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ARTICLE

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Exploration of the Activity of 7-Pyrrolidino-8-methoxyisothiazoloquinolones against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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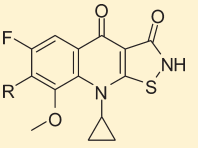
ABSTRACT: A series of 7-(3′-substituted)pyrrolidino-8-methoxyisothiazoloquinolone (ITQ) analogues were prepared, and their antibacterial potency against methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli* were compared. Many of these analogues had MIC ≤ 0.25 μg/mL against quinolone-resistant MRSA strains. The stereochemical preference was explored for a series of 1″-methyl-3′-aminomethylpyrrolidine analogues. Antibacterial activity was generally more favorable with 3′-*R*, 1″-*S* configuration. Substitution on the 3′-aminomethyl nitrogen tended to decrease activity, while potency was maintained with disubstitution or aryl substitution at the 1″-carbon. The 7-[(*R*)-3-((*S*)-1-aminoethyl)pyrrolidin-1-yl] analogue (**6a**(***R,S***)) and the (*R*)-7-[3-(2-aminopropan-2-yl)pyrrolidin-1-yl] analogue (**7a**(***R***)) were found to be the ITQs with the most promising antibacterial profiles. The MICs of these select ITQs versus a panel of clinical MRSA strains were determined, and the ITQs were found to have 8- to 16-fold greater potency than linezolid. These analogues were also evaluated for inhibition of the target enzymes, topoisomerase IV and DNA gyrase, from both wild-type and multidrug resistant strains. The ITQs were up to >30 times more inhibitory against these targets than the fluoroquinolone moxifloxacin.



INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a leading cause of nosocomial infections.[1] Over the past several years, community-associated MRSA strains have been involved in an increasing number of serious infections not originating in the hospital setting.[2] Despite the availability of several classes of antibiotics including the relatively recently introduced oxazolidinones (linezolid) and lipopeptides (daptomycin), these infections cause significant morbidity and mortality. Resistance to a number of antibacterial agents has slowly, but steadily, increased over time including reports of clinical isolates resistant to vancomycin, a widely used antistaphylococcal drug.[3,4] The current clinical landscape suggests that both the prudent use of existing drugs coupled with new antibacterial discovery are required to combat these serious medical issues.[5] A valuable new addition to our antibacterial arsenal for MRSA infections would be a new bactericidal, oral/iv agent effective against resistant isolates. The quinolone class of antibacterials could fill this need; however, most MRSA clinical isolates are sufficiently resistant to quinolones, rendering them ineffective in successfully curing many infections.

Isothiazoloquinolones (ITQs, Figure 1), originally reported by Chu and co-workers,[6−9] are a class of potent antibacterial agents related to the quinolones[10] that also inhibit the type II bacterial topoisomerases DNA gyrase (GyrA/GyrB) and topoisomerase IV (GrlA/GrlB).[11,12] These compounds probably resemble quinolones in their mechanism of action in that they form stable ternary complexes with the topoisomerases and cleaved DNA, thus acting as a "poison" and inhibiting DNA replication.[13] This leads to relatively rapid bacterial cell death by several possible mechanisms still under investigation.[13] The ITQs, best represented by the direct ciprofloxacin analogue A-62824[9] (**1**, Figure 1), were previously shown to be more potent against *S. aureus* than their quinolone counterparts. However, these initial compounds possessed selectivity and toxicity issues[14] and none were advanced into clinical development. Recently, we reported new variations of ITQs and related compounds containing functionalized aryls and heteroaryls attached via a C−C bond at the 7-position[15,16] and structural modifications at positions 4a, 6, 8, and 9.[17,18] Many of these compounds demonstrated (i) excellent in vitro antibacterial activities against MRSA, (ii) strong inhibitory activities against DNA gyrase and topoisomerase IV, (iii) weak activity against human topoisomerase II, and (iv) decreased cytotoxicity against human cell lines relative to previously reported ITQs. These data indicate that addition of an 8-methoxy group affected an increase in activity against MRSA and a decrease in cytotoxicity against human Hep2 laryngeal carcinoma cells. In our continuing efforts to develop ITQs with potent anti-MRSA and improved pharmacological properties, we tested the extension of this finding to the more traditional saturated aminocyclic type of 7-position substituents, particularly pyrrolidines that are known to enhance Gram-positive activity.[11,19,20] Here we report the synthesis, anti-MRSA activity, cytotoxicity, and target enzyme activity of 8-methoxy ITQs with 7-aminocyclic substituents, focusing on a

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potent series of 7-(3′-aminomethylpyrrolidine) ITQs and the effects of various substitutions and stereochemistry.

CHEMISTRY

Overview. The target ITQs were prepared according to general Scheme 1 by nucleophilic aromatic substitution commonly used in the preparation of quinolones.[11] The key ITQ intermediate **2** was prepared according to published methods.[18,21] The 7-position substituents of the corresponding target compound sets **3**−**12** are listed in Figure 2 with a letter designation for the appended 3′-N-substitution pattern in the included table. The stereochemical configurations of compounds with chiral 3′- and 1″-positions are designated in parentheses (see, for example, compound **6b**(*R,S*) shown in Figure 2). In some cases the appended 3-position amine or alkylamine was Boc-protected prior to the substitution reaction to improve workup and purification of the 7-substituted ITQ and was then subsequently deprotected with standard conditions to give the target ITQ analogue. In no case of using an unprotected appended amine did alkylation result in the unwanted regioisomer under these conditions. Also, the substitution reaction resulted in varying degrees (up to 20%) of demethylation of the starting ITQ core **2**. This side product was removed during final purification via preparative reverse-phase HPLC.

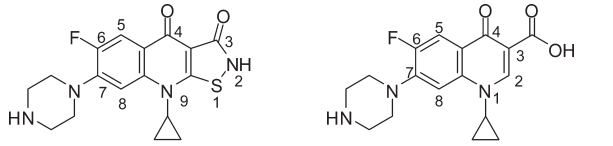
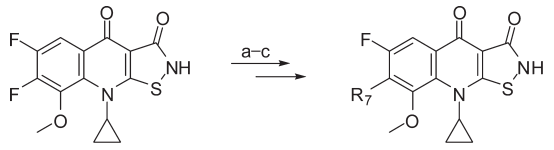


Figure 1. Numbering scheme of isothiazoloquinolone **1** and quinolone ciprofloxacin.

Scheme 1[a]

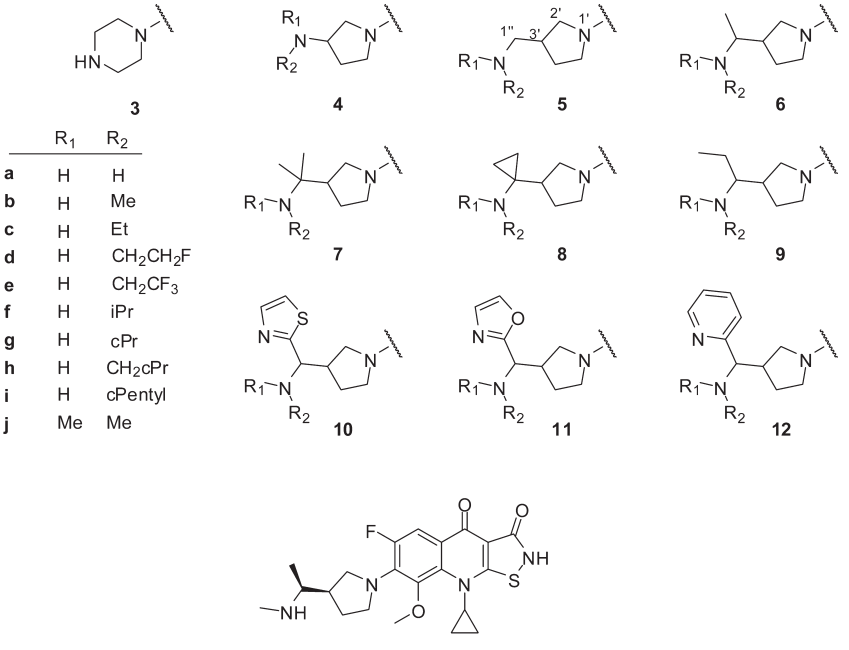


[a] Reagents and conditions: (a) piperazine or various pyrrolidines (1.1−1.2 equiv), *N,N*-diisopropylethylamine (5 equiv), DMSO, 120 °C, 15 h; (b) deprotection if needed; (c) purification by preparative HPLC, 10−44% yield overall.

Preparation of *N*-Alkyl-Substituted Pyrrolidines. Pyrrolidines used to make target compound sets **4**−**12** and their isomers were obtained from commercial sources, prepared following literature procedures[22−30] or synthesized in manners similar to the representative examples outlined below.

Reductive amination of 1-protected 3-aminomethylpyrrolidines with formic acid and formaldehyde[24] led to dimethylation and was used to prepare the pyrrolidines needed for **5k**(*S*), **6k**(*R, rac*), **6k**(*R,S*), and **7k**(*R*). The monomethyl amines (**b** series compounds) were prepared by simple reduction of the Boc-protected amine.[25] The monoethylamines (**c**) were prepared by alkylation in the presence of cesium hydroxide according to the procedure of Salvatore et al.[31] The *N*-fluoroethyl intermediates needed for preparation of **5d**(*S*), **6d**(*R,R*), **6d**(*R,S*), and **7d**(*R*) were made by treatment of the primary amines with 2-fluoroethyl 4-methylbenzenesulfonate.[32]

N-Protected pyrrolidine **13** was prepared according to a published procedure[22] and then used to synthesize pyrrolidine intermediates **14**−**17** (Scheme 2). Compound **13** was alkylated by reductive amination using commercially available cyclopropanecarboxaldehyde to afford *N*-(cyclopropylmethyl)-1-[(*R*)-1-((*S*)-1-phenylethyl)pyrrolidin-3-yl]ethanamine **14** in 93% yield.



	R_1	R_2
a	H	H
b	H	Me
c	H	Et
d	H	CH_2CH_2F
e	H	CH_2CF_3
f	H	iPr
g	H	cPr
h	H	CH_2cPr
i	H	cPentyl
j	Me	Me

Figure 2. Structures and numbering of the R_7 substituents of ITQ series **3**−**12**.

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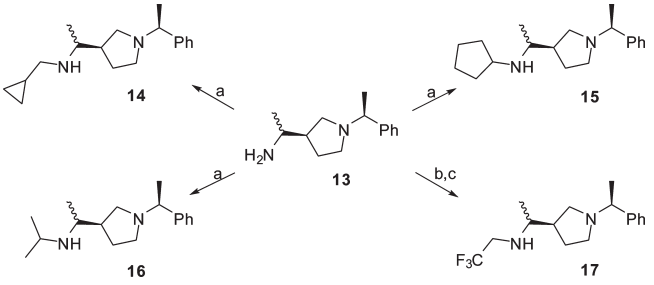
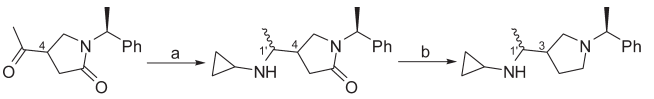
Scheme 2[a]



[a] Reagents and conditions. (a) For **14**: *c*-PrCHO. For **15**: $(CH_2)_4CO$. For **16**: CH_3COCH_3 (1.05 equiv). $NaBH(OAc)_3$ (1.4 equiv), AcOH (1 equiv), DCE, room temp, 6−24 h, 90−93%. (b) $(CF_3CO)_2O$ (1.3 equiv), $CHCl_3$, room temp, 15 h, quant; (c) LAH (2 equiv), THF, room temp, 70 °C, 3 days, 87%.

Scheme 3[a]



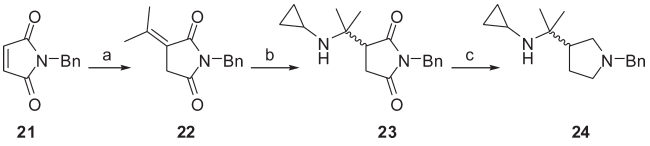
18a: (4-*R*) **18b; (4-*S*)** **19a: (4-*R*,1'-*rac*)** **19b: (4-*S*,1'-*rac*)** **20a: (3-*R*,1'-*rac*)** **20b: (3-*S*,1'-*rac*)**

[a] Reagents and conditions: (a) *c*-$PrNH_2$ (1 equiv), $NaBH(OAc)_3$ (1.4 equiv), AcOH (1 equiv), DCE, room temp, 24 h, 97% for **19a** and 96% for **19b**; (b) $BH_3 \cdot THF$ (5 equiv), THF, room temp, 24 h, 94% for **20a** and 88% for **20b**.

The same conditions were used to prepare cyclopentylamine **15** and isopropylamine **16** from cyclopentanone and acetone in 93% and 90% yield, respectively. The trifluoroethylamine compound **17** was obtained by treatment of **13** with trifluoroacetic anhydride in chloroform at room temperature to provide the trifluoroacetamide intermediate in quantitative yield. This intermediate was subsequently reduced with lithium aluminum hydride in refluxing tetrahydrofuran to give **17** in 87% yield. The intermediates **14**, **15**, **16**, and **17** were debenzylated under standard reductive conditions (hydrogenolysis over Pearlman's catalyst) and were then used to prepare compounds **6i**(*R,rac*), **6j**(*R,rac*), **6f**(*R,rac*), and **6e**(*R,rac*), respectively, shown in Scheme 1.

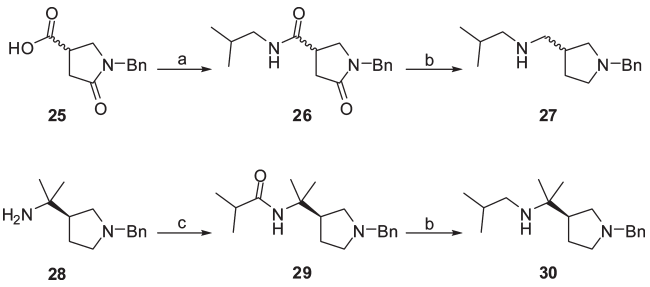
Preparation of the 3-(*N*-cyclopropyl-1-aminoethyl)pyrrolidine side chain for compound **6h**(*R,rac*) and **6h**(*S,rac*) could not be accomplished with this same reductive amination strategy, nor could it be prepared from alkylation of the primary amine by nucleophilic substitution with a halocyclopropane. Rather, *S*-phenylethylpyrrolidinones **18a** and **18b**, prepared according to previously published methods,[22] were separately treated with cyclopropylamine, sodium triacetoxyborohydride,[33] and acetic acid in 1,2-dicholoroethane to yield the *N*-cyclopropylpyrrolidinones **19a** and **19b** (Scheme 3). Further reduction with a solution of borane−tetrahydrofuran complex provided the secondary amines **20a** and **20b** in high yield (91% and 88%, respectively, over two steps). Debenzylation and subsequent treatment with **2** gave compounds **6h**(*R,rac*) and **6h**(*S,rac*), respectively. The corresponding pyrrolidine for preparation of *N*-cyclopropyl analogue **5h**(*S*) was prepared from α-methylbenzyl protected (*R*)-3-carboxy-2-pyrrolidone according to the procedure of Takemura.[34]

Scheme 4[a]



[a] Reagents and conditions: (a) 2-nitropropane (excess), K_2CO_3 (0.1 equiv), EtOH, reflux; (b) cyclopropylamine (excess, neat), room temp; (c) $BH_3 \cdot Me_2S$, room temp.

Scheme 5[a]



[a] Reagents and conditions: (a) EDCI (1.5 equiv), HOBt (1.3 equiv), 2-methylpropan-1-amine (1.1 equiv) in DMF, room temp, 18 h, 84%; (b) LAH (4 equiv) in THF reflux 18 h, quant; (c) EDCI (1.5 equiv), HOBt (1.3 equiv), isobutyric acid (1 equiv) in DMF, room temp, 18 h, 76%.

The *N*-cyclopropyl substituted pyrrolidine used for analogue **7h**(*rac*) was prepared from maleimide (**21**) according to Scheme 4. Reaction with 2-nitropropane gave the exocyclic α,β-unsaturated maleimeide (**22**), which reacted slowly with cyclopropylamine to produce adduct **23**. This was reacted without isolation with borane dimethyl sulfide complex to give the desired intermediate **24**, which was later debenzylated and treated with ITQ core **2**.

The isobutyl substituted pyrrolidine intermediates for analogues **5g**(*rac*) and **7g**(*R*) were prepared from the commercially available carboxy-substituted pyrrolidone **25** and intermediate **28**, respectively, according to Scheme 5 below.

The 3-1′-aminopropylpyrroldine diastereomers **9b**(*R,S*) and **9b**(*R,R*) were prepared from the common intermediate **32** (Scheme 6). Reduction of the cyclopropyl intermediate **31**, prepared according to Miyauchi[35] and used for the synthesis of **8a**(*R*), did not produce the expected *N*-methylcyclopropyl intermediate but instead also effected ring-opening to give **32** as a mixture of diastereomers. This mixture was used directly to prepare **9b**(*R,S*) and **9b**(*R,R*), which were separated/purified by preparative HPLC.

Preparation of Heterocycle-Substituted Pyrrolidine Analogues. Reports[36] of bulkier and/or more lipophilic 7-substitutents leading to increased activity against Gram-positive strains prompted exploration of a series of heterocycles, such as thialzolyl, oxazolyl, and pyridyl moieties, in the 1″-position of the 3′-aminomethylpyrrolidines. We employed literature procedures[37−40] to provide the heterocycle-containing pyrrolidines necessary for ITQs **10a**(*R,rac*), **11a**(*R,rac*), **12a**(*R,rac*) shown in Figure 2.

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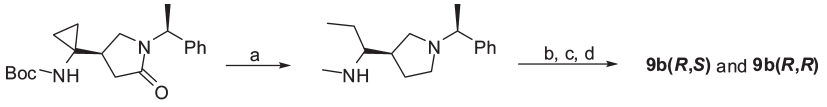
Scheme 6[a]



31 32

[a] Reagents and conditions: (a) LAH (4 equiv), THF, 0 °C → reflux, 18 h; (b) H_2 (1 atm), $Pd(OH)_2$ (cat.), EtOH, 45 °C; (c) **2** (0.9 equiv), *N*,*N*-diisopropylethylamine (5 equiv), DMSO, 120 °C, 3 h; (d) preparative HPLC to separate isomers, 20% for **9b**(*R*,*S*) and 7% for **9b**(*R*,*R*).

BIOLOGICAL EVALUATION AND DISCUSSION

Each compound was screened for antibacterial activity using standard techniques[41] against methicillin-sensitive *S. aureus* (MSSA) strain and a methicillin-resistant and quinolone-resistant *S. aureus* (MQRSA) strain and compared to the marketed quinolones moxifloxacin (MXF) and ciprofloxacin (CIP) as well as the previously reported ITQ, **1** (Table 1). Breadth of spectrum was addressed through testing against *E. coli*. Microbial specificity was determined with a 72 h cytotoxicity assay using human Hep2 cells (laryngeal carcinoma).[42]

To understand if the addition of an 8-methoxy group would provide the same benefit previously reported[16] of reducing cytotoxicity while maintaining or increasing Gram-positive potency, we first prepared compound **3**, the 8-methoxy version of **1**. There was a 4-fold improvement in cytotoxicity, while the Gram-positive potency remained the same; however, there was a loss of potency against *E. coli*. Exploring other aminocyclics at the 7-position, we found that the 3-aminopyrrolidines **4a** and **4b** were slightly more potent against MQRSA, consistent with reported SAR of standard quinolones showing enhanced Gram-positive activity of 7-pyrrolidines versus other aminocyclics.[11,19,20,28] There was a slight stereochemical preference for the 3′-*S*-isomers. Alkyl substitution on the nitrogen (**4b**) both decreased potency and improved cyctotoxicity.

Insertion of a methylene between the 3′-nitrogen and the pyrrolidine ring, compounds **5a**(*S*) and **5a**(*R*), improved MQRSA activity. Monoalkylation of the terminal amine of the 3′-aminomethylpyrrolidine reduced cytotoxicity as seen with **5b**(*S*), **5f**(*S*), and **5h**(*S*) while maintaining or improving MQRSA activity. ITQ **5h**(*S*) was exceptionally potent versus MSSA and MQRSA with MICs of 0.004 and 0.06 μg/mL, respectively. The increasing potency seen with increased bulk prompted the synthesis of the isobutyl analogue **5g**(*rac*); however, MQRSA potency was greatly decreased to 4 μg/mL (at best 2 μg/mL considering 2× dilution as the racemic mixture). Dimethylation (**5k**(*S*)) also decreased anti-MQRSA activity.

Substitution on the 1″-methylene of the 3′-aminomethylpyrrolidines to give the diastereomer series of compounds, **6a**, resulted in a differential in stereochemical preference. We observed a strong preference for the 3′-*R*-isomer, showing a 4- to 16-fold improvement in MQRSA MIC over the 3′-(*S*) isomers. Of all the diastereomers, the **6a**(*R*,*S*) isomer was the most potent with an MIC of 0.06 μg/mL against the highly resistant *S. aureus* strain. The stereochemical preference of the 1″-carbon was pronounced for the more potent 3′-*R* isomer, with the 1″-*S* isomer showing a 4-fold improvement against MQRSA (**6a**(*R*,*R*) vs **6a**(*R*,*S*)). However, no preference was seen when comparing the less active 1″-*S* isomers **6a**(*S*,*R*) and **6a**(*S*,*S*). The stereochemistry did have an impact on cytotoxicity for both diaseteromers, with the 1″-*S* isomers showing lower CC_{50} (the concentration of drug that results in toxicity to 50% of the cells), demonstrating that although cytotoxicity tracks with MIC, it can be separated.

Alkyl substitution on the 1″-nitrogen of the 3′-(1″-methyl) aminomethylpyrrolidine series (compound series **6**) offered improvement in cytotoxicity but did not improve Gram-positive potency as seen for the 3′-aminomethylpyrrolidine series **5**. Potency generally decreased with increased bulk of the substituent, and disubstitution of the nitrogen also decreased MQRSA potency as demonstrated by comparing **6k**(*R*,*S*) to its monosubstituted counterpart **6a**(*R*,*S*). Compound **6h**(*R*,*rac*) with a cyclopropyl substituent was equipotent to its 1″-desmethyl counterpart **5h**(*S*) and its nonsubstituted counterpart **6a**(*R*,*rac*) with a greater than 2-fold advantage in cytotoxicity.

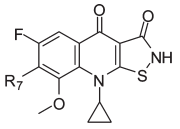
The 1″-dimethyl series **7** analogues were also highly potent anti-MQRSA agents. Comparison with corresponding compounds in the monomethyl series **6** showed a mixed trend for MICs, with MQRSA moving slightly up or down but Gram-negative MICs generally increasing. Cytotoxicity was diminished (higher CC_{50}) for all except **7d**(*R*). The 1″-cyclopropyl analogue **8a**(*R*) had the strongest MSSA activity of all the compounds with equally impressive MQRSA and *E. coli* MIC. The 1″-ethyl isomers **9b**(*R*,*S*) and **9b**(*R*,*R*) had slightly less antibacterial activity than the corresponding 1″-methyl series (**6**) compounds.

Interestingly, substitution with aryl groups on the 1″-methylene did not significantly impact MQRSA potency as exemplified by compound **10a**(*R*,*rac*) with an MIC of 0.06 μg/mL compared to 0.06 μg/mL of the corresponding 1″-methyl analogue **6a**(*R*,*rac*). One drawback to the series of 1″-aryl susbstituted analogues was the greater degree of cytotoxicity.

Further Evaluation of Select ITQs. Compounds **6a**(*R*,*S*) and **7a**(*R*) were chosen for further in vitro profiling because they exhibited strong anti-MQRSA MICs of 0.06 μg/mL. We evaluated their in vitro antibacterial activities by performing susceptibility testing against two panels of recent clinical isolates of *S. aureus* (one panel of 10 strains and one panel of 70 strains). Table 2 lists (i) the MIC ranges of the selected ITQs and reference compounds against these panels of *S. aureus* strains, (ii) the MICs at which 50% of the isolates are inhibited (MIC_{50}), and (iii) the MICs at which 90% of the isolates are inhibited (MIC_{90}). From a comparison of activity in the 10-strain panel, the MIC_{50} of ITQ **6a**(*R*,*S*) was 2-fold better than **7a**(*R*), though their MIC_{90} values were the same. Additional susceptibility testing against a collection of 70 recent *S. aureus* clinical isolates showed that **7a**(*R*) was 256 times more potent than levofloxacin (LFX) and 4- to 16-fold better than the clinical standard of care against MRSA, vancomycin (VAN).

We further evaluated dual activities of **7a**(*R*) and **6a**(*R*,*S*) along with the N-alkylated ITQs **6b**(*R*,*S*) and **6d**(*R*,*S*) against their target enzymes topoisomerase IV and DNA gyrase. Antibacterial agents having dual activities against these biochemical targets are desirable, as they should reduce the selection of

Table 1. Antibacterial and Cytotoxic Activities of 6-Fluoro-8-methoxy-ITQs[a]



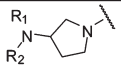
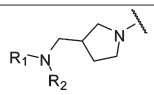
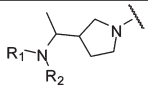
Compound	R_1	R_2	MIC[b] MSSA	MIC[b] MQRSA	MIC[b] Ec	CC_{50}[c] Hep2	R_7 preparation Reference[d]
CIP			0.25	32	0.02	>100	NA[e]
MXF			0.06	4	0.03	>100	NA
1			0.06	4	0.004	7	10
3			0.06	4	0.03	27	com[f]
4 R_7 = 							
4a(*R*)	H	H	0.03	2	0.008	25	com
4a(*S*)	H	H	0.008	0.5	0.008	19	com
4b(*R*)	H	Me	0.006	4	0.06	42	22
4b(*S*)	H	Me	0.03	2	0.06	41	22, 23
5 R_7 = 							
5a(*R*)	H	H	0.008	0.25	0.03	11	28, 22
5a(*S*)	H	H	0.008	0.25	0.004	11	28, 22
5b(*S*)	H	Me	0.008	0.5	0.06	28	28
5d(*S*)	H	CH_2CH_2F	0.008	0.13	0.03	18	32
5f(*S*)	H	iPr	0.02	0.5	0.06	33	28
5g(*rac*)	H	iBu	0.06	4	0.13	46	Scheme 5
5h(*S*)	H	cPr	0.004	0.06	0.03	23	34
5k(*S*)	Me	Me	0.06	4	0.13	>100	28
6 R_7 = 							
6a(*R*,*rac*)	H	H	0.004	0.06	0.02	9	22, 24, 25
6a(*R*,*R*)	H	H	0.02	0.25	0.03	33	22, 24, 25
6a(*R*,*S*)	H	H	0.002	0.06	0.008	6	22, 24, 25
6a(*S*,*rac*)	H	H	0.03	1	0.06	n.e.	22, 24, 25
6a(*S*,*R*)	H	H	0.02	1	0.06	51	22, 24, 25
6a(*S*,*S*)	H	H	0.03	1	0.06	15	22, 24, 25
6b(*R*,*rac*)	H	Me	0.008	0.25	0.03	42	22, 24, 25
6b(*R*,*R*)	H	Me	0.02	0.5	0.06	29	22, 24, 25
6b(*R*,*S*)	H	Me	0.008	0.25	0.03	12	22, 24, 25
6b(*S*,*rac*)	H	Me	0.06	2	0.13	>100	22, 24, 25
6c(*R*,*rac*)	H	Et	0.008	0.25	0.06	40	22, 24, 25
6c(*R*,*S*)	H	Et	0.004	0.13	0.06	27	22, 24, 25
6d(*R*,*R*)	H	CH_2CH_2F	0.004	0.25	0.06	74	32
6d(*R*,*S*)	H	CH_2CH_2F	0.002	0.13	0.06	73	32

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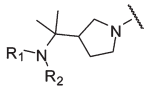
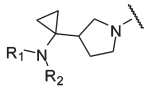
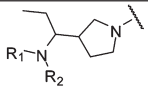
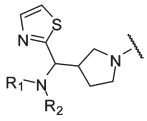
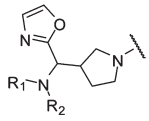
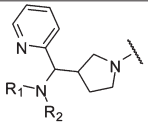
Table 1. Continued

Compound	R_1	R_2	MIC[b] MSSA	MIC[b] MQRSA	MIC[b] Ec	CC_{50}[c] Hep2	R_7 preparation Reference[d]
6e(*R*,*rac*)	H	CH_2CF_3	0.13	4	n.e.	41	Scheme 2
6f(*R*,*rac*)	H	iPr	0.02	0.5	0.5	51	Scheme 2
6h(*R*,*rac*)	H	cPr	0.004	0.06	0.06	25	Scheme 3
6h(*S*,*rac*)	H	cPr	0.02	0.13	0.13	43	Scheme 3
6i(*R*,*rac*)	H	CH_2-cPr	0.03	0.5	0.25	43	Scheme 2
6j(*R*,*rac*)	H	c-Pent	0.06	4	n.e.	42	Scheme 2
6k(*R*,*rac*)	Me	Me	0.03	1	0.06	40	24
6k(*R*,*S*)	Me	Me	0.008	0.5	0.06	39	24
7 R_7 = 							
7a(*R*)	H	H	0.004	0.06	0.06	9	27
7a(*S*)	H	H	0.03	2	0.13	61	27
7b(*R*)	H	Me	0.008	0.13	0.13	28	27, 31
7c(*R*)	H	Et	0.02	0.5	0.13	50	24, 27
7d(*R*)	H	CH_2CH_2F	0.004	0.06	0.06	19	27, 32
7g(*R*)	H	iBu	0.06	2	1	88	Scheme 5
7h(*rac*)	H	cPr	0.02	0.25	0.25	54	Scheme 4
7k(*R*)	Me	Me	0.06	1	0.13	90	24
8 R_7 = 							
8a(*R*)	H	H	0.001	0.06	0.008	4	35
9 R_7 = 							
9b(*R*,*S*)	H	Me	0.008	0.5	0.06	29	Scheme 6
9b(*R*,*R*)	H	Me	0.03	2	0.13	58	Scheme 6
10 R_7 =  **11** R_7 =  **12** R_7 = 							
10a(*R*,*rac*)	H	H	0.004	0.06	0.13	5	39
11a(*R*,*rac*)	H	H	0.004	0.25	0.06	5	40
12a(*R*,*rac*)	H	H	0.008	0.13	0.06	1	39

[a] Abbreviations: MIC, minimum inhibitory concentration; hep2, Hep2 laryngeal carcinoma cells; MSSA, methicillin-sensitive *Staphylococcus aureus* ATCC 29213; MQRSA, methicillin- and quinolone-resistant *Staphylococcus aureus* ATCC 700699; Ec, *Escherichia coli* ATCC 25922; CIP, ciprofloxacin; MXF, moxifloxacin; n.e., not evaluated. [b] Minimum inhibitory concentrations (MICs) are expressed in μg/mL. [c] 72 h cytotoxic activities (CC_{50}) are expressed in μM. [d] Reference for the method of preparation of the 7-position pyrrolidine. [e] Not applicable. [f] Commercially available.

resistant organisms and should remain active against preexisting strains with mutations in one of the two targets. Their activities were compared with those of the fluoroquinolones CIP, gemifloxacin (GEM), and moxifloxacin (MFX). The in vitro results reported in Table 3 are (i) MICs against *S. aureus* and *E. coli* and (ii) inhibitory activities against their target enzymes from *S. aureus*, wild-type topoisomerase IV and DNA gyrase, as well as the corresponding enzymes from staphylococcal mutants expressing fluoroquinolone resistance. The mutant enzymes possess previously reported mutations in their quinolone-resistance-determining regions (QRDRs).[11] The select analogues displayed stronger inhibition activities than the comparator quinolones against the target enzymes especially against enzymes from resistant bacteria. Notably, when compared with ciprofloxacin, **7a(*R*)** and **6a(*R*,*S*)** with MRSA MIC of 0.09 and 0.06 μg/mL, respectively, showed a 91-fold increase in inhibition of wild-type DNA gyrase activity for **7a(*R*)**, and **6a(*R*,*S*)** showed a 75-fold increase in inhibition of wild-type topoisomerase IV activity for **6a(*R*,*S*)**. Similar improvements in inhibition of enzymes from resistant bacteria were seen with >48-fold and >62-fold increases

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against mutant DNA gyrase for **7a**(*R*) and **6a**(*R*,*S*), respectively, and 95-fold and 121-fold increases in inhibition observed for mutant topoisomerase IV for **7a**(*R*) and **6a**(*R*,*S*), respectively. Alkylated analogues **6b**(*R*,*S*) and **6d**(*R*,*S*) were similarly active against target enzymes compared with the selected analogues **7a**(*R*) and **6a**(*R*,*S*). We note that this result demonstrated a significant improvement in inhibition of the *S. aureus* enzymes and provided a further basis for additional in vitro profiling. This potent inhibition of both the gyrase and topoisomerase IV target enzymes probably accounts for the excellent antibacterial activity even against quinolone-resistant strains and suggests that resistance development may be slow to emerge among clinical isolates.

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

		MIC (μg/mL)		
compd	organism (no. of strains)	range	50%	90%
6a(*R*,*S*)	MRSA (10)	0.004−0.5	0.06	0.5
7a(*R*)	MRSA (10)	0.008−1	0.13	0.5
7a(*R*)	MRSA (70)	0.008−0.5	0.06	0.25
LFX	MRSA (70)	0.12 to >16	16	>16
LZD	MRSA (70)	1−2	1	2
OXA	MRSA (70)	4 to >16	>16	>16
QPS/DPS	MRSA (70)	0.25−1	0.5	0.5
VAN	MRSA (70)	0.5−2	1	1

[a] Abbreviations: LFX, levofloxacin; LZD, linezolid; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; OXA, oxacillin; QPS, quinupristin; DPS, dalfopristin; VAN, vancomycin; 50% and 90%, MIC_{50} and MIC_{90}, respectively.

SUMMARY OF RESULTS

In conclusion, we described the syntheses and antibacterial activities of 8-methoxy ITQs having structural modifications at the 7-position. The structure−activity and structure−toxicity relationships are summarized in Figure 3. We found that 7-(3′-aminomethylpyrrolidino) ITQs were generally more potent than 7-(3′-aminopyrrolidine) analogues and that the *R*-isomer of the 3′-methylaminopyrrolidines were more potent (up to 16-fold) than the corresponding *S*-isomer. Substitution at the 1″-carbon of the 3′-methylaminopyrrolidines improved potency, and the *S*-1″-methyl analogues were slightly more potent than the corresponding *R*-isomers. The dimethyl substitution series (**7**) and the cyclopropyl series (**8**) were also highly potent compounds. Bulky substitution with aryl groups at the 1″-position (**9**, **10**, and **11**) did not significantly diminish MRSA potency but reduced bacterial selectivity as measured by toxicity against hep2 laryngeal cells. While bulk seemed tolerable at the 1″-position, N-substitution was generally not favorable except for *N*-cyclopropyl

Table 3. In Vitro Activities of Select ITQs[a]

	Sa enzyme Inhibition[b]				MIC[c]		
compd	TopoIV WT	TopoIV Ser80Phe[d]	DNA gyrase WT	DNA gyrase Ser84Leu[d]	MSSA	MRSA	Ec
CIP	3	57	62	>200	0.25	32	64
GEM	0.4	9.8	5.6	>200	0.03	2	4
MXF	1	11.9	7.7	>200	0.06	2	4
6a(*R*,*S*)	0.04	0.47	0.68	3.2	0.002	0.06	0.02
6b(*R*,*S*)	0.11	1.16	1.1	18.7	0.008	0.25	0.03
6d(*R*,*S*)	0.06	0.66	1.32	12	0.002	0.13	0.06
7a(*R*)	0.12	0.6	0.68	4.2	0.004	0.06	0.06

[a] Abbreviations: CIP, ciprofloxacin; Ec, *Escherichia coli* ATCC 25922; GEM, gemifloxacin; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 700699; MSSA, methicillin-sensitive *Staphylococcus aureus* ATCC 29213; MXF, moxifloxacin. [b] Inhibitions of wild-type and mutant *S. aureus* topoisomerase IV (TopoIV) decatenation (IC_{50}) and DNA gyrase supercoiling (IC_{50}) are expressed in μM. The values listed are the averages of multiple experiments. [c] Minimum inhibitory concentration (MIC) is expressed in μg/mL. The values listed are the modal values of multiple experiments. [d] Indicates position of substituted amino acid due to mutation in gene that encodes enzyme product.

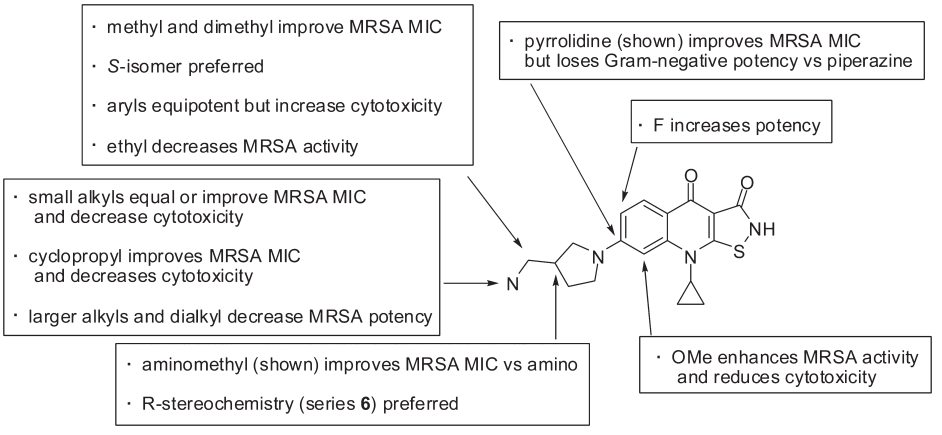

Figure 3. Summary of structure−activity and structure−toxicity relationships of 7-(3′-aminomethylpyrrolidino) ITQs (substitution effects relative to unsubstituted).

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and *N*-2-fluoroethyl analogues, particularly for more bulky substituents and N-dimethylation.

Exceptionally potent compounds **6a(*R*,*S*)** (MIC = 0.06 μg/mL) and **7a(*R*)** (MIC = 0.09 μg/mL) against MQRSA emerged from this study. The MIC_{50} and MIC_{90} against a panel of MRSA strains proved their superior efficacy against commercially established Gram-positive antibacterial agents. This activity tracks with their inhibition of target enzymes topoisomerase IV and DNA gyrase from MRSA strains. These compounds seem to be much less affected by mutations in the QRDRs of the target enzymes than conventional quinolones, and this translates to improved antibacterial activity against quinolone-resistant isolates.

Generally, isothiazoloquinolones are a viable class of antibacterial agents with exceptional potency against MRSA strains, even those that are quinolone resistant. Further in vitro and in vivo profiling studies for **6a(*R*,*S*)** and **7a(*R*)** are underway in our laboratories to evaluate their antibacterial activities and other pharmaceutical properties. From preliminary single-dose studies (unpublished results), data for **7a(*R*)** indicated efficacy in both mouse sepsis and thigh infection models with reductions in bacterial numbers equivalent to or greater than those of positive control compound.

EXPERIMENTAL SECTION

General. All nonaqueous reactions were performed under an atmosphere of dry Ar (99.99%) using oven-dried glassware and anhydrous solvents. 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 mm × 4.6 mm, 3.5 μm column (method 1); (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 mm × 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. NMR spectra were recorded using a Bruker Avance 300 spectrometer (^1H 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 ^1H decoupled. The chemical shifts for ^1H 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 $CFCl_3$. $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, $^1H-^{13}C$ HMBC, and ^{13}C APT spectra were used routinely for assignment of signals.

Boc Protection of 1″-Position Amines. The general procedure to protect primary or secondary 1″-position amines of the pyrrolidines was done as follows. The 1″-position amine (1.0 equiv) was taken in dichloromethane, and a solution of potassium carbonate (2.10 equiv) in water (same volume to dichloromethane) was added. Di-*tert*-butyl dicarbonate (1.05 equiv) was added to the solution, and the resulting solution was stirred 18 h at room temperature. After completion of the reaction, the layers were separated and the aqueous layer was extracted with dichloromethane (three times). The combined organic layer was dried over sodium sulfate and concentrated.

Debenzylation of *N*-Benzylpyrrolidine. The general procedure to remove the protective benzyl group of pyrrolidine analogues was done as follows. A solution of each same amount (wt to wt ratio) of *N*-benzylpyrrolidine and palladium hydroxide (20 wt % of Pd on carbon, 60% moisture) in ethanol (0.05 M solution) was heated on an oil bath at 40 °C for 12 h under hydrogen atmosphere and cooled to room temperature. The resulting mixture was filtered through a Büchner funnel on a layer of Celite, washing with methanol. The filtrate was evaporated under reduced pressure to afford debenzylated pyrrolidinylamine product in quantitative yields. The residue obtained was used in the next coupling reaction without further purification.

General Coupling Procedure for Compounds 3−12. The general procedures for preparation and purification (preparative RP-HPLC using mass-based fraction collection) of ITQ analogues are outlined as follows. *tert*-Butyl (*S*)-1-((*R*)-pyrrolidin-3-yl)ethylcarbamate (0.95 g, 4.44 mmol) and diisopropylethylamine (3.52 mL, 20.20 mmol) were added to a suspension of 9-cyclopropyl-6,7-difluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione (1.31 g, 4.04 mmol) in dimethylsulfoxide (12.0 mL). The reaction mixture was heated on an oil bath for 15 h at 120 °C and cooled to room temperature. The resulting zsolution was purified by preparative RP-HPLC using mass-based fraction collection to provide the desired mass only fractions. The fractions of product were combined and lyophilized. The resulting solid was suspended in ethyl acetate, treated with concentrated HCl or a solution of 1.25 M HCl in methanol. The suspension was sonicated, centrifuged, and decanted to remove solvents. This consecutive step of acidification and solidification was repeated three times. The solid product obtained was dried under vacuum, dissolved in water, and lyophilized to afford the desired product (596 mg, 30%, HCl salt) as a brown solid. Yields and analytical data for the analogues described in this report are listed below.

9-Cyclopropyl-6-fluoro-8-methoxy-7-(piperazin-1-yl)isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (3). Yield: 32%. ^1H NMR (DMSO-d_6) δ 9.32 (br, 1H), 7.59 (d, $J_{H,F}$ = 12.2 Hz, 1H), 3.74 (m, 1H), 3.70 (s, 3H), 3.48 (br, 4H), 3.18 (br, 4H), 1.08 (m, 2H), 0.90 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.3 (s). LCMS: *m*/*z* calcd for $C_{18}H_{19}FN_4O_3S$ ($[M]^+$) 390; found 391 ($[M + H]^+$).

(*R*)-7-(3-Aminopyrrolidin-1-yl)-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (4a(*R*)). Yield: 26%. ^1H NMR (DMSO-d_6) δ 8.25 (br s, 2H), 7.58 (d, $J_{H,F}$ = 13.8 Hz, 1H), 3.89−3.60 (m, 6H), 3.56 (s, 3H), 2.32−2.26 (m, 1H), 2.07−2.01 (m, 1H), 1.15−1.12 (m, 2H), 0.95 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.5 (s). LCMS: *m*/*z* calcd for $C_{18}H_{19}FN_4O_3S$ ($[M]^+$) 390; found 391 ($[M + H]^+$).

(*S*)-7-(3-Aminopyrrolidin-1-yl)-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (4a(*S*)). Yield: 30%. ^1H NMR (TFA-*d*) δ 7.92 (d, $J_{H,F}$ = 13.2 Hz, 1H), 4.41−4.27 (m, 3H), 4.16 (m, 3H), 3.65 (s, 3H), 2.68−2.54 (m, 1H), 2.52−2.39 (m, 1H), 1.54−1.46 (m, 2H), 1.28−1.21 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.5 (s). LCMS: *m*/*z* calcd for $C_{18}H_{19}FN_4O_3S$ ($[M]^+$) 390; found 391 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-[3-(methylamino)pyrrolidin-1-yl]isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (4b(*R*)). Yield: 20%. ^1H NMR (DMSO-d_6) δ 9.06 (br s, 1H), 7.59 (d, $J_{H,F}$ = 13.5 Hz, 1H), 3.87−3.66 (m, 6H), 3.67 (s, 3H), 2.65 (br s, 3H), 2.39−2.24 (m, 1H), 2.18−2.06 (m, 1H), 1.17−1.11 (m, 2H), 0.98−0.93 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.3 (s). LCMS: *m*/*z* calcd for $C_{19}H_{21}FN_4O_3S$ ($[M]^+$) 404; found 405 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-[3-(methylamino)pyrrolidin-1-yl]isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (4b(*S*)). Yield: 18%. ^1H NMR (DMSO-d_6) δ 9.28 (br s, 2H), 7.58 (d, $J_{H,F}$ = 14.1 Hz, 1H), 3.85−3.68 (m, 6H), 3.57 (s, 3H), 2.63 (t, *J* = 5.1 Hz, 3H), 2.39−2.28 (m, 1H), 2.20−2.11 (m, 1H), 1.18−1.12 (m, 2H), 0.98−0.92 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: *m*/*z* calcd for $C_{19}H_{21}FN_4O_3S$ ($[M]^+$) 404; found 405 ($[M + H]^+$).

(*S*)-7-[3-(Aminomethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5a(*S*)). Yield: 18%. ^1H NMR (D_2O) δ 7.29 (d, $J_{H,F}$ = 15 Hz, 1H), 3.87−3.47 (m, 8H), 3.18−3.06 (m, 2H), 2.67−2.57

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(m, 1H), 2.27−2.14 (m, 1H), 1.82−1.67 (m, 1H), 1.25−1.16 (m, 2H), 1.05−0.95 (m, 2H). ^{19}F NMR (D_2O) δ −123.9 (s). LCMS: *m/z* calcd for $C_{19}H_{21}FN_4O_3S$ ($[M]^+$) 404; found 405 ($[M + H]^+$).

(*R*)-7-[3-(Aminomethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5a(*R*)). Yield: 22%. ^{1}H NMR (D_2O) δ 7.19 (d, $J_{H,F}$ = 14.1 Hz, 1H), 3.71−3.31 (m, 5H), 3.39 (s, 3H), 3.13−2.99 (m, 2H), 2.61−2.44 (m, 1H), 2.20−2.06 (m, 1H), 1.75−1.58 (m, 1H), 1.23−1.06 (m, 2H), 1.01−0.84 (m, 2H). ^{19}F NMR (D_2O) δ −124.1 (s). LCMS: *m/z* calcd for $C_{19}H_{21}FN_4O_3S$ ($[M]^+$) 404; found 405 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-(3-((methylamino)-methyl)pyrrolidin-1-yl)isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5b(*S*)). Yield: 14%. ^{1}H NMR (DMSO-d_6) δ 8.98 (br s, 1H), 7.55 (d, *J* = 13.2 Hz, 1H), 3.58 (m, 3H), 3.46 (s, 3H), 2.74 (m, 1H), 2.51 (t, *J* = 5.2 Hz, 2H), 2.50 (s, 3H), 2.09 (m, 1H), 1.73 (m, 1H), 0.98 (m, 4H). ^{19}F NMR (DMSO-d_6) δ −125.6 (s). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-6-fluoro-7-(3-((2-fluoroethylamino)-methyl)pyrrolidin-1-yl)-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5d(*S*)). Yield: 13%. ^{1}H NMR (DMSO-d_6) δ 9.37 (br, 1H), 9.31 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 4.87 (t, *J* = 4.6 Hz, 1H, -CH_2F), 4.72 (t, *J* = 4.6 Hz, 1H, -CH_2F), 3.81−3.68 (m, 2H), 3.62 (m, 2H), 3.52 (s, 3H, methoxy), 3.48 (m, 1H), 3.38 (m, 1H), 3.29 (m, 1H), 3.11 (m, 2H), 2.68 (m, 1H), 2.17 (m, 1H), 1.80 (m, 1H), 1.12 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.6 (s). LCMS: *m/z* calcd for $C_{21}H_{24}F_2N_4O_3S$ ($[M]^+$) 450; found 451 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-6-fluoro-7-(3-((isopropylamino)methyl)-pyrrolidin-1-yl)-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5f(*S*)). Yield: 25%. ^{1}H NMR (CD_3OD) δ 7.53 (d, *J* = 13.8 Hz, 1H), 3.79−3.29 (m, 6H), 3.48 (s, 3H), 3.14−3.04 (m, 2H), 2.63−2.48 (m, 1H), 2.27−2.14 (m, 1H), 1.83−1.65 (m, 1H), 1.29 (d, *J* = 4.5 Hz, 6H), 1.21−1.08 (m, 2H), 0.98−0.87 (m, 2H). ^{19}F NMR (CD_3OD) δ −125.9 (s). LCMS: *m/z* calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-7-(3-((isobutylamino)methyl)-pyrrolidin-1-yl)-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2H,9H)-dione Hydrochloride (5g(*rac*)). Yield: 15%. ^{1}H NMR (CD_3OD) δ 7.49 (d, *J* = 14.1 Hz, 1H), 3.72−3.51 (m, 5H), 3.46 (s, 3H), 3.17−3.08 (m, 2H), 2.87 (d, *J* = 6.3 Hz, 2H), 2.67−2.57 (m, 1H), 2.24−2.17 (m, 1H), 2.04−1.98 (m, 1H), 1.81−1.75 (m, 1H), 1.15 (d, *J* = 6.3 Hz, 2H), 0.99−0.88 (m, 8H). ^{19}F NMR (CD_3OD) δ −125.6 (s). LCMS: *m/z* calcd for $C_{23}H_{29}FN_4O_3S$ ($[M]^+$).460; found 461 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-7-(3-((cyclopropylamino)methyl)pyrrolidin-1-yl)-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (5h(*S*)). Yield: 15%. ^{1}H NMR (DMSO-d_6) δ 9.46 (br, 2H), 7.53 (d, $J_{H,F}$ = 14.0 Hz, 1H), 7.16 (br, 1H), 3.81−3.66 (m, 2H), 3.61 (m, 2H), 3.55 (s, 3H, methoxy), 3.52−3.42 (m, 1H), 3.13 (m, 2H), 2.70 (m, 2H), 2.16 (m, 1H), 1.80 (m, 1H), 1.13 (m, 2H, *c*-Pr), 0.96 (m, 4H, *c*-Pr), 0.73 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −120.8 (s). LCMS (APCI): *m/z* calcd for $C_{22}H_{25}FN_4O_3S$ ($[M]^+$) 444; found 445 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-7-(3-((dimethylamino)methyl)pyrrolidin-1-yl)-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2H,9H)-dione Hydrochloride (5k(*S*)). Yield: 27%. ^{1}H NMR (DMSO-d_6) δ 7.50 (d, *J* = 14.2 Hz, 1H), 3.71 (m, 5H), 3.48 (m, 7H), 3.17 (t, *J* = 6.3 Hz, 1H), 2.74 (m, 4H), 2.18 (m, 1H), 1.71 (m, 1H), 1.07 (m, 2H), 0.89 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.7 (s). LCMS: *m/z* calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

(*R*)-7-[3-(1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*R*,*rac*)). Yield: 38%. ^{1}H NMR (DMSO-d_6) δ 8.23 (br, 1H), 8.20 (br, 1H), 7.56 (d, $J_{H,F}$ = 14.0 Hz, 1H), 6.20 (br, 2H), 3.82−3.50 (m, 3H), 3.60−3.45 (m, 2H), 3.52 (s, 3H, methoxy), 3.28 (m, 1H), 2.41 (m, 1H), 2.14 (m, 1H), 1.76 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H, methyl), 1.26 (d, *J* = 6.5 Hz, 3H, methyl epimer), 1.14 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.5 (s, 1F). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

7-[(*R*)-3-((*R*)-1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*R*,*R*)). Yield: 22%. ^{1}H NMR (DMSO-d_6) δ 8.28 (br, 3H), 7.54 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.82−3.50 (m, 2H), 3.51 (s, 3H, methoxy), 3.50−3.43 (m, 3H), 3.27 (m, 1H), 2.41 (m, 1H), 2.19 (m, 1H), 1.76 (m, 1H), 1.25 (d, *J* = 6.5 Hz, 3H, methyl), 1.23 (m, 2H, *c*-Pr), 0.93 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.5 (s). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

7-[(*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*R*,*S*)). Yield: 30%. ^{1}H NMR (DMSO-d_6) δ 8.15 (br, 3H), 7.55 (d, $J_{H,F}$ = 13.9 Hz, 1H), 3.86−3.51 (m, 3H), 3.52 (s, 3H, methoxy), 3.51−3.45 (m, 2H), 3.29 (m, 1H), 2.42 (m, 1H), 2.09 (m, 1H), 1.75 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H, methyl), 1.14 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

(*S*)-7-[3-(1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*S*,*rac*)). Yield: 22%. ^{1}H NMR (DMSO-d_6) δ 8.13 (br, 3H), 7.56 (d, $J_{H,F}$ = 14.0 Hz, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H, ArH epimer), 3.83−3.34 (m, 5H), 3.52 (s, 3H, methoxy), 3.27 (m, 1H), 2.48−2.03 (m, 2H), 1.76 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H, methyl), 1.24 (d, *J* = 6.5 Hz, 3H, methyl epimer), 1.24 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.5 (s, 1F). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

7-[(*S*)-3-((*R*)-1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*S*,*R*)). Yield: 10%. ^{1}H NMR (DMSO-d_6) δ 8.20 (br, 2H), 7.55 (d, $J_{H,F}$ = 13.9 Hz, 1H), 3.86−3.51 (m, 3H), 3.52 (s, 3H, methoxy), 3.51−3.45 (m, 2H), 3.29 (m, 1H), 2.42 (m, 1H), 2.09 (m, 1H), 1.75 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H, methyl), 1.14 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −121.0 (s). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

7-[(*S*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6a(*S*,*S*)). Yield: 35%. ^{1}H NMR (DMSO-d_6) δ 7.42 (d, $J_{H,F}$ = 14.1 Hz, 1H), 3.63 (m, 2H), 3.45−3.33 (m, 3H), 3.36 (s, 3H, methoxy), 2.97 (m, 1H), 2.23−1.99 (m, 2H), 1.64 (m, 1H), 1.08 (d, *J* = 5.7 Hz, 3H, methyl), 1.04 (m, 2H, *c*-Pr), 0.82 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −126.0 (s). LCMS: *m/z* calcd for $C_{20}H_{23}FN_4O_3S$ ($[M]^+$) 418; found 419 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-{3-[1-(methylamino)ethyl]pyrrolidin-1-yl}isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6b(*R*,*rac*)). Yield: 39%. ^{1}H NMR (DMSO-d_6) δ 9.08 (br, 1H), 8.89 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 6.30 (br, 1H), 3.81−3.64 (m, 3H), 3.55 (m, 2H), 3.52 (s, 3H, methoxy), 3.29 (m, 1H), 2.56 (s, 3H, -NMe), 2.55 (s, 3H, -NMe epimer), 2.28−2.03 (m, 2H), 1.78 (m, 1H), 1.29 (d, *J* = 6.6 Hz, 3H, methyl), 1.25 (d, *J* = 6.6 Hz, 3H, methyl epimer), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: *m/z* calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-8-methoxy-7-{(*R*)-3-[(*R*)-1-(methylamino)ethyl]pyrrolidin-1-yl}isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6b(*R*,*R*)). Yield: 18%. ^{1}H NMR (DMSO-d_6) δ 8.84 (br, 1H), 8.69 (br, 1H), 7.56 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.81−3.64 (m, 3H), 3.59−3.47 (m, 2H), 3.53 (s, 3H, methoxy), 3.29 (m, 1H), 2.58 (s, 3H, -NMe), 2.28−2.03 (m, 2H), 1.77 (m, 1H), 1.24 (d, *J* = 6.6 Hz, 3H, methyl), 1.14 (m, 2H, *c*-Pr), 0.96 (m, 2H, *c*-Pr).

^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-8-methoxy-7-{(*R*)-3-[(*S*)-1-(methylamino)ethyl]pyrrolidin-1-yl}isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6b(*R*,*S*)). Yield: 23%. ^{1}H NMR (DMSO-d_6) δ 9.07 (br, 1H), 8.86 (br, 1H), 7.55 (d, $J_{H,F}$ = 13.9 Hz, 1H), 3.82−3.64 (m, 3H), 3.61−3.45 (m, 2H), 3.55 (s, 3H, methoxy), 3.29 (m, 1H), 2.78−2.66 (m, 1H), 2.55 (s, 3H, -NMe), 2.07 (m, 1H), 1.75 (m, 1H), 1.29 (d, J = 6.6 Hz, 3H, methyl), 1.14 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-{3-[1-(methylamino)ethyl]pyrrolidin-1-yl}isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6b(*S*,*rac*)). Yield: 19%. ^{1}H NMR (DMSO-d_6) δ 8.99 (br, 1H), 8.70 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.82−3.61 (m, 3H), 3.60−3.37 (m, 2H), 3.52 (s, 3H, methoxy), 3.29 (m, 1H), 2.79−2.67 (m, 1H), 2.56 (s, 3H, -NMe), 2.55 (s, 3H, -NMe epimer), 2.29−2.04 (m, 1H), 1.76 (m, 1H), 1.29 (d, J = 6.6 Hz, 3H, methyl), 1.25 (d, J = 6.6 Hz, 3H, methyl epimer), 1.14 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-7-{3-[1-(ethylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6c(*R*,*rac*)). Yield: 25%. ^{1}H NMR (DMSO-d_6) δ 9.04 (br, 1H), 8.73 (br, 1H), 7.56 (d, $J_{H,F}$ = 14.0 Hz, 1H), 6.52 (br, 1H), 3.81−3.68 (m, 3H), 3.56 (m, 2H), 3.52 (s, 3H, methoxy), 3.34 (m, 1H), 3.03−2.96 (m, 2H), 2.59 (m, 1H), 2.26−2.04 (m, 1H), 1.79 (m, 1H), 1.33−1.19 (m, 6H), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.3 (s, 1F), −125.4 (s, 1F). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

9-Cyclopropyl-7-{(*R*)-3-[(*S*)-1-(ethylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6c(*R*,*S*)). Yield: 27%. ^{1}H NMR (DMSO-d_6) δ 9.15 (br, 1H), 8.81 (br, 1H), 7.49 (d, $J_{H,F}$ = 14.0 Hz, 1H), 7.20 (br, 1H), 3.79−3.59 (m, 3H), 3.49 (m, 2H), 3.48 (s, 3H, methoxy), 3.30 (m, 1H), 2.95 (m, 2H), 2.61 (m, 1H), 2.06 (m, 1H), 1.78 (m, 1H), 1.27 (d, J = 6.6 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H), 1.09 (m, 2H, *c*-Pr), 0.90 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-7-{(*R*)-3-[(*R*)-1-(2-fluoroethylamino)-ethyl]pyrrolidin-1-yl}-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6d(*R*,*R*)). Yield: 29%. ^{1}H NMR (DMSO-d_6) δ 9.41 (br, 1H), 9.19 (br, 1H), 7.55 (d, $J_{H,F}$ = 13.9 Hz, 1H), 6.20 (br, 1H), 4.91 (m, 1H, -CH_2F), 4.75 (m, 1H, -CH_2F), 3.82−3.63 (m, 2H), 3.60−3.25 (m, 6H), 3.53 (s, 3H, methoxy), 2.63 (m, 1H), 2.26 (m, 1H), 1.81 (m, 1H), 1.30 (d, J = 6.6 Hz, 3H), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{22}H_{26}F_2N_4O_3S$ ($[M]^+$) 464; found 465 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-7-{(*R*)-3-[(*S*)-1-(2-fluoroethylamino)-ethyl]pyrrolidin-1-yl}-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6d(*R*,*S*)). Yield: 14%. ^{1}H NMR (DMSO-d_6) δ 9.32 (br, 1H), 9.09 (br, 1H), 7.55 (d, $J_{H,F}$ = 13.9 Hz, 1H), 4.88 (m, 1H, -CH_2F), 4.72 (m, 1H, -CH_2F), 3.82−3.64 (m, 3H), 3.61−3.25 (m, 5H), 3.53 (s, 3H, methoxy), 2.62 (m, 1H), 2.09 (m, 1H), 1.80 (m, 1H), 1.34 (d, J = 6.6 Hz, 3H), 1.16 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.3 (s). LCMS: m/z calcd for $C_{22}H_{26}F_2N_4O_3S$ ($[M]^+$) 464; found 465 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-{3-[1-(2,2,2-trifluoroethylamino)ethyl]pyrrolidin-1-yl}isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6e(*R*,*rac*)). Yield: 15%. ^{1}H NMR (DMSO-d_6) δ 7.56 (d, $J_{H,F}$ = 14.0 Hz, 1H), 4.09−3.16 (m, 9H), 3.52 (s, 3H, methoxy), 2.26−1.99 (m, 2H), 1.76 (m, 1H), 1.28 (d, J = 6.5 Hz, 3H, methyl), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.5 (s). LCMS: m/z calcd for $C_{22}H_{24}F_4N_4O_3S$ ($[M]^+$) 500; found 501 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-7-{3-[1-(isopropylamino)ethyl]-pyrrolidin-1-yl}-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6f(*R*,*rac*)). Yield: 17%. ^{1}H NMR (DMSO-d_6) δ 9.01 (br, 1H), 8.44 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.82−3.62 (m, 3H), 3.60−3.32 (m, 3H), 3.53 (s, 3H, methoxy), 2.52 (m, 1H), 2.36−2.04 (m, 2H), 1.79 (m, 1H), 1.37−1.19 (m, 9H), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{23}H_{29}FN_4O_3S$ ($[M]^+$) 460; found 461 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-7-{3-[1-(cyclopropylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (6h(*R*,*rac*)). Yield: 37%. ^{1}H NMR (DMSO-d_6) δ 9.40 (br, 1H), 9.18 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.82−3.64 (m, 3H), 3.62−3.39 (m, 3H), 3.52 (s, 3H, methoxy), 2.70 (m, 2H), 2.31−2.05 (m, 1H), 1.81 (m, 1H), 1.38 (d, J = 6.6 Hz, 3H, methyl), 1.35 (d, J = 6.7 Hz, 3H, methyl epimer), 1.17−0.75 (m, 8H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.2 (s, 1F), −125.4 (s, 1F). LCMS: m/z calcd for $C_{23}H_{27}FN_4O_3S$ ($[M]^+$) 458; found 459 ($[M + H]^+$).

(*S*)-9-Cyclopropyl-7-{3-[1-(cyclopropylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (6h(*S*,*rac*)). Yield: 26%. ^{1}H NMR (DMSO-d_6) δ 9.32 (br, 1H), 9.12 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 7.20 (br, 1H), 3.81−3.64 (m, 3H), 3.64−3.38 (m, 3H), 3.52 (s, 3H, methoxy), 2.73 (m, 2H), 2.30−2.05 (m, 1H), 1.79 (m, 1H), 1.38 (d, J = 6.7 Hz, 3H, methyl), 1.35 (d, J = 6.7 Hz, 3H, methyl epimer), 1.17−0.75 (m, 8H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{23}H_{27}FN_4O_3S$ ($[M]^+$) 458; found 459 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-7-{3-[1-(cyclopropylmethylamino)ethyl]-pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6i(*R*,*rac*)). Yield: 12%. ^{1}H NMR (DMSO-d_6) δ 9.08 (br, 1H), 8.82 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.81−3.62 (m, 2H), 3.61−3.46 (m, 2H), 3.52 (s, 3H, methoxy), 3.38 (m, 1H), 2.99−2.51 (m, 3H), 2.49 (m, 2H), 2.30−2.03 (m, 1H), 1.79 (m, 1H), 1.31 (d, J = 6.6 Hz, 3H, methyl), 1.27 (d, J = 6.7 Hz, 3H, methyl epimer), 1.13 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr), 0.58 (m, 2H, *c*-Pr), 0.39 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s). LCMS: m/z calcd for $C_{24}H_{29}FN_4O_3S$ ($[M]^+$) 472; found 473 ($[M + H]^+$).

(*R*)-7-{3-[1-(Cyclopentylamino)ethyl]pyrrolidin-1-yl}-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6j(*R*,*rac*)). Yield: 44%. ^{1}H NMR (DMSO-d_6) δ 9.27 (br, 1H), 8.78 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 6.53 (br, 1H), 3.92−3.46 (m, 5H), 3.52 (s, 3H, methoxy), 3.37 (m, 1H), 2.68 (m, 1H), 2.50−1.91 (m, 4H), 1.89−1.61 (m, 5H), 1.54 (m, 2H), 1.33 (d, J = 6.6 Hz, 3H, methyl), 1.30 (d, J = 6.6 Hz, 3H, methyl epimer), 1.16 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.3 (s, 1F), −125.4 (s, 1F). LCMS: m/z calcd for $C_{25}H_{31}FN_4O_3S$ ($[M]^+$) 486; found 487 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-7-{3-[1-(dimethylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (6k(*R*,*rac*)). Yield: 36%. ^{1}H NMR (DMSO-d_6) δ 10.17 (br, 1H), 7.55 (d, $J_{H,F}$ = 14.0 Hz, 1H), 3.82−3.70 (m, 3H), 3.70−3.44 (m, 3H), 3.52 (s, 3H, methoxy), 2.75 (m, 3H), 2.69 (m, 3H), 2.56 (m, 1H), 2.39−2.03 (m, 1H), 1.76 (m, 1H), 1.30 (d, J = 6.7 Hz, 3H, methyl), 1.25 (d, J = 6.7 Hz, 3H, methyl epimer), 1.15 (m, 2H, *c*-Pr), 0.94 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.5 (s, 1F). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

9-Cyclopropyl-7-{(*R*)-3-[(*S*)-1-(dimethylamino)ethyl]pyrrolidin-1-yl}-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2H,9H)-dione Hydrochloride (6k(*R*,*S*)). Yield: 31%. ^{1}H NMR (DMSO-d_6) δ 10.07 (br, 1H), 7.52 (d, $J_{H,F}$ = 14.4 Hz, 1H), 3.73 (m, 2H), 3.57−3.41 (m, 2H), 3.49 (s, 3H, methoxy), 3.19−2.96 (m, 1H), 2.31−1.84 (m, 3H), 2.16 (s, 6H, *N*,*N*-dimethyl), 1.61−1.35 (m, 1H),

1.11 (m, 2H), 0.98−0.82 (m, 5H). ^{19}F NMR (DMSO-d_6) δ −126.1 (s). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

(*R*)-7-[3-(2-Aminopropan-2-yl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (7a(*R*)). Yield: 21%. ^{1}H NMR (TFA-*d*) δ 8.00 (d, $J_{H,F}$ = 13.0 Hz, 1H, aromatic H-5), 4.20 (m, 1H, pyrrolidine H-5′), 4.13−3.94 (m, 4H, *c*-Pr CH, pyrrolidine H-2, H-2′, and H-5), 3.75 (s, 3H, methoxy), 3.00 (m, 1H, pyrrolidine H-3), 2.42 (m, 1H, pyrrolidine H-4′), 2.16 (m, 1H, pyrrolidine H-4), 1.62 (s, 3H, pyrrolidine CH_3), 1.60 (s, 3H, pyrrolidine CH_3), 1.48 (m, 2H, *c*-Pr), 1.22 (m, 2H, *c*-Pr). ^{19}F NMR (D_2O): δ −124.4 (s). LCMS: m/z calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

(*S*)-7-[3-(2-Aminopropan-2-yl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (7a(*S*)). Yield: 28%. ^{1}H NMR (CD_3CO_2D) δ 7.80 (d, $J_{H,F}$ = 13.9 Hz, 1H), 4.00−3.78 (m, 3H), 3.69 (m, 1H), 3.65 (s, 3H, methoxy), 2.77 (m, 1H), 2.24 (m, 1H), 2.15−1.81 (m, 4H), 1.55 (s, 6H, dimethyl), 1.31 (m, 2H, *c*-Pr), 1.14 (m, 2H, *c*-Pr). ^{19}F NMR (CD_3CO_2D) δ −124.6 (s). LCMS: m/z calcd for $C_{21}H_{25}FN_4O_3S$ ($[M]^+$) 432; found 433 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-8-methoxy-7-(3-(2-(methylamino)-propan-2-yl)pyrrolidin-1-yl)isothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (7b(*R*)). Yield: 45%. ^{1}H NMR (DMSO-d_6) δ 8.93 (br s, 1H), 7.55 (d, J = 13.8 Hz, 1H), 3.77 (m, 3H), 3.61 (m, 1H), 3.54 (s, 3H), 3.54 (m, 2H), 2.63 (m, 1H), 2.47 (s, 3H), 2.04 (m, 1H), 1.81 (m, 1H), 1.32 (s, 3H), 1.29 (s, 3H), 1.34 (m, 2H), 0.93 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.2 (s). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ ($[M]^+$) 446; found 447 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-7-(3-(2-(ethylamino)propan-2-yl)pyrrolidin-1-yl)-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (7c(*R*)). Yield: 34%. ^{1}H NMR (DMSO-d_6) δ 8.75 (br s, 1H), 7.55 (d, J = 14.9 Hz, 1H), 3.61 (m, 5H), 3.53 (s, 3H), 2.99 (m, 2H), 2.65 (m, 1H), 2.05 (m, 1H), 1.81 (m, 1H), 1.36 (s, 3H), 1.33 (s, 3H), 1.27 (t, J = 7.3 Hz, 3H), 1.14 (m, 2H), 0.94 (m, 2H). ^{19}F NMR (DMSO-d_6) δ −125.1 (s). LCMS: m/z calcd for $C_{23}H_{29}FN_4O_3S$ ($[M]^+$) 460; found 461 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-7-(3-(2-(2-fluoroethylamino)propan-2-yl)pyrrolidin-1-yl)-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (7d(*R*)). Yield: 35%. ^{1}H NMR (DMSO-d_6) δ 7.49 (d, J = 13.8 Hz, 1H), 4.85 (t, J = 4.2 Hz, 1H), 4.69 (t, J = 6.0 Hz, 1H), 3.48 (s, 3H), 3.74−3.27 (m, 7H), 2.69−2.58 (m, 1H), 2.06−1.96 (m, 1H), 1.84−1.69 (m, 1H), 1.33 (d, J = 7.5 Hz, 6H), 1.12−1.05 (m, 2H), 0.92−0.85 (m, 2H). ^{19}F NMR (DMSO-d_6) δ 18.3 (s, 1F), −125.2 (s, 1F). LCMS: m/z calcd for $C_{23}H_{28}F_2N_4O_3S$ ($[M]^+$) 478; found 479 ($[M + H]^+$).

(*R*)-9-Cyclopropyl-6-fluoro-7-(3-(2-(isobutylamino)propan-2-yl)pyrrolidin-1-yl)-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (7g(*R*)). Yield: 5%. ^{1}H NMR (CD_3OD) δ 7.65 (d, J = 9.3 Hz, 1H), 3.97−3.57 (m, 5H), 3.57 (s, 3H), 2.97 (d, J = 6.3 Hz, 2H), 2.84−2.75 (m, 1H), 2.17−1.88 (m, 3H), 1.51 (s, 3H), 1.48 (s, 3H), 1.33−1.24 (m, 2H), 1.10 (d, J = 6.3 Hz, 8H). ^{19}F NMR (CD_3OD) δ −124.6 (s). LCMS: m/z calcd for $C_{25}H_{33}FN_4O_3S$ ($[M]^+$) 488; found 489 ($[M + H]^+$).

9-Cyclopropyl-7-(3-(2-(cyclopropylamino)propan-2-yl)pyrrolidin-1-yl)-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (7h(*rac*)). A mixture of 1-benzyl-1*H*-pyrrole-2,5-dione **21** (5.0 g, 26.7 mmol), 2-nitropropane (50 mL), K_2CO_3 (0.5 g, 3.6 mmol), and EtOH (25 mL) was refluxed for 15 h. The volatiles were then removed under reduced pressure, and the remaining residue was treated with $CHCl_3$ (30 mL) and H_2O (30 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2) to afford 2.5 g (41%) of 1-benzyl-3-(propan-2-ylidene)pyrrolidine-2,5-dione (**22**). ^{1}H NMR ($CDCl_3$) δ 7.42−7.25 (m, 5H), 4.69 (s, 2H), 3.20 (t, J = 1.5 Hz, 2H), 2.34 (t, J = 1.2 Hz, 3H), 1.86 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.5, 169.4, 149.7, 136.3, 128.8, 128.6, 127.7, 119.0, 41.9, 34.1, 24.1, 20.7. A mixture of **22** (2.0 g, 8.7 mmol) and cyclopropylamine (10 mL) was stirred for 50 days at room temperature. Excess cyclopropylamine was then removed in vacuo. To the remaining residue (crude **23**) was added $BH_3 \cdot Me_2S$ (10 mL, neat), and the mixture was stirred for 30 days at room temperature. To the reaction mixture was added H_2O and aqueous NaOH. The mixture was then refluxed for 2 h and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The remaining residue (crude **24**) was dissolved in CH_2Cl_2 (20 mL) and treated with Boc_2O (2.09 g, 9.6 mmol) and DMAP (0.05 g). The reaction mixture was stirred for 15 h at room temperature and then treated with H_2O. The mixture was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was treated with EtOH (20 mL) and 20% $Pd(OH)_2$/C (1.0 g). Then the mixture was stirred for 15 h at 60 °C under an atmosphere of H_2 (∼1 atm). The supported catalyst was removed by filtration and the solvent removed under reduced pressure. The remaining residue was treated with **2** (0.45 g, 1.4 mmol), *i*-Pr_2NEt (1 mL), and DMSO (10 mL), and the mixture was heated for 7 h at 120 °C. The solvent was removed in vacuo and the remaining residue was purified by HPLC to give the TFA salt. This material was treated with AcOEt (10 mL) and concentrated HCl (0.3 mL), then dried in vacuo to afford 180 mg of the title compound. ^{1}H NMR (DMSO-d_6) δ 9.22 (bs, NH, 1H), 9.04 (bs, NH, 1H), 7.57 (d, $J_{H,F}$ = 13.2 Hz, H-5, 1H), 3.83−3.70 (m, *c*-Pr CH, 2H), 3.59−3.48 (m, CH_2, 2H), 3.17 (s, OCH_3, 3H), 2.85−2.71 (m, CH_2, 2H), 2.14−2.02 (m, CH_2, 1H), 1.91−1.74 (m, CH_2, 1H), 1.42 (s, CH_3, 3H), 1.40 (s, CH_3, 3H), 1.36−0.70 (m, *c*-Pr CH_2, 8H). ^{19}F NMR (DMSO-d_6) δ −125.1 (s).

(*R*)-7-[3-(1-Aminocyclopropyl)pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (8a(*R*)). Yield: 10%. ^{1}H NMR (CD_3OD) δ 7.69 (d, $J_{H,F}$ = 14.1 Hz, 1H), 3.89−3.59 (m, 5H), 3.60 (s, 3H), 2.83−2.56 (m, 1H), 2.20−2.13 (m, 1H), 1.81−1.67 (m, 1H), 1.27−1.25 (m, 2H), 1.04−1.00 (m, 6H). ^{19}F NMR (CD_3OD) δ −126.2 (s). LCMS: m/z calcd for $C_{21}H_{23}FN_4O_3S$ ($[M]^+$) 430; found 431 ($[M + H]^+$).

9-Cyclopropyl-6-fluoro-8-methoxy-7-((*R*)-3-((*S*)-1-(methylamino)propyl)pyrrolidin-1-yl)isothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (9b(*R*,*S*)). Amide **31** (1.2 g, 3.5 mmol) in THF (10 mL) was added dropwise to a suspension of LAH (0.53 g, 14.0 mmol) in THF (10 mL) at 0 °C. After the addition was complete, the reaction mixture was refluxed for 18 h, cooled to 0 °C, and quenched carefully by the addition of 10% aqueous NaOH. The resulting white precipitate was removed by filtration, and the filtrate was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic extract and layer were combined, dried (Na_2SO_4), and evaporated under reduced pressure to give 0.8 g of amine **32** as a light yellow oil. This crude material (mixture of diastereomers) was used without purification for the synthesis of **9b(*R*,*S*)** and **9b(*R*,*R*)** via subsequent debenzylation, treatment with **2**, and HPLC purification. Yield: 20%. ^{1}H NMR (CD_3OD) δ 7.54 (d, J = 14.4 Hz, 1H), 3.85−3.45 (m, 6H), 3.50 (s, 3H), 2.68 (s, 3H), 2.62−2.48 (m, 1H), 2.15−2.10 (m, 1H), 1.94−1.71 (m, 3H), 1.15−1.14 (m, 2H), 1.02 (t, J = 7.5 Hz, 3H), 0.98−0.84 (m, 2H). ^{19}F NMR (CD_3OD) δ −126.0. LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ 446 ($[M^+]$); found 447 ($[M + H]^+$,).

9-Cyclopropyl-6-fluoro-8-methoxy-7-((*R*)-3-((*R*)-1-(methylamino)propyl)pyrrolidin-1-yl)isothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hyrdochloride (9b(*R*,*R*)). Yield: 7%. ^{1}H NMR (CD_3OD) δ 7.57 (d, J = 14.1 Hz, 1H), 3.90−3.49 (m, 6H), 3.50 (s, 3H), 2.69 (m, 3H), 2.59−2.51 (m, 1H), 2.24−2.15 (m, 1H), 1.86−1.64 (m, 3H), 1.21−1.12 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H), 0.98−0.88 (m, 2H).

^{19}F NMR (CD_3OD) δ −126.2 (s). LCMS: m/z calcd for $C_{22}H_{27}FN_4O_3S$ 446 ($[M^+]$); found 447 ($[M + H]^+$).

(*R*)-7-{3-[Amino(thiazol-2-yl)methyl]pyrrolidin-1-yl}-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4-(2*H*,9*H*)-dione Hydrochloride (10a(*R*,*rac*)). Yield: 33%. ^{1}H NMR (DMSO-d_6) δ 8.90 (m, 1H), 7.97 (m, 1H), 7.88 (m, 1H), 7.55 (d, $J_{H,F}$ = 14.3 Hz, 1H), 5.18 (br, 1H), 4.97 (m, 1H), 3.73 (m, 2H), 3.61−3.44 (m, 2H), 3.50 (s, 3H, methoxy), 3.32 (m, 1H), 2.88 (m, 1H), 2.26 (m, 1H), 2.25 (m, 1H), 1.90 (m, 1H), 1.11 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.7 (s, 1F). LCMS: m/z calcd for $C_{22}H_{22}FN_5O_3S_2$ ($[M]^+$) 487; found 488 ($[M + H]^+$).

(*R*)-7-{3-[Amino(oxazol-2-yl)methyl]pyrrolidin-1-yl}-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (11a(*R*,*rac*)). Yield: 25%. ^{1}H NMR (DMSO-d_6) δ 8.96 (m, 1H), 8.03 (m, 1H), 7.55 (m, 1H), 7.39 (d, $J_{H,F}$ = 14.2 Hz, 1H), 4.80 (m, 1H), 3.80 (br, 1H), 3.75 (m, 2H), 3.52 (s, 3H, methoxy), 3.47 (m, 1H), 2.88 (m, 1H), 2.54 (m, 1H), 2.26 (m, 1H), 1.95 (m, 1H), 1.70 (m, 1H), 1.14 (m, 2H, *c*-Pr), 0.95 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.6 (s, 1F). LCMS: m/z calcd for $C_{22}H_{22}FN_5O_4S$ ($[M]^+$) 471; found 472 ($[M + H]^+$).

(*R*)-7-{3-[Amino(pyridin-2-yl)methyl]pyrrolidin-1-yl}-9-cyclopropyl-6-fluoro-8-methoxyisothiazolo[5,4-*b*]quinoline-3,4(2*H*,9*H*)-dione Hydrochloride (12a(*R*,*rac*)). Yield: 21%. ^{1}H NMR (DMSO-d_6) δ 8.75−8.58 (m, 3H), 7.91−7.83 (m, 1H), 7.60−7.37 (m, 3H), 5.00 (br, 1H), 4.45 (m, 1H), 3.69 (m, 2H), 3.51 (m, 1H), 3.40 (s, 3H, methoxy), 3.06 (m, 1H), 2.74 (m, 1H), 2.21 (m, 1H), 1.90 (m, 1H), 1.60 (m, 1H), 1.05 (m, 2H, *c*-Pr), 1.05 (m, 2H, *c*-Pr). ^{19}F NMR (DMSO-d_6) δ −125.4 (s, 1F), −125.8 (s, 1F). LCMS: m/z calcd for $C_{24}H_{24}FN_5O_3S$ ($[M]^+$) 481; found 482 ($[M + H]^+$).

***N*-(Cyclopropylmethyl)-1-[(*R*)-1-((*S*)-1-phenylethyl)pyrrolidin-3-yl]ethanamine (14).** The amine **11** (0.11 g, 0.52 mmol), sodium triacetoxyborohydride (0.16 g, 0.73 mmol), and acetic acid (29.7 μL, 0.52 mmol) were successively added to a solution of cyclopropanecarboxaldehyde (41.4 μL, 0.55 mmol) in 1,2-dichloroethane (5 mL). The reaction mixture was allowed to stir for 6 h under an argon atmosphere at room temperature. The resulting solution was quenched by adding 1 N NaOH (10 mL). After being stirred for 5 min, the aqueous solution was extracted with dichloromethane (3 × 10 mL). The organic layer was washed with brine (15 mL) and dried over sodium sulfate. The solvent was evaporated to provide the desired product **12** (0.13 g, 93%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 5H, ArH), 3.10 (m, 1H, -NCHPh), 2.79−1.35 (m, 12H), 1.29 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 0.93 (d, J = 6.3 Hz, 3H, -*c*-PrCNCCH_3), 0.87 (d, J = 6.3 Hz, 3H, -*c*-PrCNCCH_3, epimer), 0.48−0.25 (m, 2H, *c*-Pr), 0.05 (m, 2H, *c*-Pr). ^{13}C NMR ($CDCl_3$) δ 145.5, 128.1, 127.0, 126.6, 65.7, 56.5, 52.7, 43.7, 27.5, 27.0, 23.1 (23.0, epimer), 18.4 (18.3, epimer), 11.4, 10.3, 3.5, 3.1. LCMS: m/z calcd for $C_{18}H_{28}N_2$ ($[M]^+$) 272; found 273 ($[M + H]^+$).

***N*-{1-[(*R*)-1-((*S*)-1-Phenylethyl)pyrrolidin-3-yl]ethyl}cyclopentanamine (15).** The procedure for the preparation of **12** was used for this compound from cyclopentanone. The product **13** was obtained as an oil. Yield: 93%. ^{1}H NMR ($CDCl_3$) δ 7.24 (m, 5H, ArH), 3.05 (m, 1H, -NCHPh), 2.90−2.52 (m, 3H), 2.38−2.07 (m, 3H), 2.06−1.63 (m, 4H), 1.62−1.32 (m, 6H), 1.29 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 1.24−1.02 (m, 2H), 0.94 (d, J = 6.2 Hz, 3H, -*c*-pentyl-NCCH_3), 0.88 (d, J = 6.3 Hz, 3H, -*c*-pentyl-NCCH_3, epimer). ^{13}C NMR ($CDCl_3$) δ 145.6, 128.1, 126.9, 126.5, 65.6, 56.9, 55.5, 52.6, 44.0 (43.7, epimer), 34.0, 32.8, 27.9, 23.6, 23.0, 18.9 (18.7, epimer). LCMS: m/z calcd for $C_{19}H_{30}N_2$ ($[M]^+$) 286; found 287 ($[M + H]^+$).

***N*-{1-[(*R*)-1-((*S*)-1-Phenylethyl)pyrrolidin-3-yl]ethyl}propan-2-amine (16).** The procedure for the preparation of **12** was used for this compound from acetone. The product **14** was obtained as a light yellow oil. Yield: 90%. ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 5H, ArH), 3.08 (m, 1H, -NCHPh), 2.77 (m, 1H), 2.63 (m, 1H), 2.48 (m, 1H), 2.41−2.13 (m, 2H), 2.12−1.62 (m, 3H), 1.54−1.32 (m, 2H), 1.29 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 1.04−0.79 (m, 9H). ^{13}C NMR ($CDCl_3$) δ 145.6, 128.1, 127.0, 126.5, 65.7, 56.4, 54.1, 52.7, 45.9, 44.1 (43.8, epimer), 27.5, 24.4, 22.7, 19.3 (19.1, epimer). LCMS: m/z calcd for $C_{17}H_{28}N_2$ ($[M]^+$) 260; found 261 ($[M + H]^+$).

2,2,2-Trifluoro-*N*-{1-[(*R*)-1-((*S*)-1-phenylethyl)pyrrolidin-3-yl]ethyl}ethanamine (17). A solution of amine **11** (0.13 g, 0.60 mmol) and trifluoroacetic anhydride (0.11 mL, 0.77 mmol) in chloroform (5 mL) was stirred for 15 h under an argon atmosphere at room temperature. To the resulting solution was added water (10 mL), and the appropriate layer was extracted with dichloromethane (3 × 10 mL). The organic layer was washed with a 5% aqueous solution of $NaHCO_3$ (15 mL) and dried over sodium sulfate. The solvent was evaporated to afford the trifluoroacetate (0.19 g, quantitative) as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 9.02 (br, 1H), 7.35 (m, 5H, ArH), 4.02−3.75 (m, 1H, -NCHPh, epimers), 3.35−3.02 (m, 2H), 2.87−2.62 (m, 1H), 2.42−1.59 (m, 5H), 1.46 (d, J = 6.6 Hz, 3H, -COCF_3NCCH_3), 1.40 (d, J = 6.6 Hz, 3H, -COCF_3NCCH_3, epimer), 1.32 (d, J = 6.5 Hz, 3H, -NCCH_3Ph), 1.32 (d, J = 6.5 Hz, 3H, -NCCH_3Ph, epimer). ^{13}C NMR ($CDCl_3$) δ 157.1, 144.2, 128.3, 127.5, 127.2, 126.9, 65.1, 56.8, 53.1 (52.5, epimer), 51.7 (51.2, epimer), 40.5, 27.9, 24.4 (22.4, epimer), 18.7 (17.5, epimer). ^{19}F NMR ($CDCl_3$) δ −75.6 (s, 1F). LCMS: m/z calcd for $C_{16}H_{21}F_3N_2O$ ($[M]^+$) 314; found 315 ($[M + H]^+$). A 1.0 M solution of lithium aluminum hydride (1.14 mL, 1.14 mmol) in tetrahydrofuran was added to a solution of the trifluoroacetate (0.18 g, 0.57 mmol) in dry tetrahydrofuran (5 mL) at room temperature. The mixed solution was heated for 3 days at 70 °C and then cooled to room temperature. The reaction mixture was quenched by cautious addition of water (1 mL), and to the mixture was added a 15% aqueous solution of NaOH (10 mL). The solution was filtered through a Büchner funnel to remove immersions and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over sodium sulfate and concentrated to give the desired product **15** (0.15 g, 87%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5H, ArH), 3.28−3.08 (m, 2H), 2.72−2.52 (m, 2H), 2.47−2.28 (m, 3H), 2.11 (m, 1H), 1.95 (m, 1H), 1.69 (m, 1H), 1.64 (s, 2H, -NCH_2CF_3), 1.46 (s, 2H, -NCH_2CF_3, epimer), 1.40 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 1.39 (d, J = 6.3 Hz, 3H, -NCCH_3Ph, epimer), 1.05 (d, J = 6.3 Hz, 3H, -NHCCH_3), 0.98 (d, J = 6.7 Hz, 3H, -NHCCH_3, epimer). ^{13}C NMR ($CDCl_3$) δ 145.2, 128.2, 127.7, 126.8, 115.0, 65.7, 56.6, 52.7, 43.7 (43.3, epimer), 31.1 (30.2, epimer), 27.6, 26.2, 22.8 (22.7, epimer), 18.4. ^{19}F NMR ($CDCl_3$) δ −71.6 (s, 3F), −71.9 (s, 3F). LCMS: m/z calcd for $C_{16}H_{23}F_3N_2$ ($[M]^+$) 300; found 301 ($[M + H]^+$).

(*R*)-4-[1-(Cyclopropylamino)ethyl]-1-((*S*)-1-phenylethyl)-pyrrolidin-2-one (19a). Cyclopropylamine (44.9 μL,0.65 mmol), sodium triacetoxyborohydride (0.19 g, 0.91 mmol), and acetic acid (36.7 μL, 0.65 mmol) were successively added to a solution of **8a** (0.15 g, 0.65 mmol) in 1,2-dichloroethane (5 mL). The reaction mixture was stirred for 24 h under an argon atmosphere at room temperature and then quenched by adding 1 N NaOH (10 mL). After being stirred for 5 min, the resulting aqueous solution was extracted with dichloromethane (3 × 10 mL). The organic layer was washed with brine (15 mL) and dried over sodium sulfate. The solvent was evaporated to give the desired product **9a** (0.17 g, 97%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 5H, ArH), 5.47 (m, 1H, -NCHPh), 3.40−2.89 (m, 2H, -pyrrolidine CH_2, C-5), 2.75−1.94 (m, 6H), 1.50 (d, J = 7.2 Hz, 3H, -NCCH_3Ph), 0.96 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3), 0.86 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3, epimer), 0.49−0.20 (m, 4H, *c*-Pr). ^{13}C NMR ($CDCl_3$) δ 173.6, 140.0, 128.4, 127.4, 127.3, 56.5, 48.7, 44.4, 37.5, 34.9, 28.1, 18.0, 16.0 (15.9, epimer), 7.1. LCMS: m/z calcd for $C_{17}H_{24}N_2O$ ($[M]^+$) 272; found 273 ($[M + H]^+$).

(*S*)-4-[1-(Cyclopropylamino)ethyl]-1-((*S*)-1-phenylethyl)-pyrrolidin-2-one (19b). The procedure for the preparation of **9a** was used for this compound from starting material **8b**. The product **9b** was obtained as an oil. Yield: 96%. ^{1}H NMR ($CDCl_3$) δ 7.25 (m, 5H, ArH), 5.49 (m, 1H, -NCHPh), 3.40−2.87 (m, 2H, -pyrrolidine CH_2,

C-5), 2.75−1.95 (m, 6H), 1.48 (d, J = 7.2 Hz, 3H, -NCCH_3Ph), 1.08 (d, J = 6.4 Hz, 3H, -*c*-PrNCCH_3), 1.02 (d, J = 6.4 Hz, 3H, -*c*-PrNCCH_3, epimer), 0.49−0.20 (m, 4H, *c*-Pr). ^{13}C NMR ($CDCl_3$) δ 173.4, 140.1, 128.2, 127.1, 126.7, 56.6, 48.6, 45.7, 37.3, 34.8, 27.9, 18.1, 15.8 (15.7, epimer), 6.9. LCMS: m/z calcd for $C_{17}H_{24}N_2O$ ([M]$^+$) 272; found 273 ([M + H]$^+$).

***N*-{1-[(*R*)-1-((*S*)-1-Phenylethyl)pyrrolidin-3-yl]ethyl}cyclopropanamine (20a).** A 1.0 M solution of borane−tetrahydrofuran complex (3.30 mL, 3.30 mmol) in tetrahydrofuran was added to a solution of **9a** (0.18 g, 0.66 mmol) in tetrahydrofuran (10 mL), and the mixture was allowed to stir for 24 h at room temperature. The solvent was evaporated under reduced pressure, and dichloromethane (20 mL) was added to the residue. The mixture was washed with a saturated sodium chloride aqueous solution (15 mL) and dried over sodium sulfate. The solvent was evaporated, and a 5 N sodium hydroxide aqueous solution (5 mL) was added to the residue. The solution was heated for 3 h on an oil bath at 100 °C, cooled to room temperature, and extracted with dichloromethane (3 × 15 mL). The organic layer was dried over sodium sulfate and evaporated to provide the desired product **10a** (0.16 g, 94%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.32−7.18 (m, 5H, ArH), 3.14 (m, 1H, -NCHPh), 2.74 (m, 1H, -*c*-PrNCH), 2.65−2.24 (m, 3H, -pyrrolidine CH_2, C-2, and -NH), 2.19−1.96 (m, 3H, -pyrrolidine CH_2, C-5, and -pyrrolidine CH, C-3), 1.89 (m, 1H, -*c*-Pr CH), 1.61−1.35 (m, 2H, -pyrrolidine CH_2, C-4), 1.34 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 1.05 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3), 0.99 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3, epimer), 0.49−0.15 (m, 4H, *c*-Pr). ^{13}C NMR ($CDCl_3$) δ 145.8, 128.3, 127.1, 126.5, 65.9, 58.0, 56.8, 52.8, 43.8, 28.5, 27.6, 23.3, 19.0 (18.7, epimer), 7.5, 6.0. LCMS: m/z calcd for $C_{17}H_{26}N_2$ ([M]$^+$) 258; found 259 ([M + H]$^+$).

***N*-{1-[(*S*)-1-((*S*)-1-Phenylethyl)pyrrolidin-3-yl]ethyl}cyclopropanamine (20b).** The procedure for the preparation of **10a** was used for this compound from starting material **9b**. The product **10b** was obtained as an oil. Yield: 88%. ^{1}H NMR ($CDCl_3$) δ 7.33−7.18 (m, 5H, ArH), 3.15 (m, 1H, -NCHPh), 2.73 (m, 1H, -*c*-PrNCH), 2.63−2.24 (m, 3H, -pyrrolidine CH_2, C-2 and -NH), 2.19−1.97 (m, 3H, -pyrrolidine CH_2, C-5, and -pyrrolidine CH, C-3), 1.85 (m, 1H, -*c*-Pr CH), 1.59−1.38 (m, 2H, -pyrrolidine CH_2, C-4), 1.35 (d, J = 6.6 Hz, 3H, -NCCH_3Ph), 1.06 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3), 0.99 (d, J = 6.3 Hz, 3H, -*c*-PrNCCH_3, epimer), 0.49−0.16 (m, 4H, *c*-Pr). ^{13}C NMR ($CDCl_3$) δ 145.6, 128.0, 126.9, 126.5, 65.7, 57.6, 56.6, 52.7, 43.4, 28.3, 27.4, 23.1, 18.8 (18.5, epimer), 7.1, 6.0. LCMS: m/z calcd for $C_{17}H_{26}N_2$ ([M]$^+$) 258; found 259 ([M + H]$^+$).

***N*-((1-Benzylpyrrolidin-3-yl)methyl)-2-methylpropan-1-amine (27).** To a stirred solution of acid **25** (2.2 g, 10.0 mmol) in DMF (20 mL) were added EDCI (2.89 g, 15.1 mmol) and HOBt (1.77 g, 13.1 mmol). 2-Methylpropan-1-amine (1.11 mL, 11.1 mmol) was added, and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then diluted with H_2O (200 mL) and extracted with $CHCl_3$. The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure. The remaining crude material was purified by silica gel flash column chromatography (eluent 0−2% MeOH in $CHCl_3$) to give 2.3 g (84%) of **26** as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.35−7.22 (m, 5H), 5.87 (br s, 1H), 4.54 (d, J = 14.7 Hz, 1H), 4.34 (d, J = 14.7 Hz, 1H), 3.51 (dd, J = 9.6, 7.2 Hz, 1H), 3.39 (t, J = 8.7 Hz, 1H), 3.08−3.01 (m, 3H), 2.78 (dd, J = 16.8, 9.6 Hz, 1H), 2.62 (dd, J = 16.8, 9.6 Hz, 1H), 1.78−1.66 (m, 1H), 0.88 (d, J = 6.3 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 172.6, 172.0, 136.0, 128.8, 128.1, 127.7, 49.2, 47.1, 46.6, 37.8, 35.0, 28.4, 20.0. LC−MS m/z calcd for $C_{16}H_{22}N_2O_2$ 274 ([M$^+$]); found 275 ([M + H]). Anal. Calcd for $C_{16}H_{22}N_2O_2$ C 70.04, H 8.08, N 10.21; found C 70.01, H 8.20, N 10.25. Amide **26** (2.3 g, 8.4 mmol) in THF (20 mL) was added dropwise to a suspension of LAH (1.27 g, 33.5 mmol) in THF (20 mL) at 0 °C. After the addition was complete, the reaction mixture was refluxed for 18 h, cooled to 0 °C, and quenched carefully by the addition of 10% aqueous NaOH. The resulting white precipitate was removed by filtration, and the filtrate was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic extract and layer were combined, dried (Na_2SO_4), and evaporated under reduced pressure to give 2 g (quant) of amine **27** as a light yellow oil. This crude material was used without purification in the next synthetic step. An analytical sample was obtained by preparative TLC (solvent system $CHCl_3$/MeOH/28% aq NH_4OH 100:10:1). ^{1}H NMR ($CDCl_3$) δ 7.32−7.21 (m, 5H), 3.59 (d, J = 17.4, 12.6 Hz, 2H), 2.77 (dd, J = 9.0, 7.8 Hz, 1H), 2.66−2.58 (m, 1H), 2.57 (d, J = 6.9 Hz, 2H), 2.52−2.46 (m, 1H), 2.39 (d, J = 6.9 Hz, 2H), 2.40−2.26 (m, 1H), 2.47 (dd, J = 9.1, 6.4 Hz, 1H), 2.05−1.92 (m, 1H), 1.77−1.68 (m, 1H), 1.51−1.42 (m, 2H), 0.88 (d, J = 6.6 Hz, 6H).

***(R)*-*N*-(2-(1-Benzylpyrrolidin-3-yl)propan-2-yl)-2-methylpropan-1-amine (30).** **30** was prepared using procedures analogous to those described above for **27**. Amide **29** was isolated as a pale yellow oil (1.0 g, 76% yield). ^{1}H NMR ($CDCl_3$) δ 7.32−7.24 (m, 5H), 6.87 (br s, 1H), 3.63 (d, J = 12.6 Hz, 1H), 3.51 (d, J = 12.9 Hz, 1H), 2.98−2.92 (m, 1H), 2.89 (d, J = 9.0 Hz, 1H), 2.29−2.10 (m, 4H), 1.91−1.73 (2H, m), 1.34 (d, J = 9.6 Hz, 6H), 1.10 (t, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 177.1, 138.8, 128.7, 128.3, 127.1, 60.2, 55.7, 55.3, 54.6, 47.6, 36.6, 25.7, 25.6, 24.4, 19.8. LC−MS m/z calcd for $C_{18}H_{28}N_2O$ 288 ([M$^+$]); found 289 ([M + H]). As described above for compound **26**, amide **29** (1 g, 3.47 mmol) was reduced using LAH (0.53 g, 13.9 mmol) to give 0.7 g of amine **30** as a light yellow oil. The isolated crude material was used without purification in the next synthetic step. An analytical sample was obtained by preparative TLC (solvent system $CHCl_3$/MeOH/28% aq NH_4OH 100:10:1). ^{1}H NMR ($CDCl_3$): δ 7.32−7.23 (m, 5H), 3.66 (d, J = 12.9 Hz, 1H), 3.53 (d, J = 12.9 Hz, 1H), 2.69−2.60 (m, 2H), 2.45−2.26 (m, 5H), 1.83−1.76 (m, 1H), 1.68−1.51 (m, 2H), 0.99 (d, J = 2.5 Hz, 6H), 0.88 (dd, J = 6.6, 0.6 Hz, 6H). LC−MS m/z calcd for $C_{18}H_{30}N_2$ 274 ([M$^+$]); found 275 ([M + H]).

Biological Assays. Antimicrobial susceptibility, cytotoxicity, topoisomerase IV assays, and DNA gyrase assays were conducted using methods described previously.[18]

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ABBREVIATIONS USED

ITQ, isothiazoloquinolone; CIP, ciprofloxacin; GEM, gemifloxacin; LVX, levofloxacin; LFX, levofloxacin; MXF, moxifloxacin; VAN, vancomycin; LZD, linezolid; OXA, oxacillin; QPS/DPS, quinupristin/dalfopristin; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MQRSA, methicillin- and quinolone-resistant *Staphylococcus aureus*; Ec, *Escherichia coli*

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(42) Cytotoxicity is reported as CC_{50}, defined as the concentration of drug that results in toxicity to 50% of the cells compared to untreated control cells. The degree of cytotoxicity was measured by Alamar Blue reduction. The amount of fluorescence or absorbance is proportional to the number of living cells and corresponds to the cells metabolic activity. Damaged and nonviable cells have lower innate metabolic activity and thus generate a proportionally lower signal than healthy cells. The Hep2 human laryngeal carcinoma cell line was incubated with drug concentrations at 37 °C for 72 h to generate eight-point CC_{50} data.