Gatifloxacin–Isatin Hybrids and Their Antimycobacterial Activities

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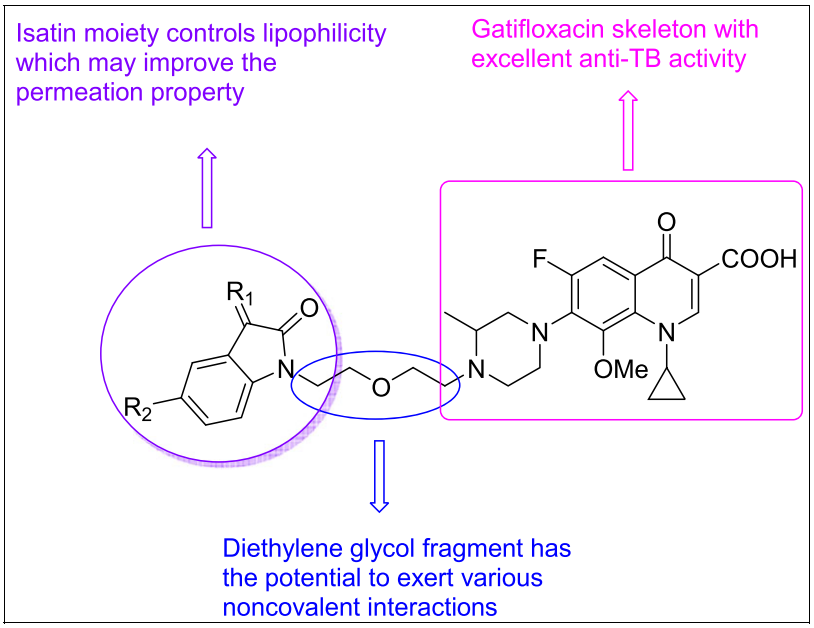

We report herein the design, synthesis, and antimycobacterial activity of a series of diethylene glycol tethered gatifloxacin–isatin hybrids **5a–o** in this paper. Results revealed that all hybrids showed promising activity against both drug-sensitive and multidrug-resistant *Mycobacterium tuberculosis* strains with minimum inhibitory concentration (MIC) in a range of 1–128 μg/mL. Particularly, hybrid **5j** with low cytotoxicity in VERO cell line was comparable with the parent gatifloxacin (MIC: 0.78 and 1 μg/mL) against MTB H37Rv and MDR-TB strains, and ≥32-fold more potent than isoniazid and rifampicin (MIC: >128 and 32 μg/mL, respectively) against MDR-TB, suggesting it may serve as a new and promising candidate for further study.

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INTRODUCTION

Tuberculosis (TB) is a disease that is caused by a bacterium called *Mycobacterium tuberculosis* (MTB), which kills 5000 people every day [1]. Approximately one-third of the world's population carry MTB, and around 10% of these people will likely develop active disease during their lifetime [2]. The global pandemic of drug-sensitive TB and the increasing threat from drug-resistant TB, together with HIV-TB coinfection, create an urgent need to develop novel, safe, fast-acting, and more effective anti-TB drugs.

Many fluoroquinolone derivatives were found to possess promising anti-TB potential and have been widely studied in recent year [3,4]. Some early fluoroquinolones have been recommended as the second-line agents by the World Health Organization for the treatment of TB mainly in cases involving resistance or intolerance to first-line anti-TB therapy because MTB isolates expressing resistance to both isoniazid (INH) and rifampicin (RIF) are susceptible to fluoroquinolones generally [2]. Moreover, some fluoroquinolones that are exemplified by moxifloxacin are potential first-line anti-TB agents, and moxifloxacin is under study for this indication currently [5,6]. Thus, fluoroquinolone derivatives are of great importance in the development of new anti-TB agents.

In spite of gatifloxacin (GTFX) has been withdrawn from the therapy because of dysglycemia, its derivatives

still worth to be investigated attribute to their excellent *in vitro* and *in vivo* anti-TB profiles [7–9]. In the meantime, installation of isatin moiety into C-7 position of GTFX was proved to be a promising strategy to develop new anti-TB candidates. As the most emblematic example, GTFX–isatin hybrid **1** (Fig. 1) demonstrated higher *in vitro* (16-fold and 64-fold against MTB H37Rv and multidrug-resistant/MDR-TB) and *in vivo* activities than the parent GTFX [7]. The structure–activity relationship revealed that the linker between the GTFX and isatin played a pivotal role in the exertion of the anti-TB activity, and the linker bearing noncovalent bind interactions favored the activity [10,11].

As a part of an ongoing program to discover new anti-TB candidates, we recently synthesized a series of 1,2,3-triazole tethered GTFX–isatin hybrids, and some of them exhibited excellent inhibitory activity against MTB H37Rv and MDR-TB strains [8,9]. Inspired by the aforementioned research results, we intended to introduce diethylene glycol as the linker between GTFX and isatin, because diethylene glycol fragment has the potential to exert various noncovalent interactions that may facilitate binding with active site. All of the synthesized GTFX–isatin hybrids were screened for their *in vitro* antimycobacterial activity against drug-sensitive MTB H37Rv and MDR-TB strains, and the cytotoxicity was also tested in VERO cells. Our primary objective was to identify optimized linker between GTFX and isatin to facilitate the further development. The illustration of the design strategy is depicted in Figure 2.

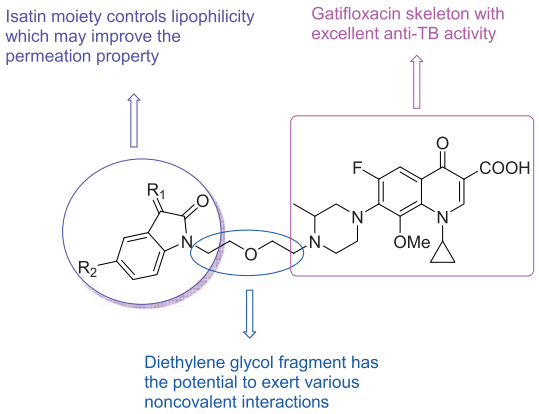


Figure 2. The design strategy of diethylene glycol tethered gatifloxacin–isatin hybrids. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

Detailed synthetic route for diethylene glycol tethered GTFX–isatin hybrids **5a–o** was described in Scheme 1. Intermediate **2** was obtained by treatment of diethylene glycol **1** with tosyl chloride in the presence of triethylamine, and then alkylation of C-5 substituted isatins **3** with intermediate **2** provided the key intermediates **4a–d**. Introduction of **4a–d** into GTFX was performed with K_2CO_3 as base yielded the desired GTFX–isatin hybrids **5a–d**, which was consequently condensation with the corresponding amine hydrochloride gave the other hybrids **5e–o**.

Compared with the parent GTFX (Log *P*: 1.51), all diethylene glycol tethered GTFX–isatin hybrids **5a–o** (Log *P*: 2.07–3.62) exhibited great lipophilicity, and this character may improve their permeation properties toward mycobacterial cell membrane. All hybrids **5a–o** along with the references GTFX, INH, and RIF were examined for their *in vitro* antimycobacterial activities against MTB H37Rv and MDR-TB (resistant to INH, RIF, and ethambutol) strains, and the results were presented in Table 1. The minimum inhibitory concentration (MIC) is defined as the lowest concentration that inhibits the visible bacterial growth.

As shown in Table 1, all diethylene glycol tethered GTFX–isatin hybrids **5a–o** displayed moderate to excellent activity against both drug-sensitive and multidrug-resistant MTB strains with MIC in a range of 1–128 μg/mL but were less active than the references GTFX, INH, and RIF (MIC: 0.78, 0.05, and 0.39 μg/mL, respectively) against drug-sensitive MTB H37Rv. Compared with the parent GTFX, the antimycobacterial activity of the GTFX–isatin hybrids **5a–o** did not increase with the incensement of the lipophilicity, suggesting simply increasing the lipophilicity of could not improve the antimycobacterial activity accordingly [8,9]. The structure–activity relationship revealed that the antimycobacterial activity of the synthesized hybrids was influenced greatly by substituents on C-3 and C-5 positions of isatin moiety, which was in accordance with our previous studies [7,8,12–17]. For C-3 position, the

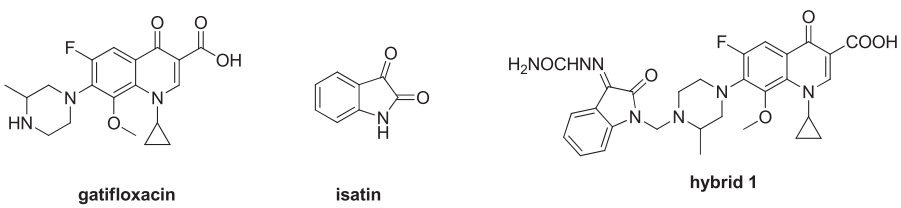


Figure 1. Chemical structures of gatifloxacin, isatin, and gatifloxacin–isatin hybrid **1**.

Scheme 1. Synthetic route for diethylene glycol tethered gatifloxacin–isatin hybrids **5a–o**.

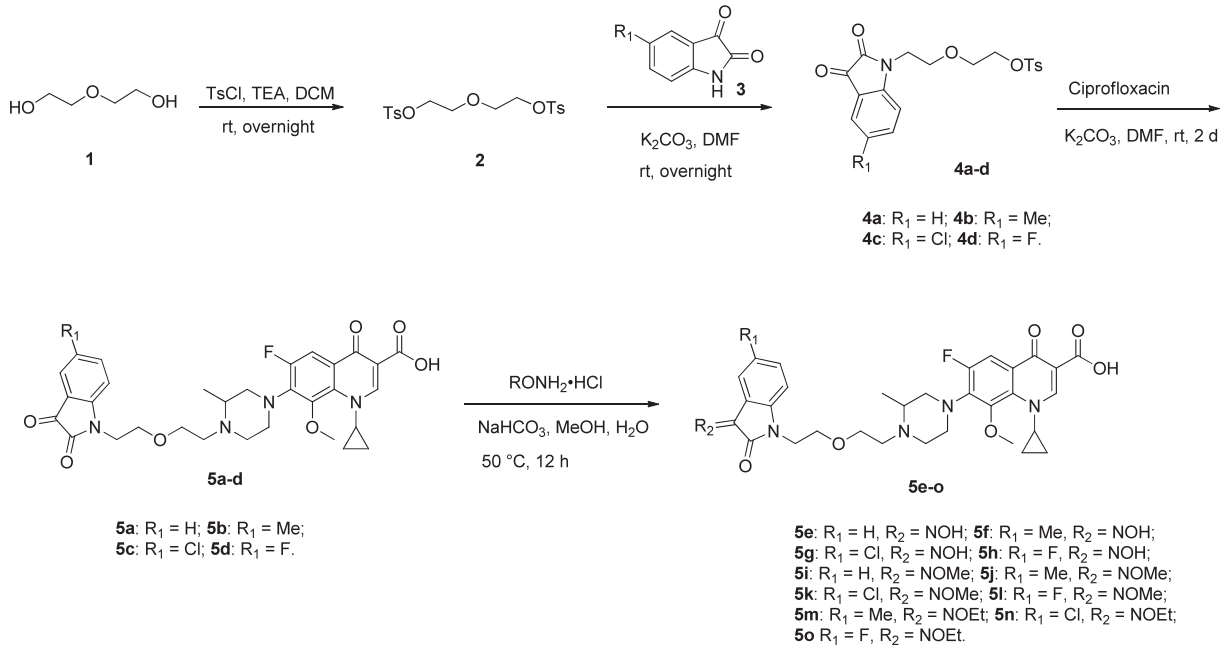
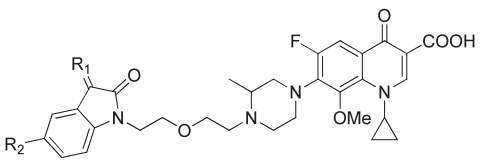


Table 1

Structures, lipophilicity, and *in vitro* antimycobacterial activities of diethylene glycol tethered GTFX–isatin hybrids **5a–o**.



Compd.	R_1	R_2	Log P[a]	MIC (μg/mL) MTB $H_{37}Rv$	MIC (μg/mL) MDR-TB	CC_{50} (μg/mL)[b]
5a	O	H	2.07	6.25	16	250
5b	O	Me	2.56	3.12	4	125
5c	O	Cl	2.63	12.5	32	125
5d	O	F	2.23	6.25	32	31.2
5e	NOH	H	2.46	50	64	125
5f	NOH	Me	2.94	50	32	250
5g	NOH	Cl	3.01	100	128	500
5h	NOH	F	2.61	100	64	125
5i	NOMe	H	2.72	6.25	8	62.5
5j	NOMe	Me	3.21	1.56	1	250
5k	NOMe	Cl	3.28	12.5	4	125
5l	NOMe	F	2.88	12.5	2	125
5m	NOEt	Me	3.54	25	32	31.2
5n	NOEt	Cl	3.62	50	64	250
5o	NOEt	F	3.22	100	64	62.5
GTFX			1.51	0.78	1.0	125
INH			−0.67	0.05	>128	125
RIF			3.71	0.39	32	500

GTFX, gatifloxacin; MIC, minimum inhibitory concentration.
[a]The Log *P* is calculated with CHEMOFFICE 2012 software.
[b]CC_{50}: The 50% cytotoxic concentration in a mammalian VERO cell line.

relative contribution of imines of the Schiff's bases to the activity was –NOMe ≥ –O > –NOEt > –NOH; introduction of electron-donating –Me was favorable to the activity, while electron-withdrawing –F and –Cl were detrimental to the activity generally. Particularly, hybrid **5j** (MIC: 1.56 and 1 μg/mL), which was found to be the most active against MTB H37Rv and MDR-TB, was comparable with the parent GTFX (MIC: 0.78 and 1 μg/mL) against the tested two strains and ≥32-fold more potent than INH and RIF (MIC: >128 and 32 μg/mL, respectively) against MDR-TB.

The diethylene glycol tethered GTFX–isatin hybrids **5a**–**o** were subsequently examined for toxicity (CC_{50}) in a mammalian VERO cell line by MTT assay [8,9], and the results were reported in Table 1. All GTFX–isatin hybrids (CC_{50}: 31.2–500 μg/mL) showed excellent cytotoxic profiles, and some of them were less toxic than the parent GTFX (CC_{50}: 125 μg/mL). The most active hybrid **5j** (CC_{50}: 250 μg/mL) also displayed lower cytotoxicity than GTFX and could act as an ideal starting point for further optimization.

CONCLUSIONS

In conclusion, 15 diethylene glycol tethered GTFX–isatin hybrids were designed and synthesized as new anti-TB agents. All the synthesized hybrids exhibited promising activity against both drug-sensitive and multidrug-resistant MTB strains as well as low cytotoxicity in VERO cell line. In particular, hybrid **5j** not only showed great potency against the tested two strains (MIC: 1.56 and 1 μg/mL) but also displayed low cytotoxicity (CC_{50}: 250 μg/mL), worth to be further optimization.

EXPERIMENTAL

Synthesis. ***The general procedure for preparing hybrids 5a–d.*** To a mixture of diethylene glycol **1** (100 mmol) and triethylamine (500 mmol) in dichloromethane (DCM) (1 L), tosyl chloride (300 mmol) was added. The mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel chromatography eluted with PE : EA = 1:2 to give the desired product **2**. The mixture of intermediate **2** (1.5 mmol), potassium carbonate (5 mmol), and isatins **3** (1 mmol) in dimethylformamide (30 mL) was stirred at room temperature overnight. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with PE : EA = 1:2 to give the intermediates **4**. A mixture of GTFX (1 mmol), intermediates **4** (1 mmol), and potassium carbonate (10 mmol) in dimethylformamide (10 mL) was stirred at room temperature for 2 days. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with DCM : MeOH = 5:1 to give the desired diethylene glycol tethered GTFX–isatin hybrids **5a**–**d**.

1-Cyclopropyl-7-(4-(2-(2-(2,3-dioxoindolin-1-yl)ethoxy) ethyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5a). Yellow solid, yield: 17%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97–1.07 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, –CH_3), 3.14–3.44 (7H, m, piperazine-5H and –NCH_2), 3.68–3.74 (4H, m, piperazine-2H and –OCH_2), 3.80 (3H, s, –OCH_3), 3.88 (2H, t, –OCH_2), 3.97–3.99 (1H, m, cyclopropyl–CH), 4.20 (2H, t, –OCH_2), 6.97 (1H, d, Ar–H), 7.06 (1H, t, Ar–H), 7.43–7.51 (2H, m, Ar–H), 7.73 (1H, s, Ar–H), 8.67 (1H, s, C2–H), 14.88 (1H, brs, COOH). ESI-MS *m/z*: 593 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{30}FN_4O_7$: C, 62.83; H, 5.61; N, 9.45. Found: C, 62.71; H, 5.47; N, 9.25.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(2-(2-(5-methyl-2,3-dioxoindolin-1-yl)ethoxy)ethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5b). Light yellow solid, yield: 21%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.21 (3H, d, –CH_3), 2.22 (3H, s, –CH_3), 3.11–3.42 (7H, m, piperazine-5H and –NCH_2), 3.67–3.78 (4H, m, piperazine-2H and –OCH_2), 3.78 (3H, s, –OCH_3), 3.86 (2H, t, –OCH_2), 3.99–4.01 (1H, m, cyclopropyl–CH), 4.19 (2H, t, –OCH_2), 6.84 (1H, d, Ar–H), 7.34 (1H, s, Ar–H), 7.38 (1H, d, Ar–H), 7.71 (1H, d, Ar–H), 8.76 (1H, s, C2–H), 14.91 (1H, brs, COOH). ESI-MS *m/z*: 607 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}FN_4O_7$: C, 63.36; H, 5.82; N, 9.24. Found: C, 63.18; H, 5.73; N, 9.16.

7-(4-(2-(2-(5-Chloro-2,3-dioxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5c). Light yellow solid, yield: 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.13 (4H, m, 2 × cyclopropyl–CH_2), 1.21 (3H, d, –CH_3), 3.13–3.45 (7H, m, piperazine-5H and –NCH_2), 3.67–3.74 (4H, m, piperazine-2H and –OCH_2), 3.79 (3H, s, –OCH_3), 3.84 (2H, t, –OCH_2), 3.98–4.00 (1H, m, cyclopropyl–CH), 4.21 (2H, t, –OCH_2), 6.94 (1H, d, Ar–H), 7.52–7.56 (2H, m, Ar–H), 7.71–7.76 (1H, m, Ar–H), 8.81 (1H, s, C2–H), 14.91 (1H, brs, COOH). ESI-MS *m/z*: 627 $[M+H]^+$, 629 $[M+2+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{32}FClN_4O_7$: C, 59.38; H, 5.14; N, 8.93. Found: C, 59.19; H, 5.01; N, 8.77.

1-Cyclopropyl-6-fluoro-7-(4-(2-(2-(5-fluoro-2,3-dioxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5d). Light yellow solid, yield: 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03–1.14 (4H, m, 2 × cyclopropyl–CH_2),

1.24 (3H, d, $-CH_3$), 3.11–3.46 (7H, m, piperazine-5H and $-NCH_2$), 3.69–3.78 (4H, m, piperazine-2H and $-OCH_2$), 3.79 (3H, s, $-OCH_3$), 3.84 (2H, t, $-OCH_2$), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.20 (2H, t, $-OCH_2$), 6.93 (1H, s, Ar–H), 7.51–7.72 (3H, m, Ar–H), 8.69 (1H, s, C2–H), 14.96 (1H, brs, COOH). ESI-MS *m/z*: 611 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{32}F_2N_4O_7$: C, 60.98; H, 5.28; N, 9.18. Found: C, 60.76; H, 5.03; N, 9.03.

The general procedure for preparing targets 5e–o. To a solution of substituted amine hydrochlorides (6 mmol) and sodium bicarbonate (6 mmol) dissolved in water (10 mL) and methanol (10 mL) was added **5a–d**. 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 $v_{DCM} : v_{MeOH} = 5:1$ to give the title compounds **5e–o**.

1-Cyclopropyl-6-fluoro-7-(4-(2-(2-(3-(hydroxyimino)-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5e). Light yellow solid, yield: 39%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.06 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, $-CH_3$), 3.13–3.41 (7H, m, piperazine-5H and $-NCH_2$), 3.63–3.71 (4H, m, piperazine-2H and $-OCH_2$), 3.79 (3H, s, $-OCH_3$), 3.89 (2H, t, $-OCH_2$), 3.98–3.99 (1H, m, cyclopropyl–CH), 4.19 (2H, t, $-OCH_2$), 6.91 (1H, d, Ar–H), 7.03 (1H, t, Ar–H), 7.31 (1H, t, Ar–H), 7.73 (1H, d, Ar–H), 7.90 (1H, d, Ar–H), 8.71 (1H, s, C2–H), 13.46 (1H, brs, NOH), 14.92 (1H, brs, COOH). ESI-MS *m/z*: 608 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{34}FN_5O_7$: C, 61.28; H, 5.64; N, 11.53. Found: C, 61.13; H, 5.39; N, 11.31.

1-Cyclopropyl-6-fluoro-7-(4-(2-(2-(3-(hydroxyimino)-5-methyl-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5f). Light yellow solid, yield: 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.15 (4H, m, 2 × cyclopropyl–CH_2), 1.23 (3H, d, $-CH_3$), 2.22 (3H, s, $-CH_3$), 3.14–3.42 (7H, m, piperazine-5H and $-NCH_2$), 3.69–3.78 (4H, m, piperazine-2H and $-OCH_2$), 3.79 (3H, s, $-OCH_3$), 3.87 (2H, t, $-OCH_2$), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.20 (2H, t, $-OCH_2$), 6.78 (1H, d, Ar–H), 7.11 (1H, d, Ar–H), 7.70–7.78 (2H, m, Ar–H), 8.69 (1H, s, C2–H), 13.46 (1H, brs, NOH), 14.72 (1H, brs, COOH). ESI-MS *m/z*: 622 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{36}FN_5O_7$: C, 61.83; H, 5.84; N, 11.27. Found: C, 61.71; H, 5.65; N, 11.13.

7-(4-(2-(2-(5-Chloro-3-(hydroxyimino)-2-oxoindolin-1-yl) ethoxy)ethyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5g). Light yellow solid, yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.23 (3H, d, $-CH_3$), 3.15–3.46 (7H, m, piperazine-5H and $-NCH_2$), 3.68–3.78 (4H, m, piperazine-2H and $-OCH_2$), 3.79 (3H, s, $-OCH_3$), 3.86 (2H, t, $-OCH_2$), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.21 (2H, t, $-OCH_2$), 6.87 (1H, d, Ar–H), 7.31 (1H, d, Ar–H), 7.70 (1H, d, Ar–H), 7.82 (1H, t, Ar–H), 8.69 (1H, s, C2–H), 13.76 (1H, brs, NOH), 14.94 (1H, brs, COOH). ESI-MS *m/z*: 642 $[M+H]^+$, 644 $[M+2+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{33}FClN_5O_7$: C, 57.99; H, 5.18; N, 10.91. Found: C, 57.76; H, 5.06; N, 11.68.

1-Cyclopropyl-6-fluoro-7-(4-(2-(2-(5-fluoro-3-(hydroxyimino)-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5h). Light yellow solid, yield: 34%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.00–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, $-CH_3$), 3.15–3.46 (7H, m, piperazine-5H and $-NCH_2$), 3.67–3.79 (4H, m, piperazine-2H and $-OCH_2$), 3.80 (3H, s, $-OCH_3$), 3.85 (2H, t, $-OCH_2$), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.21 (2H, t, $-OCH_2$), 6.86 (1H, d, Ar–H), 7.16 (1H, t, Ar–H), 7.29 (1H, t, Ar–H), 7.61 (1H, d, Ar–H), 7.73 (1H, d, Ar–H), 8.70 (1H, s, C2–H), 13.68 (1H, brs, NOH), 14.92 (1H, brs, COOH). ESI-MS *m/z*: 626 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{33}F_2N_5O_7$: C, 59.51; H, 5.32; N, 11.19. Found: C, 59.37; H, 5.23; N, 11.04.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(4-(2-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5i). Light yellow solid, yield: 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.00–1.12 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, $-CH_3$), 3.18–3.46 (7H, m, piperazine-5H and $-NCH_2$), 3.64–3.76 (4H, m, piperazine-2H and $-OCH_2$), 3.79 (3H, s, $-OCH_3$), 3.86 (2H, t, $-OCH_2$), 3.98–3.99 (1H, m, cyclopropyl–CH), 4.13 (3H, s, $NOCH_3$), 4.20 (2H, t, $-OCH_2$), 6.89 (1H, d, Ar–H), 7.02 (1H, t, Ar–H), 7.31 (1H, t, Ar–H), 7.69 (1H, d, Ar–H), 7.81 (1H, d, Ar–H), 8.70 (1H, s, C2–H), 14.94 (1H, brs, COOH). ESI-MS *m/z*: 593 $[M+H]^+$. Elemental *Anal*. Calcd (%) for $C_{31}H_{30}FN_4O_7$: C, 62.83; H, 5.61; N, 9.45. Found: C, 62.71; H, 5.47; N, 9.25.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(4-(2-(2-(3-(methoxyimino)-5-methyl-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5j). Light yellow solid, yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–0.94 (2H, m, cyclopropyl–CH_2), 1.05–1.07 (2H, m, cyclopropyl–CH_2), 1.22 (3H, d, $-CH_3$), 2.22 (3H, d, $-CH_3$), 3.13–3.44 (7H, m, piperazine-5H and $-NCH_2$), 3.68–3.74 (4H, m, piperazine-2H and $-OCH_2$), 3.80 (3H, s, $-OCH_3$), 3.87 (2H, t, $-OCH_2$), 3.97–3.99 (1H, m, cyclopropyl–CH), 4.12 (3H, s, $NOCH_3$), 4.20 (2H, t, $-OCH_2$), 6.95 (1H, t, Ar–H), 7.12 (1H, d, Ar–H), 7.34 (1H, t, Ar–H), 7.54 (1H, d, Ar–H), 7.76 (1H, d, Ar–H), 8.68 (1H, s, C2–H), 14.92 (1H, brs, COOH). ESI-MS *m/z*: 636 [M

+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{33}H_{38}FN_5O_7$: C, 62.35; H, 6.03; N, 11.02. Found: C, 62.17; H, 5.86; N, 10.88.

7-(4-(2-(2-(5-Chloro-3-(methoxyimino)-2-oxoindolin-1-yl) ethoxy)ethyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5k). Light yellow solid, yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.23 (3H, d, –CH_3), 3.20–3.43 (7H, m, piperazine-5H and –NCH_2), 3.68–3.76 (4H, m, piperazine-2H and –OCH_2), 3.79 (3H, s, –OCH_3), 3.87 (2H, t, –OCH_2), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.12 (3H, s, $NOCH_3$), 4.21 (2H, t, –OCH_2), 6.89 (1H, d, Ar–H), 7.34 (1H, d, Ar–H), 7.71–7.78 (2H, m, Ar–H), 8.69 (1H, s, C2–H), 14.92 (1H, brs, COOH). ESI-MS *m/z*: 656 [M+H]$^+$, 658 [M +2+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}FClN_5O_7$: C, 58.58; H, 5.38; N, 10.67. Found: C, 58.37; H, 5.19; N, 10.42.

1-Cyclopropyl-6-fluoro-7-(4-(2-(2-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5l). Light yellow solid, yield: 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.12 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, –CH_3), 3.17–3.48 (7H, m, piperazine-5H and –NCH_2), 3.66–3.77 (4H, m, piperazine-2H and –OCH_2), 3.80 (3H, s, –OCH_3), 3.85 (2H, t, –OCH_2), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.21 (2H, t, –OCH_2), 6.92 (1H, d, Ar–H), 7.26 (1H, t, Ar–H), 7.58 (1H, d, Ar–H), 7.71 (1H, d, Ar–H), 8.70 (1H, s, C2–H), 14.94 (1H, brs, COOH). ESI-MS *m/z*: 640 [M+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{33}F_2N_5O_7$: C, 60.09; H, 5.52; N, 10.95. Found: C, 59.93; H, 5.31; N, 10.72.

1-Cyclopropyl-7-(4-(2-(2-(3-(ethoxyimino)-5-methyl-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5m). Light yellow solid, yield: 18%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, –CH_3), 1.27–1.35 (3H, m, $NOCH_2\underline{CH_3}$), 2.24 (3H, d, –CH_3), 3.13–3.44 (7H, m, piperazine-5H and –NCH_2), 3.65–3.76 (4H, m, piperazine-2H and –OCH_2), 3.79 (3H, s, –OCH_3), 3.86 (2H, t, –OCH_2), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.13 (3H, s, $NOCH_3$), 4.20 (2H, t, –OCH_2), 4.38–4.43 (2H, m, $NO\underline{CH_2}CH_3$), 6.76 (1H, d, Ar–H), 7.14 (1H, d, Ar–H), 7.65 (1H, s, Ar–H), 7.73 (1H, d, Ar–H), 8.69 (1H, s, C2–H), 14.95 (1H, brs, COOH). ESI-MS *m/z*: 650 [M+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{34}H_{40}FN_5O_7$: C, 62.85; H, 6.21; N, 10.78. Found: C, 62.57; H, 6.03; N, 10.59.

7-(4-(2-(2-(5-Chloro-3-(ethoxyimino)-2-oxoindolin-1-yl) ethoxy)ethyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5n). Light yellow solid, yield: 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98–1.14 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, –CH_3), 1.32–1.35 (3H, m, $NOCH_2\underline{CH_3}$), 3.21–3.45 (7H, m, piperazine-5H and –NCH_2), 3.66–3.79 (4H, m, piperazine-2H and –OCH_2), 3.80 (3H, s, –OCH_3), 3.85 (2H, t, –OCH_2), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.12 (3H, s, $NOCH_3$), 4.21 (2H, t, –OCH_2), 4.43–4.48 (2H, m, $NO\underline{CH_2}CH_3$), 6.87 (1H, d, Ar–H), 7.39 (1H, d, Ar–H), 7.60–7.76 (2H, m, Ar–H), 8.69 (1H, s, C2–H), 14.95 (1H, brs, COOH). ESI-MS *m/z*: 670 [M+H]$^+$, 672 [M+2+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{33}H_{37}FClN_5O_7$: C, 59.15; H, 5.57; N, 10.45. Found: C, 59.02; H, 5.29; N, 10.31.

1-Cyclopropyl-7-(4-(2-(2-(3-(ethoxyimino)-5-fluoro-2-oxoindolin-1-yl)ethoxy)ethyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5o). Light yellow solid, yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03–1.13 (4H, m, 2 × cyclopropyl–CH_2), 1.24 (3H, d, –CH_3), 1.32–1.36 (3H, m, $NOCH_2\underline{CH_3}$), 3.18–3.49 (7H, m, piperazine-5H and –NCH_2), 3.65–3.77 (4H, m, piperazine-2H and –OCH_2), 3.80 (3H, s, –OCH_3), 3.89 (2H, t, –OCH_2), 3.99–4.00 (1H, m, cyclopropyl–CH), 4.21 (2H, t, –OCH_2), 4.41–4.46 (2H, m, $NO\underline{CH_2}CH_3$), 6.87 (1H, d, Ar–H), 7.18–7.25 (1H, m, Ar–H), 7.57 (1H, d, Ar–H), 7.69 (1H, d, Ar–H), 8.69 (1H, s, C2–H), 14.94 (1H, brs, COOH). ESI-MS *m/z*: 654 [M+H]$^+$. Elemental *Anal*. Calcd (%) for $C_{33}H_{37}F_2N_5O_7$: C, 60.63; H, 5.71; N, 10.71. Found: C, 60.53; H, 5.45; N, 10.49.

Minimum inhibitory concentration determination. The GTFX–isatin hybrids **5a–o** along with the references GTFX, RIF, and INH were screened for their *in vitro* antimycobacterial activities against MTB H37Rv and MDR-MTB by rapid direct susceptibility test technique [16,17]. The wells of a sterile 48-well plate were filled with 100 mL two-fold diluted tested compounds and 100 mL MTB H37Rv or MDR-MTB 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 and 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 90% inhibition of bacterial growth.

Cytotoxicity. The GTFX–isatin hybrids **5a–o** together with the references GTFX, RIF, and INH were further assessed for their toxicity in VERO cell line dissolved in DMSO at concentrations from 1000 to 7.8 μg/mL. The VERO cells were maintained in culture medium at 37°C under 5% CO_2. Cells were seeded in 96-well plates at the plating density of 1×10^4 cells per well and allowed to recover for 24 h. Culture media was replaced by assay

medium containing the tested hybrid or drug free. After 72 h of exposure, cells were harvested, and cell viability was assessed by MTT assay. The CC_{50} was calculated by Bliss analyses.

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