Isothiazoloquinolones with Enhanced Antistaphylococcal Activities against Multidrug-Resistant Strains: Effects of Structural Modifications at the 6-, 7-, and 8-Positions

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We describe the biological evaluation of isothiazoloquinolones (ITQs) having structural modifications at the 6-, 7-, and 8-positions. Addition of a methoxy substituent to C-8 effected an increase in antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and a decrease in cytotoxic activity against Hep2 cells. Removal of fluorine from C-6 or replacement of the C-8 carbon with a nitrogen compromised anti-MRSA activity. When the groups attached at C-7 were compared, the anti-MRSA activity decreased in the order 6-isoquinolinyl > 4-pyridinyl > 5-dihydroisoindolyl > 6-tetrahydroisoquinolinyl. The compound with the most desirable in vitro biological profile was 9-cyclopropyl-6-fluoro-8-methoxy-7-(2-methylpyridin-4-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione (**7g**). This ITQ demonstrated (i) strong in vitro anti-MRSA activity (MIC_{90} = 0.5 μg/mL), (ii) strong inhibitory activities against *S. aureus* DNA gyrase and topoisomerase IV, with weak activity against human topoisomerase II, (iii) weak cytotoxic activities against three cell lines, and (iv) efficacy in an in vivo murine thigh model of infection employing MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA[a]) has emerged as the predominant nosocomial Gram-positive pathogen.[1] Therapies consisting of the glycopeptide vancomycin (VAN) remain the last line of defense against life-threatening infections due to MRSA. Ever-increasing resistance of MRSA to VAN,[2−4] however, underscores the need to urgently expand the existing arsenal of anti-MRSA agents. Recently, we reported our initial investigations of isothiazoloquinolones (ITQs),[5,6] an under-explored subclass of quinolones first reported by Chu and co-workers at Abbott in the late 1980s,[7,8] and related isothiazolopyridones[9] as potent antibacterial agents. These ITQs have tricyclic structures comprising a quinolone nucleus with an annelated isothiazolone ring, which replaces the archetypal 3-carboxyl group (Figure 1). Remarkably, after more than 40 years of widespread research devoted to the quinolones, ITQs represent one of the few examples of replacement of the 3-carboxyl group to generate analogues with equal or increased antibacterial activity.[10] Similar to the quinolone class of bactericidal agents,[11] ITQs inhibit type II topoisomerases such as DNA gyrase and topoisomerase IV. Unlike the original ITQs reported by Chu and co-workers,[7,8,12,13] our compounds reported recently[5,6] (i) contained aromatic groups at the 7-position attached via a C−C bond (carbon-coupled) rather than a C−N bond (nitrogen-coupled), (ii) showed diminished cytotoxic activities against a human cell line, and (iii) displayed strong antibacterial activities against a clinical isolate of MRSA with intermediate-level resistance to VAN. We now report the anti-MRSA evaluation of (i) new carbon-coupled ITQs that have novel structural modifications at the 6- and 8-positions (i.e., removal of fluorine at C-6 and addition of a methoxy substituent at C-8) and (ii) new ITQs that have previously described modifications at the 7- and 8-positions (e.g., addition of a pyridinyl group at C-7 and replacement of the C-8 carbon with a nitrogen).

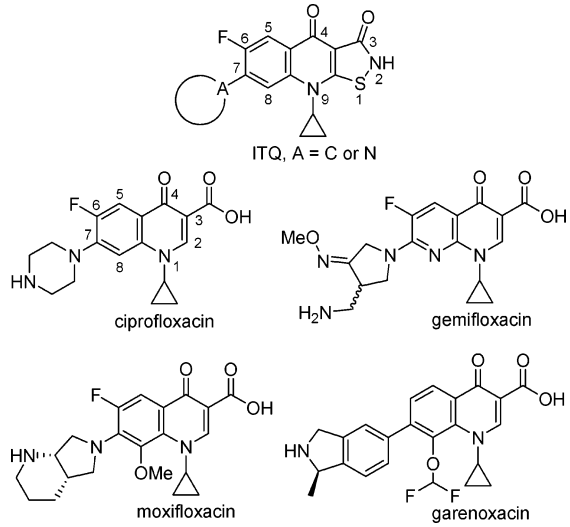

Figure 1. Examples and numbering schemes of ITQs (top) and quinolones.

Based on known structure−activity relationships of the related quinolones, we anticipated that additional modification of the ITQ nucleus at the 8-position would further improve their desired biological activities. We chose to replace the ITQ nucleus with an isothiazolonaphthyridone (replacement of the C-8 carbon with a nitrogen)—a documented strategy that yielded improved in vitro antibacterial activities and in vivo efficacies (pharmacokinetic properties) for naphthyridones compared with quinolones.[10,14−17] In addition, we elected to incorporate a methoxy group at C-8 for its known propensity in quinolones to (i) lower the emergence of resistance in *S. aureus*[18,19] and (ii) increase Gram-positive activity while reducing undesired toxicities.[11,20] In combination with addition of the methoxy group at C-8, we chose to remove the fluorine at C-6, because

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[a] Abbreviations: CFU, colony-forming units; DMA, dimethylacetamide; ITQ, isothiazoloquinolone; LVX, levofloxacin; LZD, linezolid; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; VAN, vancomycin.

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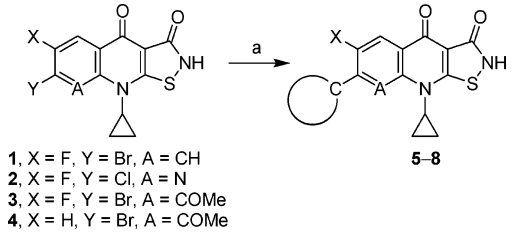
Scheme 1[a]



[a] Reagents and conditions: (a) $ArB(OH)_2$ or ArBR where Ar = aryl or heteroaryl and R = pinacolato (3−4 equiv), $NaHCO_3$ (10 equiv), $Pd(PPh_3)_4$ (5−10 mol %), DMF/H_2O, MWI (130 °C), 10−20 min, 5−68% yield after purification by preparative HPLC.

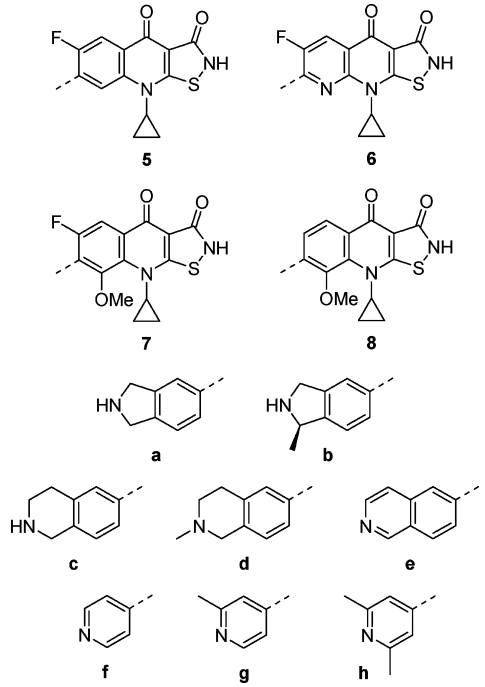


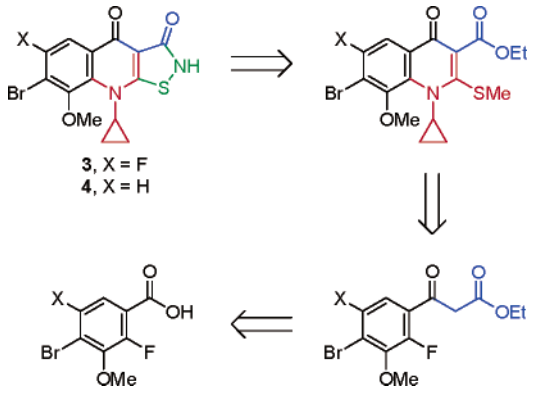
Figure 2. Numbering of the ITQs (**5**−**8**) and lettering of their C-7 substituents (**a**−**h**) used in this study. Dashed lines represent points of attachment between the ITQ nuclei and substituents.

substitutions at C-6 and C-8 confer an interdependent influence on structure−activity relationships of quinolones.[10] This strategy is especially encouraging when considering garenoxacin,[21] a potent antistaphylococcal desfluoroquinolone that also contains a heterocyclic substituent at C-7 attached via a C−C bond (Figure 1). The C-7 substituents that we selected for this study include the chiral dihydroisoindolyl group of garenoxacin, related (one-carbon homologated) tetrahydroisoquinolines, and 4-pyridines.

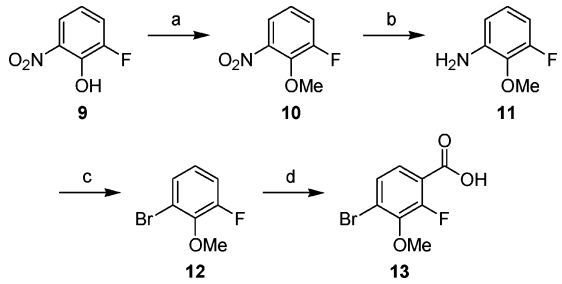
Chemistry

Overview. We prepared the target carbon-coupled ITQs via Suzuki−Miyaura cross-coupling of halides **1**−**4** with the desired boronic acids or dioxaborolanes (Scheme 1 and Figure 2). ITQ halides **1**[5] and **2**[22] were prepared according to published methods, whereas halides **3** and **4** were prepared as outlined below. The boronic acids and dioxaborolanes used in this study were either purchased from commercial suppliers, prepared using published procedures, or synthesized as described below. Products **5**−**8** were isolated (typically in 40−70% yield) via preparative HPLC. Some bromide−dioxaborolane reactant combinations, however, generated the cross-coupled products in much lower isolated yields (e.g., reaction of the nonfluorinated ITQ bromide **4** with dihydroisoindolyl dioxaborolanes). In such cases, the predominant product was that of dehalogenation.

Scheme 2



Scheme 3[a]

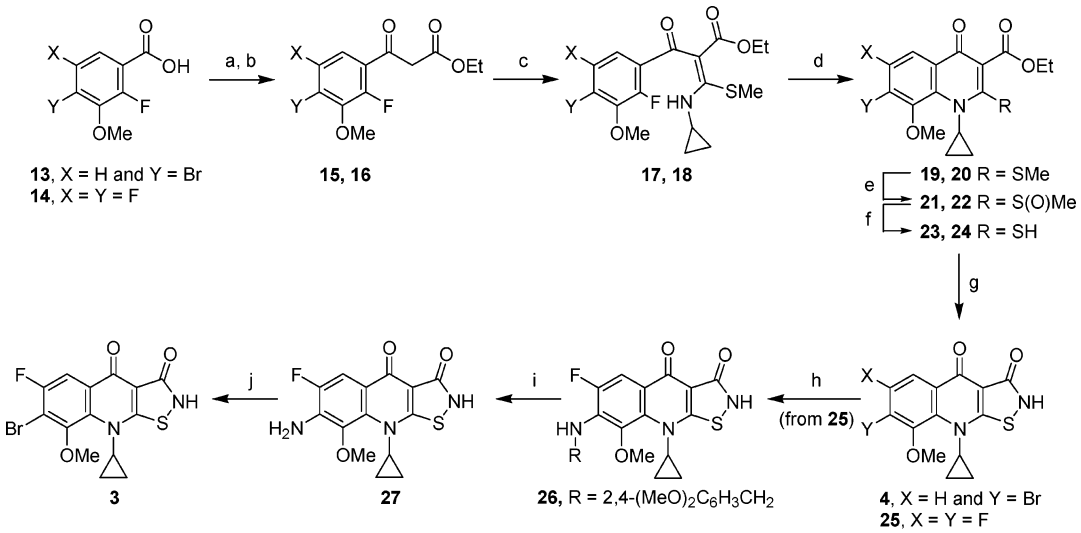


[a] Reagents and conditions: (a) Me_2SO_4 (2 equiv), K_2CO_3 (2 equiv), DMF, 80 °C, 6 h, 99%; (b) H_2 (1 atm), 10% Pd/C, MeOH, rt, 27 h, 93%; (c) $NaNO_2$ (1.05 equiv), 48% HBr, H_2O, 0−5 °C, 1.5 h, then added CuBr (0.66 equiv), 48% HBr, 0−5 °C, then heated at 60 °C until evolution of gas ceased (~2.5 h), 93%; (d) LDA (1.2 equiv), THF, −78 °C, 1.5 h, then added dry ice, −78 °C → rt, 1 h, 77%.

Preparation of ITQ Bromides. From a retrosynthetic perspective, we envisioned the preparation of ITQ bromides **3** and **4** to proceed directly via the corresponding bromo carboxylic acids (Scheme 2). Considering, however, that the bromo carboxylic acid that corresponded to **3** was not commercially available and that the synthesis of this acid was potentially cost-ineffective, we elected, instead, to employ the widely available 2,4,5-trifluoro-3-methoxybenzoic acid as starting material—a synthetic route that required conversion of fluoride to bromide later in the process (vide infra). The bromo carboxylic acid that was necessary to directly prepare the corresponding ITQ bromide **4** was synthesized as outlined in Scheme 3. Commercially available 2-fluoro-6-nitrophenol (**9**) was heated with dimethylsulfate in the presence of potassium carbonate to generate **10** in high yield. Compound **10** was reduced with hydrogen gas over palladium on carbon to give aniline **11**, which was then converted to bromide **12** via the Sandmeyer reaction. Regioselective low-temperature metalation of bromide **12** with lithium diisopropylamide (LDA), followed by reaction of the generated lithium anion with carbon dioxide (dry ice) gave the requisite carboxylic acid **13** in 77% yield (66% overall yield from **9**).

ITQ bromides **3** and **4** were constructed from the appropriate carboxylic acids using the sequence of synthetic transformations shown in Scheme 4. The syntheses of ITQs commenced with conversion of the carboxylic acids **13** and **14** to the corresponding *β*-keto esters **15** and **16** using the following classic two-step procedure: (i) conversion of the carboxylic acids to the acid chlorides using oxalyl chloride, and (ii) treatment of the generated acid chlorides with ethyl malonate in the presence of *n*-butyllithium at low temperature followed by acidic workup.[23] *β*-Keto esters **15** and **16** were reacted with cyclopropyl isothiocyanate in the presence of sodium hydride followed by

Scheme 4[a]



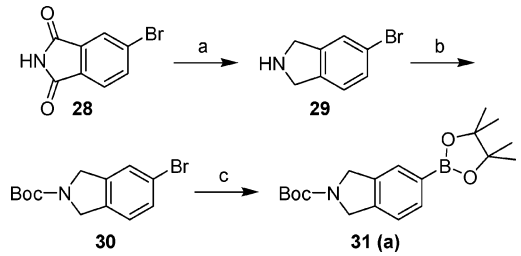
[a] Reagents and conditions: (a) $(COCl)_2$ (2 equiv), DMF (cat.), CH_2Cl_2, rt until evolution of gas ceased (∼1 h); (b) ethyl malonate (2 equiv), *n*-BuLi (3.8−5.9 equiv), THF, −78 °C → ∼5 °C, then added RCOCl, −78 °C → 10 °C, 30 min, 69−89% (2 steps); (c) *c*-PrNCS (1.7−3.0 equiv), DMF, then NaH (1.1 equiv), 0 °C→ rt, 18.5−20 h, then MeI (1.7−6.3 equiv), rt, 4−24 h, 76−92%; (d) NaH (1.1 equiv), DMF, 75 °C, 3 d; (e) *m*-CPBA (∼1 equiv), CH_2Cl_2, rt, 1 h, 56−58% (2 steps); (f) NaSH (1.5−2.6 equiv), DMF, 50 °C, 1−2 h; (g) H_2NOSO_3H (4.2−5.1 equiv), $NaHCO_3$ (10 equiv), H_2O/THF (1:1 v/v), 2.5−5 h, 85−90% (2 steps); (h) 2,4-$(MeO)_2C_6H_3CH_2NH_2$ (5 equiv), DMA, 90 °C, 24 h; (i) TFA (excess), CH_2Cl_2, rt, 18 h; (j) *t*-BuONO (2.9 equiv), $CuBr_2$ (3.6 equiv), CH_3CN, reflux, 0.5 h, 55% (3 steps).

alkylation of the generated thiolates with methyl iodide to give ketene *N*,*S*-acetals **17** and **18**, respectively. After chromatographic purification, **17** and **18** were heated in the presence of sodium hydride to generate quinolones **19** and **20**. We oxidized thioethers **19** and **20** with *m*-chloroperbenzoic acid (*m*-CPBA) to give the corresponding sulfoxides **21** and **22**. We next displaced the methyl sulfinyl groups of **21** and **22** with sodium hydrosulfide to afford the mildly air-sensitive thiols **23** and **24**, respectively. To prevent oxidative degradation of **23** and **24**, we reacted these thiols directly with hydroxylamine-*O*-sulfonic acid under basic conditions to generate the corresponding ITQ bromide **4** and ITQ fluoride **25**.

We next focused our efforts on converting fluoride **25** to the desired bromide **3**, which was required for subsequent Suzuki−Miyaura cross-coupling reactions (Scheme 1). We undertook a three-step conversion that employed aniline **27** in a Sandmeyer-type reaction. Unfortunately, our attempts to introduce the 7-amino group directly from **25** failed (e.g., reaction with ammonium hydroxide or ammonia in methanol), generating the phenol analogue of **25** via demethylation. The low susceptibility of the 7-fluoro substituent of **25** toward nucleophilic displacement (caused by the proximal 8-methoxy group[24]) was overcome by the use of 2,4-dimethoxybenzylamine in dimethylacetamide (DMA) to generate **26** exclusively. We used DMA rather than dimethylformamide (DMF) because we found that the dimethylamine present from decomposition of DMF[25] also displaced the C-7 fluoride of **25** to give the 7-dimethylamino derivative in varying amounts (concentration dependent). Compound **26** was debenzylated with trifluoroacetic acid to give aniline **27**, which was treated subsequently with *tert*-butyl nitrite in the presence of cupric bromide[26] to give the desired bromide **3**.

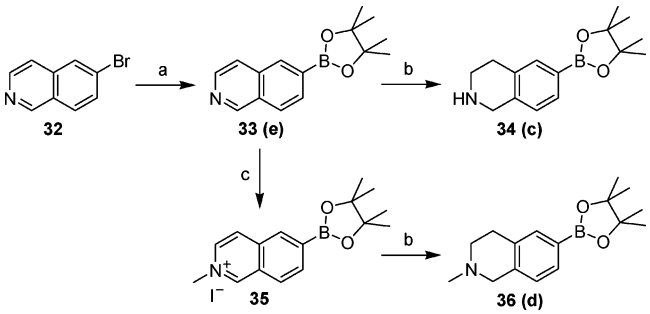
Preparation of Dioxaborolanes. Custom-made dioxaborolanes used in this study were prepared using the methods illustrated in Schemes 5 and 6. Commercially available 5-bromoisoindole-1,3-dione (**28**) was reduced with diborane, generated in situ by reaction of sodium borohydride and boron trifluoride, to give dihydroisoindole **29** in 69% yield (Scheme 5). Boc-protection of **29** to give **30**, followed by palladium-catalyzed cross-coupling of **30** with bis(pinacolato)diboron,[27]

Scheme 5[a]



[a] Reagents and conditions: (a) $NaBH_4$ (10 equiv), $BF_3{\cdot}Et_2O$ (10 equiv), THF, −10 °C → reflux, 18 h, 69%; (b) $(Boc)_2O$ (1.5 equiv), DMAP (cat.), DMF, rt, 15 h, 80%; (c) bis(pinacolato)diboron (1.1 equiv), KOAc (3 equiv), $Pd(PPh_3)_4$ (3 mol %), DMSO, 90 °C, 16 h, 80%.

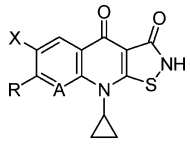
Scheme 6[a]



[a] Reagents and conditions: (a) bis(pinacolato)diboron (1.1 equiv), KOAc (3 equiv), $PdCl_2$(dppf) (30 mol %), DMF, 80 °C, 17 h, 54%; (b) H_2 (3 atm), PtO_2, EtOH, rt, 24 h, quant; (c) MeI (excess), rt, 8 h, quant.

generated **31** in 64% yield (2 steps). Dioxaborolane **31** was cross-coupled with the desired ITQ halides (Scheme 1), and the resulting products were deprotected with trifluoroacetic acid prior to purification by preparative HPLC. Isoquinoline **32**[28] was cross-coupled catalytically with bis(pinacolato)diboron to afford **33** in 54% yield (Scheme 6). Dioxaborolane **33** was used to prepare (i) the corresponding target ITQ analogues directly via Suzuki−Miyaura cross-coupling (Scheme 1), (ii) dioxaborolane **34** via reduction with hydrogen gas over Adam's catalyst,

Table 1. Antibacterial and Cytotoxic Activities of ITQs[a]



5–8

compd	X	A	R	R substn	MIC[b] (MRSA)	cytotox[c] (Hep2)
CIP					32	>100
GEM					2	46
MXF					2	>100
5a	F	CH	5-DHI	none	2	8
7a	F	COMe	5-DHI	none	0.5	19
8a	H	COMe	5-DHI	none	2	97
5b	F	CH	5-DHI	1-Me	1	2
7b	F	COMe	5-DHI	1-Me	0.5	14
8b	H	COMe	5-DHI	1-Me	2	33
5c	F	CH	6-THIQ	none	2	7
7c	F	COMe	6-THIQ	none	2	57
8c	H	COMe	6-THIQ	none	4	89
7d	F	COMe	6-THIQ	2-Me	4	61
8d	H	COMe	6-THIQ	2-Me	8	>100
5e[d]	F	CH	6-isoquinolinyl	none	0.5	21
7e	F	COMe	6-isoquinolinyl	none	0.12	39
8e	H	COMe	6-isoquinolinyl	none	0.12	45
5f[d]	F	CH	4-pyridinyl	none	1	62
6f	F	N	4-pyridinyl	none	2	>100
7f	F	COMe	4-pyridinyl	none	1	>100
8f	H	COMe	4-pyridinyl	none	0.5	>100
5g[d]	F	CH	4-pyridinyl	2-Me	0.5	36
6g	F	N	4-pyridinyl	2-Me	8	>100
7g	F	COMe	4-pyridinyl	2-Me	0.12	>100
8g	H	COMe	4-pyridinyl	2-Me	1	>100
5h[d]	F	CH	4-pyridinyl	2,6-Me_2	0.25	50
6h	F	N	4-pyridinyl	2,6-Me_2	64	>100
7h	F	COMe	4-pyridinyl	2,6-Me_2	0.25	64
8h	H	COMe	4-pyridinyl	2,6-Me_2	1	>100

[a] Abbreviations: CIP, ciprofloxacin; DHI, 2,3-dihydro-1*H*-isoindolyl; GEM, gemifloxacin; ITQ, isothiazoloquinolone; MIC, minimum inhibitory concentration; MXF, moxifloxacin; MRSA, methicillin-resistant *Staphylococcus aureus* (ATCC 700699, quinolone-resistant and vancomycin-intermediate-resistant); THIQ, 1,2,3,4-tetrahydroisoquinolinyl. [b] Minimum inhibitory concentrations (MICs) are expressed in μg/mL. [c] 72-h Cytotoxic activities (CC_{50}) are expressed in μM. [d] These analogues were described previously (see ref 6).

and (iii) dioxaborolane **36** via alkylation with methyl iodide followed by catalytic hydrogenation.

Biological Results and Discussion

In Vitro Screening. Analogues **5**−**8** were screened in vitro for antibacterial activities against a clinical strain of MRSA with reduced susceptibility to VAN (MIC = 8 μg/mL).[3] In addition, we tested these compounds for cytotoxic activities against human Hep2 laryngeal carcinoma cells to ensure microbial specificity and because this class of compounds was also explored previously as antineoplastic agents.[29] The results of these assays were compared with those of the contemporary quinolones gemifloxacin and moxifloxacin, as well as with the classic quinolone ciprofloxacin. Before consideration for further in vitro evaluation, we required that any particular ITQ analogue demonstrated a balance of strong antibacterial activity against MRSA (preferably, ≤0.5 μg/mL) and, ideally, restricted cytotoxic activity (CC_{50} ≥ 50 μM) against Hep2 cells. Many of the ITQs that we tested (Table 1) were more active than the comparator quinolones against MRSA, with several analogues exhibiting MIC values of ≤0.5 μg/mL. In general, introducing a methoxy substituent at the 8-position of analogues **5** provided two benefits to the resulting analogues **7**: equal or increased antibacterial activities (up to 4-fold) and decreased cytotoxic activities (up to 8-fold). Removal of fluorine from **7** to give analogues **8** maintained low cytotoxic activities but compromised antibacterial activities (typically, effecting a 2−4-fold decrease). Introducing nitrogen at the 8-position of **5** generated analogues **6** having unacceptable MIC values (≥2 μg/mL). Comparing the aromatic groups attached at the 7-position (**a**−**h**, Figure 2), the antibacterial activities of the corresponding analogues **5**−**8** decreased generally in the order 6-isoquinolinyl (**e**) > 4-pyridinyl (**f**, **g**, and **h**) > 5-dihydroisoindolyl (**a** and **b**) > 6-tetrahydroisoquinolinyl (**c** and **d**). Although the ITQ analogues having the 6-isoquinolinyl group at C-7 demonstrated some of the strongest activities against MRSA, these compounds also exhibited moderate cytotoxic activities. Several analogues containing 4-pyridinyl groups, however, showed strong antibacterial activities with low to moderate cytotoxic activities. Of these pyridinyl-containing analogues, we chose **7g** and **7h** for further in vitro profiling because they exhibited reduced cytotoxicities and anti-MRSA activities with MICs below 0.5 μg/mL. For contrasting purposes, we also chose to further evaluate the potent, but more cytotoxic, 5-dihydroisoindolyl-containing analogues **7a** and **7b**.

Table 2. Activities of Selected ITQs against Staphylococcal Clinical Isolates[a]

compd	organism (no. of strains)	MIC (μg/mL) range	50%	90%
7a	*S. aureus* (52)	0.004−2	0.5	1
	MRSA (38)	0.004−2	0.5	2
7b	*S. aureus* (52)	0.008−>2	0.5	2
	MRSA (39)	0.06−>2	1	2
7g	*S. aureus* (52)	0.002−2	0.25	0.5
	MRSA (39)	0.03−2	0.25	0.5
7h	*S. aureus* (52)	≤0.001−2	0.12	0.5
	MRSA (38)	≤0.001−2	0.12	1
LVX	*S. aureus* (52)	0.12−>16	16	>16
	MRSA (39)	0.12−>16	>16	>16
LZD	*S. aureus* (52)	1−>16	2	2
	MRSA (39)	1−>16	2	2
VAN	*S. aureus* (52)	0.5−>32	1	8
	MRSA (39)	0.5−>32	1	8

[a] Abbreviations: ITQ, isothiazoloquinolone; LVX, levofloxacin; LZD, linezolid; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; VAN, vancomycin; 50 and 90%, MIC_{50} and MIC_{90}, respectively.

In Vitro Evaluation of Selected Analogues. We further evaluated the in vitro antibacterial activities of **7a**, **7b**, **7g**, and **7h** by performing susceptibility testing against a collection of 52 recent clinical isolates of *S. aureus*. Table 2 lists (i) the range of MICs of the selected ITQs and reference compounds against this panel of *S. aureus* strains, (ii) the MICs at which 50% of the isolates are inhibited (MIC_{50}s), and (iii) the MICs at which 90% of the isolates are inhibited (MIC_{90}s). Pyridinyl ITQ **7g** was the most effective anti-MRSA agent (MIC_{90} = 0.5 μg/mL), whereas dihydroisoindolyl ITQ **7b** was the least effective (MIC_{90} of 2 μg/mL). In comparison, the MIC_{90}s of linezolid (LZD) and VAN against this panel of MRSA strains were 2 and 8 μg/mL, respectively. The distribution of MICs illustrated in Figure 3 clearly distinguishes **7g** from the comparator drugs against most strains of MRSA. Furthermore, **7g** demonstrated strong activity against VAN nonsusceptible MRSA isolates (Table 3).

Antibacterial agents with dual activity against the well-established quinolone target enzymes DNA gyrase and topoisomerase IV are desirable as they may reduce the selection of resistant organisms. We, therefore, tested compounds **7a**, **7b**, **7g**, and **7h** for activities against these biochemical targets. The selected ITQ analogues utilized *S. aureus* DNA gyrase and topoisomerase IV as dual targets better than the comparator

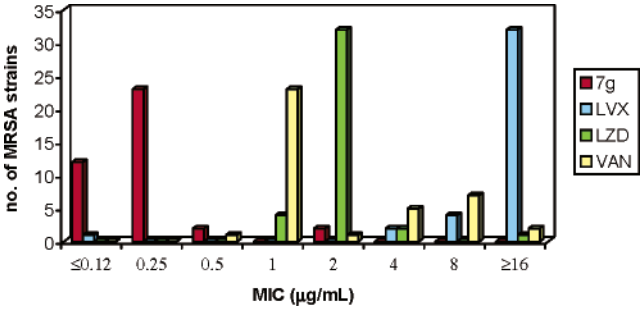


Figure 3. Antibacterial activity of **7g** against clinical isolates of MRSA compared with that of LVX, LZD, and VAN.

Table 3. Antibacterial Activities of **7g** and VAN against Vancomycin Non-Susceptible MRSA[a]

	MIC (μg/mL)	
strain	**7g**	VAN
VRSA		
F-800236	0.25	>32
F-800238	0.12	>32
VISA		
F-977210	0.12	8
F-977215	0.25	8
F-977218	0.25	8
F-988644	0.25	8
F-988647	0.25	8
F-988650	0.5	8
F-988653	0.25	8
VSSA[b]		
F-988633	0.25	2

[a] Abbreviations: MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; VAN, vancomycin; VISA, vancomycin-intermediate-resistant *Staphylococcus aureus*; VRSA, vancomycin-resistant *Staphylococcus aureus*; VSSA, vancomycin-sensitive *Staphylococcus aureus*. [b] Vancomycin-sensitive *Staphylococcus aureus* with resistance to levofloxacin (MIC = 16 μg/mL) and linezolid (MIC > 16 μg/mL).

Table 4. In Vitro Activities of Selected ITQs[a]

	Sa enzyme inhibition[b]			MICs[c]			cytotoxicity[d]		
compd	topo IV	DNA gyrase	human topo II[e]	MSSA	MRSA	FQR *Sa*[f]	Hep2	HepG2	rat hepatocytes
CIP	1.0	62	>150	0.25	32	64	>100	>100	ND
GEM	0.3	5.6	>150	0.03	2	4	46	35	>100
MXF	0.8	28	>150	0.06	2	4	>100	>100	>100
7a	0.1	2.0	75	0.008	0.5	0.5	19	35	75
7b	0.2	2.1	35	0.008	0.5	0.5	14	14	77
7g	0.7	1.6	100	0.002	0.12	0.25	>100	>100	>100
7h	0.9	3.2	55	0.004	0.25	0.25	64	65	>100

[a] Abbreviations: CIP, ciprofloxacin; FQR *Sa*, fluoroquinolone-resistant *Staphylococcus aureus*; GEM, gemifloxacin; MXF, moxifloxacin; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 700699; MSSA, methicillin-sensitive *Staphylococcus aureus* ATCC 29213. [b] Inhibitions of wild-type *S. aureus* topoisomerase IV (Topo IV) decatenation (IC_{50}) and DNA gyrase supercoiling (IC_{50}) are expressed in μM. [c] Minimum inhibitory concentrations (MICs) are expressed in μg/mL. [d] 72-h (Hep2 and HepG2) and 48-h (rat hepatocyte) cytotoxic activities (CC_{50}) are expressed in μM. [e] Inhibitions of human topoisomerase II (EC_2) are expressed in μM. The EC_2 value is defined as the effective concentration of drug required to enhance enzyme-mediated cleavage of double-stranded DNA 2-fold (see ref 31). [f] Fluoroquinolone-resistant *Staphylococcus aureus* (273/T/T/T) having Ser80-Phe and Ser84-Leu mutations in GrlA and GyrA, respectively (see ref 30)

quinolones, that is, **7a**, **7b**, **7g**, and **7h** inhibited topoisomerase IV at levels comparable with those of the comparator quinolones but inhibited DNA gyrase to a greater extent (Table 4). The strongest inhibitors of *S. aureus* DNA gyrase generally displayed the strongest antibacterial activities against methicillin-sensitive *Staphylococcus aureus*, fluoroquinolone-resistant MRSA, and

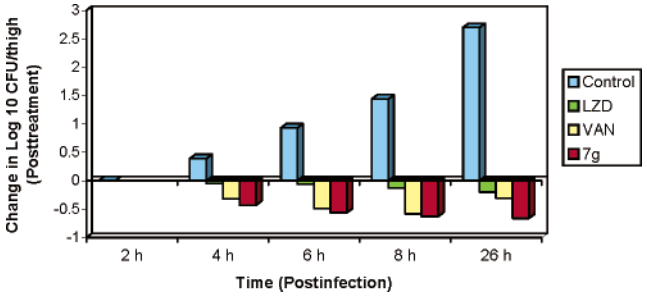


Figure 4. MRSA in vivo efficacy study in a murine thigh model of infection. Mice were dosed sc 2 h following thigh infection with 20 mg/kg LZD, 10 mg/kg VAN, or 10 mg/kg **7g**. CFU were enumerated at 0, 2, 4, 6, and 24 h post-treatment (2, 4, 6, 8, and 26 h postinfection) and expressed as log CFU per thigh.

fluoroquinolone-resistant *S. aureus* 273T/T/T[30] (a laboratory gyrase−topoisomerase IV double mutant that contained Ser84-Leu and Ser80-Phe mutations in GyrA and GrlA, respectively). We note that the ITQs listed in Table 4 also inhibited bacterial enzymes selectively, exhibiting weak to moderate inhibitory activities against human topoisomerase II. The weakest inhibitor of human topoisomerase II (**7g**) exhibited the weakest cytotoxic activities against human HepG2 (hepatocellular carcinoma) and Hep2 cells, as well as rat hepatocytes. In contrast, the compound that exhibited the strongest inhibition of human topoisomerase II (**7b**) also showed the strongest cytotoxic activities against these cell lines. Other workers have reported[29,31] that topoisomerase II-mediated DNA breakage may be linked to mammalian cytotoxic activity.

In Vivo Efficacy Model. Considering the results of the in vitro assays described above, we selected **7g** as the candidate for an in vivo study in a neutropenic-mouse thigh model of infection (Figure 4). Initial inocula were 5.13 ± 0.10 $\log_{10}$ colony-forming units (CFU)/thigh of MRSA. Test (**7g**) and control (VAN and LZD) compounds were administered subcutaneously 2 h postinfection. LZD and **7g** were dosed at 20 mg/kg, whereas VAN was dosed at 10 mg/kg. Thigh bacterial counts were determined at 2, 4, 6, 8, and 26 h postinfection. Figure 4 shows the change in population of MRSA in untreated and treated mice. The organisms expanded 2.71 $\log_{10}$ CFU/thigh after 24 h in untreated control mice. Animals treated with LZD, VAN, and **7g** showed a decrease in bacterial growth of 0.20, 0.31, and 0.66 $\log_{10}$ CFU/thigh, respectively, 24 h post-treatment (26-h timepoint). The preliminary single-dose data (Figure 4) indicate, at all time points, that compound **7g** demonstrated reductions in infection equivalent to or greater than those of the negative (untreated mice) and positive (LZD and VAN) controls.

Conclusion

We evaluated the anti-MRSA activities of novel ITQs having structural modifications at the 6-, 7-, and 8-positions. The most beneficial alteration of the ITQ nucleus was addition of a methoxy substituent to C-8, which effected increased anti-MRSA activities and decreased cytotoxic activities. Other modifications of the ITQ nucleus, that is, removal of fluorine from C-6 or replacement of the C-8 carbon with a nitrogen, compromised activities against MRSA. We observed for groups attached at C-7 that the activities against MRSA decreased in the order 6-isoquinolinyl > 4-pyridinyl > 5-dihydroisoindolyl > 6-tetrahydroisoquinolinyl. Compound **7g** (an ITQ analogue having a methoxy substituent at C-8 and a 2-methylpyridin-4-yl group at C-7) had the most attractive biological profile, demonstrating (i) excellent in vitro antibacterial activities against

a panel of clinical isolates of MRSA ($MIC_{90} = 0.5$ μg/mL), (ii) good selectivity for *S. aureus* enzymes over human topoisomerase II, and (iii) a desirable cytotoxicity profile. As proof of concept, **7g** also demonstrated inhibition of bacterial infection in a murine in vivo model employing MRSA. Work is now in progress to further evaluate **7g** in vivo as a viable anti-MRSA agent.

Experimental Section

General. All nonaqueous reactions were performed under an atmosphere of dry Ar (99.99%) using oven-dried glassware and anhydrous solvents. Elemental analyses were performed at Atlantic Microlab, Inc. (Norcross, GA). The purity of all target compounds (>95%) was verified via HPLC-MS using the following two methods: (i) 20-min gradient elution of increasing concentrations of CH_3CN in water (5−95%) containing 0.1% TFA with a flow rate of 1.0 mL/min and UV detection at 254 nm on a Waters X-bridge C18 150 × 4.6 mm 3.5 μm column (method 1); and (ii) 20-min gradient elution of increasing concentrations of MeOH in water (5−95%) containing 0.1% TFA with a flow rate of 1.0 mL/min and UV detection at 254 nm on a Waters X-bridge C8 150 × 4.6 mm 3.5 μm column (method 2). Low-resolution mass spectra were recorded on a Thermo Finnigan Surveyor MSQ instrument (operating in APCI mode) equipped with a Gilson liquid chromatograph. Unless noted otherwise, the quasi-molecular ions, $[M + H]^+$, observed in the low-resolution mass spectra, were the base peaks. High-resolution mass spectrometric analyses (ESI using NaI as internal standard) were performed at the W. M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT). NMR spectra were recorded using a Bruker Avance 300 spectrometer (^{1}H at 300.1 MHz, ^{13}C at 75.5 MHz, and ^{19}F at 282.4 MHz). All ^{13}C and ^{19}F NMR spectra were broadband ^{1}H decoupled. The chemical shifts for ^{1}H and ^{13}C are reported in parts per million (δ) relative to external TMS and were referenced to signals of residual protons in the deuterated solvent. The chemical shifts for ^{19}F are reported in parts per million (δ) relative to external $CFCl_3$. ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC, ^{1}H−^{13}C HMBC, and ^{13}C APT spectra were used routinely for assignment of signals. ITQ halides **1**[5] and **2**[22] (Scheme 1), dioxaborolane **b**[32] (Figure 2), and isoquinoline **32**[28] (Scheme 6) were prepared as described previously. Dioxaborolane **f** (Figure 2) was purchased from Acros, and boronic acids **g** and **h** (Figure 2) were purchased from Asymchem.

7-Bromo-9-cyclopropyl-6-fluoro-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione (3). A suspension of the crude TFA salt of **27** (~18 mmol, vide infra) in CH_3CN (100 mL) was added dropwise to a refluxing solution of *t*-BuONO (6 mL, 67 mmol) and $CuBr_2$ (15 g, 67 mmol) in CH_3CN (600 mL). The reaction mixture was refluxed for 0.5 h after the addition of **27** was complete, allowed to cool to rt, and diluted with a saturated aq solution of NH_4Cl (300 mL). The product was extracted with $CHCl_3$ (2 × 500 mL). The combined organic layers were washed with brine (2 × 500 mL), dried over Na_2SO_4, and evaporated under reduced pressure. A mixture of the remaining oil in 10% v/v MeOH in EtOAc (600 mL) was refluxed for 20 min with stirring. The remaining solid was removed at rt by filtration and washed with 50% v/v EtOAc in hexanes (2 × 80 mL). The filtrate was evaporated under reduced pressure to give **3** (4.1 g, 55% from **25**, 3 steps) as a red solid. ^{1}H NMR (DMSO-d_6): δ 1.01 (m, 2H, *c*-Pr-CH_2), 1.19 (m, 2H, *c*-Pr-CH_2), 3.83 (s, 3H, OCH_3), 3.85 (m, 1H, *c*-Pr-CH), 7.84 (d, $J_{H-F} = 8.5$ Hz, 1H, aromatic H-5). ^{19}F NMR (DMSO-d_6): δ −110.2 (s). HRMS *m*/*z* calcd for $C_{14}H_{10}BrFN_2NaO_3S$ $[M + Na]^+$, 406.9472; found, 406.9473.

7-Bromo-9-cyclopropyl-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione (4). The title compound was prepared using the following two-step procedure. (a) Anhydrous NaSH (Alfa Aesar, 53.3 mg, 0.95 mmol) was added in one portion to a solution of DMF (4.0 mL) containing **21** (158.1 mg, 0.37 mmol) at rt. The resulting solution was heated at 50 °C for 1 h and allowed to cool to rt. The reaction mixture was quenched by the addition of a 5% aq solution of HCl (50 mL) and was extracted with EtOAc (100 mL). The organic extract was washed with brine (50 mL) and evaporated to dryness under reduced pressure to give crude ethyl 7-bromo-1-cyclopropyl-2-mercapto-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**23**). LC-MS *m*/*z* calcd for $C_{16}H_{16}BrNO_4S$ ($[M]^+$), 397; found, 398 ($[M + H]^+$). (b) A solution of $NaHCO_3$ (316.9 mg, 3.77 mmol) in water (7.5 mL) was added to a solution of **23** (from above, ~0.37 mmol) in THF (7.5 mL) at rt. To this mixture was added H_2NOSO_3H (214.7 mg, 1.90 mmol) portionwise as a solid. The resulting amber solution was stirred at rt for 2.5 h and quenched by the addition of a 5% aq solution of HCl (50 mL). The solid that formed was collected by filtration, washed with 5% HCl (3 × 10 mL), washed with water (3 × 10 mL), and dried in vacuo to give **4** (121.9 mg, 90%, 2 steps) as a tan solid. ^{1}H NMR (DMSO-d_6): δ 1.00 (m, 2H, *c*-Pr-CH_2), 1.20 (m, 2H, *c*-Pr-CH_2), 3.79 (s, 3H, OCH_3), 3.85 (m, 1H, *c*-Pr-CH), 7.66 (d, $J = 8.5$ Hz, 1H, aromatic H-6), 7.93 (d, $J = 8.5$ Hz, 1H, aromatic H-5). ^{13}C NMR (DMSO-d_6): δ 11.5 (*c*-Pr-CH_2), 35.1 (*c*-Pr-CH), 61.9 (OCH_3), 107.7 (C-3a), 122.9 (CH, C-5), 123.5 (C−Br, C-7), 127.9 (CH, C-6), 128.0 (C-4a), 136.5 (C-8a), 146.6 (*C*-OCH_3, C-8), 164.5 (C-3), 171.1 (C═O, C-4), 171.2 (br, C-9a). HRMS *m*/*z* calcd for $C_{14}H_{11}BrN_2NaO_3S$ ($[M + Na]^+$), 388.9571; found, 388.9577.

Compounds 5−8. The general procedures for preparing (Suzuki−Miyaura cross-coupling) and purifying (preparative HPLC using mass-based fraction collection) ITQ analogues are outlined elsewhere.[5] Yields and analytical data for the analogues described in this report are listed below.

9-Cyclopropyl-7-(2,3-dihydro-1*H*-isoindol-5-yl)-6-fluoro-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5a). Yield: 10%. ^{1}H NMR (DMSO-d_6): δ 1.26 (m, 2H), 1.35 (m, 2H), 3.62 (m, 1H), 4.61 (m, 4H), 7.58−7.75 (m, 3H), 8.02 (d, $J = 10.5$ Hz, 1H), 8.08 (d, $J = 6.5$ Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −123.8 (s). HPLC: t_R 8.33 min, 95.1% purity (method 1); t_R 12.29 min, 99.3% purity (method 2).

9-Cyclopropyl-6-fluoro-7-((*R*)-1-methyl-2,3-dihydro-1*H*-isoindol-5-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5b). Yield: 29%. ^{1}H NMR (DMSO-d_6): δ 1.27 (m, 2H), 1.35 (m, 2H), 1.63 (d, $J = 7.0$ Hz, 3H), 3.62 (m, 1H), 4.61 (m, 2H), 5.01 (m, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.68−7.76 (m, 2H), 8.02 (d, $J = 10.5$ Hz, 1H), 8.09 (d, $J = 6.5$ Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −123.4 (s). HPLC: t_R 8.70 min, 99.3% purity (method 1); t_R 12.88 min, 97.1% purity (method 2).

9-Cyclopropyl-6-fluoro-7-(1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5c). Yield: 24%. ^{1}H NMR (DMSO-d_6 + DCl): δ 1.20 (m, 2H), 1.31 (m, 2H), 3.09 (t, $J = 6.5$ Hz, 2H), 3.35 (t, $J = 6.5$ Hz, 2H), 4.28 (s, 2H), 7.37 (d, $J = 8.5$ Hz, 1H), 7.51 (m, 2H), 7.94 (d, $J = 10.5$ Hz, 1H), 8.04 (d, $J = 6.5$ Hz, 1H). ^{19}F NMR (DMSO-d_6 + DCl): δ −123.7 (s). HPLC: t_R 8.49 min, 95.8% purity (method 1); t_R 12.39 min, 97.1% purity (method 2).

9-Cyclopropyl-6-fluoro-7-isoquinolin-6-yl-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5e). Yield: 24%. ^{1}H NMR (DMSO-d_6): δ 1.30 (m, 2H), 1.39 (m, 2H), 3.65 (m, 1H), 8.09 (d, $J = 10.5$ Hz, 1H), 8.20 (m, 1H), 8.29 (d, $J = 6.5$ Hz, 1H), 8.38 (d, $J = 6.0$ Hz, 1H), 8.52−8.60 (m, 2H), 8.70 (d, $J = 6.0$ Hz, 1H), 9.78 (s, 1H). ^{19}F NMR (DMSO-d_6): δ −123.1 (s). HPLC: t_R 8.30 min, 97.2% purity (method 1); t_R 12.59 min, 96.9% purity (method 2).

9-Cyclopropyl-6-fluoro-7-pyridin-4-yl-9*H*-isothiazolo[5,4-*b*]-quinoline-3,4-dione Hydrochloride (5f). Yield: 60%. ^{1}H NMR (DMSO-d_6): δ 1.14 (m, 2H), 1.26 (m, 2H), 3.45 (m, 1H), 7.71 (d, $J_{H-H} = 6.0$ Hz, 2H), 8.00 (d, $J_{H-F} = 11.0$ Hz, 1H), 8.12 (d, $J_{H-F} = 6.0$ Hz, 1H), 8.75 (d, $J_{H-H} = 6.0$ Hz, 2H). ^{19}F NMR (DMSO-d_6): δ −125.8 (s). HPLC: t_R 6.80 min, 99.1% purity (method 1); t_R 10.27 min, 99.5% purity (method 2). Anal. ($C_{18}H_{12}FN_3O_2S$·HCl·H_2O) C, H, N.

9-Cyclopropyl-6-fluoro-7-(2-methylpyridin-4-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5g). Yield: 68%. ^{1}H NMR (DMSO-d_6): δ 1.18 (m, 2H), 1.31 (m, 2H), 2.57 (s, 3H), 3.47 (m, 1H), 7.49 (m, 1H), 7.56 (m, 1H), 8.00 (d, $J_{H-F} = 11.0$ Hz, 1H), 8.10 (d, $J_{H-F} = 6.5$ Hz, 1H), 8.61 (d, $J_{H-H} = 5.5$ Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −125.4 (s). HPLC: t_R 7.28 min,

99.4% purity (method 1); t_R 10.67 min, 99.7% purity (method 2). Anal. ($C_{19}H_{14}FN_3O_2S$·HCl·1.8H_2O) C, H, N.

9-Cyclopropyl-7-(2,6-dimethylpyridin-4-yl)-6-fluoro-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (5h). Yield: 42%. ^{1}H NMR (DMSO-d_6): δ 1.18 (m, 2H), 1.31 (m, 2H), 2.52 (s, 6H), 3.48 (m, 1H), 7.33 (s, 2H), 8.00 (d, J_{H-F} = 11.0 Hz, 1H), 8.07 (d, J_{H-F} = 6.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −124.5 (s). HPLC: t_R 7.41 min, 96.5% purity (method 1); t_R 10.77 min, 96.7% purity (method 2).

9-Cyclopropyl-6-fluoro-7-pyridin-4-yl-9*H*-1-thia-2,8,9-triazacyclopenta[*b*]naphthalene-3,4-dione Hydrochloride (6f). Yield: 60%. ^{1}H NMR (DMSO-d_6): δ 1.27 (m, 2H), 1.35 (m, 2H), 3.54 (m, 1H), 8.26 (m, 2H), 8.52 (d, J = 10.5 Hz, 1H), 8.93 (m, 2H). ^{19}F NMR (DMSO-d_6): δ −128.2 (s). HPLC: t_R 7.07 min, 99.2% purity (method 1); t_R 10.88 min, 99.7% purity (method 2). Anal. ($C_{17}H_{11}FN_4O_2S$·HCl·0.4H_2O) C, H, N.

9-Cyclopropyl-6-fluoro-7-(2-methylpyridin-4-yl)-9*H*-1-thia-2,8,9-triazacyclopenta[*b*]naphthalene-3,4-dione Hydrochloride (6g). Yield: 42%. ^{1}H NMR (DMSO-d_6): δ 1.21 (m, 2H), 1.30 (m, 2H), 2.75 (s, 3H), 3.48 (m, 1H), 8.25 (m, 1H), 8.29 (m, 1H), 8.51 (d, J = 10.5 Hz, 1H), 8.85 (d, J = 6.0 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −127.4 (s). HPLC: t_R 7.20 min, 95.9% purity (method 1); t_R 10.84 min, 98.2% purity (method 2). Anal. ($C_{18}H_{13}FN_4O_2S$·HCl·1.3H_2O) C, H, N.

9-Cyclopropyl-7-(2,6-dimethylpyridin-4-yl)-6-fluoro-9*H*-1-thia-2,8,9-triazacyclopenta[*b*]naphthalene-3,4-dione Hydrochloride (6h). Yield: 41%. ^{1}H NMR (DMSO-d_6): δ 1.21 (m, 2H), 1.31 (m, 2H), 2.73 (s, 6H), 3.47 (m, 1H), 8.15 (m, 2H), 8.52 (d, J = 10.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −127.1 (br). HPLC: t_R 7.45 min, 96.1% purity (method 1); t_R 11.41 min, 95.2% purity (method 2).

9-Cyclopropyl-7-(2,3-dihydro-1*H*-isoindol-5-yl)-6-fluoro-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7a). Yield: 25%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.18 (m, 2H), 3.39 (s, 3H), 3.82 (m, 1H), 4.59 (m, 4H), 7.49−7.60 (m, 3H), 7.79 (d, J = 9.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −118.7 (s). HPLC: t_R 8.51 min, 99.7% purity (method 1); t_R 12.25 min, 99.9% purity (method 2). Anal. ($C_{22}H_{18}FN_3O_3S$·HCl·2.0H_2O) C, H, N.

9-Cyclopropyl-6-fluoro-8-methoxy-7-((*R*)-1-methyl-2,3-dihydro-1*H*-isoindol-5-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7b). Yield: 58%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.18 (m, 2H), 1.65 (d, J = 7.0 Hz, 3H), 3.39 (s, 3H), 3.81 (m, 1H), 4.48−4.67 (m, 2H), 5.00 (m, 1H), 7.55 (m, 3H), 7.79 (d, J = 9.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −118.7 (s). HPLC: t_R 8.84 min, 99.3% purity (method 1); t_R 13.08 min, 98.7% purity (method 2). Anal. ($C_{23}H_{20}FN_3O_3S$·HCl·2.0H_2O) C, H, N.

9-Cyclopropyl-6-fluoro-8-methoxy-7-(1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7c). Yield: 44%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.18 (m, 2H), 3.09 (m, 2H), 3.40 (s, 3H), 3.43 (m, 2H), 3.81 (m, 1H), 4.35 (m, 2H), 7.38 (m, 3H), 7.78 (d, J = 9.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −118.7 (s). HPLC: t_R 8.71 min, 97.2% purity (method 1); t_R 12.83 min, 98.6% purity (method 2).

9-Cyclopropyl-6-fluoro-8-methoxy-7-(2-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7d). Yield: 50%. ^{1}H NMR (DMSO-d_6, 80 °C): δ 1.08 (m, 2H), 1.22 (m, 2H), 2.98 (s, 3H), 3.20 (m, 2H), 3.45 (s, 3H), 3.55 (m, 2H), 3.85 (m, 1H), 4.46 (m, 2H), 7.34−7.45 (m, 3H), 7.81 (d, J_{H-F} = 9.5 Hz, 1H). ^{19}F NMR (DMSO-d_6, 80 °C): δ −117.8 (s). HPLC: t_R 8.75 min, 99.2% purity (method 1); t_R 12.55 min, 98.7% purity (method 2).

9-Cyclopropyl-6-fluoro-7-isoquinolin-6-yl-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7e). Yield: 21%. ^{1}H NMR (DMSO-d_6): δ 1.12 (m, 2H), 1.21 (m, 2H), 3.39 (s, 3H), 3.84 (m, 1H), 7.89 (d, J = 9.5 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 8.34 (d, J = 6.5 Hz, 1H), 8.39 (s, 1H), 8.53 (d, J = 8.5 Hz, 1H), 8.69 (d, J = 6.5 Hz, 1H), 9.76 (s, 1H). ^{19}F NMR (DMSO-d_6): δ −119.1 (s). HPLC: t_R 8.90 min, 99.9% purity (method 1); t_R 12.37 min, 92.3% purity (method 2).

9-Cyclopropyl-6-fluoro-8-methoxy-7-pyridin-4-yl-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7f). Yield: 51%. ^{1}H NMR (DMSO-d_6): δ 1.11 (m, 2H), 1.19 (m, 2H), 3.44 (s, 3H), 3.83 (m, 1H), 7.86 (d, J_{H-F} = 9.5 Hz, 1H), 8.07 (d, J_{H-H} = 5.5 Hz, 1H), 9.00 (d, J_{H-H} = 5.5 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −119.1 (s). HPLC: t_R 7.34 min, 98.9% purity (method 1); t_R 10.84 min, 99.9% purity (method 2). Anal. ($C_{19}H_{14}FN_3O_3S$·HCl·1.2H_2O) C, H, N.

9-Cyclopropyl-6-fluoro-8-methoxy-7-(2-methylpyridin-4-yl)-9*H*-isothiazolo[5,4-b]quinoline-3,4-dione Hydrochloride (7g). Yield: 45%. ^{1}H NMR (DMSO-d_6): δ 1.01 (m, 2H), 1.10 (m, 2H), 2.50 (s, 3H), 3.37 (s, 3H), 3.76 (m, 1H), 7.28 (d, J_{H-H} = 5.0 Hz, 1H), 7.35 (s, 1H), 7.75 (d, J_{H-F} = 9.5 Hz, 1H), 8.55 (d, J_{H-H} = 5.0 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −119.0 (s). HPLC: t_R 7.58 min, 99.5% purity (method 1); t_R 10.82 min, 99.8% purity (method 2). Anal. ($C_{20}H_{16}FN_3O_3S$·HCl·1.1H_2O) C, H, N.

9-Cyclopropyl-7-(2,6-dimethylpyridin-4-yl)-6-fluoro-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (7h). Yield: 45%. ^{1}H NMR (DMSO-d_6): δ 1.05 (m, 2H), 1.14 (m, 2H), 2.77 (s, 6H), 3.45 (s, 3H), 3.77 (m, 1H), 7.77 (d, J_{H-F} = 9.5 Hz, 1H), 7.88 (br s, 2H). ^{19}F NMR (DMSO-d_6): δ −118.9 (s). HPLC: t_R 7.82 min, 98.7% purity (method 1); t_R 13.08 min, 98.7% purity (method 2). Anal. ($C_{21}H_{18}FN_3O_3S$·HCl·1.8H_2O) C, H, N.

9-Cyclopropyl-7-(2,3-dihydro-1*H*-isoindol-5-yl)-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8a). Yield: 5%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.22 (m, 2H), 3.35 (s, 3H), 3.85 (m, 1H), 4.59 (m, 4H), 7.40 (d, J = 8.5 Hz, 1H), 7.55 (m, 1H), 7.65 (m, 2H), 8.06 (d, J = 8.5 Hz, 1H). HPLC: t_R 8.42 min, 99.5% purity (method 1); t_R 12.29 min, 96.0% purity (method 2).

9-Cyclopropyl-8-methoxy-7-((*R*)-1-methyl-2,3-dihydro-1*H*-isoindol-5-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8b). Yield: 12%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.22 (m, 2H), 1.64 (d, J = 6.5 Hz, 3H), 3.36 (s, 3H), 3.85 (m, 1H), 4.58 (m, 2H), 5.00 (m, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.66 (m, 2H), 8.07 (d, J = 8.0 Hz, 1H). HPLC: t_R 8.75 min, 97.9% purity (method 1); t_R 12.88 min, 95.6% purity (method 2).

9-Cyclopropyl-8-methoxy-7-(1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8c). Yield: 10%. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H), 1.22 (m, 2H), 3.10 (m, 2H), 3.36 (s, 3H), 3.43 (m, 2H), 3.85 (m, 1H), 4.35 (m, 2H), 7.36 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.50 (m, 1H), 7.56 (m, 1H), 8.05 (d, J = 8.5 Hz, 1H). HPLC: t_R 8.63 min, 99.3% purity (method 1); t_R 12.52 min, 98.0% purity (method 2).

9-Cyclopropyl-8-methoxy-7-(2-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8d). Yield: 38%. ^{1}H NMR (DMSO-d_6, 80 °C): δ 1.07 (m, 2H), 1.25 (m, 2H), 2.97 (s, 3H), 3.21 (m, 2H), 3.42 (s, 3H), 3.54 (m, 2H), 3.89 (m, 1H), 4.44 (m, 2H), 7.33 (m, 1H), 7.41 (m, 1H), 7.49−7.60 (m, 2H), 8.09 (m, 1H). HPLC: t_R 9.25 min, 99.0% purity (method 1); t_R 12.20 min, 99.5% purity (method 2).

9-Cyclopropyl-7-isoquinolin-6-yl-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8e). Yield: 22%. ^{1}H NMR (DMSO-d_6): δ 1.13 (m, 2H), 1.26 (m, 2H), 3.37 (s, 3H), 3.88 (m, 1H), 7.60 (d, J = 8.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 8.24 (dd, J = 8.5 Hz, 2.0 Hz, 1H), 8.46 (d, J = 6.5 Hz, 1H), 8.52 (m, 1H), 8.58 (d, J = 8.5 Hz, 1H), 8.71 (d, J = 6.5 Hz, 1H), 9.86 (s, 1H). HPLC: t_R 8.51 min, 95.7% purity (method 1); t_R 12.20 min, 96.3% purity (method 2).

9-Cyclopropyl-8-methoxy-7-pyridin-4-yl-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8f). Yield: 55%. ^{1}H NMR (DMSO-d_6): δ 1.10 (m, 2H), 1.22 (m, 2H), 3.42 (s, 3H), 3.86 (m, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 6.0 Hz, 2H), 8.13 (d, J = 8.5 Hz, 1H), 8.88 (d, J = 6.0 Hz, 2H). HPLC: t_R 7.12 min, 99.4% purity (method 1); t_R 10.08 min, 99.4% purity (method 2). Anal. ($C_{19}H_{15}N_3O_3S$·HCl·1.5H_2O) C, H, N.

9-Cyclopropyl-8-methoxy-7-(2-methylpyridin-4-yl)-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8g). Yield: 50%. ^{1}H NMR (DMSO-d_6): δ 1.09 (m, 2H), 1.22 (m, 2H), 2.74 (s, 3H), 3.44 (s, 3H), 3.86 (m, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.96 (m, 1H),

8.02 (m, 1H), 8.13 (d, J = 8.5 Hz, 1H), 8.81 (d, J = 6.0 Hz, 1H). HPLC: t_R 7.46 min, 99.2% purity (method 1); t_R 10.64 min, 99.4% purity (method 2).

9-Cyclopropyl-7-(2,6-dimethylpyridin-4-yl)-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione Hydrochloride (8h). Yield: 44%. ^{1}H NMR (DMSO-d_6): δ 1.07 (m, 2H), 1.21 (m, 2H), 2.76 (s, 6H), 3.45 (s, 3H), 3.85 (m, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.93 (s, 2H), 8.12 (d, J = 8.5 Hz, 1H). HPLC: t_R 7.75 min, 98.7% purity (method 1); t_R 11.07 min, 99.4% purity (method 2). Anal. ($C_{21}H_{19}N_3O_3S$·HCl·2.5H_2O) C, H, N.

1-Fluoro-2-methoxy-3-nitrobenzene (10). K_2CO_3 (59.25 g, 0.43 mol) was added slowly to a solution of DMF (200 mL) at rt that contained **9** (33.63 g, 0.21 mol) and Me_2SO_4 (41.0 mL, 0.43 mol). The orange mixture was stirred at 80 °C for 6 h. The resulting yellow mixture was cooled to rt, diluted with water (500 mL), and extracted with hexanes (3 × 500 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to give **10** (36.30 g, 99%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 4.08 (d, J_{H-F} = 2.0 Hz, 3H, OCH_3), 7.13 (apparent t of d, J_{H-H} = 8.5 Hz, J_{H-F} = 5.0 Hz, 1H, H-5), 7.34 (ddd, J_{H-F} = 10.5 Hz, J_{H-H} = 8.5 Hz, J_{H-H} = 1.5 Hz, 1H, H-6), 7.58 (d of apparent t, J_{H-H} = 8.5 Hz, J_{H-H} = 1.5 Hz, J_{H-F} = 1.5 Hz, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 62.6 (d, J_{C-F} = 5.5 Hz, OCH_3), 120.2 (d, J_{C-F} = 3.5 Hz, C-4), 121.1 (d, J_{C-F} = 19.5 Hz, C-6), 123.2 (d, J_{C-F} = 8.0 Hz, C-5), 142.2 (d, J_{C-F} = 14.5 Hz, C-2), 144.8 (br, C-3), 156.2 (d, J_{C-F} = 251.5 Hz, C-1). ^{19}F NMR ($CDCl_3$): δ −126.7 (s).

3-Fluoro-2-methoxyphenylamine (11). A mixture containing **10** (36.30 g, 0.21 mol), 10% Pd/C (8 g), and MeOH (200 mL) was stirred under an atmosphere of H_2 (1 atm) for 27 h at rt. The mixture was filtered, and the resulting solution was evaporated to dryness under reduced pressure to give **11** (27.94 g, 93%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 3.75 (br, 2H, NH_2), 3.91 (d, J_{H-F} = 1.5 Hz, 3H, OCH_3), 6.46 (m, 2H, overlapping H-4 and H-6), 6.79 (apparent t of d, J_{H-H} = 8.0 Hz, J_{H-F} = 5.5 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ 60.7 (d, J_{C-F} = 5.0 Hz, OCH_3), 106.1 (d, J_{C-F} = 19.5 Hz, C-4), 110.9 (d, J_{C-F} = 2.5 Hz, C-6), 123.7 (d, J_{C-F} = 9.5 Hz, C-5), 134.9 (d, J_{C-F} = 13.0 Hz, C-2), 141.3 (d, J_{C-F} = 5.0 Hz, C-1), 154.4 (d, J_{C-F} = 244.0 Hz, C-3). ^{19}F NMR ($CDCl_3$): δ −132.5 (s). LC-MS m/z calcd for C_7H_8FNO ($[M]^+$), 141; found, 142 ($[M + H]^+$).

1-Bromo-3-fluoro-2-methoxybenzene (12). HBr (48% in water, 140 mL) was added slowly to **11** (14.33 g, 101.5 mmol) that was cooled to 0 °C. The resulting solid was broken up with a glass rod and stirred vigorously at 0 °C for 10 min. A solution of $NaNO_2$ (7.40 g, 107.2 mmol) in water (50 mL) was added slowly (∼1.5 h) to the stirred slurry containing **11** and HBr, maintaining the temperature of the reaction mixture below 5 °C. A purple solution of CuBr (9.62 g, 67.1 mmol) in HBr (48% in water, 50 mL) was added dropwise to the reaction mixture, maintaining the temperature of the reaction mixture below 5 °C. The resulting reaction mixture was heated at 60 °C until the evolution of gas ceased (∼2.5 h). The reaction mixture was cooled to rt, and the product was extracted with Et_2O (6 × 150 mL). The combined organic extracts were washed with brine (3 × 150 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give **12** (19.44 g, 93%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 3.95 (d, J_{H-F} = 1.5 Hz, 3H, OCH_3), 6.88 (apparent t of d, J_{H-H} = 8.0 Hz, J_{H-F} = 5.5 Hz, 1H, H-5), 7.04 (ddd, J_{H-F} = 10.5 Hz, J_{H-H} = 8.0 Hz, J_{H-H} = 1.5 Hz, 1H, H-4), 7.30 (d of apparent t, J_{H-H} = 8.0 Hz, J_{H-H} = 1.5 Hz, J_{H-F} = 1.5 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$): δ 61.4 (d, J_{C-F} = 5.0 Hz, OCH_3), 116.2 (d, J_{C-F} = 19.5 Hz, C-4), 117.7 (d, J_{C-F} = 3.0 Hz, C-1), 124.5 (d, J_{C-F} = 8.0 Hz, C-5), 128.5 (d, J_{C-F} = 3.5 Hz, C-6), 145.7 (d, J_{C-F} = 12.5 Hz, C-2), 156.2 (d, J_{C-F} = 250.5 Hz, C-3). ^{19}F NMR ($CDCl_3$): δ −127.7 (s).

4-Bromo-2-fluoro-3-methoxybenzoic Acid (13). LDA was formed by dropwise addition of *n*-BuLi (1.6 M in hexanes, 56.0 mL, 89.6 mmol) to a stirred solution of *i*-Pr_2NH (13.7 mL, 96.9 mmol) in THF (150 mL) at −78 °C. The resulting solution was stirred at −78 °C for 5 min and at 0 °C for 15 min and then recooled to −78 °C. To this solution was added dropwise a solution of **12** (15.28 g, 74.5 mmol) in THF (40 mL) over a period of 30 min to give an amber solution. After stirring this solution at −78 °C for 1.5 h, dry ice (∼125 g) was added, and the resulting mixture was allowed to warm slowly (∼1 h) to rt with stirring as it degassed. The reaction mixture was acidified to pH ∼1 by addition of a 5% aq solution of HCl (∼500 mL), and the product was extracted with Et_2O (6 × 100 mL). The combined organic extracts were washed with brine (100 mL), and the product was extracted with a saturated aq solution of $NaHCO_3$ (3 × 100 mL). The combined aqueous extracts (pH ∼9) were washed with Et_2O (3 × 100 mL) and acidified slowly to pH ∼1 by addition of a 37% aq solution of HCl (∼50 mL). The product was extracted with Et_2O (3 × 200 mL), and the combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure to give **13** (14.22 g, 77%) as an off-white solid; mp 168−170 °C. ^{1}H NMR (CD_3OD): δ 3.92 (d, J_{H-F} = 1.0 Hz, 3H, OCH_3), 7.44 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 1.5 Hz, 1H, H-5), 7.55 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 7.0 Hz, 1H, H-6). ^{13}C NMR (CD_3OD): δ 62.1 (d, J_{C-F} = 4.5 Hz, OCH_3), 121.5 (d, J_{C-F} = 8.5 Hz, C-1), 123.6 (d, J_{C-F} = 2.0 Hz, C-4), 128.0 (s, C-6), 129.0 (d, J_{C-F} = 4.5 Hz, C-5), 147.7 (d, J_{C-F} = 13.5 Hz, C-3), 157.1 (d, J_{C-F} = 263.5 Hz, C-2), 166.3 (d, J_{C-F} = 3.0 Hz, CO_2H). ^{19}F NMR (CD_3OD): δ −127.0 (s). HRMS m/z calcd for $C_8H_6BrFNaO_3$ ($[M + Na]^+$), 270.9382; found, 270.9377.

Ethyl 3-(4-bromo-2-fluoro-3-methoxyphenyl)-3-oxopropionate (15). Prepared using the general two-step method of Wierenga and Skulnick.[23] (a) DMF (5 drops) was added by Pasteur pipet to a mixture containing **13** (5.30 g, 21.3 mmol) and $(COCl)_2$ in CH_2Cl_2 (2.0 M, 21.3 mL, 42.6 mmol) at rt. The resulting mixture was stirred until an amber solution formed and the evolution of gas ceased (1 h). The solution was concentrated under reduced pressure to give the intermediate acid chloride as an off-white solid that was used directly in the following step. (b) *n*-BuLi (1.6 M in hexanes) was added to a cooled (−78 °C) solution of THF (50 mL) containing ethyl hydrogen malonate (5.62 g, 42.5 mmol) and bpy (8.2 mg as indicator). The temperature of the reaction mixture was allowed to rise to ∼0 °C during the addition of *n*-BuLi. Sufficient *n*-BuLi (∼50 mL) was added until a pink color persisted at ∼5 °C for 5−10 min. A solution of the acid chloride (vide supra) in CH_2Cl_2 (20 mL) was added in one portion to the reaction mixture that was recooled to −78 °C. The resulting mixture was allowed to warm to 10 °C (∼30 min), and quenched with a 1 M aq solution of HCl (100 mL). The reaction mixture was extracted with Et_2O (3 × 100 mL). The combined organic extracts were washed with a saturated aq solution of $NaHCO_3$ (3 × 100 mL), washed with brine (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product. This material was purified by flash column chromatography on silica (eluting with 1:6 v/v EtOAc/hexanes; R_f 0.43) to give **15** (4.70 g, 69%) as a pale orange oil that solidified upon standing; mp 52−53 °C. The title compound existed as a mixture of keto (major) and enol (minor) tautomers at rt in $CDCl_3$. ^{1}H NMR ($CDCl_3$): δ 1.27 (t, J_{H-H} = 7.0 Hz, keto $CO_2CH_2CH_3$), 1.34 (t, J_{H-H} = 7.0 Hz, enol $CO_2CH_2CH_3$), 3.96 (m, overlapping keto OCH_3, enol OCH_3, and keto $C(O)CH_2CO_2CH_2CH_3$), 4.22 (q, J_{H-H} = 7.0 Hz, keto $CO_2CH_2CH_3$), 4.27 (q, J_{H-H} = 7.0 Hz, enol $CO_2CH_2CH_3$), 5.81 (d, J_{H-F} = 0.5 Hz, enol C(OH)=$CHCO_2CH_2CH_3$), 7.39 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 1.5 Hz, enol aromatic H-5), 7.43 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 1.5 Hz, keto aromatic H-5), 7.47 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 7.0 Hz, enol aromatic H-6), 7.53 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 7.0 Hz, keto aromatic H-6), 12.67 (s, enol OH). ^{19}F NMR ($CDCl_3$): δ −126.3 (s, enol), −125.9 (s, keto). HRMS m/z calcd for $C_{12}H_{12}BrFNaO_4$ ($[M + Na]^+$), 340.9801; found, 340.9797.

Ethyl 3-oxo-3-(2,4,5-trifluoro-3-methoxyphenyl)propionate (16). Prepared in a manner analogous to that described above for **15**. Yield: 89%. Compound **16** existed as a mixture of enol (major) and keto (minor) tautomers at room temperature in $CDCl_3$. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J_{H-H} = 7.0 Hz, keto $CO_2CH_2CH_3$), 1.36 (t, J_{H-H} = 7.0 Hz, enol $CO_2CH_2CH_3$), 3.97 (d, J_{H-F} = 4.0 Hz, keto CH_2CO_2Et), 4.06 (t, J_{H-F} = 1.0 Hz, enol OCH_3), 4.08 (t, J_{H-F} = 1.0 Hz, keto OCH_3), 4.24 (q, J_{H-H} = 7.0 Hz, keto $CO_2CH_2CH_3$),

4.30 (q, J_{H-H} = 7.0 Hz, enol $CO_2CH_2CH_3$), 5.83 (s, enol C(OH)=C*H*CO_2Et), 7.43 (ddd, J_{H-F} = 11.0 Hz, 8.5 Hz, 6.5 Hz, enol aromatic H-6), 7.50 (ddd, J_{H-F} = 10.5 Hz, 8.5 Hz, 6.0 Hz, keto aromatic H-6), 12.72 (s, enol OH). ^{19}F NMR ($CDCl_3$): δ −146.3 (dd, J_{F-F} = 20.5 Hz, 10.0 Hz, enol), −141.3 (dd, J_{F-F} = 20.5 Hz, 11.5 Hz, keto), −139.7 (dd, J_{F-F} = 20.5 Hz, 14.0 Hz, enol), −138.3 (dd, J_{F-F} = 20.5 Hz, 14.5 Hz, keto), −130.9 (dd, J_{F-F} = 14.0 Hz, 10.0 Hz, enol), −129.7 (dd, J_{F-F} = 14.5 Hz, 11.5 Hz, keto).

Ethyl 2-(4-Bromo-2-fluoro-3-methoxybenzoyl)-3-cyclopropylamino-3-methylsulfanylacrylate (17). NaH (60% in mineral oil, 73.7 mg, 1.92 mmol) was added portionwise to a cooled (0 °C) solution containing **15** (569 mg, 1.78 mmol), *c*-PrNCS (500 μL, 5.40 mmol), and DMF (5.0 mL). The resulting mixture was allowed to warm to rt with stirring overnight (18.5 h). MeI (700 μL, 11.22 mmol) was added to the resulting solution to give a precipitate within minutes, and the mixture continued to stir for 24 h. The reaction mixture was quenched by the addition of a saturated aq solution of NH_4Cl (50 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine (200 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product. This material was purified by flash column chromatography on silica (eluting with 1:9 v/v EtOAc/CH_2Cl_2; R_f 0.59) to give **17** (586.0 mg, 76%) as a viscous yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.86 (m, 2H, *c*-Pr-CH_2), 0.89 (t, J_{H-H} = 7.0 Hz, $CO_2CH_2CH_3$), 0.98 (m, 2H, *c*-Pr-CH_2), 2.52 (s, 3H, SCH_3), 3.01 (m, 1H, *c*-Pr-CH), 3.90 (q, J_{H-H} = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 3.94 (d, J_{H-F} = 1.5 Hz, 3H, OCH_3), 6.97 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 6.5 Hz, 1H, aromatic H-6), 7.30 (dd, J_{H-H} = 8.5 Hz, J_{H-F} = 1.5 Hz, 1H, aromatic H-5), 11.91 (br, 1H). ^{13}C NMR ($CDCl_3$): δ 8.6, 13.5, 18.1, 28.5, 60.3, 61.4 (d, J_{C-F} = 5.0 Hz), 104.2, 118.3 (d, J_{C-F} = 2.5 Hz), 123.4 (d, J_{C-F} = 3.5 Hz), 127.6 (d, J_{C-F} = 3.5 Hz), 131.9 (d, J_{C-F} = 14.5 Hz), 145.1 (d, J_{C-F} = 13.5 Hz), 152.6 (d, J_{C-F} = 253.0 Hz), 167.7, 174.5, 185.5. ^{19}F NMR ($CDCl_3$): δ −130.4 (s). HRMS *m*/*z* calcd for $C_{17}H_{20}BrFNO_4S$ ([M + H]$^+$), 432.0280; found, 432.0276.

Ethyl 3-Cyclopropylamino-3-methylsulfanyl-2-(2,4,5-trifluoro-3-methoxybenzoyl)acrylate (18). Prepared in a manner analogous to that described above for **17**. Yield: 92%. ^{1}H NMR ($CDCl_3$): δ 0.87 (m, 2H, *c*-Pr-CH_2), 0.98 (m, 5H, overlapping $CO_2CH_2CH_3$ and *c*-Pr-CH_2), 2.53 (s, 3H, SCH_3), 3.02 (m, 1H, *c*-Pr-CH), 3.97 (q, J_{H-H} = 7.5 Hz, 2H, $CO_2CH_2CH_3$), 4.02 (t, J_{H-F} = 1.0 Hz, 3H, OCH_3), 6.97 (m, 1H, aromatic H-6), 11.75 (br, 1H). ^{19}F NMR ($CDCl_3$): δ −149.5 (dd, J_{F-F} = 20.5 Hz, 7.0 Hz, 1F), −140.9 (dd, J_{F-F} = 20.5 Hz, 13.5 Hz, 1F), −135.2 (dd, J_{F-F} = 13.5 Hz, 7.0 Hz, 1F).

Ethyl 7-Bromo-1-cyclopropyl-8-methoxy-2-methylsulfanyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (19). NaH (60% in mineral oil, 51.9 mg, 1.30 mmol) was added portionwise to a solution of **17** (527.6 mg, 1.22 mmol) in DMF (5.0 mL) at rt. The reaction mixture was heated at 75 °C for 3 d, cooled to rt, and quenched by the addition of a saturated aq solution of NH_4Cl (75 mL). The mixture was extracted with EtOAc (3 × 75 mL). The combined organic extracts were washed with brine (75 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give **19** as a tan solid. This product was of sufficient purity (>95% by NMR spectroscopy) to use directly in the next synthetic step. ^{1}H NMR ($CDCl_3$): δ 0.70 (m, 2H, *c*-Pr-CH_2), 1.18 (m, 2H, *c*-Pr-CH_2), 1.39 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.63 (s, 3H, SCH_3), 3.68 (m, 1H, *c*-Pr-CH), 3.80 (s, 3H, OCH_3), 4.40 (q, *J* = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 7.54 (d, *J* = 8.5 Hz, 1H, aromatic H-6), 7.88 (d, *J* = 8.5 Hz, 1H, aromatic H-5). ^{13}C NMR ($CDCl_3$): δ 12.4 (br, *c*-Pr-CH_2), 14.2 ($CO_2CH_2CH_3$), 18.4 (SCH_3), 37.0 (*c*-Pr-CH), 60.8 (OCH_3), 61.8 ($CO_2CH_2CH_3$), 122.7 (CH, C-5), 123.1 (C−Br, C-7), 123.6 (C-3), 129.2 (C-4a), 129.3 (CH, C-6), 140.0 (C-8a), 147.9 (*C*−OCH_3, C-8), 156.3 (*C*−SCH_3, C-2), 165.5 ($CO_2CH_2CH_3$), 173.6 (C=O, C-4). LC-MS *m*/*z* calcd for $C_{17}H_{18}BrNO_4S$ ([M]$^+$), 411; found, 412 ([M + H]$^+$). HRMS *m*/*z* calcd for $C_{17}H_{18}BrNNaO_4S$ ([M + Na]$^+$), 434.0038; found, 434.0031.

Ethyl 1-Cyclopropyl-6,7-difluoro-8-methoxy-2-methylsulfanyl-4-oxo-1,4-dihydro-quinoline-3-carboxylate (20). Prepared in a manner analogous to that described above for **19**. ^{1}H NMR ($CDCl_3$): δ 0.73 (m, 2H, *c*-Pr-CH_2), 1.19 (m, 2H, *c*-Pr-CH_2), 1.38 (t, J_{H-H} = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.66 (s, 3H, SCH_3), 3.74 (m, 1H, *c*-Pr-CH), 4.08 (d, J_{H-F} = 2.5 Hz, 3H, OCH_3), 4.40 (q, J_{H-H} = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 7.76 (dd, J_{H-F} = 10.0 Hz, 8.5 Hz, 1H, aromatic H-5). ^{19}F NMR ($CDCl_3$): δ −137.7 (d, J_{F-F} = 21.0 Hz, 1F), −146.8 (d, J_{F-F} = 21.0 Hz, 1F).

Ethyl 7-Bromo-1-cyclopropyl-2-methanesulfinyl-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (21). *m*-CPBA (≤77%, 273.5 mg, 1.22 mmol) was added in one portion to a solution of **19** (from above ~1.22 mmol) in CH_2Cl_2 (5.0 mL) at rt. The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2 (10 mL), and washed with a saturated aq solution of $NaHCO_3$ (25 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give the crude product. This material was purified by flash column chromatography on silica (eluting with EtOAc; R_f 0.37) to give **21** (290.9 mg, 56%, 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$): δ 0.54 (m, 1H, *c*-Pr-CH_2 (A)), 0.93 (m, 1H, *c*-Pr-CH_2 (B)), 1.12 (m, 1H, *c*-Pr-CH_2 (A)), 1.28 (m, 1H, *c*-Pr-CH_2 (B)), 1.38 (t, *J* = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 3.26 (s, 3H, $S(O)CH_3$), 3.83 (s, 3H, OCH_3), 3.92 (m, 1H, *c*-Pr-CH), 4.40 (m, 2H, overlapping CO_2CHHCH_3), 7.58 (d, *J* = 8.5 Hz, 1H, aromatic H-6), 7.87 (d, *J* = 8.5 Hz, 1H, aromatic H-5). ^{13}C NMR ($CDCl_3$): δ 10.8 (br, *c*-Pr-CH_2 (A)), 13.9 (br, *c*-Pr-CH_2 (B)), 14.1 ($CO_2CH_2CH_3$), 35.1 (*c*-Pr-CH), 41.4 ($S(O)CH_3$), 61.1 (OCH_3), 62.1 ($CO_2CH_2CH_3$), 118.9 (C-3), 122.8 (CH, C-5), 123.9 (C−Br, C-7), 129.5 (C-4a), 130.0 (CH, C-6), 138.2 (C-8a), 148.3 (*C*−OCH_3, C-8), 164.0 ($CO_2CH_2CH_3$), 164.1 (br, *C*−$S(O)CH_3$, C-2), 174.6 (C=O, C-4). LC-MS *m*/*z* calcd for $C_{17}H_{18}BrNO_5S$ ([M]$^+$), 427; found, 428 ([M + H]$^+$). HRMS *m*/*z* calcd for $C_{17}H_{18}BrNNaO_5S$ ([M + Na]$^+$), 449.9987; found, 449.9977.

Ethyl 1-Cyclopropyl-6,7-difluoro-2-methanesulfinyl-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylate (22). Prepared in a manner analogous to that described above for **21**. Yield: 58% (2 steps). ^{1}H NMR ($CDCl_3$): δ 0.62 (m, 1H, *c*-Pr-CH_2), 1.00 (m, 1H, *c*-Pr-CH_2), 1.13 (m, 1H, *c*-Pr-CH_2), 1.29 (m, 1H, *c*-Pr-CH_2), 1.36 (t, J_{H-H} = 7.5 Hz, 3H, $CO_2CH_2CH_3$), 3.22 (s, 3H, $SOCH_3$), 3.85 (m, 1H, *c*-Pr-CH), 4.09 (d, J_{H-F} = 2.5 Hz, 3H, OCH_3), 4.37 (q, J_{H-H} = 7.5 Hz, 2H, $CO_2CH_2CH_3$), 7.75 (dd, J_{H-F} = 10.0 Hz, 8.5 Hz, 1H, aromatic H-5). ^{19}F NMR ($CDCl_3$): δ −136.2 (d, J_{F-F} = 21.0 Hz, 1F), −145.2 (d, J_{F-F} = 21.0 Hz, 1F).

9-Cyclopropyl-6,7-difluoro-8-methoxy-9*H*-isothiazolo[5,4-*b*]-quinoline-3,4-dione (25). Prepared in a manner analogous to that described above for **4**. Yield: 85% (2 steps). ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H, *c*-Pr-CH_2), 1.20 (m, 2H, *c*-Pr-CH_2), 3.86 (m, 1H, *c*-Pr-CH), 4.02 (d, J_{H-F} = 1.5 Hz, 3H, OCH_3), 7.86 (dd, J_{H-F} = 10.5 Hz, 8.5 Hz, 1H, aromatic H-5). ^{19}F NMR (DMSO-d_6): δ −145.9 (d, J_{F-F} = 22.5 Hz, 1F), −139.8 (d, J_{F-F} = 22.5 Hz, 1F).

9-Cyclopropyl-7-(2,4-dimethoxybenzylamino)-6-fluoro-8-methoxy-9*H*-isothiazolo[5,4-*b*]quinoline-3,4-dione (26). 2,4-Dimethoxybenzylamine (14 mL, 96 mmol) was added to a solution of **25** (6.2 g, 19 mmol) in DMA (200 mL). The reaction mixture was stirred at 90 °C for 24 h, allowed to cool to rt, and diluted with a mixture of $CHCl_3$ and EtOAc (5:1 v/v, 600 mL). The organic layer was separated and (i) washed with a 2 N aq solution of HCl (4 × 400 mL) to remove excess 2,4-dimethoxybenzyl amine, (ii) washed with brine (2 × 400 mL), (iii) dried over Na_2SO_4, and (iv) concentrated under reduced pressure to afford **26** (~8.3 g) as a dark brown oil. This material was used directly in the next step (synthesis of **27**) without purification. ^{1}H NMR (DMSO-d_6): δ 0.88 (m, 2H, *c*-Pr-CH_2), 1.13 (m, 2H, *c*-Pr-CH_2), 3.61 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.74 (m, 1H), 3.79 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2Ar), 6.42 (dd, J_{H-H} = 8.5 Hz, 1.5 Hz, 1H, benzyl H-5), 6.53 (d, J_{H-H} = 1.5 Hz, 1H, benzyl H-3), 7.11 (d, J_{H-H} = 8.5 Hz, 1H, benzyl H-6), 7.53 (d, J_{H-F} = 13.0 Hz, 1H, aromatic H-5). ^{19}F NMR (DMSO-d_6): δ = −131.3 (s).

7-Amino-9-cyclopropyl-6-fluoro-8-methoxy-9*H*-isothiazolo-[5,4-*b*]quinoline-3,4-dione (27). TFA (5 mL) was added to a solution of **26** (from above, ~8.3 g) in CH_2Cl_2 (150 mL). The reaction mixture was stirred at rt for 6 h and evaporated under reduced pressure to give **27** as a red oil. This material was used directly in the next step (synthesis of **3**, vide supra) without

purification. ^{1}H NMR (DMSO-d_6): δ 0.97 (m, 2H, *c*-Pr-CH_2), 1.15 (m, 2H, *c*-Pr-CH_2), 3.69 (s, 3H, OCH_3), 3.81 (m, 1H, *c*-Pr-CH), 7.58 (d, $J_{H-F} = 11.5$ Hz, 1H, aromatic H-5). ^{19}F NMR (282 MHz, DMSO-d_6): δ −133.9 (s, 1F), −73.8 (s, 3F, TFA).

5-Bromo-2,3-dihydro-1*H*-isoindole (29). To a stirred solution of **28** (1.15 g, 5.1 mmol) in THF (50 mL) at rt was added $NaBH_4$ (2 g, 53 mmol). The reaction mixture was cooled to −10 °C, and to it BF_3·Et_2O (7.5 mL, 59 mmol) was added slowly. The reaction mixture was then refluxed (∼70 °C) with stirring for 3 h, allowed to cool, and quenched slowly with cold water (10 mL) at 0−5 °C. The mixture was diluted with EtOAc (80 mL) and made alkaline (pH ∼10) upon slow addition of a 6 N aq solution of NaOH at 0−5 °C. The organic layer was separated, washed with brine (4 × 40 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting crude oil was diluted with Et_2O (30 mL) and acidified (pH ∼2) with 6 N HCl while stirring at rt. The aq layer was separated, made alkaline (pH ∼10) by the addition of 6 N aq NaOH, and extracted with EtOAc (40 mL). The organic layer was separated, washed with brine (3 × 40 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give 620 mg (69%) of **29** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 2.68 (br, 1H), 4.14 (s, 2H), 4.17 (s, 2H), 7.07 (d, $J = 8.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.34 (s, 1H).

***tert*-Butyl 5-Bromo-1,3-dihydro-isoindole-2-carboxylate (30).** To a stirred solution of **29** (500 mg, 2.5 mmol) in DMF (7 mL) was added $(Boc)_2O$ (3.0 mmol), followed by a few (catalytic) crystals of DMAP. The reaction mixture was stirred at rt for 15 h and diluted with EtOAc (25 mL), washed with brine (5 × 15 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash column chromatography on silica (eluting with 3:7 v/v EtOAc/hexanes) to give 600 mg (80%) of **30**. ^{1}H NMR ($CDCl_3$): δ 1.51 (s, 9H), 4.61 (m, 4H), 7.11 (m, 1H), 7.38 (m, 2H).

***tert*-Butyl 5-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-1,3-dihydro-isoindole-2-carboxylate (31).** To a stirred solution of **30** (60 mg, 0.20 mmol) in DMSO (1 mL) was added bis-(pinacolato)diboron (56 mg, 0.22 mmol), $Pd(PPh_3)_4$ (7 mg, 0.006 mmol), and KOAc (59 mg, 0.60 mmol). The reaction mixture was stirred at 80 °C overnight (∼18 h), allowed to cool to rt, and filtered. The filtrate was diluted with EtOAc (30 mL) and water (15 mL). The organic layer was separated, washed with brine (3 × 15 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification of the crude material by preparative TLC on silica (eluting with 3:7 v/v EtOAc/hexanes) afforded 50 mg (80%) of **31** as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 12H), 1.52 (s, 9H), 4.66 (m, 4H), 7.25 (m, 1H), 7.70 (m, 2H).

6-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)isoquinoline (33). A mixture of isoquinoline **32**[28] (315 mg, 1.5 mmol), $PdCl_2(dppf)$·CH_2Cl_2 (333 mg, 0.4 mmol), KOAc (445 mg, 4.5 mmol), and bis(pinacolato)diboron (422 mg, 1.7 mmol) in DMF (10 mL) was heated at 80 °C for 17 h. The reaction mixture was cooled to rt and filtered. The filtrate was evaporated under reduced pressure, and the remaining residue was purified by flash column chromatography on silica (eluting with 1:4 v/v EtOAc/hexanes) to afford 210 mg (54%) of **33**. ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 12H), 7.72 (d, $J = 5.5$ Hz, 1H), 7.96 (m, 2H), 8.32 (s, 1H), 8.47 (d, $J = 5.5$ Hz, 1H), 9.24 (s, 1H). LC-MS *m*/*z* calcd for $C_{15}H_{18}BNO_2$ ($[M]^+$), 255; found, 256 ($[M + H]^+$).

6-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-1,2,3,4-tetrahydroisoquinoline (34). A mixture containing dioxaborolane **33** (75 mg) and PtO_2 (10 mg) in EtOH (5 mL) was stirred under H_2 (3 atm) for 24 h at rt. PtO_2 was removed by filtration, and the filtrate was evaporated under reduced pressure to give **34** quantitatively. ^{1}H NMR ($CDCl_3$): δ 1.34 (s, 12H), 3.05 (t, $J = 6.0$ Hz, 2H), 3.35 (t, $J = 6.0$ Hz, 2H), 4.25 (s, 2H), 7.08 (d, $J = 7.5$ Hz, 1H), 7.61 (m, 2H). LC-MS *m*/*z* calcd for $C_{15}H_{22}BNO_2$ ($[M]^+$), 259; found, 260 ($[M + H]^+$).

2-Methyl-6-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-1,2,3,4-tetrahydroisoquinoline (36). Dioxaborolane **33** (50 mg) was dissolved in MeI (5 mL) and stirred at rt for 8 h. Excess MeI was removed under reduced pressure, and the remaining residue was redissolved in EtOH (2 mL) and stirred for 24 h under H_2 (3 atm) at rt in the presence of PtO_2 (10 mg). The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to give the iodide salt of **36** quantitatively. LC-MS *m*/*z* calcd for $C_{16}H_{24}BNO_2$ ($[M]^+$), 273; found, 274 ($[M + H]^+$). The recovered residue was used directly in the subsequent Suzuki−Miyaura cross-coupling reactions to form the desired ITQ analogues.

Antimicrobial Susceptibility Testing. Antibacterial activity was determined by the microdilution test method in cation-adjusted Mueller Hinton broth (CAMHB), according to the standard guidelines of the Clinical and Laboratory Standards Institute (CLSI) for broth microdilution.[33] The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent that resulted in no visible growth after 24 h at 37 °C.

Cytotoxicity Assays. Hep2 cells were plated at 3500 cells/well in MEMα media containing 10% FBS, 1X pen/strep, and 1.5 g/L $NaHCO_3$ in a standard 96-well tissue culture plate. HepG2 cells were plated at 6000 cells/well in RPMI1640 containing 10% FBS and 1X pen/strep. Cells were incubated at 37 °C, 5% CO_2 for 24 h. Postincubation, test compound (diluted in media containing 0.5% DMSO) was added and incubated for 72 h at 37 °C, 5% CO_2. Cytotoxicity was determined by incubation with Alamar Blue per manufacturer's instructions (Biosource, Camarillo, CA). Fresh rat hepatocytes (CellzDirect, Pittsboro, NC) were cultured per manufacturer's instructions. Test compound was diluted in supplied hepatocyte media containing 0.5% DMSO and incubated with cells for 48 h at 37 °C, 5% CO_2. Cytotoxicity was determined with the CellTiter-Glo Luminescent Cell Viability Kit per manufacturer's instructions (Promega, Madison, WI). The CC_{50} was defined as the concentration of drug that was lethal to 50% of the cells.

Topoisomerase IV Assay. Enzyme activity was measured by a decatenation assay that monitored the ATP-dependent unlinking of DNA minicircles from kinetoplast DNA. Specifically, 0.1 μg of catenated kDNA was incubated with 2 units of *S. aureus* topoisomerase IV for 30 min at 37 °C in 20 μL of the following buffer: 1 mM ATP, 5 mM DTT, 5 mM $MgCl_2$, 50 μg/mL of molecular grade BSA, 50 mM Tris-HCl pH 7.5, and 250 mM potassium glutamate. Reactions were stopped with 2 μL of 0.5 M EDTA, 3μL of DNA loading buffer was added, and the total reaction was loaded onto a 1% agarose/TBE gel. Gel electrophoresis proceeded for 16 h at 25 V. Gels were stained with 0.5 μg/mL ethidium bromide in TBE buffer for 45 min and destained with water for 1 h. DNA was visualized with an Alpha Imager 2200 Analysis System, and the IC_{50} was determined by nonlinear regression analysis with Graphpad Prism software. *S. aureus* topoismerase IV enzyme subunits, GrlA and GrlB, were purified to homogeneity from pET vector overexpression constructs in *E. coli*.

DNA Gyrase Assay. Enzyme activity was measured by a supercoiling assay that monitored the ATP-dependent conversion of relaxed pBR322 DNA to the supercoiled form. Specifically, 0.1 μg of relaxed pBR322 DNA was incubated with 1 unit of *S. aureus* DNA gyrase for 60 min at 37 °C in 20 μL of the following buffer: 2 mM ATP, 7.5 mM DTT, 30 mM KCl, 7.5 mM $MgCl_2$, 75 μg/mL of molecular grade BSA, 75 mM Tris-HCl pH 7.5, and 300 mM potassium glutamate. Reactions were stopped with 10 μL of 0.5% SDS, 6 mM EDTA, 5.35% glycerol, and 0.013% bromo blue, and the total reaction was loaded onto a 1% agarose/TBE gel. Gel electrophoresis proceeded for 16 h at 25 V. Gels were stained with 0.5 μg/mL of ethidium bromide in TBE buffer for 45 min and destained with water for 1 h. DNA was visualized with an Alpha Imager 2200 Analysis System, and the IC_{50} was determined by nonlinear regression analysis with Graphpad Prism software. *S. aureus* DNA gyrase enzyme subunits, GyrA[34] and GyrB, were purified to homogeneity from pET vector overexpression constructs in *E. coli*.

Human Topoisomerase II Assay. Enzyme activity was measured by a DNA cleavage assay that monitored generation of linear DNA from supercoiled pBR322 DNA catalyzed by human topoisomerase II (TopoGen, Port Orange, FL). Compounds of various concentrations were incubated with 250 ng of supercoiled pBR322 DNA and 4 units of human topo II in 1X cleavage buffer (30 mM

Tris-HCl pH 7.6, 8 mM $MgCl_2$, 60 mM NaCl, 15 mM mercaptoethanol, and 3 mM ATP) in 20 μL at 37 °C for 30 min. The reaction was stopped by adding 2 μL of 10% SDS and 2 μL of 600 ng/μL DNase-free protease K and incubated at 37 °C for 20 min to degrade DNA-bound human topoisomerase II protein. An amount equal to 12 μL of reaction with 1X loading buffer was analyzed by electrophoresis through 1% agarose gel in 1X TAE running buffer with 0.5 μg/mL of ethidium bromide and then visualized and quantified with Alpha Imager 2200 (Alpha Innotech Corporation, San Leandro, CA). The EC_2 value was defined as the effective concentration of drug required to enhance enzyme-mediated cleavage of double-stranded DNA 2-fold.[31]

Mouse Thigh Model of Infection.[35] *S. aureus* ATCC 33591 was employed as the infectious organism. Eight-week-old female CD-1 mice (Charles River Laboratories, Wilmington, MA; range of weights: 19−26 g) were made neutropenic by injecting cyclophosphamide intraperitoneally (150 mg/kg of body weight) 4 days and 1 day before infection. Inoculum was prepared by transferring colonies from a 20-h tryptic soy agar (TSA) culture to sterile PBS, and the density was adjusted to approximately 10^6 CFU/mL, with the aid of a spectrophotometer. The inoculum concentration was determined by the dilution plate count method on TSA. Mice were anesthetized with isoflurane and inoculated by injecting intramuscularly each posterior thigh with 0.1 mL of inoculum ($\sim 10^5$ CFU/thigh). Two hours after inoculation, the positive controls (LZD and VAN) and **7g** were administered. Thighs were harvested (removed aseptically, muscle and bone) from groups of three sacrificed animals (CO_2 asphyxiation) at 2, 4, 6, 8, and 26 h after inoculation. The thighs were homogenized using a tissue homogenizer (Tekmar Tissumizer, Model SDT-1810) for ∼20 s and decimally diluted in sterile PBS. Aliquots (0.1 mL) of serial dilutions were plated on TSA; plates were incubated at 37 °C for 20 h. Colony counts were used to calculate CFU/thigh.

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Supporting Information Available: List of purity data (elemental analyses and HPLC results) for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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