Design, Synthesis, and *In Vitro* Anti-mycobacterial Activities of Propylene-Tethered Gatifloxacin-Isatin Hybrids

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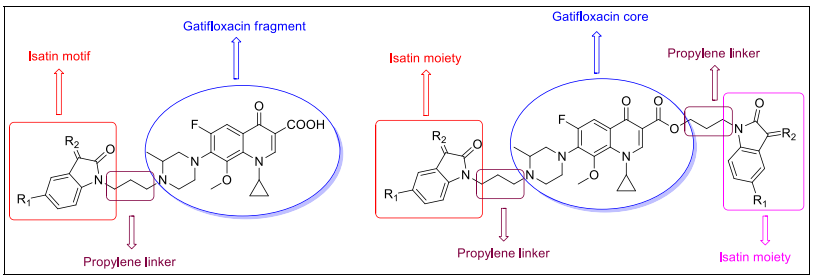

A series of propylene-tethered mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f** were designed, synthesized, and evaluated for their *in vitro* anti-mycobacterial activities against *Mycobacterium tuberculosis* (MTB) H37Rv and multidrug-resistant tuberculosis (MDR-TB) as well as cytotoxicity against VERO cell line. The results indicated that all hybrids exhibited promising anti-mycobacterial activities against MTB H37Rv and MDR-TB with MIC ranging from 0.25 to 16 μg/mL. In particular, the mono-isatin-gatifloxacin hybrid **3e** (MIC: 0.25 and 0.25 μg/mL) was found to be most active against MTB H_{37}Rv and MDR-TB strains, which was twofold more active than the parent gatifloxacin (MIC: 0.5 μg/mL) and comparable with rifampicin (**RIF**) (MIC: 0.25 μg/mL) against MTB H_{37}Rv, and 4- > 512 times more potent than the three references gatifloxacin (MIC: 1.0 μg/mL), **RIF** (MIC: 64 μg/mL), and isoniazid (>128 μg/mL) against MDR-TB, could act as a starting point for further optimization.

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INTRODUCTION

Tuberculosis (TB), which is caused mainly by *Mycobacterium tuberculosis* (MTB), affects dominantly the lungs (pulmonary TB) apart from other vital organs, has been a scourge of humanity for thousands of years [1,2]. According to the global tuberculosis report 2017 by World Health Organization (WHO), TB is the ninth leading cause of death throughout the world and the leading cause from a single infectious agent [3]. The WHO has estimated that around 10.4 million newly TB infected cases occurred in the year 2016 and, among them, led to 1.67 million deaths [3].

The first-line drugs such as isoniazid (**INH**), rifampicin (**RIF**), pyrazinamide, and ethambutol play a pivotal role for the treatment of drug-susceptible MTB infected patients [4,5]. However, the evolution of MTB new virulent forms such as drug-resistant TB (DR-TB), multidrug-resistant TB (MDR-TB) and extremely drug-resistant TB (XDR-TB) make the first-lineanti-TB drugs more and more ineffective. Therefore, there is an urgent need to develop new anti-TB agents active against various virulent forms of MTB with low toxicity, short therapy duration, and new action mechanism.

Fluoroquinolones are a family of synthetic broad spectrum antibiotics with extensive indications for infections including upper and lower respiratory infections, gastrointestinal infections, gynecologic infections, sexually transmitted diseases, prostatitis, and some skin, bone, and soft tissue infections, and their value and role in the treatment of bacterial infections continue to expand [6,7]. Fluoroquinolones also exhibit promising anti-TB activities; although they are presently recommended as second-line anti-TB agents used to treat primarily in cases involving resistance or intolerance to first-line anti-TB therapy by WHO, these drugs are potential first-line agents [7]. Moreover, MTB isolates that are resistant to both **INH** and **RIF** are still susceptible to fluoroquinolones generally. The recent research demonstrated that the lipophilicity of the fluoroquinolones plays an important role in the penetration of these compounds into bacterial cells, suggesting that increasing the lipophilic character at C-7 position may favor the anti-TB activity [8–11]. Isatin (1*H*-indole-2,3-dione, Fig. 1) derivatives endow with a broad range of biological properties such as anti-TB activity [12,13]. Therefore, incorporation of isatin into fluoroquinolones may lead to more active candidates.

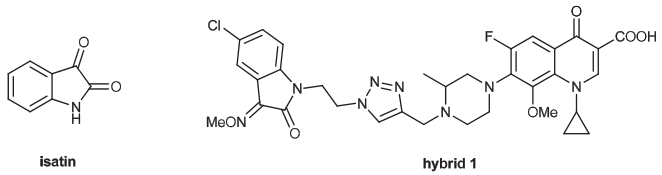


Figure 1. Chemical structures of isatin and isatin-gatifloxacin hybrid **1.**

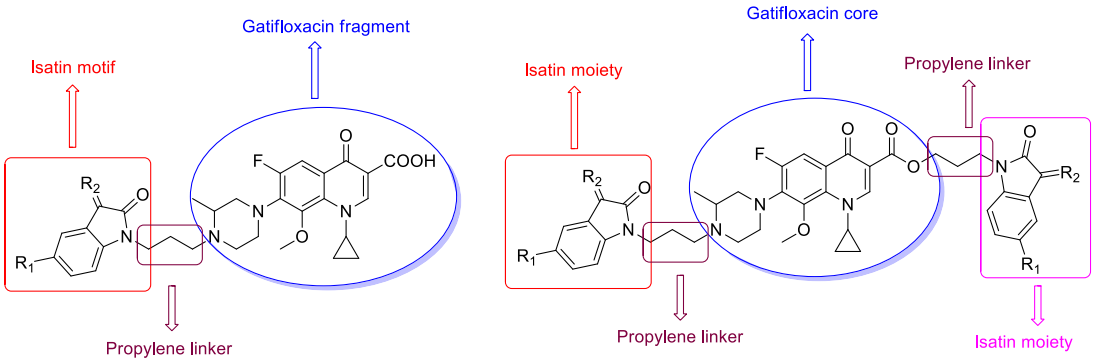
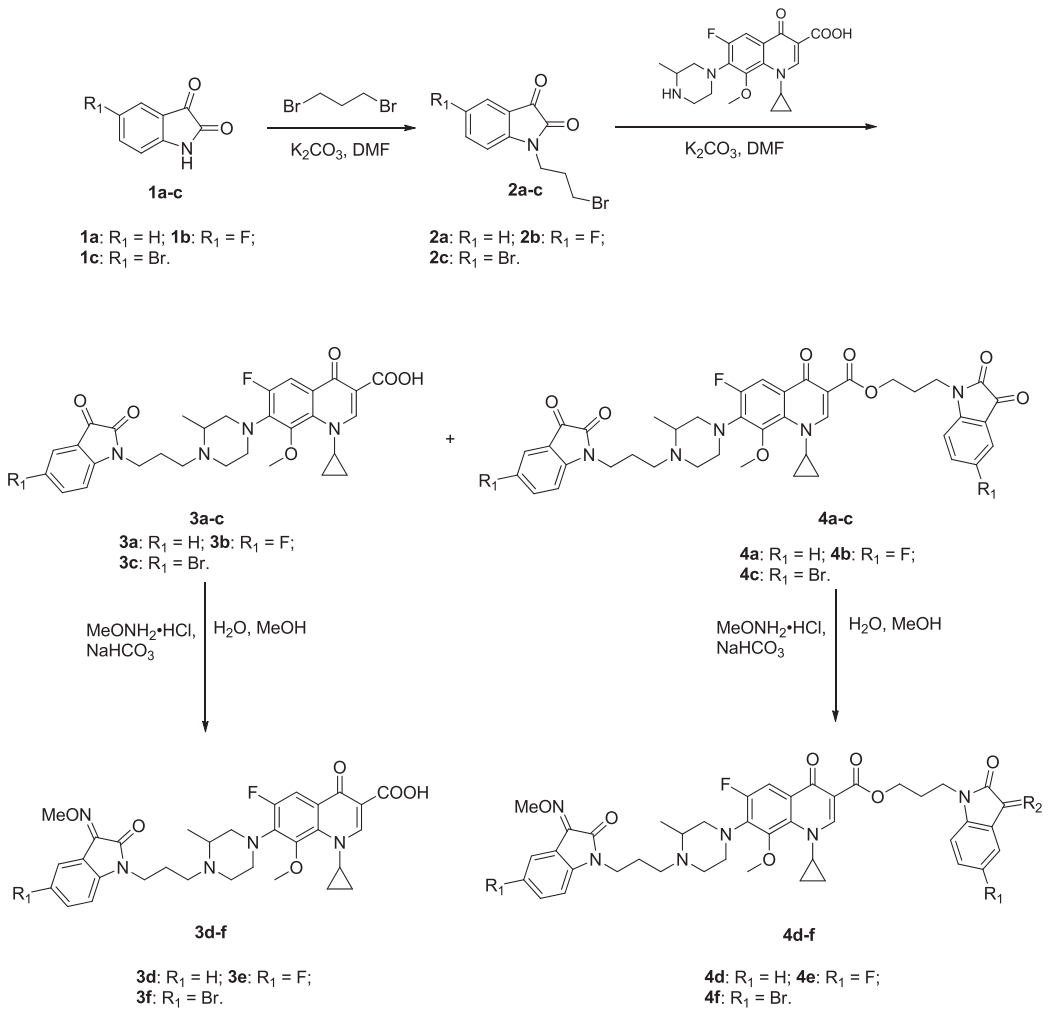


Figure 2. Illustration of design strategy. [Color figure can be viewed at wileyonlinelibrary.com]

Scheme 1. Synthetic route for mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f.**



Various fluoroquinolones-isatin hybrids tethered through different linkers such as methylene, ethylene, acetyl, carbohydrazide, and 1,2,3-triazole have been synthesized and screened for their anti-TB activities by our group, and some of them demonstrated considerable activity [14–21]. In particular, gatifloxacin-isatin hybrid **1** (MIC: 0.10 and 0.25 μg/mL) were fourfold to eightfold more active *in vitro* than the parent gatifloxacin (MIC: 0.78 and 1.0 μg/mL) against MTB H37Rv and MDR-TB strains, represents a new class of lead warrant further investigation [15].

Based on the aforementioned research results and as a continuous research program to optimize the linker between fluoroquinolones and isatin, a series of novel propylene-tethered mono-/bis-isatin-gatifloxacin hybrids were designed, synthesized, and assessed for their *in vitro* anti-mycobacterial activities against MTB H37Rv and MDR-TB as well as cytotoxicity against VERO cell line in this study. The design strategy is illustrated in Figure 2.

RESULTS AND DISCUSSION

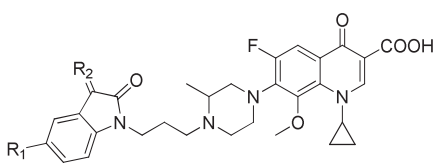
The synthetic pathways for propylene-tethered mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f** are outlined in Scheme 1. Alkylation of C-5 substituted isatins **1a–c** was performed with 1,3-dibromopropane in presence of K_2CO_3 to provide *N*-(3-bromopropyl) isatins **2a–c**, which were then incorporated into gatifloxacin core to afford the desired mono-isatin-gatifloxacin **3a–c** and bis-isatin-gatifloxacin hybrids **4a–c**. Subsequently, condensation of hybrids **3a–c** or **4a–c** with methylhydroxylamine hydrochloride in the presence of sodium bicarbonate provided other conjugates **3d–f** and **4d–f**.

All propylene-tethered mono-/bis-isatin-gatifloxacin **3a–f** and **4a–f** were assessed for their *in vitro* anti-mycobacterial activity against MTB $H_{37}Rv$ and MDR-TB as well as cytotoxicity against VERO cell line [15]. From Table 1, it can be concluded that all hybrids exhibited considerable activity against the tested two strains with

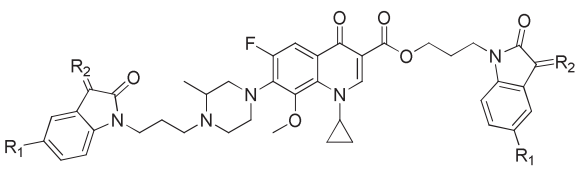
Table 1

Structures, anti-mycobacterial activity, and cytotoxicity of hybrids **3a–f** and **4a–f.**

4a-f



3a-f



4a-f

Compd.	R_1	R_2	MIC (μg/mL) MTB $H_{37}Rv$	MIC (μg/mL) MDR-TB	CC_{50}[a] (μg/mL)
3a	H	O	2	2	256
3b	F	O	0.5	0.5	64
3c	Br	O	1	2	128
3d	H	NOMe	1	2	128
3e	F	NOMe	0.25	0.25	32
3f	Br	NOMe	0.5	0.5	128
4a	H	O	16	16	512
4b	F	O	8	16	64
4c	Br	O	16	16	128
4d	H	NOMe	16	8	128
4e	F	NOMe	8	8	32
4f	Br	NOMe	8	8	256
Gatifloxacin			0.5	1	128
INH			0.06	>128	512
RIF			0.25	64	1024

INH, isoniazid; MDR-TB, multidrug-resistant tuberculosis; MTB, *Mycobacterium tuberculosis*; **RIF**, rifampicin.
[a]CC_{50}: The 50% cytotoxic concentration in a mammalian VERO cell line.

MIC in a range of 0.25 to 16 μg/mL against MTB $H_{37}Rv$ and MDR-TB. The structure–activity relationship revealed that all mono-isatin-gatifloxacin hybrids were more potent than the corresponding bis-isatin-gatifloxacin analogs, which was in accordance with the previous study that carboxylic acid at C-3 position is essential for gyrase binding and bacterial membrane transport [6]; substituents at C-3 and C-5 positions of isatin moiety have significant influence on the anti-mycobacterial activity, and introduction of methoxyimino at C-3 position as well as electron-withdrawing -F at C-5 position of isatin moiety could boost up the activity. In particular, the most potent hybrid **3e** with MIC of 0.25 and 0.25 μg/mL against MTB $H_{37}Rv$ and MDR-TB was twofold more active than the parent gatifloxacin (MIC: 0.5 μg/mL) and comparable with **RIF** (MIC: 0.25 μg/mL) against MTB $H_{37}Rv$, and 4- $>$ 512 times more potent than the three references gatifloxacin (MIC: 1.0 μg/mL), **RIF** (MIC: 64 μg/mL), and **INH** ($>$128 μg/mL) against MDR-TB.

The propylene-tethered isatin-CPFX hybrids **3a–f** and **4a–f** were subsequently examined for toxicity (CC_{50}) in a mammalian VERO cell line [14]. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product, and the results are reported in Table 1. All hybrids (CC_{50}: 32–512 μg/mL) showed acceptable cytotoxicity, and the structure–cytotoxicity relationship revealed that hybrids with -F at C-5 position showed higher cytotoxicity in VERO cell line. The cytotoxicity of the most potency **4e** (CC_{50}: 32 μg/mL) displayed acceptable cytotoxicity.

CONCLUSION

In summary, a series of novel propylene-tethered isatin-gatifloxacin hybrids were synthesized and evaluated for their *in vitro* anti-mycobacterial activity against MTB $H_{37}Rv$ and MDR-TB as well as cytotoxicity. All hybrids exhibited considerable inhibitory activity against the tested strains, and the most active conjugate **3e** with acceptable cytotoxicity was more potent than the parent **GTFX** and **RIF** against MTB $H_{37}Rv$ and MDR-TB, warrant further investigations.

EXPERIMENTAL SECTION

Synthesis. ***General procedure for the preparation of 3a–c and 4a–c.*** N-(3-bromopropyl) isatins **2a–c** were obtained *via* literature reported method [14]. To a mixture of N-(3-bromopropyl) isatins **2a–c** (5 mmol) and **GTFX** (3 mmol) in DMF (50 mL), K_2CO_3 (20 mmol) was added. The mixture was stirred at room temperature for 3 days. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase column with formic acid as additive to provide the desired targets **3a–c** and bis-isatin-GTFX hybrids **4a–c**.

1-Cyclopropyl-7-(4-(3-(2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3a). Yellow solid, yield: 38%. H NMR (400 MHz, DMSO-d_6) δ 0.95–1.09 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.99–2.05 (2H, m, –CH_2–), 2.96–3.31 (7H, m, piperazine–7H), 3.77 (3H, s, OCH_3), 3.87 (2H, t, –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.24 (2H, t, –CH_2–), 7.09 (1H, t, Ar–H), 7.32 (1H, d, Ar–H), 7.46 (1H, d, Ar–H), 7.60–7.63 (2H, m, Ar–H), 8.31 (1H, s, CHO), 8.47 (1H, s, C2–H). ESI-MS m/z: 563 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{33}FN_4O_8$: C, 61.18; H, 5.47; N, 9.21; found: C, 60.94; H, 5.21; N, 9.07.

7-(4-(3-(5-Bromo-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3b). Yellow solid, yield: 31%. H NMR (400 MHz, DMSO-d_6) δ 0.84–0.96 (4H, m, 2 × cyclopropyl–CH_2), 1.08 (3H, d, CH_3), 1.98–2.05 (2H, m, –CH_2–), 2.94–3.31 (7H, m, piperazine–7H), 3.79 (3H, s, OCH_3), 3.88 (2H, t, –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.20 (2H, t, –CH_2–), 7.24 (1H, d, Ar–H), 7.58–7.62 (2H, m, Ar–H), 7.88 (1H, s, Ar–H), 8.26 (1H, s, CHO), 8.36 (1H, s, C2–H). ESI-MS m/z: 641 $[M + H]^+$, 643 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{32}FBrN_4O_8$: C, 54.16; H, 4.69; N, 8.15; found: C, 53.89; H, 4.47; N, 8.01.

7-(4-(3-(5-Fluoro-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3c). Yellow solid, yield: 27%. H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.99–2.04 (2H, m, –CH_2–), 2.93–3.32 (7H, m, piperazine–7H), 3.79 (3H, s, OCH_3), 3.89 (2H, t, –CH_2–), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.19 (2H, t, –CH_2–), 7.30–7.32 (2H, m, Ar–H), 7.57–7.64 (2H, m, Ar–H), 8.28 (1H, s, CHO), 8.40 (1H, s, C2–H). ESI-MS m/z: 581 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{32}F_2N_4O_8$: C, 59.46; H, 5.15; N, 8.94; found: C, 59.29; H, 5.02; N, 8.77.

3-(2,3-Dioxoindolin-1-yl) propyl 1-cyclopropyl-7-(4-(3-(2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4a). Yellow solid, yield: 14%. H NMR (400 MHz, DMSO-d_6) δ 0.96–1.08 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 2.02–2.12 (4H, m, 2 × –CH_2–), 2.99–3.64 (9H, m, piperazine–7H and –CH_2–), 3.76–3.88 (7H, m, OCH_3 and 2 × –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.24 (2H, t, –CH_2–), 7.07–7.10 (1H, m, Ar–H), 7.17 (1H, t, Ar–H), 7.28–7.33 (2H, m, Ar–H), 7.46 (1H, d, Ar–H), 7.60–7.73 (4H, m, Ar–H), 8.48 (1H, s, C2–H). ESI-MS

m/z: 772 $[M + Na]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{40}FN_5O_8$: C, 65.68; H, 5.38; N, 9.34; found: C, 65.47; H, 5.32; N, 9.06.

3-(5-Bromo-2,3-dioxoindolin-1-yl) propyl 7-(4-(3-(5-bromo-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4b). Yellow solid, yield: 11%. H NMR (400 MHz, DMSO-d_6) δ 0.92–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.77–1.80 (2H, m, –CH_2–), 1.99–2.01 (2H, m, –CH_2–), 2.27–2.30 (2H, m, –CH_2–), 2.91–3.30 (7H, m, piperazine–7H), 3.71–3.90 (7H, m, OCH_3 and 2 × –CH_2–), 4.00–4.01 (1H, m, cyclopropyl–CH), 4.18–4.20 (2H, m, –CH_2–), 7.20–7.27 (2H, m, Ar–H), 7.58–7.61 (2H, m, Ar–H), 7.68 (1H, d, Ar–H), 7.87 (1H, s, Ar–H), 7.99 (1H, s, Ar–H), 8.36 (1H, s, C2–H). ESI-MS m/z: 906 $[M + H]^+$, 908 $[M + 2 + H]^+$, 910 $[M + 4 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{38}FBr_2N_5O_8$: C, 54.26; H, 4.22; N, 7.72; found: C, 53.93; H, 4.01; N, 7.52.

3-(5-Fluoro-2,3-dioxoindolin-1-yl) propyl 1-cyclopropyl-6-fluoro-7-(4-(3-(5-fluoro-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4c). Yellow solid, yield: 17%. H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.78–1.80 (2H, m, –CH_2–), 1.99–2.04 (2H, m, –CH_2–), 2.37–2.40 (2H, m, –CH_2–), 2.85–3.27 (7H, m, piperazine–7H), 3.74–3.89 (7H, m, OCH_3 and 2 × –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.17–4.20 (2H, m, –CH_2–), 7.24–7.44 (4H, m, Ar–H), 7.56–7.62 (2H, m, Ar–H), 7.69–7.71 (1H, m, Ar–H), 8.40 (1H, s, C2–H). ESI-MS m/z: 808 $[M + Na]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{38}F_3N_5O_8$: C, 62.67; H, 4.87; N, 8.91; found: C, 62.31; H, 4.56; N, 8.63.

The general procedure for preparing targets 3d–f and **4d–f**. To a solution of methylhydroxylamine hydrochloride (15 mmol) and sodium bicarbonate (15 mmol) dissolved in water (10 mL) and methanol (10 mL) was added **3a–c** or **4a–c** (5 mmol). The reaction mixture was stirred at room temperature for 24 h. After removal of the solvent, the residue was diluted with water (20 mL) and stirred for 10 min and then filtered. The solid crude product was purified by column chromatography (silica gel) eluted with DCM to *v* (DCM):*v* (MeOH) = 10:1 to give the title **3d–f** and **4d–f**.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(4-(3-(3-(methoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3d). Yellow solid, yield: 41%. H NMR (400 MHz, DMSO-d_6) δ 0.94–1.07 (4H, m, 2 × cyclopropyl–CH_2), 1.16 (3H, d, CH_3), 1.98–2.04 (2H, m, –CH_2–), 3.01–3.36 (7H, m, piperazine–7H), 3.80 (3H, s, OCH_3), 3.89 (2H, t, –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.16–4.20 (5H, m, NOMe and –CH_2–), 7.03–7.07 (1H, m, Ar–H), 7.27 (1H, d, Ar–H), 7.44 (1H, t, Ar–H), 7.62 (1H, d, Ar–H), 7.66 (1H, d, Ar–H), 8.27 (1H, s, CHO), 8.42 (1H, s, C2–H). ESI-MS m/z: 592 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{36}FN_5O_8$: C, 60.27; H, 5.69; N, 10.98; found: C, 60.09; H, 5.41; N, 10.74.

7-(4-(3-(5-Bromo-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3e). Yellow solid, yield: 37%. H NMR (400 MHz, DMSO-d_6) δ 0.84–0.96 (4H, m, 2 × cyclopropyl–CH_2), 1.06 (3H, d, CH_3), 1.98–2.04 (2H, m, –CH_2–), 2.90–3.30 (7H, m, piperazine–7H), 3.80 (3H, s, OCH_3), 3.87–3.90 (2H, m, –CH_2–), 3.98–4.00 (1H, m, cyclopropyl–CH), 4.17–4.20 (5H, m, NOMe and –CH_2–), 7.26 (1H, d, Ar–H), 7.59–7.64 (2H, m, Ar–H), 7.88 (1H, s, Ar–H), 8.24 (1H, s, CHO), 8.39 (1H, s, C2–H). ESI-MS m/z: 670 $[M + H]^+$, 672 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}FBrN_5O_8$: C, 53.64; H, 4.92; N, 9.77; found: C, 53.59; H, 4.77; N, 9.54.

1-Cyclopropyl-6-fluoro-7-(4-(3-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid formic acid salt (3f). Yellow solid, yield: 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.08 (4H, m, 2 × cyclopropyl–CH_2), 1.14 (3H, d, CH_3), 1.97–2.03 (2H, m, –CH_2–), 3.00–3.34 (7H, m, piperazine–7H), 3.80 (3H, s, OCH_3), 3.88 (2H, t, –CH_2–), 3.90–3.99 (1H, m, cyclopropyl–CH), 4.11–4.20 (5H, m, NOMe and –CH_2–), 7.21–7.31 (2H, m, Ar–H), 7.76–7.63 (2H, m, Ar–H), 8.40 (1H, s, C2–H). ESI-MS m/z: 610 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}F_2N_5O_8$: C, 58.62; H, 5.38; N, 10.68; found: C, 58.39; H, 5.10; N, 10.51.

3-(3-(Methoxyimino)-2-oxoindolin-1-yl) propyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-(3-(3-(methoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4d). Yellow solid, yield: 67%. H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.80–1.82 (2H, m, –CH_2–), 2.00–2.03 (2H, m, –CH_2–), 2.30–2.33 (2H, m, –CH_2–), 2.85–3.25 (7H, m, piperazine–7H), 3.74–3.91 (7H, m, OCH_3 and 2 × –CH_2–), 4.00–4.02 (1H, m, cyclopropyl–CH), 4.14–4.21 (8H, m, 2 × NOMe and –CH_2–), 7.05–7.10 (2H, m, Ar–H), 7.22–7.29 (2H, m, Ar–H), 7.40–7.50 (2H, m, Ar–H), 7.62 (1H, d, Ar–H), 7.82 (1H, d, Ar–H), 7.89 (1H, d, Ar–H), 8.41 (1H, s, C2–H). ESI-MS m/z: 808 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{43}H_{46}FN_7O_8$: C, 63.93; H, 5.74; N, 12.14; found: C, 63.69; H, 5.51; N, 11.87.

3-(5-Bromo-3-(methoxyimino)-2-oxoindolin-1-yl) propyl 7-(4-(3-(5-bromo-3-(methoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4e). Yellow solid, yield: 54%. H NMR (400 MHz, DMSO-d_6) δ 0.92–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.76–1.81 (2H, m, –CH_2–), 1.99–2.02 (2H, m, –CH_2–), 2.29–2.33 (2H, m, –CH_2–), 2.90–3.23 (7H, m, piperazine–7H), 3.71–3.90 (7H, m, OCH_3 and 2 × –CH_2–), 3.98–4.00 (1H, m, cyclopropyl–CH), 4.16–4.24 (8H, m, 2 × NOMe

and $-CH_2-$), 7.19–7.25 (2H, m, Ar–H), 7.57–7.62 (2H, m, Ar–H), 7.71 (1H, d, Ar–H), 7.88 (1H, s, Ar–H), 8.00 (1H, s, Ar–H), 8.39 (1H, s, C2–H). ESI-MS m/z: 964 $[M + H]^+$, 966 $[M + 2 + H]^+$, 968 $[M + 4 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{43}H_{44}FBr_2N_7O_8$: C, 53.48; H, 4.59; N, 10.15; found: C, 53.21; H, 4.22; N, 9.89.

3-(5-Fluoro-3-(methoxyimino)-2-oxoindolin-1-yl) propyl 1-cyclopropyl-6-fluoro-7-(4-(3-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4f). Yellow solid, yield: 66%. H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl–CH_2 and CH_3), 1.79–1.82 (2H, m, $-CH_2-$), 1.97–2.03 (2H, m, $-CH_2-$), 2.28–2.34 (2H, m, $-CH_2-$), 2.83–3.24 (7H, m, piperazine–7H), 3.74–3.91 (7H, m, OCH_3 and 2 × $-CH_2-$), 3.98–4.00 (1H, m, cyclopropyl–CH), 4.16–4.23 (8H, m, 2 × NOMe and $-CH_2-$), 7.25–7.32 (4H, m, Ar–H), 7.57–7.71 (3H, m, Ar–H), 8.40 (1H, s, C2–H). ESI-MS m/z: 844 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{43}H_{44}F_3N_7O_8$: C, 61.20; H, 5.26; N, 11.62; found: C, 60.95; H, 5.03; N, 11.37.

Anti-mycobacterial MIC determination. Conjugates **3a–f**, **4a–f** along with GTFX, RIF, and INH were dissolved in DMSO and evaluated *in vitro* activity against MTB H37Rv and MDR-TB *via* rapid direct susceptibility test technique [15]. The wells of a sterile 48-well plate were filled with 100-mL twofold diluted tested compounds and 100-mL MTB H37Rv or MDR-TB suspension containing 4×10^{-3} mg cells. Pure medium replaced the diluted compounds in two wells as the positive control of growth and deionized water instead of the culture in other two wells as the negative control of growth in the plates. The plates were covered and sealed, then incubated at 37°C in a wet box. The positive and negative control wells should show obvious difference after 3 days. The MIC was determined by observing the quantity and state of the cells in each test well by a continuous visual high magnification system and redetermined 7 days later. The MIC is defined as the concentration of the compound required to give complete inhibition of bacterial growth.

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