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ABSTRACT

There is an emerging global need for new and more effective antibiotics against multi-resistant bacteria. This situation has led to massive industrial investigations on novel bacterial topoisomerase inhibitors (NBTIs) that target the vital bacterial enzymes DNA gyrase and topoisomerase IV. However, several of the NBTI compound classes have been associated with inhibition of the hERG potassium channel, an undesired cause of cardiac arrhythmia, which challenges medicinal chemistry efforts through lengthy synthetic routes. We herein present a solid-phase strategy that rapidly facilitates the chemical synthesis of a promising new class of NBTIs. A proof-of-concept library was synthesized with the ability to modulate both hERG affinity and antibacterial activity through scaffold substitutions.

The extensive use of β-lactam antibiotics has resulted in development of resistance among bacteria, such as the Gram-positive methicillin-resistant *S. aureus* (MRSA). The mortality rate for humans infected with MRSA is 15–60%, and in Europe 25,000 people die every year from infections caused by multidrug-resistant bacteria.[1] MRSA is resistant to β-lactams, and some strains of MRSA are also resistant to tetracyclines, macrolides, lincosamides, aminoglycosides, trimethoprim, and in some cases also to fluoroquinolones.[2] Vancomycin has traditionally been the drug of last resort, but emergence of vancomycin resistance in MRSA is now on the rise.[3]

Recently, a new class of promising antibiotics targeting bacterial topoisomerase IIA enzymes has been developed, often referred to as Novel Bacterial Topoisomerase Inhibitors (NBTIs, Fig. 1).[4,5] There are several advantages of topoisomerase inhibitors, as illustrated by the successful applications of ciprofloxazin as a widely used antibiotic against Gram-negative bacteria. Bacteria have two type IIA topoisomerases which are named DNA gyrase (topoisomerase II) and topoisomerase IV. The active sites of gyrase and topoisomerase IV are close to identical, thus one antibiotic can potentially target two distinct enzymes, and the development of resistance would require mutations in both of the corresponding genes (*gyrA* for gyrase, and *parC* for topoisomerase IV). Additionally, topoisomerase inhibitors may be effective against both Gram-positive and Gram-negative bacteria since the topoisomerase genes are highly conserved in both species.[6]

Human topoisomerases are structurally different from the bacterial counterpart and thus generally not affected by bacterial topoisomerase inhibitors.[7]

NXL101 (**1**) was one of the first NBTIs to reach clinical investigation, but the compound was found to disturb heart rhythm by prolongation of the QT interval in healthy subjects.[8] As this scenario may lead to cardiac arrhythmia, and even sudden death, NXL101 was discontinued. The typical cause of QT prolongation is blocking of the potassium channel in the heart encoded by the *human ether-à-go-go related gene* (hERG), commonly referred to as the hERG channel.[9] Thus, there is a medical need for safe and efficacious antibiotics that are highly effective towards pathogenic resistant bacterial strains, with minimal inhibition of the hERG potassium channel. Several pharmaceutical companies are engaged in NBTI development (Fig. 1), and hERG liability has been observed as a recurring challenge.[5]

The overall yield reported for the synthesis of NXL101 by Baque and

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Fig. 1. Novel bacterial topoisomerase inhibitors (NBTIs).

Scheme 2. Selective protection of 2-carboxy piperazine. Reagents and conditions: (a) Cu(OAc)$_2$, NaOH, Acetone:H$_2$O (2:1), 10 min, rt, then Boc$_2$O, 90 min, rt. (b) EDTA, Na$_2$CO$_3$, H$_2$O:dioxane (1:1), 10 min, rt, then Boc$_2$O (1.3 equiv), rt, overnight, then AcCl (7.6 equiv), MeOH, 4 h, reflux.

co-workers was below 1%,[5a] which dramatically limits the access to structural variants and valuable structure activity relation (SAR) data. We envisioned an unexplored potential of significantly simpler, yet equipotent, NXL101 analogs, and set out to develop a new synthesis strategy that would allow the rapid generation of relevant compound libraries. In order to reduce the structural complexity, we decided to replace a stereogenic carbon atom with a nitrogen atom, thus creating a central piperazine core. This would, in turn, enable development of strategies where either parts of the molecule could be varied independently. It was envisioned, that a solid-phase synthesis (SPS) strategy would enable an easy and rapid synthesis of such compounds, by attachment of the piperazine core to a solid support through a carboxylic acid moiety (Scheme 1). Different capping groups could then be introduced via the N1 and N4 nitrogen atoms, through combinatorial synthesis.

For the SPS-strategy we decided to use the orthogonal protecting groups Boc and Fmoc. The selective protection of carboxypiperazine was achieved using copper(II) as a temporary protection group, followed by Boc-protection to give the copper(II) intermediates 6-*R* and 6-*S*. The cupper was subsequently released by addition of EDTA, which enabled the protection of the 2-amino group using FmocCl to afford the desired bis-protected compounds 7-*R* and 7-*S* in excellent yield, with high purities without the use of chromatography (Scheme 2).
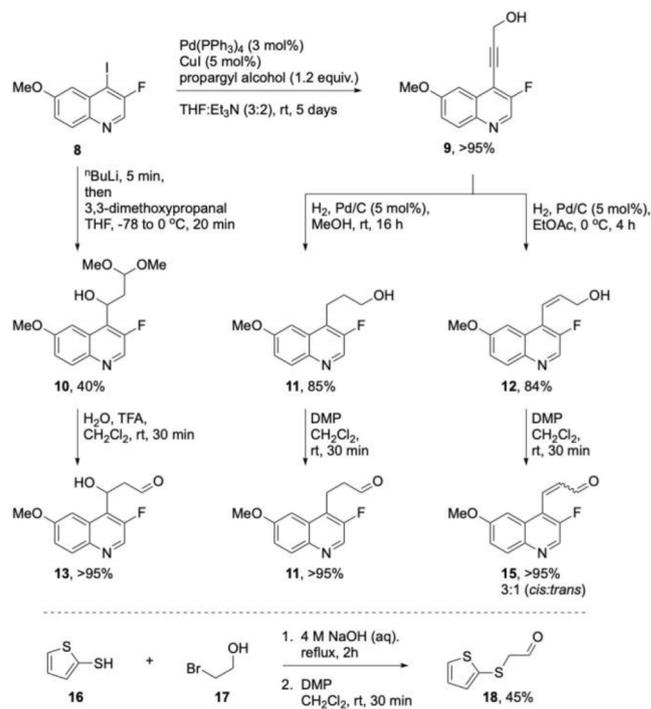
The quinoline building blocks were made from the common precursor iodide 8, which was synthesized according to Flagstad et al. (Scheme 3).[10] The alcohol-containing aldehyde building block 13 was synthesized via lithiation of the iodide 8 followed by treatment with 3,3-dimethoxypropanal. The protected aldehyde was then released under acidic conditions to give the aldehyde 13, which was used immediately immediately after hydrolysis. To access alkene and alkane-substituted quinolines, a Sonogashira cross-coupling protocol was employed. The iodide was coupled with propargyl alcohol using catalytic amounts of Pd (PPh$_3$)$_4$ and CuI, which provided the alkyne 9 in near quantitative yield (>95%). The triple bond was subsequently either fully reduced to give alcohol 11 or selectively reduced to the *cis*-alkene 12 both in good yields (85% and 84%, respectively). The alcohols were subsequently oxidized to the corresponding aldehydes 14 and 15 in excellent yields (both > 95%) using Dess-Martin periodinane (DMP) as the oxidant. The *cis*-alkene isomerized partly during the oxidation and a mixture of *cis/trans* (3/1) was obtained. The thiophene building block 18 was obtained from


Scheme 3. Synthesis of aldehyde building blocks.

2-thiophenethiol 16 by alkylation with bromoethanol 17 followed by DMP oxidation (Scheme 3).

With the bis-protected carboxy-piperazines 7-*R* and 7-*S* and the aldehyde building blocks 13–15 and 18 in hand, we set out to synthesize the first piperazine-based NBTI.

The bis-protected carboxypiperazine 7-*R* or 7-*S* was attached to a HMBA-functionalized ChemMatrix® resin using MSNT as the coupling reagent. The immobilized piperazine was then Fmoc-deprotected using piperidine followed by derivatization with quinoline building blocks. The Boc-group was then removed using TFA and the amine was reductively alkylated using the thiophene aldehyde 18. The NBTI analogs were finally released from the solid support using NaOH (aq) (Scheme

Scheme 1. Outline of solid-phase synthesis strategy for the generation of carboxypiperazine-based NBTIs.
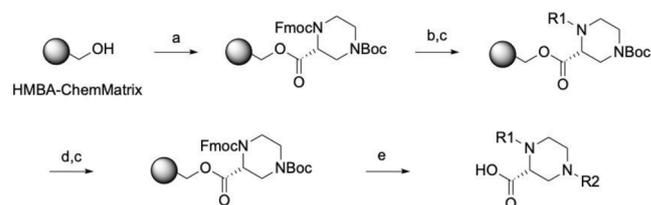
Scheme 4. SPS of piperazine-based NBTIs. Reagents and conditions: (a) 7-*R* or 7-*S*, MSNT, MeIm, CH$_2$Cl$_2$, rt, 1 h. (b) 20% piperidine (DMF), rt, 2 + 5 min (c) RCHO, NaBH(OAc)$_3$, CH$_2$Cl$_2$, rt, 4 h. (d) 50% TFA (CH$_2$Cl$_2$), rt, 1 h. (e) 0.1 M NaOH (aq), 16 h, rt, then 0.1 M HCl.

4).

All of the title compounds were characterized by 1H NMR, 13C NMR, LC-MS, HRMS and melting point. Description of detailed synthetic procedures and spectroscopic data and analysis can be found in Supporting Information.

To evaluate the potential of the solid-phase strategy as a useful template for developing NBTI analogs with improved properties, the antibiotic activity was determined against two bacterial strains (methicillin-susceptible *S. aureus*, MSSA and MRSA) by MIC. The hERG channel inhibition was measured by the Predictor[TM] hERG Fluorescence Polarization Assay test kit (catalog no. PV5365) from Invitrogen (Carlsbad, CA), see Tables 1 and 2.

The consequence of replacing the core piperidine with a piperazine and removing the hydroxyl group was readily assessed and satisfyingly, the antibiotic effect was retained. This confirmed the simplified piperazine NBTI (Table 1, entry 3) as a useful template for a SAR study of the antibiotic activity and the hERG affinity. Notably, removal of the

Table 1

Biological evaluation of piperazine-based NBTIs.

Entry	Compound	MIC (μM (μg/mL))		hERG affinity EC$_{50}$ (μM)
		MSSA[b]	MRSA[c]	
1	1 (NXL101)	0.8 (0.4)	0.4 (0.2)	24
2	19	24.7 (12.5)	1.6 (0.8)	<30
3	20	24.7 (12.5)	12.3 (6.2)	ND
4	21	0.8 (0.4)	0.4 (0.2)	17
5	22	3.1 (1.5)	1.6 (0.8)	<30
6	23	25.6 (12.5)	6.4 (3.1)	<30
7	24	51.5 (25)	25.7 (12.5)	<30
8	25	6.3 (3.1)	3.3 (1.6)	26
9	26	6.3 (3.1)	3.3 (1.6)	<30
10[d]	27	1.6 (0.8)	NA	2
11[d]	28	21.8 (12.5)	21.8 (12.5)	0.5
12[d]	29	>99.3 (>50)	>99.3 (>50)	2
13[d]	30	>99.3 (>50)	>99.3 (>50)	4

a FQ = 3-fluoro-6-methoxyquinoline (attached in the 4-position), NA = not applicable, ND = not determined.
[b] Methicillin-susceptible *S. aureus* NCTC 8325-4.
[c] Methicillin-resistant *S. aureus* CC398.
[d] Prepared in solution (experimental procedures are provided in the supporting information).

Table 2

Biological evaluation of piperazine-based NBTIs.

21, 31-39
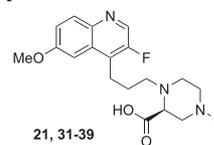
Entry	R	MIC (μM (μg/mL))		hERG affinity EC$_{50}$ (μM)
		MSSA[a]	MRSA[b]	
1	21	0.8 (0.4)	0.4 (0.2)	17
2	31	1.7 (0.8)	0.9 (0.4)	17
3	32	1.7 (0.8)	0.4 (0.2)	38
4	33	>114.3 (>50)	NA	25
5	34	14.0 (6.3)	6.9 (3.1)	87
6	35	12.9 (6.2)	6.5 (3.1)	21
7	36	>99.3 (>50)	>99.3 (>50)	7
8	37	>100.5 (>50)	NA	<30
9	38	>104.3 (>50)	>104.3 (>50)	NA
10[c]	39	0.4 (0.2)	0.2 (0.1)	25

[a] Methicillin-susceptible *S. aureus* NCTC 8325-4.
[b] Methicillin-resistant *S. aureus* CC398.
[c] Prepared in solution (experimental procedures are provided in the supporting information). NA = not applicable.

hydroxyl improved the antibacterial activity (Table 1, entry 4). Unsaturated analogs resulted in reduced antibiotic activity (Table 1, entry 5–7). The influence of substituents of the core piperazine was then studied, which indicated the strongest antibiotic effect when a carboxylic acid was installed in the 2-position of the piperazine with an *R*-configuration of the stereogenic center (Table 1, entry 4 vs. entry 9–13). Generally, the hERG affinity was in the same range as for NXL101, with the acid derivatives (Table 1, entry 10–13) being the strongest hERG binders. To verify the mechanism of action, ten compounds (**21–24, 31, 32, 34, 35, 39**) with MIC ≤ 50 μM against MRSA *S. aureus* were biochemically evaluated for gyrase and topoisomerase IV inhibitory activity in a microtitre plate-based assay (see Supporting Information). The enzymatic assays demonstrated a dual inhibitory activity of most compounds against gyrase and topoisomerases in the low μM-range, which may ultimately limit emerging resistance.[11]

To demonstrate the versatility of the solid-phase synthesis strategy, we synthesized a small library with variation of the N4 substituent based on the most active piperazine NBTI analog (Table 1, entry 4). The antibacterial activity was retained when replacing the thiophene moiety for a phenyl (Table 2, entry 2–3), whereas shortening or prolongation of the linker was less tolerated (Table 2, entry 4–6). Turning the piperazine amine into an amide resulted in complete loss of activity (Table 2, entry 7–9). Interestingly, a significantly weaker hERG affinity was observed for the (CH$_2$)$_3$Ph analog (Table 2, entry 5). More rewardingly, by further introducing a fluorinated substituent (Table 2, entry 10), an antibiotic (**39**) with improved antimicrobial activity and hERG affinity margin compared to NXL101 was obtained.

In conclusion, we have developed a new and easily accessible piperazine-based NBTI scaffold. The most potent compounds generated