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Biological evaluation of isothiazoloquinolones containing aromatic heterocycles at the 7-position: In vitro activity of a series of potent antibacterial agents that are effective against methicillin-resistant *Staphylococcus aureus*

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Abstract—We synthesized a diverse series of 9*H*-isothiazolo[5,4-*b*]quinoline-3,4-diones containing heteroaromatic groups at the 7-position via palladium-catalyzed cross-coupling. Many of these compounds demonstrated potent antistaphylococcal activity (MICs $\leqslant 2$ μg/mL) against a multi-drug-resistant strain (ATCC 700699) and low cytotoxic activity ($CC_{50} > 100$ μM) against the human cell line Hep2 (laryngeal carcinoma).
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Isothiazoloquinolones (ITQs) are an under-explored subclass of fluoroquinolones that were investigated initially by Chu and coworkers at Abbott.[1–3] ITQs represent one of the few examples of successful replacement of the archetypal 3-carboxylic acid of fluoroquinolones (i.e., with a ring-fused isothiazolone, Fig. 1) without compromising antibacterial activity.[4] Compound A-62824, for example, was reported as ~10-fold more potent in vitro than the corresponding fluoroquinolone ciprofloxacin.[5,6] The ITQs reported by Abbott were limited to analogues that contained amino substituents at C-7 attached directly via a C–N bond (nitrogen coupled).[7] In addition to inhibiting bacterial topoisomerase II, these potent antibacterial agents unfortunately inhibited mammalian topoisomerase II[8] causing topoisomerase II-mediated DNA breakage, which correlated with mammalian cellular toxicity.[9] We reported recently,[10] however, that several carbon-coupled ITQs (**1**, Fig. 1)—having functionalized phenyl groups at C-7—showed reduced cytotoxicity in a human cell line and

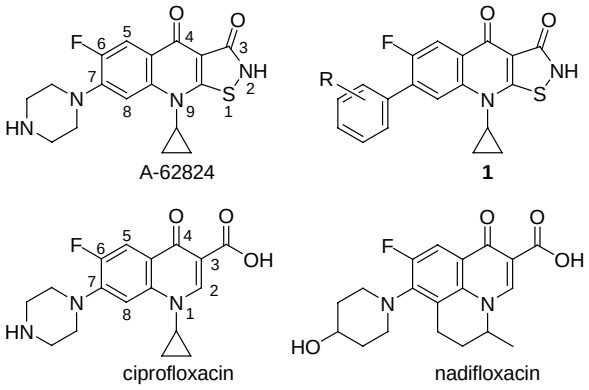

Figure 1. Examples and numbering schemes of known isothiazoloquinolones (top) and fluoroquinolones (bottom).

increased activity against methicillin-resistant *Staphylococcus aureus* (MRSA) when compared with A-62824 and contemporary fluoroquinolones (e.g., ciprofloxacin). Although analogues of **1** demonstrated potent in vitro activity against MRSA,[10] they may not necessarily perform well in vivo because most—in contrast with all marketed fluoroquinolones, except the topically administered nadifloxacin (Fig. 1)—lack the distal

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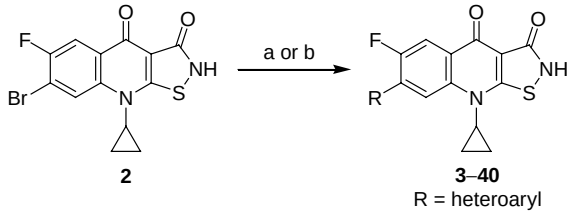
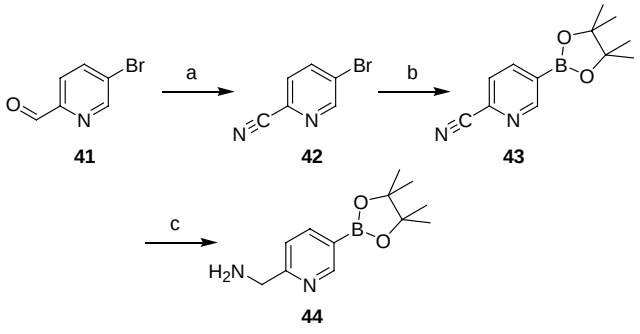
amine of the amino C-7 substituent that generally affords good in vivo efficacy.[11] We believe that substitution of non-amino functionalized phenyl groups with nitrogen-containing heteroaromatic groups at C-7 of **1** may address this potential liability. We herein report the in vitro biological evaluation of such heteroaryl analogues in an effort to fully explore carbon-coupled ITQs as potential antistaphylococcal agents.

We introduced carbon-coupled groups to C-7 of the ITQ core via palladium-catalyzed (Suzuki–Miyaura and Stille) cross-coupling of bromide **2**[10] with organoboron and organotin compounds (Scheme 1). The heteroaromatic stannanes, boronic acids, and dioxaborolanes used in this study were either (a) purchased from commercial suppliers, (b) prepared from the corresponding known[12] or commercially available bromides using standard methods,[13,14] or (c) prepared as shown in Schemes 2[15] and 3.

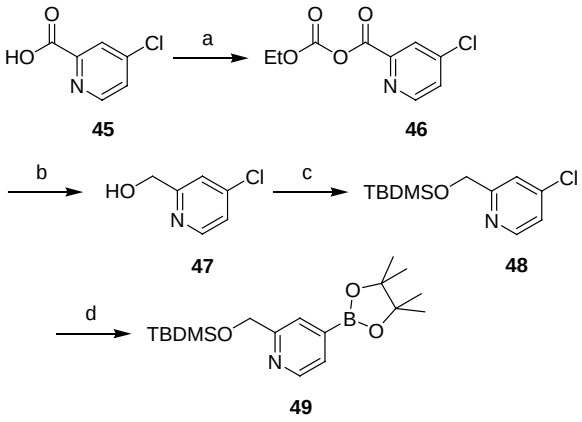
Analogues **3**–**40** (Fig. 2) were tested against Gram-negative (*Escherichia coli*) and Gram-positive (*S. aureus*) organisms (Table 1) using standard techniques.[16] These minimum inhibitory concentration (MIC) values were compared with those of ciprofloxacin (CIP) and moxifloxacin (MFX). Although the activities of several ITQs against Gram-negative bacteria were inferior to those of contemporary quinolones (CIP and MFX), several analogues displayed activities that were superior against



Scheme 1. Reagents and conditions: (a) $RB(OR')_2$ where R = heteroaryl and R′ = H or $–C(CH_3)_2–$ (3–4 equiv), $NaHCO_3$ (10 equiv), $Pd(PPh_3)_4$ (5–10 mol %), DMF/H_2O, MWI (130 °C), 10–20 min, 30–50% yield after HPLC purification; (b) $RSnBu_3$ where R = heteroaryl (2 equiv), $Pd(PPh_3)_4$ (5 mol %), DMF, MWI (130 °C), 20 min, 35% yield after HPLC purification.



Scheme 2. Reagents and conditions: (a) I_2 (1.1 equiv), NH_4OH (28% in H_2O, excess), THF, rt, 4 h, quant.; (b) bis(pinacolato)diboron (1.1 equiv), 10 mol % $PdCl_2$(dppf), KOAc (3.4 equiv), DMSO, 80 °C, 25 h, 64%; (c) H_2 (1 atm), 10% Pd/C, HOAc, rt, 16 h, quant.



Scheme 3. Reagents and conditions: (a) EtOCOCl (1.05 equiv), Et_3N (1.05 equiv), C_6H_6, rt, 1 h; (b) LAH (1.05 equiv), THF, −78 °C, 0.5 h, 66% (2 steps); (c) TBDMSCl (1.1 equiv), imidazole (5 equiv), DMF, rt, 15 h, 96%; (d) bis(pinacolato)diboron (1.1 equiv), 15 mol % $Pd(OAc)_2$, 30 mol % 1,3-bis(2,6-diisopropylphenyl)-4,5-dihydroimidazolium chloride, KOAc (2.5 equiv), THF, reflux, 15 h, used directly in subsequent Suzuki–Miyaura coupling.

methicillin-sensitive *S. aureus* (MSSA, MICs <0.02 μg/mL) and multi-drug-resistant MRSA (MICs ⩽2 μg/mL). Strong activities against MSSA tracked well with the corresponding activities against MRSA. The most active compound tested against MRSA was **21**, with an MIC of 0.25 μg/mL. Analogues containing representative 3-pyridinyl, 4-pyridinyl, 5-pyrimidinyl, and 5-quinolinyl groups at C-7 were tested against a panel of organisms (Table 2), the results of which confirmed the broad antibacterial coverage of ITQs against many Gram-positive (e.g., *S. aureus* and *S. pneumoniae*) and specific Gram-negative (i.e., *H. influenzae* and *M. catarrhalis*) pathogens. With these results in mind, we optimized (a) the potency of ITQs against a clinically important, multi-drug-resistant strain of *S. aureus* and (b) the restricted cytotoxic activity of ITQs against a mammalian cell line (Hep2).

For monocyclic heterocycles, the antibacterial activity (Table 1) decreased generally in the order 4-pyridinyl < 3-pyridinyl < 5-pyrimidinyl < 2-(1*H*-pyrrolyl) < 2-pyridinyl < 2-pyrazinyl. Typically, analogues containing amino, hydroxy, and methoxy substituents were less potent against MRSA than their parent (unsubstituted) analogues, which were, in turn, less potent than those containing methyl and fluorine substituents. Addition of a single methoxy group to 3-pyridines and 5-pyrimidines did not improve their antibacterial activity against MRSA (**24**, **25**, and **27**); however, addition of another methoxy group ortho to the biaryl linkage effected improved activity (**23** and **28**). Installation of methyl substituents at the C-2/C-6 positions of 3- and 4-pyridinyl groups generally afforded potent ITQs. There was no apparent antibacterial advantage in adding fluorine substituents to the 3- and 4-pyridinyl groups (e.g., **13** and **14**); however, the resulting ITQs maintained low cytotoxic activity comparable with their parent compounds. In contrast, addition of methyl groups to the C-2/C-6 positions (e.g., **10** and **11**) and methoxy groups to the

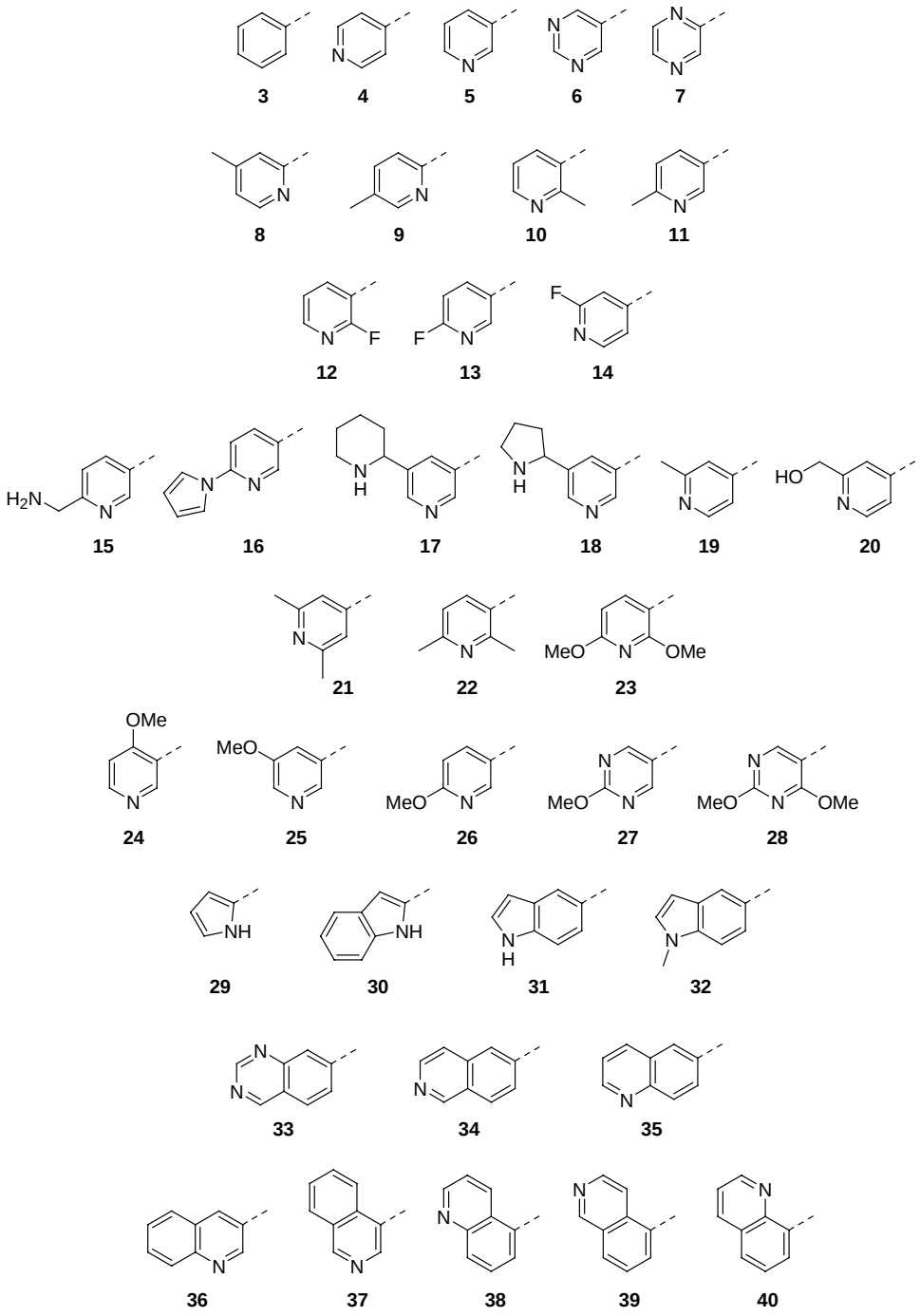


Figure 2. Heteroaryl groups and numbering of ITQs used in this study.

C-3/C-4 positions (**24** and **25**) of the heterocyclic substituents usually increased the cytotoxic activity. Further addition of a methyl group (**22**) or modification of the existing methyl group (**15** and **20**) usually caused reduced cytotoxicity at the expense of antibacterial activity against MRSA.

Of the limited number of bicyclic heterocycles that we investigated, 6-isoquinolinyl (**34**) and 6-quinolinyl (**35**) groups exhibited the best antibacterial activity, having MICs of 1 and 0.5 μg/mL, respectively, against MRSA. We also observed low cytotoxicity, generally, for ITQs with the least sterically crowded biaryl linkage (cf. **33**, **35**, and **36** with **37**, **39**, and **40**).

Several ITQs demonstrated strong inhibition of wild-type *S. aureus* topoisomerase IV that corresponded to strong antibacterial activity against susceptible *S. aureus*. We note, however, the poor correlation of in vitro enzymatic inhibition and MICs of ITQs. An obvious example is compound **21**, which showed a 64-fold increase in potency against *S. aureus* (when compared with ciprofloxacin) without a concurrent improvement in inhibition of topoisomerase IV. This poor correlation

Table 1. In vitro antibacterial and cytotoxic activities of ITQs[a]

Compound	R Group	Substitution on R group	MICs (μg/mL)			Topo IV (WT Sa) IC_{50} (μM)	Cytotox (72 h) CC_{50} (μM)
			Ec	Sa	MRSA ($M^RQ^RV^I$)		
CIP			0.02	0.25	32	1.0	>100
MFX			0.015	0.06	2.0	0.8	>100
3	Phenyl	None	0.060	0.0035	16	6.3	>100
4	4-Pyridinyl	None	0.028	0.0035	1.0	2.2	ND
5	3-Pyridinyl	None	0.0035	0.0071	2.0	0.5	>100
6	5-Pyrimidinyl	None	0.12	0.025	8.0	3.3	18
7[b]	2-pyrazinyl	None	1.0	0.12	>64	64	>91
8	2-Pyridinyl	4-Me	0.25	0.06	32	16	>100
9	2-Pyridinyl	5-Me	0.12	0.044	32	14	51
10[c]	3-Pyridinyl	2-Me	0.51	0.13	1.0	3.5	12
11[c]	3-Pyridinyl	6-Me	0.037	0.0037	1.0	8.5	31
12	3-Pyridinyl	2-F	0.015	0.015	ND	2.4	>100
13	3-Pyridinyl	6-F	0.030	0.015	2.0	12	>100
14	4-Pyridinyl	2-F	0.059	0.0074	1.0	19	>100
15[d]	3-Pyridinyl	6-H_2NCH_2	0.059	0.13	32	0. 7	>100
16[e]	3-Pyridinyl	6-(1-Pyrrolyl)	1.0	0.029	4.0	18	>100
17[e]	3-Pyridinyl	5-(2-Piperidinyl)	0.46	0.12	8.0	5.0	6
18[e]	3-Pyridinyl	5-(2-Pyrrolidinyl)	0.12	0.06	8.0	1.1	25
19	4-Pyridinyl	2-Me	0.059	0.0040	0.50	26	36
20[f]	4-Pyridinyl	2-$HOCH_2$	0.14	0.034	32	4.8	>100
21	4-Pyridinyl	2,6-Me_2	0.031	0.0038	0.25	48	50
22[c]	3-Pyridinyl	2,6-Me_2	0.5	0.13	>64	8.0	>100
23	3-Pyridinyl	2,6-$(MeO)_2$	2.0	0.046	8.0	16	98
24	3-Pyridinyl	4-MeO	0.25	0.023	4.0	10	50
25	3-Pyridinyl	5-MeO	0.061	0.03	4.0	21	34
26	3-Pyridinyl	6-MeO	0.015	0.023	16	26	>100
27	5-Pyrimidinyl	2-MeO	0.13	0.015	8.0	3.3	43
28	5-Pyrimidinyl	2,4-$(MeO)_2$	0.25	0.019	1.0	42	>100
29	2-(1*H*-Pyrrolyl)	None	1.0	0.06	16	9.9	23
30	2-(1*H*-Indolyl)	None	1.0	0.06	4.0	3.3	9
31	5-(1*H*-Indolyl)	None	0.059	0.004	2.0	>40	>82
32	5-(1*H*-Indolyl)	1-Me	1.0	0.065	8.0	3.6	>100
33	7-Quinazolinyl	None	4.0	0.06	>64	29	>100
34[c]	6-Isoquinolinyl	None	0.13	0.0020	0.50	3.6	22
35	6-Quinolinyl	None	0.13	0.0052	1.0	3.2	>100
36	3-Quinolinyl	None	0.13	0.011	8.0	50	>100
37	4-Isoquinolinyl	None	0.48	0.028	8.0	>38	51
38	5-Quinolinyl	None	0.071	0.016	2.0	33	ND
39[e]	5-Isoquinolinyl	None	0.061	0.016	>64	23	45
40	8-Quinolinyl	None	0.75	0.13	16	>40	79

[a] *Abbreviations:* CIP, ciprofloxacin; Ec, *Escherichia coli* ATCC 25922; MFX, moxifloxacin; MIC, minimum inhibitory concentration; $M^RQ^RV^I$, methicillin- and quinolone-resistant, vancomycin intermediate-resistant; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 700699; ND, not determined; Sa, *Staphylococcus aureus* ATCC 29213; WT, wild-type.
[b] Prepared from the commercially available tributylstannane.
[c] Prepared from the custom-made boronic acid or dioxaborolane derived from the previously described bromide (see Ref. 12).
[d] Prepared as shown in Scheme 1.
[e] Prepared from the custom-made boronic acid or dioxaborolane derived from the commercially available bromide.
[f] Prepared as shown in Scheme 3.

Table 2. In vitro antibacterial activity of selected ITQs[a]

Compound	MIC (μg/mL)											
	Gram-positive organisms					Gram-negative organisms						
	Sa	Efm	Efs	Spy	Spn	Hi	Mc	Sm	St	Kp	Pa	Ec
CIP	0.22	2	0.56	0.28	0.56	0.018	0.033	0.28	0.018	0.033	0.25	0.015
MFX	0.061	0.125	0.125	0.061	0.125	0.013	0.061	0.031	0.061	0.031	1	0.013
13	0.015	0.11	0.125	0.059	0.125	0.03	0.0037	1	0.125	0.125	0.5	0.03
19	0.004	0.25	0.125	0.04	0.029	0.015	0.0037	1	0.125	0.125	2	0.059
28	0.021	0.17	0.058	0.079	0.079	0.14	0.021	2	0.125	0.5	>64	0.14
38	0.016	0.25	0.125	0.25	0.061	0.028	0.0081	4	0.5	0.5	2	0.016

[a] *Abbreviations:* CIP, ciprofloxacin; Ec, *Escherichia coli* ATCC 25922; Efm, *Enterococcus faecium* ATCC49032; Efs, *Enterococcus faecalis* ATCC 29212; Hi, *Haemophilus influenzae* type b ACH-0056; Kp, *Klebsiella pneumoniae* ATCC 13883; Mc, *Moraxella catarrhalis* ATCC 8176; MFX, moxifloxacin; MIC, minimum inhibitory concentration; ND, not determined; Pa, *Pseudomonas aeruginosa* ATCC 27853; Sa, *Staphylococcus aureus* ATCC 29213; Sm, *Stenotrophomonas maltophilia* ATCC 13637; Spn, *Streptococcus pneumoniae* ATCC 49619; Spy, *Streptococcus pyogenes* ATCC 19615; St, *Salmonella typhimurium* ATCC 14028.

is likely a reflection of other contributing variables such as efflux and the presence of other targets (e.g., topoisomerase II).

To summarize, we have described novel carbon-coupled ITQs adorned by heteroaromatic groups at C-7. Several analogues displayed potent antistaphylococcal activities (MICs $\leqslant 2$ μg/mL against MRSA) that are superior to contemporary quinolones and showed low cytotoxic activity against human cells ($CC_{50} > 100$ μM). We anticipate that further modification of the heteroaromatic substituents at C-7 and the ITQ nucleus at C-8, will, in general, lower the cytotoxicity and increase the antibacterial activity of this class of compounds. Such studies are now underway in our laboratories.

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