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Optimization of TopoIV Potency, ADMET Properties, and hERG Inhibition of 5-Amino-1,3-dioxane-Linked Novel Bacterial Topoisomerase Inhibitors: Identification of a Lead with *In Vivo* Efficacy against MRSA

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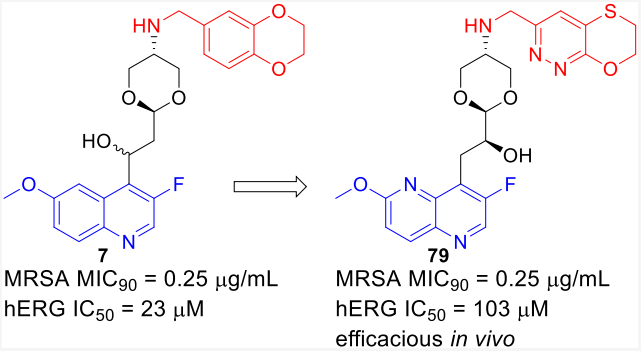
ABSTRACT: Novel bacterial topoisomerase inhibitors (NBTIs) are among the most promising new antibiotics in preclinical/clinical development. We previously reported dioxane-linked NBTIs with potent antistaphylococcal activity and reduced hERG inhibition, a key safety liability. Herein, polarity-focused optimization enabled the delineation of clear structure−property relationships for both microsomal metabolic stability and hERG inhibition, resulting in the identification of lead compound **79**. This molecule demonstrates potent antibacterial activity against diverse Gram-positive pathogens, inhibition of both DNA gyrase and topoisomerase IV, a low frequency of resistance, a favorable *in vitro* cardiovascular safety profile, and *in vivo* efficacy in a murine model of methicillin-resistant *Staphylococcus aureus* infection.



INTRODUCTION

Nearly 60 years ago, the discovery of a new class of small molecules, first exemplified by nalidixic acid, changed the practice of antibacterial therapy.[1] Subsequent research first identified DNA gyrase[2] as a key target of the quinolone class.[3] The discovery of the related enzyme topoisomerase IV (TopoIV)[4] and the demonstration that it was also inhibited by the quinolones[5] followed some years later. Generations of medicinal chemistry optimization[6] led to the identification of the fluoroquinolones, among which ciprofloxacin, levofloxacin, and moxifloxacin remain as key components of the antibacterial armamentarium. Most recently, delafloxacin gained FDA approval in 2017, including an indication for certain infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[7] Thus, decades of research firmly established the bacterial topoisomerases (DNA gyrase and TopoIV) as favored targets for antibacterial therapy and demonstrated that inhibition by small-molecule therapeutics could deliver powerful new medicines.[8] Unfortunately, decades of high prescription volumes have seeded widespread resistance to fluoroquinolones,[9] and safety concerns have also eroded the usage of these agents in certain settings.[10] Considering the relative paucity of validated antibacterial targets, it is not surprising that numerous drug discovery efforts have sought new classes of bacterial topoisomerase inhibitors[11] to overcome these limitations of existing therapies.

One such class has come to be known as the NBTIs (Novel Bacterial Topoisomerase Inhibitors).[12,13] Beginning with groundbreaking discoveries at Smithkline Beecham[14] (later GSK) and Aventis[15] (later Novexel), several compounds have advanced into late-stage preclinical or clinical development. Alongside the molecular evolution of the NBTI class have come breakthroughs in structural biology[16,17] and pharmacology[18−20] that clearly distinguish NBTIs from fluoroquinolones. Gepotidacin, advanced to Phase 3 clinical trials by GSK, represents the vanguard of NBTI development and illustrates the tremendous potential of this class of therapies.[20−23] Noting historical precedent for the success of multiple antibacterials within a given class, our laboratory and others have focused on

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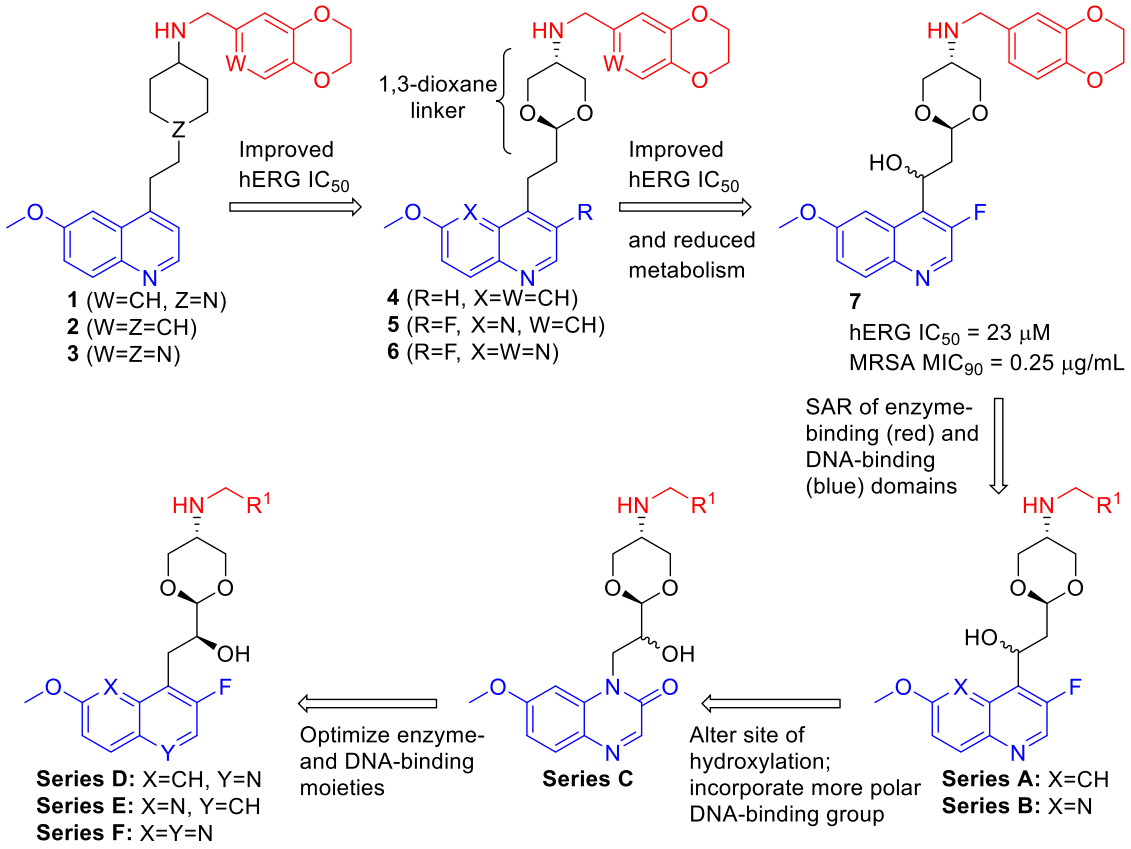

Figure 1. Optimization of dioxane-linked NBTIs leading to prototype **7** and subsequent structural diversification. Blue indicates the DNA-binding moiety, red indicates the enzyme-binding moiety, and black indicates the linker domain (see also Figure 2).

the continued optimization of NBTIs to deliver innovative new medicines.[12,13] Lowering the risk of cardiovascular toxicity, primarily through diminished inhibition of the hERG channel, has been a critical roadblock to success[24,25] and thus a key objective of several groups including our own.

Reck and colleagues demonstrated that reduced amine basicity and enhanced polarity could deliver an improved hERG profile within the NBTI class,[26−28] and we also adopted this strategy. We were particularly inspired by the amino-dioxane-containing PAK1 inhibitor reported by Ndubaku et al.,[29] believing that a 5-amino-1,3-dioxane linker moiety would achieve our rational design objectives while substantially simplifying the synthetic chemistry compared to earlier approaches. These efforts led to the identification of dioxane-linked amine-type NBTIs with potent antistaphylococcal activity and reduced hERG inhibition compared to other linkers.[30] Dioxane **4** (Figure 1) achieved a 6-fold improvement in hERG IC_{50} compared to piperidine **1** (5.1 vs 0.81 μM, respectively) and a 20-fold improvement over the more lipophilic and basic cyclohexane-linked **2** (0.26 μM). Incorporation of a fluoronaphthyridine DNA-binding moiety enhanced antistaphylococcal activity, with **5** being ∼4-fold more potent than **4** when tested against a USA300 MRSA isolate (0.125 vs 0.5 μg/mL, respectively).[31] Moreover, NBTI **5** and related analogues displayed excellent potency against fluoroquinolone-resistant (FQ^R) *S. aureus* strains and demonstrated potent *in vitro* inhibition of FQ^R *S. aureus* gyrase with the common S84L amino acid substitution. Potent antibacterial activity was likewise demonstrated against a variety of Gram-positive bacteria, including key multidrug-resistant pathogens such as MRSA and vancomycin-resistant *Enterococci* (VRE). Dioxane-linked NBTIs are more potent than a series of NBTIs with a dioxygenated linker derived from isomannide.[32] Consequently, we selected the dioxane-linked series for optimization efforts directed at five key objectives: (1) maintaining or improving whole-cell antibacterial activity, especially against MRSA, (2) enhancing TopoIV inhibition, (3) increasing potency against NBTI-resistant *S. aureus*, (4) further reducing hERG inhibition, and (5) increasing metabolic stability.

The fundamental approach was especially guided by enhancing polarity to reduce metabolism and hERG inhibition. Benzodioxane NBTI **4**[30] displayed very rapid metabolism in mouse microsomes (Table 1), such that quantitative determination of the half-life ($t_{1/2}$) and intrinsic clearance was not possible. NBTI **5**[31] was also metabolized quickly, with $t_{1/2}$ of only 2.4 min. Incorporation of the more polar

Table 1. *In Vitro* Metabolism of Select NBTIs in CD-1 Mouse Microsomes[a]

cmpd.	intrinsic Clearance (mL/min/kg)	half-life (min)	cLog P[b]
1	1940	2.81	3.77
3	2150	2.54	3.08
4	ND[c]	ND[d]	4.02
5	2243	2.43	3.90
6	ND[c]	ND[d]	3.22
7	835	6.54	2.44

[a]Charles River (Worcester, MA). [b]ChemDraw 19.1. [c]Clearance was too rapid to measure accurately. [d]Half-life was too short to measure accurately.

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pyridodioxane enzyme-binding motif in **6**[30] (ΔcLog P = −0.7 vs **5**) was insufficient to address this issue, as **6** was again very rapidly metabolized. Importantly, rapid metabolism was not limited to dioxane-linked analogues; piperidine-linked NBTIs **1** and **3**,[30] likewise displayed very short half-lives of 2.8 and 2.5 min, respectively. Reports by several groups suggested that hydroxylation of the linker domain would preserve bioactivity,[33−36] making it an attractive strategy for polarity-driven improvements to metabolic stability and hERG inhibition. Consequently, we synthesized the benzodioxane **7** as a hydroxylated prototype. Following promising results from the characterization of **7** (see below), we synthesized additional analogues to delineate structure−activity relationships of the enzyme-binding moiety R^1 (Series A) as well as several related series (B−F) that enabled the systematic exploration of the DNA-binding motif, position of hydroxylation, and additional R^1 groups.

RESULTS AND DISCUSSION

Prototype Characterization. A variety of foundational ADMET assays were conducted with **7** (Charles River, Worcester, MA, and Cleveland, OH). Mouse microsomal half-life was somewhat improved but still short ($t_{1/2}$ = 6.5 min). Notably, hERG inhibition was reduced compared to other benzodioxane NBTIs such as **5**[31] (23 vs 8.0 μM, respectively). Plasma protein binding was moderately high across species (89−94% in mouse, dog, and human). Thermodynamic solubility was 186 μM at pH 7.4 and improved somewhat in simulated intestinal fluid (340 μM at pH 6.6) and simulated gastric fluid (495 μM at pH 1.3), consistent with the weakly basic nature of the compound (calculated pK_a of 7.1, ACDLabs Percepta Classic pK_a).

An assessment of *in vitro* antibacterial activity was conducted. Compound **7** demonstrated excellent antistaphylococcal activity, with an MIC_{90} of 0.25 μg/mL against fluoroquinolone-resistant MRSA (Table S1, Supporting Information). Potent antibacterial activity was likewise observed against other Gram-positive pathogens, including vancomycin-resistant *Enterococcus faecium* (VRE) and penicillin-resistant *Streptococcus pneumoniae* strains. Highly diminished activity for *Pseudomonas aeruginosa* and no activity for *Enterobacterales* was observed. In contrast, the MIC against a lab strain of *Acinetobacter baumannii* was quite potent (0.12 μg/mL) but was somewhat weaker against three MDR *A. baumannii* isolates (0.5−8 μg/mL, see also Table 3 for additional results). We subsequently focused the majority of our antibacterial efforts on *S. aureus*.

The crystal structure of a ternary complex between a fusion construct[16] of *S. aureus* DNA gyrase, compound **7**, and a 20-bp DNA homoduplex was determined (Figure 2). The structure is characterized by three points of contact between the drug and the protein−DNA complex. The quinoline of the DNA-binding domain intercalates between base pairs 10 and 11 of the uncleaved, palindromic 20-mer dsDNA sequence. Notably, the quinoline inserts into the DNA in a level manner similar to GSK945237[37] (5IWM), rather than in a beveled manner reminiscent of GSK299423[16] (2XCR). The enzyme-binding motif fits into a hydrophobic binding pocket at the interface of the GyrA subunits analogous to previous structures. The aromatic ring stabilizes the drug in the enzyme-binding pocket through interactions with residues M121, V71, and M75. The linker provides hydrogen bonding interactions between the amine adjacent to the dioxane and residue D83 in each protein

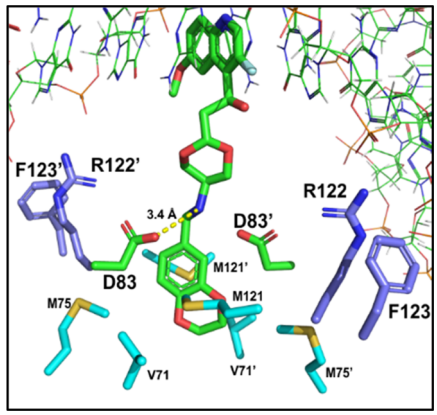

Figure 2. 2.6 Å protein crystal structure (PDB code 7MVS) showing the more favorable binding mode of compound **7** complexed with the DNA gyrase dimer (shown in sticks and annotated) and a dsDNA (shown in lines). The aspartates interacting with the inhibitor are shown in green, the residues for wild-type catalysis are shown in blue, and the hydrophobic pocket of the protein is shown in cyan. A second binding mode of compound **7** rotated 180° on the y-axis shows the linker nitrogen 3.5 Å from D83′.

monomer with distances between 3.4−3.5 Å. This is similar to the interactions of D83 with the inhibitors AM8191[36] (4PLB), GSK945237,[37] and gepotidacin[20] (6QTP). The overall binding mode is reminiscent of GSK299423,[16] where there is a symmetrical overlay of the flipped inhibitor with overlapping linker, DNA-binding, and enzyme-binding domains. However, the orientation of the inhibitor favors one binding mode over the other (demonstrating 70−80% occupancy in one conformation), rather than an equal likelihood of either binding mode.

Based on these promising results from compound **7**, we systematically explored structure−activity relationships through variations in the DNA-binding domain, enzyme-binding moiety, and site of linker hydroxylation (Series A−F, see Figure 1 and Schemes 1−6).

Chemistry. Series A and B compounds permitted the analysis of structure−activity relationships for both the DNA-binding (A versus B) and enzyme-binding moieties (diversity of R^1 groups). The synthesis (Scheme 1) was carried out according to the following general route. The previously reported[30] diol **8** was cyclized with 1,1,3,3-tetramethoxypropane to afford the dioxane **9**. Selective hydrolysis of the acyclic acetal under acidic aqueous conditions yielded aldehyde **10**; yields were higher in aqueous medium than in a mixed THF/water system (76 vs 31%, respectively). Series A alcohol **13** was prepared via lithiation of commercially available 3-fluoro-6-methoxyquinoline (**11**) with lithium diisopropylamide (LDA)[38] and subsequent trapping with aldehyde **10**, whereas the synthesis of Series B **14** employed a halogen metal exchange of the commercially available bromide **12** with *n*-hexyllithium and subsequent trapping with **10**. Low yields were at least partially attributable to competing reaction with the phthalimide protecting group. Deprotection of the phthalimide by reaction with ethanolamine in refluxing ethyl acetate[39] proceeded smoothly to provide **15** and **16**. Finally, reductive amination with a variety of commercially available aldehydes afforded **7** and **17**−**27**. Compounds from Series A and B were synthesized and tested as racemic mixtures.

Series C compounds incorporated the more polar quinoxalinone DNA-binding moiety and altered the site of

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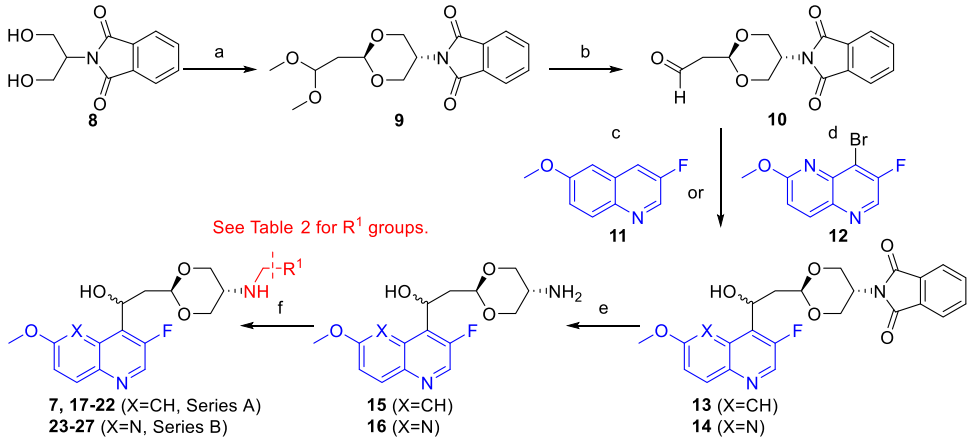
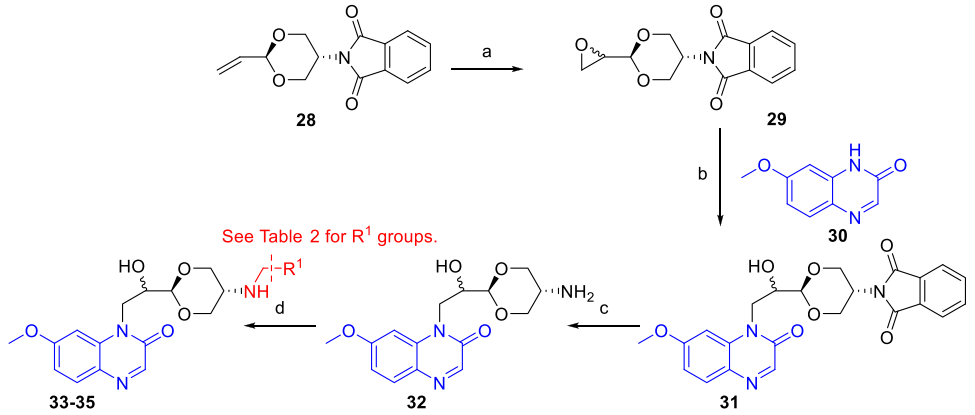
Scheme 1. Synthesis of Series A and Series B NBTIs[a]



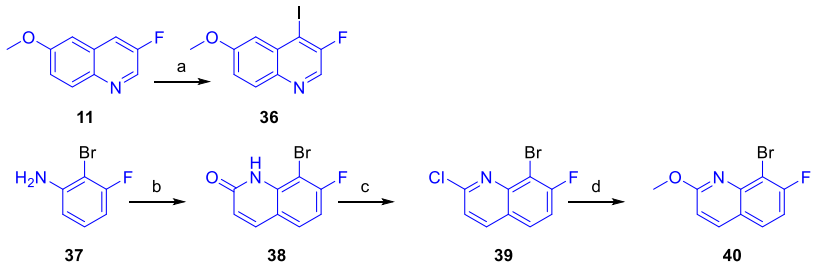
[a](a) *p*-TsOH, toluene, 85−90 °C, 26−60%. (b) PPTS, H_2O, 80 °C, 76%. (c) **11**, lithium diisopropylamide (LDA), tetrahydrofuran (THF), −78 °C, 3 h then **10**, −78 °C, 1 h, then warm to rt and quench, 19%. (d) **12**, *n*-hexyllithium, THF, −78 °C, 30 min, then **10**, 2 h, then warm to −20 °C, 3 h, and quench, 18%. (e) Excess ethanolamine, EtOAc, 70 °C, 38−73% for **15** and 66% for **16**. (f) RCHO, $ZnCl_2$, MeOH, 30 min, then $NaBH_3CN$.

Scheme 2. Synthesis of Series C NBTIs[a]



[a]Reagents: (a) *m*CPBA, CH_2Cl_2, 71%; (b) Cs_2CO_3, $Mg(OTf)_2$, *N*,*N*-dimethylformamide (DMF), 80 °C, 78%; (c) ethanolamine, EtOAc, 80 °C, 63%. d; RCHO, MeOH, CH_2Cl_2, $ZnCl_2$, 30 min, then $NaBH_3CN$.

Scheme 3. Synthesis of Halide Starting Materials for the Heck Reaction[a]



[a]For series D: (a) LDA, THF, −78 °C, 30 min, then I_2 in THF, −78 °C to rt, 42−90%. For Series E: (b) i. methyl 3,3-dimethoxypropionate, NaHMDS, THF, 0 °C to rt. ii. CH_2Cl_2, cone. H_2SO_4, 0°C, 86%; (c) DMF, toluene, 95 °C, then $POCl_3$, 88%; (d) toluene, 80 °C, then NaOMe/MeOH, 60%.

hydroxylation. Analogues were synthesized as racemic mixtures as summarized in Scheme 2. Our previously reported[30] vinyl-substituted dioxane **28** was epoxidized using *m*CPBA to afford intermediate **29**. The epoxide was then reacted with the known[26] quinoxalinone **30** under basic conditions to afford the hydroxylated intermediate **31**. The yield for this reaction was dramatically improved by adding one equivalent of magnesium triflate as a Lewis acid (78 vs 13% in its absence). The phthalimide was then removed with ethanolamine[39] as described above to afford primary amine **32**, and three analogues **33**−**35** were prepared by reductive amination.

The analogues synthesized in Series D−F enabled a more complete delineation of structure−activity relationships, including changes to lipophilicity across the DNA-binding

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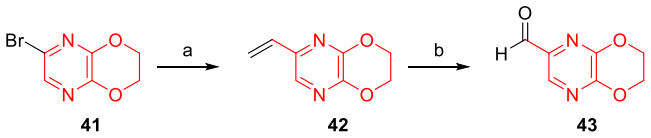
moieties in these three series. The preferred choice of the (*S*)-enantiomer of the hydroxyl group, as well as its enantioselective synthesis, followed the results and general strategy reported by Surivet et al.[40] with some modifications to protecting groups (Schemes 5 and 6). Halide starting materials for the initial Heck reaction were prepared according to Scheme 3 or purchased commercially (**12**). For Series D: fluoroquinoline **11** was metalated with LDA and trapped with iodine to afford **36**. For Series E: The commercially available aniline **37** was deprotonated with sodium bis(trimethylsilyl)-amide and reacted with methyl 3,3-dimethoxypropionate.[41] Aqueous workup followed by treatment with concentrated sulfuric acid in dichloromethane afforded intermediate **38**. Treatment of **38** with phosphoryl chloride afforded chloride **39**, and substitution of the chloride with sodium methoxide afforded compound **40**.[42]

With one exception, the required aldehydes for final analogue synthesis are commercially available or accessible by published routes. For compound **81**, aldehyde **43** was prepared according to Scheme 4. Alkene **42** was prepared by

Scheme 4. Synthesis of Aldehyde Starting Material for Analogue 81[a]



[a](a) Potassium vinyltrifluoroborate, $PdCl_2dppf$, NEt_3, *i*-PrOH, 21−30%; (b) OsO_4, $NaIO_4$, THF, H_2O, 79%.

coupling of the bromide **41** with potassium vinyltrifluoroborate under palladium catalysis. The Lemieux−Johnson oxidation of the alkene afforded aldehyde **43**.

As noted above, the synthesis of Series D−F followed the same overall route (Scheme 5). The synthesis of Series D is representative. Heck reaction of **36** with previously reported[30] vinyl-substituted dioxane **28** was carried out in anhydrous mesitylene to afford alkene **44**, which was taken without purification to the next step. Deprotection of the phthalimide provided amine **45**, and reprotection with a Boc group afforded **46**. This sequence was carried out because the low solubility of alkene **44** prevented its effective use in subsequent steps. An alternative approach beginning with synthesis of a Boc-protected 5-aminodioxane (analogous to **28**) was problematic owing to poor diastereoselectivity for the *trans*-isomer in the dioxane-forming reaction. Sharpless asymmetric dihydroxylation of alkene **46** proceeded with 90−93% ee in modest yield to afford diol **47**. Cyclization of the diol with carbonyldiimidazole (CDI) provided carbonate **48**. Benzylic reduction was effected with ammonium formate, Pd/C, and 5% $Pd/CaCO_3$ to afford alcohol **49**. The Boc group was removed with trifluoroacetic acid; a basic aqueous workup and purification by flash chromatography yielded the free primary amine **50**. Finally, reductive amination with a variety of aldehydes afforded the Series D analogues **51**−**57**.

The synthesis of Series E began with the preparation of key intermediate **40** (Scheme 3). The remaining synthetic sequence to yield Series E analogues **65**−**68** mirrored the route described above for Series D (see the Experimental Section for full details).

The synthesis of Series F initially utilized the same route as described above for Series D, beginning with bromide **12** (see the Experimental Section for full details). However, a revised shorter approach to the key primary amine **75** was then devised and implemented (Scheme 6). The key issue with the previous approach was the limited solubility of phthalimide **69**. We reasoned that a functionalized phthalimide protecting group lacking symmetry would increase solubility. We chose the 4-*tert*-butylphthalimide, reasoning that its steric bulk might also help disrupt intermolecular stacking interactions and that its lipophilicity would enhance solubility in organic solvents. To that end, the primary amine of serinol (**88**) was first protected by reaction with 4-*tert*-butylphthalic anhydride in refluxing toluene to afford diol **89**. The diol was treated with acrolein dimethyl acetal in dichloromethane in the presence of 5 Å molecular sieves to afford vinyl dioxane **90**. Heck reaction of **90** with bromonaphthyridine **12** afforded the coupled alkene **91**. Sharpless asymmetric dihydroxylation provided diol **92** in fair yield and 92−96% ee. Cyclization with CDI afforded carbonate **93**, and benzylic reduction as above afforded the homobenzylic alcohol **94**. Deprotection of the phthalimide moiety was effected with ethylenediamine[43] in refluxing ethyl acetate to afford the primary amine **75**. Final analogues in Series F were prepared using the primary amine prepared as in both Schemes 5 and 6. In the former case, the enantiopurity of the amine was lower (79−88% ee).

One analogue with a 5-methyl-dioxane was synthesized as described in Scheme 7. Bromonaphthyridine **12** was coupled with ethyl acrylate to afford enoate **95**. Reduction with diisobutylaluminum hydride afforded alcohol **96**, which was oxidized to aldehyde **97** with the Dess−Martin periodinane. Diol **98** was reacted with 4-*tert*-butylphthalic anhydride in refluxing toluene to afford the N-protected diol **99**. Condensation of diol **99** with aldehyde **97** in refluxing toluene with catalytic *p*-toluenesulfonic acid afforded the *trans*-dioxane **100**. Hydrogenation of the alkene over 10% Pd/C afforded intermediate **101**. Deprotection of the phthalimide was carried out using ethylenediamine in ethyl acetate at 70 °C to afford primary amine **102**. Finally, reductive amination of the commercially available aldehyde afforded compound **103**.

Microbiology. Following the characterization of the prototype compound **7**, several additional studies were conducted. As an initial evaluation of antistaphylococcal activity, minimum inhibitory concentrations (MICs, Table 2) were determined according to Clinical Laboratory and Standards Institute (CLSI) guidelines[44] against three strains of *S. aureus*: a laboratory reference strain (ATCC 29213), a ciprofloxacin-resistant MRSA strain (USA300),[45] and a ciprofloxacin-susceptible respiratory isolate from an individual with cystic fibrosis (CF).[32] Ciprofloxacin and compound **7** were used as controls in all assays. MICs to ciprofloxacin against ATCC 29213 were used for internal assay validation using the published quality control guidelines.[46] Compounds with benzylic alcohols (**7** and **17**−**27**) and those with a quinoxalinone DNA-binding motif (**33**−**35**) were assayed as racemic mixtures. Facile access to enantioenriched samples via Sharpless asymmetric dihydroxylation (*vide supra*) permitted evaluation of nonracemic mixtures of compounds with homobenzylic alcohols **51**−**57**, **65**−**68**, and **76**−**87**.

These NBTIs demonstrated excellent antistaphylococcal activity, with MICs of ≤0.5 μg/mL against all three strains for the great majority of compounds. MICs of up to 1−2 μg/mL were occasionally observed and tended to be associated with

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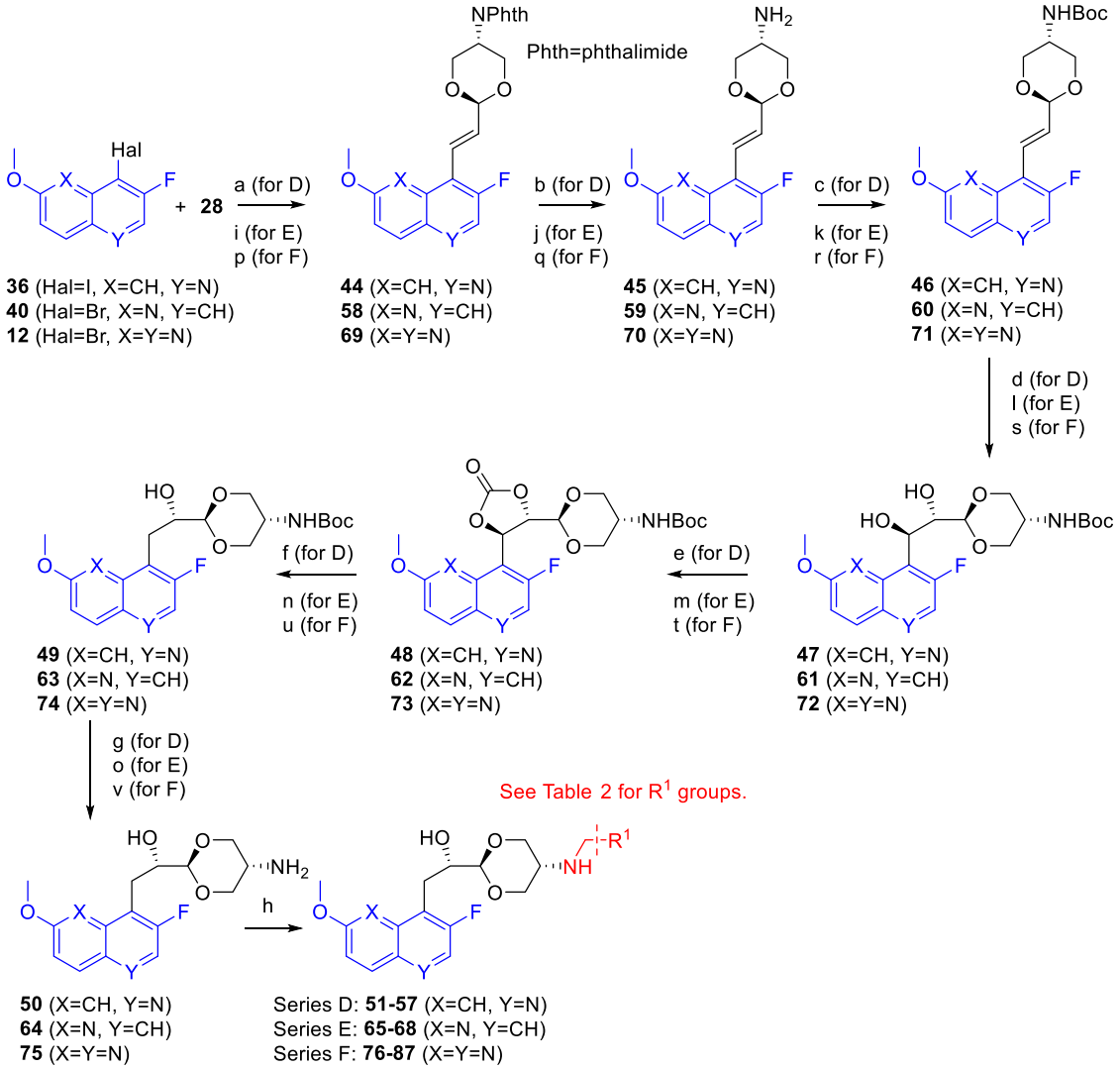
Scheme 5. Synthesis of Series D-F NBTIs[a]



[a]For Series D: (a) $Pd(OAc)_2$, PPh_3, Ag_2CO_3, mesitylene, 127 °C, carried directly to next step; (b) excess ethanolamine, EtOAc, 70 °C, 25−64%; (c) Boc anhydride, NEt_3, CH_2Cl_2, 0 °C to rt, 58−65%; (d) AD-mix *β*, *t*-BuOH/EtOAc/H_2O, $CH_3SO_2NH_2$, 26−46%; (e) CDI, NEt_3, 2-butanone, 60 °C, 42−87%; (f) $Pd/CaCO_3$, Pd/C, NH_4CHO_2, EtOH, EtOAc, 70−40 °C, 77%; (g) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C to rt, 65−81%; (h) RCHO, 4 Å mol. sieves, AcOH, THF, MeOH, 4 h, then $NaBH_3CN$. For Series E: steps i−o and h; for Series F: steps p−v and h. See the Experimental Section for details.

more polar enzyme-binding motifs, for example, pyridodioxane **17** and **24**, oxathiinopyridazines **18** and **25**, dioxanopyridazines **54** and **80**, and dioxanopyrazine **81**. Likewise, analogues bearing the more polar quinoxalinone DNA-binding motif (**33**−**35**) had generally higher MICs. NBTI **35**, which also carried the polar pyridooxazinone enzyme-binding moiety, was the least potent compound of the study. Matched-pair comparisons of DNA-binding moieties (comparing Series A to B or comparing Series D, E, and F) did not reveal other substantive differences. Despite the large disparity in susceptibility to ciprofloxacin between the ATCC 29213 and USA300 strains (0.25−0.5 vs 16 μg/mL, respectively), there was no consistent difference in activity for the NBTIs. These results support the established lack of cross-resistance in *S. aureus* between NBTIs and fluoroquinolones,[16] illustrating a key advantage of the NBTI class. MIC values for the CF isolate were likewise comparable to the ATCC strain.

A subset of analogues, chosen to capture structural and physicochemical diversity among analogues, was assayed more extensively (Table 3) against additional clinical isolates of *S. aureus*, *Enterococcus* spp., *S. pneumoniae*, and the Gram-negative pathogen *A. baumannii*. For eight of the nine compounds, MIC_{50} values were very potent (0.06−0.25 μg/mL) against both *S. aureus* and *Enterococcus* spp. and showed little difference between these pathogens. The more polar quinoxalinone derivative **34** displayed reduced activity against both organisms. The overall potency of these NBTIs against *S. pneumoniae* was weaker, with 2- to 64-fold higher MICs compared to *S. aureus* depending on the compound. MICs against *A. baumannii* were weaker still. A matched-pair comparison of the fluoroquinoline **53** (Series D) and the fluoronaphthyridine **79** (Series F) favored **79** by 2-fold.

The ubiquity of bacterial DNA gyrase opens the door for NBTIs to treat less commonly encountered pathogens, including biothreat pathogens. Indeed, gepotidacin has potent

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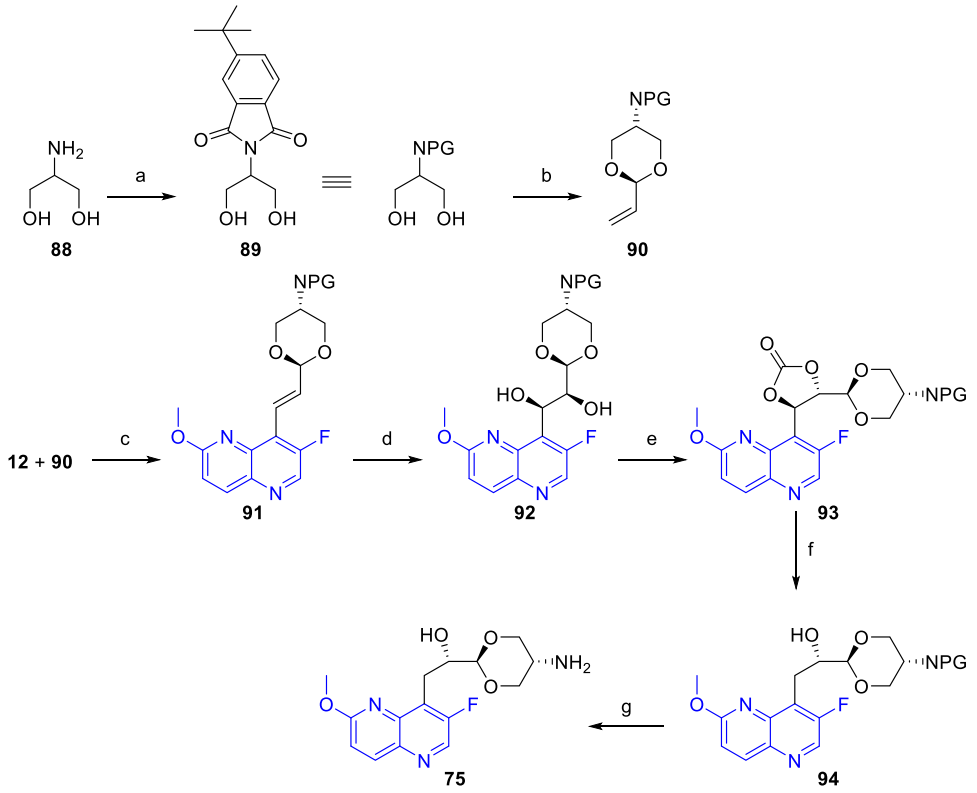
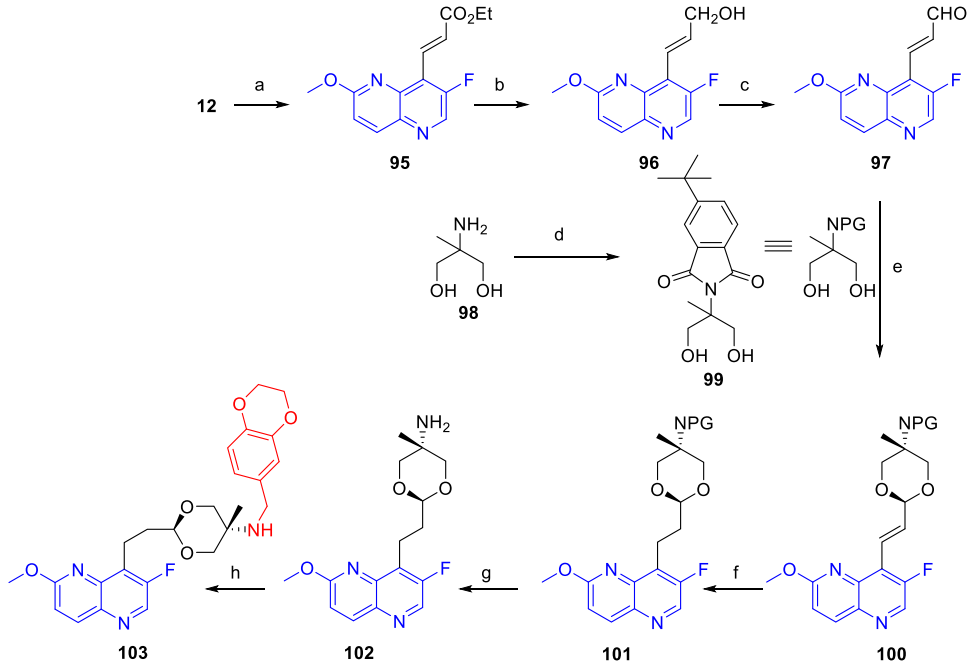
Scheme 6. Alternative Synthesis of Series F NBTIs[a]



[a](a) 4-*tert*-butylphthalic anhydride, toluene, reflux, 86%. (b) Acrolein dimethyl acetal, 5 Å mol. sieves, CH_2Cl_2, 68−87%. (c) $Pd(OAc)_2$, PPh_3, Ag_2CO_3, mesitylene, 135 °C, 41−71%. (d) AD-mix *β*, *t*-BuOH/EtOAc/H_2O, $CH_3SO_2NH_2$, 26−74%. (e) CDI, NEt_3, 2-butanone, 60 °C, 47−79%. (f) Pd/$CaCO_3$, Pd/C, NH_4CHO_2, EtOH, EtOAc, 70−40 °C, 30−59%. (g) Excess ethylenediamine, EtOAc, 70 °C, 71−87%.

Scheme 7. Synthesis of an NBTIs with a 5-Methyl Dioxane[a]



[a](a) $Pd(OAc)_2$, PPh_3, K_2CO_3, DMF, 130 °C, 54−65%; (b) DIBALH, toluene, THF, −78 °C; (c) Dess−Martin periodinane, CH_2Cl_2, 63−100% (2 steps); (d) 4-*tert*-butylphthalic anhydride, toluene, 110 °C, 87%; (e) *p*-TsOH, toluene, 110 °C, 23%; (f) H_2 (g) from H-Cube, 10% Pd/C, 90−98%; (g) ethylenediamine, EtOAc, 70 °C, 27%; (h) RCHO, 4 Å mol. sieves, AcOH, THF, MeOH, 4 h, then $NaBH_3CN$, 29%.

antibacterial activity against Gram-positive *Bacillus anthracis* and Gram-negative *Yersinia pestis* and *Francisella tularensis*, with MIC_{90} values of 0.5−1 μg/mL.[47] Noting the more potent anti-Gram-positive activity of the dioxane-linked NBTIs, MICs

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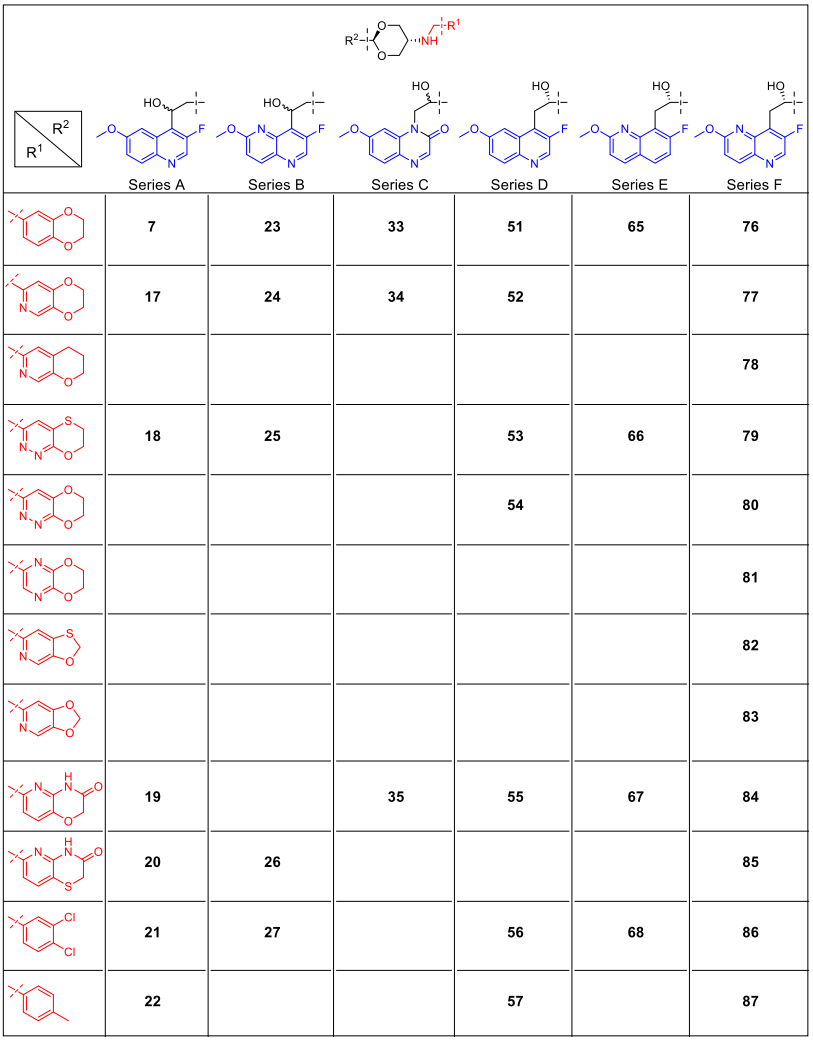
Table 2. Compound Structures and Antistaphylococcal Activity of Series A−F Compounds[a]



R^2 / R^1	Series A	Series B	Series C	Series D	Series E	Series F
	7	23	33	51	65	76
	17	24	34	52		77
						78
	18	25		53	66	79
				54		80
						81
						82
						83
	19		35	55	67	84
	20	26				85
	21	27		56	68	86
	22			57		87

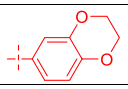
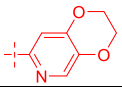
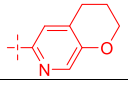
Cmpd./Series	R^1 group	*S. aureus* ATCC 29213	MRSA USA300 strain	*S. aureus* CF isolate
		MIC (μg/mL)	MIC (μg/mL)	MIC (μg/mL)
				
7/A		0.125-0.5	0.125-0.5	0.125-0.5
23/B		≤0.25	0.25	0.25
33/C		0.125-0.5	0.5-1	0.25-0.5
51/D		0.125	0.125-0.25	0.25
65/E		0.125	0.25	0.125
76/F		0.125-0.25	0.125-0.25	0.125
				
17/A		0.5-1	1	0.5
24/B		0.5-1	1	NT[b]
34/C		0.5-1	2	0.5
52/D		0.25	0.5	0.5
77/F		0.125-0.25	≤0.25	0.125-0.5
				

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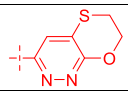
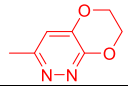
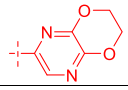
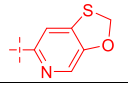
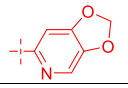
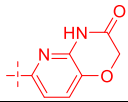
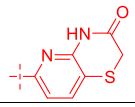
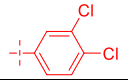
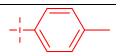
Table 2. continued

78/F		0.125	0.25	0.5
				
18/A		0.5-1	1	1-8
25/B		0.5	1	0.5
53/D		0.25	0.5	0.25
66/E		0.25-0.5	0.5	0.25
79/F		0.125-0.25	0.125-0.5	0.125-0.25
				
54/D		1	2	0.5
80/F		0.125-0.5	0.25-2	0.25-1
81/F		0.25	1	0.25-0.5
82/F		0.06-0.125	0.125	0.06-0.125
83/F		0.5	1	0.5
				
19/A		≤0.5 - 1	0.25	0.5
35/C		4-8	8	8
55/D		0.5	0.25-0.5	0.5
67/E		0.25-0.5	0.25	0.5
84/F		0.125-0.25	0.125-0.25	0.25
				
20/A		0.25-0.5	0.25	0.5
26/B		≤0.25	0.125-0.25	0.25
85/F		0.125-0.5	0.25-0.5	0.25-0.5
				
21/A		≤0.25	0.5	0.25
27/B		≤0.25	0.25	0.25
56/D		0.125-0.25	0.25	0.5
68/E		0.25	0.5	0.25
86/F		≤0.25, 0.125	0.125-0.25	0.125-0.5
				
22/A		0.125-0.5	0.5	0.5
57/D		0.25	0.5	0.25
87/F		0.125-0.25	≤0.25	0.25
gepotidacin	--	0.25-1	0.25-1	0.25-1
ciprofloxacin	--	0.25-0.5	16	0.25-0.5
vancomycin	--	1	1-2	1

[a]Minimum inhibitory concentrations (MICs) were determined in triplicate (at a minimum) according to CLSI guidelines.[44] Observed ranges are reported when appropriate. [b]Not tested.

for compounds **7**, **76**, and **79** were determined against a panel of 17 *B. anthracis* isolates (Table 3). Modest activity was observed, with **76** being the most potent ($MIC_{50/90}$ = 1/4 μg/mL) and both **7** and **79** being two-fold less active.

Target Inhibition and Resistance. Resistance to new antibacterial agents is inevitable, but minimizing the rate at which resistance emerges and spreads presents an important objective. In the case of fluoroquinolones, resistance often occurs via mutations to the genes encoding the bacterial topoisomerases, DNA gyrase and TopoIV.[9] Similar findings have been observed with NBTIs, which inhibit DNA gyrase more potently than TopoIV in *S. aureus*. Lahiri et al. have presented the most comprehensive findings on resistance to the NBTI class, with >20 reported NBTI-resistant *S. aureus* mutant strains selected by three different NBTIs.[48] The great majority of first-step mutations encoded amino acid substitutions to the GyrA domain of DNA gyrase, supporting its role as the primary target of NBTIs. The clinical relevance of

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Table 3. Antibacterial Spectrum of Select Dioxane-Linked NBTIs

cmpd.	*S. aureus* (n = 5) MIC_{50} (μg/mL)[a]	*Enterococcus* spp. (n = 5) MIC_{50} (μg/mL)[a]	*S. pneumoniae* (n = 5) MIC_{50} (μg/mL)[a]	*A. baumannii* (n = 5) MIC_{50} (μg/mL)[a]	*Bacillus anthracis* (n = 17) $MIC_{50/90}$ (μg/mL)[b]
7	0.12	0.12	1	32	2/8
34	2	0.5	8	>64	NT[c]
51	0.06	0.06	1	64	NT
52	0.25	0.12	2	64	NT
53	0.5	0.25	1	>64	NT
76	0.06	0.06	0.5	16	1/4
79	0.25	0.12	0.5	64	2/8
84	0.12	0.06	1	8	NT
86	0.06	0.06	4	>64	NT

[a]JMI Labs, North Liberty, IA. [b]Slayden lab, Colorado State University, Fort Collins, CO. [c]Not tested.

these findings is evinced by a Phase 2 clinical trial of gepotidacin in skin infections, wherein two *S. aureus* strains were identified with elevated gepotidacin MICs, each of which had a D83N amino acid substitution in DNA gyrase.[49]

The role of TopoIV as a relevant secondary target is also supported by several lines of investigation. For example, Lahiri et al. selected for second-step NBTI resistance using a first-step resistant isolate with a GyrA M121K substitution.[48] A second mutation in the *parC* gene encoding an M117K substitution to TopoIV resulted in >100-fold loss of activity versus the parent (first-step mutant) strain. We hypothesized that improving dual-target inhibition through increased potency against the secondary target TopoIV would reduce the frequency of spontaneous resistance to NBTIs. Earlier research on the role of dual-target inhibition in *Escherichia coli* supports this hypothesis. NBTI-5463[50] is a balanced inhibitor of *E. coli* DNA gyrase (IC_{50} = 5 nM) and TopoIV (IC_{50} = 2.6 nM).[51] For these investigators, it was not possible to select for resistant strains of NBTI-5463 by conventional means, consistent with an extremely low frequency of spontaneous resistance.[51]

Inhibition of DNA gyrase supercoiling and TopoIV decatenation activity was quantitated using previously reported gel-based assays with the *S. aureus* enzymes (Table 4).[31] The values obtained for the ciprofloxacin control are consistent with previously published results from Black et al.[33] In addition to determining IC_{50} values, we calculated the ratio of TopoIV IC_{50}/DNA gyrase IC_{50}. These assays are by necessity nonidentical, owing to the different functions of the enzymes. Nevertheless, we hypothesized that better dual-targeting inhibitors would be manifested by smaller ratios (i.e., more similar IC_{50} values). Given the important role played by the DNA gyrase D83 residue in binding the NBTIs, we also determined IC_{50} values against the D83N mutant DNA gyrase for a number of compounds and calculated the ratio of mutant IC_{50}/wild-type IC_{50} to ascertain the reduction in potency against the mutated enzyme.

All of the NBTIs were more potent inhibitors of DNA gyrase-mediated supercoiling than of decatenation by TopoIV, but the ratios varied substantially between compounds. We initially hypothesized that structural modifications of the amine substituent would drive the TopoIV/gyrase ratio, since it is this portion of the molecule that appears to interact most substantially with the targets[16] (hence the descriptor of enzyme-binding moiety). Prototype compound **7** (Series A) is a potent inhibitor of DNA gyrase (IC_{50} = 0.064 μM, Table 4), and our X-ray crystallography results (Figure 2) are consistent with the classical NBTI binding mode.[16] In contrast, inhibition of TopoIV by **7** is substantially weaker (IC_{50} = 5.5 μM, TopoIV/gyrase ratio = 85). The most balanced inhibitor from Series A/B is compound **27** (Series B, ratio = 2.7), differing primarily from **7** in the enzyme-binding moiety (3,4-dichlorobenzyl versus benzodioxane). At the suggestion of a reviewer, we attempted to rationalize these findings using our gyrase crystal structure and previously published[31] TopoIV homology model.

We began by docking compound **7** into the gyrase crystal structure and showed that we could recapitulate its experimentally determined binding mode (Figure 3A); the docked molecule bound slightly deeper into the pocket (RMSD = 1.04 Å). Docking compound **27** to the gyrase crystal structure afforded a similar binding pose (Figure 3B), although the Glide docking score was slightly worse than for **7** (−8.89 and −9.12, respectively), consistent with the weaker gyrase inhibition by **27**. We next docked both **7** and **27** into our previously published TopoIV homology model (Figure 3C,D). In this case, the docking score for **27** was better than that for **7** (−9.14 and −8.73, respectively), again consistent with the experimental results. Nevertheless, docking compounds into static structures from crystallography or homology modeling has inherent limitations (see the Supporting Information for supplemental discussion), and the absence of any X-ray structures of NBTIs in ternary complex with TopoIV and DNA remains a key limitation to the field.

Other enzyme-binding moieties such as the pyridooxazinone and pyridothiazinone generally afforded low TopoIV/gyrase ratios: 86% (6/7) of these analogues had ratios ≤15, while only 23% (7/30) of other compounds behaved likewise. The DNA-binding moiety and position of hydroxylation also appeared to play a role, with Series F proving to be routinely superior. Indeed, Series F afforded the most potent inhibitors of TopoIV; four compounds had IC_{50} values < 0.5 μM (**76**, **82**, **84**, and **85**). These findings, as well as other attributes (*vide infra*) made Series F our lead series and inspired the preparation of a broader set of compounds in that series.

The inhibition of the D83N mutant DNA gyrase was universally poorer (34- to 723-fold) than the wild-type enzyme but was variable across the compounds studied (Table 4). The data set for matched-pair comparisons is limited, and it is difficult to discern clear structure−activity relationships from these series. For a number of compounds (**51**, **66**, **81**, and **87**), the inhibition of the mutant gyrase was similarly potent to the inhibition of TopoIV (IC_{50} values within 3-fold).

To probe the hypothesis that superior dual-target inhibition would reduce the rate of resistance emergence, we selected four analogues (**7**, **77**, **79**, and **84**) with varying TopoIV/gyrase ratios and conducted spontaneous frequency of resistance (FoR) studies using *S. aureus* ATCC 29213. MICs were initially determined by CLSI guidelines on agar. As expected based on previous microbroth results, MICs were low for all four compounds (Table 5). Compounds **7**, **77**, and **79** displayed low frequencies of resistance at both 8× (~2 to 3 × 10^{-9}) and 16× MIC concentrations (~7 to 9 × 10^{-10}), similar to other NBTIs examined by Lahiri et al.[48] and Surivet and colleagues.[35,40] Compound **84** did not select for resistant mutants at either concentration, consistent with the low ratio for 50% inhibition of TopoIV decatenation compared to 50%

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Table 4. Target Inhibition by Dioxane-Linked NBTIs[a]

cmpd.	DNA gyrase IC_{50} (μM)[b]	TopoIV IC_{50} (μM)[c]	TopoIV/gyrase ratio	D83N gyrase IC_{50} (μM)[b]	D83N gyrase IC_{50}/WT gyrase IC_{50} ratio
Series A					
7	0.064 (n = 3)	5.5 (n = 4)	85	NT[d]	
17	0.60[e] (n = 2)	14	23	NT	
18	0.37	11 (n = 4)	30	NT	
19	0.52 (n = 2)	2.6 (n = 2)	4.9	>200 (n = 2)	>388
20	0.33	1.5	4.6	NT	
21	0.16[e] (n = 2)	8.1 (n = 2)	51	NT	
22	0.11 (n = 2)	14 (n = 6)	122	NT	
Series B					
23	0.022[e] (n = 2)	3.2	145	NT	
24	0.16[e] (n = 2)	8.8 (n = 7)	55	NT	
25	0.24	11	47	NT	
26	0.054[e] (n = 2)	2.8[e] (n = 2)	52	NT	
27	0.22	0.60	2.7	NT	
Series C					
33	0.019[e] (n = 2)	4.0	210	NT	
34	0.18[e] (n = 2)	30	165	NT	
Series D					
51	0.031 (n = 3)	2.3	73	2.2 (n = 2)	72
52	0.042	5.7	136	NT	
53	0.056 (n = 2)	4.8	87	19 (n = 3)	336
54	0.84 (n = 2)	8.5 (n = 2)	10	51 (n = 2)	60
55	0.084 (n = 2)	1.3	15	4.9 (n = 2)	59
56	0.056 (n = 2)	1.3 (n = 2)	24	NT	
57	0.014	4.1	294	NT	
Series E					
65	0.013 (n = 2)	0.87	67	NT	
66	0.12 (n = 5)	4.6	39	12 (n = 4)	99
67	0.095 (n = 4)	0.99	10	69 (n = 5)	723
68	0.043	2.3	54	NT	
Series F					
76	0.060 (n = 3)	0.38 (n = 2)	6.3	2.7 (n = 3)	45
77	0.039 (n = 3)	1.3 (n = 4)	34	13 (n = 2)	324
78	0.14 (n = 2)	1.3 (n = 2)	9.3	13 (n = 2)	98
79	0.22 (n = 5)	3.5 (n = 6)	16	15 (n = 6)	69
80	0.32 (n = 2)	4.8 (n = 3)	15	47 (n = 2)	146
81	0.36 (n = 2)	19 (n = 2)	52	29 (n = 2)	80
82	0.099 (n = 2)	0.43 (n = 2)	4.3	5.9 (n = 2)	59
83	0.85 (n = 2)	18 (n = 2)	22	73 (n = 2)	86
84	0.061 (n = 5)	0.32 (n = 4)	5.2	14 (n = 5)	229
85	0.16 (n = 2)	0.31 (n = 2)	2.0	63 (n = 2)	398
86	0.16 (n = 2)	1.1 (n = 2)	7.0	5.5 (n = 2)	34
87	0.16 (n = 2)	2.7 (n = 2)	17	6.5 (n = 2)	41
gepotidacin (racemic)	0.17 (n = 2)	2.8 (n = 3)	17	NT	
ciprofloxacin	28	8.6	0.30	580[e,f] (n = 2)	20

[a]Values from single determinations unless otherwise indicated as replicate n values. [b]Inhibition of supercoiling activity. [c]Inhibition of decatenation activity. [d]Not tested. [e]Values determined by Inspiralis (Norwich, U.K.). [f]Black et al.[33] reported an IC_{50} > 200 μM.

inhibition of DNA gyrase supercoiling (5.2, Table 4) and in accordance with our hypothesis that a more balanced inhibitor of both enzymes will slow the rate of resistance emergence.

For each compound that afforded resistant mutants, five individual colonies were archived from each test concentration (30 total isolates). We then sequenced *gyrA* for each of these isolates to identify resistance mutations (see Table 10 in the Experimental Section for primers used). Mutations in *gyrA* were seen for 11/30 isolates. Mutations encoding the D83N and M121K amino acid substitutions were observed for each compound. Such mutations are consistent with the observed binding mode for compound **7** (Figure 2) as well as with earlier reports that these sites constituted important loci of resistance to NBTIs.[32,33,35,38,40,48,52,53]

Our research program also strove to improve the antibacterial activity against *S. aureus* strains with target-based NBTI resistance. A previously reported[32,38,52,53] strain with the D83N amino acid substitution in the GyrA domain of DNA gyrase served as the primary assay. Given the key role of D83 in binding NBTIs,[16] such resistant strains have played a key role in the optimization of the NBTI class.[32,35,38,40,52,53] Unsurprisingly, all of the compounds displayed elevated MICs

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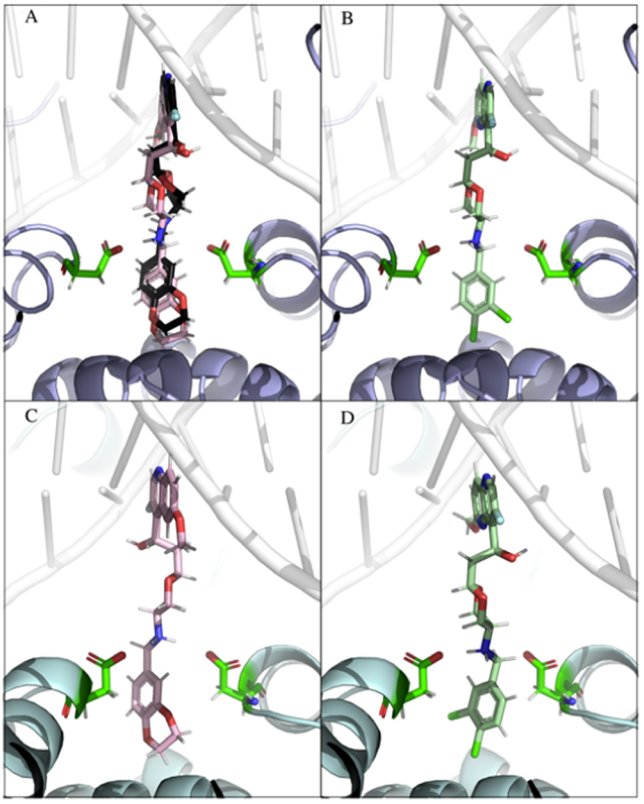

Figure 3. Docked structures of compounds **7** (pink) and **27** (light green) with crystal structure of DNA gyrase (light blue; PDB code 7MVS) from Figure 2 and homology model of TopoIV (cyan; generated using templates 4Z2C, 5IWI, 5CDN, and 3FOE). Residues D83 (gyrase) and D79 (TopoIV) are shown in bright green. DNA is shown in white. (A) Docked binding pose of **7** with DNA gyrase. The experimentally observed pose of **7** is shown in black for reference. (B) Docked binding pose of compound **27** with DNA gyrase. (C) Docked binding pose of compound **7** with TopoIV homology model. (D) Docked binding pose of compound **27** with TopoIV homology model.

with the D83N strain (Table 6). We initially hypothesized that NBTIs with more balanced inhibition of TopoIV and gyrase (i.e., more similar IC_{50} values in the two assays) would display smaller MIC elevations in the first-step mutant strain. However, while slight variations were observed between individual compounds, the MIC shift between the parent (3527) and first-step mutant strains was typically ~8- to 32-fold and was not readily correlated with the TopoIV/gyrase ratio. Numerous compounds achieved an MIC ≤ 4 μg/mL against the mutant strain and were superior to gepotidacin (MIC 8−32 μg/mL) in that regard. These results highlight both progress against NBTI-resistant *S. aureus* strains and the opportunity for continued optimization of the NBTI class.

Metabolism. To improve the metabolic stability of our NBTIs, we pursued two strategies, blocking suspected sites of metabolism and reducing overall lipophilicity. SMARTCyp[54] predictions using compound **4** were used to identify the three most likely sites of metabolism (Figure 4). Blocking the #1 site (amino methylene) as in our previously reported amide **104**[31] (Figure 4, $t_{1/2}$ = 10.6 min) did reduce metabolism compared to amine **4** ($t_{1/2}$ too short to measure), but this strategy was unfortunately not generalizable to all amide-type NBTIs (full details will be reported separately). Methylation of the #2 site (dioxane C5 position) afforded **103**, but this compound was as rapidly metabolized as the unmethylated analogue **5** ($t_{1/2}$ = 2.4 min). Given the high lipophilicity of **103** (cLog *P* = 4.4) and the observed relationship between lipophilicity and clearance for this series (*vide infra*), the rapid clearance of **103** is perhaps not surprising. Methylation at the #3 site (dioxane C2 position) also presented an appealing target, but the synthesis of the favored diastereomer was not straightforward, and we abandoned this effort in light of the findings detailed below from the polarity-driven strategy.

Our extensive efforts in optimizing dioxane-linked NBTIs afforded a solid data set to assess the relationship between lipophilicity (as cLog *P*) and intrinsic clearance from mouse microsomal studies. Compounds in this series suffered from generally short half-lives in this assay (i.e., rapid clearance, Table S2, Supporting Information).

Figure 5 presents the observed relationship for all 5-aminodioxane NBTIs in the current manuscript for which we have measured intrinsic clearance values (30 compounds, blue dots). As expected, clearance increased with lipophilicity (R^2 = 0.7315). Furthermore, 12 of 14 compounds with $t_{1/2}$ > 15 min, had cLog *P* values <1.5, suggesting a cutoff value that could prove useful. These results reinforce the importance of physicochemical property considerations in drug design,[55] as do our results on hERG inhibition (*vide infra*). As mentioned earlier, two piperidine-linked analogues (**1** and **3**, orange dots) were similar to the dioxane-linked compounds.

Safety. Cardiovascular safety is a key consideration in the development of all new drugs, and hERG inhibition presents a common obstacle, especially for NBTIs.[25] We targeted a hERG $IC_{50} \geq 100$ μM as a requirement for an antistaphylococcal lead molecule. hERG IC_{50} values for 35 compounds in the current study were determined in an automated electrophysiology assay (IonWorks Barracuda system), as previously reported,[31] and are shown in Table 7. Several compounds met our lead criteria, with the oxathiinopyridazine (**18**, **25**, and **79**) and

Table 5. Spontaneous Frequency of Resistance by Select NBTIs and Derived Mutants[a]

cmpd.	Topo IV/gyrase ratio[b]	*S. aureus* 29213 agar MIC (μg/mL)	mutation frequency at 8× MIC	amino acid substitutions in GyrA[c]	mutation frequency at 16× MIC	amino acid substitutions in GyrA[c]
7	85	0.125	2.92×10^{-9}	M121K (n = 1)	6.74×10^{-10}	D83N (n = 1)
77	34	0.125	2.02×10^{-9}	D83N (n = 2) M121K (n = 2)	8.99×10^{-10}	D83N (n = 2)
79	16	0.125	2.02×10^{-9}	M121K (n = 1)	6.74×10^{-10}	D83N (n = 1) M121K (n = 1)
84	5.2	0.25	$<2.25 \times 10^{-10}$	none	$<2.25 \times 10^{-10}$	none
Cipro	0.30	0.5	$<2.25 \times 10^{-10}$	none	$<2.25 \times 10^{-10}$	none

[a]Study carried out at Micromyx, LLC. (Kalamazoo, MI). [b]From Table 4. [c]See the Experimental Section for sequencing details.

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Table 6. Loss of Antibacterial Activity in D83N Mutant *S. aureus* Strain[a]

cmpd.	*S. aureus* parent (strain 3527) MIC (μg/mL)	*S. aureus* 1st-step mutant (GyrA D83N) MIC (μg/mL)
Series A		
7	0.125−0.5	2−16 (mode = 8)
17	1	16
18	1−2	16−32
19	≤0.5	4
20	≤0.25	4
21	≤0.25	4
22	0.5	16
Series B		
23	≤0.25	8
24	1	16
25	0.5−1	8−16
26	≤0.25	2
27	≤0.25	4
Series C		
33	0.5−1	8
34	2	16
35	8	>8
Series D		
51	0.125−0.25	4−8
52	0.5	>4, 8
53	0.5−1	8
54	2	16
55	0.25−0.5	4−8
56	0.25	4−8
57	0.5	8
Series E		
65	0.125	2
66	0.5	4
67	0.25	2
68	0.25	4
Series F		
76	0.125−0.25	4−8
77	≤0.25−0.5	4
78	0.5	8
79	≤0.25−0.5	4
80	0.25−2	2 to >8
81	1	8
82	0.125	1−2
83	1	>8
84	0.125−0.25	2−4
85	0.125	2
86	0.125−0.5	4−8
87	≤0.25	8
gepotidacin	0.25−1	8−32
ciprofloxacin	>64	64
vancomycin	1−2	1

[a]Minimum inhibitory concentrations (MICs) were determined in triplicate (at a minimum) according to CLSI guidelines.[44] Observed ranges are reported when appropriate.

dioxanopyridazine (**54** and **80**) moieties especially beneficial.[40] In contrast, pyridooxazinone (**19**, **55**, **67**, and **84**) and pyridothiazinone (**20**, **26**, and **85**) derivatives, along with those bearing highly lipophilic phenyl rings (**27**, **47**, and **69**), were the most potent hERG inhibitors (IC_{50} < 10 μM). There were no consistent or substantive differences between fluoroquinoline and fluoronaphthyridine analogues.

Exploring the relationship between lipophilicity (cLog *P*, as above) and hERG inhibition as Log_{10}[hERG IC_{50}] graphically was especially revealing (Figure 6). The 27 compounds (orange dots) lacking the oxazinone or thiazinone enzyme-binding moiety displayed a very strong relationship between lipophilicity and hERG inhibition (R^2 = 0.8662). The majority of compounds (7 of 8) meeting our lead criteria (hERG $IC_{50} \geq$ 100 μM, i.e., Log_{10}[hERG IC_{50}] ≥ 2) had cLog *P* values <1.5, again affording a useful cutoff value for design efforts. Strikingly, every oxazinone- and thiazinone-based NBTI (8 of 8 compounds, **19**, **20**, **26**, **35**, **55**, **67**, **84**, and **85**, blue dots) was a more potent inhibitor than would be predicted based on lipophilicity. While the origins of this finding are not clear, it was remarkably consistent across all six series of compounds. We demonstrated earlier that oxazinone- and thiazinone-based NBTIs were among the best enzyme-binding moieties for inhibiting TopoIV; their potent hERG inhibition illustrates the challenge of simultaneously optimizing these two properties. Using cLog $D_{7.4}$ (ACDLabs Percepta) as the measure of lipophilicity afforded similar results (data not shown). Notably, no overall correlation between pK_a (ChemDraw or ACDLabs Percepta Classic) and hERG inhibition could be discerned within this data set. In conclusion, *within the 5-amino-1,3-dioxane NBTIs* hERG inhibition is influenced most strongly by: (1) the presence or absence of a pyridooxazinone or pyridothiazinone moiety and (2) lipophilicity.

Growth inhibition was assessed for a number of representative compounds using human leukemia K562 cells and an acquired etoposide-resistant clonal subline, K/VP.5. K/VP.5 cells contain diminished levels of human topoisomerase IIα (hTopoIIα) protein compared to parental K562 cells, as described previously.[30] We sought compounds with high K562 IC_{50} values (i.e., low growth inhibitory effects/high selectivity for bacterial killing) and similar growth inhibitory effects in K/VP.5 cell lines, suggesting that hTopoIIα is not a target of these NBTIs. The NBTIs were not growth inhibitory to K562 cells, with 75% of compounds having a K562 IC_{50} > 50 μM (and the remainder >15 μM). Growth inhibitory effects in both K562 and K/VP.5 cells were similar for all compounds tested. hTopoIIα inhibition for ten compounds was also measured directly using the isolated enzyme (Inspiralis, Norwich, U.K.); none of these compounds exhibited >25% inhibition at a concentration of 100 μM (data not shown). We conclude that these dioxane-linked NBTIs do not target hTopoIIα and exhibit selective antibacterial killing activity.

Lead Selection and Advanced Profiling. Consideration of all of the above-mentioned studies led us to select Series F compound **79**, the dioxane-linked analogue of ACT-387042,[40,56] as a suitable lead molecule. Notably, **79** had potent antistaphylococcal activity (MIC_{50} = 0.25 μg/mL, Table 3), improved dual-targeting compared to prototype **7** (TopoIV/gyrase ratio of 16 vs 85, respectively, Table 4), and a low frequency of spontaneous resistance (6.74×10^{-10} at 16× MIC, Table 5). Compound **79** was more potent than gepotidacin against an NBTI-resistant *S. aureus* strain with a D83N substitution in *S. aureus* DNA gyrase (MIC = 4 μg/mL vs 8−32 μg/mL, respectively, Table 6). The hERG IC_{50} (103 μM, Table 7) met our lead criteria. Metabolism in mouse microsomes was relatively rapid, however, with $t_{1/2}$ = 18 min (Table S2, Supporting Information). Additional studies were thus conducted in several areas.

Antibacterial activity against a broader set of strains and pathogens was conducted; $MIC_{50/90}$ values and observed

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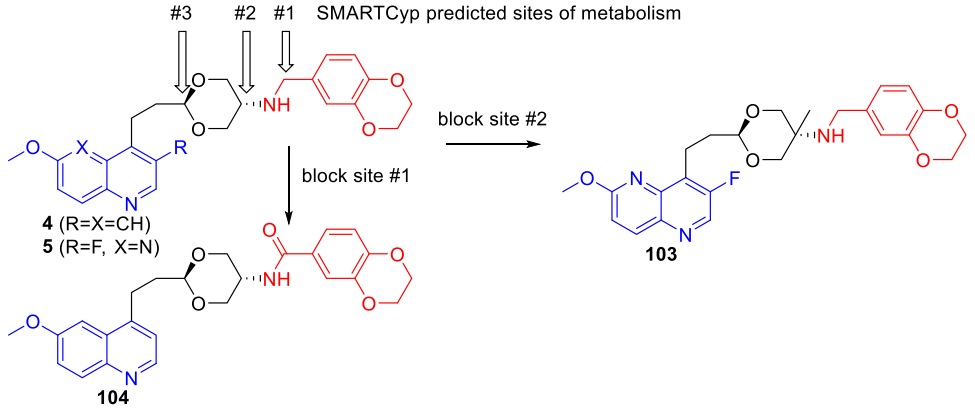

Figure 4. Predicted sites of metabolism and blocking strategies.

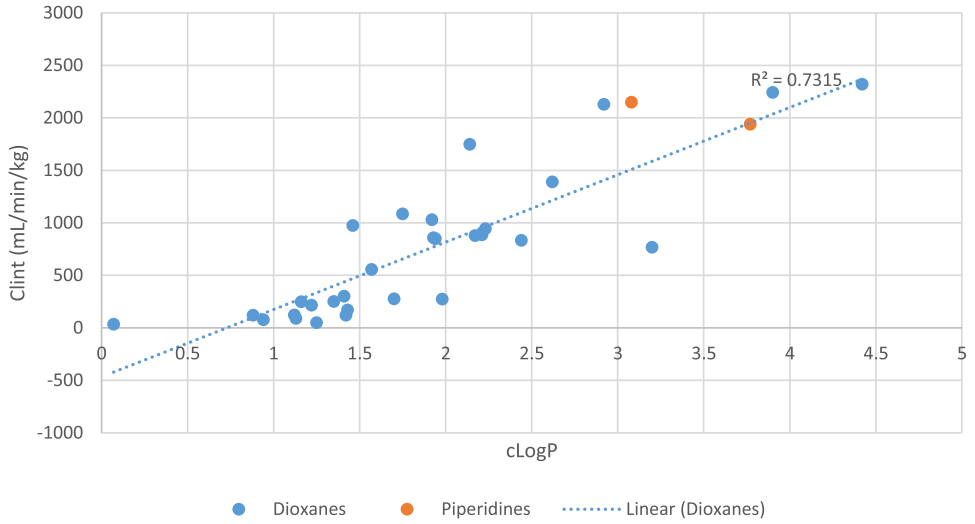

Figure 5. Impact of lipophilicity on intrinsic clearance in CD-1 mouse microsomes. Intrinsic clearance values were determined by incubation of compounds in the mouse microsomal assay at Charles River (Worcester, MA). Lipophilicity (cLog *P*) was calculated using ChemDraw 19.1.

ranges are presented in Table 8. Potent antistaphylococcal activity was observed for both fluoroquinoline-resistant (FQ^R-) MSSA and FQ^R-MRSA ($MIC_{50/90}$ = 0.12/0.25 μg/mL in both cases). A panel of FQ^R *Enterococcal* isolates with vancomycin-susceptible and vancomycin-resistant isolates of both *E. faecium* and *E. faecalis* showed slightly weaker but still potent activity ($MIC_{50/90}$ = 0.25/1 μg/mL). MICs against *Streptococcus pyogenes* were low (MIC_{50} = 0.5 μg/mL), as were those against penicillin-resistant and FQ^R-*S. pneumoniae* (MIC_{50} = 0.5 μg/mL, from Table 3). In contrast, MICs for FQ^R-*Streptococcus agalactiae* were greater (MIC_{50} = 4 μg/mL) but still superior to levofloxacin. Antibacterial activity for **79** against the Gram-negative pathogens *A. baumannii* (repeated from Table 3), *Klebsiella pneumoniae*, *Enterobacter cloacae*, *E. coli*, and *Pseudomonas aeruginosa* was universally poor. Compound **79** is thus relatively narrow in its spectrum, with little activity against Gram-negative bacteria. While new therapies for such pathogens are clearly needed, the absence of activity against *Enterobacterales* like *E. coli* could be advantageous from the perspective of diminished impact on the microbiome and reduced opportunity for resistance selection in that population.

The mechanism of action of NBTIs is typically distinct from that of other agents targeting bacterial topoisomerases such as fluoroquinolones. Aside from catalytic inhibition of prokaryotic Type II Topoisomerases, NBTIs typically induce a preponderance of DNA single-strand breaks (SSBs) compared to fluoroquinolone-induced DNA double-strand breaks (DSBs).[16−20] We recently reported that inhibition of *S. aureus* DNA gyrase by several dioxane-linked amide-type NBTIs unexpectedly resulted in accumulation of both SSBs and DSBs.[53] For lead compound **79**, we conducted pBR322 DNA cleavage assays with DNA gyrase in the presence of escalating concentrations of **79** (Figure 7A) using both gepotidacin and ciprofloxacin as controls. As we previously observed, and consistent with the results of Osheroff and colleagues,[20] gepotidacin induced SSBs (nicked DNA), whereas ciprofloxacin induced DSBs (linearized DNA). Compound **79** induced both SSBs and DSBs, with SSBs predominating at all concentrations (Figure 7B). Replicate experiments performed at 20 μM **79** demonstrated that SSB induction was 4.1-fold greater than that of DSBs (Figure 7C). The mechanism(s) involved with induction of DNA strand breakage in the presence of **79** and other NBTIs is a subject of ongoing studies in our laboratories.

A number of additional *in vitro* studies confirmed the promising safety profile of **79**. Cytotoxicity was assessed using THP-1, HepG2, and HeLa cells; IC_{50} values were >128 μg/mL (>261 μM) in each case. An ATP depletion assay in HepaRG cells (RTI International, Research Triangle Park, NC) likewise

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Table 7. *In Vitro* Safety Evaluation of Selected NBTIs

cmpd.	hERG IC_{50} (μM)[a]	K562 IC_{50} (μM)[b]	K/VP.5 IC_{50} (μM)[b]	ChemDraw cLog *P*
Series A				
7	23	23.4	23.8	2.44
17	65	>100	>100	1.75
18	>100	>200	>200	1.22
19	8.7	NI[c]	NI	0.94
20	6.5	80.5	56.0	1.16
21	11	16.7	24.5	3.82
22	13	22.9	21.7	3.02
Series B				
23	28	53.7	39.2	2.14
24	>90	186	232	1.46
25	>100	NT	NT	0.93
26	4.8	69.9	52.1	0.87
27	6.9	NT	NT	3.53
Series C				
33	59	87.7	71.1	1.57
34	102	>100	>100	0.88
35	84	NT	NT	0.07
Series D				
51	19	NT	NT	2.92
52	24	NT	NT	2.23
53	62	NT	NT	1.70
54	>200	NT	NT	1.43
55	9.8	NT	NT	1.42
56	6.6	NT	NT	4.3
Series E				
65	13	NT	NT	3.43
66	84	NT	NT	2.21
67	7.6	NT	NT	1.93
Series F				
76	18	30.4	30.5	2.62
77	39	>200	145	1.94
78	34	108	80.5	2.17
79	103	153 (n = 2)	202 (n = 2)	1.41
80	>200	>200	>200	1.13
81	>100	NT	NT	1.25
82	68	129	118	1.92
83	150	NT	NT	1.98
84	7.5	108, >200[d]	>200	1.12
85	8.4	NT	NT	1.35
86	5.6	27.9	22.2	4.01
87	NT	54.1	46.9	3.2

[a]Charles River (Cleveland, OH). [b]Values from single determinations unless otherwise indicated as replicate n values. [c]Results were not clearly interpretable to afford IC_{50} values. [d]Results from two experiments.

showed no meaningful cytotoxicity in the concentration range from 0.01 to 100 μM (17% cytotoxicity at 100 μM). Similar results in HepaRG cells (12% cytotoxicity at 100 μM) were seen for the Series B comparator **25**, which differs from **79** only in the position of the hydroxyl moiety. Off-target activity was assessed by screening a panel of 44 enzymes,[58] receptors, etc. at a concentration of 100 μM; compound **77** (differing from **79** only in the enzyme-binding moiety) was tested as a close comparator. Inhibition >45% at 100 μM **79** was only seen for two targets (5-HT_{2B} and acetyl-cholinesterase), whereas **77** had somewhat more off-target activity. Full data sets as well as follow-up determination of K_i and IC_{50} values for relevant targets (Table S3) are presented in the Supporting Information. The broad panel screening and the follow-up studies support an excellent overall profile for these dioxane-linked NBTIs, especially lead compound **79**.

As discussed above, cardiovascular safety concerns have been particularly challenging for the NBTIs.[25] Numerous studies have focused on minimizing hERG inhibition, and several recent efforts have also highlighted the role of additional ion channels in impacting cardiovascular safety.[28,32,40] To gain a broader understanding of the cardiovascular ion pharmacology of dioxane-linked NBTIs, compounds **77** and **79** were evaluated using a comprehensive *in vitro* proarrhythmia assay[59] (Table S4, Supporting Information) with several key cardiac ion channels. Lead analogue **79** showed <25% inhibition for each of the ion channels, even at concentrations up to 100 μM, consistent with a prediction of minimal preliminary CV safety risk. Translating these findings to *in vivo* safety pharmacology studies in relevant animal models will be required for further development. Compound **77** largely spared these ion channels as well, although inhibition of hERG (29.2% at 100 μM) and Nav1.5 late current (78.6% at 100 μM) was more pronounced.

An initial assessment of ADME properties of **79** was conducted, alongside **25** as a comparator (RTI International, Research Triangle Park, NC). Kinetic aqueous solubility (buffered at pH 7.4) was similar between the compounds (69.6 and 61.7 μM, respectively). Plasma protein binding for **79** was moderately high: 12 and 15% free in mouse and human plasma, respectively, and was somewhat lower for **25** (18 and 30% free). Permeability and efflux potential were assessed using CACO-2 cells. Both **79** and **25** showed moderate permeability (A → B of 5.68×10^{-6} and 7.85×10^{-6} cm/s, respectively). Higher values in the B → A direction (81.2×10^{-6} and 94.8×10^{-6} cm/s, respectively) suggested the potential for efflux. The half-life of each compound was assessed using both mouse and human microsomal preparations. In this assay, the $t_{1/2}$ for **79** in mouse microsomes was consistent with the previous study (26 min; 18 min in Table S2, Supporting Information), and the stability in human microsomes was substantially greater ($t_{1/2}$ = 94 min). Compound **25** was metabolized somewhat more rapidly in both mouse and human microsomes ($t_{1/2}$ = 21 and 52 min, respectively). Neither **79** nor **25** inhibited CYP1A2, 2B6, 2C8, 2C9, 2C19, or 2D6 by >50% at a concentration of 10 μM. The initial screening assay showed higher inhibition of CYP3A4 by **25** (72% at 10 μM), but a follow-up concentration−response assay showed the IC_{50} was >10 μM. For compound **79**, little inhibition was seen (only 13% at 10 μM in the concentration−response assay).

The ADME assays described above, coupled with microbiological results and the facile access to enantioenriched material, led to the selection of **79** for pharmacokinetic studies in CD-1 mice. Compound **79** was dosed both intravenously (IV, 10 mg/kg) and orally (PO, 50 mg/kg) in 24 mice each, and plasma concentrations were measured at 8 different timepoints, each using three mice. Key pharmacokinetic parameters are provided in Table 9. Notably, the half-life *in vivo* following IV administration was 38 min (0.63 h), slightly longer than suggested by microsomal studies (18−26 min). The observed *in vivo* half-life is comparable to half-lives reported after subcutaneous dosing of gepotidacin[60] (1.008 h at 6.25 mg/kg) or of the tetrahydropyran-linked NBTIs[56] ACT-387042 (0.31 h at 10 mg/kg) and ACT-292706 (0.19 h at 10 mg/kg). The volume of distribution was moderate at 1

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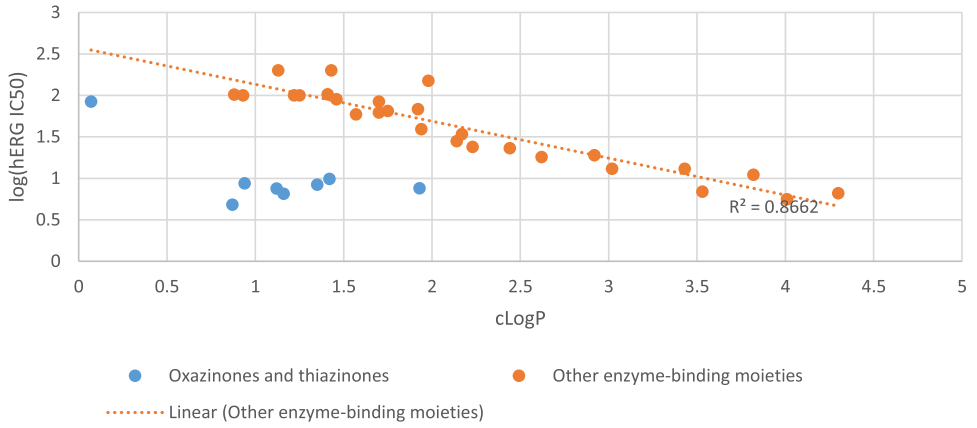

Figure 6. Impact of lipophilicity on hERG inhibition and series divergence. hERG IC_{50} values were determined at Charles River (Cleveland, OH) using the IonWorks Barracuda assay. IC_{50} values >X represented as = X. cLog *P* was calculated using ChemDraw 19.1.

Table 8. Antibacterial Spectrum of Lead NBTI Compound 79[a]

organism group (number of isolates)	**79** MIC_{50} (μg/mL)	**79** MIC_{90} (μg/mL)	**79** MIC range (μg/mL)	Levofloxacin MIC_{50}/MIC_{90} (range)
FQ^R-MSSA (15)[b]	0.12	0.25	0.06−0.25	8/64 (4 to >64)
FQ^R-MRSA (15)[b]	0.12	0.25	0.12−0.5	8/>64 (4 to >64)
Enterococcus spp. (10)[b]	0.25	1	0.06−2	64/>64 (32 to >64)
S. pyogenes (5)	0.5		0.25−0.5	0.5/-- (0.25−1)
S. pneumoniae (5)[c]	0.5		0.12−1	16/-- (8 to >64)
S. agalactiae (5)	4		4−8	32/-- (16−32)
A. baumannii (5)[c]	64		32 to >64	32/-- (8−64)
K. pneumoniae (5)	>64		>64	0.03/-- (0.015−0.5)
E. cloacae (5)	>64		64 to >64	0.12/-- (0.015−16)
E. coli (5)	>64		64 to >64	0.03/-- (0.015−16)
P. aeruginosa (5)	>64		64 to >64	1/-- (0.5−32)

[a]Assay conducted at JMI (North Liberty, IA). [b]A subset of these isolates is reported in Table 3. [c]These isolates are the same as those reported in Table 3.

L/kg. Finally, the oral bioavailability for compound **79** was 49%. These results were used to design an *in vivo* efficacy study.

Compound **79** was also tested for *in vivo* efficacy in a murine model of septicemia. Briefly, CD-1 mice were infected by IP injection of 1.3×10^8 CFUs of MRSA isolate ATCC 33591. Compound **79** displayed an MIC of 0.25 μg/mL against this strain (Pharmacology Discovery Services, Ltd., New Tapei City, Taiwan). Given the short half-life observed for **79**, animals were dosed four times intravenously at 0.5, 1.5, 2.5, and 3.5 h post injection at five different dose levels (1, 5, 10, 25, and 50 mg/kg/dose) on day one only. Survival was monitored for 7 days after infection. Untreated controls showed 0% survival, whereas >50% of mice survived at each dose level, and percent survival generally increased with dose (Figure 8 and Table S5, Supporting Information). The 25 mg/kg/dose and 50 mg/kg/dose levels led to 100% survival. Thus, compound **79** demonstrated robust *in vivo* efficacy in this initial study. Future studies will be needed to ascertain pharmacokinetic/pharmacodynamic (PK/PD) relationships[56,60] for **79** and other dioxane-linked NBTIs and to assess efficacy in tissue-based models of infection.

CONCLUSIONS

Novel bacterial topoisomerase inhibitors offer promise as potential future therapies for multidrug-resistant bacterial infections, including those caused by MRSA. Guided by the objective of reducing lipophilicity, we have optimized a series of 5-amino-1,3-dioxane-linked NBTIs to identify a lead compound, **79**, with potent activity against a broad set of Gram-positive pathogens, a low frequency of spontaneous resistance, low inhibition of hERG and other cardiac ion channels, modest activity against NBTI-resistant *S. aureus*, and *in vivo* efficacy in a mouse model of septicemia. During these studies, we delineated clear relationships between lipophilicity and both hERG inhibition and metabolic stability, establishing a target cLog *P* value of <1.5 for this series of NBTIs. Additionally, we identified NBTIs with potent inhibition of *S. aureus* TopoIV, achieving improved dual-target (TopoIV and DNA gyrase) inhibition as a result. Spontaneous frequency of resistance studies with one such compound, **84**, indicated a lower likelihood of eliciting resistance, as no resistant colonies were isolated at either 8× or 16× multiples of the MIC. Resistant colonies with gyrase D83N and M121K amino acid substitutions were obtained at low frequencies with lead compound **79**, a less potent inhibitor of TopoIV. Future efforts toward identification of a candidate for clinical development will focus on further improvements in pharmacokinetic performance while maintaining a favorable antibacterial and safety profile, alongside full characterization of PK/PD relationships.

EXPERIMENTAL SECTION

General Chemistry. Air- and/or moisture-sensitive reactions were carried out in oven-dried glassware under nitrogen protection unless otherwise noted. *N,N*-dimethylformamide (DMF), toluene, tetrahydrofuran (THF), and dichloromethane (DCM) were dried before using by passing through activated alumina under nitrogen or were purchased as anhydrous solvents. Flash chromatography was performed with a Teledyne-ISCO combiflash Rf+. ^{1}H NMR spectra were obtained at either 300 or 400 MHz using residual protiated solvent as the internal reference: $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm), DMSO-d_6 (2.50 ppm). ^{13}C NMR spectra were obtained at either 75 or 100 MHz using the solvent as the internal reference: $CDCl_3$ (77.16 ppm), CD_3OD (49.00 ppm), DMSO-d_6 (39.52 ppm). High-resolution mass spectrometry was performed using electrospray

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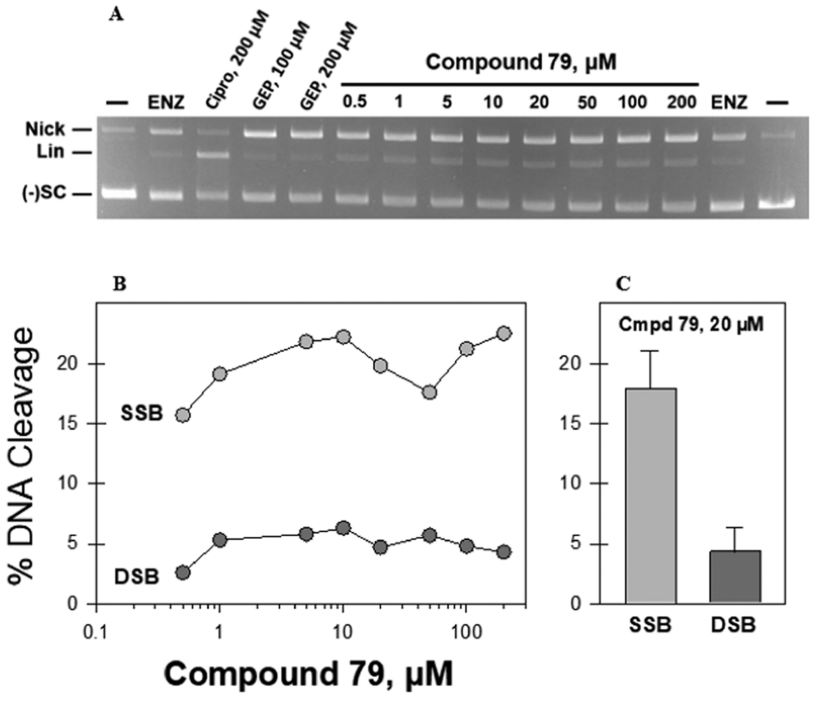

Figure 7. Gyrase-mediated DNA cleavage induced by compound **79**. (A) DNA products after cleavage reactions (30 min at 37 °C) in the presence or absence of compound **79** (0.5−200 μM). Ciprofloxacin and gepotidacin were included as controls at the indicated concentrations. (B) Compound **79** concentration-dependent induction of gyrase-mediated DNA single-strand breaks (SSB) and double-strand breaks (DSB) derived from the gel shown in (A). (C) Compound **79** (20 μM) induction of SSB and DSB, averaging results from seven separate experiments performed on separate days. Data are presented as mean ± standard deviation (SD). Quantitation of SSB and DSB DNA was accomplished by first measuring total fluorescence in each lane with corrections for differential emission in the negatively supercoiled [(−)SC] pBR322 DNA substrate compared to linearized (Lin; DSB) and nicked open circular (Nick; SSB) DNA bands.[57] Next, the percent cleavage in enzyme controls was subtracted to yield final results.

Table 9. Mouse Pharmacokinetic Parameters for NBTI 79[a]

clearance (mL/min/kg)[b]	69
half-life (min)[b]	38
AUC_{last} (μg*h/mL)[b]	2.402
Vd_{ss} (L/kg)[b]	1
C_{max} (μg/mL)[c]	7.019
T_{max} (h)[c]	0.17
AUC_{last} (μg*h/mL)[c]	5.906
oral bioavailability (%F)[c]	49

[a]Conducted by Eurofins Panlabs, Inc. (St. Charles, MO); see the Experimental Section for full details. [b]Intravenous dosing at 10 mg/kg. [c]Oral administration at 50 mg/kg.

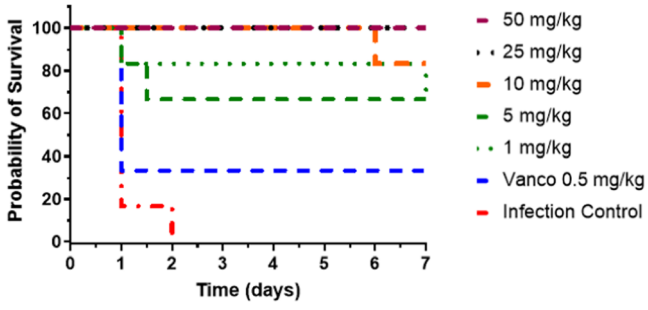

Figure 8. *In vivo* efficacy of **79** in mouse septicemia model. Mice were infected with 1.3×10^8 CFUs of MRSA strain ATCC 33591 via IP injection and treated with compound **79** at the indicated doses at 0.5, 1.5, 2.5, and 3.5 h. Compound **79** as the methanesulfonate salt was formulated in a solution of 5% dimethyl sulfoxide (DMSO) in water for injection (WFI). Vancomycin controls were treated with 0.5 mg/kg/dose at 0.5 and 3.5 h. Infection controls received no treatment. Survival was monitored for 7 days (Neosome Life Sciences, LLC, Lexington, MA).

ionization. High-performance liquid chromatography was performed using an Agilent 1260 Infinity Quaternary LC system (Agilent Technologies, Santa Clara, CA) with either an Agilent Poroshell 120 EC-C18 (2.7 μm, 3.0 mm × 150 mm) or a Syncronis C18 column (5 μm, 150 × 4.6 mm). All compounds are >95% pure by HPLC (high-performance liquid chromatography) (ELSD detection); retention time (rt) and percent purity are provided. Full details on analytical methods for each of the compounds as well as representative HPLC traces for compounds **7**, **23**, **33**, **53**, **66**, **79**, and **80** are found in the Supporting Information. Chiral chromatography was carried out using a CHIRALPAK IB N-3 column (150 × 4.6 mm i.d., 3 μm) with UV detection at 220 nm. Chromatograms are provided for synthetic intermediates **50**, **74**, and **75** as well as final compounds **53**, **79**, and **80**. 3-fluoro-6-methoxyquinoline **11** was purchased from Ambeed, Inc. 8-bromo-7-fluoro-2-methoxy-1,5-naphthyridine **12** was purchased from Alchem Pharmtech, Inc. The aldehyde starting material (6,7-dihydro-[1,4]oxathiino[2,3-*c*]pyridazine-3-carbaldehyde) for lead compound **79** was purchased from Chemspace. Several of the final analogues in this manuscript were synthesized multiple times. In these cases, a representative procedure with full characterization data is provided. All tested compounds were analyzed for the presence of pan assay interference compounds (PAINS) using https://www.cbligand.org/PAINS/search_struct.php (accessed July 12, 2021); no PAINS liabilities were detected.

*2-(2-(2,2-Dimethoxyethyl)-1,3-trans-dioxan-5-yl)isoindoline-1,3-dione (**9**).* To a stirring solution of the previously reported[30] diol 2-(1,3-dihydroxypropan-2-yl)isoindoline-1,3-dione **8** (6.0 g, 27 mmol, 1.0 equiv) and *p*-toluenesulfonic acid monohydrate (0.516 g, 2.71 mmol, 0.10 equiv) in a three-neck flask was added 1,1,3,3-tetramethoxypropane (8.91 g, 54.2 mmol, 2.0 equiv) at room

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temperature. A Dean−Stark apparatus was attached, and the temperature was raised gradually to 90 °C and the reaction was stirred for 6 h. The reaction was cooled to room temperature and quenched with triethylamine (0.20 mL), then toluene was removed by evaporation under a stream of air. The residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexanes) to give the title compound (5.23 g, 16.3 mmol, 60%) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.86−7.80 (m, 2H), 7.76−7.70 (m, 2H), 4.76 (t, J = 5.4 Hz, 1H), 4.66−4.54 (m, 2H), 4.47−4.38 (m, 2H), 4.01 (dd, J = 10.9, 4.9 Hz, 2H), 3.35 (s, 6H), 1.99 (t, J = 5.7 Hz, 2H).

*2-(5-(1,3-Dioxoisoindolin-2-yl)-1,3-trans-dioxan-2-yl)-acetaldehyde (**10**).* To a stirred solution of **9** (5.23 g, 16.3 mmol) in water was added pyridinium *p*-toluenesulfonate (PPTS) (0.409 g, 1.63 mmol, 0.100 equiv), and the reaction was stirred for 12 h at 80 °C with an attached reflux condenser. Upon consumption of starting material, the reaction was cooled to room temperature, CH_2Cl_2 was added, and the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (gradient of 1−3% methanol in dichloromethane) to give the title compound (3.38 g, 12.3 mmol, 76%) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.82 (t, J = 2.2 Hz, 1H), 7.87−7.82 (m, 2H), 7.77−7.72 (m, 2H), 5.14 (t, J = 4.6 Hz, 1H), 4.68−4.58 (m, 1H), 4.52−4.43 (m, 2H), 4.10−4.02 (m, 2H), 2.76 (dd, J = 4.6, 2.2 Hz, 2H).

*2-(2-(2-(3-Fluoro-6-methoxyquinolin-4-yl)-2-hydroxyethyl)-1,3-trans-dioxan-5-yl)isoindoline-1,3-dione (**13**).* To a stirred solution of 3-fluoro-6-methoxyquinoline **11** (0.300 g, 1.69 mmol) in THF (20 mL) at −78 °C, under nitrogen, was slowly added LDA (1.0 M solution in THF, 5.1 mL, 5.1 mmol, 3.0 equiv). After 3 h of stirring, a solution of aldehyde **10** (0.699 g, 2.54 mmol, 1.50 equiv) in THF (14 mL) was added and the reaction was stirred at −78 °C for 1 h, then continued at room temperature for 2 h. The reaction was quenched at 0 °C with saturated NH_4Cl solution, then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (gradient of 0−5% methanol in CH_2Cl_2) to give the title compound as a yellowish solid (0.1464 g, 0.3236 mmol, 19%) with a small amount of CH_2Cl_2 impurity.

^{1}H NMR ($(CD_3)_2SO$, 400 MHz): δ 8.69 (d, J = 1.6 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.89 (d, J = 2.8 Hz, 1H), 7.88−7.82 (m, 4H), 7.39 (dd, J = 9.2, 2.7 Hz, 1H), 5.95 (d, J = 4.2 Hz, 1H), 5.61−5.54 (m, 1H), 4.74 (dd, J = 6.2, 4.2 Hz, 1H), 4.34−4.15 (m, 3H), 4.09−3.99 (m, 2H), 3.90 (s, 3H), 2.40 (ddd, J = 13.8, 8.3, 4.1 Hz, 1H), 2.07 (dt, J = 13.9, 5.9 Hz, 1H).

*2-(5-Amino-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**15**).* To compound **13** (0.1607 g, 0.3552 mmol, 1.0 equiv) in ethyl acetate (12 mL) was added ethanolamine (0.32 mL, 5.3 mmol, 15 equiv) at room temperature. The reaction was heated at 70 °C for 16 h then allowed to cool to room temperature. The ethyl acetate was removed *in vacuo*, and the residue dissolved in CH_2Cl_2 and washed with brine. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layers dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (gradient of 0−5% methanol in CH_2Cl_2) to afford the title compound (0.0832 g, 0.258 mmol, 72.7%) as a yellowish solid, with a small amount of CH_2Cl_2 and other small impurities. This reaction was repeated under similar conditions on a similar scale but afforded a lower yield (38%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (d, J = 1.8 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.76 (d, J = 2.6 Hz, 1H), 7.29 (dd, J = 9.2, 2.6 Hz, 1H), 5.81 (dd, J = 9.4, 3.5 Hz, 1H), 4.68 (t, J = 4.7 Hz, 1H), 4.21−4.12 (m, 2H), 3.92 (s, 3H), 3.25 (t, J = 10.7 Hz, 1H), 3.24 (t, J = 10.8 Hz, 1H), 3.16−3.05 (m, 1H), 2.56 (ddd, J = 14.4, 9.4, 5.0 Hz, 1H), 2.08 (dt, J = 14.4, 3.9 Hz, 1H).

General Procedure for Reductive Amination with Amine 15 (Series A Analogues). To a solution of amine **15** (1.0 equiv) and the requisite aldehyde (1.2 equiv) in methanol (0.05 M amine concentration) was added $ZnCl_2$ (0.2 equiv). The reaction was stirred for 1 h at room temperature, and $NaBH_3CN$ (3.0 equiv) was added. The reaction was stirred for 12 h at room temperature, whereupon analysis by TLC indicated that it was complete. Silica gel was added, and the solvent was removed *in vacuo*. Pure product was isolated by flash chromatography on silica gel (gradient elution with methanol in CH_2Cl_2).

*2-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**7**).* Using 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde, the title compound was prepared according to the general procedure (79.3 mg, 68%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (d, J = 2.3 Hz, 1H), 7.91 (d, J = 9.3 Hz, 1H), 7.84 (d, J = 2.7 Hz, 1H), 7.34 (dd, J = 9.32, 2.7 Hz, 1H), 6.80 (d, J = 1.3 Hz, 1H), 6.78−6.72 (m, 2H), 5.69 (dd, J = 8.1, 6.0 Hz, 1H), 4.58 (dd, J = 5.8, 4.6 Hz, 1H), 4.21 (s, 4H), 4.12 (ddd, J = 10.8, 4.8, 2.2 Hz, 1H), 4.07 (ddd, J = 10.8, 4.8, 2.2 Hz, 1H), 3.93 (s, 3H), 3.60 (s, 2H), 3.31 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 3.27 (t, J = 10.7 Hz, 1H), 2.86−2.76 (m, 1H), 2.43 (ddd, J = 13.9, 8.1, 4.1 Hz, 1H), 2.12 (dt, J = 13.9, 6.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 159.90, 155.44 (d, J = 252.9 Hz), 144.97, 144.32, 142.74 (d, J = 2.4 Hz), 139.12 (d, J = 31.1 Hz), 133.98, 133.06 (d, J = 8.9 Hz), 131.53, 129.49 (d, J = 3.3 Hz), 122.31, 122.29, 118.16, 118.12, 104.61 (d, J = 5.3 Hz), 100.73, 71.83, 71.80, 65.61, 65.58, 64.02 (d, J = 3.8 Hz), 56.11, 51.18, 50.47, 42.27; HRMS (ESI) m/z calc'd for $C_{25}H_{28}FN_2O_6$ $[M + H]^+$: 471.1931; found 471.1933. This analogue was prepared several times. HPLC of a representative sample: rt: 13.757 min, purity: 99.5%; see chromatogram in the Supporting Information.

*2-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-c]pyridin-7-yl)methyl)-amino)-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**17**).* Using 2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridine-7-carbaldehyde, the title compound was prepared according to the general procedure (22.2 mg, 69%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.54 (d, J = 2.2 Hz, 1H), 7.98 (s, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.84 (d, J = 2.7 Hz, 1H), 7.35 (dd, J = 9.3, 2.7 Hz, 1H), 6.94 (s, 1H), 5.69 (dd, J = 8.0. 6.1 Hz, 1H), 4.59 (dd, J = 5.6, 4.6 Hz, 1H), 4.39−4.34 (m, 2H), 4.33−4.27 (m, 2H), 4.13 (ddd, J = 10.8, 4.7. 2.0 Hz, 1H), 4.08 (ddd, J = 10.8, 4.7. 2.0 Hz, 1H), 3.93 (s, 3H), 3.72 (s, 2H), ~3.32 (t, 1H, obscured by solvent), 3.28 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 2.85−2.74 (m, 1H), 2.43 (ddd, J = 13.9, 8.1, 4.4 Hz, 1H), 2.13 (dt, J = 13.8, 6.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 159.90, 155.47 (d, J = 253.0 Hz), 154.15, 152.67, 142.75 (d, J = 3.1 Hz), 142.19, 139.29, 138.98, 133.10 (d, J = 8.7 Hz), 131.54, 129.50 (d, J = 3.2 Hz), 122.30 (d, J = 2.6 Hz), 112.3, 104.61 (d, J = 5.3 Hz), 100.72, 71.91, 71.88, 66.52, 65.45, 64.01 (d, J = 3.8 Hz), 56.12, 52.08, 50.79, 42.27; HRMS (ESI) m/z calc'd for $C_{24}H_{27}FN_3O_6$ $[M + H]^+$: 472.1884; found: 472.1883. HPLC: rt: 11.537 min, purity: 100%.

*2-(5-(((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)methyl)-amino)-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**18**).* Using 6,7-dihydro-[1,4]oxathiino[2,3-*c*]-pyridazine-3-carbaldehyde, the title compound was prepared according to the general procedure (42.9 mg, 76%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (d, J = 2.2 Hz, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.83 (d, J = 2.7 Hz, 1H), 7.52 (s, 1H), 7.33 (dd, J = 9.3, 2.7 Hz, 1H), 5.69 (dd, J = 8.1, 6.0 Hz, 1H), 4.69−4.63 (m, 2H), 4.60 (dd, J = 5.7, 4.6 Hz, 1H), 4.17 (ddd, J = 10.8, 4.8. 2.2 Hz, 1H), 4.12 (ddd, J = 10.8, 4.8. 2.2 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 2H), 3.36−3.24 (m, 4H overlapping with solvent), 2.87−2.76 (m, 1H), 2.43 (ddd, J = 13.9, 8.1, 4.4 Hz, 1H), 2.13 (dt, J = 13.9, 6.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 161.52, 159.83, 157.88, 155.42 (d, J = 253.1 Hz), 142.70 (d, J = 2.4 Hz), 139.13 (d, J = 31.1 Hz), 133.04 (d, J = 8.9 Hz), 131.53, 129.45 (d, J = 3.3 Hz), 129.30, 127.27, 122.23 (d, J = 2.5 Hz), 104.62 (d, J = 5.3 Hz), 100.70, 71.92, 71.88, 67.85, 64.00 (d, J = 3.8 Hz), 56.13, 50.89, 50.16, 42.27, 26.36; HRMS (ESI) m/z calc'd for $C_{23}H_{26}FN_4O_5S$ $[M + H]^+$: 489.1608; found: 489.1606. HPLC: rt: 5.914 min, purity: 100%.

*6-(((2-(2-(3-Fluoro-6-methoxyquinolin-4-yl)-2-hydroxyethyl)-1,3-trans-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (**19**).* Using 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]-

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oxazine-6-carbaldehyde, the title compound was prepared according to the general procedure (21.6 mg, 51%, contaminated with a small amount of methanol). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.54 (d, J = 2.3 Hz, 1H), 7.91 (d, J = 9.2 Hz, 1H), 7.84 (d, J = 2.7 Hz, 1H), 7.34 (dd, J = 9.2, 2.7 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 5.69 (dd, J = 8.0, 6.1 Hz, 1H), 4.63 (s, 2H), 4.59 (dd, J = 5.8, 4.6 Hz, 1H), 4.16 (ddd, J = 10.8, 4.8. 2.2 Hz, 1H), 4.10 (ddd, J = 10.7, 4.8. 2.2 Hz, 1H), 3.93 (s, 3H), 3.72 (s, 2H), 3.34 (t, J = 10.6 Hz, 1H, partially obscured by solvent), 3.30 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 2.89−2.79 (s, 1H), 2.43 (ddd, J = 13.8, 8.2, 4.5 Hz, 1H), 2.14 (dt, J = 13.9, 6.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 168.10, 159.88, 155.46 (d, J = 253.1 Hz), 152.02, 142.74 (d, J = 2.3 Hz), 142.13, 139.85, 139.12 (d, J = 31.2 Hz), 133.06 (d, J = 8.9 Hz), 131.54, 129.49 (d, J = 3.5 Hz), 124.88, 122.27 (d, J = 2.5 Hz), 118.95, 104.661 (d, J = 5.2 Hz), 100.75, 71.81, 71.78, 68.12, 64.00 (d, J = 3.7 Hz), 56.11, 51.72, 50.76, 42.24; HRMS (ESI) m/z calc'd for $C_{24}H_{26}FN_4O_6$ $[M + H]^+$: 485.1836; found: 485.1835. HPLC: rt: 5.975 min, purity: 96.9%.

*6-(((2-(2-(3-Fluoro-6-methoxyquinolin-4-yl)-2-hydroxyethyl)-1,3-trans-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]thiazin-3(4H)-one (**20**).* Using 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]-thiazine-6-carbaldehyde, the title compound was prepared according to the general procedure (32.9 mg, 65%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (d, J = 2.2 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.83 (d, J = 2.7 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.33 (dd, J = 9.2, 2.7 Hz, 1H), 6.98 (d, J = 7.8 Hz, 1H), 5.69 (dd, J = 8.0, 6.1 Hz, 1H), 4.62−4.57 (m, 1H), 4.16 (ddd, J = 10.8, 4.7. 2.0 Hz, 1H), 4.11 (ddd, J = 10.8, 4.6. 2.0 Hz, 1H), 3.92 (s, 3H), 3.76 (s, 2H), 3.50 (s, 2H), 3.35 (t, J = 10.6 Hz, 1H, partially obscured by solvent), 3.30 (t, J = 10.6 Hz, 1H, partially obscured by solvent), 2.89−2.79 (m, 1H), 2.43 (ddd, J = 13.9, 8.1, 4.4 Hz, 1H), 2.13 (dt, J = 13.8, 6.1 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 168.51, 159.85, 157.25, 155.43 (d, J = 253.0 Hz), 150.13, 142.72 (d, J = 2.5 Hz), 139.12 (d, J = 31.0 Hz), 137.28, 133.04 (d, J = 8.8 Hz), 131.54, 129.47 (d, J = 3.1 Hz), 122.25 (d, J = 2.2 Hz), 118.73, 115.55, 104.62 (d, J = 5.3 Hz), 100.75, 71.76, 71.74, 63.98 (d, J = 3.7 Hz), 56.11, 51.88, 50.85, 42.23, 30.12; HRMS (ESI) m/z calc'd for $C_{24}H_{25}FN_4O_5S$ $[M + H]^+$: 501.1608; found: 501.1574. HPLC: rt: 13.957 min, purity: 99.2%.

*2-(5-((3,4-Dichlorobenzyl)amino)-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**21**).* Using 3,4-dichlorobenzaldehyde, the title compound was prepared according to the general procedure (29.5 mg, 51%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.54 (d, J = 2.3 Hz, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.84 (d, J = 2.7 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.35 (dd, J = 9.2, 2.7 Hz, 1H), 7.25 (dd, J = 8.2, 2.0 Hz, 1H), 5.70 (dd, J = 8.1, 6.1 Hz, 1H), 4.59 (dd, J = 5.8, 4.6 Hz, 1H), 4.15 (ddd, J = 10.9, 4.8. 2.2 Hz, 1H), 4.10 (ddd, J = 10.7, 4.8. 2.2 Hz, 1H), 3.93 (s, 3H), 3.73 (s, 2H), 3.33 (t, J = 10.8 Hz, 1H, partially obscured by solvent), 3.29 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 2.86−2.75 (m, 1H), 2.44 (ddd, J = 13.9, 8.1, 4.4 Hz, 1H), 2.14 (dt, J = 13.9, 6.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 159.88, 155.44 (d, J = 253.0 Hz), 144.72 (d, J = 2.4 Hz), 142.32, 139.11 (d, J = 31.1 Hz), 133.25, 133.05 (d, J = 15.0 Hz), 131.83, 131.52, 131.48, 131.24, 129.48 (d, J = 3.1 Hz), 129.06, 122.27 (d, J = 2.5 Hz), 104.62 (d, J = 5.3 Hz), 100.73, 71.92, 71.89, 64.01 (d, J = 3.8 Hz), 56.10, 50.84, 50.45, 42.26; HRMS (ESI) m/z calc'd for $C_{23}H_{24}Cl_2FN_2O_4$ $[M + H]^+$: 481.1097; found: 481.1093. HPLC: rt: 20.589 min, purity: 100%.

*1-(3-Fluoro-6-methoxyquinolin-4-yl)-2-(5-((4-methylbenzyl)-amino)-1,3-trans-dioxan-2-yl)ethan-1-ol (**22**).* Using 4-methylbenzaldehyde, the title compound was prepared according to the general procedure (27.2 mg, 76%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.52 (d, J = 2.0 Hz, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.83 (d, J = 2.5 Hz, 1H), 7.33 (dd, J = 9.2, 2.5 Hz, 1H), 7.18 (br d, J = 7.9 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 5.68 (dd, J = 8.0, 6.0 Hz, 1H), 4.61−4.55 (m, 1H), 4.16−4.04 (m, 2H), 3.91 (s, 3H), 3.67 (s, 2H), 3.31 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 3.27 (t, J = 10.7 Hz, 1H, partially obscured by solvent), 2.87−2.76 (m, 1H), 2.42 (ddd, J = 13.9, 8.2, 4.4 Hz, 1H), 2.30 (s, 3H), 2.11 (dt, J = 13.8, 5.9 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): 159.88, 155.41 (d, J = 253.0 Hz), 142.73 (d, J = 2.4 Hz), 139.11 (d, J = 31.2 Hz), 138.02, 137.82, 133.03 (d, J = 8.9 Hz), 131.53, 130.11, 129.8 (d, J = 3.3 Hz), 129.36, 122.7 (d, J = 2.5 Hz), 104.62 (d, J = 5.3 Hz), 100.72, 71.85, 71.82, 64.01 (d, J = 3.9 Hz), 56.11, 51.49, 50.62, 42.29, 21.13; HRMS (ESI) m/z calc'd for $C_{24}H_{28}FN_2O_4$ $[M + H]^+$: 427.2033; found: 427.2042. HPLC: rt: 17.045 min, purity: 100%.

The synthesis of compounds **23**−**27** was previously reported in a Ph.D. dissertation.[61] The ^{13}C NMR spectra have been reanalyzed to determine ^{19}F−^{13}C coupling constants.

*2-(2-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-hydroxyethyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**14**).* To a solution of bromide **12** (137 mg, 0.533 mmol, 1.07 equiv) in THF (6 mL), n-hexyllithium (2.5 M, 0.213 mL, 0.53 mmol, 1.1 equiv) was added slowly dropwise at −78 °C under N_2 and stirred 30 min. Then, the mixture was added dropwise slowly into a solution of compound **10** (137.6 mg, 0.5000 mmol, 1.0 equiv) in THF at −78 °C under N_2. The reaction was stirred 2 h at −78 °C, then the temperature was raised to −20 °C gradually. After 3 h, the reaction was quenched by the addition of sat. aq. NH_4Cl (5 mL). Flash chromatography on silica gel with hexane/ethyl acetate (1:1) afforded the title compound as a white solid (40.4 mg, 0.0891 mmol, 18%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.65 (s, 1H); 8.25 (d, J = 9.2 Hz, 1H); 7.83 (dd, J = 5.5, 3.0 Hz, 2H); 7.73 (dd, J = 5.5, 3.0 Hz, 2H); 7.12 (d, J = 9.1 Hz, 1H); 6.10 (d, J = 10.4 Hz, 1H); 5.68−5.56 (m, 1H); 4.98 (dd, J = 6.5, 3.8 Hz, 1H); 4.62 (m, 1H); 4.45 (t, J = 10.7 Hz, 1H); 4.43 (t, J = 10.7 Hz, 1H); 4.15−3.95 (m, 2H); 4.09 (s, 3H); 2.54 (ddd, J = 13.9, 8.8, 3.8 Hz, 1H); 2.22 (ddd, J = 13.9, 6.4, 5.2 Hz, 1H).

*2-(5-Amino-1,3-trans-dioxan-2-yl)-1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethanol (**16**).* Compound **14** (40.2 mg, 0.0891 mmol, 1.0 equiv) was suspended in ethyl acetate (2 mL), ethanolamine (0.081 mL, 1.34 mmol, 15.0 equiv) was added, and the mixture was heated to 70 °C overnight and then washed with brine. Flash chromatography on silica gel with methanol/DCM (1:20) afforded the title compound as a yellow oil (18.9 mg, 0.0585 mmol, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.62 (d, J = 0.9 Hz, 1H); 8.22 (d, J = 9.2 Hz, 1H); 7.09 (d, J = 9.1 Hz, 1H); 5.57 (dd, J = 8.7, 5.0 Hz, 1H); 4.68 (dd, J = 6.6, 3.8 Hz, 1H); 4.16−4.04 (m, 2H); 4.05 (s, 3H); 3.23 (t, J = 10.6 Hz, 1H); 3.21 (t, J = 10.5 Hz, 1H); 3.11−2.97 (m, 1H); 2.47 (ddd, J = 13.8, 8.7, 3.8 Hz, 1H); 2.15 (ddd, J = 13.9, 6.5, 5.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.01, 154.96 (d, J = 258.2 Hz), 141.27, 140.98 (d, J = 5.7 Hz), 138.89, 138.69 (d, J = 25.5 Hz), 130.59 (d, J = 12.3 Hz), 115.73 (d, J = 2.7 Hz), 99.32, 73.62, 73.53, 64.98 (d, J = 4.1 Hz), 54.40, 44.25, 43.03.

General Procedure for Reductive Amination with Amine 16 (Series B Analogues). To a solution of amine **16** (0.2 mmol) in methanol (2 mL) were added the requisite aldehyde (0.2 mmol) and zinc chloride (2 mg, 0.015 mmol, 0.07 equiv). The mixture was stirred at room temperature for 30 min, followed by the addition of sodium cyanoborohydride (40 mg, 0.6 mmol, 3.0 equiv). The reaction mixture was stirred at room temperature overnight and then purified by chromatography on silica gel with DCM/methanol (30:1). Occasionally, the product obtained from flash chromatography was contaminated by a BH_3 adduct, seen in the ^{1}H NMR spectrum as four broad peaks from 0.00 to 0.88 ppm. In these instances, the column-purified material was dissolved in methanol (2 mL) and stirred overnight at ambient temperature with Amberlite IRA743 free base (ca. 100 mg). The pure title compound was then obtained by removal of the resin by filtration and removal of the solvent under reduced pressure.

*2-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**23**).* Using 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde, the title compound was prepared in 77% yield following the general method and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.62 (s, 1H); 8.22 (d, J = 9.1 Hz, 1H); 7.10 (d, J = 9.1 Hz, 1H); 6.81−6.71 (m, 3H); 6.03 (brs, 1H); 5.57 (brs, 1H); 4.70 (dd, J = 6.5, 3.8 Hz, 1H); 4.23 (s, 4H); 4.21−4.08 (m, 2H); 4.05 (s, 3H); 3.67 (s, 2H); 3.29 (t, J = 10.6 Hz, 1H); 3.27 (t, J = 10.5 Hz, 1H); 3.02−2.89 (m, 1H); 2.46 (ddd, J = 13.9, 8.7, 3.8 Hz, 1H); 2.13 (ddd, J = 13.8, 6.4, 5.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.00, 154.96 (d, J = 258.2), 143.62, 142.85, 141.28, 141.00 (d, J = 5.7 Hz),

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138.90, 138.69 (d, J = 25.0 Hz), 133.62, 130.60 (d, J = 12.3 Hz), 121.01, 117.35, 116.88, 115.71 (d, J = 2.6 Hz), 99.63, 71.79, 71.69, 65.00 (d, J = 4.0 Hz), 64.48, 64.46, 54.41, 50.89, 49.67, 43.12. HRMS (ESI) m/z calc'd for $C_{24}H_{26}FN_3O_6Na$ $[M + Na]^+$: 494.1703; found: 494.1696. HPLC: rt: 15.996 min, purity: 99.5%; see chromatogram in the Supporting Information.

2-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-c]pyridin-7-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**24**). Using 2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridine-7-carbaldehyde, the title compound was prepared in 73% yield following the general method and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.62 (d, J = 1.0 Hz, 1H); 8.22 (d, J = 9.1 Hz, 1H); 8.08 (s, 1H); 7.09 (d, J = 9.1 Hz, 1H); 6.76 (s, 1H); 6.05 (brs, 1H); 5.57 (br dd, J = 8.4, 4.7 Hz, 1H); 4.71 (dd, J = 6.6, 3.8 Hz, 1H); 4.33−4.30 (m, 2H); 4.27−4.24 (m, 2H); 4.23−4.10 (m, 2H); 4.04 (s, 3H); 3.76 (s, 2H); 3.36 (t, J = 10.6 Hz, 1H); 3.34 (t, J = 10.6 Hz, 1H); 3.00−2.88 (m, 1H); 2.46 (ddd, J = 13.9, 8.7, 3.8 Hz, 1H); 2.13 (ddd, J = 13.9, 6.6, 4.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.01, 154.96 (d, J = 258.3 Hz), 152.77, 150.40, 141.28, 141.01 (d, J = 5.7 Hz), 140.37, 139.03, 138.90, 138.69 (d, J = 25.0 Hz), 130.59 (d, J = 12.3 Hz), 115.72 (d, J = 2.6 Hz), 110.81, 99.65, 71.58, 71.49, 65.11, 64.99 (d, J = 4.2 Hz), 64.16, 54.43, 52.02, 50.02, 43.11. HRMS (ESI) m/z calc'd for $C_{23}H_{26}FN_4O_6$ $[M + H]^+$: 473.1836; found: 473.1838. HPLC: rt: 10.795 min, purity: 99.9%.

2-(5-(((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**25**). Using 6,7-dihydro-[1,4]oxathiino[2,3-*c*]pyridazine-3-carbaldehyde, the title compound was prepared in 41% yield following the general method and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.63 (d, J = 0.9 Hz, 1H); 8.23 (d, J = 9.2 Hz, 1H); 7.26 (s, 1H); 7.10 (d, J = 9.2 Hz, 1H); 6.06 (br d, J = 9.7 Hz, 1H); 5.57 (m, 1H); 4.72 (dd, J = 6.6, 3.8 Hz, 1H); 4.69−4.61 (m, 2H); 4.27−4.14 (m, 2H); 4.05 (s, 3H); 3.96 (s, 2H); 3.36 (t, J = 10.6 Hz, 1H); 3.34 (t, J = 10.6 Hz, 1H); 3.25−3.17 (m, 2H); 3.02−2.90 (m, 1H); 2.47 (ddd, J = 13.8, 8.8, 3.8 Hz, 1H); 2.13 (ddd, J = 13.9, 6.6, 4.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.03, 160.08, 156.27, 154.95 (d, J = 258.3 Hz), 141.31, 140.98 (d, J = 5.7 Hz), 138.91, 138.71 (d, J = 25.6 Hz), 130.55 (d, J = 12.3 Hz), 125.98, 125.18, 115.76 (d, J = 2.7 Hz), 99.68, 71.49, 71.39, 66.31, 65.00 (d, J = 4.1 Hz), 54.55, 50.22, 50.19, 43.11, 25.71. HRMS (ESI) m/z calc'd for $C_{22}H_{25}FN_5O_5S$ $[M + H]^+$: 490.1560; found: 490.1573. HPLC: rt: 12.603 min, purity: 97.2%.

6-(((2-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-hydroxyethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]-thiazin-3(4H)-one (**26**). Using 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*]-[1,4]thiazine-6-carbaldehyde, the title compound was prepared in 60% yield following the general method and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.73 (brs, 1H); 8.63 (s, 1H); 8.24 (d, J = 9.1 Hz, 1H); 7.57 (d, J = 7.8 Hz, 1H); 7.10 (d, J = 9.1 Hz, 1H); 6.94 (d, J = 7.8 Hz, 1H); 6.07 (brs, 1H); 5.57 (brs, 1H); 4.72 (dd, J = 6.4, 3.8 Hz, 1H); 4.18 (m, 2H); 4.05 (s, 3H); 3.82 (s, 2H); 3.46 (s, 2H); 3.37 (t, J = 10.6 Hz, 1H); 3.35 (t, J = 10.5 Hz, 1H); 3.03−2.86 (m, 1H); 2.47 (ddd, J = 13.9, 8.7, 3.6 Hz, 1H); 2.21−2.06 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.73, 162.04, 154.95 (d, J = 258.2 Hz), 148.44, 141.31, 141.02 (d, J = 5.5 Hz), 138.91, 138.70 (d, J = 26.6 Hz), 138.53, 136.39, 130.55 (d, J = 12.4 Hz), 117.92, 115.78, 114.10, 99.70, 71.48, 71.39, 64.99 (d, J = 4.6 Hz), 54.45, 51.63, 50.12, 43.12, 29.78. HRMS (ESI) m/z calc'd for $C_{23}H_{25}FN_5O_5S$ $[M + H]^+$: 502.1560; found: 502.1573. HPLC: rt: 8.875 min, purity: 100%.

2-(5-((3,4-Dichlorobenzyl)amino)-trans-1,3-dioxan-2-yl)-1-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**27**). Using 3,4-dichlorobenzaldehyde, the title compound was prepared in 68% yield following the general method and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.62 (d, J = 0.9 Hz, 1H); 8.22 (d, J = 9.2 Hz, 1H); 7.40 (d, J = 2.0 Hz, 1H); 7.36 (d, J = 8.2 Hz, 1H); 7.12 (dd, J = 8.2, 2.0 Hz, 1H); 7.10 (d, J = 9.1 Hz, 1H); 6.07 (br d, J = 8.7 Hz, 1H); 5.57 (s, 1H); 4.71 (dd, J = 6.7, 3.8 Hz, 1H); 4.19 (ddd, J = 10.8, 4.7, 2.3, 1H), 4.14 (ddd, J = 10.8, 4.7, 2.3, 1H); 4.05 (s, 3H); 3.75 (s, 2H); 3.31 (t, J = 10.6 Hz, 1H); 3.28 (t, J = 10.6 Hz, 1H); 3.02−2.84 (m, 1H); 2.47 (ddd, J = 13.9, 8.8, 3.8 Hz, 1H); 2.13 (ddd, J = 13.9, 6.6, 4.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.02, 154.92 (d, J = 258.2 Hz), 141.29, 140.98 (d, J = 5.7 Hz), 140.64, 138.88, 138.68 (d, J = 26.9 Hz), 132.65, 131.21, 130.52 (d, J = 12.3 Hz), 130.50, 129.88, 127.28, 115.75 (d, J = 2.6 Hz), 99.67, 71.64, 71.53, 64.98 (d, J = 4.1 Hz), 54.42, 50.23, 49.95, 43.10. HRMS (ESI) m/z calc'd for $C_{22}H_{23}Cl_2FN_3O_4$ $[M + H]^+$: 482.1050; found: 482.1048. HPLC: rt: 19.733 min, purity: 99.4%.

2-(2-(Oxiran-2-yl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**29**). To a solution of previously reported[30] alkene **28** (5.60 g, 10.8 mmol, 1.0 equiv) in dichloromethane (200 mL) was added *m*CPBA (3.72 g, 21.6 mmol, 2.0 equiv) portionwise at room temperature, and the reaction mixture was stirred for 14 h. Next day, another portion of *m*CPBA (3.72 g, 21.6 mmol, 2.0 equiv) was added and mixture was stirred for 4 days at room temperature. The reaction mixture was diluted with dichloromethane and washed with NaOH (1 M, 3 × 200 mL) and brine (200 mL). The organic layer was dried over sodium sulfate and concentrated to get crude material, which was purified by flash column chromatography on silica gel with hexane/ethyl acetate (1:7) to afford the title compound as a white solid (6.40 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86−7.80 (m, 2H); 7.76−7.71 (m, 2H); 4.71−4.61 (m, 1H); 4.59 (d, J = 4.0 Hz, 1H); 4.49−4.39 (m, 2H); 4.13−4.04 (m, 2H); 3.16−3.12 (m, 1H); 2.86−2.80 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.87; 134.52; 131.64; 123.62; 100.15; 66.41; 66.38; 51.42; 44.04; 43.91. HRMS (ESI) m/z calc'd for $C_{14}H_{14}NO_5$ $[M + H]^+$: 276.0872; found: 276.0870.

2-(2-(1-Hydroxy-2-(7-methoxy-2-oxoquinoxalin-1(2H)-yl)ethyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**31**). A mixture of known[26] compound **30** (50 mg, 0.28 mmol, 1.0 equiv), epoxide **29** (117 mg, 0.43 mmol, 1.5 equiv), cesium carbonate (192 mg, 0.59 mmol, 2.1 equiv), and $Mg(OTf)_2$ (90 mg, 0.28 mmol, 1.0 equiv) in anhydrous *N,N*-dimethylformamide (5 mL) was stirred at 60 °C for 1 h and then at 80 °C for 14 h. The reaction mixture was cooled, and volatiles were removed under reduced pressure. The obtained crude material was distributed in between ethyl acetate (20 mL) and water (20 mL). Organic layer was separated, and aqueous layer was extracted with ethyl acetate (2 × 20 mL). Combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, and concentrated to get crude material, which was purified using column chromatography on silica gel with dichloromethane/methanol (3:1) to afford the title compound as an off-white solid, contaminated with DMF and DCM (100 mg, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.16 (s, 1H); 7.89−7.82 (m, 2H); 7.80 (d, J = 8.8 Hz, 1H); 7.79−7.72 (m, 2H); 6.98 (d, J = 2.4 Hz, 1H); 6.95 (dd, J = 8.8, 2.5 Hz, 1H); 4.89 (d, J = 3.6 Hz, 1H); 4.74−4.61 (m, 2H); 4.53 (t, J = 10.8 Hz, 1H); 4.52 (t, J = 10.9 Hz, 1H); 4.40 (dd, J = 14.7, 2.5 Hz, 1H); 4.19−4.07 (m, 3H); 3.92 (s, 3H). Additional characterization was carried out using material from a previous synthesis. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.87; 162.16; 156.80; 146.10; 134.57; 131.90; 131.61; 128.63; 123.67; 111.79; 101.05; 98.39; 71.23; 66.54; 66.41; 55.92; 44.56; 44.21. HRMS (ESI) m/z calc'd for $C_{23}H_{22}N_3O_7$ $[M + H]^+$: 452.1458; found: 452.1453.

1-(2-(5-Amino-trans-1,3-dioxan-2-yl)-2-hydroxyethyl)-7-methoxyquinoxalin-2(1H)-one (**32**). A mixture of phthalimide **31** (0.38 g, 0.84 mmol, 1.0 equiv), ethanolamine (1.53 mL, 25.3 mmol, 30 equiv), and ethyl acetate (30 mL) was stirred at 80 °C overnight. The solvent was removed, the mixture was dissolved in 10% methanol in dichloromethane (100 mL), and washed with brine. Aqueous layer was extracted with 10% methanol in dichloromethane (4 × 25 mL) The combined organic layers were dried over sodium sulfate, concentrated, and the crude product was purified by chromatography on silica gel with dichloromethane/methanol (9:1) to give the title compound as a white solid in ca. 90% purity (0.17 g, 0.53 mmol, 63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.03 (s, 1H); 7.73 (d, J = 8.8 Hz, 1H); 7.08 (d, J = 2.5 Hz, 1H); 6.98 (dd, J = 8.8, 2.5 Hz, 1H); 5.19 (d, J = 5.9 Hz, 1H); 4.46 (d, J = 4.2 Hz, 1H); 4.34 (dd, J = 13.9, 9.1 Hz, 1H); 4.22 (dd, J = 14.0, 3.3 Hz, 1H); 4.05−3.97 (m, 2H); 3.88 (s, 3H); 3.88−3.82 (m, 1H); 3.22 (br t, J = 10.3 Hz, 2H); 2.87−2.77 (m, 1H); 1.42 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 160.93; 154.87; 146.35; 134.95; 130.91; 127.89; 110.85; 101.38;

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99.44; 72.70; 72.66; 68.46; 55.7; 44.32; 43.57. HRMS (ESI) m/z calc'd for $C_{15}H_{20}N_3O_5$ $[M + H]^+$: 322.1403; found: 322.1401.

General Procedure for Reductive Amination with Amine 32 (Series C Analogues). To a solution of amine **32** (0.2 mmol) in methanol (4 mL) and dichloromethane (3 mL) was added the requisite aldehyde (0.2 mmol) and zinc chloride (0.2 equiv). The mixture was stirred at room temperature for 30 min, followed by the addition of sodium cyanoborohydride (3.0 equiv). The reaction mixture was stirred at room temperature overnight and then purified by chromatography on silica gel with dichloromethane/methanol (19:1).

*1-(2-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-hydroxyethyl)-7-methoxyquinoxalin-2(1H)-one (**33**).* The title compound was obtained by reaction with 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde following the general procedure. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.02 (s, 1H); 7.74 (d, J = 8.9 Hz, 1H); 7.14 (d, J = 2.4 Hz, 1H); 7.00 (dd, J = 9.0, 2.5 Hz, 1H); 6.84 (br s, 1H); 6.79 (br s, 2H); 4.59 (d, J = 3.6 Hz, 1H); 4.54 (dd, J = 14.2, 8.8 1H); 4.36 (dd, J = 14.3, 3.2 1H); 4.27−4.16 (m, 6H); 4.05−4.00 (m, 1H); 3.92 (s, 3H); 3.67 (s, 2H); 3.41 (br t, J = 11.4 Hz, 2H); 2.97−2.87 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.46; 157.53; 146.77; 144.99; 144.41; 136.03; 133.66; 132.25; 129.78; 122.35; 118.23; 118.18; 113.31; 102.92; 99.97; 71.65; 71.62; 70.54; 65.61; 65.59; 56.43, 51.15, 50.50; 45.03. HRMS (ESI) m/z calc'd for $C_{24}H_{28}N_3O_7$ $[M + H]^+$: 470.1927; found: 470.1925. HPLC: rt: 2.715 min, purity: 100%; see chromatogram in the Supporting Information.

*1-(2-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-c]pyridin-7-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-2-hydroxyethyl)-7-methoxyquinoxalin-2(1H)-one (**34**).* The title compound was obtained by reaction with 2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridine-7-carbaldehyde following the general procedure. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.01 (br s, 2H), 7.72 (d, J = 8.9 Hz, 1H); 7.12 (d, J = 1.6 Hz, 1H); 7.01−6.94 (m, 2H); 4.60 (d, J = 3.5 Hz, 1H); 4.52 (dd, J = 14.1, 8.9Hz, 1H); 4.44−4.28 (m, 5H); 4.28−4.16 (m, 2H); 4.07−3.99 (m, 1H); 3.92 (s, 3H); 3.78 (s, 2H); 3.42 (br t, J = 10.2 Hz, 1H); 2.96−2.85 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.45; 157.54; 153.86; 152.67; 146.79; 142.22; 138.99; 136.05; 132.26; 129.78; 113.29; 112.24; 102.93; 99.98; 71.74; 70.49; 66.52; 65.45; 56.43; 51.99; 50.85; 45.02. HRMS (ESI) m/z calc'd for $C_{23}H_{27}N_4O_7$ $[M + H]^+$: 471.1880; found: 471.1877. HPLC: rt: 2.469 min, purity: 100%.

*6-(((2-(1-Hydroxy-2-(7-methoxy-2-oxoquinoxalin-1(2H)-yl)-ethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]-oxazin-3(4H)-one (**35**).* The title compound was obtained by reaction with 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.17 (s, 1H); 8.02 (s, 1H); 7.72 (d, J = 8.8 Hz, 1H); 7.31 (d, J = 8.1 Hz, 1H); 7.06 (d, J = 2.4 Hz, 1H); 7.01 (d, J = 8.1 Hz, 1H); 6.97 (dd, J = 8.8, 2.4 Hz, 1H); 5.19 (d, J = 5.9 Hz, 1H); 4.61 (s, 2H); 4.49 (d, J = 4.1 Hz, 1H); 4.31 (dd, J = 14.0, 9.2 Hz, 1H); 4.25−4.08 (m, 3H); 3.90−3.80 (m, 1H), 3.86 (s, 3H), 3.70 (s, 2H); 3.31 (t, J = 10.5 Hz, partially obscured by water, 2H); 2.84−2.72 (m, 1H); 2.14 (br, s, 1H). A second sample was prepared for further characterization. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.87, 160.93, 154.87, 152.11, 146.35, 140.75, 137.64, 134.96, 130.92, 127.90, 123.39, 116.60, 110.82, 101.72, 99.47, 70.59, 68.41, 66.70, 55.75, 54.90, 50.92, 49.73, 43.52. HRMS (ESI) m/z calc'd for $C_{23}H_{26}N_5O_7$ $[M + H]^+$: 484.1832; found: 484.1828. HPLC: rt: 3.292 min, purity: 95.6%.

*3-Fluoro-4-iodo-6-methoxyquinoline (**36**).* To a stirred solution of 3-fluoro-6-methoxyquinoline **11** (2.48 g, 14.0 mmol, 1.00 equiv) in THF (35 mL) at −78 °C under nitrogen was added dropwise a solution of LDA (1 M in THF, 15.4 mL, 15.4 mmol, 1.10 equiv), and the resulting mixture was stirred at −78 °C for 30 min. Iodine (7.10 g, 28.0 mmol, 2.00 equiv) dissolved in THF (18 mL) was added dropwise by a syringe over 30 min at −78 °C under nitrogen. The mixture was allowed to slowly warm up to room temperature and stirred under nitrogen at room temperature overnight. The reaction mixture was then quenched by adding 30 mL of saturated aqueous Na_2SO_3. After diluting with DCM/water, the aqueous layer was extracted with DCM (30 mL × 3), and the combined organic layers were washed with water and brine, dried over Na_2SO_4, and decanted. The solvent was removed *in vacuo* to afford the title compound as a light yellow solid (3.82 g, 12.6 mmol, 89.8%). The reaction was run five times under similar conditions with an average yield of 72.2% (range 42.0−89.9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.64 (s, 1H), 7.99 (d, J = 9.1 Hz, 1H), 7.34 (dd, J = 9.1, 2.7 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 4.01 (s, 3H).

*2-(2-((E)-2-(3-Fluoro-6-methoxyquinolin-4-yl)vinyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**44**).* Iodoquinoline **36** (5.46 g, 18.0 mmol, 1.0 equiv), alkene **28** (4.7 g, 18 mmol, 1.0 equiv), palladium acetate (0.808 g, 3.60 mmol, 0.20 equiv), triphenylphosphine (1.89 g, 5.60 mmol, 0.40 equiv), and silver carbonate (10.0 g, 36.3 mmol, 2.0 equiv) were charged into a three-neck flask, which was evacuated and refilled with nitrogen three times. Anhydrous mesitylene (150 mL) was sparged with nitrogen and added by a syringe. The resulting mixture was heated at 127 °C and stirred under nitrogen overnight. The mixture was cooled down to room temperature, filtered through a pad of celite, and concentrated *in vacuo*. The crude product was used for the next step without further purification.

*2-((E)-2-(3-Fluoro-6-methoxyquinolin-4-yl)vinyl)-trans-1,3-dioxan-5-amine (**45**).* To a solution of phthalimide **44** (7.82 g, 18.0 mmol, 1.00 equiv) in 100 mL of ethyl acetate was added ethanolamine (13 mL, 220 mmol, 12 equiv) at room temperature. The resulting mixture was heated at 70 °C and stirred under nitrogen overnight, then allowed to cool to room temperature, and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed with saturated aqueous Na_2CO_3 and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−25% gradient) to afford the title compound in ca. 90% purity as a white solid (3.3 g, 11.53 mmol, 64.04%). This reaction was run three times under similar conditions with an average yield of 48.2% (range 24.9−64.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.61 (d, J = 2.2 Hz, 1H), 7.97 (d, J = 9.0 Hz, 1H), 7.31 (dd, J = 9.0, 2.6 Hz, 1H), 7.28 (d, J = 2.5 Hz, 1H), 7.08 (dd, J = 16.4, 1.2 Hz, 1H), 6.52 (dd, J = 16.6, 3.8 Hz, 1H), 5.15 (dd, J = 3.9, 0.9 Hz, 1H), 4.26 (dd, J = 11.4, 4.9 Hz, 2H), 3.94 (s, 3H), 3.41 (t, J = 10.9 Hz, 2H), 3.23−3.15 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.89, 154.13 (d, J = 257.8 Hz), 141.77 (d, J = 2.4 Hz), 138.71 (d, J = 29.7 Hz), 135.77 (d, J = 9.3 Hz), 131.63, 128.20 (d, J = 2.4 Hz), 124.21 (d, J = 9.4 Hz), 121.70, 120.67 (d, J = 2.7 Hz), 102.39 (d, J = 5.4 Hz), 99.53, 73.63, 55.78, 44.26.

*tert-Butyl (2-((E)-2-(3-Fluoro-6-methoxyquinolin-4-yl)vinyl)-trans-1,3-dioxan-5-yl)carbamate (**46**).* To a solution of amine **45** (0.622 g, 2.04 mmol, 1.00 equiv) and di-*tert*-butyl dicarbonate (1.42 g, 6.51 mmol, 3.18 equiv) in DCM (50 mL) at 0 °C was added triethylamine (1.2 mL, 8.61 mmol, 4.2 equiv), and the mixture was allowed to slowly warm up to room temperature overnight. The mixture was diluted with DCM and water, and the aqueous layer was extracted with DCM (30 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−10% gradient) to afford the title compound in ca. 90% purity as a white solid (0.53 g, 1.31 mmol, 60.11%). This reaction was run three times under similar conditions with an average yield of 59.0% (range 51.8−65.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (d, J = 2.1 Hz, 1H), 7.98 (d, J = 9.1 Hz, 1H), 7.31 (dd, J = 9.1, 2.7 Hz, 1H), 7.27 (d, J = 2.6 Hz, 1H), 7.08 (dd, J = 16.4, 1.2 Hz, 1H), 6.52 (dd, J = 16.5, 3.7 Hz, 1H), 5.18 (d, J = 3.4 Hz, 1H), 4.44−4.36 (br m, 1H), 4.34 (dd, J = 11.3, 4.9 Hz, 2H), 4.05−3.93 (br m, 1H), 3.94 (s, 3H), 3.52 (t, J = 10.5 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.96, 155.05, 154.11 (d, J =257.8 Hz), 141.70 (d, J = 2.4 Hz), 138.59 (d, J = 29.8 Hz) 135.49 (d, J = 9.2 Hz) 131.59, 128.21 (d, J = 2.5 Hz), 124.23 (d, J = 9.5 Hz), 121.98, 120.82, 102.30 (d, J = 5.2 Hz), 99.47, 80.27, 69.90, 55.78, 43.40, 28.44.

*tert-Butyl (2-((1S,2R)-2-(3-Fluoro-6-methoxyquinolin-4-yl)-1,2-dihydroxyethyl)-trans-1,3-dioxan-5-yl)carbamate (**47**).* To a solution of alkene **46** (0.882 g, 2.18 mmol, 1.00 equiv) in a mixture of *tert*-butanol (20 mL), ethyl acetate (4 mL) and water (20 mL) were added AD-mix-beta (5.7 g, 2.6 g/mmol of alkene) and methane-

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sulfonamide (0.250 g, 2.63 mmol, 1.20 equiv) at room temperature. The resulting mixture was stirred at room temperature overnight. $NaHSO_3$ (7.5 g, 72 mmol) was added, and the mixture was stirred for 30 min. After diluting with ethyl acetate (50 mL) and water (50 mL), the aqueous layer was extracted with ethyl acetate (30 mL × 3) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−25% gradient) to afford the title compound as a light yellow solid (0.38 g, 0. 7 mmol, 40%). This reaction was run three times under similar conditions with an average yield of 37.0% (range 26−46%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with methanesulfonamide and trace DCM) δ: 8.56 (d, J = 2.3 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.51 (br s, 1H), 7.30 (dd, J = 9.2, 2.6 Hz, 1H), 5.66 (d, J = 4.7 Hz, 1H), 4.90 (br s, 1H), 4.46 (d, J = 3.4 Hz, 1H), 4. 28−4.17 (m, 3H), 4.04 (br t, J = 3.8 Hz, 1H), 3.91 (s, 3H), 3.32 (t, J = 11.0 Hz, 1H), 3.28 (t, J = 11.0 Hz, 1H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.84, 155.26 (d, J = 255.4 Hz), 154.97, 141.44, 138.42 (d, J = 30.8 Hz), 131.37, 128.01 (d, J = 3.0 Hz) 127.68, 121.28, 102.67, 100.05, 80.49, 73.80 (d, J = 1.4 Hz), 70.07, 69.96, 67.85, 55.68, 43.16, 28.40. The enantioselectivity of this reaction was determined by analysis of intermediate **50** as well as final compound **53** (range in repeat reactions from 90 to 93% ee).

*tert-Butyl (2-((4S,5S)-5-(3-Fluoro-6-methoxyquinolin-4-yl)-2-oxo-1,3-dioxolan-4-yl)-trans-1,3-dioxan-5-yl)carbamate (**48**).* To a solution of diol **47** (0.380 g, 0.867 mmol, 1.00 equiv) in 5 mL of 2-butanone were added carbonyldiimidazole (0.210 g, 1.30 mmol, 1.49 equiv) and triethylamine (0.242 mL, 1.74 mmol, 2.00 equiv). The resulting mixture was stirred at 60 °C under nitrogen overnight. The mixture was then cooled to room temperature and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) to afford the title compound as an off-white solid (0.353 g, 0.760 mmol, 87.4%). This reaction was run three times under similar conditions with an average yield of 66.5% (range 41.9−87.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.66 (d, J = 1.9 Hz, 1H), 8.03 (d, J = 9.3 Hz, 1H), 7.36 (dd, J = 9.3, 2.6 Hz, 1H), 7.16 (d, J = 2.6 Hz, 1H), 6.48 (d, J = 5.2 Hz, 1H), 4.89 (d, J = 2.8 Hz, 1H), 4.75 (dd, J = 5.2, 2.8 Hz, 1H), 4.45−4.33 (m, 2H), 4.22 (ddd, J = 10.8, 5.1, 1.9 Hz, 1H), 4.06−3.97 (br m, 1H), 3.97 (s, 3H), 3.51 (t, J = 10.9Hz, 1H), 3,42 (t, J = 10.9 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.74, 155. 47 (d, J = 260.1 Hz), 154.88, 153.89, 141.88 (d, J = 2.7 Hz), 138.52 (d, J = 28.7 Hz), 132.29, 126.94 (d, J = 1.7 Hz), 122.88 (d, J = 7.2 Hz), 121.77, 100.31 (d, J = 4.6 Hz), 97.53, 80.55, 79.26 (d, J = 1.5 Hz), 70.71 (d, J = 2.3 Hz), 70.16, 69.52, 55.80, 43.44, 28.36.

*tert-Butyl (2-((S)-2-(3-Fluoro-6-methoxyquinolin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)carbamate (**49**).* To a suspension of carbonate **48** (0.600 g, 1.29 mmol, 1.00 equiv) in ethanol (9 mL) and ethyl acetate (0.650 mL) under nitrogen was added ammonium formate (0.455 g, 7.22 mmol, 5.59 equiv). The mixture was heated to 70 °C, and 5% Pd/$CaCO_3$ (0.052 g, 0.040 g/mmol) and Pd/C (0.013 g, 0.010 g/mmol) were added. The resulting mixture was stirred at 40 °C for 12 h, then cooled to room temperature, filtered through a pad of celite, and concentrated *in vacuo*. Purification by flash chromatography on silica gel (DCM/MeOH, 0−15% gradient) afforded the title compound as an off-white solid (0.423 g, 1.00 mmol, 77.0%). This reaction was run twice under similar conditions with an average yield of 83.1% (range 77.0−89.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (br s, 1H), 7.88 (d, J = 9.1 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 7.25 (dd, J = 9.1, 2.6 Hz, 1H), 4.55−4.40 (br m, 1H), 4.45 (d, J = 4.1 Hz, 1H), 4.34−4.24 (m, 2H), 4.06−3.93 (m, 2H), 3.92 (s, 3H), 3.42−3.22 (m, 5H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.64, 155.58 (d, J = 252.6 Hz), 155.01, 141.20 (d, J = 1.8 Hz), 137.75 (d, J = 29.7 Hz), 131.23, 129.66 (d, J = 3.4 Hz), 126.82 (d, J = 12.7 Hz), 120.60, 102.21 (d, J = 4.3 Hz), 101.75, 80.21, 71.61, 69.92, 55.59, 43.24, 28.34, 26.85.

The (*S*)-stereochemistry of the hydroxyl group in intermediate **49** was established by the synthesis and ^{1}H NMR analysis of the esters derived from (*S*)-Mosher's acid and (*R*)-Mosher's acid.[62] See Table S6, Supporting Information, for a tabulation of chemical shift changes.

*(S)-1-(5-Amino-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**50**).* To a solution of Boc-amine **49** (0.585 g, 1.38 mmol, 1.00 equiv) in DCM (15 mL) at 0 °C was added dropwise trifluoroacetic acid (2.76 mL, 36.0 mmol, 26.0 equiv), and the resulting mixture was allowed to warm slowly to room temperature and stirred for 2 additional hours. The mixture was then cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$. After diluting with DCM and water, the pH was adjusted to 10 by the addition of 1 M aqueous NaOH. The two layers were separated, and the aqueous layer was extracted with DCM (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated onto silica gel, and purified by flash chromatography on silica gel (DCM/MeOH, 0−20% gradient) to afford the title compound as an off-white solid (0.3 g, 0.931 mmol, 66.5%, ee = 93.3%). This reaction was run three times under similar conditions with an average yield of 73.9% (range 66.5−81.3%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.52 (d, J = 1.4 Hz, 1H), 7.88 (d, J = 9.2 Hz, 1H), 7.41 (d, J = 2.7 Hz, 1H), 7.32 (dd, J = 9.2, 2.7 Hz, 1H), 4.49 (d, J = 3.9 Hz, 1H), 4.23−4.12 (m, 2H), 3.94 (s, 3H), 3.87 (dt, J = 8.9, 3.7 Hz, 1H), 3.40−3.32 (m, 3H), 3.23 (ddd, J = 13.9, 9.0, 0.9 Hz, 1H), 3.04−2.94 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 160.23, 157.01 (d, J = 250.9 Hz), 142.13 (d, J = 2.1 Hz), 138.50 (d, J = 30.6 Hz), 131.36, 131.21 (d, J = 3.9 Hz), 129.88 (d, J = 13.0 Hz), 122.20 (d, J = 2.4 Hz), 103.55 (d, J = 5.4 Hz), 103.33, 73.55, 72.81, 72.80, 56.12, 45.29, 27.82 (d, J = 1.5 Hz). The enantiomeric excess for this material was determined to be 93% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive); see chromatogram in the Supporting Information. A separate synthesis of intermediate **50** afforded materials of similar ee (90%, analyzed at the stage of final compound **53**).

General Procedure for Reductive Amination with Amine 50 (Series D Analogues). Primary amine **50** (1 equiv), the requisite aldehyde (1−2 equiv), and 4 Å molecular sieves (300 mg/mmol of amine) were charged into a flask, which was evacuated and refilled with nitrogen three times. THF (15 mL/mmol), MeOH (12.5 mL/mmol), and acetic acid (1.5 equiv) were added by a syringe. The resulting mixture was stirred at room temperature for 4 h. $NaBH_3CN$ (3 equiv) was added, and the mixture was stirred for another 45 min. The mixture was cooled down to 0 °C and quenched by saturated aqueous $NaHCO_3$. After diluting with DCM and water, the pH was adjusted to 10 by adding 1 M sodium hydroxide. The two layers were separated, and the aqueous layer was extracted with DCM (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated onto silica gel, and purified by flash chromatography on silica gel (DCM/MeOH, 0−20% gradient) to afford the secondary amine products listed below. Reactions were typically conducted on a 0.15−0.2 mmol scale.

*(S)-1-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**51**).* The title compound (0.040 g, 0.085 mmol, 53.1%) was afforded as a white solid following the general reductive amination method with 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde. ^{1}H NMR (300 MHz, CD_3OD) δ: 8.49 (d, J = 1.4 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H), 7.35 (d, J = 2.6 Hz, 1H), 7.29 (dd, J = 9.2, 2.6 Hz, 1H), 6.82 (br s, 1H), 6.76 (br s, 2H), 4.47 (d, J = 3.9 Hz, 1H), 4.26−4.12 (m, 6H), 3.90 (s, 3H), 3.84 (dt, J = 8.7, 3.8 Hz, 1H), 3.62 (s, 2H), 3.38 (t, J = 10.6 Hz, 1H), 3.36 (t, J = 10.6 Hz, 1H), 3.33−3.25 (m, 1H, partially obscured by solvent), 3.17 (dd, J = 13.9, 9.4 Hz, 1H), 2.95−2.82 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 160.15, 156.95 (d, J = 250.9 Hz), 144.95, 144.29, 142.10 (d, J = 2.2 Hz), 138.46 (d, J = 30.5 Hz), 134.08, 131.33, 131.16 (d, J = 3.9 Hz), 129.83 (d, J = 13.0 Hz), 122.25, 122.16 (d, J = 2.6 Hz), 118.13 (overlapping peaks), 103.61, 103.54, 72.77 (overlapping peaks), 71.85, 65.59, 65.56, 56.12, 51.21, 50.57, 27.80 (d, J = 1.2 Hz). HRMS (ESI) m/z calc'd for $C_{25}H_{27}FN_2O_6$ $[M + H]^+$: 471.1931; found: 471.1943. HPLC: rt: 17.511 min, purity: 100%. Enantiomeric excess

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(ee) presumed to be 90% based on the analysis of compound **53**, which was synthesized from the same batch of amine **50**.

*(S)-1-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-c]pyridin-7-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**52**).* The title compound as a colorless oil residue (0.043 g, 0.091 mmol, 56.9%) was prepared following the general reductive amination method with 2,3-dihydro-[1,4]dioxino[2,3-*c*]-pyridine-7-carbaldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.53 (d, J = 1.5 Hz, 1H), 8.00 (s, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.43 (d, J = 2.7 Hz, 1H), 7.33 (dd, J = 9.2, 2.7 Hz, 1H), 6.97 (s,1H), 4.50 (d, J = 3.8 Hz, 1H), 4.40−4.36 (m, 2H), 4.33−4.29 (m, 2H), 4.24 (ddd, J = 10.7, 4.8, 2.1 Hz, 1H), 4.19 (ddd, J = 10.7, 4.8, 2.1 Hz, 1H), 3.94 (s, 3H), 3.88 (dt, J = 8.8, 4.0 Hz, 1H), 3.76 (s, 2H), 3.45−3.35 (m, 3H), 3.24 (ddd, J = 13.9, 8.8, 1.0 Hz, 1H), 2.942−2.84 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 160.25, 157.05 (d, J = 250.9 Hz), 154.25, 152.68, 142.20, 142.18, 138.99, 138.51 (d, J = 30.6 Hz), 131.37, 131.26 (d, J = 3.9 Hz), 129.91 (d, J = 12.9 Hz), 122.23 (d, J = 2.6 Hz), 112.24, 103.65, 103.59, 72.79, 72.78, 71.93, 66.53, 65.46, 56.14, 52.13, 50.91, 27.80 (d, J = 1.4 Hz). HRMS (ESI) m/z calc'd for $C_{24}H_{26}FN_3O_6$ $[M + H]^+$: 472.1884; found: 472.1897. HPLC: rt: 15.111 min, purity: 100%. Enantiomeric excess (ee) presumed to be 90% based on the analysis of compound **53**, which was synthesized from the same batch of amine **50**.

*(S)-1-(5-(((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**53**).* The title compound as a colorless oil residue (0.023 g, 0.047 mmol, 23.5%) was prepared following the general reductive amination method with 6,7-dihydro-[1,4]oxathiino-[2,3-*c*]pyridazine-3-carbaldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.52 (d, J = 1.2 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.56 (s, 1H), 7.40 (d, J = 2.6 Hz, 1H), 7.32 (dd, J = 9.2, 2.6 Hz, 1H), 4.70−4.64 (m, 2H), 4.50 (d, J = 3.8 Hz, 1H), 4.27 (ddd, J = 10.7, 4.7, 2.0 Hz, 1H), 4.23 (ddd, J = 10.7, 4.7, 1.9 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 2H), 3.87 (dt, J = 8.8, 3.8 Hz, 1H), 3.41 (t, J = 10.7 Hz, 1H), 3.39 (t, J = 10.5 Hz, 1H), 3.38−3.33 (m, 1H), 3.32−3.29 (methylene adjacent to sulfur obscured by solvent peak), 3.22 (dd, J = 13.8, 8.9 Hz, 1H), 2.95−2.84 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 161.58, 160.20, 157.96, 157.02 (d, J = 250.9 Hz), 142.14 (d, J = 2.2 Hz), 138.50 (d, J = 30.6 Hz), 131.36, 131.22 (d, J = 3.9 Hz), 129.88 (d, J = 12.9 Hz), 129.40, 127.33, 122.21 (d, J = 2.4 Hz), 103.63, 103.56, 72.76 (overlapping peaks), 71.91, 67.88, 56.15, 50.96, 50.19, 27.79, 26.37. HRMS (ESI) m/z calc'd for $C_{23}H_{25}FN_4O_5S$ $[M + H]^+$: 489.1608; found: 489.1613. HPLC: rt: 13.470 min, purity: 100%; see chromatogram in the Supporting Information. The enantiomeric excess (ee) of compound **53** was determined to be 90% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive); see chromatogram in the Supporting Information.

*(S)-1-(5-(((6,7-Dihydro-[1,4]dioxino[2,3-c]pyridazin-3-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**54**).* The title compound (0.027 g, 0.057mmol, 57.0%) was prepared following the general reductive amination method with 6,7-dihydro-[1,4]dioxino[2,3-*c*]pyridazine-3-carbaldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.54 (d, J = 1.5 Hz, 1H), 7.91 (d, J = 9.2 Hz, 1H), 7.44 (d, J = 2.7 Hz, 1H), 7.34 (dd, J = 9.2, 2.7 Hz, 1H), 7.24 (s, 1H), 4.59−4.55 (m, 2H), 4.51 (d, J = 3.8 Hz, 1H), 4.47−4.43 (m, 2H), 4.28 (ddd, J = 10.8, 4.8, 2.1 Hz, 1H), 4.23 (ddd, J = 10.8, 4.8, 2.1 Hz, 1H), 3.95 and 3.94 (overlapping singlets, 5H), 3.89 (dt, J = 8.8, 3.8 Hz), 3.46−3.36 (m, 3H), 3.25 (ddd, J = 13.9, 8.8, 1.0 Hz, 1H), 2.94−2.85 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 160.67, 160.17, 157.40, 157.00 (d, J = 250.8 Hz), 146.92, 142.13 (d, J = 2.2 Hz), 138.49 (d, J = 30.5 Hz), 131.36, 131.20 (d, J = 3.9 Hz), 129.85 (d, J = 12.9 Hz), 122.17 (d, J = 2.5 Hz), 114.99, 103.63, 103.57, 72.76, 72.75, 71.92, 66.35 (overlapping peaks), 56.14, 50.90, 50.44, 27.78 (d, J = 1.2 Hz). HRMS (ESI) m/z calc'd for $C_{23}H_{25}FN_4O_6$ $[M + H]^+$: 473.1836; found: 473.1862. HPLC: rt: 10.478 min, purity: 100%. Enantiomeric excess (ee) presumed to be 93% based on the analysis of the starting amine **50**.

*6-(((2-((S)-2-(3-Fluoro-6-methoxyquinolin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (**55**).* The title compound as an off-white solid (0.020 g, 0.041 mmol, 25.6%) was prepared following the general reductive amination method with 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]-oxazine-6-carbaldehyde. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.18 (br s, 1H), 8.66 (s, 1H), 7.93 (d, J = 9.0, 1H), 7.40−7.34 (m, 2H), 7.32 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 5.08 (d, J = 6.1 Hz, 1H), 4.62 (s, 2H), 4.42 (d, J = 4.1 Hz, 1H), 4.21−4.10 (m, 2H), 3.89 (s, 3H), 3.74−3.66 (m, 1H), 3.70 (s, 2H), 3.35−3.28 (axial dioxane protons are obscured by water peak, 2H), 3.22 (br d, J = 13.4 Hz, 1H), 3.09 (dd, J = 13.6, 9.2 Hz, 1H), 2.84−2.73 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.86, 157.86, 155.14 (d, J = 251.0 Hz), 152.12, 140.94 (d, J = 2.1 Hz), 140.74, 137.93, 137.64, 131.05, 129.45 (d, J = 3.9 Hz), 127.69 (d, J = 13.1 Hz), 123.39, 120.17 (d, J = 2.4 Hz), 116.60, 103.06 (d, J = 5.2 Hz), 102.43, 70.97, 70.62, 70.61, 66.70, 55.45, 50.93, 49.77, 26.61. HRMS (ESI) m/z calc'd for $C_{24}H_{25}FN_4O_6$ $[M + H]^+$: 485.1836; found: 485.1848. HPLC: rt: 14.730 min, purity: 98.8%. Enantiomeric excess (ee) presumed to be 93% based on the analysis of the starting amine **50**.

*(S)-1-(5-((3,4-Dichlorobenzyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethan-1-ol (**56**).* The title compound as a colorless oil residue (0.039 g, 0.081 mmol, 50.6%) was prepared following the general reductive amination method with 3,4-dichlorobenzaldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.53 (d, J = 1.5 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 2.7 Hz, 1H), 7.33 (dd, J = 9.2, 2.7 Hz, 1H), 7.27 (dd, J = 8.2, 2.0 Hz, 1H), 4.50 (d, J = 3.8 Hz, 1H), 4.26 (ddd, J = 10.7, 4.8, 2.1 Hz, 1H), 4.21 (ddd, J = 10.7, 4.8, 2.1 Hz, 1H), 3.93 (s, 3H), 3.88 (dt, J = 8.8, 3.8 Hz, 1H), 3.77 (s, 2H), 3.45−3.34 (m, 3H), 3.24 (ddd, J = 13.9, 8.8, 1.0 Hz, 1H), 2.94−2.84 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 160.24, 157.03 (d, J = 250.9 Hz), 142.42, 142.17 (d, J = 2.3 Hz), 138.50 (d, J = 30.6 Hz), 133.27, 131.85, 131.51, 131.37, 131.26, 131.22, 129.87 (d, J = 13.0 Hz), 129.09, 122.21 (d, J = 2.6 Hz), 103.63, 103.62 (d, J = 5.4 Hz), 72.77, 72.76, 71.93, 56.13, 50.95, 50.51, 27.82 (d, J = 1.5 Hz). HRMS (ESI) m/z calc'd for $C_{23}H_{23}Cl_2FN_2O_4$ $[M + H]^+$: 481.1097; found: 481.1095. HPLC: rt: 20.814 min, purity: 100%. Enantiomeric excess (ee) presumed to be 90% based on the analysis of compound **53**, which was synthesized from the same batch of amine **50**.

*(S)-2-(3-Fluoro-6-methoxyquinolin-4-yl)-1-(5-(4-methylbenzyl)-amino)-trans-1,3-dioxan-2-yl)ethan-1-ol (**57**).* The title compound as a colorless oil residue (0.022 g, 0.052 mmol, 32.5%) was prepared following the general reductive amination method with 4-methylbenzaldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.52 (d, J = 0.8 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.41 (d, J = 2.6 Hz, 1H), 7.33 (dd, J = 9.2, 2.6 Hz, 1H), 7.22 (d, J = 7.9 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 4.49 (d, J = 3.9 Hz, 1H), 4.27−4.16 (m, 2H), 3.92 (s, 3H), 3.86 (dt, J = 8.8, 4.0 Hz, 1H), 3.73 (s, 2H), 3.41 (t, J = 10.7 Hz, 1H), 3.39 (t, J = 10.6 Hz, 1H), 3.3.38−3.33 (m, 1H), 3.23 (dd, J = 13.8, 8.9 Hz, 1H), 2.97−2.87 (m, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 160.25, 157.03 (d, J = 249.3 Hz), 142.17 (d, J = 2.4 Hz), 138.50 (d, J = 30.5 Hz), 138.07, 137.87, 131.37, 131.24 (d, J = 3.9 Hz), 130.15, 129.89 (d, J = 12.9 Hz), 129.40, 122.22 (d, J = 2.5 Hz), 103.64, 103.57, 72.81, 72.80, 71.84, 56.14, 51.55, 50.75, 27.80 (d, J = 1.7 Hz), 21.14. HRMS (ESI) m/z calc'd for $C_{24}H_{27}FN_2O_4$ $[M + H]^+$: 427.2033; found: 427.2060. HPLC: rt: 19.070 min, purity: 100%. Enantiomeric excess (ee) presumed to be 93% based on the analysis of the starting amine **50**.

The synthesis of compounds **65**−**68** was previously reported in a Ph.D. dissertation.[61] The ^{13}C NMR spectra have been reanalyzed to determine ^{19}F−^{13}C coupling constants. We did not determine the enantiomeric excess of compounds in this series.

*8-Bromo-7-fluoroquinolin-2(1H)-one (**38**).* To a solution of commercial 2-bromo-3-fluorophenylamine **37** (7.76 g, 40 mmol, 1.0 equiv) and commercial methyl 3,3-dimethoxypropionate (6.8 mL, 48 mmol, 1.2 equiv) in THF (80 mL), sodium bis(trimethylsilyl)amide (2 M in THF, 30 mL, 60 mmol, 1.5 equiv) was dropped slowly at 0 °C under N_2 protection. The reaction mixture lasted 16 h at room temperature. Then, the reaction was quenched by the addition of citric acid (15.48 g, 80 mmol, 2.0 equiv) aqueous solution (80 mL). The organic layer was extracted by ethyl acetate and concentrated to a brown oil. Then, the organics were dissolved in DCM (80 mL) and conc. H_2SO_4 (32 mL) was added slowly at 0 °C. The mixture was

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stirred for 30 min, and then the solvent was removed under vacuum. Ice water (450 mL) was added, and the yellow precipate obtained was collected by Buchner funnel filtration. The precipate was washed by water until the pH value of the filtrate was 7. Then, the yellow solid was rinsed by acetonitrile (10 mL × 3). After drying, pure product was obtained as a light yellow solid (8.28 g, 34.2 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.13 (brs, 1H); 7.70 (d, J = 9.6 Hz, 1H); 7.52 (dd, J = 8.7, 5.5 Hz, 1H); 7.03 (t, J = 8.4 Hz, 1H); 6.63 (d, J = 9.6 Hz, 1H).

*8-Bromo-2-chloro-7-fluoroquinoline (**39**).* To a solution of compound **38** (8.27 g, 34.15 mmol, 1.0 equiv) in DMF (3.95 mL) and toluene (75 mL), phosphoryl chloride (2.54 mL, 27.24 mmol, 0.80 equiv) was dropped slowly at 95 °C. The reaction mixture lasted 30 min at 95 °C. The reaction mixture was cooled down to 0 °C, and sodium hydroxide (4.1 g, 102.5 mmol, 3.0 equiv) aqueous solution (30 mL) was added. The mixture was washed by saturated brine and extracted by DCM three times. The combined organic layer was concentrated to dryness as crude product. Chromatography was done on silica gel with pure DCM to give the target product as a white solid (7.78 g, 29.9 mmol, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.11 (d, J = 8.5 Hz, 1H); 7.79 (dd, J = 9.0, 5.7 Hz, 1H); 7.43 (d, J = 8.5 Hz, 1H); 7.41 (dd, J = 8.9, 8.1 Hz, 1H).

*8-Bromo-7-fluoro-2-methoxyquinoline (**40**).* To a solution of compound **39** (7.78 g, 29.9 mmol, 1.0 equiv) in toluene (80 mL), NaOMe (4845.6 mg, 89.7 mmol, 3.0 equiv) in methanol (16 mL) solution was dropped at 80 °C. The reaction mixture lasted 90 min at 80 °C. The reaction mixture was cooled down to 0 °C, and then the pH value of the mixture was adjusted to 7 by the addition of HCl solution. The mixture was extracted by DCM. The combined organic layer was concentrated to dryness as crude product. Chromatography was done on silica gel with hexane/ethyl acetate (20:1) to give the target product as a white solid (4593.9 mg, 17.94 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (d, J = 8.8 Hz, 1H); 7.66 (dd, J = 8.8, 5.9 Hz, 1H); 7.21 (t, J = 8.5 Hz, 1H); 6.91 (d, J = 8.8 Hz, 1H); 4.16 (s, 3H).

*2-(2-((E)-2-(7-Fluoro-2-methoxyquinolin-8-yl)vinyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**58**).* Bromide **40** (3783 mg, 14.77 mmol, 1.0 equiv), intermediate **28** (3798 mg, 14.76 mmol, 1.0 equiv), palladium acetate (732.5 mg, 2.93 mmol, 0.2 equiv), triphenylphosphine (1611.5 mg, 5.86 mmol, 0.4 equiv), and silver carbonate (8196 mg, 29.3 mmol, 2.0 equiv) were placed in a three-neck flask under N_2 protection. Anaerobic and anhydrous mesitylene (250 mL) was added. The mixture was heated to 135 °C overnight and then filtered through celite and rinsed with DCM. The combined solution was concentrated to dryness and further purified by chromatography on silica gel with hexane/ethyl acetate (3:1) to give the title compound as a light yellow solid (906.1 mg, 2.09 mmol, 14% yield). This compound is not 100% pure. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.94 (d, J = 8.8 Hz, 1H); 7.88−7.83 (m, 2H); 7.77−7.69 (m, 3H); 7.60 (dd, J = 8.9, 5.9 Hz, 1H); 7.16 (dd, J = 10.7, 8.9 Hz, 1H); 7.05 (dd, J = 16.6, 5.2 Hz, 1H); 6.80 (d, J = 8.8 Hz, 1H); 5.38 (d, J = 5.3 Hz, 1H); 4.82−4.69 (m, 1H); 4.65−4.57 (m, 2H); 4.17 (dd, J = 10.4, 4.7 Hz, 2H); 4.13 (s, 3H).

*2-((E)-2-(7-Fluoro-2-methoxyquinolin-8-yl)vinyl)-trans-1,3-dioxan-5-amine (**59**).* A mixture of compound **58** (906.1 mg, 2.09 mmol, 1.0 equiv), ethanolamine (1886 μL, 31.3 mmol, 15 equiv), and ethyl acetate (30 mL) was stirred and heated at 70 °C overnight. The solvent was removed, and the residue was dissolved in DCM and washed with brine. The organic layer concentrated, and the crude product was purified by chromatography on silica gel with DCM/methanol (15:1) to give the title compound as a brown oil (482.1 mg, 1.58 mmol, 76% yield). This compound is not 100% pure. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.91 (d, J = 8.8 Hz, 1H); 7.67 (d, J = 16.7 Hz, 1H); 7.57 (dd, J = 8.8, 5.9 Hz, 1H); 7.13 (dd, J = 10.8, 8.9 Hz, 1H); 6.99 (dd, J = 16.4, 4.9 Hz, 1H); 6.86 (d, J = 8.8 Hz, 1H); 5.14 (d, J = 4.9 Hz, 1H); 4.26 (dd, J = 11.2, 4.8 Hz, 2H); 4.09 (s, 3H); 3.44 (t, J = 11.0 Hz, 2H); 3.24−3.14 (m, 1H). HRMS (ESI) m/z calc'd for $C_{16}H_{18}FN_2O_3$ $[M + H]^+$: 305.1301; found: 305.1279.

*tert-Butyl (2-((E)-2-(7-Fluoro-2-methoxyquinolin-8-yl)vinyl)-trans-1,3-dioxan-5-yl)carbamate (**60**).* To a solution of compound **59** (482.1 mg, 1.58 mmol, 1.0 equiv) in DCM (10 mL), triethylamine (445 μL, 3.16 mmol, 2.0 equiv) and di-*tert*-butyl dicarbonate (517.3 mg, 2.37 mmol, 1.5 equiv) were added. The mixture was stirred overnight and then purified by chromatography on silica gel with hexane/ethyl acetate (4:1). Impure title compound was obtained as a white solid (416.7 mg, 1.03 mmol, 65%) and used in the next step.

*tert-Butyl (2-((1S)-2-(7-Fluoro-2-methoxyquinolin-8-yl)-1,2-dihydroxy ethyl)-trans-1,3-dioxan-5-yl)carbamate (**61**).* Compound **60** (416.7 mg, 1.03 mmol, 1.0 equiv) was dispensed in the mixture of *tert*-butanol (7.8 mL), ethyl acetate (1.5 mL), and H_2O (7.8 mL), and methanesulfonamide (236 mg, 2.06 mmol, 2.0 equiv) and AD-mix-beta (3282 mg, 2.06 mmol, 2.0 equiv) were added. The mixture was stirred overnight, then diluted with H_2O (50 mL) and extracted by ethyl acetate three times. Chromatography was done on silica gel with DCM/methanol (30:1) to give the target product as a white solid (374 mg, 0.85 mmol, 83%). This compound is not 100% pure and contains methanesulfonamide. ^{1}H NMR (300 MHz, MeOD-d_4) δ: 8.14 (d, J = 8.9 Hz, 1H); 7.79 (dd, J = 8.9, 5.9 Hz, 1H); 7.22 (dd, J = 10.0, 9.0 Hz, 1H); 6.93 (d, J = 8.9 Hz, 1H); 6.59 (brs, 1H); 5.64 (d, J = 5.0 Hz, 1H); 4.44 (d, J = 4.8 Hz, 1H); 4.11−3.99 (m, 3H); 4.04 (s, 3H); 3.79−3.65 (m, 1H); 3.33 (t, J = 10.9 Hz, 1H); 3.28 (t, J = 10.9 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, MeOD-d_4) δ: 163.30, 161.83 (d, J = 248.8 Hz), 157.60 147.19 (d, J = 8.7 Hz), 141.11, 130.25 (d, J = 11.3 Hz), 123.45 (d, J = 1.3 Hz), 121.34 (d, J = 14.0 Hz), 114.84 (d, J = 26.6 Hz), 113.03 (d, J = 2.6 Hz), 101.71, 80.50 76.29, 70.50, 70.46, 69.88 (d, J = 3.7 Hz), 54.41, 43.28, 28.59. HRMS (ESI) m/z calc'd for $C_{21}H_{28}FN_2O_7$ $[M + H]^+$: 439.1881; found: 439.1853.

*tert-Butyl (2-((4S)-5-(7-Fluoro-2-methoxyquinolin-8-yl)-2-oxo-trans-1,3- dioxolan-4-yl)-1,3-dioxan-5-yl)carbamate (**62**).* To a solution of compound **61** (374 mg, 0.853 mmol, 1.0 equiv) in 2-butanone (9 mL), triethylamine (238 μL, 1.71 mmol, 2.0 equiv) and 1′-carbonyldiimidazole (208 mg, 1.28 mmol, 1.5 equiv) were added. The mixture was warmed up to 60 °C and stirred overnight. Chromatography was done on silica gel with DCM/methanol (30:1) to give the target product as a white solid (300.4 mg, 0.647 mmol, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.93 (d, J = 8.9 Hz, 1H); 7.71 (dd, J = 8.9, 6.1 Hz, 1H); 7.11 (t, J = 9.3 Hz, 1H); 6.85 (d, J =8.9 Hz, 1H); 6.47 (d, J =5.8 Hz, 1H); 5.05 (dd, J = 5.8, 3.3 Hz, 1H); 4.78 (d, J = 3.3 Hz, 1H); 4.54 (br d, J =5.9 Hz, 1H); 4.29 (ddd, J = 10.7, 5.0, 1.9 Hz, 1H); 4.19 (ddd, J = 10.7, 5.0, 1.8 Hz, 1H); 4.05 (s, 3H); 4.01−3.85 (m, 1H); 3.45 (t, J = 10.8 Hz, 1H); 3.38 (t, J = 10.8 Hz, 1H); 1.41 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.25, 161.87 (d, J = 253.6 Hz), 155.39, 155.05, 145.87 (d, J = 6.6 Hz), 139.25, 131.42 (d, J = 11.6 Hz), 122.33 (d, J = 1.5 Hz), 116.07 (d, J = 10.4 Hz), 113.33 (d, J = 25.9 Hz), 113.03 (d, J = 2.6 Hz), 98.23, 80.21, 79.07 (d, J = 1.3 Hz), 70.29 (d, J = 7.9 Hz), 69.88, 69.50, 54.06, 43.20, 28.34. HRMS (ESI) m/z calc'd for $C_{22}H_{26}FN_2O_8$ $[M + H]^+$: 465.1673; found: 465.1646.

*tert-Butyl (2-((S)-2-(7-Fluoro-2-methoxyquinolin-8-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)carbamate (**63**).* Compound **62** (300.4 mg, 0.647 mmol, 1.0 equiv) was dissolved in ethanol (5 mL) and ethyl acetate (0.8 mL), and ammonium formate (226 mg, 3.58 mmol, 5.5 equiv) was added. The mixture was heated to 70 °C, and 5% palladium on calcium carbonate (26 mg, 0.02 equiv) was added. Then, the mixture was cooled down to 40 °C, and 10% palladium on carbon (6.6 mg, 0.01 equiv) was added. The reaction mixture was stirred at 40 °C overnight. Chromatography was done on silica gel with hexane/ethyl acetate (30:1) to give the target product as a colorless oil (138.9 mg, 0.329 mmol, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.95 (d, J = 8.9 Hz, 1H); 7.58 (dd, J = 8.8, 6.0 Hz, 1H); 7.15 (t, J = 9.0 Hz, 1H); 6.85 (d, J = 8.8 Hz, 1H); 4.41 (d, J = 4.4 Hz, 1H); 4.32−4.21(m, 3H); 4.05−3.99 (m, 1H); 4.06 (s, 3H); 3.98−3.84 (m, 1H); 3.52 (ddd, J = 13.9, 3.5, 1.7 Hz, 1H); 3.39 (ddd, J = 13.9, 8.3, 1.9, 1H); 3.34 (t, J = 10.8, 1H); 3.33 (t, J = 10.8, 1H); 1.43 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.52, 161.88 (d, H = 247.2 Hz), 155.03, 146.74 (d, J = 9.1 Hz), 139.68, 127.46 (d, J = 11.0 Hz), 121.98, 120.26 (d, J = 15.8 Hz), 113.97 (d, J = 26.7 Hz), 112.09 (d, J = 2.8 Hz), 102.45, 80.17, 73.02, 70.10 (two overlapped peaks), 53.96,

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43.37, 28.42, 26.21. HRMS (ESI) m/z calc'd for $C_{21}H_{28}FN_2O_6$ $[M + H]^+$: 423.1931; found: 423.1904.

*(S)-1-(5-Amino-trans-1,3-dioxan-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (**64**).* Compound **63** (138.9 mg, 0.329 mmol, 1.0 equiv) was dissolved in DCM (5 mL), and trifluoroacetic acid (0.66 mL, 8.62 mmol, 26.2 equiv) was added. The reaction mixture was stirred for 2 h and then quenched by saturated Na_2CO_3 solution (10 mL). The organic layer was collected, and the aqueous layer was extracted by DCM three times. The combined organic layer was concentrated to dryness as crude product (104.4 mg, 0.323 mmol) and then used directly in the next step.

General Procedure for Reductive Amination with Amine 64 (Series E Analogues). To a solution of amine **64** (0.1 mmol) in methanol (2 mL) were added the requisite aldehyde (0.1 mmol) and zinc chloride (1 mg, 0.007 mmol, 0.07 equiv). The mixture was stirred at room temperature for 30 min, followed by the addition of sodium cyanoborohydride (20 mg, 0.3 mmol, 3.0 equiv). The reaction mixture was stirred at room temperature overnight and then purified by chromatography on silica gel with DCM/methanol (30:1). The pure title compound was then obtained.

*(S)-1-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (**65**).* The title compound was prepared in 66% yield following the general method with 2,3-dihydrobenzo[b][1,4]dioxine-6-carbaldehyde and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (d, J = 8.8 Hz, 1H); 7.58 (dd, J = 8.8, 6.0 Hz, 1H); 7.16 (t, J = 9.0 Hz, 1H); 6.85 (d, J = 8.8 Hz, 1H); 6.83−6.72 (m, 3H); 4.42 (d, J = 4.3 Hz, 1H); 4.30−4.20 (m, 2H); 4.23 (s, 4H); 4.07 (s, 3H); 4.02−3.97 (m, 1H); 3.70 (s, 2H); 3.51 (ddd, J = 13.8, 3.4, 1.6 Hz, 1H); 3.44−3.27 (m, 3H); 3.09−2.97 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.01, 161.87 (d, J = 247.2 Hz), 146.77 (d, J = 9.1 Hz), 143.64, 142.92, 139.64, 133.34, 127.42 (d, J = 10.9 Hz), 121.99, 121.10, 120.37 (d, J = 15.7 Hz), 117.38, 116.97, 113.96 (d, J = 26.8 Hz), 112.09 (d, J = 2.8 Hz), 102.65, 73.21, 71.61, 71.56, 64.49, 64.47, 53.97, 50.85, 49.76, 26.40 (d, J = 3.4 Hz). HRMS (ESI) m/z calc'd for $C_{25}H_{28}FN_2O_6$ $[M + H]^+$: 471.1931; found: 471.1930. HPLC: rt: 18.449 min, purity: 98.4%.

*(S)-1-(5-((((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)-methyl)amino)-trans-1,3-dioxan-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (**66**).* The title compound was prepared in 72% yield following the general method with 6,7-dihydro-[1,4]oxathiino-[2,3-c]pyridazine-3-carbaldehyde and obtained as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (d, J = 8.9 Hz, 1H); 7.58 (dd, J = 8.8, 6.0 Hz, 1H); 7.29 (s, 1H); 7.16 (t, J = 9.0 Hz, 1H); 6.85 (d, J = 8.8 Hz, 1H); 4.68−4.61 (m, 2H); 4.44 (d, J = 4.3 Hz, 1H); 4.36−4.25 (m, 2H); 4.06 (s, 3H); 4.04−3.94 (m, 1H); 3.99 (s, 2H); 3.51 (ddd, J = 13.9, 3.5, 1.6 Hz, 1H). 3.44−3.33 (m, 3H); 3.24−3.17 (m, 2H); 3.11−2.98 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.04, 161.87 (d, J = 247.2), 160.10, 156.14, 146.78 (d, J = 9.1 Hz), 139.67 (d, J = 0.9 Hz), 127.47 (d, J = 11.0 Hz), 126.02, 125.24, 122.01 (d, J = 1.1 Hz), 120.33 (d, J = 15.7 Hz), 113.98 (d, J = 26.7 Hz), 112.12 (d, J = 2.8 Hz), 102.68, 73.20, 71.35, 71.28, 66.31, 53.99, 50.23, 50.16, 26.36 (d, J = 3.5 Hz), 25.71. HRMS (ESI) m/z calc'd for $C_{23}H_{26}FN_4O_5S$ $[M + H]^+$: 489.1608; found: 489.1575. HPLC: rt: 14.717 min, purity: 97.1%; see chromatogram in the Supporting Information.

*6-(((2-((S)-2-(7-Fluoro-2-methoxyquinolin-8-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (**67**).* The title compound was prepared in 70% yield following the general method with 3-oxo-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine-6-carbaldehyde and obtained as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.06 (brs, 1H); 7.95 (d, J = 8.9 Hz, 1H); 7.58 (dd, J = 8.9, 6.0 Hz, 1H); 7.21 (d, J = 8.1 Hz, 1H); 7.15 (t, J = 9.0 Hz, 1H); 6.93 (d, J = 8.1 Hz, 1H); 6.85 (d, J = 8.8 Hz, 1H); 4.64 (s, 2H); 4.44 (d, J = 4.3 Hz, 1H); 4.32−4.24 (m, 2H); 4.06 (s, 3H); 4.04−3.97 (m, 1H); 3.83 (s, 2H); 3.51 (ddd, J = 13.8, 3.6, 1.6 Hz, 1H). 3.46−3.32 (m, 3H); 3.10−2.97 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.57, 163.02, 161.88 (d, J = 247.1 Hz), 151.40, 146.76 (d, J = 9.1 Hz), 140.46, 139.65 (d, J = 0.9 Hz), 138.50, 127.46 (d, J = 11.0 Hz), 124.37, 122.01, 120.34 (d, J = 15.7 Hz), 118.30, 113.96 (d, J = 26.7 Hz), 112.12 (d, J = 2.8 Hz), 102.73, 73.13, 71.42, 71.35, 67.37, 53.97, 51.34, 50.08, 26.46 (d, J = 3.4 Hz). HRMS (ESI) m/z calc'd for $C_{24}H_{26}FN_4O_6$ $[M + H]^+$: 485.1836; found: 485.1803. HPLC: rt: 14.972 min, purity: 98.9%.

*(S)-1-(5-((3,4-Dichlorobenzyl)amino)-trans-1,3-dioxan-2-yl)-2-(7-fluoro-2-methoxyquinolin-8-yl)ethan-1-ol (**68**).* The title compound was prepared in 59% yield following the general method with 3,4-dichlorobenzaldehyde and obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (d, J = 8.9 Hz, 1H); 7.59 (dd, J = 8.8, 6.0 Hz, 1H); 7.44 (d, J = 1.6 Hz, 1H); 7.39 (d, J = 8.2 Hz, 1H); 7.21−7.11 (m, 2H); 6.86 (d, J = 8.8 Hz, 1H); 4.67 (s, 1H); 4.43 (d, J = 4.3 Hz, 1H); 4.31−4.23 (m, 2H); 4.07 (s, 3H); 4.04−3.97 (m, 1H); 3.78 (s, 2H); 3.56−3.47 (m, 1H). 3.45−3.28 (m, 3H); 3.08−2.95 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.08, 161.89 (d, J = 247.1 Hz), 146.79 (d, J = 8.9 Hz), 139.71 (d, J = 0.9 Hz), 132.72, 131.35, 130.56, 130.00, 127.50 (d, J = 11.1 Hz), 127.40, 122.04 (d, J = 1.3 Hz), 120.34 (d, J = 15.7 Hz), 114.00 (d, J = 26.8 Hz), 112.16 (d, J = 2.9 Hz), 102.71, 73.24, 71.49 (overlapping peaks), 54.00, 50.22, 50.06, 26.39 (d, J = 3.5 Hz). HRMS (ESI) m/z calc'd for $C_{23}H_{24}Cl_2FN_2O_4$ $[M + H]^+$: 481.1097; found: 481.1064. HPLC: rt: 18.613 min, purity: 100%.

*2-(2-((E)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)vinyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**69**).* Bromide **12** (4.0 g, 15.6 mmol, 1.00 equiv), alkene **28** (6.05 g, 23.3 mmol, 1.50 equiv), palladium acetate (0.699 g, 3.11 mmol, 0.200 equiv), triphenylphosphine (1.64 g, 6.25 mmol, 0.402 equiv), and silver carbonate (8.57 g, 31.1 mmol, 2.00 equiv) were charged into a three-neck flask, which was evacuated and refilled with nitrogen three times. Anhydrous mesitylene (150 mL) was sparged with nitrogen and added by a syringe. The resulting mixture was heated at 125 °C under nitrogen overnight. The mixture was cooled to room temperature and filtered through a pad of celite. After diluting with DCM (100 mL) and water (100 mL), the two layers were separated and the aqueous layer was extracted with DCM (50 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−80% gradient) to afford the title compound as a yellow solid with impurities. The reaction was repeated twice and the crude material was used for the next step without further purification.

*2-((E)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)vinyl)-trans-1,3-dioxan-5-amine (**70**).* To a solution of compound **69** (13.93 g, 31.99 mmol, 1.0 equiv) in 150 mL of ethyl acetate was added ethanolamine (28 mL, 460 mmol, 14.5 equiv) at room temperature. The resulting mixture was heated at 70 °C and stirred under nitrogen overnight, then allowed to cool to room temperature, and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed with saturated aqueous Na_2CO_3 and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−25% gradient) to afford the title compound as a light yellow solid (5.11 g, 16.7 mmol, 52.3% over two steps, not entirely pure). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (d, J = 2.3 Hz, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.61 (dd, J = 16.7, 1.1 Hz, 1H), 7.18 (dd, J = 16.7, 4.5 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 5.15 (d, J = 4.5 Hz, 1H), 4.30−4.24 (m, 2H), 4.11 (s, 3H), 3.46−3.39 (m, 2H), 3.24−3.15 (m, 1H).

*tert-Butyl (2-((E)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-vinyl)-trans-1,3-dioxan-5-yl)carbamate (**71**).* To a solution of amine **70** (5.11 g, 16.7 mmol, 1.00 equiv) and di-*tert*-butyl dicarbonate (11.0 g, 50.4 mmol, 3.01 equiv) in DCM (125 mL) at 0 °C was added triethylamine (9.4 mL, 67.4 mmol, 4.0 equiv), and the mixture was allowed to slowly warm up to room temperature overnight. The mixture was diluted with DCM and water, and the aqueous layer was extracted with DCM (60 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−10% gradient) to afford the title compound as an off-white solid, not completely pure (4.66 g, 11.5 mmol, 68.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.64 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 9.0 Hz, 1H), 7.69 (dd, J = 16.7, 1.1 Hz, 1H), 7.15 (dd, J = 16.7, 4.4

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Hz, 1H), 7.07 (d, J = 9.0 Hz, 1H), 5.17 (d, J = 4.1 Hz, 1H), 4.41 (br s, 1H),4.33 (dd, J = 11.3, 4.9 Hz, 2H), 4.09 (s, 3H), 3.96 (s, 1H), 3.52 (t, J = 10.4 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.65, 156.51 (d, J = 262.4 Hz), 155.07, 140.26 (d, J = 5.0 Hz), 140.26, 138.94 (d, J = 2.4 Hz), 138.40 (d, J = 29.0 Hz), 135.70 (d, J = 11.2 Hz), 124.54 (d, J = 7.6 Hz), 122.15, 115.51 (d, J = 2.7 Hz), 100.35, 80.22, 69.75, 54.25, 43.49, 28.45.

*tert-Butyl (2-((1S,2R)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1,2-dihydroxyethyl)-trans-1,3-dioxan-5-yl)carbamate (**72**).* To a solution of alkene **71** (4.66 g, 11.5 mmol, 1.0 equiv) in a mixture of *tert*-butanol (120 mL), ethyl acetate (24 mL), and water (120 mL) were added AD-mix-beta (57.5 g, 5.0 g/mmol of alkene) and methanesulfonamide (1.32 g, 13.9 mmol, 1.21 equiv) at room temperature. The resulting mixture was stirred at room temperature overnight. Another 25.0 g of AD-mix-beta and 0.5 g of methanesulfonamide were added, and the resulting mixture was stirred at room temperature for another 24 h. Na_2SO_3 (15.0 g, 119 mmol) was added, and the mixture was stirred for 30 min. After diluting with ethyl acetate (150 mL) and water (100 mL), the aqueous layer was extracted with ethyl acetate (2 L) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (DCM/MeOH, 0−25% gradient) to afford the title compound as a light yellow solid (3.94 g, 8.97 mmol, 78.0%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with methanesulfonamide and other impurities) δ: 8.65 (s, 1H), 8.25 (d, J = 9.2 Hz, 1H), 7.11 (d, J = 9.2 Hz, 1H), 5.65 (d, J = 3.7 Hz, 1H), 4.95 (br s, 1H), 4.63 (d, J = 5.1 Hz, 1H), 4.28−4.18 (m, 2H), 4.05 (s, 3H), 3.96 (t, J = 4.6 Hz, 1H), 3.89 (br s, 1H), 3.35 (t, J = 10.9 Hz, 1H), 3.32 (t, J = 10.9 Hz, 1H), 1.42 (s, 9H). The enantioselectivity of this reaction was determined by analysis of intermediates **74** and **75** as well as final compound **79**, (range in repeat reactions from 79−88% ee).

*tert-Butyl (2-((4S,5R)-5-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-oxo-1,3-dioxolan-4-yl)-trans-1,3-dioxan-5-yl)carbamate (**73**).* To a solution of diol **72** (3.94 g, 8.97 mmol, 1 equiv) in 150 mL of 2-butanone was added carbonyldiimidazole (2.18 g, 13.4 mmol, 1.50 equiv) and triethylamine (2.5 mL, 18 mmol, 2.0 equiv). The resulting mixture was stirred at 60 °C under nitrogen overnight. The mixture was then cooled to room temperature and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) to afford the title compound as an off-white solid, not completely pure (2.69 g, 5.78 mmol, 64.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.19 (d, J = 9.1 Hz, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.41 (d, J = 5.6 Hz, 1H), 4.99 (dd, J = 5.6, 3.2 Hz, 1H), 4.83 (d, J = 3.2 Hz, 1H), 4.43 (br d, J = 6.2 Hz, 1H), 4.32 (ddd, J = 10.8, 5.0, 1.8 Hz, 1H), 4.21 (ddd, J = 10.7, 5.0, 1.8 Hz, 1H), 4.07 (s, 3H), 3.97 (br s, 1H), 3.46 (t, J = 10.9 Hz, 1H), 3.38 (t, J = 10.8 Hz, 1H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.39, 156.44 (d, J = 264.2 Hz), 154.97, 154.88, 140.58, 140.40 (d, J = 3.7 Hz), 139.36 (d, J = 2.7 Hz), 138.16 (d, J = 27.6 Hz), 123.52 (d, J = 8.0 Hz), 116.58 (d, J = 2.6 Hz), 97.78, 80.42, 78.79 (d, J = 1.0 Hz), 69.95, 69.50, 69.03, 68.96, 54.58, 28.36.

*tert-Butyl (2-((S)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)carbamate (**74**).* To a suspension of carbonate **73** (2.19 g, 4.71 mmol, 1.00 equiv) in ethanol (33 mL) and ethyl acetate (2.5 mL) under nitrogen was added ammonium formate (1.65 g, 26.2 mmol, 5.56 equiv). The mixture was heated to 70 °C, and 5% Pd/$CaCO_3$ (0.188 g, 0.040 g/mmol) and Pd/C (0.47 g, 0.010 g/mmol) were added. The resulting mixture was stirred at 40 °C for 3 h then cooled to room temperature, filtered through a pad of celite, and concentrated *in vacuo*. Purification by flash chromatography on silica gel (DCM/MeOH, 0−15% gradient) afforded the title compound as a light yellow solid, not completely pure (1.64 g, 3.87 mmol, 82.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 8.25 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 9.1 Hz, 1H), 4.45 (d, J = 4.0 Hz, 1H), 4.32−4.22 (m, 2H), 4.22−4.13 (m, 1H), 4.12−4.05 (m, 4H), 3.95 (br s, 1H), 3.54 (dd, J = 13.6, 2.2 Hz, 1H), 3.46−3.29 (m, 3H), 1.44 (s, 9H). The enantiomeric excess (ee) was 81% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive); see the Supporting Information for chromatogram. A chromatogram using racemic material is also included there for comparison.

The (*S*)-stereochemistry of the hydroxyl group in intermediate **74** was established by synthesis and ^{1}H NMR analysis of the esters derived from the (*S*)-Mosher's acid and (*R*)-Mosher's acid.[62] See Table S7, Supporting Information, for a tabulation of chemical shift changes.

*(S)-1-(5-Amino-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**75**).* To a solution of Boc-amine **74** (1.64 g, 3.87 mmol, 1.00 equiv) in DCM (30 mL) at 0 °C was added dropwise trifluoroacetic acid (7.75 mL, 101 mmol, 26.1 equiv), and the resulting mixture was allowed to warm slowly to room temperature and stirred for 2 additional hours. The mixture was then cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$. After diluting with DCM and water, the pH was adjusted to 10 by the addition of 1 M aqueous NaOH. The two layers were separated, and the aqueous layer was extracted with DCM (60 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated onto silica gel, and purified by flash chromatography on silica gel (DCM/MeOH, 0−20% gradient) to afford the title compound as a light yellow solid (0.689 g, 2.13 mmol, 55.0%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.61 (s, 1H), 8.19 (d, J = 9.1 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 4.48 (d, J = 4.0 Hz, 1H), 4.21−4.07 (m, 6H), 3.56 (ddd, J = 13.0, 4.3, 1.5 Hz, 1H), 3.37−3.28 (m, 3H, overlapped with solvent), 3.00−2.91 (m, 1H). The enantiomeric excess (ee) was 81% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive); see the Supporting Information for chromatogram.

Amine **75** was also prepared according to Scheme 6 in the manuscript as described below.

*5-(tert-Butyl)-2-(1,3-dihydroxypropan-2-yl)isoindoline-1,3-dione (**89**).* Serinol **88** (25 g, 0.27 mol, 1.0 equiv) and 4-*tert*-butylphthalic anhydride (55.96 g, 0.2740 mol, 1.0 equiv) were suspended in anhydrous toluene (350 mL) and stirred at reflux overnight. The mixture was then allowed to cool to room temperature, whereupon a large amount of precipitate was formed, and the mixture was stirred for another 3 h at room temperature. Filtration on a Buchner funnel afforded the title compound as an off-white powder (65.45 g, 0.2360 mol, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.86 (dd, J = 7.8, 1.7 Hz, 1H), 7.83 (dd, J = 1.6, 0.6 Hz, 1H), 7.77 (dd, J = 7.8, 0.6 Hz, 1H), 4.83 (dd, J = 6.6, 5.5 Hz, 2H), 4.27−4.18 (m, 1H), 3.83−3.74 (m, 2H), 3.69−3.61 (m, 2H), 1.35 (s, 9H).

*5-(tert-Butyl)-2-(2-vinyl-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**90**).* To a solution of diol **89** (10.9 g, 39.3 mmol, 1.0 equiv) and *p*-toluenesulfonic acid monohydrate (0.075 g, 0.39 mmol, 0.010 equiv) in DCM (200 mL) in the presence of 5 Å molecular sieves (39.30 g, 1 g/mmol) was added acrolein dimethyl acetal (4.7 mL, 40 mmol, 1.0 equiv) at room temperature. The resulting mixture was stirred at room temperature for 40 h. After filtration through a pad of celite, the mixture was concentrated *in vacuo* and purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−50% gradient) to afford the title compound as an off-white solid (10.3 g, 32.7 mmol, 83.1%). This reaction was run five times with yields ranging from 68.0 to 86.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (t, J = 1.12 Hz, 1H), 7.75 (app d, J = 1.1 Hz, 2H), 5.90 (ddd, J = 17.4, 10.7, 4.6 Hz, 1H), 5.53 (dt, J = 17.4, 1.2 Hz, 1H), 5.35 (dt, J = 10.7, 1.1 Hz, 1H), 5.10 (d, J = 4.6 Hz, 1H), 4.70−4.61 (m, 1H), 4.54−4.46 (m, 2H), 4.10−4.04 (m, 2H), 1.37 (s, 9H).

*5-(tert-Butyl)-2-(2-((E)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)vinyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**91**).* 8-Bromo-7-fluoro-2-methoxy-1,5-naphthyridine **12** (3.3 g, 13 mmol, 1.0 equiv), alkene **90** (4.85 g, 15.4 mmol, 1.2 equiv), palladium acetate (0.575 g, 2.56 mmol, 0.20 equiv), triphenylphosphine (1.35 g, 5.15 mmol, 0.40 equiv), and silver carbonate (7.06 g, 25.6 mmol, 2.0 equiv) were charged into a three-neck flask, which was evacuated and refilled with nitrogen three times. Anhydrous mesitylene (100 mL)

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was sparged with nitrogen and added by a syringe. The resulting mixture was heated at 135 °C under nitrogen overnight. The mixture was cooled to room temperature and filtered through a pad of celite. After diluting with DCM (100 mL) and water (100 mL), the two layers were separated, and the aqueous layer was extracted with DCM (50 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−80% gradient) to afford the title compound as a yellow solid (2.65 g, 5.39 mmol, 42%). This reaction was run four times under similar conditions with an average yield of 55% (range 41−71%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with impurities) δ: 8.65 (d, J = 2.0 Hz, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.88 (br s, 1H), 7.76 (br s, 2H), 7.64 (d, J = 16.6 Hz, 1H), 7.21 (dd, J = 16.6, 4.7 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 5.39 (d, J = 4.7 Hz, 1H), 4.78−4.68 (m, 1H), 4.60 (t, J = 10.7 Hz, 2H), 4.15 (dd, J = 10.6, 4.7 Hz, 2H), 4.13 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.44, 168.01, 162.66, 159.28, 156.54 (d, J = 262.5), 140.29 (d, J = 5.6 Hz), 140.23, 138.92, 138.38 (d, J = 29.0 Hz), 135.69 (d, J = 11.1 Hz), 131.89, 131.48, 128.99, 124.57 (d, J = 7.7 Hz), 123.45, 122.20, 120.79, 115.52 (d, J = 2.2 Hz), 100.87, 66.65, 54.35, 44.13, 35.92, 31.25.

*5-(tert-Butyl)-2-(2-((1S,2R)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1,2-dihydroxyethyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**92**).* Alkene **91** (0.491 g, 0.999 mmol, 1.00 equiv), potassium hexacyanoferrate(III) (2.8 g, 2.8 g/mmol), and potassium carbonate (1.28 g, 1.28 g /mmol) were suspended in a mixture of *tert*-butanol (6 mL), ethyl acetate (1.2 mL), and water (6 mL). Hydroquinidine 1,4-phthalazinediyl diether (22.08 mg, 22.08 mg/mmol), potassium osmate(VI) dihydrate (2 mg, 2 mg/mmol), and methanesulfonamide (115 mg, 115 mg/mmol) were added at room temperature. The resulting mixture was stirred at room temperature under nitrogen for 24 h. Na_2SO_3 (1 g, 1 g/mmol) was added, and the resulting mixture was stirred for another 30 min. Ethyl acetate (30 mL) and water (30 mL) were added, the two layers were separated, and the aqueous layer was extracted with ethyl acetate (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) to provide the title compound as an off-white solid (0.303 g, 0.577 mmol, 57.7%). This reaction was run five times under similar conditions with an average yield of 53.0% (range 25.8−74.4%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with DCM) δ: 8. 69 (br s, 1H), 8.26 (d, J = 9.1 Hz, 1H), 7.86 (app t, J = 1.1 Hz, 1H), 7.75 (app d, J = 1.1 Hz, 2H), 7.13 (d, J = 9.1 Hz, 1H), 5.70 (d, J = 4.0 Hz, 1H), 4.90 (d, J = 5.2 Hz, 1H), 4.68−4.58 (m, 1H), 4.48 (t, J = 10.9 Hz, 1H), 4.46 (t, J = 10.9 Hz, 1H), 4.09 (s, 3H), 4.09−4.03 (m, 2H), 4.03−3.99 (m, 1H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.25, 167.82, 162.07, 159.16, 155.74 (d, J = 259.2), 141.3, 141.04, 138.65, 138.50 (d, J = 26.4 Hz), 131.78, 131.38, 128.88, 128.48 (d, J = 11.2 Hz), 123.36, 120.70, 115.79 (d, J = 2.3 Hz), 100.33, 75.23, 68.20 (d, J = 2.9 Hz), 66.41, 66.35, 54.44, 44.03, 35.83, 31.16. The enantioselectivity of this reaction was determined by analysis of amine **75** below or by analysis of analogue **80**. The observed ee range was 92−96%.

*5-(tert-Butyl)-2-(2-((4S,5R)-5-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-2-oxo-trans-1,3-dioxolan-4-yl)-1,3-dioxan-5-yl)-isoindoline-1,3-dione (**93**).* To a solution of diol **92** (0.766 g, 1.46 mmol, 1.00 equiv) in 20 mL of 2-butanone was added carbonyldiimidazole (0.355 g, 2.19 mmol, 1.50 equiv) and triethylamine (0.406 mL, 2.91 mmol, 2.00 equiv). The resulting mixture was heated to 60 °C and stirred under nitrogen overnight. The mixture was then cooled to room temperature and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) to afford the title compound as an off-white solid (0.374 g, 0.678 mmol, 46.5%). This reaction was run four times under similar conditions with an average yield of 66.3% (range 46.5−78.5%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with DCM) δ: 8.72 (s, 1H), 8.23 (d, J = 9.1 Hz, 1H), 7.88 (br s, 1H), 7.76 (app d, J = 0.9 Hz, 2H), 7.13 (d, J = 9.1 Hz, 1H), 6.52 (d, J = 5.5 Hz, 1H), 5.10 (d, J = 3.1 Hz, 1H), 5.06 (dd, J = 5.4, 3.1 Hz, 1H), 4.72−4.62 (m, 1H), 4.54 (t, J = 10.8 Hz, 1H), 4.49 (t, J = 10.8 Hz, 1H), 4.18 (ddd, J = 10.4, 4.7, 1.6 Hz, 1H), 4.12 (s, 3H), 4.06 (ddd, J = 10.5, 4.8, 1.6 Hz, 1H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.27, 167.85, 163.49, 159.49, 156.53 (d, J = 264.1 Hz), 154.86, 140.57, 140.48 (d, J = 3.7 Hz), 139.37 (d, J = 2.6 Hz), 138.16 (d, 27.6 Hz), 131.75, 131.66, 128.83, 123.74 (d, J = 8.1 Hz), 123.58, 120.92, 116.72 (d, J = 2.5 Hz), 97.93, 78.80, 68.99 (d, J = 6.4 Hz), 66.48, 66.17, 54.70, 43.82, 35.96, 31.24.

*5-(tert-Butyl)-2-(2-((S)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**94**).* To a suspension of carbonate **93** (0.277 g, 0.502 mmol, 1.00 equiv) in ethanol (3.5 mL) and ethyl acetate (0.25 mL) under nitrogen was added ammonium formate (0.176 g, 2.79 mmol, 5.56 equiv). The mixture was heated to 70 °C, at which point 5% Pd/$CaCO_3$ (0.0201 g, 0.040 g/mmol) and Pd/C (0.0052 mg, 0.010 g/mmol) were added. The resulting mixture was stirred at 40 °C for 3 h, then cooled to room temperature and filtered through a pad of celite. The filtrate was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel (hexane/ethyl acetate, 0−80% gradient) to afford the title compound as an off-white solid (0.076 g, 0.15 mmol, 30%). This reaction was run five times under similar conditions with an average yield of 44% (range 30−59%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with ethyl acetate and small impurities) δ: 8.66 (s, 1H), 8.21 (d, J = 9.1 Hz, 1H), 7.86 (br s, 1H), 7.74 (app d, J = 0.9 Hz, 2H), 7.08 (d, J = 9.1 Hz, 1H), 4.72 (d, J = 3.9 Hz, 1H), 4.71−4.61 (m, 1H), 4.47 (t, J = 10.9 Hz, 1H), 4.45 (t, J = 10.9 Hz, 1H), 4.20−4.05 (m, 3H), 4.10 (s, 3H), 3.61−3.53 (m, 1H), 3.45 (dd, J = 12.8, 8.5 Hz, 1H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.41, 167.98, 163.01, 159.27, 157.65 (d, J = 256.7 Hz), 142.11 (d, J = 6.3 Hz), 140.80, 138.58, 138.35 (d, J = 28.5 Hz), 131.88, 131.48, 128.97 (d, J = 12.5 Hz), 128.97, 123.44, 120.79, 115.61 (d, J = 2.4 Hz), 102.12, 72.21, 66.47, 66.41, 54.36, 44.22, 35.92, 31.25, 26.13 (d, J = 1.8 Hz).

*(S)-1-(5-Amino-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**75**).* To a solution of protected amine **94** (0.612 g, 1.2 mmol, 1.00 equiv) in ethyl acetate (15 mL) was added ethylenediamine (1.2 mL, 18.02 mmol, 15 equiv) at room temperature. The resulting mixture was heated to 70 °C and stirred under nitrogen overnight. The mixture was cooled down to room temperature and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 0−20% gradient) to afford the title compound **58** as a white solid (0.303 g, 0.937 mmol, 78.0%). This reaction was run three times under similar conditions with an average yield of 73.3% (range 71.0−78.0%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.61 (d, J = 0.8 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 4.47 (d, J = 4.0 Hz, 1H), 4.20−4.14 (m, 1H), 4.14−4. 09 (m, 2H), 4.09 (s, 3H), 3.55 (ddd, J = 13.0, 4.3, 1.5 Hz, 1H), 3.36−3.28 (overlaps with solvent, m, 3H), 3.00- 2.92 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.99, 159.29 (d, J = 254.6 Hz), 143.17 (d, J = 6.8 Hz), 140.64, 139.28 (d, J = 2.2 Hz), 138.72 (d, J = 29.5 Hz), 131.31 (d, J = 12.7 Hz), 116.58 (d, J = 2.7 Hz), 103.66, 73.50, 72.14, 72.13, 54.53, 45.26, 27.33. The ee of amine **75** was determined to be 96% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive; see the Supporting Information for chromatogram). A separate synthesis of **75** using this route afforded material of 92% ee (see the Supporting Information for chromatogram).

General Procedure for Reductive Amination with Amine 75 (Series F Analogues). Primary amine **75** was prepared either from **94** as directly above or by deprotection of the NHBoc intermediate **74** as described earlier. Amine **75** (1 equiv), the requisite aldehyde (1−2 equiv), and 4 Å molecular sieves (300 mg/mmol of amine) were charged into a flask, which was evacuated and refilled with nitrogen three times. THF (15 mL/mmol), MeOH (12.5 mL/mmol)

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and acetic acid (1.5 equiv) were added by a syringe. The resulting mixture was stirred at room temperature for 4 h. $NaBH_3CN$ (3 equiv) was added, and the mixture was stirred for another 45 min. The mixture was cooled down to 0 °C and quenched by saturated aqueous $NaHCO_3$. After diluting with DCM and water, the pH was adjusted to 10 by adding 1 M sodium hydroxide. The two layers were separated, and the aqueous layer was extracted with DCM (30 mL × 3). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated onto silica gel, and purified by flash chromatography on silica gel DCM/MeOH, 0−20% gradient to afford the secondary amine products listed below. Reactions were typically conducted on 0.15−0.2 mmol scale.

*(S)-1-(5-(((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**76**).* The title compound as a white solid (0.149 g, 73.5%) was prepared following the general reductive amination method with 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde. This reaction was run was run twice under similar conditions with an average yield of 78.1% (range 73.5−82.7%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.60 (d, J = 1.1 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 6.83 (s, 1H), 6.77 (app d, J = 1.1 Hz, 2H), 4.47 (d, J = 3.9 Hz, 1H), 4.25−4.20 (m, 4H), 4.19−4.12 (m, 3H), 4.08 (s, 3H), 3.64 (s, 2H), 3.54 (ddd, J = 13.0, 4.5, 1.6 Hz, 1H), 3.36 (t, J = 10.7 Hz, 1H), 3.35 (t, J = 10.9 Hz, 1H), 3.34−3.27 (m, largely obscured by solvent, 1H), 2.91−2.82 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.99, 159.28 (d, J = 254.7 Hz), 144.98, 144.33, 143.17 (d, J = 6.8 Hz), 140.63, 139.27 (d, J = 2.1 Hz), 138.71 (d, J = 29.5 Hz), 134.04, 131.31 (d, J = 12.7 Hz), 122.31, 118.19, 118.12, 116.59 (d, J = 2.7 Hz), 103.93, 72.13, 72.12, 71.77, 65.62, 65.60, 54.54, 51.19, 50.55, 27.30. HRMS (ESI) m/z calc'd for $C_{24}H_{26}FN_3O_6$ $[M + H]^+$: 472.1884; found: 472.1879. HPLC: rt: 17.847 min, purity: 100%. Enantiomeric excess (ee) presumed to be 96% based on the analysis of the starting amine **75**. A separate synthesis afforded material of 88% ee.

*(S)-1-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-c]pyridin-7-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**77**).* The title compound as an off-white sticky solid (0.040 g, 0.085 mmol, 78%) was prepared following the general reductive amination method with 2,3-dihydro-[1,4]dioxino-[2,3-*c*]pyridine-7-carbaldehyde. This reaction was run twice under similar conditions with an average yield of 65% (range 51−78%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.60 (d, J = 1.1 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 8.0 (s, 1H), 7.14 (d, J = 9.1 Hz, 1H), 6.96 (s, 1H), 4.48 (d, J = 3.9 Hz, 1H), 4.39−4.35 (m, 2H), 4.32−4.29 (m, 2H), 4.20−4.13 (m, 3H), 4.08 (s, 3H), 3.75 (s, 2H), 3.54 (ddd, J = 13.0, 4.5, 1.6 Hz, 1H), 3.37 (t, J = 10.9 Hz, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.33−3.27 (partially obscured by solvent, m, 1H), 2.91−2.81 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.98, 159.29 (d, J = 253.2 Hz), 154.19, 152.67, 143.17 (d, J = 6.8 Hz) 142.19, 140.63, 139.27 (d, J = 2.1 Hz), 138.98, 138.71 (d, J = 29.4 Hz), 131.31 (d, J = 12.6 Hz), 116.58 (d, J = 2.7 Hz), 112.26, 103.94, 72.11 (d, J = 0.9 Hz), 71.85, 66.52, 65.45, 54.54, 52.11, 50.92, 27.29. HRMS (ESI) m/z calc'd for $C_{23}H_{25}FN_4O_6$ $[M + H]^+$: 473.1836; found: 473.1835. HPLC: rt: 15.493 min, purity: 100%. Enantiomeric excess (ee) presumed to be 96% based on the analysis of compound **80**, which was synthesized from the same batch of amine **75**. A separate synthesis afforded material of 88% ee.

*(S)-1-(5-(((3,4-Dihydro-2H-pyrano[2,3-c]pyridin-6-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**78**).* The title compound as a white solid (0.028 g, 0.060 mmol, 48%) was prepared twice (average yield: 68%) following the general reductive amination method with 3,4-dihydro-2*H*-pyrano[2,3-*c*]pyridine-6-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 6. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.59 (d, J = 0.6, 1H), 8.17 (d, J = 9.1 Hz, 1H), 7.95 (s, 1H), 7.16−7.12 (m, 2H), 4.48 (d, J = 3.9 Hz, 1H), 4.26−4.21 (m, 2H), 4.21−4.12 (m, 3H), 4.08 (s, 3H), 3.76 (s, 2H), 3.54 (ddd, J = 13.0, 4.4, 1.4 Hz, 1H), 3.37 (t, J = 10.9 Hz, 1H), 3.36 (t, J = 10.9 Hz, 1H), 3.33−3.27 (m, 1H, partially obscured by solvent), 2.92−2.84 (m, 1H), 2.82 (t, J = 6.5 Hz, 2H), 2.06−1.98 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.93, 159.25 (d, J = 254.7 Hz), 152.83, 151.14, 143.12 (d, J = 6.8 Hz), 140.61, 139.23 (d, J = 2.1 Hz), 138.70, 138.69 (d, J = 29.5 Hz), 133.96, 131.26 (d, J = 12.7 Hz), 124.57, 116.54 (d, J = 2.7 Hz), 103.95, 72.10, 72.09, 71.84, 67.83, 54.53, 52.00, 50.92, 27.29, 25.18, 22.63. HRMS (ESI) m/z calc'd for $C_{24}H_{27}FN_4O_5$ $[M + H]^+$: 471.2043; found: 471.2044. HPLC: rt: 16.338 min, purity: 100%. Enantiomeric excess (ee) presumed to be 96% based on the analysis of compound **80**, which was synthesized from the same batch of amine **75**.

*(S)-1-(5-(((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)-methyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**79**).* The title compound as an off-white solid (0.350 g, 0.715 mmol, 77.0%) was prepared following the general reductive amination with 6,7-dihydro-[1,4]oxathiino[2,3-*c*]pyridazine-3-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 5. This reaction was run twice under similar conditions with an average yield of 69.5% (range 58.6−77.0%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.60 (s, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.57 (s, 1H), 7.14 (d, J = 9.1 Hz, 1H), 4.70−4.65 (m, 2H), 4.48 (d, J = 3.9 Hz, 1H), 4.25−4.12 (m, 3H), 4.08 (s, 3H), 3.91 (s, 2H), 3.55 (ddd, J = 13.0, 4.4, 1.4 Hz, 1H), 3.372 (t, J = 10.8 Hz, 1H), 3.367 (t, J = 10.8 Hz, 1H), 3.34−3.27 (m, 3H, partially obscured by solvent), 2.92−2.82 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.96, 161.58, 159.27 (d, J = 254.6 Hz), 157.96, 143.15 (d, J = 6.8 Hz), 140.62, 139.26 (d, J = 2.0 Hz), 138.70 (d, J = 29.5 Hz), 131.28 (d, J = 12.5 Hz), 129.38, 127.34, 116.55 (d, J = 2.6 Hz), 103.92, 72.10 (two overlapping peaks), 71.85, 67.88, 54.52, 50.99, 50.19, 27.27, 26.36. HRMS (ESI) m/z calc'd for $C_{22}H_{24}FN_5O_5S$ $[M + H]^+$: 490.1560; found: 490.1560. HPLC: rt: 14.584 min, purity: 100% (see the Supporting Information for chromatogram). The enantiomeric excess of compound **79** prepared as described ranged from 79 to 88%, as determined by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive; see the Supporting Information for representative chromatogram).

*N-((6,7-Dihydro-[1,4]oxathiino[2,3-c]pyridazin-3-yl)methyl)-2-((S)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl-ammonium Methanesulfonate (Methanesulfonate Salt of **79**).* To a solution of compound **79** (0.0724 g, 0.148 mmol, 1.00 equiv) in DCM (4 mL) at 0 °C was added dropwise methanesulfonic acid (0.310 mL, 0.155 mmol, 1.05 equiv, 0.5 M in DCM), and the resulting mixture was stirred at 0 °C for 4 min, then concentrated *in vacuo* to afford the title compound as a light yellow solid (0.080 g, 0.137 mmol, 92.4%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.63 (d, J = 1.0 Hz, 1H), 8.21 (d, J = 9.1 Hz, 1H), 7.56 (s, 1H), 7.17 (d, J = 9.1 Hz, 1H), 4.74−4.70 (m, 2H), 4.62 (d, J = 4.0 Hz, 1H), 4.52−4.46 (m, 2H), 4.45 (s, 2H), 4.30−4.24 (m, 1H), 4.10 (s, 3H), 3.83 (t, J = 10.9, Hz, 1H), 3.81 (t, J = 10.9, Hz, 1H), 3.67−3.59 (m, 1H), 3.56 (dd, J = 13.0, 4.5, 1.6 Hz, 1H), 3.40−3.29 (m, 3H, overlapped with solvent), 2.70 (s, 3H). This salt was prepared multiple times, as was the free amine precursor. The enantiomeric excess (ee) of a representative free amine used in salt formation was 79% by chiral HPLC (60/40 hexane/ethanol with 1% diethylamine as an additive). The observed ee range from different syntheses of compound **79** was 79-88%.

*(S)-1-(5-(((6,7-Dihydro-[1,4]dioxino[2,3-c]pyridazin-3-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**80**).* The title compound was prepared in 49% yield following the general method with 6,7-dihydro-[1,4]-dioxino[2,3-*c*]pyridazine-3-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 6. ^{1}H NMR (400 MHz, (CD_3OD)) δ: 8.60 (s, 1H); 8.18 (d, J = 9.1 Hz, 1H); 7.23 (s, 1H); 7.14 (d, J = 9.1 Hz, 1H); 4.59−4.55 (m, 2H); 4.48 (d, J = 3.9 Hz, 1H); 4.46−4.23 (m, 2H); 4.25−4.18 (m, 2H); 4.16 (dt, J = 8.9, 4.2 Hz, 1H); 4.08 (s, 3H); 3.94 (s, 2H); 3.55 (ddd, J = 12.9, 4.3, 3.0 Hz, 1H), 3.38 (t, J = 10.8 Hz, 1H); 3.37 (t, J = 10.8 Hz, 1H); 3.34−3.27 (m, 1H, partially obscured by solvent); 2.93−2.82 (m, 1H). ^{13}C NMR (100 MHz, (CD_3OD)) δ: 163.98, 160.59, 159.29 (d, J = 254.7 Hz), 157.44, 146.97, 143.17 (d, J = 6.8 Hz), 140.64, 139.28 (d, J = 2.2 Hz), 138.72 (d, J = 29.6 Hz), 131.31 (d, J = 12.7 Hz), 116.58 (d, J = 2.6 Hz), 115.06, 103.95, 72.12, 72.11, 71.82, 66.38, 66.36, 54.54, 50.94,

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50.41, 27.28. HRMS (ESI) m/z calc'd for $C_{22}H_{25}FN_5O_6$ $[M + H]^+$ 474.1789; found: 474.1789. HPLC: rt: 13.907 min, purity: 100% (see the Supporting Information for chromatogram). The enantiomeric excess of compound **80** was determined by chiral HPLC to be 96% (60/40 hexane/ethanol with 1% diethylamine as an additive; see the Supporting Information for chromatogram).

The synthesis of compound **81** required the synthesis of the aldehyde starting material **43**.

*6-Vinyl-2,3-dihydro-[1,4]dioxino[2,3-b]pyrazine (**42**).* The previously described[63] 6-bromo-2,3-dihydro-[1,4]dioxino[2,3-*b*]pyrazine (**41**, 0.041 g, 0.19 mmol, 1.0 equiv), triethylamine (0.031 mL, 0.22 mmol, 1.2 equiv), potassium vinyltrifluoroborate (0.032 g, 0.24 mmol, 1.3 equiv), and $PdCl_2$dppf (0.0034 g, 0.0046 mmol, 0.025 equiv) were combined in isopropanol and heated at 100 °C in a sealed tube overnight. The mixture was allowed to cool to room temperature, concentrated *in vacuo*, and purified via flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) to afford the title compound (0.009 g, 0.05 mmol, 30%). This reaction was run twice under similar conditions with yields ranging from 21 to 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ:7.78 (s, 1H), 6.66 (dd, J = 17.3, 10.8 Hz), 6.17 (dd, J = 17.3, 1.3 Hz), 5.43 (dd, J = 10.8, 1.3 Hz), 4.48 (s, 4H).

*2,3-Dihydro-[1,4]dioxino[2,3-b]pyrazine-6-carbaldehyde (**43**).* Vinyl intermediate **42** (0.020 g, 0.12 mmol, 1.0 equiv) was added to a vial containing OsO_4 (0.030 mL, 2.5% by wt. in *tert*-butanol, 0.0030 mmol, 0.024 equiv), $NaIO_4$ (0.078 g, 0.36 mmol, 3.0 equiv), THF (1.66 mL), and water (1.66 mL). The reaction mixture was stirred overnight at room temperature, diluted with water, and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, decanted, and concentrated *in vacuo*. Purification by flash chromatography on silica gel (hexane/ethyl acetate, 0−100% gradient) afforded the title compound (0.016 g, 0.096 mmol, 79%). The ^{1}H NMR spectrum in CD_3OD showed an ∼3:1 mixture of aldehyde and the hemiacetal derived from CD_3OD addition. The ^{13}C NMR spectrum in DMSO-d_6 showed a clean aldehyde. ^{1}H NMR (400 MHz, CD_3OD) δ: 9.85 (s, 1H), 8.38 (s, 1H), 4.63−4.60 (m, 2H), 4.60−4.56 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.45, 151.14, 147.44, 138.88, 136.90, 65.51, 64.73.

*(S)-1-(5-(((2,3-Dihydro-[1,4]dioxino[2,3-b]pyrazin-6-yl)methyl)-amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**81**).* The title compound was prepared in 64% yield following the general method with aldehyde **43** and using amine **75** prepared by the synthetic route described in Scheme 6. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ: 8.73 (s, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.80 (s, 1H), 7.21 (d, J = 9.0 Hz, 1H), 4.83 (d, J = 5.8 Hz, 1H), 4.48−4.42 (m, 4H), 4.38 (d, J = 4.2 Hz, 1H), 4.14−4.07 (m, 2H), 4.02 (s, 3H), 4.01−3.94 (m, 1H), 3.69 (s, 2H), 3.36 (ddd, 12.7, 3.9, 1.5 Hz, 1H), 3.28 (t, J = 10.7 Hz, 1H, partially obscured by water), 3.27 (t, J = 10.7 Hz, 1H, partially obscured by water), 3.14 (dd, J = 12.6, 9.5 Hz, 1H), 2.82−2.70 (m, 1H), 2.13 (br s, 1H). ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 161.68, 157.4 (d, J = 254.8 Hz), 146.49, 146.17, 146.14, 141.23 (d, J = 6.9 Hz), 140.35, 137.92 (d, J = 1.9 Hz), 137.86 (d, J = 28.2 Hz), 132.72, 129.23 (d, J = 12.7 Hz), 114.96 (d, J = 2.4 Hz), 102.83, 70.57, 70.52, 70.01, 64.68, 64.53, 53.53, 49.57, 48.33, 26.14. HRMS (ESI) m/z calc'd for $C_{22}H_{25}FN_5O_6$ $[M + H]^+$ 474.1789; found: 474.1789. HPLC: rt: 14.268 min, purity: 97.4%. Enantiomeric excess (ee) presumed to be 92% based on the analysis of the starting amine **75**.

*(S)-1-(5-(((1,3]Oxathiolo[5,4-c]pyridin-6-ylmethyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-ethan-1-ol (**82**).* The title compound was prepared in 25% yield following the general method with [1,3]oxathiolo[5,4-*c*]pyridine-6-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 6. ^{1}H NMR (400 MHz, (CD_3OD)) δ: 8.60 (d, J = 1.0 Hz, 1H), 8.19 (d, J = 9.1 Hz, 1H), 7.92 (s, 1H), 7.38 (s, 1H), 7.15 (d, 9.1 Hz, 1H), 5.82 (s, 2H), 4.48 (d, J = 3.9 Hz, 1H), 4.22−4.13 (m, 3H), 4.09 (s, 3H), 3.78 (s, 2H), 3.56 (ddd, J = 13.0, 4.5, 1.6 Hz, 1H), 3.37 (t, J = 10.8 Hz, 1H), 3.37 (t, J = 10.8 Hz, 1H), 3.34−3.28 (m, 1H, partially obscured by solvent), 2.92−2.82 (m, 1H). ^{13}C NMR (400 MHz, (CD_3O)) δ: 163.98, 159.29 (d, J = 254.6 Hz), 154.85, 154.75, 143.17 (d, J = 6.8 Hz), 141.15, 140.63, 139.28 (d, J = 2.1 Hz), 138.72 (d, J = 29.5 Hz), 131.31 (d, J = 12.7 Hz), 129.93, 117.50, 116.58 (J = 2.7 Hz), 103.94, 77.28, 72.12, 72.11, 71.87, 54.54, 52.14, 50.99, 27.30. HRMS (ESI) m/z calc'd for $C_{22}H_2FN_4O_5S$ $[M + H]^+$ 475.1451; found: 475.1466. HPLC: rt: 16.227 min, purity: 100%. Enantiomeric excess (ee) presumed to be 96% based on the analysis of compound **80**, which was synthesized from the same batch of amine **75**.

*(S)-1-((2S,5R)-5-(([1,3]Dioxolo[4,5-c]pyridin-6-ylmethyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**83**).* The title compound was prepared in 37% yield following the general method with [1,3]dioxolo[4,5-*c*]pyridine-6-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 6. ^{1}H NMR (400 MHz, (CD_3OD)) δ: 8.60 (d, J = 0.9 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.94 (s, 1H), 7.14 (d, J = 9.1 Hz, 1H), 7.04 (s, 1H), 6.11 (s, 2H), 4.48 (d, J = 3.9, 1H), 4.21−4.13 (m, 3H), 4.08 (s, 3H), 3.79 (s, 2H), 3.55 (ddd, J = 13.0, 4.5, 1.5 Hz, 1H), 3.372 (t, J = 10.9 Hz, 1H), 3.365 (t, J = 11.1 Hz, 1H), 3.33−3.27 (m, 1H, partially obscured by solvent), 2.91−2.82 (m, 1H). ^{13}C NMR (100 MHz, (CD_3OD)) δ: 163.98, 159.29 (d, J = 254.6 Hz), 157.50, 156.66, 146.97, 143.17 (d, J = 6.8 Hz), 140.64, 139.28 (d, J = 2.2 Hz), 138.71 (d, J = 29.5 Hz), 131.31 (d, J = 12.7 Hz), 128.43, 116.57 (d, J = 2.7 Hz), 104.89, 104.17, 103.95, 72.13, 72.12, 71.90, 54.53, 52.60, 51.00, 27.30. HRMS (ESI) m/z calc'd for $C_{22}H_{24}FN_4O_6$ $[M + H]^+$ 459.1680; found: 459.1700. HPLC: rt: 15.152 min, purity: 95.7%. Enantiomeric excess (ee) presumed to be 96% based on the analysis of compound **80**, which was synthesized from the same batch of amine **75**.

*6-(((2-((S)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b]-[1,4]oxazin-3(4H)-one (**84**).* The title compound (0.060 g, 0.13 mmol, 82%) was prepared following the general reductive amination method with 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 5. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.18 (s, 1H), 8.73 (s,1H), 8.26 (d, J = 9.0, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 9.0 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 4.83 (d, J = 6.0 Hz, 1H), 4.61 (s, 2H), 4.38 (d, J = 4.1 Hz, 1H), 4.15−4.06 (m, 2H), 4.01 (s, 3H), 4.01−3.94 (m, 1H), 3.69 (s, 2H), 3.36 (dd, J = 12.8, 2.7 Hz, 1H), 3.280 (t, J = 10.7 Hz, 1H, partially obscured by water), 3.276 (t, J = 10.8 Hz, 1H, partially obscured by water), 3.14 (dd, J = 12.5, 9.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.88, 161.71, 157.43 (d, J = 254.8 Hz), 152.15, 141.25 (d, J = 6.9 Hz), 140.74, 140.37, 137.93 (d, J = 1.8 Hz), 137.88 (d, J = 28.4 Hz), 137.64, 129.25 (d, J = 12.7 Hz), 123.40, 116.62, 114.99 (d, J = 2.3 Hz), 102.86, 70.64, 70.60, 70.01, 66.71, 53.55, 50.92, 49.78, 26.15. HRMS (ESI) m/z calc'd for $C_{23}H_{24}FN_5O_6$ $[M + H]^+$: 486.1789; found: 486.1801. HPLC: rt: 14.639 min, purity: 100%. Enantiomeric excess (ee) presumed to be 88% based on the analysis of compound **79**, which was synthesized from the same batch of amine **75**.

*6-(((2-((S)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-trans-1,3-dioxan-5-yl)amino)methyl)-2H-pyrido[3,2-b]-[1,4]thiazin-3(4H)-one (**85**).* The title compound as a white solid (0.023 g, 0.046 mmol, 46%) was prepared twice (average yield: 41%) following the general reductive amination method with 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]thiazine-6-carbaldehyde and using amine **75** prepared by the synthetic route described in Scheme 5. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.86 (s, 1H), 8.73 (s, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 9.0 Hz, 1H), 7.07 (d, J = 7.9 Hz, 1H), 4.82 (d, J = 6.0 Hz, 1H), 4.39 (d, J = 4.1 Hz, 1H), 4.15−4.07 (m, 2H), 4.01 (s, 3H), 4.01−3.95 (m, 1H), 3.73 (br s, 2H), 3.53 (s, 2H), 3.39−3.25 (partially obscured by water, m, 3H), 3.14 (dd, J = 12.6, 9.5 Hz, 1H), 2.74 (br s, 1H), 2.17 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.09, 161.69, 157.52, 157.41 (d, J = 254.7), 148.72, 141.23 (d, J = 6.9 Hz, 1H), 140.35, 137.92 (d, J = 1.9 Hz), 137.86 (d, J = 28.4 Hz), 135.95, 129.22 (d, J = 12.7 Hz), 116.49, 114.96 (d, J = 2.3 Hz), 112.89, 102.85, 70.60, 70.56, 69.99, 53.52, 51.09, 49.84, 28.72, 26.13. HRMS (ESI) m/z calc'd for $C_{23}H_{25}FN_5O_5S$ $[M + H]^+$: 520.1560; found: 520.1570. HPLC: rt:

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15.373 min, purity: 99.6%. Enantiomeric excess (ee) presumed to be 81% based on the analysis of amine **75** and the Boc-precursor **74**.

*(S)-1-(5-((3,4-Dichlorobenzyl)amino)-trans-1,3-dioxan-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (**86**).* The title compound as a sticky solid (0.038 g, 0.079 mmol, 64%) was prepared following the general reductive amination method with 3,4-dichlorobenzaldehyde. This reaction was run twice under similar conditions with an average yield of 73% (range 64−83%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.59 (s, 1H), 8.17 (d, J = 9.1 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.27 (dd, J = 8.2, 1.9 Hz, 1H), 7.13 (d, J = 9.1 Hz, 1H), 4.48 (d, J = 3.9 Hz, 1H), 4.23−4. 12 (m, 3H), 4.07 (s, 3H), 3.75 (s, 2H), 3.53 (ddd, J = 13.0, 4.4, 1.4 Hz, 1H), 3.37 (t, J = 10.8 Hz, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.30 (dd, J = 12.5, 9.4 Hz, 1H, partially obscured by solvent), 2.92−2.81 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.92, 159.23 (d, J = 254.7 Hz), 143.11 (d, J = 6.8 Hz), 142.35, 140.58, 139.21 (d, J = 2.1 Hz), 138.67 (d, J = 29.5 Hz), 133.24, 131.82, 131.48, 131.25, 131.25 (d, J = 12.7 Hz), 129.09, 116.55 (d, J = 2.7 Hz), 103.92, 72.10, 72.09, 71.85, 54.52, 50.95, 50.48, 27.30. HRMS (ESI) m/z calc'd for $C_{22}H_{22}Cl_2FN_3O_4$ $[M + H]^+$: 482.1050; found: 482.1055. HPLC: rt: 21.415 min, purity. Enantiomeric excess (ee) presumed to be 96% based on the analysis of the starting amine **75**. A separate synthesis afforded material of 88% ee.

*(S)-2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-((2r,5S)-5-((4-methylbenzyl)amino)-1,3-dioxan-2-yl)ethan-1-ol (**87**).* The title compound (0.058 g, 0.14 mmol, 90%) was prepared following the general reductive amination method with 4-methylbenzaldehyde and using amine **75** prepared by the synthetic route described in Scheme 5. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.60 (d, J = 1.0 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 7.24−7.19 (m, 2H), 7.17−7.12 (m, 3H), 4.47 (d, J = 3.9 Hz, 1H), 4.20−4.12 (m, 3H), 4.08 (s, 3H), 3.72 (s, 2H), 3.54 (ddd, J = 13.0, 4.4, 1.6 Hz, 1H), 3.37 (t, J = 10.9 Hz, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.34−3.28 (m, 1H, partially obscured by solvent), 2.94−2. 84 (m, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 163.97, 159.27 (d, J = 254.7 Hz), 143.15 (d, J = 6.7 Hz), 140.62, 139.26 (d, J = 2.0 Hz), 138.70 (d, J = 29.5 Hz), 138.03, 137.87, 131.28 (d, J = 12.7 Hz), 130.12, 129.39, 116.57 (d, J = 2.5 Hz), 103.94, 72.13 (two overlapping peaks), 71.80, 54.53, 51.52, 50.73, 27.30, 21.13. HRMS (ESI) m/z calc'd for $C_{23}H_{26}FN_3O_4$ $[M + H]^+$: 428.1986; found: 428.1988. HPLC: rt: 19.590 min, purity: 100%. Enantiomeric excess (ee) presumed to be 88% based on the analysis of compound **79**, which was synthesized from the same batch of amine **75**.

*Ethyl (E)-3-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)acrylate (**95**).* 8-Bromo-7-fluoro-2-methoxy-1,5-naphthyridine **12** (1.0 g, 3.9 mmol, 1.0 equiv), $Pd(OAc)_2$ (0.009 g, 0.04 mmol, 0.01 equiv), PPh_3 (0.021 g, 0.078 mmol, 0.02 equiv), and K_2CO_3 (1.1 g, 7.8 mmol, 2.0 equiv) were charged into a three-neck flask, which was evacuated and refilled with nitrogen three times. Anhydrous DMF (20 mL) was sparged with nitrogen and added by a syringe, followed by syringe addition of ethyl acrylate (0.51 mL, 4.7 mmol, 1.2 equiv). The resulting mixture was heated at 130 °C and stirred under nitrogen overnight. The mixture was cooled to room temperature, filtered through a pad of celite, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane/EA, 0−60% gradient) to afford the title compound as a light yellow solid (0.7 g, 2.5 mmol, 65%). This reaction was run twice under similar conditions with an average yield of 60% (range 54−65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71 (d, J = 2.0 Hz, 1H), 8.51 (d, J = 16.5 Hz, 1H), 8.21 (d, J = 9.1 Hz, 1H), 7.33 (d, J = 16.5 Hz, 1H), 7.13 (d, J = 9.1 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.14 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H).

*(E)-3-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)prop-2-en-1-ol (**96**).* To a solution of ester **95** (1.75 g, 6.32 mmol, 1 equiv) in 50 mL of THF at −78 °C under nitrogen was added a solution of diisobutylaluminium hydride (1 M in toluene, 19 mL, 19 mmol, 3 equiv) dropwise, and the resulting was stirred at −78 °C for 15 min, then allowed to slowly warm up to room temperature, and stirred for another 1 h. The reaction mixture was then quenched by the addition of 5 mL of MeOH, and 10 mL of sat. aqueous Rochelle's salt solution was added. The mixture was stirred until a clear solution was formed. The aqueous layer was extracted with DCM (30 mL × 3), and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was used directly for the next step without further purification.

*(E)-3-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)acrylaldehyde (**97**).* To a solution of crude alcohol **96** (1.48 g, 6.32 mmol, 1.0 equiv) in 100 mL of DCM was added Dess−Martin periodinane (4.1 g, 9.7 mmol, 1.5 equiv) at room temperature, and the resulting mixture was stirred at room temperature overnight. The mixture was diluted with DCM and water. The aqueous layer was extracted with DCM (30 mL × 3), and the combined organic layers were washed with water, 1 M NaOH, and brine, then dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to afford the title compound (1.2 g, 5.2 mmol, 82%). The reaction was run three times under similar conditions with an average yield of 82% (range 63−100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.86 (dd, J = 7.7, 1.0 Hz, 1H), 8.74 (d, J = 1.9 Hz, 1H), 8.31 (d, J = 16.5 Hz, 1H), 8.22 (d, J = 9.1 Hz, 1H), 7.55 (dd, J = 16.5, 7.7 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 4.13 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 194.72, 163.37, 156.72 (d, J = 267.4 Hz), 140.56, 139.90 (d, J = 4.4 Hz), 139.75, 139.35 (d, J = 2.8 Hz), 138.48 (d, J = 28.4 Hz), 137.25 (d, J = 10.7 Hz), 122.59 (d, J = 7.4 Hz), 116.18 (d, J = 2.8 Hz), 54.48.

*5-(tert-Butyl)-2-(1,3-dihydroxy-2-methylpropan-2-yl)isoindoline-1,3-dione (**99**).* A solution of 2-amino-2-methylpropane-1,3-diol **98** (2.57 g, 24.5 mmol, 1 equiv) and 4-*tert*-butylphthalic anhydride (5 g, 24.5 mmol, 1 equiv) in 100 mL of toluene was heated to 110 °C and stirred under nitrogen for 24 h. The mixture was cooled to room temperature, concentrated onto silical gel, and purified by flash chromatography (hexane/EA, 0−80% gradient) to afford the title compound as an off-white solid (5 g, 21.3 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (br s, 1H), 7.76−7.71 (m, 2H), 4.42 (d, J = 12.0 Hz, 2H), 3.86 (d, J = 12.2 Hz, 2H), 1.41 (s, 3H), 1.38 (s, 9H).

*5-(tert-Butyl)-2-(2-((E)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)vinyl)-5-methyl-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**100**).* A solution of aldehyde **97** (1 g, 4 mmol, 1 equiv), diol **99** (1.2 g, 4.1 mmol, 1 equiv), and *p*-TsOH (0.008 g, 0.04 mmol, 0.01 equiv) in 50 mL of toluene was heated to 110 °C and stirred under nitrogen overnight. The mixture was cooled to room temperature, concentrated onto silica gel, and purified by flash chromatography (hexane/EA, 0−100% gradient) to afford the title compound as an off-white solid with small impurities (0.51 g, 1.0 mmol, 23%). The stereochemistry was assigned based on a cross peak observed between the equatorial protons at C4/C6 of the dioxane and the methyl at C5. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.66 (d, J = 1.9 Hz, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.82 (s, 1H), 7.76−7.68 (m, 2H), 7.64 (d, J = 16.7 Hz, 1H), 7.20 (dd, J = 16.7 Hz, 4.5 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 5.29 (d, J = 4.4 Hz, 1H), 5.02 (d, J = 11.3 Hz, 2H), 4.26 (d, J = 11.0 Hz, 2H), 4.11 (s, 3H), 1.86 (s, 3H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.67, 169.26, 162.63, 159.08, 156.55 (d, J = 262.4 Hz), 140.29 (d, J = 5.6 Hz), 140.21, 138.90 (d, J = 2.4 Hz), 138.40 (d, J = 29.0 Hz), 135.99 (d, J = 11.3 Hz), 132.00, 131.42, 129.16, 124.62 (d, J = 7.6 Hz), 123.03, 122.06, 120.27, 115.50 (d, J = 2.6 Hz), 101.07, 73.00, 55.81, 54.26, 35.88, 31.26, 20.36.

*5-(tert-Butyl)-2-(2-(2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-5-methyl-trans-1,3-dioxan-5-yl)isoindoline-1,3-dione (**101**).* A solution of alkene **100** (0.51 g, 1.0 mmol) in 35 mL of MeOH and 15 mL of EA was hydrogenated using an H-Cube with 10% Pd/C at 25 °C under 20 bar for 12 h. The resulting mixture was concentrated *in vacuo* to afford the title compound (0.5 g, 1 mmol, 98%). The reaction was run twice under similar conditions with an average yield of 94% (range 90−98%). ^{1}H NMR (400 MHz, $CDCl_3$, contaminated with impurities) δ: 8.62 (s, 1H), 8.23 (d, J = 9.1 Hz, 1H), 7.80 (s, 1H), 7.73 (dd, J = 7.9, 1.6 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 4.91 (d, J = 11.3 Hz, 2H), 4.59 (t, J = 4.9, 1H), 4.11 (s, 3H), 4.03 (d, J = 11.0 Hz, 2H), 3.35 (t, J = 7.4 Hz, 2H), 2.17−2.09 (m, 2H), 1.81(s, 3H), 1.36 (s, 9H).

*2-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-5-methyl-trans-1,3-dioxan-5-amine (**102**).* To a stirred solution of protected amine **101** (0.176 g, 0.347 mmol, 1.00 equiv) was added

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ethylenediamine (280 μL, 4.2 mmol, 12 equiv), and the resulting mixture was heated to 70 °C and stirred overnight. The mixture was cooled to room temperature, diluted with 30 mL EA, and washed with sat. aqueous $NaHCO_3$. The organic phase was concentrated onto celite and purified by flash chromatography with neutral alumina (DCM/MeOH, with 1% NH_4OH, 0−10% gradient) to afford the title compound as a light yellow solid with impurities (0.030 g, 0.093 mmol, 27%). ^1H NMR (400 MHz, $CDCl_3$, contaminated with impurities). δ: 8.60 (s, 1H), 8.16 (d, J = 9.0 Hz, 1H), 7.06 (d, J = 9.0 Hz, 1H), 4.42 (t, J = 5.0 Hz, 1H), 4.08 (s, 3H), 3.68 (d, J = 10.9 Hz, 2H), 3.36−3.27 (m, 4H), 2.15−2.06 (m, 2H), 1.33 (s, 3H).

*N-((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl)-2-(2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-5-methyl-trans-1,3-dioxan-5-amine (**103**).* The title compound (0.016 g, 0.034 mmol, 29%) was prepared following the general reductive amination method for Series F with amine **102** and 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.16 (d, J = 9.0 HZ, 1H), 7.06 (d, J = 9.0 Hz, 1H), 6.86−6.73 (m, 3H), 4.44 (t, J = 4.9 Hz, 1H), 4.23 (s, 4H), 4.08 (s, 3H), 3.80 (d, J = 10.8 Hz, 2H), 3.64 (s, 2H), 3.43 (d, J = 10.6 Hz, 2H), 3.30 (t, J = 7.5 Hz, 2H), 2.15−2.06 (m, 2H), 1.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.42, 157.21 (d, J = 255.5 Hz), 143.59, 142.80, 141.72 (d, J = 7.1 Hz), 140.22, 138.61 (d, J = 2.0 Hz), 138.06 (d, J = 28.3 Hz), 134.17, 131.84 (d, J = 12.6 Hz), 121.15, 117.36, 117.04, 115.27 (d, J = 2.7 Hz), 101.95, 76.32, 64.50, 64.47, 53.92, 49.38, 45.49, 33.54, 20.76, 18.33 (d, J = 1.9 Hz). HRMS (ESI) *m/z* calc'd for $C_{25}H_{29}FN_3O_5$ $[M + H]^+$: 470.2091; found: 470.2094. HPLC: rt: 20.035 min, purity: 100%.

X-ray Crystallography (Figure 2). Protein expression and purification: The *S. aureus* DNA gyrase fusion construct was designed as outlined by Bax[16] with several modifications. The cDNA encoding the DNA gyrase was codon-optimized for *E. coli* expression (GenScript) and cloned into pDB.His.MBP (DNASU), integrating an N-terminal His-tag-MBP-TEV protease cleavage motif. Expression was completed in *E. coli* at 25 °C for 24 h after induction with 100 μM IPTG. The cells were sonicated, cleared, treated with 50 μg/mL streptomycin, and then cleared a second time. The lysate was purified by Ni-NTA (Cytiva) followed by Q-Sepharose (Cytiva) chromatography. The fractions containing DNA gyrase were pooled and incubated overnight at 4 °C with DNAse I and 5 mM $MgCl_2$, then purified on a heparin column (Cytiva). The His-tag-MBP-TEV protease cleavage motif was removed by treatment with TEV protease overnight at 4 °C followed by Ni-NTA chromatography with flowthrough collected and concentrated for buffer exchange into 20 mM HEPES, pH = 7.0, 100 mM sodium sulfate, 5 mM manganese(II) sulfate on a Superdex 200 Increase column (Cytiva), yielding protein homodimer with >95% purity by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Crystallization and structure determination of the DNA gyrase ternary complex: The dsDNA 20−20 was designed with the sequence 5′-AGCCGTAGGGCCCTACGGCT-3′ based on the guidance established by Srikannathasan.[64] Compound **7** was lyophilized and resuspended in DMSO to a concentration of 5 mM. The ternary complex was formed by mixing protein:DNA:inhibitor in a 1:1.1:10 ratio and incubated at 4 °C for 3 days prior to desalting with SEC. The eluted protein was concentrated by centrifugal filtration to 111 μM and mixed with mother liquor (50 mM Bis-Tris, pH = 6.2, 15% PEG 5000 MME) at a 0.8:1.2 ratio using sitting-drop vapor diffusion plates and allowed to crystallize at 298 K. Crystals typically form within 24 h and typically complete growth in 4−7 days. Crystals were cryoprotected in a 1:5 solution of glycerol:mother liquor and flash-frozen for shipping to the Structural Biology Center at Argonne National Laboratory for data collection. Data was collected on the 19-ID beamline and images were indexed, integrated, and scaled with HKL2000 (HKL Research, Inc.) in the $P6_1$ space group with a resolution of 2.6 Å. Phases were determined by molecular replacement with 2XCS (PDB) as a model using Phaser-MR in the Phenix software package.[65] Coordinates for the inhibitors were created in PyMOL and restraints were assigned using eLBOW.[66] Refinement was performed using phenix.refine[67] in the Phenix software package, and all coordinate and electron density maps were visualized using winCOOT.[68] Data collection and refinement statistics can be found in Table S8 in the Supporting Information.

Microsomal Clearance and Half-Life (Tables 1 and S2). Metabolic stability assessments using mouse microsomes as reported in Tables 1 and S2 (Supporting Information) were conducted at Charles River (Worcester, MA). Test compounds at 2 μM concentration were incubated with CD-1 mouse microsomes (0.5 mg/mL) at 37 °C for 0, 15, 30, 60, 90, and 120 min. Aliquots at these time points were transferred to a cold quench plate and mixed. Samples were vortexed for a minimum of 10 min, centrifuged for 10 min at 3100 RPM at 4 °C, diluted with water, and analyzed by LC/MS/MS. The % remaining is calculated relative to T_0 using peak area ratios, and the half-life ($t_{1/2}$) is calculated as −0.693/slope, where ln(2) = 0.693 and slope is from ln(% remaining) vs time. Intrinsic clearance (Cl_{int}) = (1/prep conc.) × (0.693/half-life) × (mg prep/g liver) × (g liver/kg body weight).

Microbiology (Tables S1, 2, 3, 6, and 8). MICs for compound **7** in Table S1 (Supporting Information) were determined at Laboratory Specialists, Inc. (Westlake, OH) according to CLSI guidelines.[44]

S. aureus MICs presented in Tables 2 and 6 were determined in the Wozniak lab (The Ohio State University) according to CLSI guidelines.[44]

MICs in Table 3 (except *B. anthracis*, see below) and Table 8 were determined at JMI Labs (North Liberty, IA) according to CLSI guidelines.[44]

B. anthracis MICs were determined in the Slayden lab (Colorado State University) according to CLSI guidelines.

Biochemical Assays for Inhibition of DNA Gyrase (WT and D83N) and TopoIV (Table 4). Assays to measure inhibition of supercoiling by wild-type DNA gyrase and decatenation by TopoIV were carried out as previously reported.[31] Inhibition of supercoiling by the D83N mutant DNA gyrase enzyme was conducted according to the manufacturer's instructions using mutant enzyme and an assay kit purchased from Inspiralis, Ltd. (Norwich, U.K.).

TopoIV Homology Model and Ligand Docking (Figures 3 and S1, Supporting Information). A homology model for *S. aureus* TopoIV was generated previously using RosettaCM[69] with the following templates: 4Z2C, 5IWI, 5CDN, and 3FOE.[31] The crystal structure of *S. aureus* DNA gyrase with compound **7** co-crystallized (Figure 2) was also used. For both of these systems, compounds **7** and **27** were docked into the structures using Glide SP.[70,71] Prior to docking, the ligands were prepared using Ligprep[72] from Schrödinger and the protein/DNA was also prepared using Protein Preparation Wizard[73] from Schrödinger with default parameters. Figure 3 shows the top scoring models by Glide score. Additionally, the ternary structure was minimized (also using Protein Preparation Wizard) post-docking for compound **27** to the TopoIV homology model to remove clashes (Figure S1, Supporting Information).

Frequency of Resistance (Table 5). Initial MICs on agar as well as subsequent determination of spontaneous frequencies of resistance were carried out at Micromyx, LLC (Kalamazoo, MI) as described previously.[32] MICs were determined in triplicate. For frequencies of resistance, each of the test compounds (**7**, **77**, **79**, and **84**) was inoculated on two plates per test concentration.

DNA Sequencing (Table 5). DNA Isolation: *S. aureus* isolates were grown in Cation Adjusted BBL Mueller Hinton II Broth overnight at 37 °C. DNA isolation was conducted using the DNeasy Blood & Tissue Kit (Cat. No. 69506, QIAGEN, Germany) according to the manufacturer's specifications. The extracted DNA was eluted using 200 μL of ultrapure distilled water.

DNA Amplification. PCR amplification of DNA Gyrase subunit A (gyrA) was performed on the isolated DNA from the *S. aureus* isolates. Primers were designed according to the published DNA sequence of gyrA (Table 10). Q5 High-Fidelity 2× Master Mix (25 μL, Cat. No. M0492S, New England BioLabs), gyrA forward primer (2.5 μL), gyrA reverse primer (2.5 μL), *S. aureus* isolate DNA (50 ng), and ultrapure distilled water were added to 0.2 mL PCR tubes (Cat. No. PC7061, Alkali Scientific Inc.) with a total reaction volume of 50 μL. The reactions were placed in an Eppendorf MasterCycler

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Table 10. DNA Primers Used for PCR Amplification and Sequencing (Sigma-Aldrich)

primer name	DNA sequence (5′−3′)
gyrA_F	GAGTGTTATCGTTGCTCGTGC
gyrA_R	GAATATTCGTTGCCATACCTACCGC

EP Gradient Thermal Cycler (No. 5341, Eppendorf), and the following parameters were carried out: an initial 4 min at 98 °C; 30 cycles of 30 s at 98 °C, 30 s at 60 °C, and 30 s at 72 °C; and a final elongation step at 72 °C for 2 min.

DNA Purification and Sequencing. The PCR products were purified using the QIAquick PCR Purification Kit (Cat. No. 28106, QIAGEN, Germany) and eluted with 50 μL of ultrapure distilled water. The purified DNA samples of the *S. aureus* isolates were sent to GENEWIZ for Sanger Sequencing.

hERG Inhibition (Table 7). Inhibition of the hERG channel was assayed using the IonWorks Barracuda system at Charles River (Cleveland, OH) as previously described.[31] IC_{50} values were calculated from eight-point concentration−response curves with four replicates per concentration.

Cellular Growth Inhibition Assays (Table 7). Growth inhibition assays using K562 or K/VP.5 cells were carried out as previously reported.[53]

DNA Cleavage Assays (Figure 7). These assays were carried out with compound **79** using a kit from Inspiralis, Ltd. (Norwich, U.K.) according to the previously reported methods.[53]

Cytotoxicity Assays in THP-1, HepG2, and HeLa Cells. Cytotoxicity was determined for THP-1, HepG2, and HeLa cells as previously described.[74,75] Compound **79** was tested from 128 to 0.0625 μg/mL, and IC_{50} was determined from nonlinear regression analysis of inhibition curves.

Off-Target Activity (Table S3, Supporting Information). Initial screening for off-target activity against 44 targets[58] at a concentration of 100 μM was conducted at Eurofins Panlabs (St. Charles, MO) as previously reported.[32] Follow-up studies to determine K_i/IC_{50} values were likewise conducted at Eurofins Panlabs. Full results from both studies are included in the Supporting Information.

Inhibition of Cardiac Ion Channels (Table S4, Supporting Information). Results from comprehensive *in vitro* proarrhythmia assays (CiPA) were determined at Eurofins Panlabs (St. Charles, MO) as previously reported.[32]

Pharmacokinetics in Mice (Table 9). Eurofins Discovery (St. Charles, MO) performed the pharmacokinetic (PK) studies. In brief, pharmacokinetic (PK) study was performed in male ICR (CD-1) mice weighing 22 ± 2 g (provided by BioLasco Taiwan, a Charles River Laboratories Licensee) following oral (PO) and intravenous (IV) administration of **79** at 50 and 10 mg/kg, respectively. Compound **79** as the free base was formulated in DMSO/ Solutol HS-15/ 0.9% NaCl (5/5/90, v/v/v) for both PO and IV administrations. Plasma samples were collected via cardiac puncture at 10, 30, 60, 120, 240, 360, 480, and 1440 min after PO administration, and at 3, 10, 30, 60, 120, 240, 360, and 1440 min after IV administration. The exposure levels of **79** in plasma samples were determined by liquid chromatography−tandem mass spectrometry (LC-MS/MS) (AB SCIEX QTRAP 6500) and analyzed for fundamental pharmacokinetic parameters of the test compound after PO dosing (T_{max}, C_{max}, AUC_{last}, AUC_{Inf}, AUC/D, AUC_{Extr}, and MRT) and IV dosing ($t_{1/2}$, C_0, AUC_{last}, AUC_{Inf}, AUC_{Extr}, MRT, V_{ss}, and CL) were obtained from the noncompartmental analysis (NCA) of the plasma data using WinNonlin. All aspects of the work, including housing, experimentation, and disposal of animals were performed in general accordance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition (National Academy Press, Washington, D.C., 2011) in an AAALAC-accredited laboratory animal facility. This study and the related standard operating procedures (SOPs) were reviewed and approved by Pharmacology Discovery Services Taiwan, Ltd. Institutional Animals Care and Use Committee (IACUC).

LC/MS/MS conditions: Mass spectrometer: AB SCIEX QTRAP 6500 (LCMS-011); Data processor: Analyst 1.6.3; Ionization mode: Electrospray, Positive ions; Scan Mode: Multiple reaction monitoring (MRM); MRM of Analyte: 490.0/472.1 (Compound **79**); MRM of IS: 358.5/141.9 (Oxybutynin); Column: Agilent Poroshell 120 EC-C18 column 2.7μm (3.0 mm × 50 mm); Mobile phase A: Acetonitrile (CAN); Mobile Phase B: Water (H_2O)/Methanol (MeOH)/1 M $HCOONH_4$ = 99/1/0.1 (v/v/v), pH = 3.5.

***In Vivo* Efficacy (Figure 8 and Table S5, Supporting Information).** This study was performed at Neosome Life Sciences, LLC. Procedures involving the care and use of animals were reviewed and approved by the NeoSome Institutional Animal Care and Use Committee (IACUC) prior to conduct. Compound **79** as the methanesulfonate salt was formulated in a solution of 5% DMSO in water for injection (WFI). CD-1 female mice weighing 18−20 g were utilized in a mouse septicemia model. MRSA ATCC 33591 was prepared by resuspending a portion of an overnight plate culture and adjusting to an OD of 1.3 at 625 nm. The adjusted bacterial suspension was further diluted in 8% hog gastric mucin (pH 7.5) to achieve the infecting inoculum. Actual infecting CFUs were determined through serial dilution and plating on bacterial growth media. At 30 min post infection, groups of six mice each were treated with **79** or control agents via intravenous tail vein injection. Three additional doses of **79** were performed at 1 h intervals. Vancomycin was delivered IV at 0.5 and 3.5 h post infection. Treatment was delivered in a volume of 10 mL/kg. The mice were monitored for survival over 7 days.

ADME. ADME assays on compound **7** plasma protein binding, thermodynamic solubility, microsomal stability were carried out at Charles River (Worcester, MA).

ADMET assays on compounds **25** and **79** (plasma protein binding, kinetic aqueous solubility, CACO-2 permeability, microsomal stability, and cytochrome P450 inhibition, cytotoxicity in HepaRG cells) were conducted at RTI International (Research Triangle Park, NC) as previously reported.[32]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01250.

Molecular formula strings (CSV)

Figure 3A_compound_7_to_crystal_structure_gyrase (PDB)

Figure 3B_compound_27_to_crystal_structure_gyrase (PDB)

Figure 3C_compound_7_to_homology_model_TopoIV (PDB)

Figure 3D_compound_27_to_homology_model_TopoIV (PDB)

Figure S1B_compound_27_to_homology_model_TopoIV_min (PDB)

Antibacterial spectrum of compound **7**, half-life and intrinsic clearance data, off-target and cardiac ion activity of **79** and **77**, survival table for *in vivo* efficacy, Mosher ester analysis of key intermediates, and data collection and refinement statistics from X-ray crystallography (Tables S1−S8); discussion of docking results (Figure S1); HPLC conditions for purity assessment of tested compounds and chiral chromatography for the determination of enantiomeric excess (ee); representative HPLC traces for both purity assessment and ee determination; NMR methods; ^1H and ^{13}C NMR spectra for all tested compounds; full data sets for off-target activity assays; PDB coordinates of hydrogen-

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suppressed atomic models from docking experiments (PDF)

Accession Codes

The structural data of compound **7** in ternary complex with DNA gyrase and uncleaved DNA have been entered into the RCSB Protein Data Bank (http://www.rcsb.org) and can be accessed with PDB code 7MVS. The authors will release the atomic coordinates upon publication of the study.

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Notes

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■ ABBREVIATIONS USED

AcOH, acetic acid; ATCC, American Type Culture Collection; br s, broad signal; BuOH, butanol; CDI, 1,1′cabonyldiimdazole; CF, cystic fibrosis; CLSI, Clinical Laboratory and Standards Institute; dppf, 1,1′-bis(diphenylphosphino)-ferrocene; dsDNA, double-stranded DNA; EtOAc, ethyl acetate; EtOH, ethanol; FoR, frequency of resistance FQ^R, fluoroquinolone-resistant; GyrA, gyrase A subunit; *gyrA*, gene encoding the gyrase A subunit; hTopoIIα, human topoisomerase IIα; *m*CPBA, *meta*-chloroperbenzoic acid; MeOH, methanol; MSSA, methicillin-susceptible *Staphylococcus aureus*; NaHMDS, sodium bis(trimethylsilyl)amide; NaOMe, sodium methoxide; NBTI, novel bacterial topoisomerase inhibitor; OAc, acetate; OTf, triflate; Phth, phthalimide; *p*-TsOH, *para*-toluenesulfonic acid; TopoIV, topoisomerase IV

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