Isothiazolopyridones: Synthesis, Structure, and Biological Activity of a New Class of Antibacterial Agents

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Abstract: We report the syntheses of first-generation derivatives of isothiazolopyridones and their in vitro evaluation as antibacterial agents. These compounds, containing a novel heterocyclic nucleus composed of an isothiazolone fused to a quinolizin-4-one (at C-2 and C-3 of the quinolizin-4-one), were prepared using a sequence of seven synthetic transformations. The solid-state structure of 7-chloro-9-ethyl-1-thia-2,4a-diazacyclopenta[*b*]naphthalene-3,4-dione was determined by X-ray diffraction. The prepared derivatives of desfluoroisothiazolopyridones exhibited (a) antibacterial activity against Gram-negative and Gram-positive organisms, (b) inhibitory activities against DNA gyrase and topoisomerase IV, and (c) no inhibitory activity against human topoisomerase II.

Fluorinated quinolones (fluoroquinolones) are an important class of broad-spectrum antibacterial agents that operate bactericidally by inhibiting DNA gyrase[1,2] (bacterial topoisomerase II, the classical target) and topoisomerase IV[3] (a more recently recognized target, important particularly in Gram-positive pathogens).[4] Despite more than four decades of intensive investigation of quinolones, the 4-pyridone-3-carboxylic acid structure (Figure 1) remains a common feature of most potent inhibitors of DNA gyrase and topoisomerase IV. Positions C-3 and C-4 (the β-keto acid group collectively) are considered necessary for the binding of quinolones to DNA gyrase in the ternary complex.[5] Substitution at the 3-position is generally deleterious, although exceptions have been described. Replacement of the 3-carboxylic acid group of quinolones with isosteres such as sulfonic acid, acetic acid, hydroxamic acid, phosphoric acid, and sulfonamide resulted in reduced antibacterial activities.[6] One successful strategy for replacement of the 3-carboxylic acid group with retained (or enhanced) antibacterial activity employed a ring-fused isothiazolone group[7] (Figure 1, isothiazoloquinolone). These fluorinated isothiazoloquinolones (ITQs) exhibited in vitro antibacterial activity and inhibition of DNA gyrase that were superior to their 3-carboxylic acid counterparts (parent quinolones).[8,9] More recently, 2-pyridones[10] (quinolizin-4-ones)—bioisosteres of quinolones where the nitrogen atom of the quinolone core is interchanged with the bridgehead carbon atom (Figure 1)—have emerged as outstanding broad-spectrum antibacterial agents that are potent against organisms that are resistant to many of the clinically utilized fluoroquinolones.[11] Here, we report the syntheses and antibacterial evaluation of novel molecules that combine a ring-fused isothiazolone with a 2-pyridone heterocycle: first-generation isothiazolopyridones (ITPs).

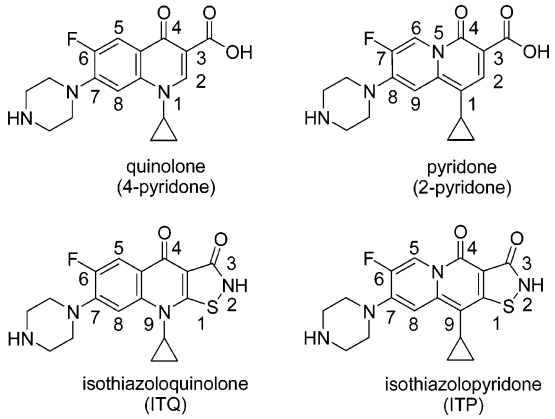

Figure 1. Structurally related antibacterial agents.

We designed our synthetic pathway to yield an intermediate compound having the novel ITP ring system with a leaving group (i.e., a halide) at C-7. We considered this approach to allow introduction of chemical diversity during the last step of the synthetic process via nucleophilic substitution of the labile group at C-7 with desired amines—a classical approach for the related quinolones to efficiently produce analogues for biological evaluation. Installation of a halide at C-7 also presents the opportunity to prepare analogues containing carbon-linked pendant groups via palladium-catalyzed cross-coupling methodologies.

The synthesis of ITPs began with low-temperature lithiation of 4-chloro-2-picoline with lithium diisopropylamide (LDA) followed by reaction of the generated lithium anion with ethyl iodide to give propylpyridine **2**[10] in 83% yield (Scheme 1). The success of the synthesis of ITPs relied on the following key steps that involved formation of quinolizinone **4** having sulfur at C-2. Reaction of ketene dithioacetal **1a**[12] (generated by successive reaction of diethyl malonate with sodium hydride, carbon disulfide, and methyl iodide) with the lithium anion of **2** (generated at low temperature using LDA as base) effected substitution of one of the thiomethyl units of **1a** to generate **3a** via a Michael addition−elimination process. Subsequent ring-closure of the Michael adduct **3a** at 120 °C in solutions of dimethyl sulfoxide (DMSO) furnished quinolizinone **4a** in 47% yield (based on **2**). Further synthetic elaboration of the quinolizinone core, namely introduction of the fused isothiazolone ring, presented a challenge. There are mild methods known for preparing fused isothiazolones (1,2-benzisothiazol-3(2*H*)-ones) from frameworks consisting of either a 2-mercaptobenzoic acid[13] or a 2-mercaptobenzoate[14] moiety. Our initial approach to prepare this necessary scaffold was manipulation of the methylated thiol **4a** using a two-step process:[14] first, oxidation of methyl thioether **4a** with *m*-chloroperbenzoic acid (*m*-CPBA) to give the corresponding sulfoxide and, second, displacement of the sulfinyl group of this sulfoxide with sodium hydrosulfide. Reaction of **4a** with *m*-CPBA in methylene chloride at room temperature generated sulfoxide **5** in 88% yield; however, reaction of **5** with sodium hydrosulfide did not effect displacement of the sulfinyl group to give **6**, but rather, effected only displacement of the chloride at C-7. The necessity of having a chloride at C-7 of the intermediate ITP for later amination

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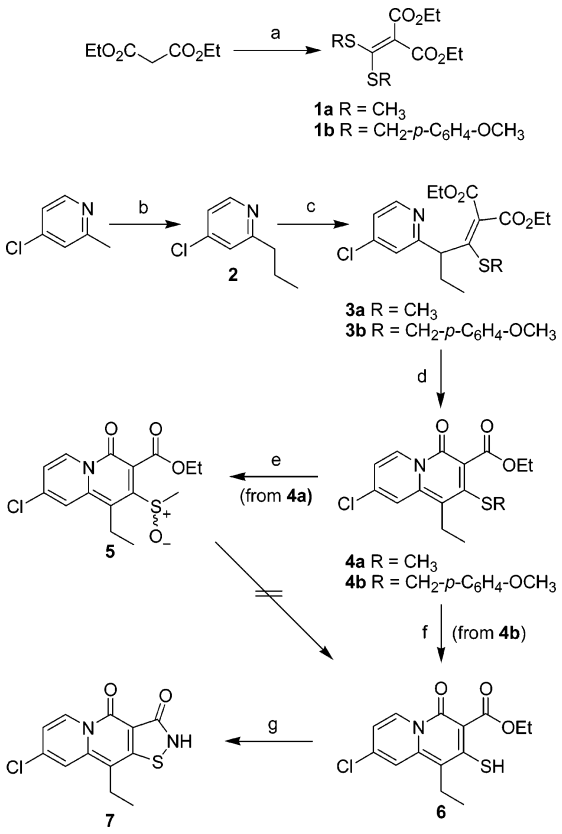
Scheme 1[a]



[a] Reagents and conditions: (a) NaH (2 equiv), DMF, 0 °C, 15−30 min, then CS_2 (2−3 equiv), 0 °C → rt, 1 h, then added alkyl halide (2−5 equiv), rt, 15 h, 69−85%; (b) LDA (1.1 equiv), THF, −78 °C, 30 min, then EtI, −78 → −30 °C, 1.5 h, 83%; (c) LDA (1.1 equiv), THF, −78 °C, 1.5 h, then added **1** (1 equiv), −78 → −15 °C → rt, 2.5−4 h; (d) DMSO, 120 °C, 5.5−42 h, 24−47% (two steps); (e) *m*-CPBA (∼1.1 equiv), CH_2Cl_2, rt, 1 h, 88%; (f) TFA/anisole, 40 °C, 23 h; (g) hydroxylamine-*O*-sulfonic acid (4 equiv), $NaHCO_3$ (10 equiv), THF/H_2O, 79% (two steps).

reactions precluded the use of this method employing sodium hydrosulfide. Our alternative strategy was replacement of the methyl group of **4a** with a protecting group that is susceptible to cleavage under mild conditions (nonnucleophilic nor nonreductive). We chose the 4-methoxybenzyl group for this purpose because it is cleaved under acidic conditions[15] and because it was adopted successfully in the synthesis of thio-containing quinolones.[16,17] The 4-methoxybenzyl thioether **4b** was prepared in 24% yield from **2** using the methods described above for the methyl analogue **4a**. Treatment of **4b** with trifluoroacetic acid containing anisole afforded the thio derivative **6**. To prevent oxidative degradation of **6**, we reacted this compound directly (without purification) with hydroxylamine-*O*-sulfonic acid under basic conditions to give the desired intermediate **7** in 79% yield (based on **4b**). Extensive 2D NMR experiments established the proton−carbon and carbon−carbon connectivities within the pyridone portion of **7**, i.e., correlations were observed for all carbons excluding the juxtaposed isothiazolo carbonyl carbon (C-3) and quaternary carbon (C-3a). To obtain further structural information, we labeled **7** with ^{15}N via reaction of **6** with [^{15}N]-hydroxylamine-*O*-sulfonic acid.[18] The ^{15}N NMR spectrum of [^{15}N]-**7** showed one resonance in the amido region[19] at 104.6 ppm, indicating the presence of an isothiazolo moiety. Furthermore, its ^{13}C NMR spectrum suggested that an isothiazolo ring had formed with one carbon atom proximal to the ^{15}N nucleus, i.e., the carbonyl resonance at 166.8 ppm was observed as a doublet (J = 3.5 Hz), indicating a ^{15}N-2-N/^{13}C-3-C coupling.

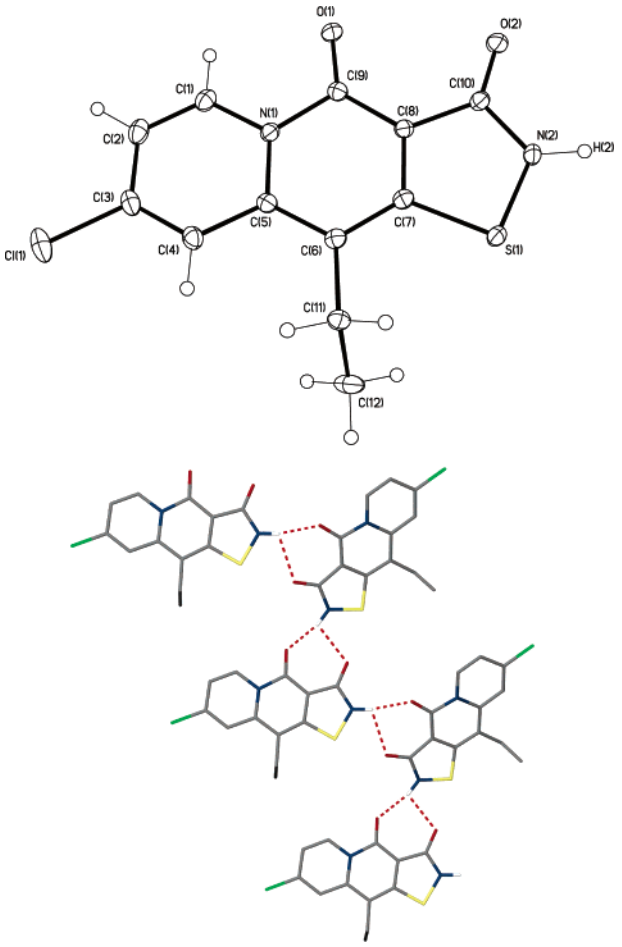
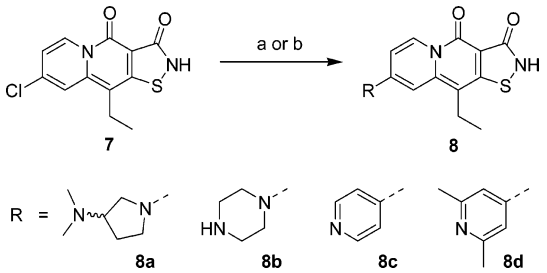


Figure 2. The solid-state structure of **7** as determined by X-ray diffraction. ORTEP view showing the atom-labeling scheme with thermal ellipsoids drawn at 30% probability (top) and view of hydrogen-bonded chains along the crystallographic *b*-axis (bottom). Selected bond lengths (Å) and angles (deg): S(1)−C(7), 1.7264(18); S(1)−N(2), 1.6913(16); N(2)−C(10), 1.377(2); O(2)−C(10), 1.227(2); C(8)−C(10), 1.466(2); C(8)−C(9), 1.412(3); O(1)−C(9), 1.231(2); N(1)−C(9), 1.449(2); C(8)−C(7)−S(1), 111.70(13); N(2)−S(1)−C(7), 90.82(8); C(10)−N(2)−S(1), 116.43(13); N(2)−C(10)−C(8), 107.91(16); C(7)−C(8)−C(10), 113.06(16).

An X-ray crystallographic study of a single crystal of **7** confirmed the proposed structure of the novel ITP heterocycle (Figure 2). The amido hydrogen of the isothiazolo moiety, H(2), was located from the electron difference map and refined to a distance of 0.93(2) Å. The NH group was hydrogen bound to the carbonyl groups of an adjacent molecule with H(2)−O interatomic distances of 2.01 and 2.48 Å for O(1) and O(2), respectively. This interaction created infinitely extending chains of hydrogen-bonded molecules propagating along the crystallographic *b*-axis. The N(2)−O interatomic distances were 2.92 Å for both O(1) and O(2) of the adjacent molecule and the N(2)−H(2)−O angles were measured at 166.1 and 109.0 ° for O(1) and O(2), respectively. In contrast, isothiazol-3-ols (the hydroxy tautomers of isothiazol-3-ones) form hydrogen-bonded dimers in the solid state.[20]

Our final synthetic step was adornment of the ITP nucleus with traditionally favored nitrogen heterocycles and heteroaromatic carbon isosteres[21] (Scheme 2). Displacement of the chloride of **7** with excess (*rac*)-3-(dimethylamino)pyrrolidine and piperazine proceeded smoothly under microwave irradiation (MWI)[22] in solutions of DMSO to give the corresponding nitrogen-coupled ITPs **8a** and **8b** in 63% and 60% yield,

Scheme 2[a]



[a] Reagents and conditions: (a) amine (6 equiv), DMSO, MWI (120 °C), 5 min, 60−63%; (b) boronic acid (5 equiv), $NaHCO_3$ (15 equiv), $Pd(PPh_3)_4$ (15 mol %), DMF/H_2O, MWI (110 °C), 15 min, 76−79%.

Table 1. In Vitro Antibacterial Activities of ITPs[a]

	E. coli		*S. aureus*	
compd	MIC	DNA gyrase	MIC	Topo IV
CIP	0.02 (0.05)	0.3	0.25 (0.68)	2.3
NOR	0.06 (0.19)	0.6	0.50 (1.6)	3.5
8a	4.0 (11)	24	8.0−16 (22−45)	79
8b	4.0 (12)	14	16 (48)	37
8c	0.25 (0.69)	0.4	0.125 (0.35)	8.1
8d	1.0 (2.6)	0.2	0.125 (0.32)	6.3

[a] MICs expressed in μg/mL (μM). *E. coli* = *Escherichia coli* ATCC 25922 (Gram negative); *S. aureus* = *Staphylococcus aureus* ATCC 29213 (Gram positive). Inhibition of DNA gyrase supercoiling (IC_{50}) and topoisomerase IV (Topo IV) decatenation (IC_{50}) are expressed in μM.

respectively. Microwave-assisted Suzuki−Miyaura cross-coupling of chloride **7** with 4-pyridinylboronic acid and 2,6-dimethyl-4-pyridinylboronic acid[23] afforded carbon-coupled ITPs **8c** and **8d** in 76% and 79% yield, respectively.

Analogues **8a**−**d** were tested against Gram-negative and Gram-positive bacteria (Table 1), and their activities were compared with those of the fluoroquinolones ciprofloxacin (CIP) and norfloxacin (NOR). The in vitro results in Table 1 are reported as (a) minimum inhibitory concentrations (MICs)[26] against *E. coli* and *S. aureus*, and (b) inhibitory activities against their respective target enzymes, DNA gyrase[27] and topoisomerase IV.[28] The nitrogen-coupled analogues (**8a** and **8b**) showed moderate antibacterial activity, having MICs of 4.0−16 μg/mL. The carbon-coupled analogues (**8c** and **8d**), however, demonstrated stronger antibacterial activity than the nitrogen-coupled analogues, having MICs of 0.125−1.0 μg/mL. In particular, the activities of the carbon-coupled analogues against *S. aureus* were 64−128-fold greater than those of the nitrogen-coupled analogues. A similar increase in activity against *S. aureus* was observed previously when the piperazinyl group of ciprofloxacin was replaced with 4-pyridinyl groups.[29] The significant differences in MICs between the nitrogen- and carbon-coupled analogues of **8** likely resulted from the corresponding differences in inhibition of the cellular targets, *E. coli* DNA gyrase and *S. aureus* topoisomerase IV (Table 1): the most potent inhibitors of DNA gyrase and topoisomerase IV demonstrated the most potent antibacterial activity. We note that none of the analogues exhibited activity in the *S. aureus* DNA gyrase supercoiling assay (IC_{50} >200 μM). In addition, analogues **8a**−**8d** inhibited bacterial enzymes selectively, displaying no activity against the mammalian counterpart human topoisomerase II (EC_2 >150 μM).[30]

Although the MICs of **8** against *E. coli* are modest by current standards, these data are similar to those of related desfluoro-2-pyridones reported in an earlier study[10] that demonstrated the necessity of a fluoride at C-6 for strong antibacterial activity (these investigators observed a ~300-fold increase in the activity of their fluoro analogues compared with their respective desfluoro analogues). We believe that installation of a fluoride at C-6—giving second-generation fluoroisothiazolopyridones—should substantially improve the antibacterial activity of this class of compounds. This strategy, involving adaptation of the concise synthetic route described in this report, is the focus of work underway in our laboratory.

Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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