Journal of Medicinal Chemistry

Article

Discovery of Novel DNA Gyrase Inhibiting Spiropyrimidinetriones - Benzisoxazole Fusion with N-Linked Oxazolidinone Substituents Leading to a Clinical Candidate (ETX0914)

Gregory S. Basarab, Peter Doig, Vincent Galullo, Gunther Kern, Amy Kimzey, Amy Kutschke, Joseph Newman, Marshall Morningstar, John Mueller, Linda Otterson, Karthick Vishwanathan, Fei Zhou, and Madhu Gowravaram

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b00863 • Publication Date (Web): 09 Jul 2015

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

Discovery of Novel DNA Gyrase Inhibiting Spiropyrimidinetriones - Benzisoxazole Fusion with N-Linked Oxazolidinone Substituents Leading to a Clinical Candidate (ETX0914)

Gregory S. Basarab,* Peter Doig, Vincent Galullo, Gunther Kern, Amy Kimzey, Amy Kutschke, Joseph P. Newman, Marshall Morningstar, John Mueller, Linda Otterson, Karthick Vishwanathan, Fei Zhou and Madhusudhan Gowravaram

AstraZeneca R&D Boston, Infection Innovative Medicines, 35 Gatehouse Drive, Waltham, Massachusetts 02451, USA

ABSTRACT

A novel class of bacterial type-II topoisomerase inhibitor displaying a spiropyrimidinetrione architecture fused to a benzisoxazole scaffold shows potent activity against Gram-positive and fastidious Gram-negative bacteria. Here, we describe a series of *N*-linked oxazolidinone substituents on the benzisoxazole that improve upon the antibacterial activity of initially described compounds of the class, show favorable PK properties and demonstrate efficacy in an in vivo *Staphylococcus aureus* infection model. Inhibition of the topoisomerases DNA gyrase and topoisomerase IV from both Gram-positive and a Gram-negative organisms was demonstrated. Compounds showed a clean in vitro toxicity profile, including no genotoxicity and no bone marrow toxicity at the highest evaluated concentrations or other issues that have been problematic for some fluoroquinolones. Compound **1u** was identified for advancement into human clinical trials for treatment of uncomplicated gonorrhoea based on a variety of beneficial attributes including the potent activity and the favorable safety profile.

INTRODUCTION

There is a critical need to discover and develop antibacterial agents with a novel mode-of-action that address highly problematic resistance issues across the most widely used classes of antibacterials including, but not limited to β-lactam and glycopeptide antibiotics that inhibit cell-wall biosynthesis, aminoglycoside and macrolide antibiotics that disrupt ribosome function, and fluoroquinolone topoisomerase inhibiting antibacterials that impede DNA replication.[1-4] Multidrug resistant *Staphylococcus aureus*, for example, represents one of a series of resistant pathogens encountered routinely in hospital settings where treatment options have become quite

limited.[5-9] Recently, an alarming spread of drug resistant *Neisseria gonorrhoeae*[10, 11] has caught the attention of the World Health Organization,[12] the Centers for Disease Control and Prevention[13] and the European Centre for Disease Control and Reponse,[14] which have implored the development of novel treatment options. To address such problems of resistance, we previously described a novel class of antibacterial agents with a benzisoxazole scaffold fused to a six-membered ring that displays a spirocyclic pyrimidinetrione pharmacophore (*e.g.* Compound **1u**, ETX0914, Figure 1 and Table 1).[15, 16] Compound **1u** showed activity against Gram-positive bacteria including *Streptococcus pneumoniae*, *Streptococcus pyogenes* and methicillin-resistant fluoroquinolone-resistant (MRQR) *S. aureus* as well as against fastidious Gram-negative bacteria including *Haemophilus influenzae* and *N. gonorrhoeae.* Like fluoroquinolones (*e.g.* ciprofloxacin **2**, Figure 1), **1u** and other spiropyrimidinetriones inhibit type II topoisomerases, DNA gyrase and topoisomerase IV (Topo IV), but through a different mode of inhibition as suggested by their potent activity against clinical bacterial strains resistant to the fluoroquinolones. Compound **1u** was also fully active against laboratory strains of *S. aureus* and *S. pneumoniae* resistant to novobiocin (**3**, Figure 1), which binds to the ATP site of DNA gyrase and Topo IV.[17, 18] It was also more active against *S. aureus* including MRSA (methicillin resistant *S. aureus*) than linezolid (**4**, Figure 1), a current standard of care for skin infections caused by the organism. Developing novel drugs that operate by a novel DNA gyrase and Topo IV inhibitory mechanism would ultimately expand the arsenal of drugs available to practicing physicians to treat bacterial infections. Preliminary structure-activity relationships around the benzisoxazole scaffold showed that the oxazolidinone substituent averted bone marrow toxicity and genotoxicity seen with other substituents, which led to the selection of **1u** for development through Phase 1 clinical trials (Clinicaltrials.gov Identifier: NCT01929629).[15] The introduction

of the oxazolidinone sp^3 centers enabled the incorporation of asymmetric centers that were not possible with a wide variety of aromatic substituents linked through a carbon or nitrogen atom to the benzisoxazole.[19] Clinical success has been correlated with increased fraction sp^3 (Fsp^3) more generally in drug candidates attributed, in part, to the potential to increase solubility and decrease off-target activity.[20] Herein, we describe a more in depth analysis of benzisoxazole spiropyrimidinetriones with N-linked oxazolidinone substituents that generated considerable import due to favorable antibacterial, physical property and pharmacokinetic (PK) attributes. Presented are the associated structure-activity relationships, pharmacology and toxicology properties that led to the selection of **1u** for human clinical trials.

RESULTS AND DISCUSSION

Synthesis. The general synthesis of fluorobenzisoxazoles with oxazolidinone and imidazolidinone substituents (Scheme 1) utilized either compound **5a**[16] or **5b** (see Supporting Information), which can be made in multigram quantities in five steps from commercial starting materials. Chlorobenzisoxazole **5c** (Supporting Information) was made similarly to **5a** and was carried on in the reaction sequence of Scheme 1. Using the chiral trans (2*R*,4*R*)-dimethylmorpholine in **5a**, **5b** and **5c** enabled the enantiospecific synthesis of final products. Treatment of the compounds with a variety of chiral oxazolidinones (X = O) and imidazolidinones (X = NH, NCH_3) and sodium hydride afforded **6a-q**, the products of S_NAr displacement of the 3-position chloride. These were generally converted to spiropyrimidinetrione final products **1a-q** by heating with barbituric acid in acetic acid-water. For two compounds, **1d** and **1i** with pendant hydroxyl groups, the final conversion was better carried out in an ethanol-aqueous HCl co-solvent to avoid by-product acylation seen with the acetic acid conditions.

Overall, the sequence of Scheme 1 enabled the efficient late stage introduction and survey of oxazolidinone/imidazolidinone substituents for SAR purposes. Compounds **1a-q** (as well as all final products herein) were purified predominately by chiral Supercritical Fluid Chromatography (SFC) to remove **7a-q**, a minor diastereomer produced in each of the reactions. Though diastereomers and not enantiomers are being separated, purifications were best achieved via such chiral chromatography. The final reaction proceeded by an in situ ketal hydrolysis to the aldehyde, a Knövenagel condensation and a tertiary amino effect reaction (T-reaction).[21-23] The T-reaction in its current form was pioneered by Reinhoudt in the 1980s and involves an irreversible internal redox or [1,5]-hydride shift reaction to form a zwitterion intermediate.[24, 25] The morpholine methyl substituent next to the iminium species can epimerize accounting for the configuration seen in compounds **1a-p**.[16, 26-27] The cyclization of the zwitterion iminium species under the reaction conditions can occur above and below the plane of either the *cis*- or *trans*-dimethylmorpholine rings in a reversible fashion to allow for four possible diastereomers. Only diastereomers **1** and **7** were seen in the crude reaction mixture, and their configurations represent the thermodynamically lowest energy of the four possible with **1** being favored over **7** in a 9:1 ratio.

In an alternative synthetic sequence utilized for the syntheses of **1r-u** (Scheme 2), the benzisoxazole and oxazolidinone were assembled before incorporation of the chiral morpholine unit, the latter introduced in the penultimate step. This proved advantageous for the scale-up of individual compounds for three reasons. First, the chiral morpholine accounted for much of the cost associated with the synthesis, which would be minimized by its late stage introduction. Second, the late stage introduction allowed for more facile diversification away from the dimethylmorpholine with alternate amines for the analog program (work not included herein).

Finally, overall yields of the reaction sequence were higher. The key intermediate **10** was prepared in four steps starting with protection of aldehyde **8** to set up a fluorine-directed *ortho*-lithiation[28, 29] followed by DMF quench to form aldehyde **9**. The aldehyde was converted to oximoyl chloride **10** to be followed by chloride displacement with chiral aminoalcohols and cyclization to benzisoxazoles **11r-u** on treatment with strong base. The oxazolidinone was made by reaction with carbonyl diimidazole (CDI), and the acetal was hydrolyzed to the aldehyde with acid affording **12 r-u**. S_NAr dimethylmorpholine displacement of the fluoride adjacent to the aldehyde afforded **13 r-u**, which was followed by Knövenagel condensation and T-reaction with barbituric acid. As before, a 9:1 ratio of diastereomers was obtained with the major materials **1r-u** being separated from the minor diastereomers. In two cases, **1t** and **1u**, the minor diastereomers were isolated and fully characterized analytically and biologically. As described earlier for other spiropyrimidinetriones,[16, 26, 27] the configurations of **1** and **7** were determined by NMR including detailed NOESY correlations around the morpholine ring (see Supporting Information for the NOESY spectra of **1u** and **7u**). The morpholine ring of both **1u** and **7u** exist primarily in a chair conformation in solution, with both methyl groups equatorial for the former and one methyl group axial, the other equatorial for the latter. A crystal structure of **1u** as a 1:1 methanol solvate confirmed the configuration, mirrored the solution conformation and showed the attachment of the oxazolidinone ring to be nearly co-planar with the benzisoxazole scaffold with a 1˚ torsion (CCDC 1025296, Figure 2). Finally, the enantiomers *ent*-**1t** and *ent*-**1u** of **1t** and **1u**, respectively, were synthesized in an independent synthetic sequence using meso *cis*-dimethylmorpholine and chiral oxazolidinones. The sequence therefore required a the separation of (2*R*,4*S*,4a*S*)- and (2*S*,4*R*,4a*R*)-morpholine diastereomers in the final products (see Supporting Information).

Structure-activity relationships Compounds were evaluated for inhibition of DNA gyrase activity using the *Escherichia coli* isozyme amenable (versus other isozymes) to a robust fluorescence polarization (FP) anisotropy-based DNA supercoiling assay (Table 1) that is considerably higher through-put than typically used gel-based assays.[30] Antibacterial activity, determined as the MIC (Table 1), did not correlate well with inhibitory potency for *E. coli* (Figure 3A) as ascertained by the R^2 factor from regression analysis being only 0.16. Deviations from linearity were likely due to variable membrane permeability and the capability of the compounds to also inhibit Topo IV (see below). However, as the logD range narrows further to reflect compounds that might have similar permeability, the R^2 continued to increase: for example, a narrow logD range of 1.5-1.9 led to an R^2 of 0.91 (Figure 3B). It was within this narrow range that the aggregate of multivariate compound properties proved best toward identifying a candidate drug. Among such properties are solubility, protein binding and in vivo clearance in addition to antibacterial activity. Four compounds (**1h**, **1o**, **1t**, and **1u**) showed MIC values <10 μM versus *E. coli*, though the values were not sufficiently low to anticipate efficacy against the pathogen at reasonable doses in an in vivo situation. Nonetheless, the robust spread of MIC data seen for *E. coli* offered a measure of differentiation among the compounds and a tool for understanding parameters that account for activity. Compounds with higher antibacterial activity against *E. coli* generally showed higher activity against the other bacteria in Table 1 demonstrating the utility of the FP assay for understanding the SAR. In contrast to *E. coli*, the other bacteria did show sufficient susceptibility to anticipate demonstration of in vivo efficacy. These include the fastidious Gram-negative pathogens *H. influenzae* and *N. gonorrhoeae* and four Gram-positive pathogens, namely *S. pneumoniae*, *Streptococcus pyogenes* and two strains of *S. aureus*, a drug susceptible strain (MSSA) and a methicillin and fluoroquinolone resistant

strain (MRQR). Two comparators, ciprofloxacin and linezolid are included in Table 1; ciprofloxacin provides a frame of reference as a fluoroquinolone DNA gyrase inhibitor, and linezolid serves as a standard of care for Gram-positive skin infections. Resistance to fluoroquinolones in the MRQR strain is due to two topoisomerase mutations, S85P in GyrA and S80Y in ParC. As a result, the MIC for ciprofloxacin against the MSSA strain was 0.78 μM and >50 μM versus the MRQR strain. In contrast, the MIC values for the spiropyrimidinetriones of Table 1 versus the MSSA and MRQR strains were similar with variations only 1- to 4-fold. This supports the differential mode-of-inhibition for spiropyrimidinetriones relative to fluoroquinolones. A balanced set of MIC values for the compounds of Table 1 were also obtained across the *Staphylococcus* and *Streptococcus* species. Compound **1t**, for example, showed MIC values of 0.2-0.4 μM across *S. pneumoniae*, *S. pyogenes* and MSSA in contrast to initial benzisoxazole spiropyrimidinetriones that showed notably higher antibacterial activity against Staphylococci relative to Streptococci.[16] Overall, the compounds with more favorable physical and PK properties critical for advancing a drug candidate also showed sufficient antibacterial potency against *S. aureus* and *S. pyogenes* to address skin infections, against *S. aureus*, *S. pneumoniae* and *H. influenzae* to address respiratory tract infections and against *N. gonorrhoeae* to address uncomplicated gonorrhea.

As comparators, the opposite enantiomers (*ent*-**1t** and *ent*-**1u**) of two compounds, **1t** and **1u**, were made, and the corresponding minor diastereomers **7t** and **7u** were separated for biological and physical property profiling (Table 2). As shown in previous publications describing spiropyrimidinetriones,[16, 26] stereochemistry around the morpholine ring had a profound impact on inhibitory potency and thereby antibacterial activity, and compounds with the (*R*,*S*,*S*)-morpholine configuration as diagrammed for **1** displayed much higher activity than

the enantiomeric antipodes (*ent*-**1t** and *ent*-**1u**) and minor diastereomers (**7t** and **7u**). Importantly, there was also a recognition element for human plasma protein binding (PPB) as the more active diastereomers **1t** and **1u** showed a 6- to 9-fold higher free fraction (or fraction unbound, f_u) than those of the respective enantiomers or the respective minor diastereomers. A higher f_u would be expected to afford higher activity in an in vivo situation as unbound drug is thought to be necessary for interaction with the target and the pathogen. As an in vitro indication of this, MIC values were determined for MSSA in the presence of 50% human serum (Table 1).[31] For the most part, the shift to a higher MIC was around 2-fold. However, more highly protein bound compounds such as **1m** with f_u = 0.01 displayed a notably larger 16-fold MIC increase. On the other hand, PPB can increase compound exposure *in vivo* due to its role in protecting a compound from clearance mechanisms; compounds with both a high f_u and a low clearance would be thus best suited as drug candidates.[32-34] Realizing maximal drug exposure and efficacy in a disease model thus relates to the free AUC with the preference being for compounds with a higher f_u and AUC (lower clearance) and a lower MIC.

Structure insights of DNA gyrase or Topo IV could not be used for optimization of spiropyrimidinetrione analogs as their binding environment has not been determined to date by X-ray diffraction or NMR as has been done for fluoroquinolones,[35-37] aminopiperidine topoisomerase inhibitors termed NBTIs (novel bacterial topoisomerase inhibitors),[35] aminocoumarins[17, 18, 38] and other ATP competitive inhibitors.[39] Hence, the latitude for substitution on the oxazolidinone and imidazolidinone ring as seen in Table 1 was determined through SAR correlations and was shown to be quite broad. Both saturated carbon positions of the oxazolidinone (4- and 5-positions, see **1a** of Table 1 for numbering) tolerated substitutions in either possible configuration. However in every matched pair, substituents below the plane of the

oxazolidinone ring as drawn in Table 1 were preferred over those above. This includes the matched pairs **1t** and **1u** that showed higher inhibitory potency and antibacterial activity than **1b** and **1g**, respectively. Substituents at each of the oxazolidinone 4- and 5-positions showed similar activity; hence, **1b**-**f** and **1t** with 5-position substituents showed similar activity to **1g**-**k** and **1u** that have respectively identical 4-position substituents. Substitution at both the 4- and 5-positions with methyl groups followed the trend that overall highest potency was seen with the orientation of both methyl groups below the plane of the oxazolidinone ring as seen by comparing **1o** with **1n**. More polar substituents, as incorporated in **1d**, **1l**, **1p**, **1q** and **1r**, diminished antibacterial activity presumably due to decreased target potency and/or bacterial membrane permeability. Importantly, addition of the substituents onto the oxazolidinone generally increased solubility (compare with **1a**, Table 1) presumably due to re-organization of crystal packing arrangements. The X-ray structure of **1u** showed the oxazolidinone methyl group to be nearly perpendicular (81˚) to the plane encompassing the benzisoxazole and oxazolidinone (Figure 2) without π-π stacking within the crystal lattice. Fluorine and chlorine substituents on the benzisoxazole ring were about equipotent; the increased lipophilicity of chlorine decreased f_u and solubility. Finally, replacement of the oxazolidinone ring oxygen atom with nitrogen (**1p** and **1q**) decreased but did not eliminate activity.

IV pharmacokinetic (PK) parameters were determined in the rat for select compounds with higher antibacterial activity, solubility and f_u (clearances shown in Table 1). Low clearance (Cl) was generally seen with methyl substituents on the oxazolidinone ring. Methoxymethyl substituents generally afforded higher Cl as seen with **1e**, **1j** and **1k**. Replacement of the fluorine atom with chlorine usually lowered Cl, as in the instances of **1c**, **1f** and **1h** relative to **1t**, **1e** and **1u**, respectively, correlating with the lower f_u. Distilling f_u, antibacterial activity and rat Cl

(normalizing AUC to a 1 mg dose) into a single number fAUC/MIC (f_u*AUC/dose*MIC),[15] showed favorable values for three compounds, **1o**, **1t** and **1u** relative to other compounds of Table 1. These three compounds were evaluated in rat and dog for both IV and oral PK (Table 3). The Cl, associated fAUC/MIC, and bioavailability for the three compounds in both species were sufficiently high that, taking cross-species hepatocyte stability into account, the expected human exposure would be favorable.

In vitro genotoxicity attributes. A link has been made between the inhibition of mammalian topoisomerase IIα (TopoIIα) and genotoxicity as manifested in a mouse micronucleus chromosome aberration assay (MMA) and a mouse lymphoma assay (MLA).[40] Indeed, the use of the fluoroquinolone gemifloxacin, a 20 μM inhibitor of TopoIIα (Table 4), has been limited due to clastogenicity in the MLA, an in vitro human lymphocyte chromosome aberration assay and an in vivo rat micronucleus chromosome aberration assay.[41] Compounds **1t** and **1u** were profiled for inhibition of human TopoIIα,[42] more broadly for human topoisomerase IIβ (TopoIIβ)[43] and for mammalian in vitro genotoxicity in the MMA and MLA (Table 4). Gemifloxacin was included as a genotoxic agent and ciprofloxacin as a fluoroquinolone with a clinically acceptable genotoxicity profile. Neither **1t** nor **1u** showed evidence of genotoxicity at the highest concentrations tested in both assays and a more favorable profile than both ciprofloxacin and gemifloxacin. The data in Table 4 reflects experiments done in the absence of metabolizing S9 liver fractions. Compounds **1t** and **1u** were also shown to be negative for genotoxicity in the presence of S9 liver fractions (data not shown). Moreover, **1t** and **1u** were less active against the human TopoIIα and TopoIIβ with multiples ranging from 210- to 2350-fold relative to inhibition of *E. coli* DNA gyrase. Overall, the data of Table 4 indicate a correlation between potent TopoIIα inhibition and mammalian in vitro genotoxicity as measured by the MMA and MLA.

Both **1t** and **1u** were tested at sub-lethal concentrations for Ames mutagenicity against a standard five strain *Salmonella typhimurium* LT2 panel (TA1535, TA1537, TA100, TA98, TA102) with and without added S9. Similar to fluoroquinolones, the compounds were positive in only the DNA repair proficient TA102 strain as would be expected with the topoisomerase mode-of-action. Consistent with these in vitro results, compound **1u** was negative in an in vivo rat micronucleus assay at the maximum tolerated oral dose of 500 mg/kg/day for two days.

Mammalian cellular toxicity. Given the low, but measurable inhibitory activity of **1t** and **1u** against the human topoisomerases, toxicity to mammalian cell lines was also investigated. Cross screening against mammalian cell cultures, oftentimes immortalized, offers an avenue to mitigate toxicity. Neither **1t** nor **1u** registered high anti-proliferative activity against the human A459 cell line, a human THLE liver cell line or a THP-1 monocyte white blood cell line. Assessing the THLE cell line was particularly important as hepatic toxicity is a major factor leading to the termination of new drug development.[44-46] Furthermore, neither **1t** nor **1u** lysed sheep red blood cells at the highest concentrations tested. The safety indicated from these assays is not unlike other on the market antibacterials including linezolid, levofloxacin and gemifloxacin as seen in Table 5. However, it should be noted that longer term dosing of linezolid and gemifloxacin in the clinic has been limited due to bone marrow suppression characterized by thrombocytopenia, which would not be foreseen monitoring cytotoxicity against mature mammalian cell lines.[41, 47, 48] Hence, the toxicity against immature bone-marrow myeloid and erythroid cell lines was evaluated (Table 5). The bone marrow suppression seen in the clinic with linezolid and gemifloxacin would be predictable based on the IC_{50} measurements against myeloids and erythroids, while **1u** notably showed no activity against either cell line at the highest 100 μM concentration tested. This is similar to the profile seen for levofloxacin, which does not suffer

from issues of thrombocytopenia in clinical use. In our opinion, the inhibition of bone marrow cell growth should be assessed more routinely as a more sensitive gauge of potential toxicity issues associated with longer term use of antibacterial agents targeting enzymes or receptors with human homologues similar to what is routinely done for oncology cytotoxic drugs.[49, 50]

In vivo efficacy. Compounds **1t** and **1u** were evaluated for efficacy in a *S. aureus* neutropenic thigh infection model in mice (Figure 4) using the MSSA strain of Tables 1 and 2 (*S. aureus* ARC516).[51] A higher Cl of 57 mL/min/kg was measured for **1u** in the mouse, despite it having a lower mouse hepatic Cl_{int} (6.9 µl/min/mg). By comparison, the mouse hepatic Cl_{int} (15 µl/min/mg) for **1t** was higher than that for **1u** while the in vivo mouse Cl (20 mL/min/kg) was lower. The mechanism of clearance for the two compounds in the mouse has not been fully elucidated. However, co-administration of the P450 inhibitor 1-aminobenzotriazole (ABT)[52] with **1u** in mice lowered Cl to 15 mL/min/kg suggesting a susceptibility to oxidative metabolism. Hence, ABT was co-administered for an efficacy study with **1u** and was not used for **1t**. The untreated control typically showed about a 3.0 log (1000-fold) growth in CFU relative to the level of $5x10^5$ CFU inoculum achieved at the time zero. Gratifyingly, both compounds demonstrated efficacy in the models. Compound **1u** showed a static response at about 10 mg/kg/day, and **1t** between 10 and 25 mg/kg/day. Maximal responses of nearly a 2-log reduction in CFU were seen at 25 and 50 mg/kg/day for **1t** and **1u**, respectively, and higher doses did not improve efficacy. Overall, **1t** and **1u** could not be differentiated from one another based on the pharmacology, and extensive side-by-side rat and dog tolerability studies (data not shown) along with the aforementioned somewhat better cytotoxicity profile (Table 5) led to **1u** being selected for pre-clinical development.

In vitro mode-of-inhibition. The selection of **1u** for pre-clinical development instigated studies to validate the mode-of-action in line with what was observed for previous spiropyrimidinetriones.[16, 53] DNA gyrase is a large tetrameric complex of two GyrA and two GyrB subunits operating as a molecular machine to alter the topological state of DNA necessary for replication. Topo IV is highly homologous to DNA gyrase containing two analogous ParC and ParE (to GyrA and GyrB, respectively) subunits. The primary function of DNA gyrase is to introduce negative supercoils ahead of the DNA replication fork while the primary role of Topo IV is to decatenate DNA strands. As with other type II topoisomerases, DNA gyrase and Topo IV cleave both strands of duplex DNA to engineer the change in topological state. Agarose gel assays from *E. coli* and *S. aureus* (Gram-negative and Gram-positive isozymes) were set up to track the conversion of relaxed to supercoiled plasmid DNA mediated by DNA gyrase and of catenated to decantanated DNA mediated by Topo IV (Table 6). Compound **1u** and the comparator ciprofloxacin inhibited *E. coli* and *S. aureus* DNA gyrase supercoiling and Topo IV decatenation activity causing double-stranded cleaved DNA to accumulate. This is in contrast to the NBTI class, for which mostly single stranded DNA cleavage was observed,[54] and to novobiocin, which does not induce DNA strand cleavage.[55] In the gel based DNA gyrase assay, only fully supercoiled DNA is quantitatively assessed, and therefore a high amount of supercoiling needs to be introduced into the test DNA. In the FP assay, a DNA oligomer's ability to form triplexes with double-stranded DNA is measured, and the amount of supercoiled DNA that abrogates triplex formation has not been determined. Hence, though the IC_{50} values between the two assays will differ, they both serve to rank order relative inhibitor potency. The gel-based assays showed that, like fluoroquinolones, **1u** has the potential for dual-target inhibition in both Gram-negative and Gram-positive bacteria. Ultimately, analysis of mutations in the targets and

their capability to confer resistance in cell growth assays is required to dissect the relative importance of the two enzyme targets to inhibition by **1u**. Indeed, spontaneous mutants to **1u** in *S. aureus* and *N. gonorrhoeae* shown to have GyrB modifications in the DNA cleavage domain are not cross-resistant to fluoroquinolone antibacterials. The data supported DNA gyrase over Topo IV being the primary target of inhibition for **1u** for the two organisms.[56, 57]

CONCLUSION

Compound **1u**[15, 58] was given the designation ETX0914 as it progressed through extensive pre-clinical investigations including toxicology, formulations development and microbiological characterization toward entering human clinical trials. The activity and pharmacokinetics of the compound support utility against skin and skin structure infections caused by Gram-positive bacteria, *S. aureus* and *S. pyogenes* in particular. The spectrum of activity suggests coverage against respiratory tract infections caused by *S. aureus, S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis* and various atypical bacterial pathogens. The high activity against *N. gonorrhoeae* including resistant isolates supports treatment of sexually transmitted uncomplicated gonorrhea.[59] To identify better analogs, optimization work closely monitored antibacterial activity, solubility, PPB and PK attributes in a multivariate fashion. In parallel, a variety of in vitro toxicology parameters were monitored to mitigate safety issues that might occur in clinical use. Among these were ion channel inhibition including hERG, bone marrow toxicity and mammalian genotoxicity. Genotoxicity in particular became of paramount concern as early spiropyrimidinetrione analogs showed positive in vitro activity; hence, the clean genotoxicity profile for **1u** and in fact, for the other oxazolidinone analogs herein that were evaluated, bodes well for progression of the drug candidate. Ultimately, gonorrhea was chosen as a gateway indication to evaluate **1u** in clinical trials to address a pressing medical need and to enable a

relatively rapid route to evaluate human tolerability and clinical efficacy, both particularly important for a novel drug class without precedence in medicine.

EXPERIMENTAL SECTION

General Considerations All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions at 300 K using a Bruker Ultrashield 300 MHz instrument or a Bruker Ultrashield 400 MHz instrument. ^{13}C NMR spectra were recorded in DMSO-d_6 solutions at 300 K and 126 MHz using a Bruker DRX-500 500 MHz instrument with a QNP cryoprobe or at 101 MHz using a Bruker Ultrashield 400 MHz instrument or at 75.5 MHz using a Brucker Ultrashield 300 MHz instrument. ^{19}F NMR spectra were recorded at 282 MHz in $CDCl_3$ or DMSO-d_6 solutions at 300 K using a Bruker Ultrashield 300 MHz instrument. Chemical shifts are reported as parts per million relative to TMS (0.00) for ^{1}H and ^{13}C NMR and $CFCl_3$ for ^{19}F NMR. High-resolution mass spectra (HRMS) were obtained using a hybrid quadrupole time-of-flight mass spectrometer (microTOFq II, Bruker Daltonics) in ESI^+ mode. Silica gel chromatographies were performed on an ISCO Combiflash Companion Instruments using ISCO RediSep Flash Cartridges (particle size: 35-70 microns) or Silicycle SiliaSep Flash Cartridges (particle size: 40-63 microns). Preparative reverse phase HPLC was carried out using YMC Pack ODS-AQ (100 × 20 mm ID, S-5 μ particle size, 12 nm pore size) on Agilent instruments. When not indicated, compound intermediates and reagents were purchased from chemical supply houses. All final compounds (Compounds **7a-u**) were determined to be greater than 95% pure via analysis by reversed phase UPLC-MS (retention times, RT, in minutes) with a Waters Acquity UPLC instrument with DAD and ELSD and a UPLC HSS T3, 2.1 x 30 mm, 1.8 um column and a gradient of 2 to 98% acetonitrile in water with 0.1% formic acid over 2.0 minutes at 1 mL/min. Injection volume was 1 μL and the column temperature was 30 °C. Detection was based on electrospray ionization (ESI) in positive and negative polarity using Waters ZQ mass spectrometer (Milford, MA, USA),

diode-array UV detector from 210 to 400 nm, and evaporative light scattering detector (Sedex 75, Sedere, Alfortville Cedex, France).

3-(5-(Dimethoxymethyl)-6-((2R,6R)-2,6-dimethylmorpholino)-7-fluorobenzo[d]isoxazol-3-yl)oxazolidin-2-one (**6a)** A solution of 2-oxazolidinone (728 mg, 8.36 mmol) in 1 mL DMF was added slowly to a stirred suspension of NaH (60% oil dispersion, 290 mg, 7.25 mmol) in 1 mL DMF at 0 °C. The mixture was stirred at rt for 10 min, and a solution of **5b** (0.25 g, 0.7 mmol, see Supplementary Materials) in 3 mL DMF was added. This mixture was heated at 80 °C for 5 h, poured into ice cooled aqueous NH_4Cl, and extracted twice with EtOAc. The organic layers were dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (20-50% EtOAc gradient in $CHCl_3$) to afford 500 mg starting material **5b** and 800 mg (35%) of the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (s, 1H), 5.74 (s, 1H), 4.45-4.73 (m, 2H), 3.93-4.25 (m, 4H), 2.71-2.93 (m, 2H), 3.06-3.35 (m, 8H), 1.23 (br s, 6H); MS (ES) MH^+: 410.4 for $C_{19}H_{25}FN_3O_6$.

(5*S*)-3-{6-[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl}-5-methyl-1,3-oxazolidin-2-one (6b) NaH (60% dispersion) (0.157 g, 3.92 mmol) was added to a solution of (*S*)-5-methyloxazolidin-2-one[60] (0.397 g, 3.92 mmol) in 6 mL DMF at rt. After stirring for 30 min, **5a** (0.7 g, 1.96 mmol) was added, and the mixture was heated at 90 °C for 4 h. The mixture was quenched with aqueous NH_4Cl and solvent was removed. The residue was taken up in Et_2O, which was washed 3 times with water and once with brine. The combined aqueous layers were extracted with Et_2O, which was washed twice more with water and once with brine. The combined Et_2O layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (0-30% gradient of EtOAc in CH_2Cl_2) to afford 230 mg starting material **5b** and 250 mg (30%) of the title compound. ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.52 (d, *J*=1.0 Hz, 1H), 6.35 (s, 1H), 4.79-5.13 (m, 1H), 4.33 (dd, *J*=8.0, 9.8 Hz, 1H), 4.12-4.26 (m, 4H), 3.97-4.12 (m, 2H), 3.84 (dd, *J*=7.0, 10.0 Hz, 1H), 3.00 (dd, *J*=5.5, 11.0 Hz, 2H), 1.62 (d, *J*=6.3 Hz, 3H), 1.35 (br. s., 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -144.46; MS (ES) MH^+: 422.1 for $C_{20}H_{25}FN_3O_6$.

(*R*)-3-(7-Chloro-6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)benzo[d]isoxazol-3-yl)-5-methyloxazolidin-2-one (6c) A solution of (*R*)-5-methyloxazolidin-2-one[61] (271 mg, 2.68 mmol) in 4 mL DMF was added slowly to a stirred suspension of NaH (60% oil dispersion, 107 mg, 2.68 mmol) in 3 mL DMF at 0 °C. The mixture was stirred at rt for 10 min, and a solution of **5c** (1.0 g, 2.68 mmol, see Supporting Information) in 5 mL DMF was added. The resulting mixture was heated at 85 °C for 2 h, poured into ice cooled aqueous NH_4Cl, and extracted twice with EtOAc. The organic layers were dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (25% EtOAc gradient in hexanes) to afford 500 mg starting material **5c** and 800 mg (35%) of the title compound. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.58 (s, 1H), 6.22 (s, 1H), 4.87-5.17 (m, 1H), 3.48 - 4.34 (m, 9H), 2.67-3.20 (m, 3H), 1.49 (d, *J*=6.4 Hz, 3H), 1.32 (br. s., 3H), 1.13 (br. s., 3H); MS (ES) MH^+: 438 for $C_{20}H_{25}ClN_3O_6$.

(*S*)-5-((*tert*-Butyldiphenylsilyloxy)methyl)-3-(6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxazolidin-2-one A solution of (*S*)-5-((*tert*-butyldiphenylsilyloxy)methyl)oxazolidin-2-one (3.0 g, 8.4 mmol, described in Supplementary Material) in 10 mL DMF was added slowly to a stirred suspension of NaH (60% dispersion, 0.37 g, 8.4 mmol) in 10 mL DMF at 0°C over 10 min. The mixture was stirred at rt for 30 min, and a solution of **5a**[15] (3.0 g, 8.4 mmol) in 10 mL DMF was added. This mixture was heated at 80 °C for 2 h and poured into ice-cooled aqueous NH_4Cl before being extracted twice with EtOAc. The organic layers were dried (Na_2SO_4) and concentrated to give a residue that was chromatographed

over silica gel (40-50% EtOAc gradient in pet. ether) to afford the title compound. Yield 2.13 g (37%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.30-7.70 (m, 10H), 6.19 (s, 1 H), 4.95-5.11 (m, 1H), 4.30 (t, *J*=9.3 Hz, 1H), 3.83-4.14 (m, 9H), 3.17-3.27 (m, 2H), 2.90 (dd, *J*=10.83, 5.4 Hz, 2H), 1.23 (d, *J*=5.4 Hz, 6H), 0.89 (s, 9H); MS (ES) MH^+: 676 for $C_{36}H_{43}FN_3O_7Si$.

(*S*)-3-(6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)-5-(hydroxymethyl)oxazolidin-2-one (6d) Acetic acid (0.89 mL, 15.5 mmol) and a solution of 1M TBAF (3.1 mL, 3.1 mmol) in THF were added sequentially to a solution of the preceding compound (2.1 g, 3.11 mmol) in 15 mL THF. The mixture was stirred at rt for 18 h before being diluted with water and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to afford material that was purified on a silica gel column (50-70% EtOAc gradient in hexanes) to give the title compound (1.33 g, 98% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.4 (s, 1H), 6.2 (s, 1H), 4.7-5.05 (m, 1H), 3.9-4.3 (m, 8H), 3.5-3.8 (m, 2H), 2.8-3.3 (m, 4H), 1.2 (d, *J*= 5.8 Hz, 6H). MS (ES) MH^+: 438.1 for $C_{20}H_{25}FN_3O_7$.

(*S*)-3-(6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)-5-(methoxymethyl)oxazolidin-2-one (6e) Prepared as described for **6c** with (*S*)-5-(methoxymethyl)oxazolidin-2-one (294 mg, 2,24 mmol), NaH (60% dispersion, 90 mg, 2.24 mmol) and **5a** (800 mg, 2.24 mmol) to afford 480 mg (47% yield) of the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (s, 1H), 6.18 (s, 1H), 4.91-5.19 (m, 1H), 4.22 (t, *J*= 8 Hz, 1H), 2 3.6-4.3 (m, 9H), 3.34 (s, 3H), 3.16-3.28 (m, 2H), 2.7-2.90 (m, 2H), 1.23 (d, *J*=6.2 Hz, 6H); MS (ES) MH^+: 452 for $C_{21}H_{27}FN_3O_7$.

(*S*)-3-(6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-chlorobenzo[d]isoxazol-3-yl)-5-(methoxymethyl)oxazolidin-2-one (6f) Prepared as described for **6h** with 1.05 g (8.04

mmol) (*S*)-5-(methoxymethyl)oxazolidin-2-one, NaH (60% dispersion, 322 mg, 8.04 mmol) and **5c** (1.0 g, 2.68 mmol) (Supplementary materials) to afford 600 mg (48%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 6.35 (s, 1 H), 4.9 (m, 1H), 4.1-4.35 (m, 6H), 3.9-4.1 (m, 2H), 3.70 (dd, *J*=10.7, 4.0 Hz, 1H), 3.67 (dd, *J*=10.7, 4.0 Hz, 1H), 3.45 (s and m, 4H), 3.25 (m, 1H), 3.05 (m, 1H), 2.7-2.85 (m, 1H), 1.45 (br. s, 3H), 1.15 (s. br, 3H); MS (ES) MH^+: 468.2 for $C_{21}H_{27}ClN_3O_7$.

(4*R*)-3-{6-[(2*R*,6*R*)-2,6-Dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl}-4-methyl-1,3-oxazolidin-2-one (6g) A solution of (4*R*)-4-methyl-1,3-oxazolidin-2-one[62] (1.0 g, 9.9 mmol) in 10 mL DMF was added slowly to a stirred suspension of NaH (60% dispersion, 0.24 g, 9.9 mmol) in 10 mL DMF at 0 °C over 10 min. The mixture was stirred at rt for 30 min, and a solution of **5a**[15] (1.1 g, 3.1 mmol) in 3 mL DMF was added. This mixture was heated at 80 °C for 12 h and poured into ice-cooled water before being extracted twice with EtOAc. The organic layers were dried (Na_2SO_4) and concentrated to give a residue that was chromatographed over silica gel (EtOAc gradient in pet. ether) to afford the title compound. Yield: 0.15 g (12%). MS (ES) MH^+: 422.4 for $C_{20}H_{25}FN_3O_6$.

(*S*)-3-(7-chloro-6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)benzo[d]isoxazol-3-yl)-4-methyloxazolidin-2-one (6h) NaH (60% dispersion, 322 mg, 8.04 mmol) was added to a solution of (*S*)-4-methyloxazolidin-2-one[62] (1.05 g, 8.04 mmol) in 10 mL DMF. After 10 min stirring, a solution of **5c** (1.0 g, 2.68 mmol, see Supporting Information) in 5 mL DMF was added, and the mixture was heated at 100 ˚C for 1 h. After quenching with saturated aqueous NH_4Cl, The mixture was extracted with EtOAc, which was washed with brine. Drying (Na_2SO_4) and removal of solvent gave a residue that was chromatographed on silica gel (20% acetone in hexanes) to afford 0.6 g (48%) of the title compound as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ

8.69 (s, 1H), 6.36 (s, 1H), 4.94 (m, 1H), 4.00-4.33 (m, 10H), 3.80-3.91 (m, 1H), 3.71-3.79 (m, 1H), 3.63-3.70 (m, 1H), 3.18-3.35 (m, 1H), 3.06 (d, *J*=10.0 Hz, 1H), 2.79 (d, *J*=10.7 Hz, 1H), 1.45 (d, *J*=5.1 Hz, 3H), 1.19-1.26 (m, 3H); MS (ES) MH^+: 468.2 for $C_{21}H_{27}ClN_3O_7$.

(*R*)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-(6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxazolidin-2-one (6i) NaH (60% dispersion) (0.056 g, 1.40 mmol) was added to a solution of (*R*)-4-(((tert-butyldimethylsilyl)oxy)methyl)oxazolidin-2-one[63] (0.324 g, 1.40 mmol) in DMF (5 mL) at room temperature. After stirring for 30 min, **5a** (0.5 g, 1.40 mmol) was added, and the mixture was heated at 80 °C for 6 h. The mixture was quenched with aqueous NH_4Cl, and solvent was removed. The residue was taken up in Et_2O, which was washed 3 times with water and once with brine. The combined aqueous layers were extracted with Et_2O, which was washed twice more with water and once with brine. The combined Et_2O layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (0-30% gradient of EtOAc in CH_2Cl_2) to isolate two materials. The first eluting material was consistent with starting material **5a** and the second eluting material with the title compound as a white solid. Yield 233 mg (30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (s, 1H), 6.23 (s, 1H), 4.74 (d, *J*=3.8 Hz, 2H), 4.43-4.54 (m, 1H), 3.96-4.26 (m, 7H), 3.80 (d, *J*=10.9 Hz, 1H), 3.31 (s, 2H), 3.27 (d, *J*=10.9 Hz, 2H), 2.95 (dd, *J*=5.65, 10.7 Hz, 2H), 1.27 (d, *J*=6.2 Hz, 6H), 0.81 (s, 9H), 0.00 (s, 3H), -0.15 (s, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -145.55; MS (ES) MH^+: 552.0 for $C_{26}H_{39}FN_3O_7Si$.

(4*S*)-3-{6-[(2*R*,6*R*)-2,6-dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl}-4-(methoxymethyl)-1,3-oxazolidin-2-one (6j) NaH (60% dispersion, 0.102 g, 2.55 mmol) was added to a solution of (*S*)-4-(methoxymethyl)oxazolidin-2-one in 5 mL DMF at rt. After stirring for 30 min, **5a** (0.7 g, 1.96 mmol) was added, and the mixture was heated at 80

°C for 6 h. The mixture was quenched with aqueous NH_4Cl and solvent was removed. The residue was taken up in Et_2O, which was washed 3 times with water and once with brine. The combined aqueous layers were extracted with Et_2O, which was washed twice more with water and once with brine. The combined Et_2O layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (0-30% gradient of EtOAc in CH_2Cl_2). Two materials were isolated. The first eluting material was consistent with starting material (221 mg) and the second eluting material with the title compound (243 mg, 27%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J*=1.0 Hz, 1H), 6.35 (s, 1H), 4.72-4.82 (m, 1H), 4.63-4.69 (m, 1H), 4.57-4.63 (m, 1H), 4.14-4.24 (m, 4H), 4.00-4.10 (m, 2H), 3.91 (dd, *J*=5.0, 10.3 Hz, 1H), 3.70 (dd, *J*=2.6, 10.2 Hz, 1H), 3.37 (s, 3H), 3.25-3.37 (m, 2H), 3.00 (dd, *J*=5.5, 10.8 Hz, 2H), 1.34 (br. s., 6H); ^{19}F NMR (282 MHz, DMSO-*d*$_6$) δ -145.51; MS (ES) MH^+: 452.3 for $C_{21}H_{27}FN_3O_7$.

(S)-3-(7-chloro-6-((2R,6R)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)benzo[d]isoxazol-3-yl)-4-(methoxymethyl)oxazolidin-2-one (6k) Prepared as described for **7c** with 703 mg (5.36 mmol) (*S*)-5-(methoxymethyl)oxazolidin-2-one, 214 mg (60% dispersion, 5.35 mmol) NaH and 2.0 g (5.36 mmol) **5c** (Supplementary Material) to afford 421 mg (17%) of the title compound. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.45 (s, 1H), 6.21 (s, 1H), 4.72-4.79 (m, 1H), 4.46 (dd, *J*=3.5, 7.6 Hz, 1H), 3.93-4.15 (m, 8H), 3.87 (dd, *J*=3.5, 10.5 Hz, 1H), 3.57 (dd, *J*=2.0, 10.5 Hz, 1H), 3.25 (s, 3H), 3.06 (br. s., 2H), 2.67-2.87 (m, 1H), 1.32 (br. s., 3H), 1.13 (br. s., 3H); MS (ES) MH^+: 468.2 for $C_{21}H_{27}ClN_3O_7$.

(4*S*)-4-[(Dimethylamino)methyl]-3-{6-[(2*R*,6*S*)-2,6-dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl}-1,3-oxazolidin-2-one (6l) Prepared from **5a** (335 mg, 0.94 mmol), NaH (60% dispersion, 38 mg, 0.94 mmol) and (135 mg, 0.94 mmol) (4*S*)-4-[(dimethylamino)methyl]-1,3-oxazolidin-2-one (Supporting Information) using the method

described for **6c** to afford 102 mg (34%) of the title compound**.** ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.25 (s, 1H), 6.1 (s, 1H), 4.7 (d, 2H), 4.45 (d, 1H), 4.1 (m, 2H), 3.75 (m, 2H), 4.0 (m, 2H), 3.1 (d, 2H), 2.85 (t, 2H), 2.5 (m, 2H), 2.2 (s, 6H), 1.1 (d, 6H); MS (ES) MH^+: 465.5 for $C_{22}H_{30}FN_4O_6$.

(*S*)-3-(6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)-4-phenyloxazolidin-2-one (6m) NaH (60% dispersion, 56 mg, 1.40 mmol) was added to a solution of (*S*)-4-phenyloxazolidin-2-one (0.229 g, 1.40 mmol) in DMF (20 mL) at rt. After stirring for 30 min, **5a** (0.5 g, 1.40 mmol) was added, and the mixture was heated at 80 °C for 24 h. The mixture was quenched with aqueous NH_4Cl and solvent was removed. The residue was taken up in Et_2O, which was washed 3 times with water and once with brine. The combined aqueous layers were extracted with Et_2O, which was washed twice more with water and once with brine. The combined Et_2O layers were dried (MgSO4) and concentrated to give an oil that was chromatographed on silica gel (0-30% gradient of EtOAc in CH_2Cl_2) to afford two components. The first eluting component was consistent with recovered starting material **3a** (225 mg). The second eluting component was identified as the title compound and was isolated as a white solid (96 mg, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J*=1.0 Hz, 1H), 7.31-7.47 (m, 5H), 6.34 (s, 1H), 5.70 (dd, *J*=6.3, 8.8 Hz, 1H), 4.96 (t, *J*=8.8 Hz, 1H), 4.47 (dd, *J*=6.3, 8.8 Hz, 1H), 3.99-4.27 (m, 7H), 2.97 (dd, *J*=5.65, 11.2 Hz, 2H), 1.29-1.45 (br. s, 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -145.2; MS (ES) MH^+: 484.1 for $C_{25}H_{27}FN_3O_6$.

(4*S*,5*R*)-3-[6-[(2*R*,6*R*)-2,6-Dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl]-4,5-dimethyl-oxazolidin-2-one (6o) and **(4*R*,5*S*)-3-[6-[(2*R*,6*R*)-2,6-dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazol-3-yl]-4,5-dimethyl-oxazolidin-2-one (6n)** A solution of *rel*-(*R*,*R*)-4,5-dimethyloxazolidin-2-one[64] (2.7 g, 18.8

mmol) in 50 mL DMF was added slowly to a suspension of NaH (60% dispersion, 752 mg, 18.8 mmol) in 10 mL DMF at rt. After 10 minutes stirring, **5a** (6.7 g, 18.8 mmol) was added and the mixture was heated in the microwave at 100 °C for 1 h. The resulting mixture was cooled and poured into ice cold aqueous NH_4Cl, and extracted with EtOAc. The organic layer was washed with water, brine, and dried (Na_2SO_4). After concentration, the residue was purified on a silica gel column (elution with 0-5% methanol in $CHCl_3$) to give a solid as a mixture of diastereomers. The diastereomers were separated by chiral HPLC using Chiralpak IC (250 x 4.6mm) column (hexane:methanol:ethanol (70:15:15) 1.0 mL/min) to afford 2 components. The first eluting component was identified as **6o** (840 mg, 10% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (s, 1H), 6.18 (s, 1H), 4.91-5.23 (m, 1H), 4.57-4.77 (m, 1H), 4.03-4.13 (m, 5H), 3.99 (dd, *J*=5.0, 8.0 Hz, 2H), 3.24 (m, 1H), 3.20 (m, 1H), 2.91 (d, *J*=4.90 Hz, 1H), 2.88 (d, *J*=4.7 Hz, 1H), 1.39 (d, *J*=6.6 Hz, 3H), 1.33 (d, *J*=6.4 Hz, 3H), 1.23 (d, *J*=6.0 Hz, 6H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -145.46; MS (ES) MH^+: 436.2 for $C_{21}H_{27}ClN_3O_6$. The material matched that made from (*R*,*R*)-4,5-dimethyloxazolidin-2-one.[65] The second eluting component was identified as **6n** (920 mg, 11% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H), 6.18 (s, 1H), 4.90-5.21 (m, 1H), 4.56-4.80 (m, 1H), 3.91-4.15 (m, 7H), 3.28 (m, 1H), 3.24 (m, 1H), 3.20 (m, 1H), 2.91 (d, *J*=6.4 Hz, 1H), 2.88 (d, *J*=5.5 Hz, 1H), 1.39 (d, *J*=6.6 Hz, 3H), 1.33 (d, *J*=6.6 Hz, 3H), 1.23 (d, *J*=5.8 Hz, 6H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -145.47; MS (ES) MH^+: 436.2 for $C_{21}H_{27}ClN_3O_6$.

1-(6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)imidazolidin-2-one (6p) Prepared from **5a** (200 mg, 0.56 mmol), NaH (60% dispersion, 33.6 mg, 0.84 mmol) and imidazolidin-2-one (72.4 mg, 0.84 mmol) using the method described for **3b** to afford 30 mg (13%) of the title compound**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J*=1.1

Hz, 1H), 6.33 (s, 1H), 5.15 (br. s., 1H), 4.2-4.3 (m, 6H), 4.0-4.1 (m, 2H), 3.7-3.8 (m, 2H), 3.25-3.4 (br. s, 2H), 2.9-3.0 (dd, *J*=11.0, 5.6 Hz, 2H), 1.32 (d, *J*=6.2 Hz, 6H). MS (ES) MH^+: 407.1 for $C_{19}H_{24}FN_4O_5$.

1-(6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)-3-methylimidazolidin-2-one (6q) NaH (60% dispersion, 0.056 g, 1.40 mmol) was added to a solution of 1-methylimidazolidin-2-one (0.140 g, 1.40 mmol) in DMF (5 mL) at rt. After stirring for 30 min, **5a** (0.5 g, 1.40 mmol) was added, and the mixture was heated at 80 °C for 6 h. The mixture was quenched with aqueous NH_4Cl and solvent was removed. The residue was taken up in Et_2O, which was washed 3 times with water and once with brine. The combined aqueous layers were extracted with Et_2O, which was washed twice more with water and once with brine. The combined Et_2O layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (0-30% gradient of EtOAc in CH_2Cl_2) to afford 216 mg (37%) of the title compound as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 6.35 (s, 1H), 4.11-4.29 (m, 5H), 3.97-4.08 (m, 5H), 3.64 (t, *J*=7.9 Hz, 2H), 3.00 (m, 5H), 1.34 (br. s., 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -145.0; MS (ES) MH^+: 421.9 for $C_{20}H_{26}FN_4O_5$.

(*R*)-2-(5-(1,3-dioxolan-2-yl)-2,3,4-trifluoro-*N*'-hydroxybenzimidamido)-3-hydroxy-*N*,*N*-dimethylpropanamide Et_3N (1.5 mL, 10.7 mmol) and **10** (2.5 g, 8.9 mmol, described in Supplementary Materials) were added to a solution of (*R*)-2-amino-3-hydroxy-N,N-dimethylpropanamide (1.4 g, 10.7 mmol, described in Supplementary Materials) in 30 mL DMF, and the mixture was stirred at rt for 16 h. The mixture was poured into water (75 mL) and extracted with 3 times with EtOAc. The combined organic layers were washed twice with water and once with brine before being dried (Na_2SO_4). Removal of solvent was followed by chromatography on silica gel (3% MeOH in $CHCl_3$) to afford the title compound as a pale yellow

oil. Yield: 3.0 g (90%).^{1}H NMR (400 MHz, DMSO-d_6) δ 2.72 (s, 3H), 2.79 (s, 3H), 3.40-3.43 (m, 1H), 3.97-4.07 (m, 6H), 4.92 (t, 1H), 5.92 (d, 1H), 6.03 (s, 1H), 7.28 (t, 1H), 10.16 (s, 1H). ^{19}F NMR (376.5 MHz, DMSO-d_6) δ -134.4 (m), -138.2 (m), -159.48 (m); MS (ES) MH^+: 378.3 for $C_{15}H_{19}F_3N_3O_5$.

(*R*)-2-((5-(1,3-dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)amino)-3-hydroxy-*N*,*N*-dimethylpropanamide (11r) A solution of the preceding compound (3.0 g, 7.95 mmol) and Cs_2CO_3 (5.7 g, 17.5 mmol) in 10 mL DMF was stirred at rt for 16 h. The mixture was poured into water (75 mL) and extracted with 3 times with EtOAc. The combined organic layers were washed with twice with water and once with brine before being dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (1% MeOH in $CHCl_3$) to afford the title compound as a pale yellow solid. Yield: 2.0 g (70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.85 (s, 3H), 3.14 (s, 3H), 3.63-3.71 (m, 2H), 4.00-4.09 (m, 4H), 4.64-4.69 (m, 1H), 5.03 (t, 1H), 6.09 (s, 1H), 7.61 (d, 1H), 8.11 (d, 1H); ^{19}F NMR (376.5 MHz, DMSO-d_6) δ -143.2 (d), -161.8 (d); MS (ES) MH^+: 358.3 for $C_{15}H_{18}F_2N_3O_5$.

(*R*)-3-(5-(1,3-dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)-*N*,*N*-dimethyl-2-oxooxazolidine-4-carboxamide A solution of **11r** (2.0 g, 5.59 mmol) in 10 mL THF was added to a stirring mixture of NaH (60% dispersion, 223 mg, 5.59 mmol) in 10 mL THF at -10°C, and stirring was continued for 10 min. A solution of CDI (1.8 g, 11.2 mmol) in 10 mL THF was added, and after stirring for another 10 min, the reaction mixture was then poured into ice water. The mixture was extracted twice with EtOAc, and the combined organic layers were washed with water and brine. Drying (Na_2SO_4) and removal of solvent was followed by chromatography on silica gel ($CHCl_3$) to afford the title compound as a pale yellow solid. Yield: 2.0 g (93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86 (s, 3H), 3.07 (s, 3H), 3.98-4.07 (m, 4H), 4.53 (dd, 1H), 4.82

(t, 1H), 5.63 (dd, 1H), 6.10 (s, 1H), 8.39 (dd, 1H). ^{19}F NMR (376.5 MHz, DMSO-d_6) δ -140.2 (d, *J*=18.8 Hz), -160.9 (d, *J*=18.8 Hz); ^{19}F NMR (376.5 MHz, DMSO-d_6) δ: -140.2 (d), -160.9 (d); MS (ES) MH^+: 384.3 for $C_{16}H_{16}F_2N_3O_6$

(*R*)-3-(6,7-difluoro-5-formylbenzo[d]isoxazol-3-yl)-N,N-dimethyl-2-oxooxazolidine-4-carboxamide (12r) A stirred solution of the preceding compound (1.9 g, 4.96 mmol) in 15 mL 1,4-dioxane and 7.5 mL 6N HCl (4.5 mL) was stirred at rt for 8 h. The mixture was poured into ice-cooled water and extracted with twice with EtOAc. The combined organic layers were washed with water and brine. Drying (Na_2SO_4) and removal of solvent afforded the title product as a solid. Yield: 1.65 g (98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86 (s, 3H), 3.08 (s, 3H), 4.57 (dd, 1H), 4.85 (t, 1H), 5.64 (dd, 1H), 8.79 (dd, 1H), 10.19 (s, 1H); ^{19}F NMR (376.5 MHz, DMSO-d_6) δ -142.9 (d, *J*=22.6 Hz), -160.2 (d, *J*=22.6 Hz); ^{19}F NMR (376.5 MHz, DMSO-d_6) δ: -140.9 (d), -160.2 (d); MS (ES) MH^+: 340.3 for $C_{14}H_{12}F_2N_3O_5$.

(*R*)-3-(6-((2*R*,6*R*)-2,6-dimethylmorpholino)-7-fluoro-5-formylbenzo[d]isoxazol-3-yl)-N,N-dimethyl-2-oxooxazolidine-4-carboxamide (13r) A solution of **12r** (1.65 g, 4.80 mmol), DIEA (1.7 mL, 9.6 mmol) and (2*R*,6*R*)-2,6-dimethylmorpholine (0.68 g, 5.8 mmol) in 20 mL CH_3CN was heated at 80 ºC for 16 h. After cooling to rt, volatiles were removed and the residue was chromatographed on silica gel (EtOAc gradient in $CHCl_3$) to give title compound as pale yellow solid. Yield: 1.95 g (92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.65 (s, 1H), 5.64 (dd, *J*=3.2, 8.7 Hz, 1H), 4.84 (t, *J*=8.8 Hz, 1H), 4.55 (dd, *J*=3.2, 8.8 Hz, 1H), 4.11-4.15 (m, 2H), 3.38-3.41 (m, 2H), 3.08 (s, 3H), 2.99-3.03 (m, 2H), 2.87 (s, 3H), 1.20 (d, *J*=6.3 Hz, 6H); ^{19}F NMR (376.5 MHz, DMSO-d_6) δ: -146.81; ES MH^+: 435.4 for $C_{20}H_{23}FN_4O_6$.

(2*R*)-1-[[5-(1,3-Dioxolan-2-yl)-6,7-difluoro-1,2-benzoxazol-3-yl]amino]propan-2-ol (11t) (*R*)-1-aminopropan-2-ol (1.3 ml, 16.6 mmol) was added to a solution of **10** (2.22 g, 7.88 mmol,

described in Supplementary Materials) 30 mL DMF at rt wherein there was a slow exotherm. After stirring for 40 min, potassium *tert*-butoxide (1.77 g, 15.8 mmol) was added all at once. After stirring for 1 h, the reaction was incomplete by LC-MS analysis and additional potassium *tert*-butoxide was added (400 mg, 3.6 mmol). The resulting mixture was stirred at rt for 1 h before being quenched with aqueous NH_4Cl. Solvent was removed, and the solid residue was taken up in water while breaking up the solid mass with a spatula, and the mixture was stirred at rt overnight. The solids were filtered and rinsed through with water before being dried in vacuo to give the title compound. Yield 2.24 g (95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.30-8.55 (m, 1H), 6.10 (s, 1H), 4.87-5.21 (m, 1H), 4.27 (t, *J*=8.9 Hz, 1H), 4.04 (m, 4H), 3.78 (dd, *J*=7.2, 9.4 Hz, 1H), 1.49 (d, *J*=6.2 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -140.64 (d, *J*=21.5 Hz), -161.34 (d, *J*=21.5 Hz); ES MH^+: 301 for $C_{13}H_{14}F_2N_2O_4$.

(*R*)-3-(5-(1,3-Dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)-5-methyloxazolidin-2-one A mixture of **11t** (500 mg, 1.67 mmol), carbonyl diimidazole (405 mg, 2.50 mmol), and DMAP (102 mg, 0.83 mmol) 10 mL THF was heated at reflux overnight (21 h). The solvent was removed and the residue was taken up in 1N HCl with stirring at rt for 90 min affording solids. The solids were filtered and rinsed well with water breaking them up with a spatula before drying *in vacuo*. The material is consistent with the title compound with about 5% impurity due to hydrolysis of the ketal to corresponding aldehyde. Yield 407 mg (75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33-8.55 (m, 1H), 6.10 (s, 1H), 4.86-5.17 (m, 1H), 4.27 (t, *J*=8.9 Hz, 1H), 4.04 (d, *J*=4.3 Hz, 5H), 3.78 (dd, *J*=7.2, 9.4 Hz, 1H), 1.49 (d, *J*=6.2 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -140.64 (d, *J*=21.5 Hz), -161.34 (d, *J*=21.5 Hz); MS (ES) MH^+: 327 for $C_{14}H_{13}F_2N_2O_5$.

(*R*)-6,7-Difluoro-3-(5-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (12t)

A solution of the preceding intermediate (404 mg, 1.24 mmol) in HCl (1.0 M in water) (10 mL, 10.0 mmol) and 10 mL THF was stirred at rt for 3 d. The reaction mixture was diluted with water and extracted twice with EtOAc, and each extract was washed with brine. The organic layers were combined and dried ($MgSO_4$) and concentrated to afford material as an off-white solid consistent to give the title compound. Yield 350 mg (100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.29 (s, 1H), 8.95 (dd, *J*=1.9, 5.8 Hz, 1H), 5.02 (m, 1H), 4.33 (dd, *J*=8.1, 10.0 Hz, 1H), 3.85 (dd, *J*=7.2, 10.0 Hz, 1H), 1.64 (d, *J*=6.4 Hz, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -142.71 (d, *J*=19.4 Hz), -158.95 (d, *J*=19.4 Hz).

6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-7-fluoro-3-((*R*)-5-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (13t) A mixture of **13t** (250 mg, 0.89 mmol), (2*R*,6*R*)-2,6-dimethylmorpholine (122 mg, 1.06 mmol) and K_2CO_3 (122 mg, 0.89 mmol) in 4.5 mL CH_3CN and 0.5 mL water was heated at 100 °C in a microwave reactor vessel for 2 h. The solvent was removed and the residue diluted with water and extracted 2 times with EtOAc with each extract being washed with brine. The organic layers were combined, dried ($MgSO_4$) and concentrated to afford material that was chromatographed on silica gel (0-30% EtOAc gradient in CH_2CH_2) to afford a white solid consistent with the title compound. Yield 245 mg (73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.40 (s, 1H), 8.67 (d, *J*=1.1 Hz, 1H), 4.53-4.95 (m, 2H), 4.03-4.41 (m, 3H), 3.44 (d, *J*=12.1 Hz, 2H), 3.05 (dd, *J*=5.65, 11.5 Hz, 2H), 1.57-1.63 (m, 3H), 1.34 (d, *J*=6.6 Hz, 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -146.0; MS (ES) MH^+: 378.0 for $C_{18}H_{21}FN_3O_5$.

(*S*)-5-(1,3-dioxolan-2-yl)-2,3,4-trifluoro-*N*'-hydroxy-*N*-(1-hydroxypropan-2-yl)benzimidamide (*S*)-2-aminopropan-1-ol (2.35 g, 31.2 mmol) was added to a solution of **10** (4.0 g, 142 mmol) in 10 mL DMF cooled in an ice water bath. After warming to rt 30 min, the mixture was diluted with water and extracted with twice with EtOAc. The combined organic

layers were dried ($MgSO_4$) and concentrated to give the title compound (3.9 g, 86%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.34 (td, *J*=7.3, 2.4 Hz, 1H), 6.03 (s, 1H), 5.72 (d, *J*=10.4 Hz, 1H), 4.66 (t, *J*=5.5 Hz, 1H), 3.93-4.11 (m, 4H), 3.24 (t, *J*=5.5 Hz, 2H), 2.86-3.05 (m, 1H), 0.98 (d, *J*=6.4 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -134.55 (dd, *J*=9.3, 21.6 Hz), -138.45 (dd, *J*=9.3, 21.6 Hz), -159.78 (t, *J*=21.6 Hz); MS (ES) MH^+: 321 for $C_{13}H_{16}F_3N_2O_4$.

(*S*)-3-(5-(1,3-dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)-4-methyloxazolidin-2-one (11u) A mixture of the preceding compound (3.82 mg, 11.9 mmol) and Cs_2CO_3 (15.5 g, 47.7 mmol) in 25 ml of DMF was stirred at rt for 3 h. CDI (1.93 g, 11.9 mmol) in 6 mL DMF was added with subsequent stirring for 1 h. The mixture was diluted with EtOAc and water. The organic layers was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated to give material that was chromatographed on silica gel (40-50% EtOAc gradient in hexanes) to give the title compound (2.06 g, 53 %) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (dd, *J*=1.8, 5.9 Hz, 1H), 6.16 (s, 1H), 4.69-4.85 (m, 2H), 4.17-4.25 (m, 3H), 4.07-4.12 (m, 2H), 1.59 (d, *J*=6.0 Hz, 3H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -139.87 (d, *J*=19.5 Hz), -160.39 (d, *J*=19.5 Hz); ES MH^+: 327 for $C_{14}H_{13}F_2N_2O_5$.

(*S*)-6,7-Difluoro-3-(4-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (12u) A solution the **11u** (456 mg, 1.3 mmol) in 1M aqueous HCl (10 mL, 10.0 mmol) and 10 mL THF was stirred at rt for 2 d. The reaction mixture was diluted with water and extracted 2 times with EtOAc. The combined organic layers were washed with water and brine before being dried ($MgSO_4$) and concentrated to give 390 mg (99%) of the title compound as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.2 (s, 1H), 8.69 (dd, *J*=6.0, 1.7 Hz, 1H), 4.64-4.84 (m, 2H), 4.18-4.34 (m, 1H) 1.46 (d, *J*=6.0 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -143.25 (d, *J*=21.6 Hz), -160.42 (d, *J*=20.5 Hz).

6-((2*R*,6*R*)-2,6-Dimethylmorpholino)-7-fluoro-3-((*R*)-5-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (13u) A mixture of the preceding intermediate (250 mg, 0.90 mmol), (2*R*,6*R*)-2,6-dimethylmorpholine (124 mg, 1.07 mmol) and K_2CO_3 (124 mg, 0.90 mmol) in butyronitrile (3 mL) and water (0.5 mL) was heated at 100 °C in a microwave reactor vessel for 1h. The solvent was removed and the residue diluted with water and extracted 2 times with EtOAc with each extract being washed with brine. The organic layers were combined, dried ($MgSO_4$) and concentrated to afford material that was chromatographed on silica gel (0-30% EtOAc gradient in CH_2CH_2) to afford a yellow solid consistent with the title compound. Yield 245 mg (73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1 H), 6.19 (s, 1H), 4.63-4.81 (m, 2H), 4.20-4.28 (m, 1H), 3.23 (d, *J*=11.3 Hz, 2H), 2.91 (dd, *J*=10.7, 5.3 Hz, 2H), 1.44 (d, *J*=6.03 Hz, 3H), 1.23 (d, *J*=5.84 Hz, 6H); NMR (282 MHz, $CDCl_3$) δ -142.0. MS (ES) MH^+: 378.0 for $C_{18}H_{21}FN_3O_5$.

(*S*)-N-(1-Cyclopropyl-2-hydroxyethyl)-5-(1,3-dioxolan-2-yl)-2,3,4-trifluoro-N'-hydroxybenzimidamide A solution of (*S*)-2-amino-2-cyclopropylethanol (1.38 g, 13.3 mmol), Et_3N (1.85 mL, 13.3 mmol) and **10** (2.50 g, 8.89 mmol) in 20 mL DMF was stirred at rt for 16 h. The mixture was poured into water and extracted with 3 times with EtOAc. The combined organic layers were washed with twice with water and once with brine. Drying (Na_2SO_4) and removal of solvent gave a residue that was chromatographed on silica gel (3% MeOH in $CHCl_3$) to afford the title compound as a pale yellow solid. Yield: 2.2 g (72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.0 (s, 1H), 7.04 (t, *J*=7.3 Hz, 1H), 6.04 (s, 1H), 5.83 (d, *J*=10.6 Hz, 1H), 4.73 (t, *J*=5.4 Hz, 1H), 3.96-4.08 (m, 4H), 3.41 (t, *J*=5.1 Hz, 2H), 2.17-2.32 (m, 1H), 0.90-0.92 (m, 1H), 0.33 (m, 2H), -0.90-0.12 (m, 1H), -0.77- -0.87 (m, 1H).

(*S*)-2-((5-(1,3-Dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)amino)-2-cyclopropylethanol

(11s) A stirred solution of the preceding compound (2.2 g, 6.4 mmol) and Cs_2CO_3 (4.6 g, 14.1 mmol) in 20 mL DMF was stirred at rt for 16 h. The mixture was poured into water and extracted with 3 times with EtOAc. The combined organic layers were washed with twice with water and once with brine. Drying (Na_2SO_4) and removal of solvent gave a residue that chromatographed on silica gel (1% MeOH in $CHCl_3$) to afford the title compound as a pale yellow solid. Yield: 1.80 g (87%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.05 (dd, *J*=1.2, 5.8 Hz, 1H), 7.16 (d, *J*=8.1 Hz, 1H), 6.08 (s, 1H), 4.75 (t, *J*=5.6 Hz, 1H), 4.03-4.10 (m, 4H), 3.64-3.65 (m, 1H), 3.53-3.56 (m, 1H), 3.03-3.05 (m, 1H), 1.01-1.12 (m, 1H), 0.37-0.45 (m, 3H), 0.23-0.25 (m, 1H); MS (ES) MH^+: 327.3 for $C_{15}H_{17}F_2N_2O_4$.

(*S*)-3-(5-(1,3-Dioxolan-2-yl)-6,7-difluorobenzo[d]isoxazol-3-yl)-4-cyclopropyloxazolidin-2-one The title compound was synthesized following the procedure described for the preparation of **12r** using **11s** (1.20 g, 3.67 mmol), Cs_2CO_3 (4.77 g, 14.7 mmol) and CDI (602 mg, 3.67 mmol). Yield: 1.10 g (85%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.21 (dd, *J*=1.4, 5.9 Hz, 1H), 6.10 (s, 1H), 4.70 (t, *J*=8.4 Hz, 1H), 4.30-4.33 (m, 1H), 4.21-4.25 (m, 1H), 4.01-4.08 (m, 4H), 1.21-1.23 (m, 1H), 0.53-0.56 (m, 2H), 0.42-0.44 (m, 1H), 0.28-0.31 (m, 1H); MS (ES) MH^+: 353.3 for $C_{16}H_{15}F_2N_2O_5$.

(*S*)-3-(4-Cyclopropyl-2-oxooxazolidin-3-yl)-6,7-difluorobenzo[d]isoxazole-5-carbaldehyde (12s) The title compound was synthesized following the procedure described for the preparation of **12r** using the preceding intermediate (1.10 g, 3.12 mmol). Yield: 0.91 g (95%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.20 (s, 1H), 8.64 (dd, *J*=1.6, 5.9 Hz, 1H), 4.72 (t, *J*=8.4 Hz, 1H), 4.32-4.36 (m, 1H), 4.24-4.28 (m, 1H), 1.21-1.25 (m, 1H), 0.56-0.62 (m, 2H), 0.44-0.49 (m, 1H), 0.30-0.34 (m, 1H); MS (ES) MH^+: 309.3 for $C_{14}H_{11}F_2N_2O_4$.

3-((*S*)-4-Cyclopropyl-2-oxooxazolidin-3-yl)-6-((2*R*,6*R*)-2,6-dimethylmorpholino)-7-fluorobenzo[d]isoxazole-5-carbaldehyde (13s) The title compound was synthesized following the procedure described for the preparation of **13r** using **12s** (0.90 g, 2.92 mmol), (2*R*,6*R*)-2,6-dimethylmorpholine (402 mg, 3.5 mmol) and K_2CO_3 (403 mg, 2.92 mmol). Yield: 1.0 g (86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.47 (s, 1H), 8.30 (s, 1H), 4.69 (t, *J*=8.0 Hz, 1H), 4.19-4.33 (m, 2H), 4.10-4.15 (m, 2H), 3.31-3.39 (m, 2H), 2.99-3.03 (m, 2H), 1.20 (d, *J*=6.4 Hz, 6H), 0.57-0.60 (m, 2H), 0.44-0.49 (m, 1H), 0.30-0.34 (m, 1H); MS (ES) MH^+: 404.4 for $C_{20}H_{23}FN_3O_5$.

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-(2-oxo-1,3-oxazolidin-3-yl)-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1a) A mixture of **6a** (800 mg, 1.95 mmol) and barbituric acid (250 mg, 1.95 mmol) in 8 mL acetic acid and 2 mL water was heated at 110 °C for 3.5 h. The solvents were removed, and the reaction mixture was purified using SFC (Chiralpak IA column with 40% isopropanol and 60% CO_2 mobile phase) to afford 571 mg (62%) of the title compound as the major eluting component. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H), 11.45 (s, 1H), 7.75 (s, 1H), 4.58 (t, *J*=8.0 Hz, 2H), 4.04-4.21 (m, 3H), 3.94 (d, *J*=8.8 Hz, 1H), 3.74-3.87 (m, 1H), 3.59-3.73 (m, 2H), 3.01-3.20 (m, 1H), 2.91 (d, *J*=14.2 Hz, 1H), 1.15 (d, *J*=6.3 Hz, 3H), 0.89 (d, *J*=6.3 Hz, 3H); ^{19}F NMR (471 MHz, DMSO-d_6) δ -158.17 (s, 1F); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.9, 167.7, 154.2, 153.7 (d, *J*=12.8 Hz), 152.8 (d, *J*=1.8 Hz), 149.5, 134.7, 133.3 (d, *J*=238.3 Hz), 122.2, 118.5 (d, *J*=1.8 Hz), 106.1, 72.1, 71.6, 64.4, 63.9, 56.2 (d, *J*=10.1 Hz), 52.9, 44.8, 38.5, 18.2, 18.1; MS RT = 2.20 min, (ES) MH^+: 474.1 for $C_{21}H_{21}FN_5O_7$. HRMS (ES) MH^+ calcd for $C_{21}H_{21}FN_5O_7$ 474.1420, found 474.1439. $[\alpha]_D^{20}$ = -177 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-[(5*S*)-5-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1b) Prepared following the procedure described for the preparation of **1a** using **6b** (243 mg, 0.58 mmol) and barbituric acid (74 mg, 0.58 mmol) to afford 148 mg (53%) of the title compound as the major eluting component from SFC ((*S*,*S*) Whelk-O1, 60% CO_2, 40% EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 11.43 (s, 1H), 7.74 (s, 1H), 4.89-5.07 (m, 1H), 4.21 (dd, *J*=8.0, 9.5 Hz, 1H), 4.10 (d, *J*=12.6 Hz, 1H), 3.97-4.04 (m, 1H), 3.94 (d, *J*=9.0 Hz, 1H), 3.63-3.82 (m, 4H), 3.11 (t, *J*=12.8 Hz, 1H), 2.92 (d, *J*=14.0 Hz, 1H), 1.46 (d, *J*=6.3 Hz, 3H), 1.15 (d, *J*=6.3 Hz, 3H), 0.89 (d, *J*=6.5 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.14;. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.6, 153.6, 153.6 (d, J_{CF}=13.2 Hz), 152.9 (d, J_{CF}=2.2 Hz), 149.4, 134.7, 133.3 (d, J_{CF}=238.6 Hz), 122.2, 118.5, 106.2, 72.5, 72.1, 71.6, 64.4, 56.2 (d, J_{CF}=9.5 Hz), 52.9, 51.1, 38.6, 19.7, 18.14, 18.12; UPLC RT = 0.98 min, MS (ES) MH^+: 488.3 for $C_{22}H_{23}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{22}H_{23}ClN_5O_7$ 488.1576, found 488.1579 ; $[\alpha]_D^{20}$ = -130 (c = 1; MeOH).

((2*R*,4*S*,4a*S*)-11-Chloro-2,4-dimethyl-8-((*R*)-5-methyl-2-oxooxazolidin-3-yl)-2,4,4a,6-tetrahydro-1H,1'H-spiro[isoxazolo[4,5-*g*][1,4]oxazino[4,3-*a*]quinoline-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (1c) Prepared following the procedure described for the preparation of **1a** using **6c** (420 mg, 0.96 mmol) and barbituric acid (123 mg, 0.96 mmol) to afford 328 mg (68%) of the title compound as the major eluting component from SFC (Chiralpak IA column, 60% CO_2, 40% MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.57 (br. s., 2H), 7.84 (s, 1H), 4.90-5.02 (m, 1H), 4.43-4.56 (m, 1H), 4.23 (dd, *J*=8.3, 9.4 Hz, 1H), 3.90-4.04 (m, 2H), 3.55-3.77 (m, 3H), 2.90-3.19 (m, 2H), 1.40-1.51 (m, 3H), 1.09-1.25 (m, 3H), 0.89 (d, *J*=6.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.8, 167.6, 162.3, 153.6, 153.0, 149.5, 144.4, 122.2, 121.5, 105.3, 97.1,

72.5, 72.4, 65.9, 56.2, 52.7, 50.9, 19.9, 18.1. UPLC RT = 0.96 min, (ES) MH^+: 504.2 for $C_{22}H_{23}ClN_5O_7$. HRMS (ES) MH^+ calcd for $C_{22}H_{23}ClN_5O_7$ 504.1281, found 504.1303. $[\alpha]_D^{20}$ = -249 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-8-[(5*S*)-4-(hydroxymethyl)-2-oxo-1,3-oxazolidin-3-yl]-2,4-dimethyl-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1d) Prepared following the procedure described for the preparation of **1a** using **6d** (100 mg, 0.23 mmol) and barbituric acid (29.3 mg, 0.23 mmol) in 3:1 EtOH-aqueous 6N HCl as solvent to afford 60 mg (52%) of the title compound as the major eluting component from SFC (Chiralpak 1B column with 30% MeOH and 75% CO_2 mobile phase). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.75 (br. s., 1 H), 11.42 (br. s., 1 H), 7.76 (s, 1 H), 5.23 (t, *J*=5.65 Hz, 1 H), 4.75-4.94 (m, 1H), 4.04-4.22 (m, 2H), 3.85-3.99 (m, 2H), 3.53-3.84 (m, 5H), 3.03-3.20 (m, 1H), 2.91 (d, *J*=14.3 Hz, 1H), 1.14 (d, *J*=6.2 Hz, 3H), 0.89 (d, *J*=6.4 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.18; UPLC RT = 0.81 min, (ES) MH^+: 504.2 for $C_{22}H_{23}FN_5O_8$. HRMS (ES) MH^+ calcd for $C_{23}H_{23}FN_5O_8$ 504.1525, found 504.1533; $[\alpha]_D^{20}$ = -165 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-8-[(5*S*)-4-(methoxymethyl)-2-oxo-1,3-oxazolidin-3-yl]-2,4-dimethyl-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1e) Prepared following the procedure described for the preparation of **1a** using **6e** (445 mg, 0.99 mmol) and barbituric acid (126 mg, 0.99 mmol) to afford 368 mg (72%) of the title compound as the major eluting component from SFC ((*S*,*S*) Whelk-O1 column with 25% of 85:15 acetonitrile-methanol and 75% CO_2 mobile phase). Yield 368 mg (72 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 1H), 1.44 (s, 1H), 7.77 (s, 1H), 4.89-5.09 (m, 1H), 3.54-4.27 (m), 2.84-3.20 (m, 2H), 1.15 (d, *J*=6.0 Hz, 3H), 0.89 (d, *J*=6.2 Hz, 3H);

^{19}F NMR (282 MHz, DMSO-d_6) δ -158.18; ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 153.8, 153.6, 152.7, 149.4, 134.8, 133.3 (d, *J*=238.8 Hz), 122.3, 118.5, 106.0, 74.1, 72.2, 72.1, 71.6, 64.4, 58.7, 56.2 (d, *J*=9.4 Hz), 52.9, 46.2, 38.6, 18.1, 18.1. MS (ES) RT = 2.26 min, MH^+: 518.2 for $C_{23}H_{25}FN_5O_8$. HRMS (ES) MH^+ calcd for $C_{23}H_{25}FN_5O_8$ 518.1682, found 518.1706; $[\alpha]_D^{20}$ = -174 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Chloro-8-[(5*S*)-4-(methoxymethyl)-2-oxo-1,3-oxazolidin-3-yl]-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-g]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1f) Prepared following the procedure described for the preparation of **1a** using **6f** (600 mg, 1.28 mmol) and barbituric acid (164 mg, 1.28 mol) to afford 360 mg (53%) of the title compound as the major eluting component from reverse phase HPLC (20-50% acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.8 (br. s.) 11.4 (br. s.) 7.9 (s) 4.9 - 5.1 (m) 4.5 (dd, *J*=13.9, 1.8 Hz) 4.2 (t, *J*=9.3 Hz) 4.0 (d, *J*=8.7 Hz) 3.9 - 4.0 (m) 3.8 (dd, *J*=9.6, 6.0 Hz) 3.6 - 3.7 (m) 3.3 (s) 3.0 (dd, *J*=14.0, 10.0 Hz) 3.0 (dd, *J*=14.4, 1.1 Hz) 1.2 (d, *J*=6.2 Hz) 0.9 (d, *J*=6.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 167.6, 162.3, 153.6, 152.8, 149.5, 144.4, 122.2, 121.4, 105.2, 97.1, 74.1, 72.5, 72.2, 65.8, 58.7, 56.2, 52.7, 46.1, 39.1, 18.1; MS RT = 2.81 min, UPLC RT = 0.93 min, (ES) MH^+: 534.2 for $C_{23}H_{24}ClN_5O_8$; HRMS (ES) MH^+ calcd for $C_{23}H_{25}ClN_5O_8$ 534.1386, found 534.1412. $[\alpha]_D^{20}$ = -243 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-[(4*R*)-4-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1g) Prepared following the procedure described for the preparation of **1a** using **6g** (100 mg, 0.24 mmol) and barbituric acid (31 mg, 0.24 mmol) to afford 34 mg (29%) of the title compound as the major eluting component from reverse phase HPLC (20-50%

acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.77 (s, 1 H) 11.46 (s, 1 H) 7.59 (s, 1 H) 4.65 (m, 2 H) 4.19 (dd, *J*=7.6, 4.2 Hz, 1 H) 4.10 (d, *J*=12.5 Hz, 1 H) 3.93 (d, *J*=8.7 Hz, 1 H) 3.69 (m, 3 H) 3.10 (t, *J*=12.7 Hz, 1 H) 2.91 (d, *J*=14.0 Hz, 1 H) 1.41 (d, *J*=5.9 Hz, 3 H) 1.14 (d, *J*=6.2 Hz, 3 H) 0.89 (d, *J*=6.2 Hz, 3 H). ^{19}F NMR (282 MHz, DMSO-d_6) δ -73.78 (s, 1F), -158.09 (s, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 154.0, 153.4 (d, *J*=13.2 Hz), 152.2 (d, *J*=2.2 Hz), 149.4, 134.7, 133.2 (d, *J*=238.8 Hz), 122.4, 118.1, 107.0, 72.1, 71.6, 70.3, 64.4, 56.3 (d, *J*=9.9 Hz), 52.8, 38.6, 18.1, 18.1, 17.5. UPLC RT = 0.99 min, (ES) MH^+: 488.0 for $C_{22}H_{22}FN_5O_7$. HRMS (ES) MH^+ calcd for $C_{22}H_{23}FN_5O_7$ 488.1576, found 488.1577. $[\alpha]_D^{20}$ = -239 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Chloro-2,4-dimethyl-8-((*S*)-4-methyl-2-oxooxazolidin-3-yl)-2,4,4a,6-tetrahydro-1H,1'H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'H)-trione (1h) Prepared following the procedure described for the preparation of **1a** using **6h** (262 mg, 0.60 mmol) and barbituric acid (77 mg, 60 mmol) to afford 102 mg (34%) of the title compound as the major eluting component from SFC (Chiralpak IA column, 60% CO_2, 40% MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.78 (s, 1H), 11.40 (s, 1H), 7.68 (s, 1H), 4.59-4.78 (m, 2H), 4.49 (d, *J*=12.5 Hz, 1H), 4.09-4.27 (m, 1H), 3.89-4.07 (m, 2H), 3.50-3.75 (m, 2H), 2.92-3.12 (m, 2H), 1.41 (d, *J*=5.9 Hz, 3H), 1.08-1.26 (m, 3H), 0.90 (d, *J*=6.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 167.6, 162.1, 154.1, 152.3, 149.4, 144.5, 122.3, 121.0, 106.0, 97.2, 72.5, 70.2, 65.8, 65.6, 58.9, 56.2, 52.7, 52.4, 20.7, 18.1, 17.3; UPLC RT = 0.95 min, (ES) MH^+: 504.3 for $C_{22}H_{22}ClN_5O_7$. HRMS (ES) MH^+ calcd for $C_{22}H_{23}ClN_5O_7$ 504.1281, found 504.1290. $[\alpha]_D^{20}$ = -176 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-8-((*S*)-4-(hydroxymethyl)-2-oxooxazolidin-3-yl)-2,4-dimethyl-2,4,4a,6-tetrahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-

pyrimidine]-2',4',6'(3'*H*)-trione (1i) A mixture of **6i** (231 mg, 0.42 mmol), 1 mL 6N HCl and barbituric acid (53.6 mg, 0.42 mmol) in 3 mL ethanol was heated at 120 °C for 1 h in a microwave reactor. The solvents were removed, and the reaction mixture was purified using SFC (Chiralpak IC column with 35% ethanol and 65% CO_2 mobile phase) to give the title compound (62 mg, 29% yield) as a solid as the major eluting component. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 11.43 (s, 1H), 7.72 (s, 1H), 5.21 (br. s., 1H), 4.54-4.68 (m, 2H), 4.46 (dd, J=3.0, 7.0 Hz, 1H), 4.10 (d, J=13.0 Hz, 1H), 3.94 (d, J=9.0 Hz, 2H), 3.74-3.85 (m, 1H), 3.63-3.71 (m, 2H), 3.54 (d, J=11.8 Hz, 1H), 3.11 (t, J=12.0 Hz, 1H), 2.93 (d, J=14.3 Hz, 1H), 1.15 (d, J=6.0 Hz, 3H), 0.89 (d, J=6.3 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) -158.3; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.7, 154.4, 153.5 (d, J_{CF}=13.2 Hz), 152.2, 149.4, 134.7, 133.3 (d, J_{CF}=238.6 Hz), 122.1, 118.6, 106.7, 72.1, 71.6, 66.2, 64.4, 57.8, 57.4, 56.3 (d, J_{CF}=9.5 Hz), 52.9, 38.6, 18.14, 18.11; UPLC RT = 0.85 min, (ES) MH^+: 504.1 for $C_{22}H_{23}fN_5O_8$; HRMS (ES) MH^+ calcd for $C_{22}H_{23}FN_5O_8$ 504.1525, found 504.1506; ; $[\alpha]_D^{20}$ = -104.7 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-8-[(4*S*)-4-(methoxymethyl)-2-oxo-1,3-oxazolidin-3-yl]-2,4-dimethyl-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1j) Prepared following the procedure described for the preparation of **1a** using **6j** (0.67 g, 1.5 mmol) and barbituric acid (0.21 g, 1.6 mmol) to afford 561 mg (72%) of the title compound as the major eluting component from SFC (Chiralpak IA column with 20% methanol and 80% CO_2 mobile phase). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.76 (br. s., 1H), 11.43 (br. s., 1H), 7.66 (s, 1H), 4.69-4.76 (m, 1H), 4.60-4.68 (m, 1H), 4.43 (dd, *J*=3.95, 8.1 Hz, 1H), 4.11 (d, *J*=12.8 Hz, 1H), 3.95 (d, *J*=9.0 Hz, 1H), 3.85 (dd, *J*=3.6, 10.4 Hz, 1H), 3.75-3.82 (m, 1H), 3.61-3.72 (m, 2H), 3.54 (dd, *J*=2.1, 10.4 Hz, 1H), 3.26 (s, 3H), 3.08-3.15 (m, 1H), 2.92 (d, *J*=14.3 Hz, 1H), 1.15 (d, *J*=6.0 Hz, 3H), 0.90 (d, *J*=6.4 Hz, 3H); ^{19}F NMR

(564 MHz, DMSO-d_6) δ -161.8; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.7, 154.2, 153.5 (d, J_{CF}=13.2 Hz), 152.1 (d, J_{CF}=2.9 Hz), 149.4, 134.8, 133.3 (d, J_{CF}=239.3 Hz), 122.4, 118.3, 106.6, 72.1, 71.7, 69.1, 66.6, 64.4, 58.8, 56.2 (d, J_{CF}=8.8 Hz), 55.9, 52.9, 38.5, 18.13, 18.09; UPLC RT = 0.96 min, (ES) MH^+: 518.4 for $C_{23}H_{24}FN_5O_8$; HRMS (ES) MH^+ calcd for $C_{23}H_{25}FN_5O_8$ 518.1682, found 518.1694; $[\alpha]_D^{20}$ = -128 (c = 1.14; MeOH).

(2*R*,4*S*,4a*S*)-11-Chloro-8-((*S*)-4-(methoxymethyl)-2-oxooxazolidin-3-yl)-2,4-dimethyl-2,4,4a,6-tetrahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (1k) Prepared following the procedure described for the preparation of **1a** using **6k** (350 mg, 0.75 mmol) and barbituric acid (96 mg, 0.75 mmol) to afford 218 mg (55%) of the title compound as the major eluting component from reverse phase HPLC (20-50% acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26-11.96 (m, 2H), 7.76 (s, 1H), 4.60-4.76 (m, 2H), 4.45-4.55 (m, 1H), 4.42 (dd, *J*=3.7, 7.7 Hz, 1H), 3.91-4.04 (m, 2H), 3.84 (dd, *J*=3.3, 10.3 Hz, 1H), 3.58-3.72 (m, 2H), 3.52 (dd, *J*=1.8, 10.3 Hz, 1H), 3.25 (s, 3H), 2.97 (d, *J*=13.2 Hz, 1H), 2.51-2.54 (m, 1H), 1.17 (d, *J*=6.2 Hz, 3H), 0.90 (d, *J*=6.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 167.6, 162.2, 154.2, 152.3, 149.4, 144.5, 122.3, 121.3, 105.7, 97.2, 72.5, 69.0, 66.6, 65.8, 58.8, 56.2, 55.7, 52.7, 39.1, 18.1, 18.1; UPLC RT = 0.96 min, (ES) MH^+: 534.2 for $C_{23}H_{25}ClN_5O_8$; HRMS (ES) MH^+ calcd for $C_{23}H_{25}ClN_5O_8$ 534.1386, found 534.1406. $[\alpha]_D^{20}$ = -195 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-8-{(4*S*)-4-[(Dimethylamino)methyl]-2-oxo-1,3-oxazolidin-3-yl}-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione, TFA Salt (1l) Prepared following the procedure described for the preparation of **1a** using **6l** (92 mg, 0.21 mmol) and barbituric acid (27 mg, 0.21 mmol) to afford 41 mg (37%) of the title compound as the major eluting component reverse

phase HPLC (20-50% acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.84 (s, 1H), 11.43 (s, 1H), 9.62 (br. s., 1H), 7.69 (s, 1H), 4.99 (br. s., 1H), 4.74 (t, *J*=8.5 Hz, 1H), 4.55 (dd, *J*=3.6, 9.0 Hz, 1H), 4.10 (d, *J*=12.6 Hz, 1H), 3.94 (d, *J*=8.8 Hz, 1H), 3.72-3.84 (m, 1H), 3.50-3.71 (m, 4H), 3.06-3.19 (m, 1H), 2.78-3.00 (m, 7H), 1.14 (d, *J*=6.0 Hz, 3H), 0.89 (d, *J*=6.3 Hz, 3H); ^{19}F NMR (471 MHz, DMSO-d_6) δ -73.6, -158.4; ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.9, 167.6, 157.8, 153.8 (d, J_{C-F}=12.8 Hz), 153.5, 152.1, 149.5, 135.0, 133.3 (d, J_{C-F}=238.3 Hz), 122.6, 118.4, 106.2, 72.1, 71.7, 68.1, 64.4, 57.5, 56.2 (d, J_{C-F} =9.2 Hz), 52.8, 51.8, 43.3, 38.5, 18.1, 18.1; UPLC RT = 0.65 min, (ES) MH^+: 531.1 for $C_{24}H_{27}FN_6O_7$; HRMS (ES) MH^+ calcd for $C_{24}H_{28}FN_6O_7$, 531.1998 found 531.2021; $[\alpha]_D^{20}$ = -99 (c = 0.1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-[(4*S*)-2-oxo-4-phenyl-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1m) Prepared following the procedure described for the preparation of **1a** using **6m** (93 mg, 0.19 mmol) and barbituric acid (24.6 mg, 0.10 mmol) to afford 52 mg (49%) of the title compound as the major eluting component from reverse phase HPLC (20-50% CH_3CN/water gradient with 0.1% TFA) purification. Yield 52 mg (49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.84 (s, 1H), 11.49 (s, 1H), 7.66 (s, 1H), 7.29-7.46 (m, 5H), 5.68 (dd, *J*=6.2, 8.7 Hz, 1H), 4.98 (t, *J*=8.8 Hz, 1H), 4.35 (dd, *J*=6.0, 8.51 Hz, 1H), 4.07 (d, *J*=13.2 Hz, 1H), 3.93 (d, *J*=8.8 Hz, 1H), 3.70-3.79 (m, 1H), 3.60-3.70 (m, 2H), 3.22 (s, 1H), 3.10 (t, *J*=12.1 Hz, 1H), 2.95 (d, *J*=14.2 Hz, 1H), 1.13 (d, *J*=6.3 Hz, 3H), 0.89 (d, *J*=6.3 Hz, 3H); ^{19}F NMR (471 MHz, DMSO-d_6) δ -158.03; ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.9, 167.7, 154.3, 153.3 (d, J_{CF} =12.8 Hz), 151.9, 151.9, 149.4, 138.0, 134.8, 133.2 (d, J_{CF} =239.2 Hz), 128.9, 128.4, 126.5, 122.5, 117.9, 106.7, 72.1, 71.7, 71.4, 64.4, 59.5, 56.2 (d, J_{CF} =9.2 Hz), 52.8, 38.5, 18.1, 18.1;

UPLC RT = 1.06 min, (ES) MH^+: 550.1 for $C_{27}H_{24}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{27}H_{25}FN_5O_7$ 550.1733, found 550.1743; $[\alpha]_D^{20}$ = -228 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-8-((4*R*,5*S*)-4,5-Dimethyl-2-oxooxazolidin-3-yl)-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1'H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'H)-trione (1n) Prepared following the procedure described for the preparation of **1a** using **6n** (920 mg, 2.11 mmol) and barbituric acid (270 mg, 2.11 mmol) to afford 740 mg (70%) of the title compound as the major eluting component from reverse phase HPLC (20-50% acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (br. s., 2H), 7.62 (s, 1H), 5.02 (quin, *J*=6.7 Hz, 1H), 4.59 (quin, *J*=6.7 Hz, 1H), 4.09 (d, *J*=12.7 Hz, 1H), 3.93 (d, *J*=8.7 Hz, 1H), 3.57-3.84 (m, 3H), 3.10 (t, *J*=12.5 Hz, 1H), 2.91 (d, *J*=14.2 Hz, 1H), 1.26-1.41 (m, 6H), 1.14 (d, *J*=6.2 Hz, 3H), 0.89 (d, *J*=6.4 Hz, 3H) **.** ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.15. ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 153.4 (d, *J*=13.2 Hz), 153.2, 152.2 (d, *J*=2.8 Hz), 149.5, 134.7, 133.3 (d, *J*=238.8 Hz), 122.3 (d, *J*=2.2 Hz), 118.2, 106.8, 75.6, 72.1, 71.6, 64.4, 56.2 (d, *J*=9.4 Hz), 56.1, 52.9, 38.6, 18.1, 18.1, 14.1, 12.0. UPLC RT = 1.02 min, (ES) MH^+: 502 for $C_{23}H_{24}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{23}H_{24}FN_5O_7$ 502.1733, found 502.1729; $[\alpha]_D^{20}$ = -117(c = 0.1; MeOH).

(2*R*,4*S*,4a*S*)-8-((4*S*,5*R*)-4,5-Dimethyl-2-oxooxazolidin-3-yl)-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1'H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'H)-trione (1o) Prepared following the procedure described for the preparation of **1a** using **6o** (840 mg, 1.93 mmol) and barbituric acid (247 mg, 1.93 mmol) to afford 690 mg (71%) of the title compound as the major eluting component from reverse phase HPLC (20-50% acetonitrile/water gradient with 0.1% TFA) purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.8 (s, 1 H) 11.4 (s, 1 H) 7.6 (s, 1 H) 5.0 (q, *J*=6.7 Hz, 1 H) 4.6 (q, *J*=6.7 Hz, 1 H) 4.1 (d, *J*=12.7 Hz,

1 H) 3.9 (d, *J*=8.7 Hz, 1 H) 3.7-3.9 (m, 1 H) 3.6-3.7 (m, 2 H) 3.0-3.2 (m, 1 H) 2.9 (d, *J*=14.0 Hz, 1 H) 1.4 (d, *J*=6.6 Hz, 3 H) 1.3 (d, *J*=6.4 Hz, 3 H) 1.1 (d, *J*=6.0 Hz, 3 H) 0.9 (d, *J*=6.4 Hz, 3 H). ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.2; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.7, 153.4 (d, J_{CF}=13.2 Hz), 153.3, 152.2 (d, J_{CF}=2.2 Hz), 149.4, 134.7, 133.3 (d, J_{CF}=238.6 Hz), 122.3, 118.2, 106.8, 75.5, 72.1, 71.6, 64.4, 56.2 (d, J_{CF}=9.5 Hz), 55.7, 52.9, 38.6, 18.1, 18.1, 14.2, 12.0; UPLC RT = 0.98 min (ES) MH^+: 502.3 for $C_{23}H_{24}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{23}H_{25}FN_5O_7$ 502.1733, found 502.1753; $[\alpha]_D^{20}$ = -221(c = 0.1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-(2-oxoimidazolidin-1-yl)-2,4,4a,6-tetrahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (1p) Prepared following the procedure described for the preparation of **1a** using **6p** (30 mg, 0.07 mmol) and barbituric acid (10.4 mg, 0.08 mmol) to afford 9.8 mg (26%) of the title compound as the major eluting component from reverse phase HPLC purification (25-40% CH_3CN gradient in water with 0.1% HCO_2H). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.5 (br. s, 2H), 7.77 (s, 1H), 7.43 (s, 1H), 6.55 (br. s, 1H), 4.02 (d, *J*= 13.6 Hz, 1H), 3.85 (m, 2H), 3.70 (m, 1H), 3.60 (m, 1H), 3.45 (t, *J*=8.0 Hz, 2H), 3.02 (m, 1H), 2.84 (d, J=13.9 Hz, 1H), 1.07 (d, *J*=6.2 Hz, 3H), 0.81 (d, *J*=6.2 Hz, 3H); MS RT = 2.04 min, (ES) MH^+: 473.3 for $C_{21}H_{21}FN_6O_6$. HRMS (ES) MH^+ calcd for $C_{21}H_{22}FN_6O_6$ 473.1579, found 473.1594. $[\alpha]_D^{20}$ = -114 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-(3-methyl-2-oxoimidazolidin-1-yl)-2,4,4a,6-tetrahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'*H*)-trione (1q) Prepared following the procedure described for the preparation of **1a** using **6q** (213 mg, 0.51 mmol) and barbituric acid (64.9 mg, 0.51 mmol) to afford 130 mg (53%) of the title compound as the major eluting component from SFC (Chiralpak IC column with 50% methanol and 60% CO_2 mobile phase). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (br. s., 1H),

11.35 (br. s., 1H), 7.83 (s, 1H), 4.02 (d, *J*=12.5 Hz, 1H), 3.85 (d, *J*=8.8 Hz, 1H), 3.75-3.82 (m, 2H), 3.65-3.74 (m, 1H), 3.46-3.64 (m, 4H), 3.18-3.23 (m, 1H), 2.98-3.09 (m, 1H), 2.84 (d, *J*=14.0 Hz, 1H), 1.07 (d, *J*=6.0 Hz, 3H), 0.81 (d, *J*=6.3 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.42; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.6, 155.7, 154.2, 153.4 (d, *J*=12.4 Hz), 149.4, 134.4, 133.4 (d, *J*=237.8 Hz), 121.4, 119.5, 106.9, 72.1, 71.6, 64.3, 56.2 (d, *J*=8.8 Hz), 53.1, 44.4, 41.8, 38.8, 30.5, 18.2, 18.1; UPLC RT = 0.90 min (ES) MH^+: 487.1 for $C_{22}H_{24}FN_6O_6$; HRMS (ES) MH^+ calcd for $C_{22}H_{24}FN_6O_6$ 487.1736, found 487.1718. ; $[\alpha]_D^{20}$ = -115.6 (c = 1; MeOH).

(*R*)-3-((2*R*,4*S*,4aS)-11-Fluoro-2,4-dimethyl-2',4',6'-trioxo-2,2',3',4,4a,4',6,6'-octahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidin]-8-yl)-*N*,*N*-dimethyl-2-oxooxazolidine-4-carboxamide (1r) Prepared following the procedure described for the preparation of **1a** using **13r** (1.95 g, 4.48 mmol) and barbituric acid (573 mg, 4.48 mmol) to afford 1.68 g (69%) of the title compound as the major eluting component from reverse phase HPLC (25-40% CH_3CN/water gradient with 0.1% HCO_2H). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.77 (s, 1H), 11.45 (s, 1H), 7.76 (s, 1H), 5.59 (dd, *J*=3.6, 8.9 Hz, 1H), 4.79 (t, *J*=8.9 Hz, 1H), 4.44 (dd, *J*=3.7, 8.8 Hz, 1H), 4.09 (d, *J*=12.8 Hz, 1H), 3.94 (d, *J*=8.9 Hz, 1H), 3.60-3.84 (m, 3H), 3.12 (m, 1H), 3.09 (s, 3H), 2.88-2.95 (m, 1H), 2.87 (s, 3H), 1.14 (d, *J*=6.2 Hz, 3H), 0.89 (d, *J*=6.2 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.28; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 167.7, 167.2, 154.0, 153.7 (d, J_{CF}=12.4 Hz), 152.2 (d, J_{CF}=2.9 Hz), 149.4, 134.3, 133.3 (d, J_{CF}=238.6 Hz), 122.5, 118.3, 106.1, 72.1, 71.7, 66.7, 64.4, 56.2 (d, *J*=8.8 Hz), 55.1, 53.0, 38.6, 36.2, 35.5, 18.1, 18.1; UPLC RT = 0.85 min, MS (ES) MH^+: 545.1 for $C_{24}H_{26}FN_6O_8$; HRMS (ES) MH^+ calcd for $C_{24}H_{26}FN_6O_8$ 545.1791, found 545.1807. $[\alpha]_D^{20}$ = -171 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-8-((*S*)-4-Cyclopropyl-2-oxooxazolidin-3-yl)-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1*H*,1'*H*-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(3'H)-trione (1s) A mixture of **13s** (1.0 g, 2.48 mmol) and barbituric acid (317 mg, 2.48 mmol) in 2 mL *i*-PrOH was heated at 130 ˚C for 2 h. After removal of solvent, the residue was stirred in a mixture of 2 mL MeOH and 5 mL water. Solid material was filtered and dried in vacuo. Yield 1.0 g (79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 11.46 (s, 1H), 7.53 (s, 1H), 4.63-4.69 (m, 1H), 4.17-4.29 (m, 3H), 4.11 (d, *J*=12.8 Hz, 1H), 3.95 (d, *J*=8.8 Hz, 1H), 3.72-3.86 (m, 1H), 3.57-3.72 (m, 2H), 3.05-3.21 (m, 1H), 2.95 (d, *J*=14.0 Hz, 1H), 1.15 (d, *J*=6.3 Hz, 3H), 0.90 (d, *J*=6.3 Hz, 3H), 0.51-0.59 (m, 2H), 0.39-0.48 (m, 1H), 0.24-0.32 (m, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.01; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 167.7, 154.3, 153.4 (d, *J*=12.4 Hz), 152.5 (d, *J*=2.2 Hz), 149.4, 134.8, 133.4 (d, *J*=239.3 Hz), 122.5, 117.7, 107.4, 72.1, 71.7, 68.1, 64.4, 59.9, 56.3 (d, *J*=8.8 Hz), 52.9, 38.5, 18.1, 18.1, 12.8, 4.5, 0.1; UPLC RT = 1.02 min, (ES) MH^+: 514.1 for $C_{24}H_{25}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{24}H_{25}FN_5O_7$ 514.1733, found 514.1722; $[\alpha]_D^{20}$ = -84.4 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-[(5*R*)-5-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1t) Prepared following the procedure described for the preparation of **7a** using **13t** (1.67 g, 3.96 mmol) and barbituric acid (508 mg, 3.96 mmol) to afford 1.56 g (81%) of the title compound as the major eluting component from SFC (Chiralpak IC column with 30% methanol and 70% CO_2 mobile phase) ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (br. s, 1 H), 11.35 (br. s, 1H), 7.8 (s, 1H), 4.9-5.1 (m, 1H), 4.3 (s, 1H), 4.1-4.2 (m, 1H), 4.0 (d, *J*=8.9 Hz, 1H), 3.7 (d, *J*=6.4 Hz, 4H), 3.1-3.3 (m, 1H), 2.9-3.1 (m, 1H), 1.5 (d, *J*=6.2 Hz, 3H), 1.2 (d, J=6.2 Hz, 3H), 0.9 (d, *J*=6.4 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.16; ^{13}C NMR (75 MHz,

DMSO-d_6) δ 170.9, 167.6, 153.6 (d, J_{CF}=12.7 Hz), 153.6, 152.8 (d, J_{CF}=2.2 Hz), 149.4, 134.7 (d, J_{CF}=1.7 Hz), 133.2 (d, J_{CF}=238.8 Hz), 122.2 (d, J_{CF}=2.2 Hz), 118.5, 106.2, 72.4, 72.1, 71.6, 64.4, 56.2 (d, J_{CF}=9.9 Hz), 52.9, 51.0, 38.6, 19.9, 18.1, 18.1; UPLC RT = 0.93 min, (ES) MH^+: 488.2 for $C_{22}H_{23}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{22}H_{23}FN_5O_7$ 488.1576 found 488.1580. $[\alpha]_D^{20}$ = -188 (c = 1; MeOH). Also isolated as the second component eluting from the SFC purification was **(2*R*,4*R*,4a*R*)-11-fluoro-2,4-dimethyl-8-[(5*R*)-4-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (7t)** Yield: 122 mg (6.3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 11.46 (s, 1H), 7.74 (s, 1H), 4.89-5.09 (m, 1H), 4.22 (dd, *J*=8.3, 9.5 Hz, 1H), 4.03-4.14 (m, 1H), 3.91-4.01 (m, 1H), 3.87 (dd, *J*=2.9, 13.2 Hz, 1H), 3.71-3.79 (m, 2H), 3.54-3.66 (m, 2H), 3.07 (d, *J*=14.8 Hz, 1H), 1.47 (d, *J*=6.3 Hz, 3H), 1.29-1.32 (d, *J*=6.3 Hz, 3H), 0.95 (d, *J*=6.3 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.16; ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.0, 168.5, 153.6, 153.2 (d, J_{CF}=13.2 Hz), 152.8, 149.6, 135.5, 133.6 (d, J_{CF}=240.0 Hz), 121.8, 118.6, 106.6, 72.5, 66.8, 65.0, 64.8, 53.2 (d, J_{CF}=9.5 Hz), 51.1, 51.0, 37.8, 19.8, 18.6, 16.5; UPLC RT = 0.91 min, (ES) MH^+: 488.2 for $C_{22}H_{23}FN_5O_7$; HRMS (ES) MH^+ calcd for $C_{22}H_{23}FN_5O_7$ 488.1576, found 488.1576; $[\alpha]_D^{20}$ = 93 (c = 1; MeOH).

(2*R*,4*S*,4a*S*)-11-Fluoro-2,4-dimethyl-8-[(4*S*)-4-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (1u) Prepared following the procedure described for the preparation of **1a** using **13u** (585 mg, 1.39 mmol) and barbituric acid (178 mg, 1.39 mmol) to afford 480 mg (71%) of the title compound as the major eluting component from SFC (Chiralpak IC column with 30% methanol and 70% CO_2 mobile phase). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 1H), 11.43 (s, 1H), 7.59 (s, 1H), 4.55-4.79 (m, 2H), 4.15-4.23 (m, 1H), 4.10 (d, *J* = 12.8 Hz, 1H),

3.94 (d, *J* = 8.8 Hz, 1H), 3.78 (br. s., 1H), 3.55-3.72 (m, 2H), 3.13 (d, *J* = 12.8 Hz, 1H), 2.94 (d, *J* = 13.9 Hz, 1H), 1.42 (d, *J* = 5.8 Hz, 3H), 1.15 (d, *J* = 6.2 Hz, 3H), 0.89 (d, *J* = 6.2 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -158.12; ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.7, 154.0, 153.4 (d, *J*=13.2 Hz), 152.1 (d, J_{CF}=2.8 Hz), 149.4, 134.8 (d, J_{CF}=1.7 Hz), 133.3 (d, J_{CF}=238.8 Hz), 122.4 (d, J_{CF}=2.2 Hz), 118.1 (d, J_{CF}=2.8 Hz), 106.9, 72.1, 71.7, 70.2, 64.4, 56.3 (d, J_{CF}=9.4 Hz), 52.9, 52.5, 38.6, 18.1, 18.1, 17.3; UPLC RT = 0.92 min, (ES) MH$^+$: 488.1 for $C_{22}H_{23}FN_5O_7$; HRMS (ES) MH$^+$ calcd for $C_{22}H_{23}FN_5O_7$ 488.1576 found 488.1597; $[\alpha]_D^{20}$ = -92 (c = 1; MeOH). Also isolated as the second component eluting from the SFC purification was **(2*R*,4*R*,4a*R*)-11-fluoro-2,4-dimethyl-8-[(4*S*)-4-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'*H*,6*H*-spiro[1,4-oxazino[4,3-*a*][1,2]oxazolo[4,5-*g*]quinoline-5,5'-pyrimidine]-2',4',6'(1'*H*,3'*H*)-trione (7u)** Yield: 35 mg (5.2%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.73 (br. s., 1 H), 11.47 (br. s., 1H), 7.58 (s, 1H), 4.55-4.74 (m, 2H), 3.71-4.22 (m, 5H), 3.51-3.65 (m, 2H), 3.06 (d, *J*=15.3 Hz, 1H), 1.40 (d, *J*=5.6 Hz, 3H), 1.29 (d, *J*=5.3 Hz, 3H), 0.95 (d, *J*=6.2 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -155.17; ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.2, 168.7, 154.1, 152.9 (d, J_{CF}=12.6 Hz), 152.1, 149.8, 135.5, 133.6 (d, J_{CF}=241.0 Hz), 122.2, 118.1, 107.4, 70.3, 66.7, 65.0, 64.8, 53.3, 52.8, 51.0, 37.8, 18.6, 17.4, 16.5; UPLC RT = 0.94 min, (ES) MH$^+$: 488.2 for $C_{22}H_{23}FN_5O_7$; HRMS (ES) MH$^+$ calcd for $C_{22}H_{23}FN_5O_7$ 488.1576 found 488.1596; $[\alpha]_D^{20}$ = 151 (c = 1; MeOH).

Animals. Compounds for PO, IV and IP dosing in mice, rats and dogs were formulated in 0.2M meglumine/20% hydroxypropyl-β-cyclodextrin in rats and dogs and DMA:TEG:saline 40/40/20% in mice. Wistar Han rats for pharmacokinetic and pharmacology studies were obtained from Charles River Laboratories (Raleigh, NC). CD-1 mice were obtained from Charles River Laboratories (Kingston, NY). All animals were housed and acclimated in the animal

facility on site before each study. All experimental procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee.

***S. aureus* neutropenic thigh infection model.** Compounds **1t** and **1u** were studied in a neutropenic mouse thigh infection model as described previously.[51] Briefly, mice were rendered neutropenic by injecting cyclophosphamide (Sigma-Aldrich, St. Louis MO) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before experimental infection. For **7u** two hours prior to infection, mice received an administration of 50 mg/kg ABT orally to inhibit cytochrome P450 activity, mice received a second 50 mg/kg administration 12h later.[52] No ABT was administered for similar experiments with **7t**. Mice were infected with *S. aureus* ARC516 to achieve a target inoculum of $5x10^5$ CFU. Groups of five animals each received intraperitoneal injections of either **1t** or **1u** on a bid, q12h regimen prepared in 2M meglumine/30% HPbCD starting 2 h after infection. An additional group of five mice received vehicle alone. Efficacy was determined 24 h after the start of treatment. Thighs were removed, weighed, homogenized and aliquots plated onto tryptic soy agar plates and incubated at 37 °C overnight for CFU determination.

Minimum Inhibitory Concentration (MIC) The bacterial strains included in these studies are maintained in the AstraZeneca Research Collection (ARC). The minimum inhibitory concentration (MIC) against each isolate was determined following the guidelines of the Clinical Laboratory Standards Institute (CLSI).[66, 67] Susceptibility testing against all species was performed using the broth microdilution method with the exception of *N. gonorrhoeae* isolates where the standard agar dilution method was used. The quality control isolates obtained from the American Type Culture Collection and used during testing were *N. gonorrhoeae* ATCC49226, *S. aureus* ATCC29213, and *S. pneumoniae* ATCC49619. Reference antimicrobials were obtained

from the US Pharmacopeial Convention (Rockville, MD; novobiocin) and MP Biomedicals (Santa Ana, CA; ciprofloxacin), and were tested in accordance with CLSI recommendations. For more potent compounds with MIC values < 6 μM, the reported data represents the averages of 3 or more replications.

Cytotoxicity assays. Experiments for assessing THLE cytotoxicity,[44] myeloid cytotoxicity[49] and erythroid cytotoxicity[50] were performed as described previously. A resazurin-based cyctotoxicity assay was used with THP1 human monocyte cells.[46] Modification of literature procedures[68] were used to assess A549 proliferation. Briefly, an A549 cell line was trypsinized, resuspended and counted. 100 μL of cells were deposited into 96-well flat bottomed plates at a cell density of 1000 cells/well and incubated for 24 h in a CO_2 atmosphere. Compounds were added to test plates in 50 μL of culture medium (RPMI w/o PR) with 10 doubling dilutions from 200 to 0.2 μM and incubated for 72 h in a CO_2 atmosphere. 20 μl of an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] solution (Promega, product #G3582) was added. The test plates which were incubated for 2 h and read in a plate reader at 650 nm and 490 nm (the reading at A650 nm is subtracted from the reading at A490 nM). The MIC is recorded as the highest compound concentration that does not give a positive value.

To assess sheep red blood cell lysis, compounds were added to test plates with 10 doubling dilutions in 2 μL DMSO. A sheep red blood cell suspension (10^7 cells/ml) diluted in 0.2% PBS buffer was added (final volume 100 μL). The plates were incubated for 24 h at rt and read by eye. MIC is the minimum concentration of compound at which the test well looks completely clear.

In vitro micronucleus assay, In vitro MLA and Ames mutagenicity Experiments for assessing micronuclei in mouse lymphoma cells, the mouse lymphoma assay and the Ames assay were performed as described previously.[69-71]

Pharmacokinetic studies. Pharmacokinetic properties of selected compounds were studied in male rats and dogs and mice of either gender. Plasma pharmacokinetics were determined from 0 to 24 hr following 15 min IV infusions at 3 mg/kg or PO administration at 10 mg/kg. Serial 200 µl samples of whole blood were taken from the jugular vein of each animal at time intervals. Concentration of compound in plasma was determined by LC-MS/MS and pharmacokinetic parameters were estimated using a non-compartmental model in WinNonLin (Pharsight). Mean results were determined for each experiment with 3 mice or 3 rats.

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

FP-based inhibition of DNA topoisomerase activity. IC_{50} values against *E. coli* DNA gyrase, human topoisomerase IIα and human topoisomerase IIβ were determined using literature procedures.[30, 42, 43]

Inhibition of DNA topoisomerase activity. Two-fold decreasing concentrations of **1u** or ciprofloxacin were prepared in DMSO and diluted 10-fold in water. A total of 3 µl of serial dilutions in 10% DMSO were transferred a 96 well polypropylene plate. DMSO served as a control for the uninhibited reaction. Next, 24 µl assay buffer containing DNA substrate and ATP were added to compound and control wells (no compound) and reactions were initiated by addition of enzyme prepared in enzyme dilution buffer. Supercoiling reactions were conducted

for 1 h at 20 ^{o}C and contained 1.8 mM spermidine, 8 mM $MgCl_2$, 24 mM KCl, 6.5% (w/v) glycerol, 0.005% Brij-35 and 2 mM dithiolthreitol. Relaxed DNA plasmid was present at 0.0013 µg/mL and DNA gyrase tetramer at 3 nM (*S. aureus*) or 2 nM (*E. coli*). Decatenation reactions for *E. coli* were 30 min at 20 ˚C and for *S. aureus* 60 min at 37 ˚C, and contained 20 mM Tris, pH 8.0, 50 mM ammonium acetate, 5 mM dithiothreitol, 8 mM $MgCl_2$, 0.5 mM EDTA, 5% w/v glycerol, 0.005% w/v Brij-35, 200 ng kinetoplast DNA, and 1 mM ATP and 2 nM *E.coli* or 20 nM *S. aureus* TopoIV tetramer. Controls were initiated with either 3 µl enzyme solution (100% reaction) or dilution buffer (no enzyme). Final DMSO concentration was 1%. Reactions were stopped by addition of 6µl 0.5M EDTA and subsequent addition of 4 µl STOP DYE. Gel electrophoresis was performed using 1% agarose buffered with 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA and run 18 to 20 h at 30V and stained using 1 µg/ml ethidium bromide solution. Gels images were captured using AlphaEase software. For supercoiling reactions, the amount of supercoiled products present in the control reactions (no compound, with and without gyrase) were quantified using AlphaEase software and were used to define conditions of no inhibition (no compound, with gyrase) and full inhibition (no compound, no gyrase). The supercoiled DNA products in reactions containing DNA gyrase and **7u** or ciprofloxacin were quantified using AlphaEase software, and % inhibition relative to the control reactions were calculated. This data was fit using the following equation (fit = (A+((B*x)/((C*(D+1))+x))) to calculate IC_{50} values. For decatenation reactions, the amount of decatenated products present in the control reactions (no compound, with and without TopoIV) were quantified using AlphaEase software and were used to define conditions of no inhibition (no compound, with TopoIV) and full inhibition (no compound, no TopoIV). The decatenated DNA products in reactions containing TopoIV and **7u** or ciprofloxacin were quantified using AlphaEase software, and %

inhibition relative to the control reactions were calculated. This data was fit using the following equation (fit = (A+((B*x)/((C*(D+1))+x))) to calculate IC_{50} values.

Plasma protein binding determination. Human, rat, and dog plasma protein binding was determined from a 10 μM compound solution in a Dianorm plasma well incubating at 37 ºC for 16 hours. Free fractions were calculated from ratios of drug concentration in buffer and plasma wells determined by LC-MS/MS.

X-ray crystallography. Colorless crystals of **1u** (MeOH solvate form) were obtained by slow evaporation from MeOH/*i*-PrOAc solution. The diffraction data were collected at 23 ºC on the Bruker Apex diffractometer (Mo source) at the University of Georgia. The crystal structure was solved and refined with the SHELXTL package. Hydrogen atoms attached on nitrogen and oxygen atoms were located in the electronic density map, and all the positions of other hydrogen atoms were calculated. Experimental details were included in the cif file (CCDC 1025296).

SUPPORTING INFORMATION

Included are the syntheses of intermediates **2b**, **2c** and **8** and of *ent*-**7t** and *ent*-**7u**. The SMILES formula, DNA gyrase inhibitory potency and select MIC values for compounds are provided in .csv format. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +27 (0)21 6505995; E-mail: greg.basarab@uct.ac.za; Present Address: Department of Chemistry, Drug Discovery and Development Centre, University of Cape Town, Rondebosch 7701, Cape Town, South Africa

Notes

The authors declare the following competing financial interest(s): We are or have been employed by AstraZeneca Pharmaceuticals.

ACKNOWLEDGMENT

We would like to thank the following individuals from AstraZeneca for their analytical services and helpful advice: Natascha Bezdenejnih, Nancy DeGrace, Dr. Camil Joubran and Sharon Tentarelli. Dr. Richard Alm, Michael Huband and Dr. Sushmita Lahiri contributed to in vitro microbiological characterizations and annotations of resistant mutants. Beth Andrews contributed to the DNA gyrase and Topo IV inhibition assays. Marie-Eve Beaudoin, Dr. Eric Gangl, Erica Manyak, Debora Roaquim, Crystal Brown and Eric Miele are acknowledged for DMPK work, and Lena Grosser and Georg Neckermann for contributing to the in vivo efficacy studies. Dr. Mick Fellows contributed to the genotoxicity studies. Dr. Dedong Wu coordinated the small molecule X-ray crystallography work. We also appreciate contributions for the synthesis of compounds and intermediates by scientists at Biocon, Inc.

ABBREVIATIONS

ABT: aminobenzotriazole; ARC: AstraZeneca Research Collection; CDI: carbonyl dimidazole; CFU: colony-forming unit; Cl: clearance; F: oral bioavailability; f_u: fraction unbound; Fsp^3: fraction sp^3; DIEA: diisopropylaminoethylamine; GyrA: the A-subunit of DNA gyrase; FP: fluorescence polarization; f_u: fraction unbound; GyrB: the B-subunit of DNA gyrase; LOEL: lowest observable effect level; MLA: mouse lymphoma assay; MMA: mouse micronucleus chromosome aberration assay; MRQR MRQR: methicillin resistant, quinolone resistant *S. aureus*; MSSA: methicillin sensitive *S. aureus*; NBTI: novel bacterial topoisomerase inhibitor; NOEL: no observable effect level; ParC: the C-subunit of topoisomerase IV; ParE: the E-subunit

of topoisomerase IV; PPB: plasma protein binding; S_NAr: nucleophilic aromatic substitution; SFC: supercritical fluid chromatography; TBDPS: *tert*-butyldimethylphenylsilyl; T-reaction: Tertiary amino effect reaction; Topo IV: topoisomerase IV.

REFERENCES

1. Livermore, D. M. Discovery research: the scientific challenge of finding new antibiotics. *J. Antimicrob. Chemother.* **2011**, *66,* 1941-1944.

2. Wise, R. The urgent need for new antibacterial agents. *J. Antimicrob. Chemother.* **2011**, *66,* 1939-1940.

3. Walsh, C. T.; Wencewicz, T. A. Prospects for new antibiotics: a molecule-centered perspective. *J. Antibiot.* **2014**, *67,* 7-22.

4. Georgopapadakou, N. H. Prospects for new antibacterials: can we do better? *Expert Opin. Investig. Drugs* **2014**, *23,* 145-148.

5. Rice, L. B. Progress and challenges in implementing the research on ESKAPE pathogens. *Infect. Cont. Hosp. Ep.* **2010**, *31,* S7-S10.

6. Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2009**, *48,* 1-12.

7. Stryjewski, M. E.; Corey, G. R. Methicillin-resistant *Staphylococcus aureus*: an evolving pathogen. *Clin. Infect. Dis.* **2014**, *58,* S10-S19.

8. Rodvolk, K. A.; McConeghy, K., W. Methicillin-resistant *Staphylococcus aureus* therapy: past, present and future. *Clin. Infect. Dis.* **2014**, *58(S1),* S20-S27.

9. Moellering, R. C. MRSA: the first half century. *J. Antimicrob. Chemother.* **2012**, *67,* 4-11.

10. Tapsall, J.; Read, P.; Carmody, C.; Bourne, C.; Ray, S.; Limnios, A.; Sloots, T.; Whiley, D. Two cases of failed ceftriaxone treatment in pharyngeal gonorrhoea verified by molecular microbiological methods. *J. Med. Microbiol.* **2009**, *58,* 683-687.

11. Lewis, D. A. The gonococcus fights back: is this time a knock out? *Sex. Transm. Infect.* **2010**, *86,* 415-421.

12. World Health Organization Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*, 2012. http://www.who.int/reproductivehealth/publications/rtis/9789241503501/en/ (accessed 07/08, 2015).

13. Centers for Disease Control and Prevention Gonorrhea Treatment Guidelines Revised Guidelines to Preserve Last Effective Treatment Option. http://www.cdc.gov/nchhstp/newsroom/docs/Gonorrhea-Treatment-Guidelines-FactSheet.pdf (accessed 07/08, 2015).

14. European Centre for Disease Prevention and Control Response plan to control and manage the threat of multidrug-resistant gonorrhoea in Europe. http://www.ecdc.europa.eu/en/publications/publications/1206-ecdc-mdr-gonorrhoea-response-plan.pdf (accessed 07/08, 2015).

15. Basarab, G. S.; Kern, G. H.; McNulty, J.; Mueller, J. P.; Lawrence, K.; Vishwanathan, K.; Alm, R. A.; Barvian, K.; Doig, P.; Galullo, V.; Gardner, H.; Gowravaram, M.; Huband, M.; Kimzey, A.; Morningstar, M.; Kutschke, A.; Lahiri, S. D.; Perros, M.; Singh, R.; Schuck, V. J. A.; Tommasi, R.; Walkup, G.; Newman, J. V. Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial Type II topoisomerases. Accepted for publication in *Scientific Reports.*

16. Basarab, G. S.; Brassil, P.; Doig, P.; Galullo, V.; Haimes, H. B.; Kern, G.; Kutschke, A.; McNulty, J.; Schuck, V. A. J.; Stone, G.; Gowravaram, M. Novel DNA gyrase inhibiting spiropyrimidinetriones with a benzisoxazole scaffold – SAR and in vivo characterization. *J. Med. Chem.* **2014**, *57*, 9087-9095.

17. Bellon, S.; Parsons, J. D.; Wei, Y.; Hayakawa, K.; Swenson, L. L.; Charifson, P. S.; Lippke, J. A.; Aldape, R.; Gross, C. H. Crystal structures of *Escherichia coli* topoisomerase IV ParE subunit (24 and 43 kilodaltons): a single residue dictates differences in novobiocin potency against topoisomerase IV and DNA gyrase. *Antimicrob. Agents Chemother.* **2004**, *48,* 1856-1864.

18. Gross, C. H.; Parsons, J. D.; Grossman, T. H.; Charifson, P. S.; Bellon, S.; Jernee, J.; Dwyer, M.; Chambers, S. P.; Markland, W.; Botfield, M.; Raybuck, S. A. Active-site residues of *Escherichia coli* DNA gyrase required in coupling ATP hydrolysis to DNA supercoiling and amino acid substitutions leading to novobiocin resistance. *Antimicrob. Agents Chemother.* **2003**, *47,* 1037-1046.

19. Barvian, K.; Basarab, G. S.; Gowravaram, M. R.; Hauck, S. I.; Zhou, F. Fused, spirocyclic heteroaromatic compounds for the treatment of bacterial infections. USP 8658641, 2011.

20. Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: Increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52,* 6752-6756.

21. D'yachenko, E. V.; Glukhareva, T. V.; Dyudya, L. V.; Eltsov, O. V.; Morzherin, Y. Y. The *tert*-amino effect in heterocyclic chemistry. Synthesis of spiro heterocycles. *Molecules* **2005**, *10,* 1101-1108.

22. Meth-Cohn, O.; Suschitzky, H. Heterocycles by ring closure of ortho-substituted t-anilines (the t-amino effect). In *Advances in Heterocyclic Chemistry;* Academic Press: Vol. Volume 14, 1972, pp 211-278.

23. Matyus, P.; Elias, O.; Tapolcsanyi, P.; Polonka-Balint, A.; Halasz-Dajka, B. Ring-closure reactions of ortho-vinyl-*tert*-anilines and (Di)aza-heterocyclic analogues via the *tert*-amino effect: recent developments. *Synthesis* **2006**, 2625.

24. Visser, G. W.; Verboom, W.; Benders, P. H.; Reinhoudt, D. N. 1-Pyrrolidin-1-ylbuta-1,3-dienes as potential 1,5-dipoles; synthesis of pyrrolizines. *J. Chem. Soc., Chem. Commun.* **1982**, 669-671.

25. Verboom, W.; Reinhoudt, D. N.; Visser, R.; Harkema, S. "Tert-amino effect" in heterocyclic synthesis. Formation of N heterocycles by ring-closure reactions of substituted 2-vinyl-N,N-dialkylanilines. *J. Org. Chem.* **1984**, *49,* 269-276.

26. Basarab, G. S.; Galullo, V.; DeGrace, N.; Hauck, S.; Joubran, C.; Wesolowski, S. S. Synthesis of a tetrahydronaphthyridine spiropyrimidinetrione DNA gyrase inhibiting antibacterial agent - differential substitution at all five carbon atoms of pyridine *Org. Lett.* **2014**, *16*, 6456-6459.

NBT

27. Ruble, J. C.; Hurd, A. R.; Johnson, T. A.; Sherry, D. A.; Barbachyn, M. R.; Toogood, P. L.; Bundy, G. L.; Graber, D. R.; Kamilar, G. M. Synthesis of (-)-PNU-286607 by asymmetric cyclization of alkylidene barbiturates. *J. Am. Chem. Soc.* **2009**, *131,* 3991-3997.

28. Bridges, A.,J.; Lee, A.; Maduakor, E. C.; Schwartz, C. E. Fluorine as an ortho-directing group in aromatic metalation: generality of the reaction and the high position of fluorine in the Dir-Met potency scale. *Tetrahedron Lett.* **1992**, *33,* 7495-7498.

29. Moyroud, J.; Guesnet, J.; Bennetau, B.; Mortier, J. Lithiations ortho-directed by carboxylic acid, fluorine, and chlorine functions. II. Regioselective synthesis of polysubstituted benzoic acids and acetophenones. *B. Soc. Chim. Fr.* **1996**, *133,* 133-141.

30. Shapiro, A.; Jahic, H.; Prasad, S.; Ehmann, D.; Thresher, J.; Gao, N.; Hajec, L. A homogeneous, high-throughput fluorescence anisotropy-based DNA supercoiling assay. *J. Biomol. Screen.* **2010**, *15,* 1088-1098.

31. Perola, E.; Stamos, D.; Grillot, A.; Ronkin, S.; Wang, T.; LeTiran, A.; Tang, Q.; Deininger, D. D.; Liao, Y.; Tian, S.; Drumm, J. E.; Nicolau, D. P.; Tessier, P. R.; Mani, N.; Grossman, T. H.; Charifson, P. S. Successful application of serum shift prediction models to the design of dual targeting inhibitors of bacterial gyrase B and topoisomerase IV with improved in vivo efficacy. *Bioorg. Med. Chem. Lett.* **2014**, *24,* 2177-2181.

32. Liu, X.; Wright, M.; Hop, C. E. C. A. Rational use of plasma protein and tissue binding data in drug design. *J. Med. Chem.* **2014**, *57*, 8238-8248.

33. Heuberger, J.; Schmidt, S.; Derendorf, H. When is protein binding important? *J. Pharm. Sci.* **2013**, *102,* 3458-3467.

34. Berezhkovskiy, L. M. On the influence of protein binding on pharmacological activity of drugs. *J. Pharm. Sci.* **2010**, *99,* 2153-2165.

35. Bax, B. D.; Chan, P. F.; Eggleston, D. S.; Fosberry, A.; Gentry, D. R.; Gorrec, F.; Giordano, I.; Hann, M. M.; Hennessy, A.; Hibbs, M.; Huang, J.; Jones, E.; Jones, J.; Brown, K. K.; Lewis, C. J.; May, E. W.; Saunders, M. R.; Singh, O.; Spitzfaden, C. E.; Shen, C.; Shillings, A.; Theobald, A. J.; Wohlkonig, A.; Pearson, N. D.; Gwynn, M. N. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* **2010**, *466,* 935-942.

36. Laponogov, I.; Sohi, M. K.; Veselkov, D. A.; Pan, X.; Sawhney, R.; Thompson, A. W.; McAuley, K. E.; Fisher, L. M.; Sanderson, M. R. Structural insight into the quinolone–DNA cleavage complex of type IIA topoisomerases. *Nature Struct. Biol.* **2009**, *16,* 667-669.

37. Laponogov, I.; Veselkov, D. A.; Crevel, I. M.-T.; Pan, X.; Fisher, L. M.; Sanderson, M. R. Structure of an 'open' clamp type II topoisomerase-DNA complex provides a mechanism for DNA capture and transport. *Nucleic Acids Res.* **2013**, *41,* 9911-9923.

38. Tsai, F. T. F.; Singh, O. M. P.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A. L.; Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; Wigley, D. B. The high-resolution crystal structure of a 24-kDa gyrase B fragment from *E. coli* complexed with one of the most potent coumarin inhibitors, clorobiocin. *Proteins* **1997**, *28,* 41-52.

39. Mayer, C.; Janin, Y. L. Non-quinolone inhibitors of bacterial type IIA topoisomerases: a feat of bioisosterism. *Chem. Rev.* **2014**, *114,* 2313-2342.

40. Boos, G.; Stopper, H. Genotoxicity of several clinically used topoisomerase II inhibitors. *Toxicol. Lett.* **2000**, *116,* 7-16.

41. EMA withdrawal assessment report for factive International http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500060988.pdf (accessed 07/08, 2015).

42. Shapiro, A. B. A high-throughput-compatible, fluorescence anisotropy-based assay for ATP-dependent supercoiled DNA relaxation by human topoisomerase IIα. *Biochem. Pharmacol.* **2013**, *85,* 1269-1277.

43. Shapiro, A. B.; Austin, C. A. A high-throughput fluorescence anisotropy-based assay for human topoisomerase II β-catalyzed ATP-dependent supercoiled DNA relaxation. *Anal. Biochem.* **2014**, *448,* 23-29.

44. Thompson, R. A.; Isin, E. M.; Li, Y.; Weidolf, L.; Page, K.; Wilson, I.; Swallow, S.; Middleton, B.; Stahl, S.; Foster, A. J.; Dolgos, H.; Weaver, R.; Kenna, J. G. In vitro approach to assess the potential for risk of idiosyncratic adverse reactions caused by candidate drugs. *Chem. Res. Toxicol.* **2012**, *25,* 1616-1632.

45. Antoine, D. J.; Williams, D. P.; Park, B. K. Understanding the role of reactive metabolites in drug-induced hepatotoxicity: state of the science. *Expert Opin. Drug Metab. Toxicol.* **200**8, *4,* 1415-1427.

46. McMillian, M. K.; Li, L.; Parker, J. B.; Patel, L.; Zhong, Z.; Gunnett, J. W.; Powers, W. J.; Johnson, M. D. An improved resazurin-based cytotoxicity assay for hepatic cells. *Cell Biol. Toxicol.* **2002**, *18,* 157-173.

47. Falagas, M. E.; Manta, K. G.; Ntziora, F.; Vardakas, K. Z. Linezolid for the treatment of patients with endocarditis: a systematic review of the published evidence. *J. Antimicrob. Chemother.* **2006**, *58,* 273-280.

48. Attassi, K.; Hershberger, E.; Alam, R.; Zervos, M. J. Thrombocytopenia associated with linezolid therapy. *Clin. Infect. Dis.* **2002**, *34,* 695-698.

49. Volpe, D. A.; Warren, M. K. Myeloid clonogenic assays for comparison of the in vitro toxicity of alkylating agents. *Toxicology in Vitro* **2003**, *17,* 271-277.

50. Boehm, D.; Bell, A. Simply red: A novel spectrophotometric erythroid proliferation assay as a tool for erythropoiesis and erythrotoxicity studies. *Biotechnol. Rep.* **2014**, *4,* 34-41.

51. Andes, D.; Craig, W. A. In vivo activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. *Antimicrob. Agents Chemother.* **1998**, *42,* 2375-2379.

52. Balani, S. K.; Li, P.; Nguyen, J.; Cardoza, K.; Zeng, H.; Mu, D.; Wu, J.; Gan, L.; Lee, F. W. Effective dosing regimen of 1-aminobenzotriazole for inhibition of antipyrine clearance in Guinea pigs and mice using serial sampling. *Drug Metab. Dispos.* **2004**, *32,* 1092-1095.

53. Miller, A. A.; Bundy, G. L.; Mott, J. E.; Skepner, J. E.; Boyle, T. P.; Harris, D. W.; Hromockyj, A. E.; Marotti, K. R.; Zurenko, G. E.; Munzner, J. B.; Sweeney, M. T.; Bammert, G. F.; Hamel, J. C.; Ford, C. W.; Zhong, W.; Graber, D. R.; Martin, G. E.; Han, F.; Dolak, L. A.; Seest, E. P.; Ruble, J. C.; Kamilar, G. M.; Palmer, J. R.; Banitt, L. S.; Hurd, A. R.; Barbachyn, M. R. Discovery and characterization of QPT-1, the progenitor of a new class of bacterial topoisomerase inhibitors. *Antimicrob. Agents Chemother.* **2008**, *52,* 2806-2812.

54. Black, M. T.; Stachyra, T.; Platel, D.; Girard, A.; Claudon, M.; Bruneau, J.; Miossec, C. Mechanism of action of the antibiotic NXL101, a novel nonfluoroquinolone inhibitor of bacterial type II topoisomerases. *Antimicrob. Agents Chemother.* **2008**, *52,* 3339-3349.

55. Robinson, M. J.; Corbett, A. H.; Osheroff, N. Effects of topoisomerase II-targeted drugs on enzyme-mediated DNA cleavage and ATP hydrolysis: evidence for distinct drug interaction domains on topoisomerase II. *Biochemistry* **1993**, *32,* 3638-3643.

56. Huband, M. D.; Bradford, P. A.; Otterson, L. A.; Basarab, G. S.; Kutschke, A.; Giacobbe, R. A.; Patey, S. A.; Johnstone, M. R.; Potter, M. E.; Miller, P. F.; Mueller, J. P. In vitro antibacterial activity of AZD0914: a new spiropyrimidinetrione DNA gyrase/topoisomerase inhibitor with potent activity against Gram-positive, fasitidious Gram-negative, and atypcial bacteria. *Antimicrob. Agents Chemother.* **2015**, *59*, 467-474.

57. Alm, R. A.; Lahiri, S. D.; Kutschke, A.; Otterson, L. G.; McLaughlin, R. E.; Whiteaker, J. D.; Lewis, L. A.; Su, X.; Huband, M. D.; Gardner, H.; Mueller, J. P. Characterization of the novel DNA gyrase inhibitor AZD0914: low resistance potential and lack of cross-resistance in *Neisseria gonorrhoeae*. *Antimicrob. Agents Chemother.* **2015**, *59*, 1478-1486.

58. Basarab, G. S.; Gowravaram, M. R.; Hauck, S. I.; Zhou, F. Preparation of spiro[oxazino[4,3-a]isoxazolo[4,5-g]quinoline-pyrimidine]trione compounds and methods for treating bacterial infections. US 20140206677, 2014.

59. Unemo, M.; Golparian, D.; Shafer, W. M. Challenges with gonorrhea in the era of multi-drug and extensively drug resistance – are we on the right track? *Expert Review of Anti-infective Therapy* **2014**, *12,* 653-656.

60. Rein, K.; Goicoechea-Pappas, M.; Anklekar, T. V.; Hart, G. C.; Smith, G. A.; Gawley, R. E. Chiral dipole-stabilized anions: experiment and theory in benzylic and allylic systems. Stereoselective deprotonations, pyramidal inversions, and stereoselective alkylations of lithiated (tetrahydroisoquinolyl)oxazolines. *J. Am. Chem. Soc.* **1989**, *111,* 2211-2217.

61. Chouhan, G.; Alper, H. Synthesis of ring-fused oxazolo- and pyrazoloisoquinolinones by a one-pot Pd-catalyzed carboxamidation and aldol-aype condensation cascade process. *J. Org. Chem.* **2009**, *74,* 6181-6189.

62. Nishiyama, T.; Matsui, S.; Yamada, F. Synthesis and spectral behavior of 5-methyl-3-phenyl-1,3-oxazolidin-2-ones using NMR. *J. Heterocycl. Chem.* **1986**, *23,* 1427-1429.

63. Berkowitz, D. B.; Sloss, D. G. Diallyl (lithiodifluoromethyl)phosphonate: a new reagent for the introduction of the (difluoromethylene)phosphonate functionality. *J. Org. Chem.* **1995**, *60,* 7047-7050.

64. Knapp, S.; Patel, D. V. Bromocyclization of unsaturated thiocarbamidates. Synthesis of (+-)-sporamine. *J. Am. Chem. Soc.* **1983**, *105,* 6985-6986.

65. Enders, D.; Kallfass, U. A short diastereo- and enantioselective synthesis of cis-4,5-disubstituted oxazolidin-2-ones. *Synlett* **2002**, 33-36.

66. Clinical and Laboratory Standards Institute *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard.* Clinical and Laboratory Standards Institute: Wayne, PA, 2012; Vol. 42, pp 88.

67. Clinical and Laboratory Standards Institute *M100-S24. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement.* Clinical and Laboratory Standards Institute: Wayne, PA, 2014; Vol. 34, pp 10.

68. Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Cancer Commun.* **1991**, *3,* 207-212.

69. Doherty, A. T.; Hayes, J.; Fellows, M.; Kirk, S.; O'Donovan, M. A rapid, semi-automated method for scoring micronuclei in mononucleated mouse lymphoma cells. *Mutat. Res.-Gen. Tox. En.* **2011**, *726,* 36-41.

70. Lloyd, M.; Kidd, D. The mouse lymphoma assay. In Parry, J. M., Parry, E. M., Eds.; Springer New York: 2012; Vol. 817, pp 35-54.

71. Mortelmans, K.; Zeiger, E. The Ames Salmonella/microsome mutagenicity assay. *Mutat. Res.-Fund. Mol. M.* **2000**, *455,* 29-60.

Figure 1. Antibacterial agents **1**-**4**.

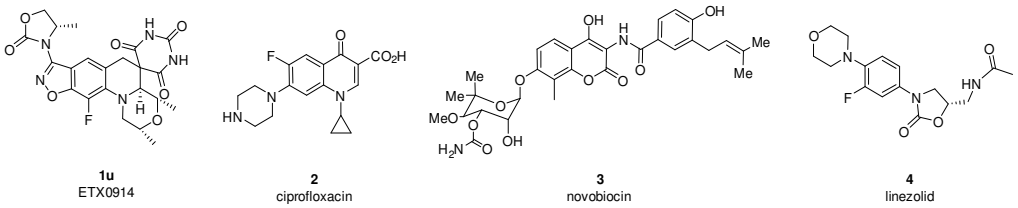


Figure 2. Crystal structure of **1u** (data in the Cambridge Crystallographic Data Centre: CCDC 1025296). Left: Ortep representation with MeOH solvate removed. Right: Side view (with hydrogen atoms also removed) showing morpholine chair conformation and perpendicular orientation of oxazolidinone methyl group.

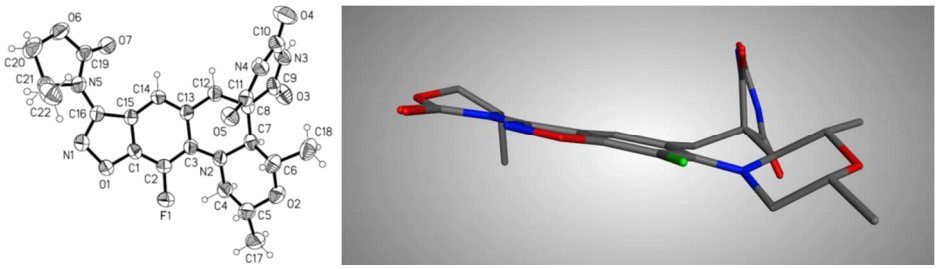


Figure 3. Correlation between *E. coli* DNA gyrase inhibitory potency and antibacterial activity: (A) Compounds within Table 1 (B) Select compounds with a narrow logD range (1.5-1.9)

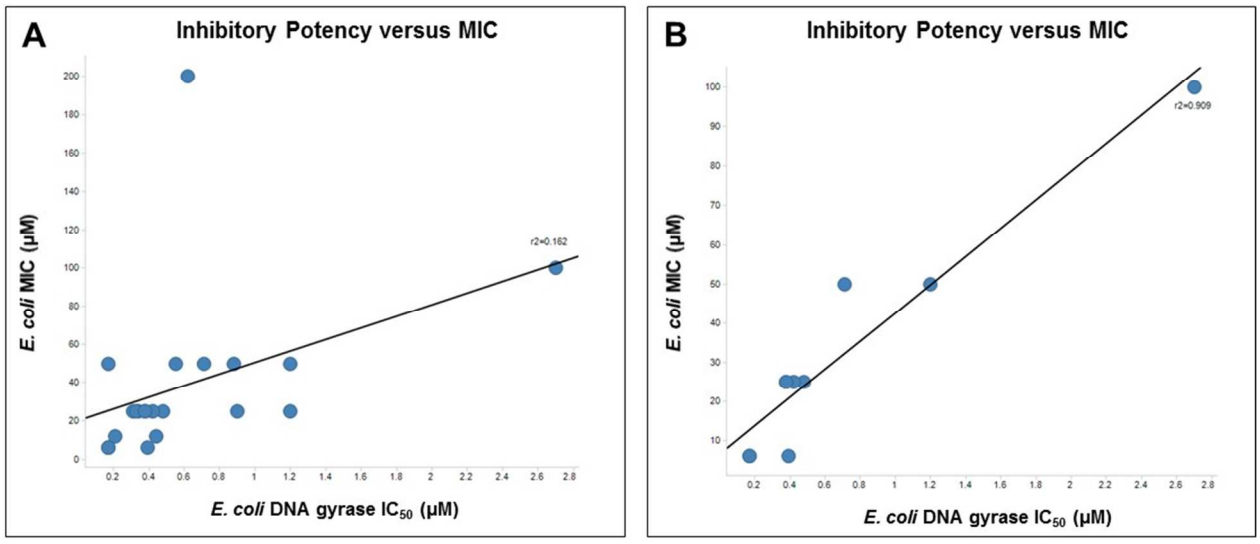
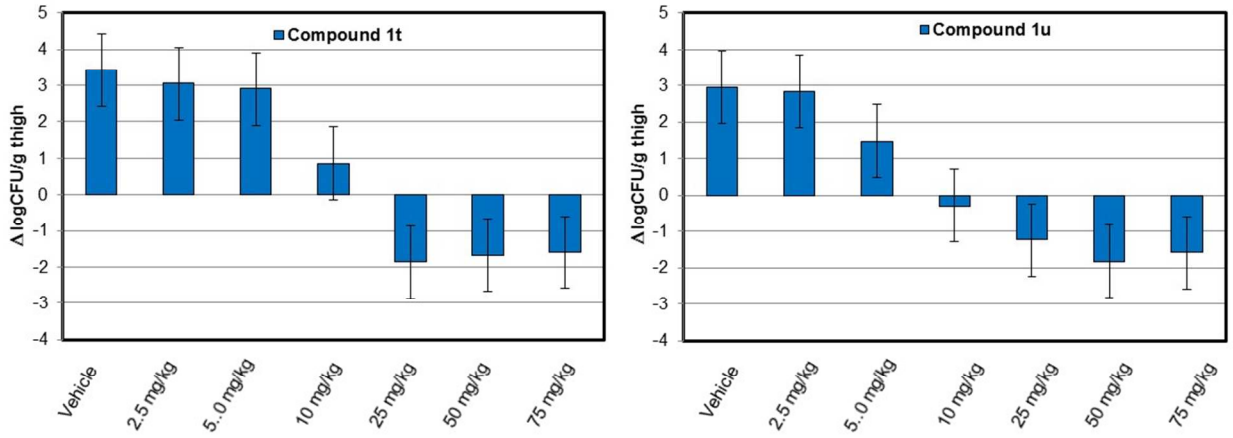
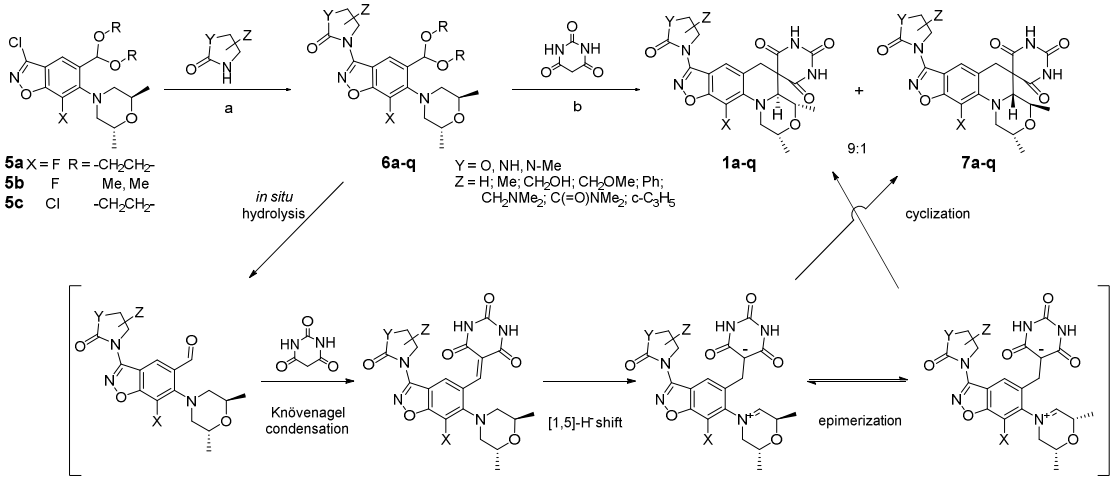


Figure 4. Dose-response of **1t** (left) and **1u** (right) in a mouse neutropenic *S. aureus* thigh infection model.

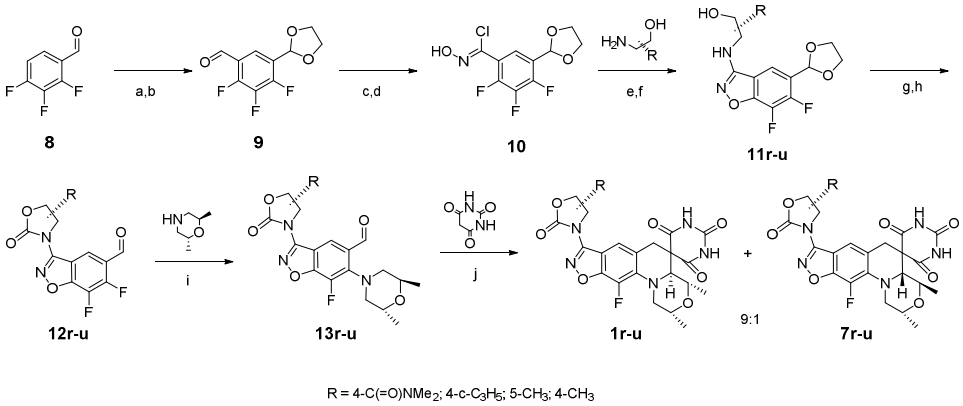


Scheme 1. General synthesis of N-linked Oxazolidinones and Imidazolidinones



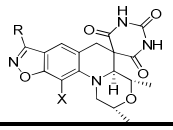
Reagents and conditions: (a) NaH, DMF, 80 ˚C, 2-16 h, 12-47%; (b) AcOH, H_2O or EtOH, 6N HCl, 120 ˚C, 1h, 15-72%.

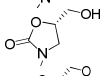
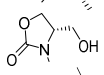
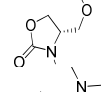
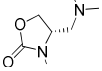
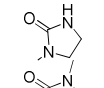
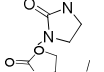
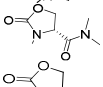
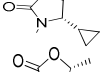
Scheme 2. Alternative synthetic scheme to N-linked Oxazolidinones



Reagents and conditions: (a) ethylene glycol, p-TsOH, refluxing toluene, 78% yield (b) *n*-BuLi, -70 ˚C, THF, DMF quench, 93% yield; (c) NH_2OH, EtOH, rt, 24 h, 80% yield; (d) NCS, DMF, rt, 4 h, 76% yield; (e) excess amine, DMF, rt, 1-3 h; (f) NaO-*t*-Bu or Cs_2CO_3, rt, 2 steps: 59-93% ; (g) CDI, DIEA, DMF, 70 ˚C, 2-3 h; (h) HCl, dioxane or THF, water, 70 ˚C, 2 steps: 75-99%; (i) K_2CO_3 or DIEA, CH_3CN, water, 80 ˚C, 4-5 h, 73-98%; (j) AcOH, water 120 ˚C, 1h, 26-81%.

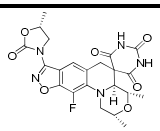
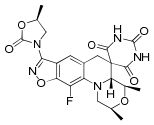
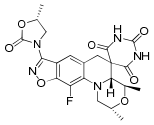
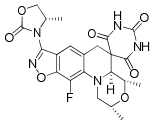
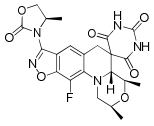
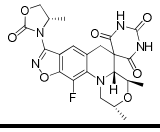
Table 1. Variation of Benzisoxazole Substituents



Cmpd	X	R	LogD	Human PPB (% f_u)[a]	Solubility (μM)	Eco IC_{50} (μM)[b]	MICs (μM)								Rat Cl (mL/min/kg)[k]
							Spn[c]	Spy[d]	MSSA[e]	MSSA +ser[f]	MRQR[g]	Hin[h]	Ngo[i]	Eco[j]	
1a	F		1.4	10	17	0.44	0.20	0.10	<0.05	0.20	0.78	0.20	0.39	12	16
1b	F		1.7	11	140	0.48	0.39	0.39	0.098	0.78	0.78	0.39	0.78	25	ND
1c	Cl		1.9	8.4	320	0.21	0.39	0.20	0.098	0.39	0.78	0.39	0.39	12	9.5
1d	F		0.46	29	32	0.17	0.39	0.20	1.6	3.1	25	0.39	0.78	50	ND
1e	F		1.5	18	580	0.31	0.39	0.20	0.20	0.39	1.6	0.39	0.78	25	53
1f	Cl		1.6	8.4	300	0.42	0.20	0.20	0.20	0.39	1.6	0.39	0.39	25	7.2
1g	F		1.7	ND[l]	>1000	2.7	6.2	1.6	1.6	6.2	6.2	1.6	3.1	100	ND
1h	Cl		1.8	5.0	510	0.17	0.39	0.20	0.20	0.78	0.78	0.39	0.39	6.2	20
1i	F		1.0	28	420	0.34	0.39	0.20	0.39	1.6	1.6	0.78	1.6	25	ND
1j	F		1.4	22	910	0.33	0.39	0.39	0.20	0.39	0.78	0.39	0.39	25	87
1k	Cl		1.6	13	370	0.38	0.39	0.78	0.20	0.39	0.78	0.39	0.39	25	63
1l	F		1.5	24	>1000	0.88	1.6	1.6	0.78	1.6	6.2	1.6	3.1	50	ND
1m	F		1.8	1	94	0.71	3.1	1.6	0.78	25	1.6	0.78	0.78	50	ND
1n	F		2.0	9.2	320	1.2	1.6	0.78	0.39	1.5	1.6	1.6	1.6	25	ND
1o	F		1.8	14	360	0.38	0.39	0.39	0.20	0.78	0.78	0.39	0.39	6.2	14
1p	F		1.4	19	100	0.55	0.78	0.39	1.56	6.2	25	0.78	3.1	50	ND
1q	F		1.7	14	370	1.2	0.78	0.39	1.56	6.2	12.5	1.6	0.78	50	ND
1r	F		0.53	24	790	0.62	0.78	0.78	3.1	3.1	12.5	0.78	1.6	200	ND
1s	F		2.4	6.4	550	0.90	0.78	0.78	0.39	3.1	0.78	0.39	0.39	25	ND
1t	F		1.7	18	590	0.39	0.39	0.39	0.20	0.39	0.78	0.39	0.39	6.2	17
1u	F		1.6	18	820	0.17	0.39	0.39	0.20	0.78	0.78	0.20	0.39	6.2	22
ciprofloxacin			-0.94	74	240	0.19	3.1	1.6	0.78	0.78	>50	0.002	0.012	0.05	ND
linezolid			0.47	72	>1000	>32	3.1	3.1	6.25	25	6.25	50	25	>50	30

[a] f_u: fraction unbound; [b] *E. coli* FP-based DNA gyrase inhibition; [c] Spn: *S. pneumoniae*; [d] Spy: *S. pyogenes*; [e] Methicillin sensitive *S. aureus*; [f] Methicillin sensitive *S. aureus* + 50% human serum; [g] Methicillin resistant, quinolone resistant *S. aureus*; [h] *H. influenzae*; [i] *N. gonorrheoea*; [j] *E. coli*; [k] in vivo clearance; [l] ND = not determined

Table 2. Influence of Stereochemistry on DNA Gyrase Inhibitory Potency, MIC Values and PPB

Cmpd	Structure	Eco IC_{50} (μM)[a]	Human PPB (% f_u)[b]	MICs (μM)					
				Spn[c]	Spy[d]	MSSA[e]	MRQR Sau[f]	Hin[g]	Eco[h]
1t		0.39	18	0.39	0.39	0.20	0.78	0.39	6.2
ent-**1t**		>17	2.0	100	50	25	100	100	>200
7t		>17	2.4	200	50	50	100	50	>200
1u		0.17	18	0.39	0.39	0.20	0.78	0.20	6.2
ent-**1u**		>17	2.8	200	100	100	200	100	>200
7u		>17	3	50	35	35	100	50	>200

[a]*E. coli* FP-based DNA gyrase inhibition; [b]f_u: fraction unbound; [c]Spn: *S. pneumoniae*; [d]Spy: *S. pyogenes*; [e]Methicillin sensitive *S. aureus*; [f]Methicillin resistant, quinolone resistant *S. aureus*; [g]*H. influenzae*; [h]*E. coli*

Table 3. In vitro and in vivo PK properties of select compound in rat, dog and human

	PPB (%f_u)[a]		Hepatocyte Cl_{int}[b] (μL/min/mg)			in vivo Cl[c] (mL/min/kg)		Bioavailability F (%)		fAUC/MIC[d]	
Cmpd	**Rat**	**Dog**	**Rat**	**Dog**	**Hu-man**	**Rat**	**Dog**	**Rat**	**Dog**	**Rat**	**Dog**
1o	ND	14	8.3	6.3	7.3	14	7.7	62	40	14	47
1t	15	22	6.0	3.0	<1	17	8.6	48	74	20	62
1u	13	14	11	2.8	6	22	3.7	31	71	12	83

[a]f_u: fraction unbound; [b]intrinsic clearance; [c]clearance; [d]free area under the curve/MIC

Table 4. Human topoisomerase inhibition and genotoxicity

Cmpd	IC_{50} (μM) topoisomerase		Mouse Micronucleus		MLA[a] (3h incubation)		MLA (24h incubation)		Ames (+)
	Human TopoIIα	Human TopoIIβ	NOEL[b] (μM)	LOEL[c] (μM)	NOEL (μM)	LOEL (μM)	NOEL (μM)	LOEL (μM)	
1t	200	84	>400	ND[d]	>510	ND	>200	ND	TA102 (+/- S9)
1u	>400	79	>400	ND	>510	ND	>200	ND	TA102 (+/- S9)
2 (Cip)[e]	110	110	200	ND	100	300	20	100	TA102 (+/- S9)
Gemi[f]	20	13	3	10	10	30	ND	ND	TA102 (+/- S9)

[a]Mouse lymphoma assay; [b]No observable effect level; [c]Lowest observable effect level; [d]not determined; [e]ciprofloxacin; [f]gemifloxacin

Table 5. Cytoxicity

Cmpd	Blood cells		Somatic cells		Bone marrow cells	
	RBC lysis IC_{50} (μM)	THP1 IC_{50} (μM)	A549 MIC (μM)	THLE MIC (μM)	Myeloid IC_{50} (μM)	Erythroid IC_{50} (μM)
1t	>200	>120	>200	185	52	>100
1u	>200	>120	>200	>300	>100	>100
Lin[a]	>200	>120	>130	>300	69	18
Levo[b]	>200	>120	>180	>300	88	79
Gemi[c]	ND[d]	72	ND	200	7	10

[a]linezolid; [b]levofloxacin; [c]gemifloxacin; [d]not determined

Table 6. Gel-based inhibition of *S. aureus* and *E. coli* topoisomerases

Compound	*S. aureus* IC_{50} (μM)		*E. coli* IC_{50} (μM)	
	DNA gyrase	Topo IV	DNA gyrase	Topo IV
1u	5.8 ± 2.5	22 ± 6	2.1 ± 0.5	16 ± 5
2 (ciprofloxacin)	39 ± 5.4	14 ± 5	1.0 ± 0.3	4.8 ± 1

Table of Contents Graphic