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Isothiazoloquinolones containing functionalized aromatic hydrocarbons at the 7-position: Synthesis and in vitro activity of a series of potent antibacterial agents with diminished cytotoxicity in human cells

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Abstract—This report describes 9*H*-isothiazolo[5,4-*b*]quinoline-3,4-diones (ITQs) containing aromatic groups at the 7-position that were prepared using palladium-catalyzed cross-coupling and tested against a panel of susceptible and resistant bacteria. In general, these compounds were more effective against Gram-positive than Gram-negative organisms. Many of the ITQs were more potent than contemporary quinolones and displayed a particularly strong antistaphylococcal activity against a clinically important, multi-drug-resistant strain. In contrast with ITQs reported previously, several of the analogues described in this Letter demonstrated low cytotoxic activity against a human cell line.

The quinolones are a well-known class of broad-spectrum bactericidal agents that inhibit essential type II bacterial topoisomerases such as DNA gyrase[1,2] and topoisomerase IV.[3] Although several generations of quinolones have emerged in the 40 years since their initial discovery, the core structure of therapeutically useful quinolones continues to incorporate the 4-pyridone-3-carboxylic acid moiety (Fig. 1).[4] Bioisosteric substitution of the carboxylic acid at C-3 is generally detrimental to the antibacterial activity of quinolones;[5] however, replacement of this group with a ring-fused isothiazolone surrogate enhanced bioactivity (2- to 10-fold based on limited published data) of the resulting isothiazoloquinolones (ITQs, Fig. 1).[6–9] These tricyclic quinolones utilized small nitrogen-containing heterocycles attached directly to the ITQ nucleus at C-7 through a C–N bond (nitrogen-coupled). Unfortunately, these potent antibacterial agents inhibited both bacterial and

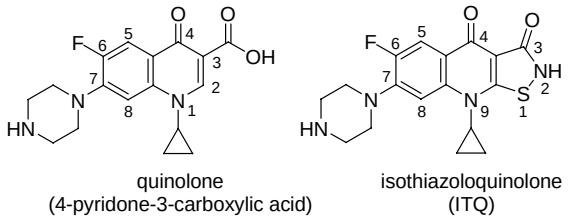

Figure 1. Structures and numbering schemes of a quinolone (ciprofloxacin) and an isothiazoloquinolone (A-62824).

mammalian topoisomerase II[10] and caused related topoisomerase II-mediated DNA breakage in cell-free assays—an activity that was correlated with mammalian cytotoxicity.[11] Another successful approach for preparing potent analogues with structures that deviate from conventional quinolones is replacement of the amino substituents at C-7—groups studied extensively because of their strong influence on potency and safety of quinolones[12]—with aromatic groups bonded directly to the quinolone nucleus through a C–C bond (carbon-coupled).[13,14] Garenoxacin, a quinolone of this subclass that was described recently,[15–17] has shown great promise in advancing toward the market due, in part, to its strong

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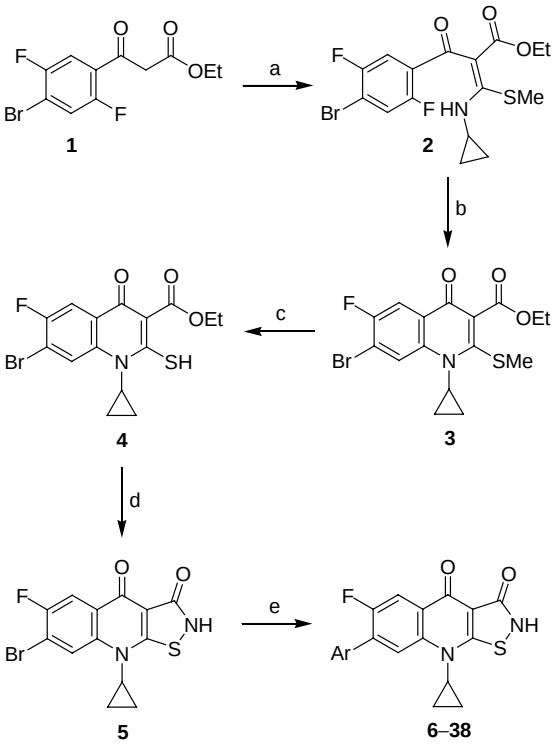
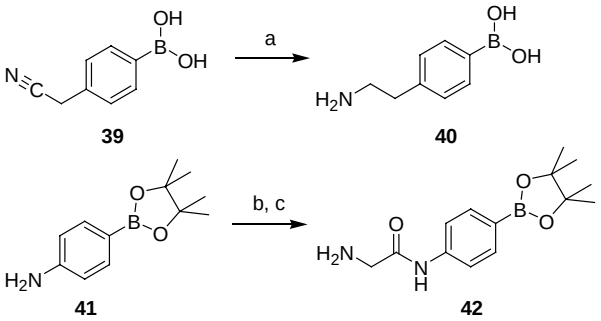
activity against quinolone-resistant Gram-positive strains. As part of our efforts to develop new quinolones with exceptional potency against emerging quinolone-resistant organisms, we describe the synthesis and antibacterial evaluation of novel ITQs that contain aromatic groups carbon-coupled to the 7-position. Specifically, we limit our discussion to analogues that contain functionalized phenyl substituents. Our primary objectives are (a) to optimize the potency of these compounds against resistant strains of *Staphylococcus aureus* and (b) to restrict their mammalian cytotoxicity to concentrations that exceed their therapeutic range.

Our synthetic strategy involved introduction of carbon-linked pendant groups via palladium-catalyzed cross-coupling of organoboron compounds with an ITQ nucleus containing a bromide at C-7. The 7-bromo ITQ nucleus was prepared from keto ester **1**[14] using the synthetic sequence outlined in Scheme 1 that followed an earlier report on the synthesis of the 7-fluoro ITQ nucleus.[18] Sequential treatment of **1** with cyclopropyl isothiocyanate, sodium hydride, and methyl iodide at room temperature generated ketene *N*,*S*-acetal **2** in 94% yield. Subsequent reaction of **2** with sodium hydride at 75 °C effected ring closure to give **3** in 93% yield. Unlike that reported for the 7-fluoro ITQ nucleus, we found that oxidation of **3** with *m*-chloroperbenzoic acid followed by displacement of the resulting methyl sulfinyl group with sodium hydrosulfide was unnecessary: methyl thioether **3** was converted directly to thiol **4** in 84% yield using excess anhydrous sodium hydrosulfide with gentle heating in tetrahydrofuran. To prevent oxidative degradation of **4**, we reacted this compound directly (without purification) with hydroxylamine-*O*-sulfonic acid under basic conditions to give the desired ITQ intermediate **5**[19] in 85% yield. Microwave-assisted Suzuki–Miyaura cross-coupling of the ITQ nucleus **5** with the desired boronic acids or dioxaborolanes furnished derivatives **6**–**38**, typically, in 30–50% yield after HPLC purification,[20] with 2-substituted phenyl derivatives having the lowest yields. The boronic acids and dioxaborolanes used in this study were either (a) purchased from commercial suppliers or (b) prepared from the corresponding commercially available bromides using standard methods.[21] The exceptions were boronic acid **40** and dioxaborolane **42** (Scheme 2) that were used to prepare ITQ analogues **35** and **36**, respectively.



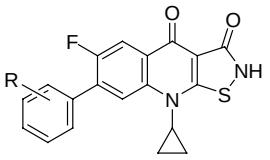
Scheme 1. Reagents and conditions: (a) *c*-PrNCS (1.7 equiv), DMF, rt, then NaH (1.07 equiv), 0 °C → rt, 20 h, then MeI (1.7 equiv), rt, 4 h, 94%; (b) NaH (1.05 equiv), DMF, 75 °C, 18 h, 93%; (c) anhydrous NaSH (1.5 equiv), THF, 45 °C, 18 h, 84%; (d) H_2NOSO_3H (4.2 equiv), $NaHCO_3$ (10 equiv), H_2O/THF, 85%; (e) $ArB(OR')_2$ where Ar = aryl and R′ = H or $–C(CH_3)_2–$ (2–3 equiv), $NaHCO_3$ (10 equiv), $Pd(PPh_3)_4$ (5 mol %), DMF/H_2O, MWI (130 °C), 10–20 min, 30–50% yield after HPLC purification.

The minimum inhibitory concentrations (MICs)[22] of ITQ analogues were determined against quinolone-susceptible and quinolone-resistant pathogens (Tables 1 and 2), and were compared directly with that of their corresponding 3-carboxylic acid counterpart ciprofloxacin (CIP). Because this class of compounds was also explored previously as antineoplastic agents,[11] we tested their cytotoxic activity in human Hep2 cells (laryngeal carcinoma).[23] Most of the ITQs were more effective against Gram-positive (*S. aureus*) than Gram-negative (*Escherichia coli*) organisms. Several ITQs were exceptionally potent (MICs $\leqslant$1 μg/mL) against methicillin-resistant *S. aureus* (MRSA, Table 1)—activities that were superior to those of contemporary quinolones such as gatifloxacin (GAT), gemifloxacin (GEM), and moxifloxacin (MFX). One of the most active compounds that we tested against MRSA was the hydroxy analogue **18**. This compound, however, showed strong inhibitory activity against human topoisomerase II[24] (EC_2 = 15 μM[25]) and strong cytotoxic activity (Table 1). Nitrogen-coupled ITQs described previously[10,11] (e.g., A-62824, Fig. 1) also showed strong activity against mammalian topoisomerase II (EC_2 = 20 μM) and strong cytotoxic activity (Table 1). Our data suggest that this undesirable activity may not be a class effect for ITQs,[26] but rather, a complex function that relies heavily on the contributions of the individual C-7 components. For example, as



Scheme 2. Reagents and conditions: (a) H_2 (3 atm), 10% Pd/C, EtOH, rt, 48 h, quant.; (b) Boc-glycine (1.5 equiv), BOP (1.5 equiv), DIEA (4.2 equiv), DMF, rt, 3 h, 70%; (c) neat TFA, rt, 1 h, quant.

Table 1. Minimum inhibitory concentrations (MICs) and cytotoxic activities of ITQs[a]



Compound	R	MIC (μg/mL)			Cytotox (72 h) CC_{50} (μM)
		Ec	Sa	MRSA ($M^RQ^RV^I$)	
CIP		0.02	0.25	32	>100
A-62824		0.004	0.05	4.0	7
GAT		0.015	0.125	8.0	67
GEM		0.015	0.03	2.0	46
MFX		0.015	0.06	2.0	>100
6	H	0.06	0.004	16	>100
7	3-$H_2NC(O)$	0.125	0.008	2.0	ND
8	3-Ac	0.125	0.004	2.0	>100
9	3-AcNH	0.125	0.004	64	ND
10	2-NC	2.0	0.125	>64	>85
11	3-NC	0.03	0.001	0.125	>100
12	4-NC	1.0	0.03	16	>100
13	4-NC-CH_2	0.02	0.002	0.50	84
14	2-F	0.125	0.015	8.0	>100
15	3-F	0.125	0.004	1.0	96
16	4-F	0.25	0.03	32	>100
17	3-HO	0.06	0.004	0.25	>100
18	4-HO	0.03	0.004	0.125	8
19	2-MeO	2.0	0.125	32	>84
20	3-MeO	0.125	0.004	2.0	26
21	4-MeO	0.125	0.015	2.0	91
22	3-MeO-4-HO	0.06	0.001	0.125	4
23	4-HO-3,5-Me_2	0.125	0.001	2.0	ND
24	3-HO-CH_2	0.125	0.008	2.0	ND
25	4-HO-CH_2	0.06	0.004	4.0	25
26	2-H_2N	0.125	0.03	64	46
27	3-H_2N	0.004	0.008	8.0	>100
28	4-H_2N	0.06	0.004	0.50	34
29	3-H_2N-4-Me	0.125	0.008	2.0	18
30	3-H_2N-4-F	0.125	0.008	0.50	28
31	3-Me_2N	2.0	0.06	32	>100
32	4-Me_2N	0.50	0.001	4.0	51
33	3-H_2N-CH_2	0.015	0.015	2.0	8
34	4-H_2N-CH_2	0.015	0.03	8.0	4
35[b]	4-$H_2N(CH_2)_2$	0.06	0.02	>64	11
36[b]	4-$H_2NCH_2C(O)NH$	1.0	0.25	64	>75
37[c]	3-(2-Piperidinyl)	0.125	0.06	8.0	4
38[c]	4-(2-Piperidinyl)	0.25	0.125	4.0	4

[a] *Abbreviations:* CIP, ciprofloxacin; Ec, *Escherichia coli* ATCC 25922; GAT, gatifloxacin; GEM, gemifloxacin; MFX, moxifloxacin; $M^RQ^RV^I$, methicillin- and quinolone-resistant, vancomycin intermediate-resistant; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 700699; ND, not determined; Sa, *Staphylococcus aureus* ATCC 29213.

[b] Prepared from the custom-made boronic acid or dioxaborolane (Scheme 2).

[c] Prepared from the custom-made dioxaborolane derived from the commercially available bromide.

shown previously for the quinolones,[24] transposition of the 4-hydroxy group of **18** to the 3-position (**17**) strongly diminished the cytotoxic activity. Several other ITQs listed in Table 1 also exhibited low cytotoxicity (CC_{50} >100 μM). Compound **15** also demonstrates that both strong cytotoxicity and inhibition of human topoisomerase II are not class effects for all ITQs: **15** exhibited a 4- to 12-fold better activity (MICs) against *S. aureus* strains and >7-fold less cytotoxic and human topoisomerase II activity than A-62824 (**15**, CC_{50} = 96 μM and EC_2 >150 μM). In addition, ITQs were active against the target enzymes, inhibiting gyrase (*E. coli*) and topoisomerase IV (*S. aureus*) at levels comparable with those of quinolones (Table 2).[27]

In general, substitution at the 2-position of the C-7 phenyl group effected significant reduction in antibacterial activity (increase in MIC) when compared directly with those of the 3- and 4-substituted counterparts (Table 1). For most substituents, the activity of 3-substituted analogues against MRSA was greater than that of the corresponding 4-substituted analogues (e.g., cf. **11** and

Table 2. Minimum inhibitory concentrations (MICs) and activities against the target enzymes gyrase and topoisomerase IV of selected ITQs[a]

Compound	Ec MIC (μg/mL)	Gyrase (WT Ec) IC_{50} (μM)	Sa MIC (μg/mL)	Topo IV (WT Sa) IC_{50} (μM)
CIP	0.02	0.3	0.25	1.0
A-62824	0.004	0.1	0.05	0.5
GAT	0.015	0.5	0.125	1.2
GEM	0.015	0.5	0.03	0.3
MFX	0.015	0.3	0.06	0.8
15	0.125	1.0	0.004	7.2
17	0.06	0.2	0.004	1.6
18	0.03	0.8	0.004	25
24	0.125	0.5	0.008	8.2
25	0.06	0.9	0.004	41
27	0.004	3.1	0.008	10
28	0.06	0.3	0.004	12
30	0.125	0.3	0.008	10
33	0.015	0.1	0.015	0.2
34	0.015	0.1	0.03	1.5
37	0.125	0.7	0.06	2.7
38	0.25	1.0	0.125	2.6

[a] *Abbreviations:* CIP, ciprofloxacin; Ec, *Escherichia coli* ATCC 25922; GAT, gatifloxacin; GEM, gemifloxacin; MFX, moxifloxacin; ND, not determined; WT, wild-type; Sa, *Staphylococcus aureus* ATCC 29213.

15 with **12** and **16**, respectively). In many instances, inclusion of OH or NH_2 into the C-7 phenyl group also imparted strong activity against susceptible *E. coli* (Table 2). Further modification of the OH and NH_2 groups (e.g., one-carbon homologation and methylation) yielded analogues with reduced overall antibacterial activity.

In summary, we described novel carbon-coupled ITQs that displayed potent antistaphylococcal activities (MICs ~0.1 μg/mL against MRSA) that are superior to those of ciprofloxacin, gatifloxacin, gemifloxacin, and moxifloxacin. Several of these ITQs exhibited cytotoxicity at concentrations (CC_{50} >100 μM) well above their projected therapeutic range. Unlike data for previously reported compounds of this class, our data suggest that high mammalian cytotoxicity is not a class effect of ITQs and can be attenuated by judicious choice of substituents placed at the 7-position. A small set of compounds (**11**, **13**, **15**, and **17**) displayed acceptable levels of cellular toxicity (CC_{50} >80 μM) and in vitro activity against MRSA (⩽1 μg/mL) that are necessary for consideration as candidates for further in vitro and in vivo profiling.

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19. **5**: ^{1}H NMR (300 MHz, DMF-d_7, 60 °C): δ 1.36 (m, 2H), 1.48 (m, 2H), 3.70 (m, 1H), 8.04 (d, $J_{H\text{-}F}$ = 8.5 Hz, 1H), 8.45 (d, $J_{H\text{-}F}$ = 5.5 Hz, 1H). $^{13}C\{^1H\}$ NMR (75 MHz, DMF-d_7, 60 °C): δ 9.5, 32.7, 108.0, 112.3 (d, $J_{C\text{-}F}$ = 24.0 Hz), 115.3 (d, $J_{C\text{-}F}$ = 23.0 Hz), 123.0, 126.6 (d, $J_{C\text{-}F}$ = 5.5 Hz), 140.1 (d, $J_{C\text{-}F}$ = 2.0 Hz), 155.7 (d, $J_{C\text{-}F}$ = 244.5 Hz), 165.3, 170.4, 172.6 (d, $J_{C\text{-}F}$ = 2.0 Hz). $^{19}F\{^1H\}$ NMR (282 MHz, DMF-d_7, 60 °C): δ −115.4 (s). HRMS *m/z* calcd for $C_{13}H_8{}^{79}BrFN_2NaO_2S$ 376.9372 ($[M+Na]^+$); found 376.9367. Anal. Calcd for $C_{13}H_8BrFN_2O_2S$: C, 43.96; H, 2.27; N, 7.89. Found: C, 43.73; H, 2.50; N, 7.54.
20. General procedure: Under an atmosphere of argon, a reaction vessel was charged with 7-bromo-9-cyclopropyl-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione (0.1 mmol), dimethylformamide (4 mL), tetrakis(triphenylphosphine)palladium(0) (0.005 mmol), the desired boronic acid or dioxaborolane (0.2–0.3 mmol), and a 1 M aqueous solution of sodium bicarbonate (1 mmol). The resulting mixture was irradiated with microwaves (CEM Discover) at 130 °C for 10–20 min, allowed to cool, and evaporated to dryness under reduced pressure. The isolated residues were purified using preparative HPLC to give the desired products. Preparative HPLC was performed using a YMC Pack Pro C18 150 × 20.0 mm × 5 μm column with an isocratic elution of 0.35 min at 90:10 H_2O/CH_3CN containing 0.1% TFA followed by a 23-min linear gradient elution from 90:10 to 10:90 at a flow rate of 18.9 mL/min with UV detection at 254 nm. The purified products were isolated as TFA salts and were converted to the corresponding hydrochloride salts by addition of a solution of hydrogen chloride (~1.25 M in methanol) followed by evaporation; this process was repeated twice.
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testing: 11th informational supplement. Vol. 21, no. 1, M100-S11. National Committee for Clinical Laboratory Standards, Wayne, PA). The MIC was defined as the lowest concentration of each compound resulting in inhibition of visible growth of bacteria after incubation at 37 °C for 18–24 h.

23. The CC_{50} value is defined as the concentration of drug that is lethal to 50% of the cells. The Hep2 human laryngeal carcinoma cell line was incubated with drug at 37 °C for 72 h to generate eight-point CC_{50} data.
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27. The IC_{50} value is defined as the concentration of drug required to inhibit 50% of the supercoiling (decatenation) of double-stranded DNA catalyzed by gyrase (topoisomerase IV).