Bioorganic & Medicinal Chemistry Letters 27 (2017) 5014–5021

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

4-Hydroxy-2-pyridones: Discovery and evaluation of a novel class of antibacterial agents targeting DNA synthesis

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ARTICLE INFO

Article history:
Received 3 August 2017
Revised 27 September 2017
Accepted 4 October 2017
Available online 5 October 2017

Keywords:
Antibacterial
Gram-negative
4-Hydroxy-2-pyridone
Structure-activity relationship
DNA synthesis inhibitor

ABSTRACT

The continued emergence of bacteria resistant to current standard of care antibiotics presents a rapidly growing threat to public health. New chemical entities (NCEs) to treat these serious infections are desperately needed. Herein we report the discovery, synthesis, SAR and *in vivo* efficacy of a novel series of 4-hydroxy-2-pyridones exhibiting activity against Gram-negative pathogens. Compound **1c**, derived from the *N*-debenzylation of **1b**, preferentially inhibits bacterial DNA synthesis as determined by standard macromolecular synthesis assays. The structural features of the 4-hydroxy-2-pyridone scaffold required for antibacterial activity were explored and compound **6q**, identified through further optimization of the series, had an MIC_{90} value of 8 μg/mL against a panel of highly resistant strains of *E. coli*. In a murine septicemia model, compound **6q** exhibited a PD_{50} of 8 mg/kg in mice infected with a lethal dose of *E. coli*. This novel series of 4-hydroxy-2-pyridones serves as an excellent starting point for the identification of NCEs treating Gram-negative infections.

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Decades of antibiotic misuse coupled with a paucity of new drugs has led to the rapid rise of bacteria resistant to current standard of care antibiotics. Infections caused by these drug resistant bacteria represent a rapidly growing threat to human health. In 2014, the Wellcome Trust and UK Department of Health commissioned a Review of Antimicrobial Resistance (AMR), and estimated that globally at least 700,000 deaths per year were attributable to drug resistant bacteria.[1] Multiple agencies, including the CDC[2] and WHO,[3] have begun to sound the alarm on this growing health crisis. Despite the urgent medical need for new chemical entities (NCEs) to treat these serious infections, there has been a steady decline in the number of new antibiotics developed and approved over the past three decades.[4] The problem is even more acute for NCEs that target Gram-negative infections.[5] Clearly there is an increasing need for novel antibacterial agents that target these drug resistant pathogens. Herein we report on the *in vitro* and *in vivo* antibacterial activity of compounds belonging to a new antibacterial scaffold, 4-hydroxy-2-pyridones, that target Gram-negative pathogens and the development of a structure-activity relationship (SAR) for these compounds.

As part of a program aimed at discovering new Gram-positive antibacterial agents, pyranoquinoline **1a** (Fig. 1) was identified through an intensive SAR optimization effort.[6] Bacterial minimum inhibitory concentrations (MIC) were determined by following CLSI guidelines with the exception that organisms were grown in brain-heart-infusion media.[7] Although **1a** possessed a moderate MIC of 3.1 μg/mL against wild-type *Staphylococcus aureus* ATCC 29213 (*S. aureus*^{WT}), it had greatly reduced activity (MIC > 12.5 μg/mL) against wild-type *Escherichia coli* ATCC 25922 (*E. coli*^{WT}). Structural modification aimed at improving the activity of these compounds against Gram-positive agents as well as improving their ADME properties led, after excision of the pyrone ring in **1a**, to the *N*-benzyl-2-pyridone **1b**. 2-Pyridone **1b** provided a 2-fold improvement in the MIC against *S. aureus*^{WT} although it continued to lack activity against *E. coli*^{WT}. Further structural modification, which resulted in the removal of the *N*-benzyl group in **1b**, led to the *NH*-2-pyridone **1c**. This structural modification, which resulted in no significant net change in activity against *S. aureus*^{WT}, resulted in a marked improvement in activity against *E. coli*^{WT} (MIC = 0.8 μg/mL) as well as modest activity (MIC = 15.6 μg/mL) against the highly fluoroquinolone resistant *E. coli* strain SKM18 (*E. coli*^{R}).[8] Although compound **1c** is structurally distinct from a series of broad spectrum 2-pyridone inhibitors of bacterial DNA synthesis reported ear-

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https://doi.org/10.1016/j.bmcl.2017.10.006
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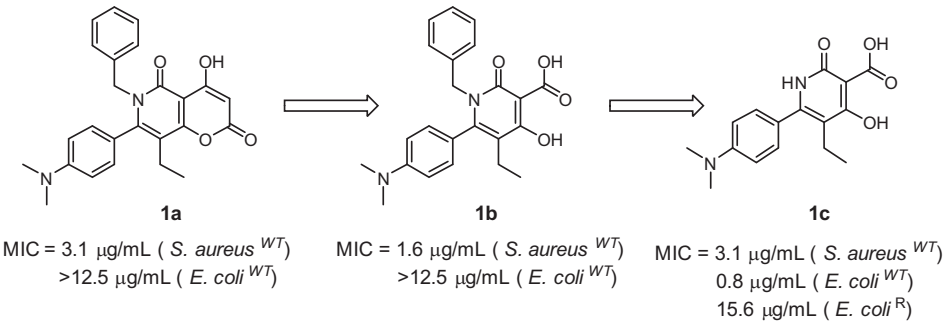

Fig. 1. Structural evolution of pyrano[3,2-*c*]pyridine-2,5-diones to 4-hydroxy-2-pyridones.

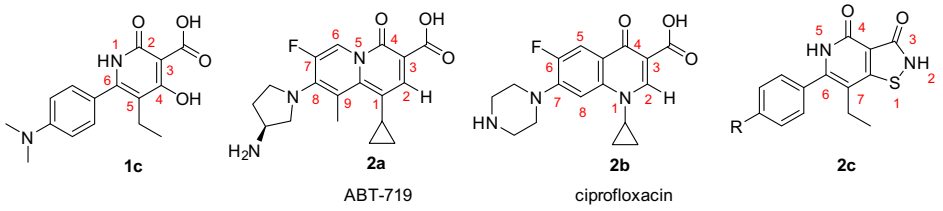

Fig. 2. Representative structures of 4-hydroxy-2-oxo-1,2-dihydropyridines (4-hydroxy-2-pyridones, **1c**), 4-*H*-4-oxoquinolizines (ABT-719, **2a**), 4-oxo-1,4-dihydroquinolone (ciprofloxacin, **2b**) and isothiazolo[4,5-*c*]pyridine-3,4(2*H*,5*H*)-dione **2c**.

lier[9,10] (**2a**, Fig. 2), the two series share common structural motifs including the 2-pyridone core and pendant carboxylic acid. Further, the 2-pyridone cores in both **1c** and **2a** are isomeric with the 4-pyridone core present in the fluoroquinolone class of bacterial DNA synthesis inhibitors (e.g., ciprofloxacin, **2b**).[10,11] Recently, a series of isothiazolone bacterial type II topoisomerase inhibitors (e.g., **2c**) have been reported.[12]

To explore the SAR in the 4-hydroxy-2-pyridone scaffold, three synthetic routes were developed (Scheme 1, Methods A–C). A generally applicable synthetic route, Method A, was developed to convert readily available aryl ketones **1d** to the corresponding *tert*-butylimines **1e** via treatment with excess *tert*-butylamine under dehydrative conditions. The 2-pyridone esters **1f** were then generated through annulation with trimethyl methane tricarboxylate under thermal conditions. Finally, nucleophilic cleavage of the methyl esters **1f** was carried out efficiently with lithium iodide to afford the 4-hydroxy-2-pyridone-3-carboxylates **1g**.

For 4-hydroxy-2-pyridones possessing amine substitution on the aryl ring, Method B was developed. This route utilized readily available 4-halo-ketones **1h** (X = Cl or Br). Treatment of **1h** with 2,4-dimethoxybenzylamine in the presence of $TiCl_4$ gave imines **1i**. Employing the same annulation conditions used in Method A afforded 2-pyridones **1j**. For effective introduction of amine substitution into the aryl ring, prior protection of the 2-pyridones **1j** was required. A two-step protection/hydrolytic process was accomplished under Mitsunobu conditions followed by ester hydrolysis providing dibenzyloxypyridine carboxylates **1k** in good yield. Amine functionality was installed utilizing Buchwald-Hartwig amination in the presence of NaO*t*-Bu and JohnPhos precatalyst followed by global deprotection using hydrogenolysis conditions, affording the final amino substituted 4-hydroxy-2-pyridones **1l**.

For compounds that were incompatible with the high temperature annulation conditions employed in Methods A and B, a third route, Method C, was developed which employed Suzuki coupling of chloropyridine intermediate **1p** as a key step. Condensation of readily available nitriles **1m** with malonyl chloride afforded chloropyridines **1n**. Mitsunobu reaction of **1n** with benzyl alcohol gave dibenzyloxy chloropyridines **1o**. Introduction of the carboxylate functionality was accomplished by metallation of **1o** with *n*-BuLi followed by trapping the aryllithium species with $ClCO_2Bn$, providing key tribenzylated chloropyridine intermediates **1p**. Intermediates **1p** were then coupled with pinacol boronates **1r**, derived from the corresponding aryl bromides **1q**, via Suzuki coupling ($Pd_2(dba)_3$/*t*-Bu_3P-HBF_4) to afford the tribenzylated pyridines **1s**. Hydrogenolysis with palladium on carbon gave the 4-hydroxy-2-pyridones **1g** in good yields.

The structural modification leading from *N*-benzyl-2-pyridone **1b** to *NH*-2-pyridone **1c** resulted in a striking (greater than 16-fold) improvement in the MIC against *E. coli*^{WT}. This prompted an investigation of the mechanism of action (MOA) of these compounds. Standard macromolecular synthesis assays were conducted using radiolabeled precursors of DNA, RNA, protein, cell wall and fatty acid synthesis to characterize the activity of compounds **1b** and **1c** against *S. aureus*^{WT} and *E. coli*^{WT} (Fig. 3).[13] *N*-Benzyl-4-hydroxy-2-pyridone **1b** was evaluated at multiple concentrations against *S. aureus*^{WT}. At concentrations at or near the MIC value (1.6 μg/mL), **1b** displayed preferential inhibition of protein synthesis. At concentrations greater than the MIC, multiple macromolecular pathways were affected, likely due to cell death as a result of inhibiting protein synthesis. The macromolecular synthesis assay for **1c** was conducted against both *S. aureus*^{WT} and *E. coli*^{WT}. Although compounds **1b** and **1c** differ structurally only by the substituent on the pyridone nitrogen (benzyl vs. H), we were surprised to discover the striking difference between the two compounds in their effect on the macromolecular pathways. Against *S. aureus*^{WT}, **1c** showed preferential inhibition of DNA synthesis at concentrations below the MIC value (data not shown). Against *E. coli*^{WT}, **1c** displayed selective inhibition of DNA synthesis. Further, we determined that compound **1c** showed modest inhibition of *E. coli* DNA gyrase A (gyrA) with an IC_{50} of 11 μM.[14] However, reduced activity against *E. coli* topoisomerase IV (parC) was observed (~30% inhibition at a concentration of 100 μM). The identification of compounds with improved activity against Gram-negative pathogens and the striking change in mechanism observed between **1b** and **1c** prompted further investigation of these novel 4-hydroxy-2-pyridones.

The unique characteristics of the Gram-negative bacterial cell wall present a formidable obstacle to the optimization of antibac-

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