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Graphical Abstract

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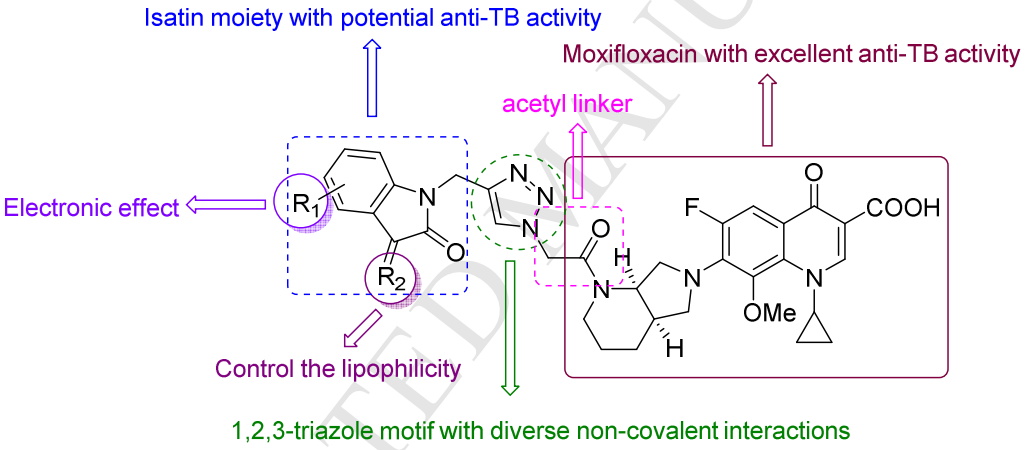

Synthesis and biological evaluation of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids as potential anti-tubercular agents against both drug-susceptible and drug-resistant *Mycobacterium tuberculosis* strains

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Abstract: Herein, synthesis and biological evaluation of fourteen moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids as potential anti-tubercular agents against both drug-susceptible (MTB H_{37}Rv), rifampicin-resistant and multidrug-resistant *Mycobacterium tuberculosis* strains were reported, and cytotoxicity towards VERO cells as well as inhibitory activity against MTB DNA gyrase were also discussed in this paper. The structure-activity relationship and structure-cytotoxicity relationship demonstrated that substituents on the C-3 and C-5/C-7 positions of isatin framework were closely related with the anti-mycobacterial activity and cytotoxicity. The most active hybrids **8h** and **8l** (MIC: 0.12-0.5 μg/mL) showed excellent activity which was no inferior to the parent moxifloxacin against the tested drug-susceptible, rifampicin-resistant and multidrug-resistant *Mycobacterium tuberculosis* strains, demonstrating their potential application as novel anti-tubercular candidates.

Keywords: moxifloxacin; 1,2,3-triazole; isatin; anti-tubercular; drug-resistant *Mycobacterium tuberculosis*; structure-activity relationship; structure-cytotoxicity relationship

1. Introduction

The World Health Organization (WHO) End TB Strategy aimed to halt the global tuberculosis (TB) epidemic by 2035, with the target to decline TB deaths by 95% and to reduce new cases by 90% [1,2]. An estimated 54 million lives were saved between 2000 and 2017 with the technology development of disease detection and the

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improvement of treatment success rates [3,4]. However, TB continues to ravage the world, leading to tremendous morbidity and mortality as evidenced by that over 10 million new TB cases (1 million children) and 1.6 million (230,000 children) deaths occurred in 2017 [5]. The main obstacle to elimination of TB is the widely spread of drug-resistant TB (DR-TB, resistance to at least one first-line anti-TB drugs) especially multi-drug resistant TB (MDR-TB, resistance to at least both isoniazid and rifampicin) [6], and 3.5% of new cases and 18% of previous treated cases were MDR-TB or rifampicin-resistant (RR) TB patients with 580,000 incident cases and 230,000 deaths in 2017 [3]. The second-line anti-TB agents (such as levofloxacin) and newly developed anti-TB agents (such as bedaquiline and delamanid) occupy an important position in the treatment of DR-TB and MDR-TB patients, but these agents are generally less effective (treatment success remains low, at 55% globally in 2017) and more toxic [7,8]. Moreover, there are only 20 drugs in Phase I, II or III trials for the treatment of drug-susceptible TB, MDR-TB or latent TB infection, and they are still not sufficient [3]. All the above facts made an urgent demand to develop new anti-TB agents with unique mechanisms and favorable profiles such as high efficacy, low toxicity and short therapy duration.

Moxifloxacin (7-[(4aS,7aS)-octahydro-1*H*-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid), as the fourth generation of fluoroquinolone antibiotic, could act on both DNA gyrase and topoisomerase IV, possess broad-spectrum antimicrobial activity [9,10]. Moxifloxacin exhibits excellent *in vitro* and *in vivo* potency against drug-susceptible and drug-resistant *Mycobacterium tuberculosis* (MTB) strains including various levels of fluoroquinolones resistance strains [11-13], and the clinical trial demonstrated that the efficacy of moxifloxacin was no inferior to the standard regimen in early phase of treatment TB patients [14,15]. Currently, moxifloxacin is under phase III clinical trial, and it may be used in the near future for the treatment of TB, even MDR-TB. However, the moxifloxacin-resistant MTB strains have already emerged, and serious adverse effects like irreversible peripheral neuropathy [16], hepatitis [17], and phototoxicity reactions [18] may occur as a result of moxifloxacin therapy. To overcome the resistance and reduce the adverse effects, modification of moxifloxacin is needed.

Isatin and 1,2,3-triazole derivatives exhibit a variety of pharmacological properties such as antibacterial [19,20], anticancer [21,22], and anti-TB [23,24] activities which may be attributed to that these heterocycles can serve as a useful tool to manipulate lipophilicity, polarity, and hydrogen bonding capacity of molecules, and consequently improve pharmacological, pharmacokinetic, toxicological, and physicochemical properties of drug candidates and the ultimate drugs. Moreover, several isatin- or 1,2,3-triazole-based compounds are under clinical evaluations for the treatment of various diseases including TB [23,24], demonstrating isatin and 1,2,3-triazole moieties are useful pharmacophores in the development of new drugs.

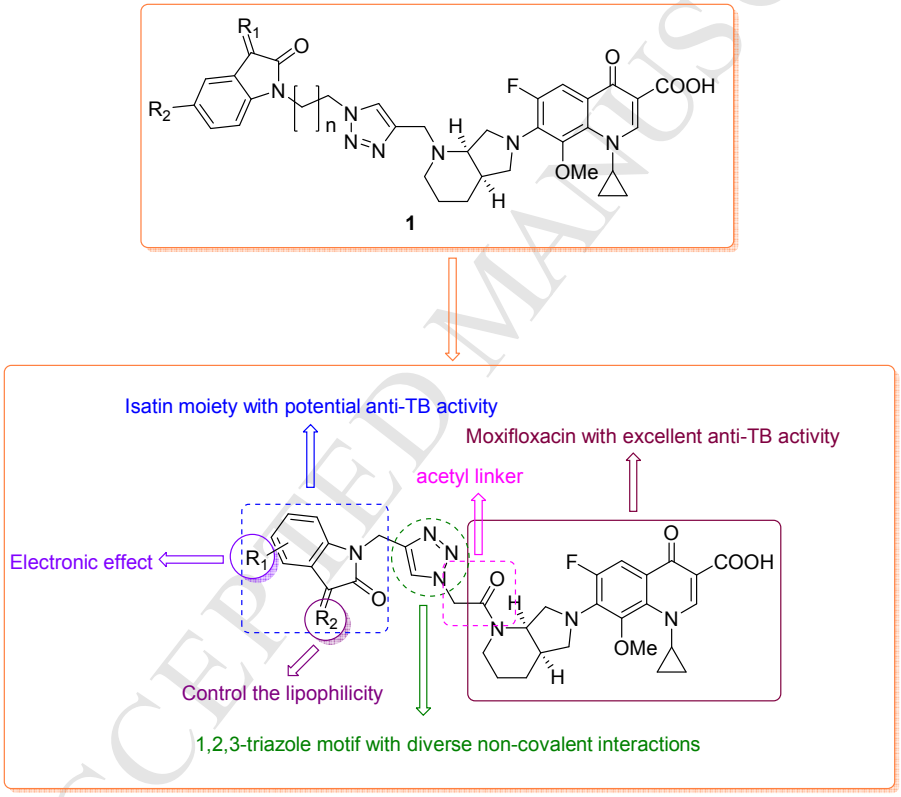


Figure 1 Design strategy for moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids

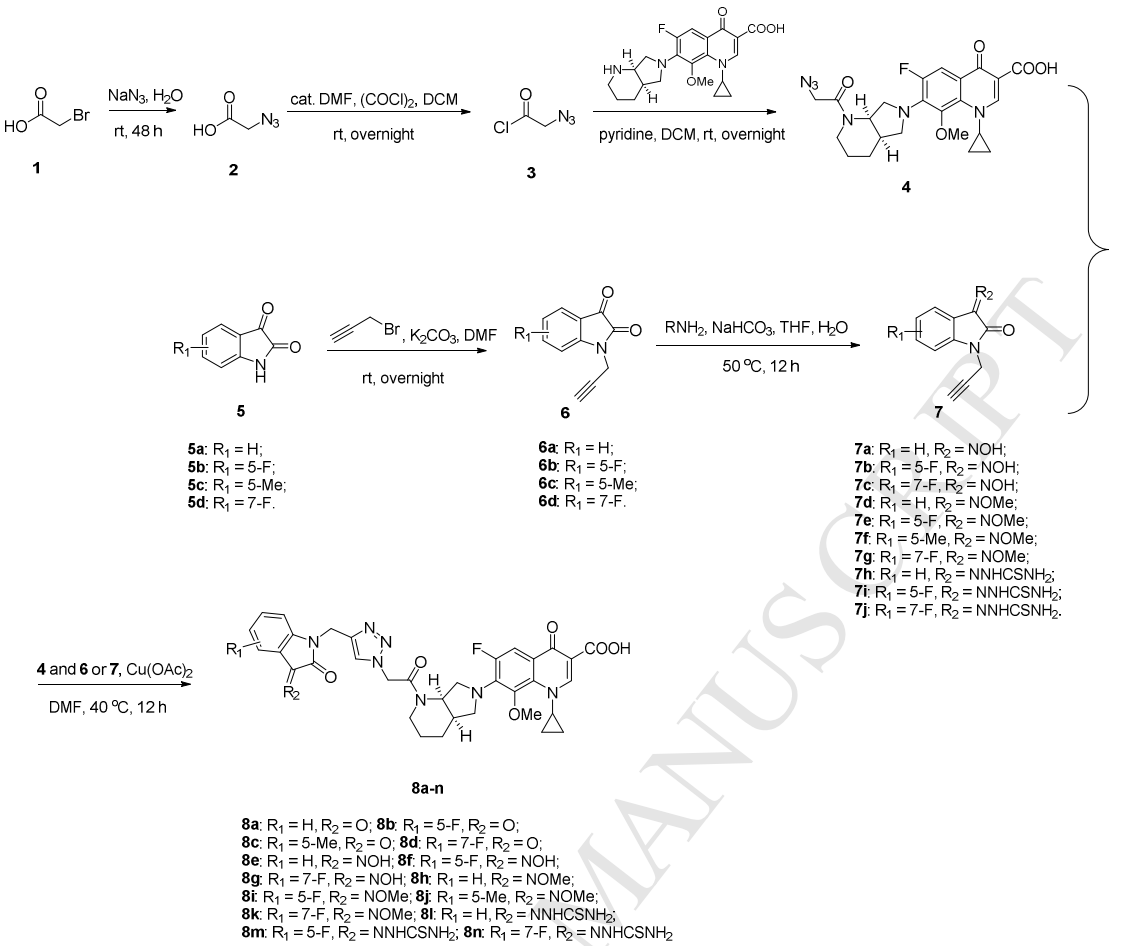
Moxifloxacin-1,2,3-triazole-isatin **1** (**Figure 1**) tethered *via* various alkyl linkers displayed promising anti-TB activity against both drug-susceptible and drug-resistant (DR) MTB strains [25,26], and the structure-activity relationship (SAR) as well as structure-cytotoxicity relationship demonstrated that the linker between the

fluoroquinolone and 1,2,3-triazole frameworks as well as substituents on the isatin fragment influenced the anti-TB activity and cytotoxicity significantly [27-30]. Thus, further optimization of the linker between the moxifloxacin and 1,2,3-triazole moieties and substituents on the isatin motif may provide more effective anti-TB candidates which are highly active against both drug-susceptible and drug-resistant MTB strains.

Based on the above facts, a series of novel moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids were designed, synthesized and evaluated for their *in vitro* antimycobacterial activity against drug-susceptible (MTB $H_{37}Rv$) and DR including MDR MTB strains. The design strategy was depicted in **Figure 1**.

2. Results and discussion

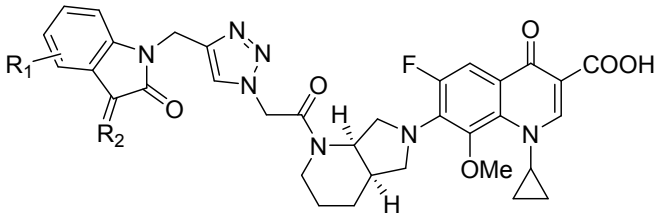
The synthetic pathway for the desired moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n** was depicted in **Scheme 1**. 2-Bromoacetic acid **1** was converted to 2-azidoacetic acid **2** by treatment with sodium azide in H_2O, and then 2-azidoacetyl chloride **3** was obtained by treatment of 2-azidoacetic acid **2** with oxalyl chloride in presence of catalytic amount of DMF in DCM. Acylation reaction between moxifloxacin and 2-azidoacetyl chloride **3** with pyridine as base in DCM provided the 2-azidoacetyl moxifloxacin **4**. Isatin/5-methylisatin/5-fluoroisatin/7-fluoroisatin **5a-d** reacted with propargyl bromide with K_2CO_3 as base in DMF, giving *N*-propargyl isatin intermediates **6a-d**. Condensation of *N*-propargyl isatins **6a-d** with the requested amine hydrochlorides in presence of $NaHCO_3$ in a mixture of THF and H_2O yielded isatin intermediates **7a-j**. Finally, cyclization of 2-azidoacetyl moxifloxacin **4** with *N*-propargyl isatin intermediates **6a-d** or **7a-j** with copper acetate ($Cu(OAc)_2$) as catalyst generated the desired moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n**.



Scheme 1 Synthesis of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n**

The chemical structures and yields of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n** were presented in **Table 1**.

Table 1. Chemical structures and yields of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n**



8a-n

Compd	R_1	R_2	Yield (%)
8a	H	O	41%
8b	5-F	O	43%
8c	5-Me	O	36%
8d	7-F	O	24%
8e	H	NOH	28%
8f	5-F	NOH	37%
8g	7-F	NOH	19%
8h	H	NOMe	35%
8i	5-F	NOMe	39%
8j	5-Me	NOMe	46%
8k	7-F	NOMe	27%
8l	H	$NNHCSNH_2$	19%
8m	5-F	$NNHCSNH_2$	15%
8n	7-F	$NNHCSNH_2$	11%

Moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n** together with references gatifloxacin, moxifloxacin, isoniazid and rifampicin were initially evaluated for their *in vitro* anti-mycobacterial activity against MTB $H_{37}Rv$ and cytotoxicity towards mammalian VERO cells, and MIC as well as CC_{50} values were presented in **Table 2**. The selectivity index (SI) expressed in terms of CC_{50}/MIC, and the results were also listed in **Table 2**.

Table 2. *In vitro* anti-mycobacterial activity against MTB $H_{37}Rv$, cytotoxicity and selectivity index of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n**

Compd.	$MIC_{MTB\ H37Rv}$ (μg/mL)	CC_{50} (μg/mL)	SI
8a	0.25	32	128
8b	1	16	16
8c	2	64	32
8d	8	32	4
8e	1	32	32
8f	2	16	8
8g	16	16	1
8h	0.5	128	256
8i	1	32	32
8j	4	64	16
8k	16	16	1
8l	0.12	16	128
8m	0.25	8	32
8n	8	8	1
Gatifloxacin	0.5	64	128
Moxifloxacin	0.12	128	1,024
Isoniazid	0.12	128	1,024
Rifampicin	0.06	>128	>512

From **Table 2**, it can be seen that all moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n** (MIC: 0.12-16 μg/mL) showed considerable activity against MTB H_{37}Rv, and the anti-mycobacterial activities of hybrids **8a**, **8h**, **8l** and **8m** (MIC: 0.06-0.5 μg/mL) were in the same level with those of the references gatifloxacin, moxifloxacin, isoniazid and rifampicin (MIC: 0.12-0.5 μg/mL). The SAR indicated that introduction of thiosemicarbazone at C-3 position of isatin skeleton was favorable to the activity, while oxime and methyloxime was harmful to the activity when compared with the ketone, and the relative contribution order of the substituents was thiosemicarbazone > ketone > methyloxime > oxime. Hybrids with either electron-donating methyl or electron-withdrawing fluoro at C-5 position of isatin moiety exhibited reduced activity when compared with unsubstituted analogues, and movement of fluoro to C-7 position led to great loss of activity, suggesting this position may not suitable for modification.

All hybrids **8a-n** (CC_{50}: 8-64 μg/mL) except **8h** (CC_{50}: 128 μg/mL) were more toxic than the parent moxifloxacin (CC_{50}: 128 μg/mL), and the structure-cytotoxicity relationship revealed that installation of oxime and thiosemicarbazone at C-3 position or fluoro at either C-5 or C-7 position of isatin fragment could increase the cytotoxicity, while methyloxime at C-3 position and methyl at C-5 position of isatin motif could decrease cytotoxicity to some extent.

The SI of the synthesized hybrids were in a range of 1 to 256, and three of them **8a**, **8h** and **8l** were comparable to or higher than gatifloxacin (SI: 128), but all of them were lower than moxifloxacin, isoniazid and rifampicin (SI: >512). The most active four hybrids **8a**, **8h**, **8l** and **8m** (SI: 32-256) which also showed relatively high SI were selected for further evaluation of their inhibitory activity against MTB DNA gyrase and anti-mycobacterial activity against one rifampicin-resistant (RR-MTB) and two MDR (resistant to isoniazid and rifampicin) MTB strains, and the results were presented in **Table 3** and **4**, respectively.

Table 3. *In vitro* inhibitory activity of hybrids **8a**, **8h**, **8l** and **8m** against MTB DNA gyrase

Comp.	IC_{50} (μg/mL)
8a	4.0
8h	6.0
8l	5.0
8m	8.0
Gatifloxacin	5.0
Moxifloxacin	5.5

DNA gyrase is one of the targets for the anti-TB agents, and isatin structural scaffolds as well as moxifloxacin could bind DNA gyrase [31,32]. Thus, hybridization of isatin with moxifloxacin may enhance of the inhibitory activity against DNA gyrase, and consequently improve the anti-TB activity. From **Table 3**, all the selected four hybrids with IC_{50} values of 4.0-8.0 μg/mL were in the same level with the references gatifloxacin (IC_{50}: 5.0 μg/mL) and moxifloxacin (IC_{50}: 5.5 μg/mL) against MTB DNA gyrase, and the most active hybrid **8a** (IC_{50}: 4.0 μg/mL) was slightly more active than the two references.

Table 4. *In vitro* anti-mycobacterial activity of hybrids **8a**, **8h**, **8l** and **8m** against RR and MDR MTB strains

Compd.	MIC (μg/mL)		
	RR-MTB	MDR-MTB1	MDR-MTB2
8a	0.5	0.5	0.25
8h	1	0.5	1
8l	0.25	0.25	0.5

8m	1	1	2
Gatifloxacin	1	0.25	0.5
Moxifloxacin	0.5	0.25	0.25
Isoniazid	0.5	64	>128
Rifampicin	>128	>128	>128

From **Table 4**, all of the four selected hybrids showed promising activity against the tested RR-MTB and two MDR-MTB strains with MIC values ranging from 0.25 to 2 μg/mL, and they were far more potent than the first-line anti-TB agents isoniazid and rifampicin (MIC: ≥64 μg/mL) against two MDR-MTB strains. Two of them **8h** and **8l** with MIC of 0.25-0.5 μg/mL were no inferior to the fluoroquinolone references gatifloxacin (MIC: 0.25-1 μg/mL) and moxifloxacin (MIC: 0.25-0.5 μg/mL) against the tested three strains, demonstrating their potential to fight against DR-TB even MDR-TB.

The two hybrids **8h** and **8l** with promising activity against drug-susceptible, rifampicin-resistant and MDR MTB strains, together with low cytotoxicity towards CHO cells were selected for further investigation on their *in vivo* pharmacokinetics in mice (100 mg/kg, subcutaneous injection/s.c. administration), and the results were presented in **Table 5**.

Table 5 The *in vivo* pharmacokinetic values of hybrids **8h** and **8l** and moxifloxacin in mice

Compd.	Pharmacokinetics (s.c.)			
	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (min)	$AUC_{0\text{-}inf}$ (ng•h/mL)
8h	3,247	3.1	39	6,435
8l	1,398	4.3	52	3,269
moxifloxacin	10,783	2.6	27	19,243

C_{max}: the peak concentration;

$t_{1/2}$: half-life;

T_{max}: the time to reach peak concentration;

AUC: the area under the curve.

From **Table 5**, the *in vivo* pharmacokinetic profiles of the selected hybrids **8h** and **8l** were worse than the parent moxifloxacin in terms of the peak concentration (C_{max}: 3,247 and 1,398 ng/mL *vs* 10,783 ng/mL), the time to reach peak concentration (T_{max}: 39 and 52 min *vs* 27 min) and the area under the curve ($AUC_{0\text{-}inf}$: 6,435 and 3,269 ng•h/mL *vs* 19,243 ng•h/mL), and this may be attributed to that the existence of 1,2,3-triazole and amide functional groups led to poor solubility and bioavailability. However, the half-life ($t_{1/2}$: 3.1 and 4.3 h *vs* 2.6 h) was longer than moxifloxacin.

3. Conclusions

In summary, fourteen moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids were designed, synthesized and assessed for their *in vitro* anti-mycobacterial activity against drug-susceptible (MTB $H_{37}Rv$), RR and MDR MTB strains. Cytotoxicity towards VERO cells as well as inhibitory activity against MTB DNA gyrase of the hybrids were also investigated in this study. Among them, the most active hybrids **8h** and **8l** (MIC: 0.12-0.5 μg/mL, CC_{50}: 128 and 16 μg/mL) showed excellent activity which was no inferior to the parent moxifloxacin against all tested drug-susceptible and drug-resistant MTB strains, and acceptable cytotoxicity towards VERO cells, suggesting these two hybrids could act as a platform for further investigation.

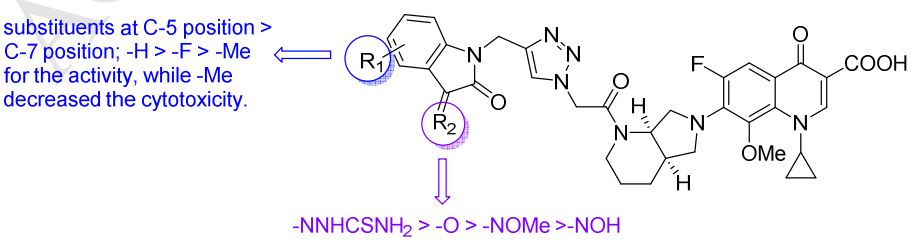


Figure 2 The SAR and structure-cytotoxicity relationship of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids

The SAR and structure-cytotoxicity relationship (**Figure 2**) revealed that the substituents on the C-3, C-5 and C-7 positions of isatin motif influenced the activity and cytotoxicity remarkably, and the enriched SAR and structure-cytotoxicity relationship may help global efforts for identification of new chemical entities as potent anti-TB agents.

4. Experimental section

4.1 Chemistry

All chemicals for this study were chemically pure or analytical grade. TLC were performed on silica plates (Merk, ART5554 60F254). ^{1}H NMR spectra were determined on a Varian Mercury-400 instrument in $CDCl_3$ or DMSO-d_6. HRMS-ESI were obtained on an MDSSCIEX Q-Tap mass spectrometer.

4.2 Synthesis

2-Bromoacetic acid **1** (20 mmol) and sodium azide (30 mmol) in H_2O (100 mL) was stirred at room temperature for 48 h, and then aq. HCl (12 mol/L, 10 mL) was added dropwise. After stirring for additional 30 min, the mixture was extracted with methyl tert-butyl ether (100 mL×3). The combined organic layers were washed with H_2O (100 mL), brine (100 mL×2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give 2-azidoacetic acid **2** (yield: 94%) as yellow oil.

To a solution of 2-azidoacetic acid **2** (18 mmol) and oxalyl chloride (50 mL) in DCM (200 mL), 5 drops of DMF was added. The mixture was stirred at room temperature overnight, and then evaporated under reduced pressure to give crude 2-azidoacetyl chloride **3** as brown oil which was used directly in the next step.

To a solution of moxifloxacin (10 mmol) in a mixture of pyridine (50 mL) and DCM (200 mL), a solution of 2-azidoacetyl chloride **3** (18 mmol) in DCM (50 mL) was added dropwise. The mixture was stirred at room temperature overnight, and then evaporated under reduced pressure. The residue was purified by silica gel chromatography eluted with DCM:MeOH=10:1 to give the key intermediate 2-azidoacetyl moxifloxacin **4** (yield: 44%) as yellow solid.

The suspension of isatin/5-methylisatin/5-fluoroisatin/7-fluoroisatin **5a-d** (10 mmol), propargyl bromide (20 mmol) and K_2CO_3 (30 mmol) in DMF (50 mL) was stirred at room temperature overnight, and then the mixture was poured into ice-water (200 g). The solid was filtered, washed with H_2O (100 mL) and petroleum ether (20 mL) to give crude *N*-propargyl isatin intermediates **6a-d** as brown solid which was used directly in the next step.

The mixture of *N*-propargyl isatins **6a-d** (5 mmol), the requested amine hydrochlorides (6 mmol) and $NaHCO_3$ (10 mmol) in a mixture of THF (30 mmol) and H_2O (30 mmol) was stirred at 50 ^oC for 12 h, and then cooled to room temperature. The mixture was extracted with ethyl acetate (50 mL×2), and the combined organic layers were washed with brine (50 mL×3), and then concentrated under reduced pressure to yield isatin intermediates **7a-j** as yellow solid which was used directly in the next step.

Finally, cyclization of *N*-propargyl isatin precursors **6a-d** or **7a-j** (1.1 mmol) with 2-azidoacetyl moxifloxacin **4** (1 mmol) by "click chemistry" in presence of copper acetate ($Cu(OAc)_2$, 0.1 mmol) in DMF (10 mL) at 40 ^oC for 12 h. The mixture was filtered, and the filtrated was concentrated under reduced pressure. The residue was purified by Prep-HPLC to generate the desired moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n** (yield: 12-46%).

4.2.1

1-cyclopropyl-7-((4aR,7aR)-1-(2-(4-((2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6*H*-pyrrolo[3,4-b]pyridin-6-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8a**)

Yellow solid, yield: 41%. ^1H NMR (400 MHz, DMSO-d_6) δ 0.85-1.52 (m, 7H), 1.77-1.80 (m, 2H), 2.24-2.27 (m, 2H), 3.27-3.31 (m, 1H), 3.60 (d, 3H), 3.80-4.42 (m, 4H), 4.57 (s, 1H), 4.96 (s, 2H, -CH_2- linker), 5.41-5.67 (m, 2H, -CH_2- linker), 7.14 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.18 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.57 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.62-7.66 (m, 2H, Ar-H), 8.09 (s, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 15.14 (brs, 1H, COOH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.56, 181.55, 176.46, 175.27, 166.35, 158.82, 158.51, 158.28, 150.78, 150.66, 141.54, 140.54, 138.60, 134.95, 128.15, 125.85, 124.94, 123.88, 118.01, 111.72, 106.81, 81.90, 61.94, 61.51, 51.72, 50.74,

41.14, 24.18, 24.06, 23.92, 10.40, 8.52. HRMS-ESI: m/z Calcd. for $C_{34}H_{33}FN_7O_7$ $[M+H]^+$: 670.2420; Found: 670.2411.

4.2.2

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((5-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8b**)

Yellow solid, yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86-1.53 (m, 6H), 1.77-1.80 (m, 3H), 2.32-2.44 (m, 1H), 2.74-2.78 (m, 1H), 3.60 (d, 3H), 3.80-4.04 (m, 4H), 4.14-4.16 (m, 1H), 4.86-4.88 (m, 1H), 5.00 (s, 2H, -CH_2- linker), 5.41-5.71 (m, 2H, -CH_2- linker), 7.20 (d, 1H, *J* = 4.0 Hz, Ar-H), 7.48-7.53 (m, 2H, Ar-H), 7.64-7.68 (m, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 15.15 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{32}F_2N_7O_7$ $[M+H]^+$: 688.2326; Found: 688.2319.

4.2.3

1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-(2-(4-((5-methyl-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8c**)

Yellow solid, yield: 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-1.44 (m, 6H), 1.91-1.95 (m, 3H), 2.19 (s, 3H, -Me), 3.30-3.37 (m, 2H), 3.53-3.63 (m, 4H), 3.82-4.06 (m, 4H), 4.58-4.60 (m, 1H), 5.04 (s, 2H, -CH_2- linker), 5.16-5.35 (m, 2H, -CH_2- linker), 7.11-7.22 (m, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.60 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.82 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.80 (s, 1H, Ar-H), 14.96 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{35}H_{35}FN_7O_7$ $[M+H]^+$: 684.2577; Found: 684.2568.

4.2.4

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((7-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8d**)

Yellow solid, yield: 24%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-1.60 (m, 8H), 2.42-2.48 (m, 1H), 3.30-3.49 (m, 3H), 3.59 (d, 3H), 3.82-4.02 (m, 4H), 4.52-4.60 (m, 1H), 5.13-5.38 (m, 4H, 2×-CH_2- linker), 7.07-7.09 (m, 1H, Ar-H), 7.32-7.34 (m, 1H, Ar-H), 7.40 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.72-7.86 (m, 2H, Ar-H), 8.09 (s, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 15.00 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{32}F_2N_7O_7$ $[M+H]^+$: 688.2326; Found: 688.2319.

4.2.5

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((3-(hydroxyimino)-2-oxoindolin-1-yl) methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-met hoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8e**)

Yellow solid, yield: 28%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87-1.53 (m, 7H), 1.76-1.80 (m, 2H), 2.25-2.28 (m, 2H), 3.29-3.33 (m, 1H), 3.62 (d, 3H), 3.82-4.14 (m, 4H), 4.61 (s, 1H), 4.98 (s, 2H, -CH_2- linker), 5.42-5.61 (m, 2H, -CH_2- linker), 7.12 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.16 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.54 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.64-7.68 (m, 2H, Ar-H), 8.12 (s, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 12.42 (brs, 1H, NOH), 15.12 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{34}FN_8O_7$ $[M+H]^+$: 685.2529; Found: 685.2524.

4.2.6

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((5-fluoro-3-(hydroxyimino)-2-oxoindol in-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8f**)

Yellow solid, yield: 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88-1.52 (m, 6H), 1.78-1.80 (m, 3H), 2.35-2.45 (m, 1H), 2.76-2.79 (m, 1H), 3.61 (d, 3H), 3.80-4.08 (m, 4H), 4.14-4.16 (m, 1H), 4.85-4.88 (m, 1H), 5.01 (s, 2H, -CH_2- linker), 5.44-5.65 (m, 2H, -CH_2- linker), 7.18 (d, 1H, *J* = 4.0 Hz, Ar-H), 7.39-7.46 (m, 2H, Ar-H), 7.62-7.65 (m, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 8.65 (s, 1H, Ar-H), 12.38 (brs, 1H, NOH), 15.18 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{33}F_2N_8O_7$ $[M+H]^+$: 703.2435; Found: 703.2427.

4.2.7

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((-7-fluoro-3-(hydroxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8g**)

Yellow solid, yield: 19%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87-1.79 (m, 8H), 2.25-2.42 (m, 1H), 3.18-4.12 (m, 9H), 5.06-5.09 (m, 3H), 5.38-5.61 (m, 2H, -CH_2- linker), 7.09-7.11 (m, 1H, Ar-H), 7.29-7.34 (m, 1H, Ar-H), 7.62-7.66 (m, 1H, Ar-H), 7.88 (d, 1H, *J* = 4.0 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 15.14 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{33}F_2N_8O_7$ $[M+H]^+$: 703.2435; Found: 703.2427.

4.2.8

1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-(2-(4-((3-(methoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8h**)

Yellow solid, yield: 35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81-1.42 (m, 6H), 2.25-2.39 (m, 3H), 3.29-3.50 (m, 3H), 3.60 (d, 3H), 3.79-4.09 (m, 3H), 4.28 (d, 3H, NOMe), 4.53-4.56 (m, 1H), 5.04-5.37 (m, 4H, 2×-CH_2- linker), 7.05 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.15-7.19 (m, 1H, Ar-H), 7.35-7.39 (m, 1H, Ar-H), 7.53-7.78 (m, 2H, Ar-H), 7.62-7.66 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.81 (s, 1H, Ar-H), 15.02 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{35}H_{36}FN_8O_7$ $[M+H]^+$: 699.2686; Found: 699.2678.

4.2.9

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8i**)

Yellow solid, yield: 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-1.52 (m, 8H), 2.32-2.39 (m, 1H), 3.30-3.36 (m, 2H), 3.51-3.62 (m, 4H), 3.82-4.06 (m, 4H), 4.31 (d, 3H, NOMe), 4.54-4.58 (m, 1H), 5.07 (s, 2H, -CH_2- linker), 5.27-5.38 (m, 2H, -CH_2- linker), 7.08-7.18 (m, 2H, Ar-H), 7.66 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.72-7.79 (m, 2H,

Ar-H), 8.78 (s, 1H, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.72, 173.46, 167.17, 164.69, 163.14, 161.47, 149.83, 142.52, 139.07, 134.35, 133.05, 124.96, 119.04, 116.15, 115.45, 115.19, 110.57, 108.14, 107.90, 107.59, 105.63, 65.10, 61.24, 56.31, 51.49, 51.07, 49.27, 48.20, 41.12, 40.50, 35.46, 24.93, 18.73, 10.51, 8.59. HRMS-ESI: m/z Calcd. for $C_{35}H_{35}F_2N_8O_7$ $[M+H]^+$: 717.2591; Found: 717.2582.

4.2.10

1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-(2-(4-((3-(methoxyimino)-5-methyl-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8j**)

Yellow solid, yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-1.60 (m, 8H), 2.32 (s, 4H), 3.30-3.34 (m, 2H), 3.53-3.89 (m, 5H), 4.00-4.09 (m, 2H), 4.30 (d, 3H, NOMe), 4.54-4.58 (m, 1H), 5.07 (s, 2H, -CH_2- linker), 5.15-5.32 (m, 3H), 7.06 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.20 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.77-7.83 (m, 3H), 8.80 (s, 1H, Ar-H), 14.98 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{36}H_{38}FN_8O_7$ $[M+H]^+$: 713.2842; Found: 713.2836.

4.2.11

1-cyclopropyl-6-fluoro-7-((4aR,7aR)-1-(2-(4-((7-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8k**)

Yellow solid, yield: 27%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-1.92 (m, 8H), 2.30-2.32 (m, 1H), 3.29-3.32 (m, 2H), 3.50-3.61 (m, 4H), 3.83-4.00 (m, 4H), 4.30 (d, 3H, NOMe), 4.52-4.58 (m, 1H), 5.14-5.34 (m, 5H), 6.98-7.02 (m, 1H, Ar-H), 7.11-7.16 (m, 1H, Ar-H), 7.75-7.78 (m, 3H, Ar-H), 8.77 (s, 1H, Ar-H), 14.99 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{35}H_{35}F_2N_8O_7$ $[M+H]^+$: 717.2591; Found: 717.2587.

4.2.12

7-((4aR,7aR)-1-(2-(4-((3-(2-carbamothioylhydrazono)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8l**)

Yellow solid, yield: 19%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-1.93 (m, 9H), 3.30-3.35 (m, 2H), 3.52-3.61 (m, 4H), 3.82-4.12 (m, 4H), 5.08 (s, 2H, -CH_2- linker), 5.26-5.39 (m, 2H, -CH_2- linker), 6.71 (s, 1H, NH), 7.09-7.12 (m, 1H, Ar-H), 7.25-7.27 (m, 1H, Ar-H), 7.39-7.41 (m, 1H, Ar-H), 7.52-7.55 (m, 2H, Ar-H and NH), 7.79-7.86 (m, 2H, Ar-H), 8.79 (s, 1H, Ar-H), 12.78 (s, 1H, NNHCS), 14.98 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{35}H_{36}FN_{10}O_6S$ $[M+H]^+$: 743.2513; Found: 743.2519.

4.2.13

7-((4aR,7aR)-1-(2-(4-((3-(2-carbamothioylhydrazono)-5-fluoro-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8m**)

Yellow solid, yield: 19%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-1.64 (m, 8H), 2.23-2.24 (m, 1H), 3.30-3.35 (m, 2H), 3.52-3.62 (m, 4H), 3.74-4.05 (m, 4H), 4.58-4.59 (m, 1H), 5.07 (s, 2H, -CH_2- linker), 5.26-5.37 (m, 2H, -CH_2- linker), 6.71 (s, 1H, NH), 7.09-7.11 (m, 1H, Ar-H), 7.22-7.27 (m, 2H, Ar-H), 7.54-7.56 (m, 1H, Ar-H), 7.79-7.87 (m, 2H, Ar-H and NH), 8.80 (s, 1H, Ar-H), 12.78 (s, 1H, NNHCS). HRMS-ESI: m/z Calcd. for $C_{35}H_{35}F_2N_{10}O_6S$ $[M+H]^+$: 761.2424; Found: 761.2421.

4.2.14

7-((4aR,7aR)-1-(2-(4-((3-(2-carbamothioylhydrazono)-7-fluoro-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8n**)

Yellow solid, yield: 11%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83-1.96 (m, 8H), 2.33-2.34 (m, 1H), 3.30-3.35 (m, 2H), 3.52-3.62 (m, 4H), 3.82-4.10 (m, 4H), 4.70-4.72 (m, 1H), 5.24-5.38 (m, 4H, 2×-CH_2- linker), 6.68 (s, 1H, NH), 7.02-7.14 (m, 2H, Ar-H), 7.27-7.29 (m, 1H, Ar-H), 7.58 (s, 1H, NH), 7.80-7.89 (m, 2H, Ar-H), 8.80 (s, 1H, Ar-H), 12.76 (s, 1H, NNHCS), 14.97 (brs, 1H, COOH). HRMS-ESI: m/z Calcd. for $C_{35}H_{35}F_2N_{10}O_6S$ $[M+H]^+$: 761.2424; Found: 761.2422.

4.3. MIC determination

The *in vitro* activities of moxifloxacin-acetyl-1,2,3-1*H*-triazole-methylene-isatin hybrids **8a-n**, along with the references gatifloxacin, moxifloxacin, isoniazid and rifampicin against MTB $H_{37}Rv$, RR-MTB and MDR-MTB were evaluated *via* rapid direct susceptibility test technique [25,26]. The wells of a sterile 48-well plate were filled with 100 mL two-fold diluted tested compounds and 100 mL MTB $H_{37}Rv$ (or MDR-TB) suspension containing 4×10^{-3} mg cells. The plates were incubated at 37 °C in a wet box. 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 re-determined 7 days later.

4.4. Cytotoxicity

The hybrids **8a-n** were dissolved in DMSO and examined for toxicity (CC_{50}) in a mammalian CHO cell line at concentrations from 128 to 4 μg/mL [33]. The VERO cells were maintained in culture medium (Minimum Essential Medium with Earle's salt, supplemented with 10% fetal bovine serum) at 37 °C under 5% CO_2. Cells were seeded in 96-well plates at the plating density of 1×104 cells per well and allowed to recover for 24 h. Culture medium was replaced by assay medium containing the compound to be tested or drug-free. After 72 h of exposure, cells were harvested and cell viability was assessed by MTT assay. The CC_{50} values were calculated by Bliss analyses.

4.5 Pharmacokinetic Profiles determination

SPF female ICR mice (20-25 g) were used in the pharmacokinetic study [34,35]. Total area under the concentration time curve (AUC), the elimination half-time ($t_{1/2}$), the peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of samples were etermined directly from the experimental data using WinNonlin V6.2.1.

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1. Hybrids **8h** and **8l** (MIC: 0.12-0.5 μg/mL) were no inferior to the parent moxifloxacin against all tested strains
2. The structure-activity relationship and structure-cytotoxicity relationship were enriched