Cl) in presence of aqueous sodium bicarbonate ($NaHCO_3$). Subsequent oxidation of secondary alcohols using Parikh-Doering conditions (combining sulfur trioxide-pyridine complex, dimethylsulfoxide and *N*,*N*-diisopropylethylamine (DIPEA)) in dichloromethane (DCM) led to ketones **13a-c**. A reductive amination reaction, performed with ammonium acetate and sodium cyanoborohydride gave rise to free amines that were further N-Boc protected upon treatment with di-*tert*-butyl carbonate (Boc_2O). Removal of the benzyloxycarbonyl (Cbz) protecting groups under normal hydrogen atmosphere in presence of 20% palladium hydroxide on carbon (20% $Pd(OH)_2$ on C) as a catalyst afforded free amines **14a,c**. Subsequently, amines **14a,b** were reductively aminated using aldehyde **10** to afford compounds **15a,b** as equimolar mixtures of C_β-epimers. Intermediates **15a,b** were treated with TFA to afford NBTIs **16a,b**, as equimolar mixtures of C_β-epimers.

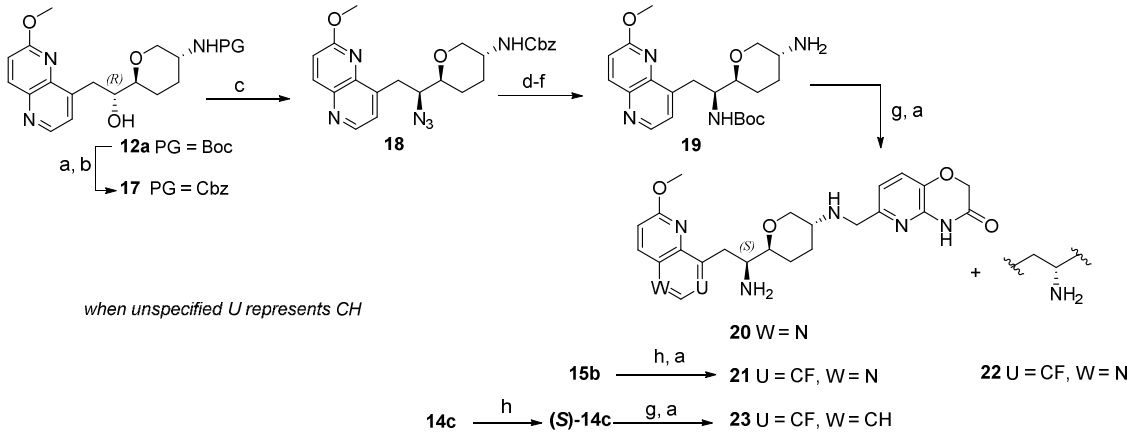
Scheme 2[a]



[a]Reagents: (a) TFA, rt (b) Cbz-Cl, $NaHCO_3$, EtOAC-H_2O, rt (c) Pyr.SO_3, DIPEA, DMSO, DCM, 0°C to rt, (d) NH_4OAc, $NaBH_3CN$, MeOH-DCM, rt (e) Boc_2O, DCM, rt (f) H_2, 20% $Pd(OH)_2$ on C, EtOAc-MeOH, rt (g) **10**, 3Å MS, DCE-MeOH, 50°C then $NaBH_4$, 0°C.

As described in Scheme 3, the asymmetric synthesis of **20**, the (*S*)-configured epimer of **16a** was prioritized over its (*R*)-epimer based on our previous findings.[18] Starting from alcohol **12a**, N-Boc protecting group was exchanged to N-Cbz via a deprotection (TFA) - protection (Cbz-Cl, $NaHCO_3$) sequence to afford alcohol **17**.

Scheme 3[a]

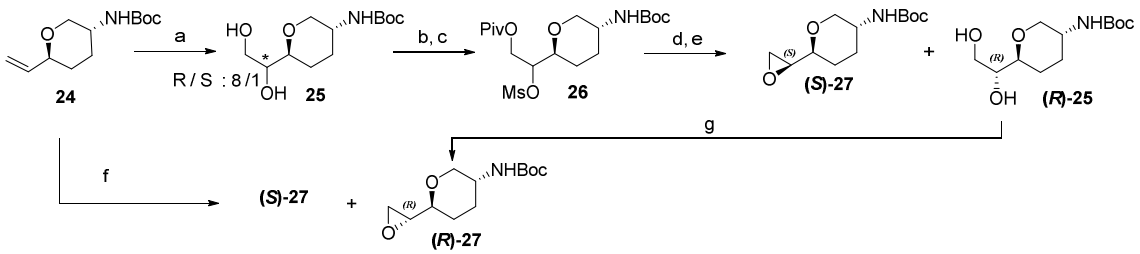


[a]Reagents: (a) TFA, rt (b) Cbz-Cl, $NaHCO_3$, $EtOAc-H_2O$, rt (c) DPPA, DIAD, PPh_3, THF, rt (d) PPh_3, aq. THF, 60°C (e) Boc_2O, DCM, rt (f) H_2, 20% $Pd(OH)_2$ on C, EtOAc-MeOH, rt (g) **10**, MS 3Å, DCE-MeOH, 50°C then $NaBH_4$, 0°C (h) chiral HPLC separation.

Gratifyingly, when treated with diphenyl phosphoryl azide (DPPA) under typical Mitsunobu reaction conditions (combining PPh_3 and diisopropyl azodicarboxylate (DIAD)), alcohol **17** afforded azide **18** as a single (*S*)-configured enantiomer. Subsequent Staudinger reduction (PPh_3 in aqueous THF) of azide **18**, Boc-protection (Boc_2O) and hydrogenolysis (H_2, 20% $Pd(OH)_2$on C) of the remaining N-Cbz group led to intermediate **19**. The latter was transformed to corresponding NBTI **20** via reductive

amination reaction employing aldehyde **10** and Boc deprotection (TFA). Unfortunately, this sequence did not lead to analogous NBTIs **21** and **23,** as attempted Mitsunobu reactions on alcohols **12b,c** or their N-Cbz protected analogs (not shown) failed, returning only elimination products. Therefore, previous intermediate **15b** was subjected to a chiral HPLC separation, and resulting C_β epimers were N-Boc deprotected (TFA), giving rise to NBTIs **21** and **22**. Considering that the ^{1}H NMR spectrum recorded for **20** showed an excellent fit with the one of compound **21** (comparatively to the ^{1}H NMR spectrum obtained for **22**),[25] the absolute stereochemistry of the carbon bearing the primary amine in compound **21** was similarly assigned to (*S*). Intermediate **14c** was subjected to chiral HPLC separation to afford (*S*)-**14c**.[26] Latter amine was alkylated via a reductive amination with aldehyde **10** and treated with TFA, affording NBTI **23**.

Scheme 4[a]

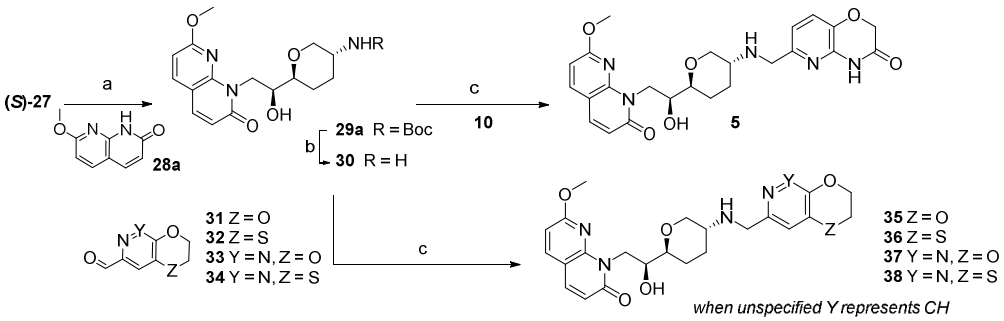


[a]Reagents: (a) AD-mix-α, *t*BuOH, 0°C (b) Piv-Cl, DMAP, DCM, 0°C (c) Ms-Cl, TEA, 0°C (d) MeONa, MeOH, rt (e) cat. (*R*,*R*)-salen-Co(III)-OTs, H_2O (20 mol%), rt (f) *m*-CPBA, $NaHCO_3$, DCM, rt (g) $MeC(OMe)_3$, *p*TsOH, DCM, rt then TMS-Cl, DCM, rt then MeONa, MeOH, rt.

The asymmetric synthesis of N-linked THP-based NBTIs relies on chiral epoxides (*R*)-**27** and (*S*)-**27**. As illustrated in Scheme 4, alkene **24** easily available in large amounts[18] was chosen as a starting material. Direct epoxidation with *m*-CPBA gave rise to a mixture of (*R*) and (*S*)-epimers of epoxide **27**. Both epimers could be separated by tedious chromatography to afford (*R*)-**27** and (*S*)-**27** in 21 and 29% yield, respectively. Sharpless asymmetric dihydroxylation of alkene **24** using commercial AD-mix-β gave rise to diol **25** in a low diastereoselectivity (de ~30%) in favor of the enantiomer (*S*)-**25**. On the other hand, the use of commercial AD-mix-α returned diol **25** with an acceptable level of diastereoselectivity (de ~70%) in favor of the enantiomer (*R*)-**25**. Diol **25** was then treated with pivaloyl chloride (Piv-Cl) in presence of DMAP to cleanly mask the primary alcohol. Subsequent reaction with methanesulfonyl chloride (Ms-Cl) in presence of triethylamine (TEA) led to compound

26. Upon treatment with sodium methoxide in methanol, **26** formed the intermediate primary sodio alcoholate that displaced via a clean S_N2 reaction the adjacent mesylate, closing the epoxide **27**, finally isolated as a 8 to 1 mixture of epimers. The minor epimer (*R*)-**27** was converted back to diol (*R*)-**25** via its selective hydrolysis using (*R*,*R*)-Jacobsen's catalyst,[27] thereby allowing the isolation of enantiomerically pure epoxide (*S*)-**27**. Diol (*R*)-**25** was transformed to epoxide (*R*)-**27** using the one-pot methodology developed by Sharpless[28] (for experimental details see Supporting Information).

Scheme 5[a]



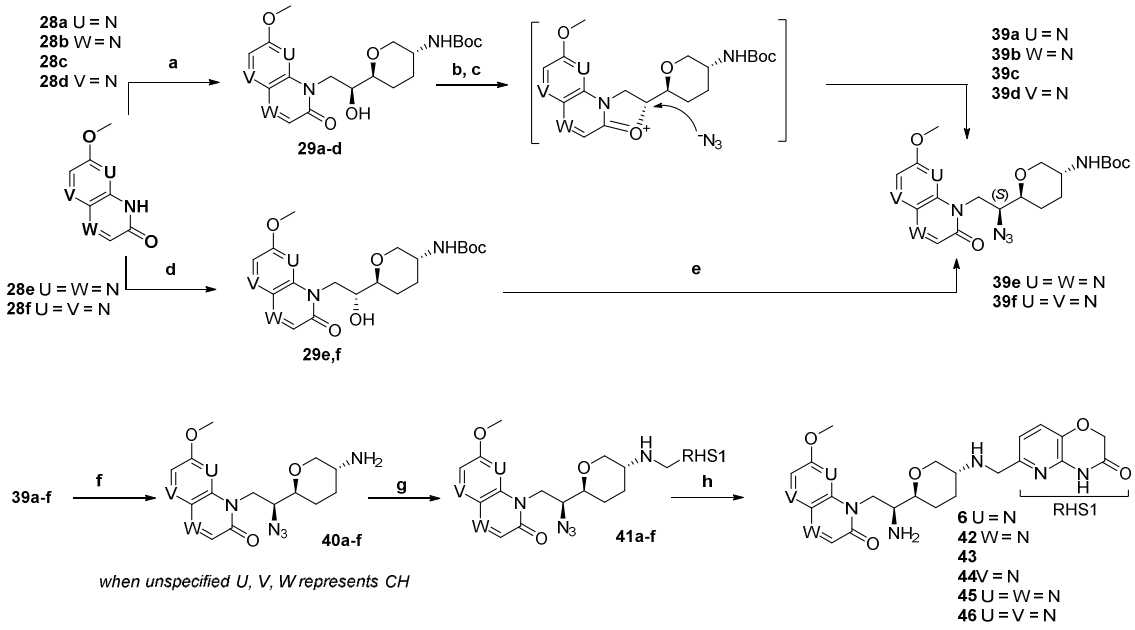
[a]Reagents: (a) Cs_2CO_3, DMF, 80°C (b) TFA, rt (c) **10** or **31-34**, 3Å MS, DCE-MeOH, 50°C, then $NaBH_4$, 0°C.

As shown in Scheme 5, epoxide (*S*)-**27** and naphthyridinone **28a**[29] were reacted together in hot DMF in presence of cesium carbonate to afford alcohol **29a**. After Boc deprotection using TFA, the resulting amine **30** was subjected to reductive amination reactions with aldehydes **10** or **31-34**, giving rise to NBTIs **5** or **35-38**, respectively.

As shown in Scheme 6, the synthesis of NBTIs **6** and **42-44** started from alcohols **29a-d** obtained by opening of epoxide **(*S*)-27** by naphthyridinones **28a,b**, quinolinone **28c**, or naphthyridinone **28d**, respectively, using cesium carbonate in hot DMF. Alcohols **29a-d** were transformed to their corresponding mesylates by treatment with Ms-Cl in presence of TEA. The latter intermediates reacted with NaN_3 in hot DMF gave rise to azides **39a-d**. The nucleophilic substitution reaction took place with an overall net retention of configuration as the intramolecular formation of an oxonium species occurs faster than the subsequent backside attack by the azide anion. However, this sequence failed in delivering azides **39e,f**. Therefore, the synthesis of NBTIs **45-46** had to start from alcohols **29e,f** obtained in this instance by opening of epoxide (*R*)-**27** by pyrido-pyrazinones **28e,f** respectively using potassium carbonate in hot DMF. A subsequent Mitsunobu-type reaction (DIAD, PPh_3) using DPPA

as an azide source was performed on (*R*)-diols **29e,f**, returning (*S*)-configured azides **39e,f** in low yields. Azides **39a-f** were Boc-deprotected under acidic conditions, affording amines **40a-f**, which after reductive amination with aldehyde **10** gave rise to intermediates **41a-f**. Staudinger reduction led to NBTIs **6** and **42-46**, isolated as single diastereomers.[30]

Scheme 6[a]

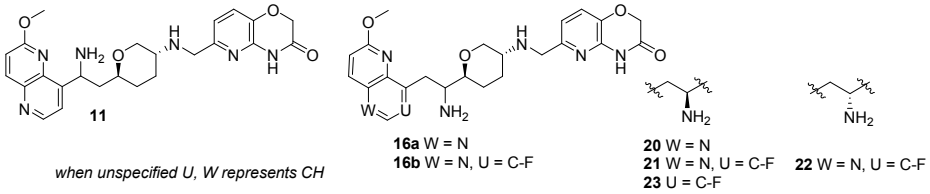


[a]Reagents: (a) **(*S*)-27**, Cs_2CO_3, DMF, 80°C (b) Ms-Cl, TEA, DCM, 0°C (c) NaN_3, DMF, 80°C (d) **(*R*)-27**, K_2CO_3, DMF, 80°C (e) DPPA, DIAD, PPh_3, THF, rt (f) TFA, rt (g) **10**, 3A MS, DCE-MeOH, 50°C, then $NaBH_4$, 0°C (h) PPh_3, THF-H_2O, 50°C.

RESULTS AND DISCUSSION

For all novel chemical entities, antibacterial activity, expressed as MIC (minimal inhibitory concentration, [μg/mL]), was measured against a panel of bacterial strains including drug-susceptible *Staphylococcus aureus* (*S. aureus*), *E. coli,* and *P. aeruginosa* strains and a quinolone-resistant *A. baumannii*. Inhibition of DNA gyrase and Topo IV enzymes isolated from wild-type *E. coli, A. baumannii* and *P. aeruginosa* was determined.

Table 2: Antibacterial and topoisomerases activities of NBTIs **2**, **11**, **16a,b** and **20-23**



	MIC (μg/mL)				Gyrase SCIA (IC_{50}, μM)			TopoIV RIA (IC_{50}, μM)		
	Sa[a]	***Ec***[b]	***Ab***[c]	***Pa***[d]	***Ec***[e]	***Ab***[f]	***Pa***[g]	***Ec***[e]	***Ab***[f]	***Pa***[g]
2	≤0.03	>8	2	>8	8	2	0.5	0.125	0.125	0.125
11	2	1	8	8	8	2	0.5	0.125	0.125	0.125
16a	0.125	0.125	0.5	2	0.5	0.5	0.5	0.5	0.5	0.5
16b	0.03	0.25	0.25	4	0.125	0.125	0.03	0.03	0.03	0.03
20	0.06	0.125	0.25	0.5	0.125	0.125	0.125	0.03	0.03	0.03
21	0.03	0.125	0.125	1	0.125	0.125	0.03	0.03	0.125	0.03
22	1	1	1	>8	0.5	0.5	0.125	0.125	0.125	0.125
23	0.03	≤0.03	≤0.03	1	0.03	0.03	0.03	≤0.01	0.03	0.03

[a] *Staphylococcus aureus ATCC 29213.* [b] *Escherichia coli* ATCC 25922. [c] *Acinetobacter baumannii* T6474. [d] *Pseudomonas aeruginosa* ATCC 27853. [e] wild-type (wt) *Escherichia coli* enzyme. [f] wt *Acinetobacter baumannii* enzyme. [g] wt *Pseudomonas aeruginosa* enzyme.

Our investigations on dibasic C-linked THP-based NBTIs started with the characterization of compound **11**, built on a -$C_{\alpha}H(NH_2)$-CH_2- linker. As shown in Table 2, compound **11**, a mixture of two epimeric amines at the benzylic position, displayed an inhibitory profile similar to NBTI **2**, with potent activities against Topo IV enzymes isolated from Gram-negatives (~0.125 μM), and more scattered activities against DNA gyrase enzymes with IC_{50}s ranging from 0.5 to 8 μM. However, in contrast to NBTI **2**, compound **11** showed a promising MIC of 1 μg/mL against wild-type *E. coli*. Despite potent inhibition of *A. baumannii* and *P. aeruginosa* Topo IV enzymes, limited antibacterial activity against *A. baumannii* and *P. aeruginosa* strains was observed with **11** (MIC = 8 μg/mL). The antibacterial activity of **11** against *S. aureus* was also weaker than expected (MIC = 2 μg/mL). Both epimers **11a** and **11b** showed activities comparable to **11**, behaving as dual but unbalanced DNA gyrase-Topo IV inhibitors (data not shown). Naphthyridine-based derivatives **16a** (U = CH) and **16b** (U = CF), featuring -CH_2-$C_{\beta}H(NH_2)$- linkers were also evaluated as mixtures of two epimeric amines. Both **16a** and **16b** elicited dual and balanced inhibitory activity against DNA gyrases and Topo IV

enzymes. DNA topoisomerase inhibition for all species was tightly distributed in comparison to those observed for **11**, with IC_{50}s for **16a** and **16b** ranging between 0.03 μM and 0.5 μM. The slightly more potent DNA topoisomerase inhibition observed for compound **16b** in comparison to **16a** did not translate into a better antibacterial activity. Both compounds **16a** and **16b** inhibited growth of *P. aeruginosa* with MICs = 2 and 4 μg/mL respectively. For the first time in the series, we could observe decent anti-pseudomonal activities for biochemically potent compounds. Compounds **20** and **21**, (*S*)-configured epimers at C_β (the carbon bearing the primary amine), present in compound **16a** and **16b**, show potent, dual and balanced inhibitory activities against all DNA topoisomerases with IC_{50}s within a narrow range (0.03 to 0.125 μM). Gratifyingly, exquisite antibacterial activities against *S. aureus*, *E. coli* and *A. baumannii* (all MICs < 0.25 μg/mL) as well as good antibacterial activity against *P. aeruginosa* were measured for compounds **20** and **21,** with MICs = 0.5 and 1 μg/mL, respectively. The (*R*)-configured epimer **22** was found to be a >10-fold less potent antibiotic against Gram-negative strains than **21.** This could in part be due to its 4-fold lower inhibitory potency against DNA topoisomerases compared to **21**. Compound **23** featuring a fluoroquinoline LHS displayed exquisite inhibitory activity against DNA topoisomerases and good antibacterial activity again *P. aeruginosa*. From this limited dataset, we could establish that the (*S*)-configured -CH_2-$C_\beta H(NH_2)$- linker shows superior coverage of both Gram-positive and Gram-negative bacteria as compared to the (*R*)-configured linker and -$C_\alpha H(NH_2)$-CH_2- linkers. We could also confirm the absence of cross-resistance with FQs in this sub-series as demonstrated with good MICs measured against a FQ-resistant *A. baumannii* strain. Analogues of NBTIs **20** or **21**, featuring dioxino- or oxathiino-pyridine (or pyridazine) RHS were also prepared but displayed MICs ≥ 8 μg/mL against *P. aeruginosa* despite potent DNA topoisomerases inhibition (see Supporting Information, table S-9).

To understand if cytoplasmic accumulation drives the better cellular activity of NBTIs featuring the (*S*)-configured -CH_2-$C_\beta H(NH_2)$- linker and a pyrido-oxazinone RHS over their -CH_2-CH(OH)- analogs, compounds **4** and **21** sharing a similar topoisomerase inhibitory profile on *E. coli* enzymes, were chosen for MIC determination against a panel of genetically defined *E. coli* mutants. These *E. coli* mutants possess either a decreased ability to expel compounds (*tolC* and *acrAB* knockout

mutants) or an increased permeability to lipophilic substances due to a truncated lipopolysaccharide (LPS) layer (rfaC).

Table 3: Antibacterial activities of compounds **4** and **21** against a panel of genetically modified strains

	log$D_{7.4}$	*E.coli* wt[a]	*E.coli* Δ*rfaC*[b] *Permeability* ↑		*E.coli* Δ*tolC*[c] *Efflux* ↓;		*E.coli* Δ*acrAB*[d] *Efflux* ↓	
		MIC[e]	MIC[e]	ΔMIC[f]	MIC[e]	ΔMIC[f]	MIC[e]	ΔMIC[f]
4	2.0	0.5	0.031	↓, 16 x	0.004	↓, 100 x	0.004	↓, 100 x
21	0.9	0.063	0.031	↓, 2 x	0.008	↓, 8 x	0.008	↓, 8 x

[a] *E. coli* MG1655, wild-type strain. [b] Derivative of *E. coli* MG1655 with truncated LPS. [c] Derivative of *E. coli* MG1655 lacking the efflux system TolC of the outer membrane. [d] Derivative of *E. coli* MG1655 lacking the AcrAB efflux system of the cytoplasmic membrane. [e] MIC in μg/mL. [f] Fold change compared to MIC measured on wild-type strain.

Compound **4** displayed good antibacterial activity against wild-type *E. coli*, but its amine-containing analog compound **21,** was still 8-fold more potent with a MIC of 0.063 μg/mL (Table 3). Firstly, the marked potency difference observed against wild-type strains between compounds **4** and **21** , was absent with an *E. coli* permeable mutant as MICs measured for both compounds were identical (0.031 μg/mL). Therefore ΔMIC for compound **4** is significantly larger (16-fold difference) than the one observed for compound **21** (2-fold change) and, in contrast to compound **4**, compound **21** retains more of its activity when an intact outer membrane (OM) is present. Secondly, there was only a little difference in potency against *E. coli* strains with impaired efflux systems (MICs of 0.008 and 0.04 μg/mL, respectively). ΔMIC for compound **4** (100-fold difference) was again larger with these pairs of strains than the one observed for compound **21** (8-fold change), suggesting that compound **21** is less affected by efflux via the AcrAB-TolC system than the corresponding hydroxyl analog **4**. As indicated in Table 3, primary amine **21** is more polar ($logD_{7.4} = 0.9$) than its hydroxyl counterpart **4** ($logD_{7.4} = 2.0$). Furthermore, the measured pK_a of the primary amine present in compound **21** was 1-log higher (pK_a = 8.4) than the one of the secondary amine present in both compounds (pK_a = 7.4). Consequently, compound **21** is expected to be mostly protonated (> 90%) at pH 7.4, whereas compound **4** is only 50% charged at this pH. The better entry into the cytoplasm of the GN *E. coli* of dibasic compounds such as **21** may be the result of either specific uptake through porins or self-promoted uptake through the outer membrane of Gram-negative bacteria.[31,32] Overall, the enhanced antibacterial activity of compound **21** on wild-type *E. coli* over its analog **4** can probably be ascribed

to higher cytoplasmic accumulation, resulting from the combination of a better permeability through the cell envelope and a lower propensity to be effluxed.

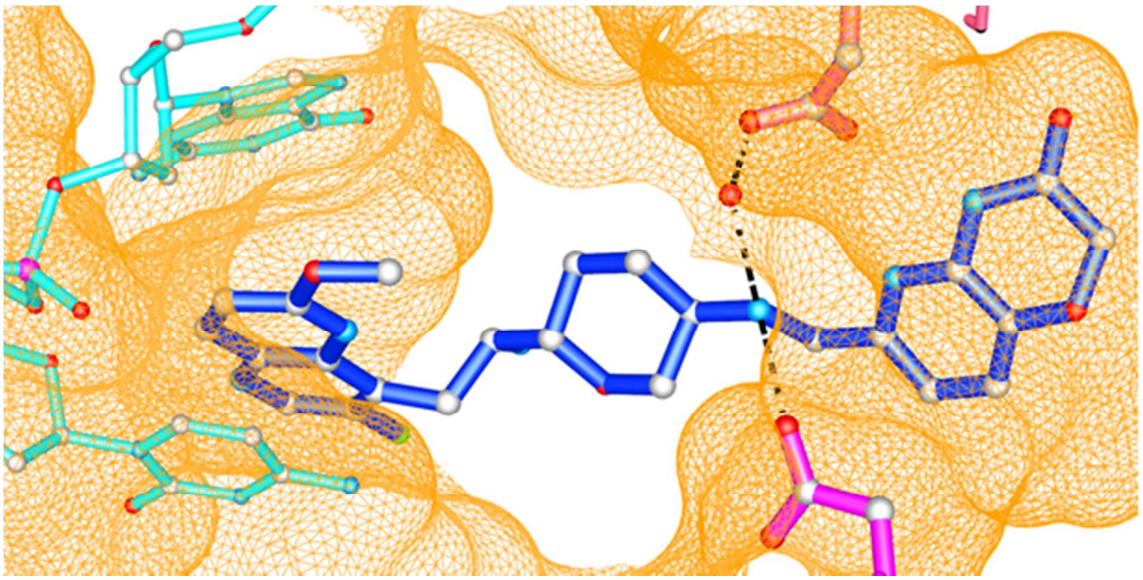
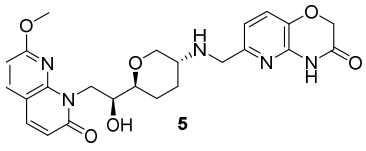


Figure 3: Compound **21** (blue) is docked into the DNA – gyrase dimer. (DNA cyan; chain 1 red; chain 2 magenta). The surface of the active site is mimicked by an orange net. Hydrogen bond interactions are shown in black dotted lines.

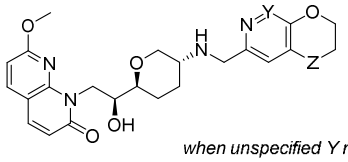
We tried to rationalize the broad enzymatic inhibitory activity of compound **21** by docking it into the X-ray structure of **3** complexed to a dimer of *S. aureus* DNA gyrase A[33] and a 20-bp DNA duplex (PDB accession code: 4PLB, see Supporting Information)[20] using the software Moloc.[34] Molecule **21** runs along the internal C2-symmetry axis of the GyrA-DNA complex with the LHS being intercalated between a GpC pair of base pairs while the RHS is embedded in a cavity located at the interface of the two GyrA monomers. A linker with appropriate length, geometry and flexibility carrying functional groups is connecting these two units. The protonated primary amine present in the linker of **21** improves solubility a lot (>100μg/mL at pH varying from 1 to 9) and increases polarity but has no obvious H-bond partner in the protein DNA complex unlike a hydroxyl group in **3** located in an identical position in the template structure. However, the secondary amine in the linker is in an ideal position to form a strong H-bond with Asp83 of one GyrA monomer and a second water mediated H-bond with the same amino acid of the second protein monomer. The pyrido-oxazinone RHS nicely complements the shape of a hydrophobic pocket establishing strong van der Waals interactions but clearly lacking any direct H-bonds with the protein. All structural features involved in binding of **21**

are present in both DNA gyrase and topoIV and are well conserved across key bacterial species thereby providing the basis for its broad antibacterial activity. This structural insight also provides a rational for the lack of cross-resistance of NBTIs with FQs that bind at a different site.[33]

Table 4: Antibacterial and topoisomerases activities of NBTIs **5, 35-38**.



5



35 Z = O
36 Z = S
37 Y = N, Z = O
38 Y = N, Z = S

when unspecified Y represents CH

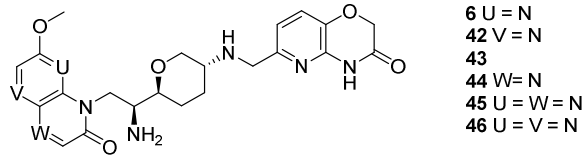
	MIC (μg/mL)				Gyrase SCIA (IC_{50}, μM)			TopoIV RIA (IC_{50}, μM)		
	Sa[a]	***Ec***[b]	***Ab***[c]	***Pa***[d]	***Ec***[e]	***Ab***[f]	***Pa***[g]	***Ec***[e]	***Ab***[f]	***Pa***[g]
5	≤0.03	0.5	≤0.03	8	0.125	0.125	0.125	0.03	0.125	0.03
35	≤0.03	4	0.5	>8	8	0.125	0.125	0.125	0.125	0.125
36	≤0.03	4	0.5	>8	0.5	0.125	0.5	0.03	0.03	≤0.01
37	0.06	>8	>8	>8	32	8	8	2	8	0.5
38	0.031	>8	8	>8	8	2	0.5	0.125	0.5	0.125

[a] *Staphylococcus aureus ATCC 29213.* [b] *Escherichia coli* ATCC 25922. [c] *Acinetobacter baumannii* T6474. [d] *Pseudomonas aeruginosa* ATCC 27853. [e] wild-type (wt) *Escherichia coli* enzyme. [f] wt *Acinetobacter baumannii* enzyme. [g] wt *Pseudomonas aeruginosa* enzyme.

As our working hypothesis seemed to hold some promise, we turned our attention to NBTIs featuring a polar LHS. Thanks to a modular synthesis we had a rapid access to such novel N-linked THP-based molecules built on a -CH_2-CH(OH)- linker. A set of compounds, all featuring the 1,8-naphthyridinone LHS, was synthesized to study within this limited sub-series, the relationship that exists between the RHS and the antibacterial coverage. As illustrated in Table 4, these compounds, *i.e* compounds **5** and **35-38**, displayed good antibacterial activities against *S. aureus* wild-type strains. However, as in the C-linked THP-based series, only compound **5**, featuring a pyrido-oxazinone RHS exhibited activity against the Gram-negative *E. coli* and *A. baumannii,* as a result of dual and balanced inhibitory activity against DNA topoisomerases (IC_{50}s ranged between 0.03 and 0.125 μM). However its antibacterial activity against *P. aeruginosa* remained weak (MIC = 8 μg/mL). Compounds **35** and **36**, featuring a dioxino-pyridine and an oxathiino-pyridine RHS, were also dual DNA gyrase- Topo IV inhibitors, although some variations in IC_{50}s were observed. Both inhibited *A. baumannii* growth potently (MIC = 0.5 μg/mL) but were mostly inactive against *E. coli* and *P. aeruginosa*. Compound

37, featuring a dioxino-pyridazine showed weak to no inhibitory activity against DNA topoisomerases isolated from Gram-negative strains, failing in turn to inhibit GNB growth. Compound **38**, a close analog of our preclinical candidate **2**, shared with it similar enzymatic and antibacterial properties, being only active against *S. aureus* in this panel. The measured $logD_{7.4}$ values for **3** and **5** were identical ($logD_{7.4} = 2.0$). Introduction of a supposed polar LHS did not translate into NBTIs with increased polarity (lower $logD_{7.4}$). Compounds **4** and **5**, with identical inhibitory potency on DNA topoisomerases and similar physicochemical properties, exhibited ultimately a similar antibacterial potency and spectrum. The lack of activity against Gram-negative strains for DNA topoisomerases inhibitors **5**, **35, 36** and **38** could be attributed to a poor cytoplasmic accumulation because, similarly to compound **4**, MICs recorded on *E. coli* mutants with decreasing efflux capability or increased permeability were dramatically improved, when compared to MICs obtained on wild-type *E. coli* strains (see Supporting Information, table S-11).

Table 5: Antibacterial and topoisomerases activities of NBTIs **6, 42-46**.



6 U = N
42 V = N
43
44 W= N
45 U = W = N
46 U = V = N

when unspecified U, V, W represents CH

	MIC (µg/mL)				Gyrase SCIA (IC_{50}, µM)			TopoIV RIA (IC_{50}, µM)		
	Sa[a]	***Ec***[b]	***Ab***[c]	***Pa***[d]	***Ec***[e]	***Ab***[f]	***Pa***[g]	***Ec***[e]	***Ab***[f]	***Pa***[g]
6	0.25	0.25	1	1	0.125	0.125	0.125	0.03	0.125	0.03
42	1	1	4	4	0.5	0.03	0.03	0.03	0.125	0.03
43	0.25	0.25	2	4	0.125	0.125	0.125	0.03	0.125	0.5
44	0.063	0.25	2	4	0.5	0.03	0.03	0.03	0.125	0.03
45	0.125	0.5	2	2	0.5	0.125	0.125	0.125	0.125	0.03
46	0.5	0.5	2	1	0.5	0.125	0.125	0.03	0.03	0.03

[a] *Staphylococcus aureus ATCC 29213.* [b] *Escherichia coli* ATCC 25922. [c] *Acinetobacter baumannii* T6474. [d] *Pseudomonas aeruginosa* ATCC 27853.[e] wild-type (wt) *Escherichia coli* enzyme. [f] wt *Acinetobacter baumannii* enzyme. [g] wt *Pseudomonas aeruginosa* enzyme.

To strengthen our earlier findings on the beneficial effect of increasing polarity by modification of the two-atom linker, a series of dibasic N-linked THP-based molecules was synthetized and evaluated. As

shown in Table 5, compounds **6** and **42-46**, featuring the pyrido-oxazinone RHS and various LHS linked to the (*S*)-configurated -CH_2-$C_\beta H(NH_2)$- motif through a nitrogen atom, were all dual and balanced DNA gyrase and TopoIV inhibitors with activities below 0.5 μM. Gratifyingly, compound **6** displayed good antibacterial activity against *S. aureus* and *E. coli* with MICs = 0.25 mg/L and difficult-to treat Gram-negative pathogens *A. baumannii* and *P. aeruginosa* with MICs = 1 μg/mL. NBTIs **42-46** were not as potent antibiotics as **6** against *P. aeruginosa* and *A. baumannii***,** but consistently displayed MICs ≤ 4 μg/mL against these strains. Despite potent and dual inhibitory activities against *E. coli* DNA topoisomerases, NBTI **42** exhibited a slightly weaker antibacterial activity against wild-type *E. coli* (MICs = 1 μg/mL) than its congeners NBTIs **43-46** (MICs ≤ 0.5 μg/mL).[35] A weaker antibacterial activity against *S. aureus* was also observed for NBTI **42**. Compound **6** exhibits a measured $logD_{7.4}$ of 0.3, significantly lower than that of NBTI **4** ($logD_{7.4}$ = 2.0). A large proportion (>95%) of **6** is positively charged at pH 7.4 as measured pK_as of its primary and secondary amines were 8.7 and 7.0 respectively.[36] The direct comparison of antibacterial activity of compounds **5** and **6** confirmed unambiguously that the amine function present in the linker ameliorates the activity against Gram-negative pathogens,[35] by favoring cytoplasmic accumulation. As demonstrated with compound **21**, MICs recorded for compounds **6** and **42**-**46** on *E. coli* mutants with impaired efflux systems or increased permeability were less improved than those measured with compound **5** on these strains (see ΔMIC in Supporting Information, table S-10). It turned out that in case of THP-based NBTIs featuring a pyrido-oxazinone RHS, our working hypothesis of increasing concomitantly polarity and basicity by addition of an amino group at a position not detrimental for on-target activity, indeed led to significant gain in antibacterial activity against difficult-to-treat GNB, thanks to a sufficient cytoplasmic accumulation.

Preliminary microbiological profile of two representative molecules

Compounds **6** and **21** were selected as representative molecules of the N- and C-linked THP-based series respectively, and were evaluated in vitro against a panel of clinically relevant Gram-positive and Gram-negative organisms, including multidrug resistant ones. This microbiological evaluation was performed to compare the broadness of their antibacterial spectrum and their in vitro potency (MIC data and bacteria resistance phenotypes are detailed in Supporting Information).

As shown in Table 6, compound **21,** featuring a fluoronaphthyridine-LHS, exhibited a broad and potent cellular activity against a wide range of organisms. MICs against representative Gram-positive strains of *S. aureus*, enterococci *(E. faecium* and *E. faecalis),* streptococci *(S. agalatiae, S. pneumoniae*, and *S. pyogenes*) were ≤ 2 μg/mL. Remarkably, with the only exception of one *Serratia marcescens* strain, antibacterial activity against Gram-negative pathogens such as *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*, with clinically relevant resistance phenotypes (FQ-resistance, carbapenem-resistance), were also good, all strains being susceptible to **21** with MICs ≤ 4 μg/mL.

Table 6: MIC ranges obtained for NBTIs **6** and **21** against selected strains (MIC in μg/mL).

Strains	Nb of strains	Ciprofloxacin	6	21
S. aureus	5	0.25 - >16	0.25 - 2	0.125 - 0.5
Enterococcus faecium	2	> 16	1, 16	0.063, 0.5
Enterococcus faecalis	1	1	1	0.25
Streptococcus agalactiae	1	1	4	2
Streptococcus pneumoniae	2	1, >16	0.25, 8	0.125, 2
Streptococcus pyogenes	1	0.25	≤0.03	≤0.03
E. coli	4	≤0.03 - >16	0.5 - 2	0.125 - 0.5
Citrobacter freundii	1	≤0.03	1	0.25
Enterobacter cloacae	3	≤0.03 - 0.5	2 - 16	0.25 - 4
Klebsiella pneumoniae	7	0.06 - >16	8 - 16	1 - 4
P. aeruginosa	4	0.125 - >16	1 - 2	1 - 2
A. baumannii	4	> 16	1 - > 16	0.125 - 4
Serratia marcescens	2	0.06, 1	8, >16	1, 8

In comparison, naphthyridinone-based compound **6** which also exhibited broad antibacterial spectrum, was generally less potent against Gram-positive and Gram-negative bacteria (roughly 2- to 4-fold lower). While compound **6** had weak or no activity against some MDR *E. faecium* and *A. baumannii*

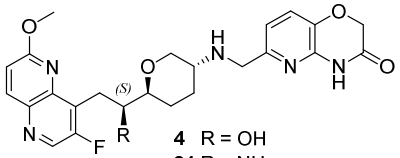
strains (MICs ≥ 16 μg/mL), it surprisingly showed similar potency than compound **21** against *P. aeruginosa* strains (same MIC range 1 - 2 μg/mL).

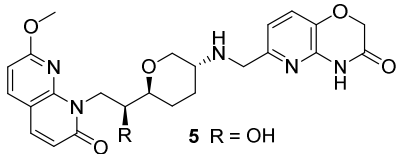
In vivo efficacy screening experiments with NBTI 16b, 21 and 6

The antibacterial activity of compounds **6** and **21** was explored in neutropenic murine thigh infection models in vivo using Gram-positive and Gram-negative pathogens. Because compound **21** was only available in limited amounts, in vivo efficacy experiments were mostly performed with compound **16b** as a surrogate (**16b** is a 1-1 mixture of compound **20** and **21**, see table 2). As summarized in Table 7, pharmacokinetic properties of compounds **6, 16b** and **21** were determined in rodents. In mouse, compounds **6** and **21** showed comparable profiles with high clearance and large volume of distribution resulting in a long terminal half-life (~6 H). Using the methicillin-sensitive *S. aureus* (MSSA) strain as an infecting pathogen, and administering subcutaneously (sc) compound **16b** (MIC against the MSSA infecting strain = 0.06 μg/mL) at a dose of 40 mg/kg, a net 2-log reduction of colony forming units (CFU) in thighs was observed compared to CFU at treatment start when measured 24h post-infection (22h post treatment). To confirm that the strong anti-staphylococcal activity of compound **16b** actually resulted from its more active epimer compound **21**, the latter was assayed using the same experimental set-up (dose and duration), and it reduced bacterial load by more than 2.5-log unit in thighs. From a pharmacokinetic-pharmacodynamic (PK-PD) standpoint, the AUC/MIC ratio associated with compound **21** at the dose of 40mg/kg/day is 23.5 (derived from iv PK data generated at 10mg/kg). Subsequently, compound **16b** was tested under the same conditions against the methicillin-resistant *S. aureus* (MRSA) (MIC against the MRSA in vivo strain = 0.03 μg/mL). The net bacterial load was reduced by 1-log unit in thighs after sc administration of a 40 mg/kg dose. To explore the broadness of the antibacterial coverage, *in vivo* efficacy of compound **16b** was also determined against a Gram-negative *K. pneumoniae* strain. After sc administration of a dose of 30 mg/kg, compound **16b** (MIC against *K. pneumoniae* in vivo strain = 0.125 μg/mL) nearly exhibited a net static efficacy. At a dose of 90mg/kg, compound **16b** elicited a 1-log reduction in colony forming unit (CFU) in thighs compared to CFUs at treatment start. These efficacy data were determined 7.5h post infection (5.5h post-treatment) to ensure that during the dosing interval (5.5h), the free drug exposure of **16b** was sufficient.

Because of its lower in vitro potency against *K. pneumoniae*, in vivo efficacy of compound **6** was assessed against a susceptible Gram-negative *E. coli* strain. When administered subcutaneously at doses of 120 and 360 mg/kg, compound **6** (MIC against *E. coli* in vivo strain = 0.125 μg/mL) showed a net static and 2-log reduction in colony forming units in thighs respectively, when measured 7.5 h post infection. Technical procedures, graphical representations and statistical details of in vivo experiments are reported in Supporting Information.

Table 7: Mouse, Rat and Dog Pharmacokinetic Parameters of selected THP-Based NBTIs.





Compounds	4	5	6	16b	21
Mouse [a]					
AUC_n (μg.h/(mL.dose)) [b]			186	260	236
f_u			0.369	0.154	0.15
$fAUC_n$ (μg.h/(mL.dose)) [b]			68.6	40.0	35.4
Cl/F (mL/min/kg)			86	64	71
Vd_{ss} (L/kg)			n.d	6.0	12.3
$t_{1/2}$ (h)			5.2	1.2	5.6
Rat [c]					
AUC_n (μg.h/(mL.dose)) [b]	457	378	145		194
f_u	0.086	0.167	0.236		0.15
$fAUC_n$ (μg.h/(mL.dose)) [b]	39	63	34		29
Cl (mL/min/kg)	37	44	110		86
Cl_u (mL/min/kg)	430	263	144		573
Vd_{ss} (L/kg)	2.5	2.5	21		25
$t_{1/2}$ (h)	1.3	1	9.1		8.8
Dog [d]					
AUC_n (μg.h/(mL.dose)) [b]			376		34
Cl (mL/min/kg)			45		490
Vd_{ss} (L/kg)			2.5		61
$t_{1/2}$ (h)			1.3		2.9

[a] Mouse dosed iv at 10 mg/kg (compounds **16b** and **21**) or sc at 120 mg/kg (compound **6**). [b] Dose-normalized AUC obtained after sc or iv dosing. [c] Rat dosed iv at 10mg/kg (compounds **6** and **21**) or 1mg/kg (compounds **4** and **5**). [d] Dog dosed iv at 1mg/kg (compounds **6** and **21**).

The pharmacokinetic profiles of **6** and **21** in rat were also comparable with both dibasic compounds displaying high clearances, long half-lives and large volumes of distribution. Compounds **4** and **5**, their respective monobasic analogs featuring a -CH_2-C_βH(OH)- linker, shared also comparable pharmacokinetic parameters in rat, with moderate clearances (Cl 37-44 mL/min/kg), moderate volume of distribution and short terminal half-life. Free AUC, the parameter that, together with MIC, is

inferred to drive the therapeutic dose,[19] was similar for **4**, **6** and **21**, and only slightly advantageous for compound **5**. The high clearances of the dibasic compounds **6** and **21** observed in rodents were replicated in the dog, raising concerns of potentially inferior pharmacokinetic profiles, necessitating non-viable dosing regimens in the clinic.

Cardiovascular safety assessment of representative NBTIs 6 and 21

We already reported that, contrary to compound **2**, compound **4** featuring a pyrido-oxazinone RHS, exhibited an unsatisfactory cardiovascular safety profile, partly due to hERG K^+ channel inhibition.[18] As shown in Table 8**,** a block of the hERG K^+ channel was also observed for compounds **5**, **6** and **21**, with 71%, 37% and 86% inhibition at 30μM compound concentration, respectively. The cardiovascular safety profile of these compounds was assessed further in vitro using cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CM) and in vivo with anesthetized guinea pigs.

Table 8: Effects of selected THP-based NBTIs on hERG K^+ channels, on the spontaneous rhythm of human induced-pluripotent-stem-cell-derived-cardiomyocytes (iPSC-CM), and on MAP, HR and ECG parameters in anesthetized guinea pigs.

Compounds	2	4	5	6	21
hERG block (%) [a]	20	92	71	37	86
hERG IC_{50}/IC_{20} (μM)	128/32	2.0/0.5	15/3.7	52/13	5/1.2
iPSC-CM (μM) [b,e]	13 (22)	0.21 (0.42)	0.86 (1.4)	0.27-0.81 (0.31-0.93)	0.28 (0.50)
QT (μM) [b,c]	>9.0 (>39)	0.36 (2.1)	1.5 (3.5)	2.7 (5.0)	0.53 (1.6)
HR (μM) [b,c]	>9.0 (>39)	1.5 (8.9)	6 (14)	11 (19)	0.53(1.6)
MAP (μM) [b,c]	>9.0 (>39)	4.8 (29)	21 (49)	28 (53)	1.8 (5.6)
QRS (μM) [b,c]	>9.0 (>39)	1.5 (8.9)	21 (49)	2.7 (5.0)	1.8 (5.6)
PR (μM) [b,c]	>9.0 (>39)	4.8 (29)	>21 (>49)	2.7 (5.0)	1.8 (5.6)
f_u [d]	0.23	0.168	0.43	0.54	0.33

[a] Reduction of K^+ current measured at 30 μM compound concentration. [b] Lowest active free (total) concentration (see methods in Supporting Information for the definition of active) [c] Compared to vehicle-treated animals. [d] Unbound fraction measured in guinea pig plasma by rapid equilibrium dialysis. [e] Free/total corrected based on the presence of 20% serum in the test solution.

In line with their hERG-blocking activities, compound **2** affected the spontaneous rhythm of iPSC-CM only at high concentrations (~13 μM free compound) while compound **4** was already inhibiting at ~0.21 μM free compound (Table 8). While a similar observation could be made for compounds **5** and **21**, compound **6** appears different in that it affected iPSC-CM much more potently than hERG K^+ channels. During the in vivo experiment, doses of 3, 10 and 30 mg of compound per kg of bodyweight were administered intravenously over three consecutive 20-min periods. Mean arterial pressure (MAP), heart rate (HR) and several ECG parameters (including PR, QRS, RR and QT intervals) were recorded continuously, and blood samples were taken at the end of each period for determination of drug levels. For each cardiovascular parameter, Table 8 reports the lowest drug plasma concentration (free and total) at which the parameter is different between compound-treated and vehicle-treated animals. At the highest dose tested, compound **2** had no effect on any parameter while compounds **4** and **21**, based on naphthyridine-LHS, affected QT-interval already at sub-micromolar free drug concentrations and compounds **5** and **6**, based on naphthyridinone-LHS**,** at an intermediate concentration, in line with their hERG-blocking activities. HR is also changed potently by compounds

4 and **21** but more weakly by compounds **5** and **6**, and all four compounds affect MAP only at higher drug concentrations. Compounds **4**, **5**, and **21** also increased PR- and QRS-intervals more weakly than QT-interval whereas compound **6** was equipotent on the three parameters suggesting that it affects additional cardiovascular targets as potently as hERG; this could be related to its remarkably potent activity on iPSC-CM.

The nature and magnitude of these cardiovascular effects were of particular concern and did not support the advancement of one of these compounds toward preclinical development. Among all THP-based NBTIs displaying the desired broad antibacterial spectrum, we were not yet able to identify compounds displaying a cleaner cardiovascular safety profile. The introduction of an extra primary amine, essential feature in this chemical series to enable anti Gram-negative activity, led so far to dibasic compounds with no improved cardiovascular safety profile in comparison to earlier compounds carrying the pyrido-oxazinone RHS such as **4**. Further investigation on the RHS have to be carried out in order to improve the cardiovascular safety profile while keeping broad antibacterial coverage.

CONCLUSIONS

We have reported herein efforts to broaden the antibacterial spectrum of THP-based NBTIs towards problematic Gram-negative pathogens, especially *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii* strains. Notably, we could demonstrate that dibasic compounds, designed by introducing an extra primary amino function within the flexible 2-atom linker on the NBTI scaffold, led to improved Gram-negative whole cell activity. For broad and potent DNA topoisomerases inhibitors, the data we presented here suggests that the better antibacterial activity against Gram-negative organisms, and notably *P. aeruginosa* could be primarily attributed to a higher cytoplasmic accumulation compared to earlier compounds. The mechanism facilitating a better permeation across membranes of these chemicals remains unknown, although it is tempting to speculate that their two cationic groups (protonated amines) could displace divalent metals (Mg^{2+} or Ca^{2+}) needed to stabilize the LPS layer, thereby promoting self-uptake of these molecules.[32] Two subsets of compounds differing in the nature of the LHS, but featuring the optimized (*S*)-configured -CH_2-$CH(NH_2)$- linker could be synthesized. For each subset, a representative compound with good potency against *P. aeruginosa* was studied in more depth. Compound **21**, belonging to the C-linked THP-based NBTI subset, displayed a broad and potent antibacterial spectrum, including MDR pathogens. Compound **6**, on the other hand, a representative compound of the N-linked THP-based NBTI subset, had a narrower spectrum and a lower potency against certain Gram-positive and negative strains. Compounds **6** and **21** not only showed in vitro activity but were also efficacious in murine models against relevant Gram-negative organisms. Pharmacokinetic properties of both compounds **6** and **21** were characterized by high clearances in rodent species that were not detrimental to *in vivo* efficacy. Within our proprietary chemical space delineated around the pyrido-oxazinone RHS (chosen to ensure broad antibacterial coverage), compounds devoid of cardiovascular safety liabilities could not yet be identified and further optimization around the THP-based NBTI series is necessary.[37] A better understanding of parameters driving cytoplasmic drug accumulation within GNB as well as tools for its reliable evaluation are required to accelerate the discovery and the optimization of urgently needed novel antibacterials.[38, 39]

EXPERIMENTAL SECTION

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) were recorded on a Bruker Ascend 500 is 500MHz (125MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26ppm, DMSO-d_6 2.50ppm), 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), broad singlet (br s). Melting points were determined using a Büchi B-540 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-EtOAc mixture or DCM-MeOH (doped with 1% v/v of aqueous NH_4OH for basic compounds). Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F254 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 MeCN in water containing 0.04% of TFA; or on a Waters XBridge C18, OBD, 5 μm, 4.6 mm × 50 mm (Waters, Switzerland), eluting with a gradient of 5−95% of MeCN in water containing 13 mM of NH_4OH UV at 230 nm and 254nm. Purity and identity were further confirmed by NMR spectroscopy.

***tert*-Butyl ((3*R*,6*S*)-6-((*RS*)-2-bromo-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (8).** To a suspension of **7**[17] (0.6 g, 1.55 mmol) in CCl_4 (10 mL) were added NBS (0.42 g, 2.32 mmol) and AIBN (0.03 g, 0.16 mmol). The mixture was heated at 80°C under a sunlamp beam for 6 h. The reaction mixture was cooled to rt and was diluted with DCM (20 mL), washed with 10% aq $Na_2S_2O_3$ (2 x 15 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (DCM-MeOH gradient) to afford **8** (0.55 g, 76% yield) as a light yellow foam; ^{1}H NMR (DMSO-d_6) mixture of isomers δ: 8.83 (d, J = 4.6 Hz, 0.5 H), 8.82 (d,

J = 4.6 Hz, 0.5 H), 8.32 (d, *J* = 9.0 Hz, 0.5 H), 8.31 (d, *J* = 9.1 Hz, 0.5 H), 7.91 (d, *J* = 4.6 Hz, 0.5 H), 7.89 (d, *J* = 4.6 Hz, 0.5 H), 7.34 (d, *J* = 9.0 Hz, 0.5 H), 7.33 (d, *J* = 9.0 Hz, 0.5 H), 6.77 (d, *J* = 8.0 Hz, 0.5 H), 6.63 (d, *J* = 8.0 Hz, 0.5 H), 6.38 (dd, *J* = 3.7, 10.7 Hz, 0.5 H), 6.33 (dd, *J* = 6.8, 8.9 Hz, 0.5 H), 4.05 (s, 1.5 H), 4.04 (s, 1.5 H), 3.82 (m, 0.5 H), 3.70 (m, 0.5 H), 3.49 (m, 0.5 H), 3.30 (overlaid m, 0.5 H), 2.99 (t, *J* = 10.8 Hz, 0.5 H), 2.95 (overlaid m, 0.5 H), 2.67 (t, *J* = 10.8 Hz, 0.5 H), 2.62 (overlaid m, 0.5 H), 2.20-2.14 (m, 1 H), 1.89-1.70 (m, 3 H), 1.41-1.21 (overlaid m, 1.5 H), 1.38 (s, 4.5 H), 1.35 (s, 4.5 H), 1.24 (m, 0.5 H); ESI-MS $(M+H)^+$ m/z 466.

(3*R*,6*S*)-6-((2*RS*)-2-Azido-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-*2H*-pyran-3-amine (9). To a solution of **8** (0.5 g, 1.07 mmol) in DMF (7 mL) was added NaN_3 (0.21 g, 3.22 mmol). The resulting mixture was heated at 80°C for 2 h. Water (50 mL) and Et_2O (20 mL) were added, two layers were separated and the aq layer was extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The residue was dissolved in TFA (2.1 mL), and stirred at rt for 15 min. After evaporation to dryness, the residue was partitioned between saturated aq $NaHCO_3$ (10 mL) and a DCM-MeOH mixture (9-1, 15 mL). The two layers were separated and the aq layer was extracted twice more with DCM-MeOH (9-1, 2 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness to afford **9** (0.29 g, 82% yield, 2 steps) as a yellow viscous oil; ^{1}H NMR (DMSO-d_6) mixture of isomers δ: 8.84 (d, *J* = 4.5 Hz, 0.5 H), 8.82 (d, *J* = 4.5 Hz, 0.5 H), 8.32 (d, *J* = 9.1 Hz, 0.5 H), 8.31 (d, *J* = 9.0 Hz, 0.5 H), 7.71 (d, *J* = 4.5 Hz, 0.5 H), 7.70 (d, *J* = 4.5 Hz, 0.5 H), 7.33 (d, *J* = 9.0 Hz, 0.5 H), 7.32 (d, *J* = 9.0 Hz, 0.5 H), 5.94 (dd, *J* = 4.9, 9.1 Hz, 0.5 H), 5.86 (t, *J* = 7.1 Hz, 0.5 H), 4.06 (s, 3 H), 3.80 (ddd, *J* = 2.1, 4.5, 10.6 Hz, 0.5 H), 3.71 (ddd, *J* = 2.1, 4.6, 10.6 Hz, 1 H), 3.42 (m, 0.5 H), 3.12 (m, 0.5 H), 2.90 (t, *J* = 10.5 Hz, 0.5 H), 2.71 (t, *J* = 10.5 Hz, 0.5 H), 2.54 (overlaid m, 1 H), 2.13 (m, 0.5 H), 2.08-1.98 (m, 1.5 H), 1.89-1.81 (m, 1 H), 1.75 (m, 0.5 H), 1.65 (m, 0.5 H), 1.58-1.37 (br s, 2 H), 1.34-1.14 (m, 1.5 H), 1.07 (m, 0.5 H); ESI-MS $(M+H)^+$ m/z 329.

6-((((3*R*,6*S*)-6-((2*RS*)-2-Amino-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-*2H*-pyran-3-yl)amino)methyl)-*2H*-pyrido[3,2-*b*][1,4]oxazin-3(*4H*)-one (11). To a solution of **9** (0.15 g, 0.46 mmol) in dry MeOH (4 mL) was added **10** (0.11 g, 0.59 mmol). The mixture was stirred at rt

overnight. The reaction mixture was cooled to 0°C and $NaBH_4$ (0.07 g, 1.83 mmol) was added. The mixture was stirred 15 min then DCM (6 mL) was added. The reaction proceed for 30 min at 0°C. Water (8 mL) was carefully added to the clear solution, followed by DCM-MeOH (9-1, 10 mL) and the two layers were separated. The aq layer was extracted twice with DCM-MeOH (9-1, 15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The residue was dissolved in THF (5.4 mL) was added polymer-bound PPh_3 (0.18 g) and water (1.1 mL) were added. The resulting mixture was heated at 60°C for 2 h. The reaction mixture was concentrated to dryness and the residue was purified by chromatography (DCM-MeOH gradient) to afford **11** (0.09 g, 41% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) mixture of isomers δ: 8.74 (d, J = 4.5 Hz, 0.5 H), 8.73 (d, J = 4.5 Hz, 0.5 H), 8.26 (d, J = 9.1 Hz, 0.5 H), 8.24 (d, J = 9.0 Hz, 0.5 H), 7.74 (d, J = 4.5 Hz, 0.5 H), 7.72 (d, J = 4.5 Hz, 0.5 H), 7.29 (two overlaid d, J = 8.1 Hz, 2 x 0.5 H), 7.25 (d, J = 9.0 Hz, 0.5 H), 7.24 (t, J = 9.0 Hz, 0.5 H), 7.00 (two overlaid d, J = 8.1 Hz, 2 x 0.5 H), 4.92 (dd, J = 4.3, 8.9 Hz, 0.5 H), 4.87 (dd, J = 5.3, 8.3 Hz, 0.5 H), 4.60 (s, 2 H), 4.01 (s, 3 H), 3.91-3.85 (m, 1 H), 3.70-3.63 (m, 2 H), 3.41-3.25 (overlaid m, 2 H), 2.89 (t, J = 10.5 Hz, 0.5 H), 2.85 (t, J = 10.5 Hz, 0.5 H), 2.45 (overlaid m, 1 H), 2.25-1.91 (m, 3 H), 1.89-1.81 (m, 1 H), 1.79-1.71 (m, 1.5 H), 1.65 (m, 0.5 H), 1.29-1.09 (m, 2 H); ESI-MS $(M+H)^+$ m/z 465; HR ESI-MS $(M+H)^+$ m/z = 465.2257 (calc. for $C_{24}H_{29}N_6O_4$: 465.2250); ^{t}R = 0.39.

General Procedure A: N-Cbz derivatives formation. A solution of N-Boc protected amine (1 equiv) in TFA (4 mL/mmol) was stirred at rt for 10 min. After evaporation to dryness, the residue was partitioned between 1M NaOH (3.5 mL/mmol) and a DCM-MeOH mixture (9-1, 25 mL/mmol). The aq layer was extracted with the same mixture (2 x 25 mL/mmol). The combined extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was taken up in EtOAc (5 mL/mmol) and water (5 mL/mmol). $NaHCO_3$ (0.25 g/mmol) and benzyl chloroformate (1.1 equiv) were added. The reaction proceeded at rt for 30 min. The two layers were decanted and the aq layer was extracted once with EtOAc. The combined organic layers were concentrated to dryness and the residue was purified by chromatography (heptane-EtOAc gradient) to afford the corresponding N-Cbz protected amine.

General Procedure B: Oxidation to ketone. To a suspension of N-Cbz derivative (1 equiv) in DCM (4 mL/mmol) cooled to 0°C was added DIPEA (3.1 equiv). A mixture of py.SO_3 complex (0.4 g/mmol) in DMSO (1 mL/mmol) was then added drop wise. The reaction mixture was stirred overnight at rt. Saturated $NaHCO_3$ solution (15 mL/mmol) and DCM (10 mL/mmol) were added. The two layers were separated and the aq layer was extracted with DCM (2 x 10 mL/mmol). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The crude was purified by column chromatography (DCM-MeOH gradient) to afford the corresponding ketone.

Benzyl ((3*R*,6*S*)-6-(2-(6-Methoxy-1,5-naphthyridin-4-yl)acetyl)tetrahydro-2*H*-pyran-3-yl)carbamate (13a). Alcohol **12a**[18] (2.68 g, 6.64 mmol) was reacted following general procedures A (72% yield) and B (60% yield) to afford **13a** (1.24 g, 60% yield) as an off-white solid.[18]

Benzyl ((3*R*,6*S*)-6-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)acetyl)tetrahydro-2*H*-pyran-3-yl)carbamate (13b). Alcohol **12b**[18] (2.43 g, 5.77 mmol) was reacted following general procedure A (96% yield) and B (> 95% yield) to afford **13b** (2.53 g) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 8.83 (s, 1 H); 8.30 (d, *J* = 9.1 Hz, 1 H); 7.41-7.31 (m, 6 H); 7.24 (d, *J* = 9.1 Hz, 1 H); 5.10-4.99 (m, 2 H); 4.52-4.38 (m, 2 H); 4.10-4.01 (m, 2 H); 3.99 (s, 3 H); 3.52 (m, 1 H); 3.18 (t, *J* = 10.6 Hz, 1 H); 2.09-1.91 (m, 2 H); 1.65-1.50 (m, 2 H); ESI-MS $(M+H)^+$ m/z 454.

Benzyl ((3*R*,6*S*)-6-(2-(7-Fluoro-2-methoxyquinolin-8-yl)acetyl)tetrahydro-2*H*-pyran-3-yl)carbamate (13c). Alcohol **12c**[18] (2.7 g, 6.42 mmol) was reacted following general procedure A (94% yield) and B (>95% yield) to afford **13c** (2.73 g) as an off-white solid; ^{1}H NMR ($CDCl_3$) δ: 7.94 (d, *J* = 8.8 Hz, 1 H), 7.62 (dd, *J* = 6.2, 8.9 Hz, 1 H), 7.28-7.42 (m, 5 H), 7.16 (t, *J* = 8.9 Hz, 1 H), 6.84 (d, *J* = 8.8 Hz, 1 H), 5.17-5.06 (m, 2 H), 4.57 (m, 1 H), 4.51-4.36 (m, 2 H), 4.25 (m, 1 H), 3.98 (s, 3 H), 3.92 (dd, *J* = 2.8, 10.9 Hz, 1 H), 3.83-3.66 (m, 1 H), 3.15 (t, *J* = 10.4 Hz, 1 H), 2.22-2.04 (m, 2 H), 1.73 (m, 1 H), 1.43 (m, 1 H); ESI-MS $(M+H)^+$ m/z 453.

General Procedure C: Reductive amination with ammonium acetate. To a solution of ketone (1 equiv) in DCM (4 mL/mmol) and MeOH (7 mL/mmol) was added ammonium acetate (25 equiv).

After 5 min, and $NaBH_3CN$ (2 equiv) was added. The reaction proceeded at rt until completion. The reaction mixture was diluted with saturated $NaHCO_3$ (10 mL/mmol) and DCM (10mL/mmol). The two layers were decanted and the aq layer was extracted with a DCM-MeOH mixture (9-1, 3 x 10 mL/mmol). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was chromatographed (DCM-MeOH containing 0.5% NH_4OH gradient) to afford the corresponding free amine.

General Procedure D: Boc protection of amines. To a solution of amine (1 equiv) in DCM (2mL/mmol) was added a solution of Boc_2O (2 eq) in DCM (3mL). The reaction proceeded for 2 h 30 at rt. The solvent was evaporated and the crude was directly subjected to chromatography (heptane-EtOAc) to afford the corresponding N-Boc protected amine.

General Procedure E: Hydrogenolysis of N-Cbz carbamates. To a solution of N-Boc amine (1 equiv) in EtOAc (20 mL) and MeOH (2 mL) was added 20% $Pd(OH)_2$ on C (moisturized, 0.3 g/mmol). The reaction proceeded 1 h at rt under normal hydrogen atmosphere (balloon). The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was chromatographed (DCM-MeOH containing 0.5% NH_4OH gradient) to afford the corresponding deprotected amine.

***tert*-Butyl (1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)carbamate (14a).** Ketone **13a** (1.24 g, 2.85 mmol) was reacted following general procedures C to E to afford **14a** (0.491 g, 43% yield) as a white foam; ^{1}H NMR ($CDCl_3$) mixture of diastereomers δ: 8.64 (two overlaid d, *J* = 4.4 Hz, 1 H), 8.19 (two overlaid d, *J* = 9.0 Hz, 1 H), 7.41 (m, 1 H), 7.11 (two overlaid d, *J* = 9.0 Hz, 1 H), 5.45 (m, 0.5 H), 5.08 (m, 0.5 H), 4.10 (s, 1.5 H), 4.09 (s, 1.5 H), 4.17-3.90 (overlaid m, 2 H), 3.50 (m, 1 H), 3.36-3.14 (m, 2 H), 3.02-2.71 (m, 2 H), 2.07 (m, 1 H), 1.84-1.09 (m, 5 H), 1.21 (s, 4.5 H), 1.12 (s, 4.5 H); ESI-MS $(M+H)^+$ m/z 403.

***tert*-Butyl (1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)carbamate (14b).** Ketone **13b** (2.52 g, 5.56 mmol) was reacted following general procedures C to E to afford **14b** (1.35 g, 57% yield) as a white foam; ^{1}H NMR ($CDCl_3$)

mixture of diastereomers δ: 8.60 (two overlaid s, 1 H), 8.16 (two overlaid d, *J* = 9.1 Hz, 1 H), 7.06 (two overlaid d, *J* = 9.1 Hz, 1 H), 5.33 (m, 0.5 H), 5.05 (m, 0.5 H), 4.11 (s, 1.5 H), 4.09 (s, 1.5 H), 4.09-3.85 (m, 2 H), 3.54-3.25 (m, 3 H), 3.03 (t, *J* = 10.4 Hz, 0.5 H), 2.97 (t, *J* = 10.4 Hz, 0.5 H), 2.89-2.71 (m, 2 x 0.5 H), 2.07 (m, 1 H), 1.91-1.49 (m, 2.5 H), 1.43-1.20 (m, 2.5 H), 1.21 (s, 4.5 H), 1.12 (s, 4.5 H); ESI-MS $(M+H)^+$ m/z 421.

General Procedure F: Reductive amination using aldehydes RHS-CHO. 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 aldehyde RHS-CHO (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 gradient) to afford the corresponding alkylated product.

General Procedure G: Boc deprotection. A solution of N-Boc protected amine (1 equiv) in TFA (5 mL/mmol) was stirred at rt for 25 min. The solvent was evaporated and the mixture was taken up in a DCM-MeOH mixture (9-1, 20 mL/mmol and pH was adjusted to 10 adding 2M NaOH carefully. The two layers were separated and the aq layer was extracted twice with a DCM-MeOH mixture (9-1, 3 x 20 mL/mmol). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (DCM-MeOH containing 1% NH_4OH gradient) to afford the corresponding NBTI.

6-((((3*R*,6*S*)-6-((1*RS*)-1-Amino-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-*2H*-pyran-3-yl)amino)methyl)-*2H*-pyrido[3,2-*b*][1,4]oxazin-3(*4H*)-one (16a). Amine **14a** (0.1 g, 0.25 mmol) and aldehyde **10** were reacted following general procedure F. The resulting product was then reacted following general procedure G to afford **16a** (0.057 g, 49% yield, two steps) as an off-white solid; mp = 135.8±0.2°C; ^{1}H NMR (DMSO-d_6) mixture of diastereomers δ: 10.80 (br s, 1 H), 8.64 (d, *J* = 4.4 Hz, 1 H), 8.21 (d, *J* = 9.0 Hz, 1 H), 7.52 (t, *J* = 4.1 Hz, 1 H), 7.28 (d, *J* = 8.1 Hz, 1 H), 7.21 (d, *J* =

9.0 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 4.58 (s, 3 H), 3.99 (s, 3 H), 3.95 (overlaid m, 1 H), 3.73-3.62 (m, 2 H), 3.48 (m, 0.5 H), 3.38 (m, 0.5 H), 3.14-3.01 (m, 2 H), 2.96-2.85 (m, 1.5 H), 2.84 (dd, *J* = 9.1, 13.0 Hz, 0.5 H), 2.47 (overlaid m, 1 H), 2.19-1.58 (br s, 1 H), 2.02 (m, 1 H), 1.82 (m, 0.5 H), 1.68 (m, 0.5 H), 1.47 (m, 1 H), 1.29-1.13 (m, 2 H); ESI-MS $(M+H)^+$ m/z 465; HR ESI-MS $(M+H)^+$ m/z = 465.2250 (calc. for $C_{24}H_{29}N_6O_4$: 465.2250); ^{t}R = 0.61.

6-((((3*R*,6*S*)-6-((1*RS*)-1-Amino-2-(3-fluoro-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 (16b). Amine **14b** (0.12 g, 0.285 mmol) and aldehyde **10** were reacted following general procedure F. The resulting product was then reacted following general procedure G to afford **16b** (0.073 g, 52% yield, two steps) as an off-white solid; ^{1}H NMR (DMSO-d_6) mixture of diastereomers δ: 10.80 (br s, 1 H), 8.73 (s, 1 H), 8.25 (d, *J* = 9.1 Hz, 1 H), 7.28 (d, *J* = 8.1 Hz, 1 H), 7.20 (d, *J* = 9.0 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 4.59 (s, 2 H), 4.00 (s, 3 H), 3.95 (overlaid m, 1 H), 3.72-3.62 (m, 2 H), 3.32 (m, 1 H), 3.14-2.86 (m, 5 H), 2.10-1.79 (br s, 1 H), 2.02 (m, 1 H), 1.86 (m, 1 H), 1.65 (m, 1 H), 1.45 (m, 1 H), 1.26-1.09 (m, 2 H); ESI-MS $(M+H)^+$ m/z 483; HR ESI-MS $(M+H)^+$ m/z = 483.2159 (calc. for $C_{24}H_{28}FN_6O_4$: 483.2156); ^{t}R = 0.44.

Benzyl ((3*R*,6*S*)-6-((1*R*)-1-hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (17). Alcohol **12a**[18] (2.68 g, 6.64 mmol) was reacted following general procedure A to afford **17** (2.09 g, 72% yield) as a white solid; ^{1}H NMR ($CDCl_3$) δ: 8.70 (d, *J* = 4.4 Hz, 1 H), 8.24(d, *J* = 9.0 Hz, 1 H), 7.45 (d, *J* = 4.5 Hz, 1 H), 7.37-7.31 (m, 5 H), 7.26 (d, *J* = 9.1 Hz, 1 H, 1 H), 5.19-5.04 (m, 2 H), 4.96 (m, 1 H), 4.45 (m, 1 H), 4.15 (m, 1 H), 4.07 (s, 3 H), 3.91 (m, 1 H), 3.70 (m, 1 H), 3.55 (dd, *J* = 2.7, 13.7 Hz, 1 H), 3.30 (dd, *J* = 7.2, 13.7 Hz, 1 H); 2.99 (overlaid m, 1 H), 2.97 (t, J = 10.5 Hz, 1 H), 2.13 (m, 1 H), 2.01 (m, 1 H), 1.52 (m, 1 H), 1.24 (m, 1 H); ESI-MS $(M+H)^+$ m/z 438.

Benzyl ((3*R*,6*S*)-6-((1*S*)-1-azido-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (18). To a mixture of alcohol **17** (2.75 g, 6.29 mmol) and PPh_3 (2.14 g, 8.17 mmol) in THF (30 mL), cooled to -10°C, was drop wise DPPA (1.77 mL, 8.17 mmol) and then DIAD (1.74 mL,

8.8 mmol). The reaction mixture was then warmed to 0°C and DCM (5mL) was added. The reaction proceeded for 2h. Saturated sodium bicarbonate (150 mL) and EtOAc (150 mL) were added. The aq layer was extracted with EtOAc (150 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was chromatographed (heptane-EtOAc 1-2) to afford **18** (1.5 g, 52% yield) as a white solid; ^{1}H NMR ($CDCl_3$) δ: 8.69 (d, *J* = 4.4 Hz, 1 H), 8.21 (d, *J* = 9.1 Hz, 1 H), 7.44 (d, *J* = 4.4 Hz, 1 H), 7.37-7.31 (m, 5 H), 7.13 (d, *J* = 9.1 Hz, 1 H), 5.12-5.09 (m, 2 H), 4.44 (m, 1 H), 4.20 (m, 1 H), 4.05 (s, 3 H), 3.89 (m, 1 H), 3.72 (m, 1 H), 3.56 (dd, *J* = 6.1, 12.8 Hz, 1 H), 3.34 (m, 1 H), 3.23 (m, 1 H), 2.99 (t, *J* = 10.4 Hz, 1 H), 2.15 (m, 1 H), 1.88-1.62 (m, 2 H), 1.28 (m, 1 H); ESI-MS $(M+H)^+$ m/z 463.

***tert*-Butyl ((*S*)-1-((2*S*,5*R*)-5-aminotetrahydro-*2H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)carbamate (19).** To a solution of azide **18** (1.5 g, 3.2 mmol) in THF (40 mL) was added PPh_3 (1.27g, 4.86 mmol). The mixture was heated at 60°C for 30 min, and water (5mL) was added. The reaction proceeded overnight. After cooling, the solvent was removed in vacuo. The residue was chromatographed (DCM-MeOH 9-1 containing 1% NH_4OH gradient) to afford the amine (1.32 g, 93% yield) as a white solid. The latter was reacted following general procedures D and E to afford **19** as a white solid; mp = 149.6±0.1°C; ^{1}H NMR ($CDCl_3$) δ: 8.65 (d, *J* = 4.4 Hz, 1 H), 8.18 (d, *J* = 9.0 Hz, 1 H), 7.40 (d, *J* = 4.4 Hz, 1 H), 7.11 (d, *J* = 9.0 Hz, 1 H), 5.08 (m, 1 H), 4.11 (s, 3 H), 4.11 (overlaid m, 1H), 3.96 (m, 1 H), 3.46 (dd, *J* = 4.4, 12.7 Hz, 1 H), 3.31 (m, 1 H), 3.21 (m, 1 H), 2.93 (t, *J* = 10.6 Hz, 1 H), 2.80 (m, 1 H), 2.00 (m, 1 H), 1.64-1.48 (m, 3 H), 1.28 (overlaid m, 1 H), 1.28 (s, 9 H), 1.19 (overlaid m, 1 H); ESI-MS $(M+H)^+$ m/z 403.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-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 (20). Amine **19** (0.4 g, 1 mmol) and aldehyde **10** were reacted following general procedure F. The resulting product was then reacted following general procedure G to afford **20** (0.29 g, 62% yield over two steps) as an off-white solid; mp = 155.3±0.2°C; ^{1}H NMR (DMSO-d_6) δ: 8.64 (d, *J* = 4.4 Hz, 1 H), 8.21 (d, *J* = 9.0 Hz, 1 H), 7.51 (d, *J* = 4.5 Hz, 1 H), 7.28 (d, *J* = 8.1 Hz, 1 H), 7.21 (d, *J* = 9.0 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 4.59 (s, 2 H), 4.03 (s, 3 H), 4.02 (overlaid m, 1 H), 3.72-3.63 (m, 2 H), 3.36 (m, 1 H), 3.01-3.09 (m, 2 H),

2.95-2.85 (m, 2 H), 2.44 (overlaid m, 1 H), 2.11-1.51 (br. s, 3 H), 2.02 (m, 1 H), 1.68 (m, 1 H), 1.45 (m, 1 H), 1.20 (m, 1 H), 1 NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 161.3, 152.9, 148.0, 146.1, 141.4, 141.2, 141.1, 141.0, 138.0, 125.9, 123.8, 117.1, 116.4, 81.1, 72.5, 67.2, 55.3, 53.9, 53.5, 51.5, 35.7, 30.9, 27.2; HR ESI-MS $(M+H)^+$ m/z = 465.2253 (calc. for $C_{24}H_{29}N_6O_4$: 465.2250); ^{t}R = 0.61.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(3-fluoro-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) and 6-((((3*R*,6*S*)-6-((1*R*)-1-amino-2-(3-fluoro-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 (22). Amine **14b** (1.54 g, 3.67 mmol) and aldehyde **10** were reacted following general procedure F. The resulting mixture **15b** was separated on a ChiralCel OD-H column with 80% hexane and 20% EtOH containing 0.1% of diethylamine. The (*S*)-enantiomer eluted first (^{t}R = 11.06 min.) followed by the (*R*)-enantiomer (^{t}R = 15.5 min). Each enantiomer was then reacted following general procedure G to afford **21** (0.465 g) and **22** (0.31 g) as off-white solids. For **21**: mp = 156.±0.1°C; ^{1}H NMR (DMSO-d_6) δ: 8.75 (s, 1 H), 8.27 (d, *J* = 9.0 Hz, 1 H), 7.30 (d, *J* = 8.0 Hz, 1 H), 7.22 (d, *J* = 9.0 Hz, 1 H), 7.02 (d, *J* = 8.0 Hz, 1 H) 4.61 (s, 2 H), 4.03 (s, 3 H), 4.02 (overlaid m, 1 H), 3.73-3.65 (m, 2 H), 3.30 (overlaid m, 1 H), 3.13-3.03 (m, 3 H), 2.95 (t, *J* = 10.4 Hz, 1 H), 2.44 (overlaid m, 1 H), 2.11-1.51 (br. s, 3 H), 2.04 (m, 1 H), 1.68 (m, 1 H), 1.50 (m, 1 H), 1.21 (m, 1 H), 1 NH missing; ESI-MS $(M+H)^+$ m/z 483; HR ESI-MS $(M+H)^+$ m/z = 483.2153 (calc. for $C_{24}H_{28}FN_6O_4$: 483.2156); ^{t}R = 0.44. For **22**: ^{1}H NMR (DMSO-d_6) δ: 8.72 (s, 1 H), 8.25 (d, *J* = 9.0 Hz, 1 H), 7.28 (d, *J* = 8.0 Hz, 1 H), 7.20 (d, *J* = 9.0 Hz, 1 H), 6.99 (d, *J* = 8.0 Hz, 1 H) 4.58 (s, 2 H), 4.00 (s, 3 H), 3.91 (m, 1 H), 3.72-3.62 (m, 2 H), 3.35 (m, 1 H), 3.12-2.94 (m, 3 H), 2.92 (t, *J* = 10.4 Hz, 1 H), 2.44 (overlaid m, 1 H), 2.03 (m, 1 H), 1.84 (m, 1 H), 1.37 (m, 1 H), 1.18 (m, 1 H), 4 NH missing; ESI-MS $(M+H)^+$ m/z 483; HR ESI-MS $(M+H)^+$ m/z = 483.2153 (calc. for $C_{24}H_{28}FN_6O_4$: 483.2156); ^{t}R = 0.45.

***tert*-Butyl ((*S*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethyl)carbamate ((*S*)-14c).** Ketone **13c** (2.73 g, 6.0 mmol) was reacted following general procedures C to E to afford **14c** (1.73 g, 68% yield) as a white foam; ^{1}H NMR ($CDCl_3$) mixture of diastereomers δ: 7.92 (two overlaid d, *J* = 8.8 Hz, 1 H), 7.57-7.52 (m, 1 H), 7.11 (m, 1 H), 6.84 (two

overlaid d, J = 8.8 Hz, 1 H), 5.49 (m, 0.5 H), 5.13 (m, 0.5 H), 4.09 (s, 1.5 H), 4.08 (s, 1.5 H), 4.07-3.85 (m, 2 H), 3.52-3.23 (m, 3 H), 3.02 (t, J = 10.4 Hz, 0.5 H), 2.97 (t, J = 10.4 Hz, 0.5 H), 2.90-2.73 (m, 1.5 H), 2.07 (m, 1 H), 1.87-1.51 (m, 2.5 H), 1.43-1.20 (m, 2.5 H), 1.20 (s, 4.5 H), 1.11 (s, 4.5 H). Amine **14c** (0.38 g) was separated on a ChiralPak AD-H column with 90% hexane and 10% EtOH containing 0.1% of diethylamine to afford first the (*R*)-enantiomer (^{t}R = 11.2 min) and then (*S*)-**14c** (0.185 g, 47% yield) (^{t}R = 13.15 min.) as a white foam; ^{1}H NMR (DMSO-d_6) δ: 8.20 (d, J = 8.8 Hz, 1 H), 7.76 (dd, J = 6.1, 8.8 Hz, 1H), 7.25 (t, J = 9.2 Hz, 1 H), 6.96 (d, J = 8.8 Hz, 1 H), 6.05 (d, J = 9.7 Hz, 1H), 4.00 (s, 3 H), 3.90 (m, 1H), 3.78 (m, 1 H), 3.26 (overlaid m, 1 H), 3.22-3.09 (m, 2H), 2.80 (t, J = 10.4 Hz, 1 H), 1.85 (m, 1 H), 1.50 (m, 1 H), 1.42-1.29 (m, 3 H), 1.22 (m, 1H), 1.12 (s, 9 H), 1.09 (overlaid m, 1 H); ESI-MS $(M+H)^+$ m/z 420.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(7-fluoro-2-methoxyquinolin-8-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (23). Amine (*S*)-**14c** (0.18 g, 0.43 mmol) was reacted following general procedures F and G to afford **23** (0.165 g, 79% yield) as an off-white solid; mp = 161.6±0.1°C; ^{1}H NMR (DMSO-d_6) δ: 8.21 (d, J = 8.9 Hz, 1 H), 7.77 (dd, J = 6.3, 8.9 Hz, 1 H), 7.30-7.24 (m, 2 H), 6.99 (d, J = 8.1 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 4.58 (s, 2 H), 4.01 (s, 3 H), 4.01 (overlaid m, 1 H), 3.72-3.62 (m, 2 H), 3.30 (overlaid m, 1 H), 3.07-2.97 (m, 3 H), 2.92 (t, J = 10.4 Hz, 1 H), 2.43 (overlaid m, 1 H), 1.99 (m, 1 H), 1.64 (m, 1 H), 1.50 (m, 1 H), 1.18 (m, 1 H), 4 NH missing; ESI-MS $(M+H)^+$ m/z 482; HR ESI-MS $(M+H)^+$ m/z = 482.2209 (calc. for $C_{25}H_{29}FN_5O_4$: 482.2203); ^{t}R = 0.68.

***tert*-Butyl ((3*R*,6*S*)-6-(1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (25).** Alkene **24**[17] was dissolved in a 2-methyl-2 propanol-water mixture (1:1, 190 mL). The mixture was cooled to 0°C and AD-mix-α ® (26.30 g) was added. After stirring overnight at 0°C, $NaHSO_3$ (28.18 g) was added. The two layers were decanted and the aq layer was extracted twice with EtOAc (2 x 150 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed (DCM-MeOH gradient), affording diol **25** (8-1 mixture of diastereomers, 3.92 g, 80% yield) as a white solid. For major diastereomer (*R*)-**25**: ^{1}H NMR ($CDCl_3$), δ: 4.23 (br. s, 1 H), 4.09 (ddd, J = 2.4, 5.1, 10.5 Hz, 1 H), 3.68-3.74 (m, 2 H), 3.52-3.66 (m,

2 H), 3.35 (ddd, *J* = 2.4, 5.1, 11.4 Hz, 1 H), 2.98 (t, *J* = 10.8 Hz, 1 H), 2.51 (br. d, *J* = 6.0 Hz, 1 H), 2.09-2.21 (m, 2 H), 1.78 (m, 1 H), 1.54 (m, 1 H), 1.43 (s, 9 H), 1.22-1.36 (m, 1 H); ESI-MS $(M+H)^+$ m/z 262.

2-((2*S*,5*R*)-5-((*tert*-Butoxycarbonyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-((methylsulfonyl)oxy)ethyl pivalate (26). To a solution of **25** (3.92 g, 15 mmol) and DMAP (3.67 g, 30 mmol) in DCM (75 mL), cooled to 0°C, was added pivaloyl chloride (2.4 mL, 19.5 mmol). The reaction proceeded for 1 h. The reaction mixture was partitioned between saturated $NaHCO_3$ (100 mL) and EtOAc (150 mL). The aq layer was extracted with EtOAc (100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (Hept-EtOAc 1-1) affording the title compound as a white solid (3.68 g, 71 % yield); MS (ESI) m/z 346 (MH^+). The latter was dissolved in DCM (55 mL). After cooling to 0°C, TEA (3 mL, 2 eq.) and Ms-Cl (1 mL, 1.2 eq.) were added. The reaction was stirred at 0°C for 1 h. Saturated $NaHCO_3$ (185 mL) and DCM (185 mL) were added. The two layers were decanted and the organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (heptane-EtOAc 1-1), affording **26** as a colorless foam (4.23 g, 94 % yield); ^{1}H NMR ($CDCl_3$), major diastereomer δ: 4.81 (m, 1 H), 4.40 (m, 1 H), 4.24 (m, 1 H), 4.18-4.07 (m, 3 H), 3.51-3.64 (m, 1 H), 3.46 (ddd, *J* = 2.3, 4.3, 11.3 Hz, 2 H), 3.07 (s, 3 H), 2.98 (t, *J* = 10.4 Hz, 1 H), 2.15 (m, 1 H), 1.82 (m, 1 H), 1.65 (m, 1 H), 1.40 (s, 9 H), 1.29 (m, 1 H), 1.20 (s, 9 H); ESI-MS $(M+H)^+$ m/z 424.

***tert*-Butyl ((3*R*,6*S*)-6-((*S*)-oxiran-2-yl)tetrahydro-2*H*-pyran-3-yl)carbamate ((*S*)-27).** To a solution of **26** (4.12 g, 9.73 mmol) in THF (50 mL) was added sodium methylate (25 wt% solution in MeOH, 4.5 mL). The mixture was stirred at rt for 15 min. The reaction mixture was partitioned between 10% aq $NaHSO_4$ (100 mL) and EtOAc (200 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford a pale yellow solid (2.36 g, >95 yield). The latter (6.19 g, 25.44 mmol), dissolved in a minimum amount of THF (11.8 mL). Water (0.092 mL, 5.08 mmol) and (*R*,*R*)-(salen)Co-OTs[27] (0.1g, 0.127mmol) were added. The reaction proceeded 2 h. The solution was diluted in EtOAc (30 mL) and dried over $MgSO_4$, filtered, and concentrated under

reduced pressure. The residue was purified by chromatography (heptane-EtOAc 1-1) to afford first (*S*)-**27** (4.88 g) as a white solid, and then diol (*R*)-**25** (0.99 g, 16% yield) as a white solid. For (*S*)-**27**: ^{1}H NMR ($CDCl_3$) δ: 4.21 (m, 1 H), 4.11 (ddd, *J* = 2.2, 4.7, 10.8 Hz, 1 H), 3.61 (m, 1 H), 3.11-3.291 (m, 3 H), 2.78 (t, *J* = 10.4 Hz, 1 H), 2.64 (dd, *J* = 2.6, 4.9 Hz, 1 H), 2.14 (m, 1 H), 1.76-1.52 (m, 2 H), 1.43 (s, 9 H), 1.28 (qd, *J* = 4.5, 12.4 Hz, 1 H), ^{13}C NMR ($CDCl_3$) δ: 155.3, 79.7, 77.9, 71.3, 53.9, 46.5, 44.2, 30.2, 28.4 (3C), 27.2; ESI-MS $(M+H)^+$ m/z 244.3; For (*R*)-**25** (*vide supra*).

***tert*-Butyl ((3*R*,6*S*)-6-((*R*)-oxiran-2-yl)tetrahydro-2*H*-pyran-3-yl)carbamate ((*R*)-27).** To an ice-chilled solution of alkene **24** (0.5 g, 2.2 mmol) in DCM (14 mL) were added $NaHCO_3$ (0.37 g, 4.4 mmol) and *m*-CPBA (0.8 g, 4.4 mmol). The reaction proceeded at rt overnight. The reaction mixture was diluted in DCM (70mL) and 10% aq $Na_2S_2O_3$ (15 mL) was added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness. The crude product was purified by chromatography (heptane-EtOAc 2-1) to afford first (*R*)-**27** (0.12 g, 21% yield) and then (*S*)-**27** (0.15 g, 29% yield) as white solids. For (*R*)-**27**: ^{1}H NMR ($CDCl_3$) δ: 4.23 (m, 1 H), 4.09 (ddd, *J* = 2.1, 4.6 Hz, 10.8 Hz, 1 H), 3.60 (m, 1 H), 3.18 (ddd, *J* = 2.3, 4.6 Hz, 11.1 Hz, 1 H), 2.99 (t, *J* = 10.4 Hz, 1 H), 2.93 (overlaid m, 1H), 2.77 (dd, *J* = 4.7, 5.8 Hz,, 1H), 2.71 (dd, *J* = 2.6, 5.2 Hz, 1 H), 2.13 (m, 1 H), 1.82 (m, 1H), 1.55 (m, 1 H), 1.43 (s, 9 H), 1.28 (m, 1 H); ESI-MS $(M+H)^+$ m/z 244.3.

General Procedure H: Epoxide opening. A solution of epoxide (*S*)-**27** or (*R*)-**27** (1 equiv) in DMF (1.5 mL/mmol) was added to a suspension of aromatic **28a-f** (1.1 equiv) in DMF (2 mL/mmol) and Cs_2CO_3 (2 equiv.) (in case of epoxide (*R*)-**27**, K_2CO_3 was used). The reaction mixture was stirred at 80°C until completion. The solvent was removed under reduced pressure, then the residue was partitioned between water (20 mL/mmol) and EtOAc (20 mL/mmol). The aq layer was extracted once more with EtOAc (20 mL/mmol). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by chromatography (heptane-EtOAc gradient) to afford **29a-f**.

***tert*-Butyl ((3*R*,6*S*)-6-((1*S*)-1-hydroxy-2-(7-methoxy-2-oxo-1,8-naphthyridin-1(2*H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29a).** Epoxide (*S*)-**27** (2.65 g, 15 mmol) and

naphthyridinone **28a** were reacted following general procedure H to afford **29a** (3.50 g, 56% yield) as a white solid; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 8.5 Hz, 1 H), 7.82 (d, J = 9.4 Hz, 1 H), 6.69 (d, J = 8.5 Hz, 1 H), 6.69 (overlaid m, 1 H), 6.47 (d, J = 9.4 Hz, 1 H), 4.65 (dd, J = 8.8, 12.6 Hz, 1 H), 4.43 (d, J = 7.0 Hz, 1 H), 4.32 (dd, J = 4.1, 12.6 Hz, 1 H), 3.96 (s, 3 H), 3.91 (overlaid m, 1 H), 3.80 (m, 1 H), 3.30 (m, 1 H), 3.13 (m, 1 H), 2.90 (t, J = 10.5 Hz, 1 H), 1.86 (m, 1 H), 1.53-1.63 (m, 2 H), 1.34 (s, 9 H), 1.33 (overlaid m, 1 H); ESI-MS $(M+H)^+$ m/z 420.

***tert*-Butyl ((3*R*,6*S*)-6-((1*S*)-1-hydroxy-2-(7-methoxy-2-oxoquinoxalin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29b).** Epoxide (*S*)-**27** (0.2 g, 0.8 mmol) and quinaxolinone **28b** were reacted following general procedure H to afford **29b** (0.26 g, 80% yield) as a white solid; ^{1}H NMR (DMSO-d_6) δ: 8.04 (s, 1 H), 7.73 (d, J = 8.8 Hz, 1 H), 7.11 (d, J = 2.5 Hz, 1 H), 6.99 (dd, J = 2.5, 8.8 Hz, 1 H), 6.77 (d, J = 8.0 Hz, 1 H), 5.02 (d, J = 6.3 Hz, 1H,-OH), 4.31 (dd, J = 8.4, 13.8 Hz, 1 H), 4.19 (dd, J = 3.9, 13.8 Hz, 1 H), 3.88 (s, 3 H), 3.87 (overlaid m, 1 H), 3.80 (m, 1 H), 3.37(m, 1 H), 3.30 (m, 1 H), 2.96 (t, J = 10.6 Hz, 1 H), 1.89 (m, 1 H), 1.56-1.64 (m, 2 H), 1.33-1.39 (m, 10 H); ESI-MS $(M+H)^+$ m/z 420.

***tert*-Butyl ((3*R*,6*S*)-6-((1*S*)-1-hydroxy-2-(7-methoxy-2-oxoquinolin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29c).** Epoxide (*S*)-**27** (1.04 g, 4.27 mmol) and quinolinone **28c** were reacted following general procedure H to afford **29c** (0.78 g, 44% yield) as a white solid; ^{1}H NMR ($CDCl_3$) δ: 7.65 (d, J = 9.4 Hz, 1 H), 7.46 (d, J = 8.6 Hz, 1 H), 7.08 (d, J = 2.1 Hz, 1 H), 6.83 (dd, J = 2.3, 8.6 Hz, 1 H), 6.56 (d, J = 9.3 Hz, 1 H), 5.35 (br s, 1 H), 4.54 (dd, J = 8.1, 12.8 Hz, 1 H), 4.36 (dd, J = 2.6, 12.8 Hz, 1 H), 4.27 (m, 1 H), 4.18 (m, 1 H), 4.02 (m, 1 H), 3.89 (s, 3 H), 3.65 (m, 1 H), 3.42 (m, 1 H), 3.08 (t, J = 10.4 Hz, 1 H), 2.17 (m, 1 H), 1.99 (m, 1 H), 1.63 (m, 1 H), 1.45 (s, 9 H), 1.31(m, 1 H); ESI-MS $(M+H)^+$ m/z 419.

***tert*-Butyl ((3*R*,6*S*)-6-((1*S*)-1-hydroxy-2-(7-methoxy-2-oxo-1,5-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29d).** Epoxide (*S*)-**27** (2.50 g, 10.27 mmol) and naphthyridinone **28d** were reacted following general procedure H to afford **29d** (1.82 g, 42% yield) as a white solid; ^{1}H NMR ($CDCl_3$) δ: 8.29 (d, J = 2.4 Hz, 1 H), 7.90 (d, J = 9.5 Hz, 1 H), 7.41 (d, J =

2.2 Hz, 1 H), 6.76 (d, *J* = 9.7 Hz, 1 H), 4.52-4.36 (m, 2 H), 4.26 (m, 1 H), 4.16 (m, 1 H), 3.97 (m, 1 H), 3.94 (s, 3 H), 3.72-3.52 (m, 2 H), 3.42 (m, 1 H), 3.07 (t, *J* = 10.4 Hz, 1 H), 2.17 (m, 1 H), 1.99 (m, 1 H), 1.63 (m, 1 H), 1.44 (s, 9 H), 1.32 (m, 1 H); ESI-MS $(M+H)^+$ m/z 420.

***tert*-Butyl ((3*R*,6*S*)-6-((*R*)-1-hydroxy-2-(6-methoxy-3-oxopyrido[2,3-b]pyrazin-4(*3H*)-yl)ethyl)tetrahydro-*2H*-pyran-3-yl)carbamate (29e).** Epoxide (*R*)-**27** (0.487 g, 2mmol) and pyridopyrazinone **28e** were reacted following general procedure H to afford **29e** (0.43 g, 51% yield) as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 8.12 (d, *J* = 8.6 Hz, 1 H), 8.10 (s, 1 H), 6.83 (d, *J* = 8.6 Hz, 1 H), 6.74 (d, *J* = 8.0 Hz, 1 H), 4.94 (d, *J* = 6.2 Hz, 1 H), 4.50 (dd, *J* = 8.5 , 12.7 Hz, 1 H), 4.35 (dd, J = 4.7, 12.7 Hz, 1 H), 3.99 (s, 3 H), 3.91 (m, 1 H), 3.65 (m, 1 H), 3.29 (m, 1 H), 3.16 (m, 1 H), 2.86 (t, *J* = 10.6 Hz, 1 H), 1.92-1.85 (m, 2 H), 1.37 (s, 9 H), 1.37-1.32 (overlaid m, 2 H); ESI-MS $(M+H)^+$ m/z 421.

***tert*-Butyl ((3*R*,6*S*)-6-((1*R*)-1-hydroxy-2-(3-methoxy-6-oxopyrido[2,3-*b*]pyrazin-5(*6H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29f).** Epoxide (*R*)-**27** (0.487 g, 2 mmol) and pyrido-pyrazinone **28f** were reacted following general procedure J to afford **29f** (0.44 g, 52% yield) as a white solid; ^{1}H NMR (DMSO-d_6) δ: 8.21 (s, 1 H), 7.93 (d, *J* = 9.6 Hz, 1 H), 6.75 (d, *J* = 8.0 Hz, 1 H), 6.71 (d, *J* = 9.7 Hz, 1 H), 4.87 (d, *J* = 6.3 Hz, 1 H), 4.51 (dd, *J* = 8.2, 12.9 Hz, 1 H), 4.38 (dd, *J* = 8.2, 12.9 Hz, 1 H), 4.03 (s, 3 H), 3.85 (m, 1 H), 3.64 (m, 1 H), 3.29 (m, 1 H), 3.14 (m, 1 H), 2.83 (t, *J* = 10.6 Hz, 1 H), 1.92-1.85 (m, 2 H), 1.37 (s, 9 H), 1.37-1.32 (overlaid m, 2 H); ESI-MS $(M+H)^+$ m/z 421.

1-((1*S*)-2-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-hydroxyethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (30). Carbamate **29a** (2.1 g, 5 mmol) was reacted following general procedure G to afford **30** (1.1 g, 69% yield) as a colorless foam; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, *J* = 8.4 Hz, 1 H), 7.82 (d, *J* = 9.4 Hz, 1 H), 6.70-6.67 (d, *J* = 8.4 Hz, 1 H), 6.46 (d, *J* = 9.4 Hz, 1 H), 4.64 (dd, *J* = 8.8, 12.9 Hz, 1 H), 4.36 (m, 1 H) 4.32 (dd, *J* = 4.2, 12.9 Hz, 1 H), 3.93 (s, 3 H), 3.91 (m, 1 H), 3.76 (m, 1 H), 3.16-3.08 (m, 2 H), 2.78 (t, *J* = 10.4 Hz, 1 H), 2.52 (m, 1 H), 1.87 (m 1 H), 1.58-1.48 (m, 2 H), 1.32 (br s, 1 H), 1.10 (m, 1 H); ESI-MS $(M+H)^+$ m/z 320.

6-((((3*R*,6*S*)-6-((1*S*)-1-Hydroxy-2-(7-methoxy-2-oxo-1,8-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (5). Amine **30** (0.1 g, 0.31 mmol) and aldehyde **10** were reacted following general procedure F to afford **5** (0.020 g, 14% yield) as an off-white solid; mp = 174.3±0.2°C; ^{1}H NMR (DMSO-d_6) δ: 11.15 (s, 1 H), 8.01 (d, *J* = 8.4 Hz, 1 H), 7.83 (d, *J* = 9.4 Hz, 1 H), 7.29 (d, *J* = 8.1 Hz, 1 H), 7.00 (d, *J* = 8.1 Hz, 1 H), 6.69 (d, *J* = 8.4 Hz, 1 H), 6.47 (d, *J* = 9.4 Hz, 1 H), 4.65 (dd, *J* = 8.8, 12.8 Hz, 1 H), 4.59 (s, 2 H), 4.37 (d, *J* = 6.6 Hz, 1 H), 4.31 (dd, *J* = 3.9, 12.6 Hz, 1 H), 3.95-3.87 (overlaid m, 2 H), 3.93 (s, 3 H), 3.73-3.63 (m, 2 H), 3.17 (m, 1 H), 2.89 (t, *J* = 10.5 Hz, 1 H), 2.42 (m, 1 H), 2.06-1.94 (m, 2 H), 1.62-1.48 (m, 2 H), 1.18 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ: 166.4, 163.7, 163.1, 152.9, 149.3, 141.2, 140.4, 138.2, 138.0, 123.8, 118.9, 117.1, 109.7, 105.9, 79.2, 72.5, 70.0, 67.2, 54.2, 53.4, 51.4, 43.8, 30.8, 26.3; ESI-MS $(M+H)^+$ m/z 482.

1-((1*S*)-2-((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-hydroxyethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (35). Amine **30** (0.1 g, 0.31 mmol) and aldehyde **31** were reacted following general procedure F to afford **35** (0.088 g, 61% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, *J* = 8.4 Hz, 1 H), 7.99 (s, 1 H), 7.83 (d, *J* = 9.4 Hz, 1 H), 6.91 (s, 1 H), 6.69 (d, *J* = 8.4 Hz, 1 H), 6.47 (d, *J* = 9.4 Hz, 1 H), 4.65 (dd, *J* = 8.8, 12.8 Hz, 1 H), 4.38 (d, *J* = 6.6 Hz, 1 H), 4.34-4.25 (m, 5 H), 3.93 (s, 3 H), 3.9-3.85 (overlaid m, 2 H), 3.70-3.60 (m, 2 H), 3.16 (m, 1 H), 2.88 (t, *J* = 10.5 Hz, 1 H), 2.42 (m, 1 H), 2.08-1.94 (m, 2 H), 1.61-1.45 (m, 2 H), 1.17 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ: 166.7, 163.1, 154.7, 150.3, 149.3, 140.4, 140.2, 138.2, 138.1, 123.8, 110.2, 109.7, 105.8, 79.2, 72.5, 70.0, 65.4, 64.4, 54.2, 53.2, 51.7 43.8, 30.8, 26.2; ESI-MS $(M+H)^+$ m/z 469; HR ESI-MS $(M+H)^+$ m/z = 469.2086 (calc. for $C_{24}H_{29}N_4O_6$: 469.2087); ^{t}R = 0.76.

1-((1*S*)-2-((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-hydroxyethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (36). Amine **30** (0.11 g, 0.34 mmol) and aldehyde **32** were reacted following general procedure F to afford **36** (0.085 g, 51% yield) as an off-white solid; mp = 132.9°C; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, *J* = 8.4 Hz, 1 H), 7.92 (s, 1 H), 7.82 (d, *J* = 9.4 Hz, 1 H), 7.13 (s, 1 H), 6.68 (d, *J* = 8.4 Hz, 1 H), 6.46 (d, *J* = 9.4 Hz,

1 H), 4.64 (dd, *J* = 8.8, 12.7 Hz, 1 H), 4.38-4.28 (m, 4 H), 3.94 (s, 3 H), 3.94-3.86 (overlaid m, 2 H), 3.70-3.60 (m, 2 H), 3.26-3.21 (m, 2 H), 3.16 (m, 1 H), 2.88 (t, *J* = 10.5 Hz, 1 H), 2.42 (m, 1 H), 2.07 (m, 1 H), 1.99 (m, 1 H), 1.61-1.47 (m, 2 H), 1.19 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ: 164.7, 164.2, 150.3, 149.0, 147.6, 139.3, 139.2, 137.7, 129.5, 120.2, 118.8, 109.8, 106.6, 78.7, 72.9, 72.1, 64.8, 54.1, 53.2, 51.3, 45.2, 30.2, 26.3, 25.7; ESI-MS $(M+H)^+$ m/z 485; HR ESI-MS $(M+H)^+$ m/z = 485.1855 (calc. for $C_{24}H_{29}N_4O_5S$: 485.1858); ^{t}R = 0.79.

1-(2-((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-hydroxyethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (37). Amine **30** (0.1 g, 0.31 mmol) and aldehyde **33** were reacted following general procedure F to afford **37** (0.039 g, 27% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, *J* = 8.4 Hz, 1 H), 7.83 (d, *J* = 9.4 Hz, 1 H), 7.17 (s, 1 H), 6.69 (d, *J* = 8.4 Hz, 1 H), 6.47 (d, *J* = 9.4 Hz, 1 H), 4.65 (m, 1 H), 4.51-4.46 (m, 2 H), 4.41-4.34 (m, 3 H), 4.30 (m, 1 H), 3.93 (s, 3 H), 3.96-3.86 (overlaid m, 2 H), 3.88-3.78 (m, 2 H), 3.16 (m, 1 H), 2.88 (t, *J* = 10.5 Hz, 1 H), 2.40 (m, 1 H), 2.27 (m, 1 H), 1.98 (m, 1 H), 1.62-1.45 (m, 2 H), 1.19 (m, 1 H); ESI-MS $(M+H)^+$ m/z 470; HR ESI-MS $(M+H)^+$ m/z = 470.2044 (calc. for $C_{23}H_{28}N_5O_6$: 470.2039); ^{t}R = 0.71.

1-((1*S*)-2-((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-hydroxyethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (38). Amine **30** (0.1 g, 0.31 mmol) and aldehyde **34** were reacted following general procedure F to afford **38** (0.020 g, 14% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 8.00 (d, *J* = 8.4 Hz, 1 H), 7.82 (d, *J* = 9.4 Hz, 1 H), 7.52 (s, 1 H), 6.68 (d, *J* = 8.4 Hz, 1 H), 6.46 (d, *J* = 9.4 Hz, 1 H), 4.64 (dd, *J* = 8.5, 12.6 Hz, 1 H), 4.58-4.55 (m, 2 H), 4.37 (d, *J* = 6.6 Hz, 1 H), 4.31 (dd, *J* = 3.9, 12.6 Hz, 1 H), 3.96 (s, 3 H), 3.96-3.85 (overlaid m, 2 H), 3.85-3.75 (m, 2 H), 3.31-3.24 (m, 2 H), 3.16 (m, 1 H), 2.87 (t, *J* = 10.5 Hz, 1 H), 2.42 (m, 1 H), 2.10 (m, 1 H), 2.00 (m, 1 H), 1.62-1.46 (m, 2 H), 1.18 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ: 163.7, 163.1, 160.1, 157.9, 149.3, 140.4, 138.2, 126.0, 125.5, 118.9, 109.7, 105.9, 79.1, 72.4, 70.0, 66.6, 54.2, 53.1, 49.6, 43.8, 30.7, 26.2, 25.5; ESI-MS $(M+H)^+$ m/z 486; HR ESI-MS $(M+H)^+$ m/z = 486.1813 (calc. for $C_{23}H_{28}N_5O_5S$: 486.1811); ^{t}R = 0.73.

General Procedure I: Mesylate and azide formation. To an ice-chilled suspension of alcohol **29a-d** (1 equiv) in DCM (3.5 mL/mmol), were added TEA (2 equiv) and Ms-Cl (1.2 equiv). The reaction was stirred at the same temperature for 40 minutes. Saturated $NaHCO_3$ (6mL/mmol) and DCM (10 mL/mmol) were added. The two layers were decanted and the organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. A mixture of the latter crude mesylate (1 equiv) and NaN_3 (3 equiv) in DMF (8 mL/mmol) was heated at 80°C for 20 h. The solvent was removed in vacuo, and the residue was partitioned between water (10 mL/mmol) and EtOAc (25 mL/mmol). The aq layer was extracted with EtOAc (2 x 12 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 gradient) to afford corresponding azide **39a-d**.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*)-1-Azido-2-(7-methoxy-2-oxo-1,8-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (39a).** Alcohol **29a** (0.872 g, 2.0 mmol) was reacted following general procedure I to afford the azide **39a** (0.62 g, 67% yield) as a white foam. ^{1}H NMR (DMSO-d_6) δ: 8.05 (d, J = 8.4 Hz, 1 H), 7.88 (d, J = 9.5 Hz, 1 H), 6.77 (d, J = 7.6 Hz, 1 H), 6.73 (d, J = 8.4 Hz, 1 H), 6.50 (d, J = 9.4 Hz, 1 H), 4.94 (dd, J = 9.8, 13.1 Hz, 1 H), 4.36 (dd, J = 4.0, 13.2 Hz, 1 H), 3.97 (s, 3 H), 3.86 (m, 1 H), 3.74 (m, 1 H), 3.48 (m, 1 H), 3.34 (m, 1 H), 2.99 (t, J = 10.7 Hz, 1 H) 2.42 (m, 1 H), 1.90 (m, 1 H), 1.75 (m, 1 H), 1.58 (m, 2 H), 1.41 (m, 1 H), 1.36 (s, 9 H); ESI-MS $(M+H)^+$ m/z 445.

1-((1*S*)-2-((2*S*,5*R*)-5-Aminotetrahydro-2*H*-pyran-2-yl)-2-azidoethyl)-7-methoxy-1,8-naphthyridin-2(*1H*)-one (40a). N-Boc protected amine **39a** (0.61 g, 1.38 mmol) was reacted following general procedure G to afford amine **40a** (0.47 g, > 95% yield) as a colorless foam; ^{1}H NMR (DMSO-d_6) δ: 8.05 (d, J = 8.4 Hz, 1 H), 7.88 (d, J = 9.4 Hz, 1 H), 6.73 (d, J =8.4 Hz, 1 H), 6.50 (d, J = 9.4 Hz, 1 H), 4.93 (dd, J = 9.6, 13.2 Hz, 1 H), 4.36 (dd, J = 4.0, 13.2 Hz, 1 H), 3.96 (s, 3 H), 3.82 (ddd, J = 2.1, 4.6, 10.6 Hz, 1 H), 3.72 (m, 1 H), 3.46 (ddd, J = 2.3, 4.0, 11.1 Hz, 1 H), 2.88 (t, J = 10.5 Hz, 1 H), 2.57 (m, 1 H), 1.89 (m, 1 H), 1.69 (m, 1 H), 1.54 (m, 1 H), 1.40-1.31 (br s, 2 H), 1.17 (m, 1 H); ESI-MS $(M+H)^+$m/z 445.

6-((((3*R*,6*S*)-6-((1*S*)-1-Azido-2-(7-methoxy-2-oxo-1,8-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (41a). Amine **40a** (0.09 g, 0.26 mmol) and aldehyde **10** were reacted following general procedure F to afford **41a** (0.116 g, 87% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1 H), 8.08 (d, J = 8.4 Hz, 1 H), 7.90 (d, J = 9.5 Hz, 1 H), 7.31 (d, J = 8.1 Hz, 1 H), 7.03 (d, J = 8.1 Hz, 1 H), 6.76 (d, J = 8.4 Hz, 1 H), 6.53 (d, J = 9.4 Hz, 1 H), 4.95 (dd, J = 9.7, 13.2 Hz, 1 H), 4.62 (s, 2 H), 4.37 (dd, J = 3.9, 13.3 Hz, 1 H), 4.03 (m, 1 H), 3.98 (s, 3 H), 3.76 (m, 1 H), 3.75-3.65 (m, 2 H), 3.52 (ddd, J = 2.0, 4.0, 11.2 Hz, 1 H), 3.01 (t, J = 10.6 Hz, 1 H), 2.54 (m, 1 H), 2.06 (m, 1 H), 1.74 (m, 1 H), 1.53 (m, 1 H), 1.19-1.33 (m, 2 H); ESI-MS $(M+H)^+$ m/z 507.

General Procedure J: Azide reduction. To a solution of azide derivative **40a-f** (1 equiv) in THF (10 mL/mmol) were added PPh_3 (1.5 equiv) and water (0.5 mL/mmol). The mixture was heated at 60°C overnight. The volatiles were removed under reduced pressure and the residue was purified by chromatography (DCM-MeOH containing 0.5% NH_4OH gradient) to afford corresponding NBTI **6**, **42-46**.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(7-methoxy-2-oxo-1,8-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (6). Azide **41a** (0.11 g, 0.22 mmol) was reacted following general procedure J to afford **6** (0.096 g, 93 % yield) as an off-white solid; mp = 162.1±0.2°C; ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 8.4 Hz, 1 H), 7.82 (d, J = 9.4 Hz, 1 H), 7.28 (d, J = 8.0 Hz, 1 H), 6.99 (d, J = 8.1 Hz, 1 H), 6.68 (d, J = 8.4 Hz, 1 H), 6.46 (d, J = 9.4 Hz, 1 H), 4.58 (s, 2 H), 4.50 (dd, J = 9.0, 12.5 Hz, 1 H), 4.29 (dd, J = 5.7, 12.5 Hz, 1 H), 3.91 (overlaid m, 1 H), 3.92 (s, 3 H), 3.72-3.62 (m, 2 H), 3.06-3.14 (m, 2 H), 2.93 (t, J = 10.5 Hz, 1 H), 2.42 (m, 1 H), 2.04-1.96 (m, 2 H), 1.60-1.48 (m, 2 H), 1.21 (m, 1 H), 3 NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 163.8, 163.2, 152.9, 149.3, 141.2, 140.4, 138.2, 138.0, 123.8, 118.9, 117.1, 109.7, 105.9, 79.6, 72.6, 67.2, 54.1, 53.5, 53.4, 51.5, 44.7, 30.9, 27.0; ESI-MS $(M+H)^+$ m/z 481; HR ESI-MS $(M+H)^+$ m/z = 481.2202 (calc. for $C_{24}H_{29}N_6O_5$: 481.2199); ^{t}R = 0.61.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(7-methoxy-2-oxo-1,5-naphthyridin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (42). Alcohol **29b** (0.65 g, 1.55 mmol) was reacted sequentially following general procedures I, F and J to afford NBTI **42** (0.090 g, overall yield: 12% yield) as an off-white solid; mp = 135.6±0.1°C; ^{1}H NMR (DMSO-d_6) δ: 11.1 (br s, 1 H), 8.04 (s, 1 H), 7.74 (d, *J* = 8.9 Hz, 1 H), 7.31 (d, *J* = 8.0 Hz, 1 H), 6.97-7.10 (m, 3 H), 4.61 (s, 2 H), 4.29 (dd, *J* = 8.5, 14.0 Hz, 1 H), 4.09 (dd, *J* = 5.1, 14.0 Hz, 1 H), 4.00 (m, 1 H), 3.89 (s, 3 H), 3.74-3.66 (m, 2 H), 3.13 (m, 1 H), 3.01 (m, 1 H), 2.93 (t, *J* = 10.5 Hz, 1 H), 2.47 (m, 1 H), 2.06-1.93 (m, 2 H), 1.60-1.53 (m, 2 H), 1.18 (m, 1 H), 2NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 162.0, 156.7, 152.8, 141.2, 140.7, 138.0, 137.9, 134.8, 131.8, 123.9, 121.8, 117.1, 105.7, 77.6, 72.5, 67.2, 56.5, 53.3, 53.0, 51.4, 45.1, 30.6, 26.4; ESI-MS $(M+H)^+$ m/z 481; HR ESI-MS $(M+H)^+$ m/z = 481.2200 (calc. for $C_{23}H_{28}N_5O_5S$: 481.2199); ^{t}R = 0.57.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(7-methoxy-2-oxoquinolin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (43). Alcohol **29c** (0.78 g, 1.87 mmol) was reacted sequentially following general procedures I, F and J to afford NBTI **43** (0.105 g, overall yield: 23% yield) as an off-white solid; mp = 102.5±0.1°C; ^{1}H NMR (DMSO-d_6) δ: 7.78 (d, *J* = 9.4 Hz, 1 H), 7.60 (d, *J* = 8.7 Hz, 1 H), 7.28 (d, *J* = 8.1 Hz, 1 H), 7.06 (d, *J* = 1.6 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 6.85 (m, 1 H), 6.39 (d, *J* = 9.4 Hz, 1 H), 4.59 (s, 2 H), 4.29 (m, 1 H), 4.10-4.01 (m, 2 H), 3.97 (m, 1 H), 3.83 (s, 3 H), 3.07 (m, 1 H), 2.98 (m, 1 H), 2.89 (t, *J* = 10.5 Hz, 1 H), 2.45 (overlaid m, 1 H), 1.98 (m, 1 H), 1.62-1.48 (m, 2 H), 1.06-1.29 (m, 2 H), 4NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 162.7, 161.9, 152.7, 141.5, 141.2, 140.0, 138.1, 131.0, 123.9, 118.2, 117.1, 115.1, 110.4, 99.3, 77.1, 72.4, 67.2, 56.1, 53.3, 52.9, 51.4, 44.2, 30.5, 26.4; ESI-MS $(M+H)^+$ m/z 481; HR ESI-MS $(M+H)^+$ m/z = 480.2245 (calc. for $C_{25}H_{30}N_5O_5$: 480.2246); ^{t}R = 0.62.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(7-methoxy-2-oxoquinoxalin-1(*2H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (44). Alcohol **29d** (1.6 g, 3.81 mmol) was reacted sequentially following general procedures I, F and J to afford NBTI **44** (0.08 g, overall yield: 30% yield) as an off-white solid; mp = 99.8±0.1°C; ^{1}H NMR (DMSO-d_6) δ: 8.25 (d, *J* = 2.4 Hz, 1 H), 7.84 (d, *J* = 9.7 Hz, 1 H), 7.48 (d, *J* = 2.3 Hz, 1 H), 7.28 (d, *J* = 8.1 Hz, 1 H),

6.99 (d, *J* = 8.1 Hz, 1 H), 6.63 (d, *J* = 9.7 Hz, 1 H), 4.59 (s, 2 H), 4.29 (m, 1 H), 4.09 (m, 1 H), 3.95 (m, 1 H), 3.83 (s, 3 H), 3.73-3.63 (m, 2 H), 3.05 (m, 1 H), 2.95 (m, 1 H), 2.86 (t, *J* = 10.5 Hz, 1 H), 2.45 (overlaid m, 1 H), 1.98 (m, 1 H), 1.62-1.48 (m, 2 H), 1.16 (m, 1 H), 4 NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 161.7, 155.7, 152.7, 147.0, 141.2, 138.1, 134.8, 131.7, 128.4, 123.9, 117.1, 111.6, 99.1, 77.5, 72.4, 67.2, 56.4, 53.3, 52.6, 51.4, 44.6, 30.5, 26.4; ESI-MS $(M+H)^+$ m/z 481; HR ESI-MS $(M+H)^+$ m/z = 481.2201 (calc. for $C_{24}H_{29}N_6O_5$: 481.2199); ^{t}R = 0.59.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(6-methoxy-3-oxopyrido[2,3-*b*]pyrazin-4(*3H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (45). Alcohol **29e** (0.41 g, 1 mmol) was reacted sequentially following the procedure described for the preparation of azide **18**, general procedures F and J to afford NBTI **45** (0.075 g, overall yield: 3% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 11.1 (br s, 1 H), 8.13 (d, *J* = 8.6 Hz, 1 H), 8.11 (s, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.02 (d, *J* = 8.1 Hz, 1 H), 6.82 (d, *J* = 8.6 Hz, 1 H), 4.61 (s, 2 H), 4.48 (dd, *J* = 9.4, 12.6 Hz, 1 H), 4.24 (dd, *J* = 4.8, 12.6 Hz, 1 H), 3.98 (overlaid m, 1 H), 3.96 (s, 3 H), 3.73-3.66 (m, 2 H), 3.17 (m, 1 H), 3.13 (m, 1 H), 2.95 (t, *J* = 10.5 Hz, 1 H), 2.46 (overlaid m, 1 H), 2.10-1.86 (m, 2 H), 1.63-1.53 (m, 2 H), 1.49-1.17 (m, 2 H), 1NH missing; ESI-MS $(M+H)^+$ m/z 482; HR ESI-MS $(M+H)^+$ m/z = 482.2147 (calc. for $C_{23}H_{28}N_7O_5$: 482.2151); ^{t}R = 0.59.

6-((((3*R*,6*S*)-6-((1*S*)-1-Amino-2-(3-methoxy-6-oxopyrido[2,3-*b*]pyrazin-5(*6H*)-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (46). Alcohol **29f** (0.29 g, 0.7 mmol) was reacted sequentially following the procedure described for the preparation of azide **18**, general procedures F and J to afford NBTI **46** (0.012 g, overall yield: 6% yield) as an off-white solid; ^{1}H NMR (DMSO-d_6) δ: 11.10 (br s, 1 H), 8.21 (s, 1 H), 7.93 (d, *J* = 9.6 Hz, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.02 (d, *J* = 8.1 Hz, 1 H), 6.71 (d, *J* = 9.6 Hz, 1 H), 4.62 (s, 2 H), 4.49 (dd, *J* = 9.2, 12.7 Hz, 1 H), 4.26 (dd, *J* = 4.9, 12.8 Hz, 1 H), 4.01 (s, 3 H), 3.96 (overlaid m, 1 H), 3.73-3.66 (m, 2 H), 3.15 (m, 1 H), 3.07 (m, 1 H), 2.97 (t, *J* = 10.5 Hz, 1 H), 2.44 (overlaid m, 1 H), 2.10-1.84 (m, 2 H), 1.62-1.52 (m, 2 H), 1.49-1.00 (m, 2 H), 1NH missing; ^{13}C NMR (DMSO-d_6) δ: 166.4, 162.5, 159.1, 152.9, 145.3, 141.2, 139.0, 138.0, 130.3, 126.6, 123.8, 122.1, 117.1, 79.3, 72.6,

67.2, 54.6, 53.4, 53.2, 51.4, 44.7, 30.8, 26.9; ESI-MS $(M+H)^+$ m/z 482; HR ESI-MS $(M+H)^+$ m/z = 482.2152 (calc. for $C_{23}H_{28}N_7O_5$: 482.2151); ^tR = 0.58.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

All authors declare no competing financial interest.

ACKNOWLEDGMENTS

Guillaume Brunet, Jonathan Delers, Marina Dos Santos, Mika Gaertner, Hakim Hadana, Verena Kägi, Andreja Knezevic, Stéphanie Kraemer, François Le Goff, Kerya Long, Gregor Meier, Etienne Ochala, Daniela Sabato, Romain Sube and Maria Weiss are warmly thanked for the technical assistance they provided during the work described in the manuscript. We are grateful to the DMPK, Preformulation and Preclinical Galenics teams as well as to our colleagues from Process Research for their dedicated and continuous support. Dr Luboš Remeň is thanked for a helpful discussion on the transformation of **29a-d** to **39a-d**.

ABBREVIATIONS USED

GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; FQ, fluoroquinolone; NBTI, novel (non-fluoroquinolone) bacterial type II topoisomerase inhibitor; CRE, Carbapenem-resistant *Enterobacteriaceae*; LHS, bicyclic aromatic left-hand side; RHS, aromatic right-hand side (as positioned in Figure 1); DPPA, diphenyl phosphoryl azide; DIAD, diisopropyl azodicaroxylate; DIPEA, *N*,*N*-diisopropylethylamine; iPSC-CM, cardiomyocytes derived from human induced pluripotent stem cells; CFU, colony forming unit; RIA, Relaxation Inhibitory Activity; SCIA, Supercoiling Inhibitory Activity; MeCN, acetonitrile; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; Et_2O, diethyl ether; TEA, triethylamine; wt, wild-type.

ASSOCIATED CONTENT

*** Supporting Information**

Experimental details on MIC testing; DNA gyrase and Topo IV inhibition assays; iPSC-CM assay; safety pharmacology in guinea pigs; mice, in life procedures, graphical representations and statistical details; pharmacokinetics in the mouse, in the rat and in the dog; preparation of **11a, 11b** and alternative preparation of (*R*)**-27**; preparation, antibacterial and topoisomerases activities of analogues of **20** and **21** featuring alternative RHS (**S1, S2** and **S3)**; preparation and antibacterial activities of the analogue of **42** featuring a (*S*)-configured -CH_2-CH(OH)- linker (**S4**); MIC determination of compounds **5, 6, 20, 22, 23, 35-38** and **42-46** against *E. coli* mutants. PDB coordinates for computation model of **21** docked to DNA-*S. aureus* gyrase dimer (4PLB). Molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.organic

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

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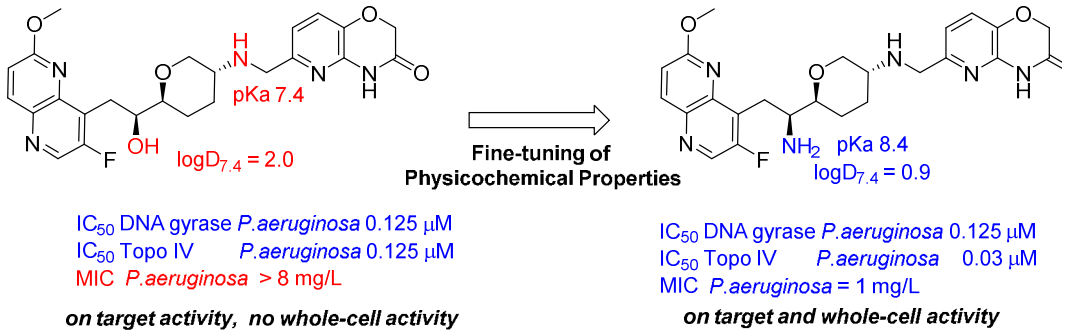
Table of Contents graphic



IC_{50} DNA gyrase *P.aeruginosa* 0.125 μM
IC_{50} Topo IV *P.aeruginosa* 0.125 μM
MIC *P.aeruginosa* > 8 mg/L

on target activity, no whole-cell activity

IC_{50} DNA gyrase *P.aeruginosa* 0.125 μM
IC_{50} Topo IV *P.aeruginosa* 0.03 μM
MIC *P.aeruginosa* = 1 mg/L

on target and whole-cell activity