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Exploring Left-Hand-Side substitutions in the benzoxazinone series of 4-amino-piperidine bacterial type IIa topoisomerase inhibitors

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ABSTRACT

An SAR survey at the C-6 benzoxazinone position of a novel scaffold which inhibits bacterial type IIa topoisomerase demonstrates that a range of small electron donating groups (EDG) and electron withdrawing groups (EWG) are tolerated for antibacterial activity. Cyano was identified as a preferred substituent that affords good antibacterial potency while minimizing hERG cardiac channel activity.

Due to the continued decline of novel antibacterials being launched onto the market and the rise of antibiotic-resistant infections, the medical community has realized that there is a significant global public health threat. The medical need is urgent to effectively treat infections caused by multi-drug-resistant (MDR) Gram positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE), as well as multi-drug resistance Gram-negative enterobacteriacea and *Pseudomonas aeruginosa*.[1–3] In principal, a new antibacterial agent having a novel mechanism of action and low spontaneous mutation frequency would be less susceptible to the rapid emergence of resistance. While the debate on the pros and cons of exploiting the inhibition of novel bacterial targets identified via genomic analysis versus the inhibition of historical validated targets continues, we believe that a balanced portfolio of targets would be desirable to secure future success. As such, a discovery program was initiated to pursue an antibacterial scaffold that inhibits a well-established target, bacterial type IIa topoisomerase. Type IIa bacterial DNA topoisomerases (DNA gyrase and topoisomerase IV) are essential enzymes for DNA replication, transcription and recombination, and are well-conserved across bacterial pathogens. DNA gyrase is responsible for introducing negative supercoils and topoisomerase IV decatenates and relaxes supercoiled DNA. Even though bacterial DNA gyrase is related to mammalian topoisomerase II, DNA gyrase and topoisomerase IV have proven to be excellent targets for identifying novel antibacterial agents, for example, the quinolones.[4] Among these efforts, there have been intensive discovery activities across the pharmaceutical industry since the late 1990s[5a–d] to identify a novel dual-targeting, non-quinolone GyrA/ParC inhibitor, represented by the prototype Viquidacin (NXL101). However, development of this agent was discontinued at the Phase I clinical trial stage due to significant QT interval prolongation seen in healthy human subjects.[5e]

Based on a scaffold identified from the patent literature,[6] we created a novel series that incorporated an N-linked '4-aminopiperidine' linker between a Left-Hand-Side (LHS) benzoxazinone subunit and a Right-Hand-Side (RHS) pyridooxazinone moiety (Fig. 1). Herein we describe the SAR studies conducted toward optimization of the substituent X at the C-6 position of the LHS fragment, conducted with the aid of a computational pharmacophore model. The rationale for using this particular LHS was partly due to synthetic feasibility, which allowed a quick survey of a broad range of substituents, X. Our assumption was that the SAR obtained could be transferable to other desirable fused bicyclic LHS systems (or even tricyclic systems), such as quinolones, naphthyridones,

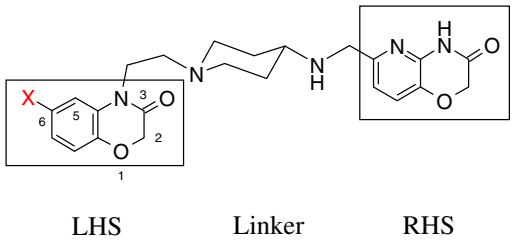

Figure 1. Type IIa topoisomerase inhibitors.

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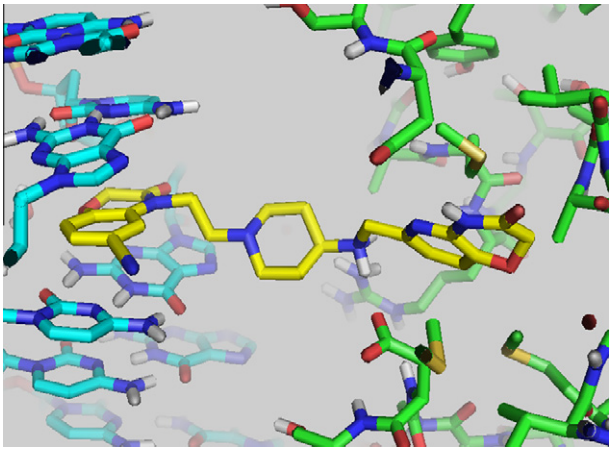
quinoxalinones, etc., that we were actively pursuing in parallel, to further co-optimize antibacterial activity, DMPK properties and safety profile, especially focusing on reducing the hERG inhibition

The titled series of compounds were easily synthesized in a convergent fashion by alkylating the key LHS intermediate, II, with (4-Boc-aminopiperidinyl)ethyl mesylate, followed by Boc deprotection of the primary amine and reductive amination with RHS aldehyde (Scheme 1). The desired substituents were incorporated either directly from the corresponding starting materials (I or IV), or installed through standard functional group transformations either at the intermediate stage (V), or on the final product (VI).[7] Selected aza versions of the final product, VI (Y or Z = N) could also be prepared in a similar fashion.

A group at GlaxoSmithKline recently published a 2.1 Å co-crystal structure of this class of inhibitor, GSK299423, in complex with *S. aureus* DNA gyrase and a 20-bp DNA duplex,[9] which provided a number of intriguing structural insights into the binding mode of these agents. Interestingly, the bicyclic LHS of the inhibitor intercalates two stretched central DNA base pairs while the RHS portion binds into a hydrophobic pocket at the gyrase A dimer interface. For comparison, a docking pose of our inhibitor, **14**, is shown in Picture 1 to illustrate the orientation and potential interaction of the central linker amine and Asp83. This highlighted structural work provided additional insights for further optimization of this class of inhibitors, which were integrated into our pharmacophore model. It is envisioned that this series interacts with the DNA base pairs G–C and C–G in the same fashion as the GSK inhibitor, GSK299423, stabilizing the DNA-bound enzyme.

The data in Table 1 show that a wide variety of substitutions, beyond the initial methoxy group are well tolerated at the C-6 position. The exact nature of the interaction of the C-6 X group with the target DNA strands is unknown. From the docking Picture 1 (X = CN, compound **14**) it can be imagined that there is ample room to accommodate larger substituents. Indeed, some aromatic substituents, such as the 2-furyl moiety (compound **18**), confer potent enzymatic and antibacterial activities to the scaffold. However, in general, smaller-sized groups are favored, and there seems to be a steric limit around the atom directly linked to the bicyclic ring (compounds **2**, **8**). Cyano and fluoro appear to be excellent alternatives to the initial methoxy appendage (compound **17**). Therefore, all three of these groups have remained as preferred C-6 substituents in our later discovery efforts. It was also observed that any branching at the C-2 position, even with a small methyl group, diminished enzymatic and antibacterial activity (data not shown). This further confirmed to us that the space between DNA base pairs clearly prefers a planar structure with correct size, similar

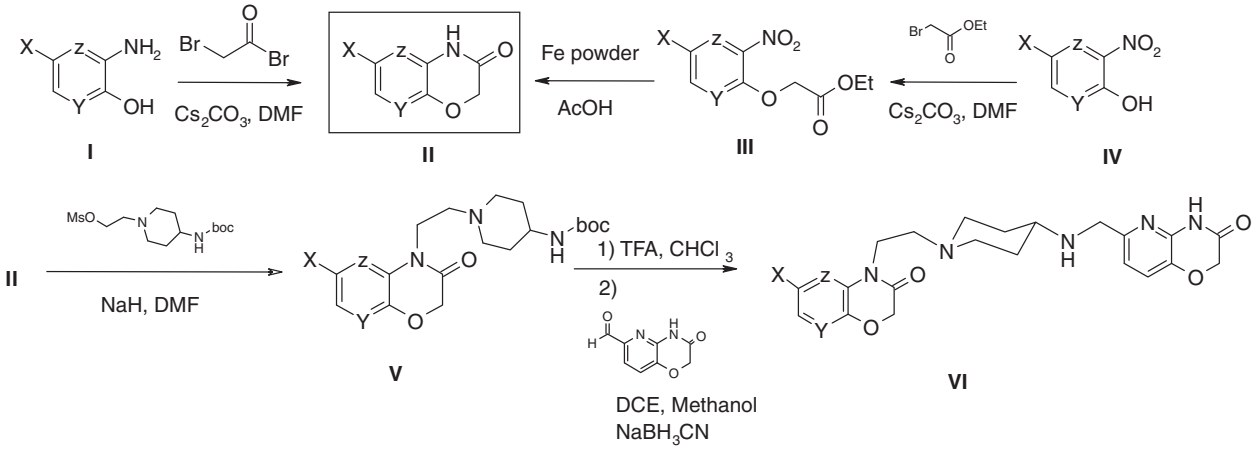


Scheme 2. Docking of compound **14** with X-ray structure (color keys: inhibitor carbon: yellow; enzyme carbon: green; DNA carbon: cyan; nitrogen: blue; oxygen: red; hydrogen: grey).

to many DNA intercalating agents. The required size is preferably similar to fused bicyclic nucleic acid bases. It is obvious that the electron density and molecular volume of the LHS group are also important factors for forming favorable interactions.[10] These observations were later proved to be consistent in other LHS ring systems that we explored.[11]

Topo IV (ParC)[12] enzymatic activities correlate reasonably well with bacterial growth inhibition, if a compound has suitable physical properties. It is well known that antibacterial activity of a particular compound is the result of a combination of target enzymatic potency and its physical properties, which largely govern the cell membrane penetration capability and efflux potential. Gratifyingly, with this current scaffold, a relatively broad antibacterial spectrum is achievable (see compounds**14, 15, 16, 17**) as long as the compound's log *D* falls within the required range. Especially valuable activity against difficult to treat Gram-negative pathogens, such as *Pseudomonas aeruginosa*, is attainable. Of the species tested, *Klebsiella pneumoniae* is the most difficult species to cover with current series. The efflux potential was evaluated through the use of isogenic *Escherichia coli* strains, with or without tolC, as well as other pump knockout strains. Generally, a clear trend of efflux was evident, with over a 20-fold difference in MIC values were often being observed (see compounds **4**, **14**, **15**).

A good correlation between log *D*, and hERG inhibition was also observed. Increasing log *D* to greater than 1 generally resulted in



Scheme 1. Synthesis of type IIa topoisomerase inhibitors.

Table 1
Variation of C-6 substitution X (Y = H, Z = H)

Compound	X	IC_{50} (μM) hERG	Log *D*	MIC (μM)									IC_{50} (nM) Eco ParC
				Sau	Spn	Spy	Hin	Mca	Pae	Eco	Eco (Tolc-)	Kpn	
1	*c*-Tetrazol	>100	−0.8	>10.9	>10.9	>10.9	>10.9	>10.9	>10.9	>10.9	>10.9	>10.9	353
2	1-Hydroxyethyl	>100	0.3	>16.6	>16.6	>16.6	4.2	>16.6	>16.6	>16.6	1.1	>16.6	NT
3	OH	>100	0.5	17.7	8.8	1.1	0.3	0.1	17.7	4.4	0.1	>17.7	36
4	Br	2.8	1.4	0.1	0.3	NV	0.1	<0.015	7.7	0.5	<0.02	3.9	18
5	CF_3CONH–	>100	0.2	>16.1	>16.1	>16.1	16.1	16.1	>16.1	>16.1	8.1	>16.1	NT
6	Carboxyl	>100	−1.6	>13.5	>13.5	>13.5	>13.5	>13.5	>13.5	>13.5	>12.7	>13.5	4680
7	Methoxycarbonyl	14.8	0.8	1	8.1	2	0.5	0.1	>16.1	4	0.1	16.1	78
8	Dimethylaminosulfonyl	NT	0.2	>14.6	>14.6	>14.6	14.7	>14.6	>14.6	>14.6	7.3	NT	NT
9	Methylsulfonyl	50.7	−0.5	>15.5	15.5	15.5	3.9	3.9	>15.5	>15.5	1	NT	NT
10	Nitro	24.3	NT	0.3	0.5	0.3	0.1	0	5.9	0.5	<0.02	NT	NT
11	Ethylsufonyl	>100	−0.1	>15.1	>15.1	15.1	7.6	7.6	>15.1	>15.1	1.9	NT	NT
12	CF_3O–	1.4	1.8	1	1	1	0.1	0.1	15.3	3.8	0.1	NT	NT
13	Acetyl	49.1	0.4	1	2.1	1	0.3	0.3	16.7	2.1	0.1	NT	NT
14	CN	>44	0.2	0.3	0.8	0.3	0.1	0	3.8	0.5	<0.02	4.3	36
15	Cl	3	1.3	0.3	0.5	0.5	0.1	0.1	4.2	0.5	<0.02	NT	23
16	F	8.5	0.9	0.6	1.1	0.5	0.1	0	6.2	0.6	0.1	NT	NT
17	MeO–	12.9	0.9	0.3	1.1	NV	0.1	0.1	8.6	0.5	0.1	NT	NT
18	2-Furyl	1.3	2.2	0.5	0.5	2.0	0.3	0.06	15.9	2.0	0.06	15.9	18

Notes for table 1: hERG: Ionworks electrophysiological assay; Log *D*: experimental value measured by shake flask method with LC–MS detection; minimum inhibitory concentration (MIC) was determined according to CLSI protocols[8]; Sau: *Staphylococcus aureus;* Spn: *Streptococcus pneumoniae;* Spy: *Streptococcus pyogenes;* Hin: *Haemophilus influenzae;* Mca: *Moraxella catarrhalis;* Pae: *Pseudomonas aeruginosa;* Eco: *Escherichia coli;* Kpn: *Klebsiella pneumoniae;* IC_{50} was determined using the protocols in Ref. 12b.

potent, low single-digit μM hERG inhibition (such as compounds **4**, **15, 18**), a known issue in this series of inhibitors across the industry. In this current survey, the cyano substitution (compound **14**, log *D* 0.2) seems to be ideal, giving balanced hERG profile and potent antibacterial activity.

Based on the overall structural novelty, the antibacterial potency/spectrum, and drug-like physical properties, including aqueous solubility (the majority of compounds have >1 mM solubility), this scaffold was deemed to be an excellent series for further exploration to identify novel antibacterial agents with a desirable spectrum. The SAR obtained from the benzoxazinone LHS has proven to be applicable to other LHS fused bicyclic ring systems. The series exhibits rapid bactericidal activity and has shown no cross-resistance with marketed clinical antibacterial agents (e.g., fluoroquinolones) at the target level. Further modification of the bicyclic LHS, linker and RHS will be reported in due course.[11]

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Compound **1** was synthesized from VI where X = CN; NaN_3, TEA.HCl, NMP.
Compound **2** was synthesized from VI where X = acetyl; $NaBH_4$, MeOH, TEA.HCl, NMP.
Compound **3** was synthesized from V where X = OCH_3; BBr_3 (1M), DCM.
Compound **4** was synthesized from I where X = Br, commercially available.
Compound **5** was synthesized from V where X = NO_2, $NH_4.HCO_2$, Pd/C, acetyl chloride, DCM.
Compound **6** was synthesized from VI where X = MeOCO-; LiOH, MeOH.
Compound **7** was synthesized from I where X = MeOCO-.
Compound **8** was synthesized from I where X = Me_2NSO_2-.
Compound **9** was synthesized from I where X = $MeSO_2$-, which was prepared through demethylation of 2-MeO–5-$MeSO_2$ aniline with BBr_3.
Compound **11** was synthesized from I where X = $EtSO_2$-.
Compound **12** was synthesized from IV where X = CF_3O.
Compound **13** was synthesized from IV where X = acetyl-.
Compound **14** was synthesized from V where X = CN. MS (ES): 463(MH^+) for $C_{24}H_{26}N_6O_4$ (MW462.51); ^1H NMR ($CDCl_3$) δ: 1.49 (m, 2H); 1.94 (m, 2H); 2.14 (m, 2H); 2.56 (m, 1H); 2.59 (t, *J* = 6.7 Hz, 2H); 2.94 (m, 2H); 3.84 (s, 2H); 4.03 (t, 2H); 4.62 (s, 2H); 4.67(s, 2H); 6.94 (d, 1H); 7.03 (d, 1H); 7.19 (d, 1H); 7.30 (dd, 1H); 7.46 (d, 1H).
Compound **15** was synthesized from I = Cl, commercially available.
Compound **16** was synthesized from I = F, commercially available.
Compound **17** was synthesized from IV = MeO.
Compound **18** was synthesized from VI = Br, through Suzuki coupling with 2-furyl boronic acid.
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production of inorganic phosphate, a product of the ATPase reaction. Inorganic phosphate was quantified using the ammonium molybdate/malachite green-based detection system. For determination of IC_{50} values, assays were performed in 384-well microtiter plates. Each well contained a dilution range of the compound dissolved in DMSO. In addition, each well contained: 20 mM Tris pH 8.0, 50 mM ammonium acetate, 0.16 mM ATP, 0.005% Brij-35, 8.0 mM magnesium chloride, 0.5 mM EDTA, 2.5% v/v glycerol, 5 mM dithiothreitol, 0.005 mg/mL sheared salmon sperm DNA, 0.5 nM *E. coli* ParC protein, 0.5 nM *E. coli* ParE protein. Final volume of assays was 30 μL. Reactions were incubated 24 h at room temperature and then quenched with the addition of 30 μL malachite green reagent {{183 Lanzetta,P.A. 1979;}} via a bulk reagent dispenser. Plates were incubated 3–5 min at room temperature, and then absorbance at 650 nM was measured using a Spectramax 384 plate reader.; (c) Gyrase (inhibition of supercoiling activity data not shown) and Topo IV (ParC) enzymatic activities correlate reasonably well. In general, the gyrase potency is weaker than ParC in our assay conditions. Later in the program, using a modified version of ParC enzyme assay[12b], the retested IC_{50}s of selected compounds are even more potent. For example, 3.2 nm for compound **14** and 5.0 nM for compound **17**.