Bioorganic & Medicinal Chemistry Letters 28 (2018) 2477–2480

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-staphylococcal activity of novel bacterial topoisomerase inhibitors with a 5-amino-1,3-dioxane linker moiety

Linsen Li[a], Antony Okumu[a], Sheri Dellos-Nolan[b], Zoe Li[c], Soumendrakrishna Karmahapatra[c], Anthony English[c], Jack C. Yalowich[c], Daniel J. Wozniak[b,d], Mark J. Mitton-Fry[a,*]

[a] Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210, USA
[b] Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA
[c] Division of Pharmacology, The Ohio State University, Columbus, OH 43210, USA
[d] Department of Microbiology, The Ohio State University, Columbus, OH 43210, USA

ARTICLE INFO

Keywords:
MRSA
NBTI
Gyrase
Topoisomerase
TopoIV
Antibacterial

ABSTRACT

Novel bacterial type II topoisomerase inhibitors (NBTIs) constitute a promising new class of antibacterial agents. We report a series of NBTIs with potent anti-staphylococcal activity and diminished hERG inhibition. Dioxane-linked compound **9** demonstrated MICs ≤1 μg/mL against both methicillin-susceptible (MSSA) and -resistant *Staphylococcus aureus* (MRSA), accompanied by reduced hERG inhibition as compared to cyclohexane- or piperidine-linked analogs.

The continuous evolution and dissemination of multidrug-resistant bacteria presents a significant threat to human health.[1] Within this context, methicillin-resistant *Staphylococcus aureus* (MRSA) is a particularly important pathogen in both community and hospital settings,[2] and the incidence of MRSA in individuals with cystic fibrosis is also increasing.[3] Indeed, according to a 2013 report issued by the Centers for Disease Control, MRSA ranks as the number one cause of mortality from antibiotic-resistant infections in the United States.[4] Several recent drug approvals hold promise for the treatment of MRSA, including ceftaroline,[5] tedizolid,[6] and delafloxacin.[7] Nevertheless, these antibiotics are analogs of well-established drug classes, and fully addressing the threat of multidrug-resistant bacterial infections requires the development of entirely new therapeutic classes.[8] Unfortunately, achieving this goal has proved particularly challenging, especially in light of reduced pharmaceutical investment.[9]

Against this backdrop, Novel Bacterial Type II Topoisomerase Inhibitors (NBTIs) have emerged as a promising new class.[10] The NBTIs exert antibacterial activity through inhibition of both DNA gyrase and topoisomerase IV (TopoIV), the biological targets of the well-established fluoroquinolone antibiotics. Crystallographic[11] and mutational[12,13] studies have elucidated a binding mode for NBTIs that is distinct from the fluoroquinolones, thus circumventing resistance to these important medicines. Finally, several recent reports have raised the exciting possibility that, in addition to excellent activity against Gram-positive bacteria, NBTI spectrum of activity might also be extended to Gram-negative pathogens.[14,15]

X-ray crystallographic studies of the NBTI GSK299423 (**1**, Fig. 1) in ternary complex with *S. aureus* DNA gyrase and DNA revealed a three part pharmacophore.[11] The bicyclic quinoline moiety binds DNA and is connected to the enzyme-binding oxathiolopyridine motif through a linker domain. Additional structural efforts by these authors[16,17] and others[18,19] have established the generality of this binding mode for NBTIs. Intriguingly, the linker does not appear to make critical contacts with either the DNA or enzyme, with the exception of a characteristic amine that contacts an aspartate residue (D83) at the entrance of a hydrophobic pocket of DNA gyrase. Consequently, the linker has been the focus of substantial medicinal chemistry efforts and has proved amenable to considerable structural innovation.

Several NBTIs have been advanced to human clinical trials.[10,17,20–22] Gepotidacin (**2**, Fig. 1) has recently demonstrated efficacy in a Phase 2 trial involving acute bacterial skin and skin structure infections, with a substantial proportion of isolates being MRSA.[23] Potential limitations of gepotidacin include thrice daily dosing and the clinical observation of *S. aureus* strains with reduced susceptibility, likely through a single amino acid substitution in DNA gyrase (D83N). This mutation has been reported to confer resistance to a number of NBTIs, which commonly inhibit DNA gyrase much more potently than TopoIV.[24–28] Interestingly, although gepotidacin only minimally inhibits hERG (IC_{50} = 588 μg/mL), significant (> 10 ms) prolongation of the cardiac QT interval has been observed in clinical trials.[29] At least

* Corresponding author.
E-mail address: mitton-fry.1@osu.edu (M.J. Mitton-Fry).

https://doi.org/10.1016/j.bmcl.2018.06.003
Received 12 March 2018; Received in revised form 14 May 2018; Accepted 1 June 2018
Available online 01 June 2018
0960-894X/

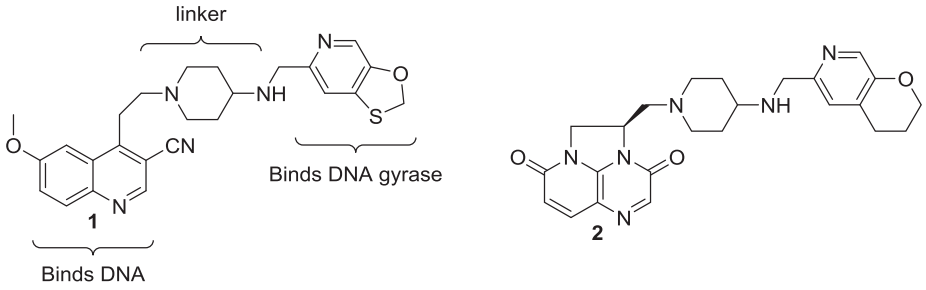


Fig. 1. Examples of previously reported NBTIs: GSK299423 (**1**) and gepotidacin (**2**).

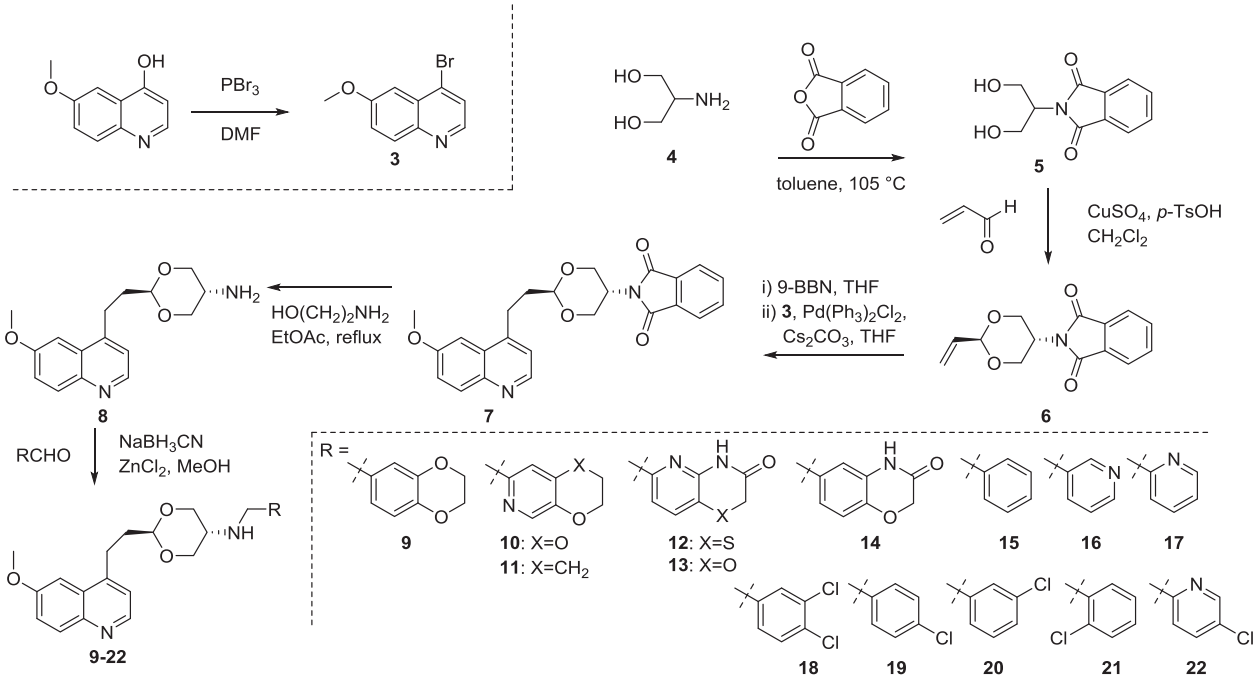
one NBTI has previously been eliminated from clinical trials due to QT-prolongation.[30] The minimization of hERG inhibition has thus been a key objective of multiple medicinal chemistry programs. Of particular interest is the report by Reck and colleagues, who showed attenuation of hERG inhibition by NBTIs through modulation of physicochemical properties such as amine basicity and lipophilicity.[22]

Ndubaku and coworkers described the use of a 5-amino-1,3-dioxane moiety as a more polar and less basic replacement for a piperidine in a series of PAK1 inhibitors, in part to address hERG activity.[31] Noting the observed reductions in hERG inhibition,[31] we hypothesized that a *trans*-configured dioxane ring would serve as a suitable surrogate for previously reported linkers and that its reduced amine basicity would confer potential safety benefits through diminished hERG inhibition. Prior reports describing NBTIs with tetrahydropyran[15,24,26] and oxabicyclooctane[18,19,27] linkers suggested that oxygenation in this domain would be well tolerated. Facile synthetic access was expected to be highly enabling to the development of structure-activity relationships (SAR). In this regard, improved dual-target inhibition, through optimization of TopoIV activity would be expected to confer advantages in terms of a reduced propensity for resistance emergence.[32]

In order to establish proof of principle, a series of NBTIs possessing a 6-methoxyquinoline DNA binding moiety and the 5-amino-1,3-dioxane linker was prepared using a short synthetic route (Scheme 1). Quinoline intermediate **3** was synthesized on multigram scale via bromination[33] of commercially available 4-hydroxy-6-methoxyquinoline with PBr_3 and was isolated by simple filtration in high yield (95%) and purity (> 95%). Serinol (**4**) was efficiently protected as the phthalimide derivative (**5**) in good yield (91%) and purity (95%) on > 75 g scale without chromatographic purification. Condensation of diol **5** with acrolein proceeded smoothly to afford alkene **6**. Hydroboration of alkene **6** with 9-BBN and Suzuki-Miyaura reaction of the resulting intermediate with quinoline bromide **3** afforded the coupled intermediate **7**. Deprotection of the phthalimide with ethanolamine in refluxing ethyl acetate[34] yielded primary amine **8** as the key intermediate. Finally, reductive amination installed a series of enzyme-binding motifs in compounds **9–22**.

Minimal inhibitory concentrations (MICs) for all analogs were determined using the *S. aureus* reference strain ATCC 29213 (Table 1). Ciprofloxacin served as a positive control in each assay. A variety of bicyclic enzyme-binding moieties were explored in the initial proof of concept efforts, since they have shown considerable promise in prior reports.[35] The following general trends were notable. Benzodioxine **9** afforded potent whole cell activity (≤ 1 μg/mL) against the control *S. aureus* strain that was eroded only slightly in the aza analogs **10** and **11**. Pyridothiazinone **12** and pyridooxazinone **13** also showed potent whole cell activity. In contrast, the monocyclic phenyl analog **15** and 3-pyridyl derivative **16** lost all activity, while the 2-pyridyl analog **17** demonstrated only a modest MIC (8 μg/mL).

Inspired by a recent disclosure of NBTIs with anti-mycobacterial activity,[36] we also incorporated a 3,4-dichlorophenyl moiety in compound **18**. Gratifyingly, this analog showed whole cell activity on par with the bicyclic analogs. The monosubstituted 4-chlorophenyl analog



Scheme 1.