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## Graphical Abstract

# Azide-alkyne cycloaddition towards 1H-1,2,3-triazole-tethered gatifloxacin and isatin conjugates: design, synthesis and *in vitro* anti-mycobacterial evaluation

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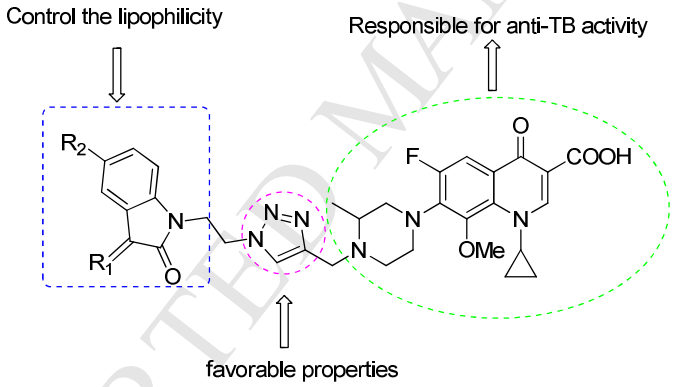


A new class of 1H-1,2,3-triazole-tethered gatifloxacin isatin conjugates (MIC: 0.10-8 $\mu$g/mL) with greater lipophilicity compared with gatifloxacin exhibited excellent inhibitory activity against MTB $H_{37}Rv$ and MDR-TB.

# Azide-alkyne cycloaddition towards 1H-1,2,3-triazole-tethered gatifloxacin and isatin conjugates: design, synthesis and *in vitro* anti-mycobacterial evaluation

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**Abstract:** Twelve novel 1H-1,2,3-triazole-tethered gatifloxacin (**GTFX**) isatin conjugates **5a-l** with greater lipophilicity compared with **GTFX** were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against *M. tuberculosis* (MTB) $H_{37}$Rv and MDR-TB as well as cytotoxicity. The preliminary results showed that all the targets (MIC: 0.10-8 $\mu$g/mL) exhibited excellent inhibitory activity against MTB $H_{37}$Rv and MDR-TB, but eight of them ($CC_{50}$: 7.8-62.5 $\mu$g/mL) were much more toxic than the parent **GTFX** ($CC_{50}$: 125 $\mu$g/mL). Among them, **5g** (MIC: 0.10 $\mu$g/mL) was 4-8 times more potent *in vitro* than the references **GTFX** (MIC: 0.78 $\mu$g/mL) and **RIF** (MIC: 0.39 $\mu$g/mL) against MTB $H_{37}$Rv, but less active than **INH** (MIC: 0.05 $\mu$g/mL). The most potent **5g** and **5h** (MIC: 0.25 $\mu$g/mL) were 4->512 times more active than the three references (MIC: 1.0->128 $\mu$g/mL) against MDR-TB. Unfortunately, both of the two hybrids ($CC_{50}$: 7.8 $\mu$g/mL) were much more cytotoxic than the other derivatives, need to be further optimized.

**Keywords:** Gatifloxacin; Isatin; Triazole; Conjugates; Anti-tuberculosis activity; Anti-mycobacterial activity; Structure-activity relationship

## 1. Introduction

Tuberculosis (TB) remains one of the most widespread and leading deadliest diseases result in 1.4 million deaths and 10.4 million clinical cases, and is in continual increase,

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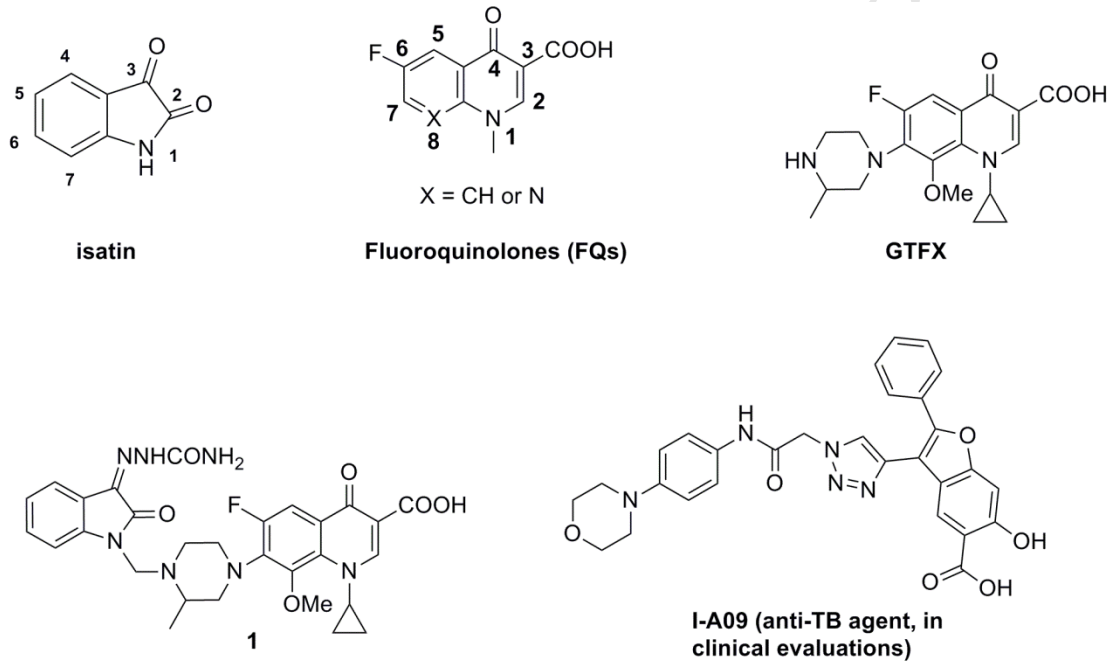
especially in developing countries according to the World Health Organization (WHO) 2016 report [1]. The emergence of drug-resistant TB (DR-TB), multidrug-resistant TB (MDR-TB, only 40-80% cure rate, results in 0.48 million deaths in 2015), extensively drug-resistant TB (XDR-TB) and the recently cases of totally drug resistant (TDR) as well as co-infection with HIV (a major cause of death among people living with HIV/AIDS, leads to 0.4 million deaths in 2015) alarming the serious problem in TB control and demanding the need for new drugs more potent than earlier with safe ADME profile. However, no new chemical entity has been emerged in last 50 years after the discovery of rifampicin (**RIF**), creating an urgent need to develop new drugs and strategies for efficient treatment. Several strategies are being pursued in order to identify new leads, although only a few leads are being optimized to generate drug candidates. Most of the efforts have been directed towards making new analogues or modifying old drugs or existing compounds with an established activity for shortening and improving TB treatment, since it's the most promising strategy to develop new anti-TB agents in the short time.

The isatin (1H-indole-2,3-dione, **Fig. 1**) moiety is a privileged scaffold for chemical modification and is responsible for a broad spectrum of biological properties such as anti-TB, anti-bacterial, anti-fungal, anti-virus, anti-tumor, anti-HIV, and well tolerance in humans [2-4]. The traditional antibiotics fluoroquinolones (FQs, **Fig. 1**) exhibited excellent anti-bacterial activity, and some of them are currently recommended as the second-line agents by the WHO for the treatment of TB [5]. Moreover, the lipophilicity of the FQs plays an important role in the penetration of these compounds into bacterial cells, which suggests that increasing the lipophilic character at C-7 position could also increase the anti-TB activity [6]. Therefore, several series of FQs-isatin derivatives with remarkable improvement in lipophilicity have been synthesized and their anti-TB activity has been explored [7-12]. Among these derivatives, gatifloxacin (**GTFX**, **Fig. 1**, has been withdrawn from the therapy because of its side effects (dysglycaemia), but still worth to be investigated attribute

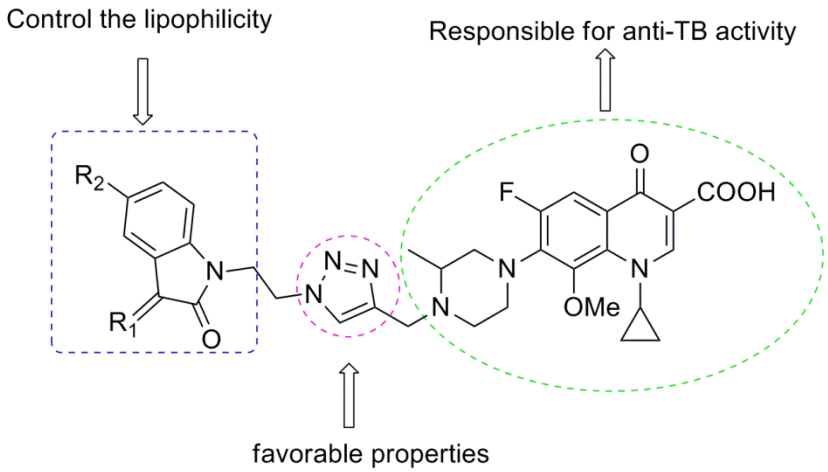
to its excellent *in vitro* and *in vivo* anti-TB activities) methylene isatin hybrid **1** exhibited higher *in vitro* (16 and 64 folds against MTB $H_{37}Rv$ and MDR-TB) and *in vivo* potency than the parent **GTFX** [6]. Further investigation indicated that the anti-TB activity of these derivatives was greatly influenced by the linkers between FQs and isatin [7-12]. Chemical structures of isatin, FQs, **GTFX**, GTFX-isatin derivative **1** and **I-A09** are shown in **Fig. 1**.




< **Fig 1.** Chemical structures of isatin, FQs, GTFX, GTFX-isatin derivative 1 and I-A09>

Azoles, are one of the most important classes of nitrogen containing heterocycles, exhibited various biological activities. In particular, 1,2,3-triazole and its derivatives, which are easily to be obtained by 'Click chemistry', have attracted continuous interest. Indeed this particular application of 'Click chemistry' has become one of the mainstays of currently favored approaches for the introduction of structural diversity in medicinal chemistry. Moreover, some drugs currently in use are based on 1,2,3-triazole moiety such as anti-HIV agent **TSAO**, antibiotic **Cefatrizine**, anti-bacterial agent **Tazobactum** as well as anti-cancer agent **CAI** [13]. In addition, **I-A09** is in clinical evaluations currently, and may be used to treat TB infection in the

near future [14]. Obviously, the favorable properties of 1,2,3-triazole ring like moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions are responsible for their enhanced biological activities [15].



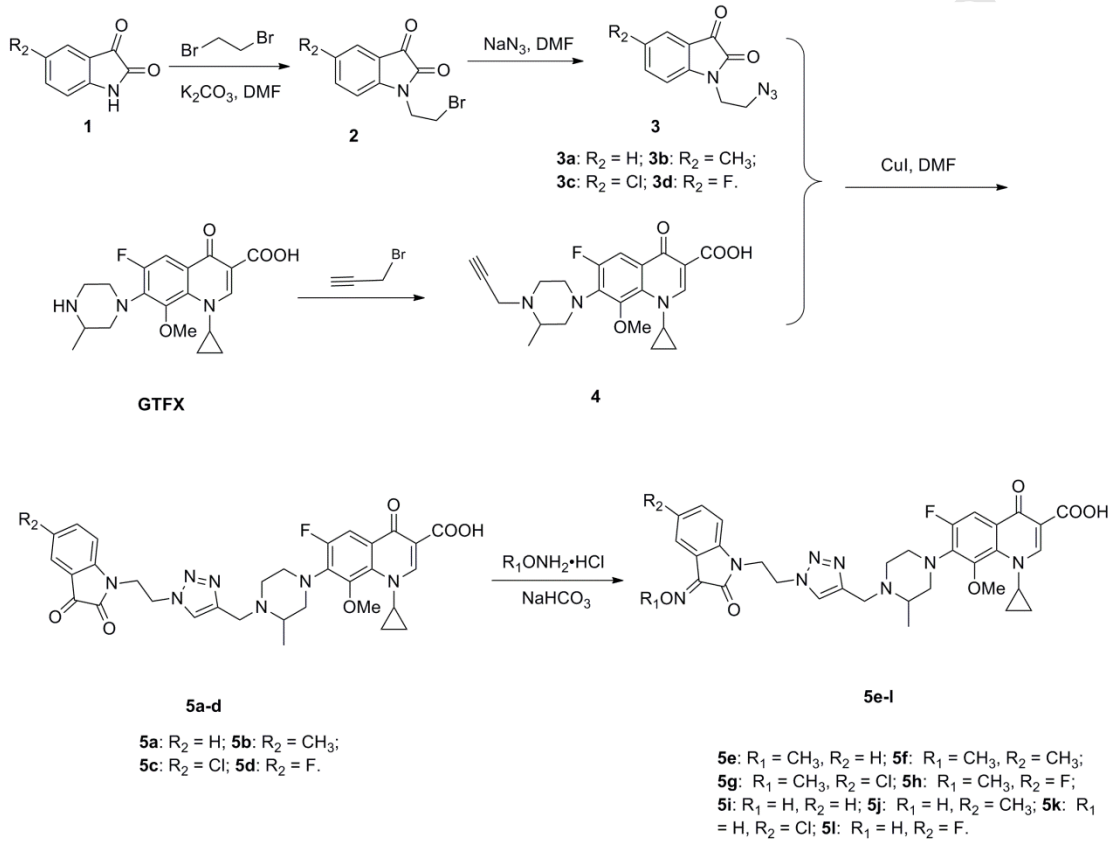

< **Fig 2.** Illustration of the design strategy for triazole-tethered GTFX-isatin derivatives>

Inspired by the above research results, we designed and synthesized a series of triazole-tethered GTFX-isatin derivatives in this study. Illustration of the design strategy for triazole-tethered GTFX-isatin derivatives is depicted in **Fig 2**. These derivatives were initially evaluated for their *in vitro* anti-mycobacterial activity against MTB $H_{37}$Rv and MDR-TB strains, with Isoniazid (**INH**), rifampicin (**RIF**) and **GTFX** as references.

## 2. Results and discussion

Detailed synthetic pathways to triazole-tethered GTFX-isatin derivatives **5a-l** are depicted in Scheme 1. C-5 substituted isatins and **GTFX** were alkylated with 1,2-dibromoethane and propargyl bromide, respectively, in the presence of anhydrous potassium carbonate to give the corresponding *N*-(2-bromoethyl)isatins **2a-d** (yield: 51-67%) and propargyl GTFX **4** (yield: 39%) *via* literature methods [10, 16, 17]. The subsequent treatment of C-5 substituted *N*-(2-bromoethyl)isatins **2a-d** with sodium azide at 60 °C resulted in the formation of the desired azido precursors **3a-d** [18]. The

precursors **3a-d** and **4** were utilized for the synthesis of desired 1,2,3-triazole-tethered conjugates *via* Cu-promoted azide-alkyne cycloaddition reaction in the presence of CuI in DMF gave targets **5a-d** (yield: 28-39%) [17]. Subsequent condensations of targets **5a-d** with requisite substituted amine hydrochlorides in the presence of sodium bicarbonate formed other derivatives **5e-l** (43-67%) [10].




< **Scheme 1** Synthesis of 1H-1,2,3-triazole-tethered GTFX isatin conjugates **5a-l**>

All hybrids **5a-l** (Log *P*: 2.32-3.53) with greater lipophilicity compared with **GTFX** (Log *P*: 1.51), and this profile may be rendering them more capable of penetrating various biomembrane, consequently improving their permeation properties toward mycobacterial cell membrane. The conjugates **5a-l** were initially evaluated for their *in vitro* anti-mycobacterial activity against MTB $H_{37}Rv$ and MDR-TB by rapid direct susceptibility test technique [10]. The MDR-TB strain was resistant to **INH**, **RIF** and ethambutol (**EMB**). The minimum inhibitory concentration (MIC) is defined as the

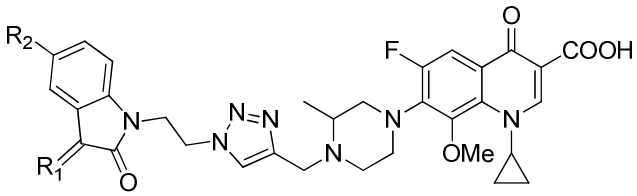
minimum concentration of compound required to give 90% inhibition of bacterial growth and MICs of the compounds are reported in Table 1. All triazole-tethered GTFX-isatin conjugates exhibited considerable *in vitro* activity against MTB $H_{37}Rv$ and MDR-TB with MIC ranging from 0.10 to 8 $\mu$g/mL. Against MTB $H_{37}Rv$, three of them (**5c**, **5g** and **5h**) were more active than that of the parent **GTFX** (MIC: 0.78 $\mu$g/mL), particularly, **5g** (MIC: 0.10 $\mu$g/mL) was 4-8 times more potent *in vitro* than the references **GTFX** (MIC: 0.78 $\mu$g/mL) and **RIF** (MIC: 0.39 $\mu$g/mL) against MTB $H_{37}Rv$, but less active than **INH** (MIC: 0.05 $\mu$g/mL). Against MDR-TB, five conjugates (**5c, 5d, 5f, 5g** and **5h**) with MIC of 0.25-0.5 $\mu$g/mL were more active than the references **GTFX** (MIC: 1.0 $\mu$g/mL), **INH** (MIC: 32 $\mu$g/mL) and **RIF** (MIC: >128 $\mu$g/mL), and the most potent derivatives **5g** and **5h** (MIC: 0.25 $\mu$g/mL) were 4->512 times more active than the three references.

The lipophilicity of the synthesized targets (Clog *P*: 2.32-3.46) was much greater than the parent **GTFX** (Clog *P*: 1.51), to some extent, increasing the lipophilicity of targets could increase the anti-TB activity as evidenced by the fact that the most lipophilic targets (**5c**, **5g** and **5k**) in each series also exhibited the highest activity. However, in general, the anti-TB activity did not boost up with the incensement of the lipophilicity, indicating simply increasing lipophilicity of the tested compounds does not improve the anti-mycobacterial activity accordingly which could also be associated with other steric factors [10,11].

The resistance index (RI: $MIC_{MDR\text{-}TB}$ : $MIC_{MTB\ H_{37}Rv}$) of the targets was in a range of 0.32 to 2.56, and eight of them showed RI around 1. In particular, the RI of **5b**, **5d** and **5f** was less than 1, indicated this kind of hybrids could reduce the cross-resistant to some extent.

**Table 1**

Structures, lipophilicity, anti-mycobacterial activity and cytotoxicity of compounds **5a-l**



| Compd. | $R_1$ | $R_2$ | Clog *P* [a] | MIC ($\mu$g/mL) | | $CC_{50}$ [c]($\mu$g/mL) |
|---|---|---|---|---|---|---|
| | | | | MTB $H_{37}Rv$ | [b]MDR-TB | |
| **5a** | O | H | 2.32 | 1.56±0.52 | 4.0±1.3 | 125±41.7 |
| **5b** | O | Me | 2.81 | 1.56±0.52 | 1.0±0.33 | 250±83.3 |
| **5c** | O | Cl | 2.88 | 0.39±0.13 | 0.5±0.17 | 62.5±20.8 |
| **5d** | O | F | 2.48 | 0.78±0.26 | 0.5±0.17 | 125±41.7 |
| **5e** | $NOCH_3$ | H | 2.97 | 0.78±0.26 | 1.0±0.33 | 62.5±20.8 |
| **5f** | $NOCH_3$ | Me | 3.46 | 1.56±0.52 | 0.5±0.17 | 31.2±10.4 |
| **5g** | $NOCH_3$ | Cl | 3.53 | 0.10±0.03 | 0.25±0.08 | 7.8±2.6 |
| **5h** | $NOCH_3$ | F | 3.13 | 0.20±0.06 | 0.25±0.08 | 7.8±2.6 |
| **5i** | NOH | H | 2.71 | 3.12±1.04 | 8.0±2.7 | 250±83.3 |
| **5j** | NOH | Me | 3.20 | 6.25±2.08 | 8.0±2.7 | 62.5±20.8 |
| **5k** | NOH | Cl | 3.27 | 0.78±0.26 | 2.0±0.67 | 31.2±10.4 |
| **5l** | NOH | F | 2.85 | 1.56±0.52 | 1.0±0.33 | 7.8±2.6 |
| **GTFX** | | | 1.51 | 0.78±0.26 | 1.0±0.33 | 125±41.7 |

| | | | | |
|---|---|---|---|---|
| **INH** | -0.67 | 0.05±0.16 | >128 | 125±41.7 |
| **RIF** | 3.71 | 0.39±0.13 | 32±10.7 | 500±167 |

[a]The Clog *P* is calculated with ChemOffice 2004 software.

[b]MDR-TB: resistant to INH, RIF and EMB.

[c]$CC_{50}$: The 50% cytotoxic concentration in a mammalian VERO cell line.

Against MTB $H_{37}Rv$, the relatively order of substituents at C-5 position on isatin affecting activity was -Cl > -F > -H > -Me, while against MDR-TB, the order was -F > -Cl > -Me > -H. The relative contribution of imines/carbonyl of Schiff's base to activity against both strains is as follows: methyloxime > carbonyl > oxime. The SAR revealed that conjugates with electron-withdrawing groups (-Cl and -F) at C-5 position on isatin boost up the activity against both MTB $H_{37}Rv$ and MDR-TB strains, suggesting further modification should focus on introduction of various electron-withdrawing groups like $-NO_2$.

The conjugates **5a-l** were subsequently examined for toxicity ($CC_{50}$) in a mammalian VERO cell line [10]. 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. Eight of them ($CC_{50}$: 7.8-62.5 $\mu$g/mL) were much more toxic than the parent **GTFX** ($CC_{50}$: 125 $\mu$g/mL), and four hybrids ($CC_{50}$: 125-250 $\mu$g/mL) were equal to or less toxic than the parent. Unfortunately, the cytotoxicity of the most potency **5g** and **5h** ($CC_{50}$: 7.8$\mu$g/mL) is much more than the other derivatives. In general, the carbonyl hybrids were less toxic than methyloxime and oxime derivatives, indicating the introduction of imines increased the cytotoxicity. In addition, halogen atoms at C-5 position on isatin also increased the cytotoxicity.

In summary, a series of novel triazole-tethered GTFX isatin conjugates with greater lipophilicity compared with **GTFX** were synthesized and evaluated for their *in vitro* anti-mycobacterial activity against MTB $H_{37}Rv$ and MDR-TB. All the targets

exhibited excellent inhibitory activity against the tested MTB $H_{37}Rv$ and MDR-TB, and the most active derivatives were more potent than the parent **GTFX**, **INH** and **RIF** against both strains. The SAR of this kind of hybrids was enriched, and the results warrant further development of the anti-TB properties of 1,2,3-triazole-tethered **FQs** isatin conjugates.

## 3. Experimental section

### *3.1.Synthesis*

#### *3.1.1. General Procedure for the Preparation of* **5a-d**

*N*-(2-azidoethyl)isatins **3a-d** and propargyl GTFX **4** (yield: 39%) were prepared *via* literature methods [10, 16, 17]. To a mixture of *N*-(2-azidoethyl)isatins **3a-d** (1.0 mmol) and propargyl GTFX **4** (1.0 mmol) in DMF (50 mL), CuI (30 mg) was added under $N_2$ atmosphere. The mixture was allowed to react for 4 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography eluted with DCM to *v*(DCM):*v*(MeOH)=10:1.

*3.1.1.1. 1-cyclopropyl-7-(4-((1-(2-(2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl) methyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**5a**)

Yellow solid, yield: 36%. Mp: 167-169 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.02-1.22 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.37 (2H, m, piperazine-2H), 2.62-2.64 (1H, m, piperazine-1H), 2.94-3.26 (4H, m, piperazine-4H), 3.61-3.83 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.01-4.14 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.66 (2H, s, -$CH_2$ of linker), 6.91 (1H, d, Ar-H), 7.04 (1H, t, Ar-H), 7.49-7.57 (2H, m, Ar-H), 7.70 (1H, s, Ar-H), 8.13 (1H, s, triazole-H), 8.69 (1H, s, C2-H), 14.91 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.38, 16.10, 41.19, 47.33, 48.13, 50.98, 51.25, 54.32, 57.46, 63.43, 106.92, 107.12, 110.71, 117.74, 121.21, 123.65, 124.93, 125.17, 125.34, 134.56, 138.59, 139.36, 139.49, 143.27, 146.16, 150.76, 150.97, 158.54, 166.21, 176.67, 183.39. ESI-MS *m/z*: 630 $[M+H]^+$.

*3.1.1.2. 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-((1-(2-(5-methyl-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquin*

*oline-3-carboxylic acid* (**5b**)

Light yellow solid, yield: 28%. Mp: 154-155 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 1.03-1.13 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.20 (3H, s, -$CH_3$), 2.33-2.36 (2H, m, piperazine-2H), 2.62 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.23-3.31 (3H, m, piperazine-3H), 3.70 (3H, s, -$OCH_3$), 3.73 (2H, q, -$CH_2$ chain), 4.10-4.16 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.63-4.66 (2H, m, -$CH_2$ of linker), 6.76 (1H, d, Ar-H), 7.32 (1H, s, Ar-H), 7.36 (1H, d, Ar-H), 7.73 (1H, d, Ar-H), 8.10 (1H, s, triazole-H), 8.77 (1H, s, C2-H), 14.95 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 8.92, 15.58, 19.92, 40.79, 46.91, 47.67, 50.48, 50.50, 50.79, 53.83, 56.99, 62.97, 106.50, 110.01, 117.21, 120.63, 120.72, 124.66, 132.49, 134.13, 138.34, 138.97, 139.09, 142.81, 145.75, 148.17, 150.48, 154.17, 156.65, 158.10, 165.64, 176.27, 183.11. ESI-MS *m/z*: 664 $[M+H]^+$.

*3.1.1.3. 7-(4-((1-(2-(5-chloro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5c**)*

Light yellow solid, yield: 39%. Mp: 182-183°C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 1.02-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.35 (2H, m, piperazine-2H), 2.64 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.21-3.30 (3H, m, piperazine-3H), 3.64-3.83 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.15-4.17 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.65 (2H, s, -$CH_2$ of linker), 6.89 (1H, d, Ar-H), 7.53-7.58 (2H, m, Ar-H), 7.71-7.74 (1H, m, Ar-H), 8.15 (1H, s, triazole-H), 8.81 (1H, s, C2-H), 14.95 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.45, 16.07, 41.27, 47.41, 48.12, 48.52, 50.97, 51.31, 54.24, 57.44, 63.51, 107.00, 112.38, 113.27, 119.08, 121.21, 124.40, 125.30, 127.82, 134.60, 137.39, 139.46, 143.25, 146.26, 149.31, 150.96, 154.67, 157.15, 158.33, 166.14, 176.74, 182.29. ESI-MS *m/z*: 664 $[M+H]^+$.

*3.1.1.4. 1-cyclopropyl-6-fluoro-7-(4-((1-(2-(5-fluoro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5d**)*

Light yellow solid, yield: 33%. Mp: 171-174 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 0.92-1.08 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.37 (2H, m, piperazine-2H), 2.62 (1H, d, piperazine-1H), 2.90-2.93 (1H, m, piperazine-1H), 3.19-3.33 (3H, m, piperazine-3H), 3.64-3.83 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.01-4.14 (3H, m,

cyclopropyl-CH and -$CH_2$ of linker), 4.65 (2H, s, -$CH_2$ of linker), 6.90 (1H, s, Ar-H), 7.42-7.72 (3H, m, Ar-H), 8.13 (1H, s, triazole-H), 8.69 (1H, s, C2-H). ESI-MS *m/z*: 648 $[M+H]^+$.

*3.1.2. The general procedure for preparing targets* **5e-l**

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)=10:1 to give the title compounds **5e-l** (43-67%).

*3.1.2.1. 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-((1-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5e**)*

Light yellow solid, yield: 67%. Mp: 136-137 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.03-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.37 (2H, m, piperazine-2H), 2.63 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.21-3.31 (3H, m, piperazine-3H), 3.70 (3H, s, -$OCH_3$), 3.72 (2H, q, -$CH_2$ chain), 4.09-4.17 (6H, m, cyclopropyl-CH, -$CH_2$ of linker and $NOCH_3$), 4.66 (2H, s, -$CH_2$ of linker), 6.88 (1H, d, Ar-H), 6.99 (1H, t, Ar-H), 7.33 (1H, t, Ar-H), 7.73 (1H, d, Ar-H), 7.81 (1H, d, Ar-H), 8.02 (1H, s, triazole-H), 8.70 (1H, s, C2-H), 14.96 (1H, brs, COOH). ESI-MS *m/z*: 659 $[M+H]^+$.

*3.1.2.2. 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-((1-(2-(3-(methoxyimino)-5-methyl-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5f**)*

Light yellow solid, yield: 58%. Mp: 126-129 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.03-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.20-2.30 (5H, m, -$CH_3$ and piperazine-2H), 2.63 (1H, d, piperazine-1H), 2.98 (1H, t, piperazine-1H), 3.17-3.26 (3H, m, piperazine-3H), 3.59-3.81 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.12-4.20 (6H, m, cyclopropyl-CH, -$CH_2$ of linker and $NOCH_3$), 4.68 (2H, s, -$CH_2$ of linker), 6.70-6.86 (1H, m, Ar-H), 7.11-7.31 (1H, m, Ar-H), 7.69-7.77 (2H, m, Ar-H), 8.04 (1H, s, triazole-H), 8.71 (1H, s, C2-H), 14.91 (1H, brs, COOH). ESI-MS *m/z*: 644 $[M+H]^+$.

*3.1.2.3. 7-(4-((1-(2-(5-chloro-3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-tri*

*azol-4-yl)methyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**5g**)*

Light yellow solid, yield: 61%. Mp: 156-158 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 1.03-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.34 (2H, m, piperazine-2H), 2.61 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.22-3.31 (3H, m, piperazine-3H), 3.66-3.79 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.13-4.20 (6H, m, cyclopropyl-CH, -$CH_2$ of linker and $NOCH_3$), 4.65 (2H, d, -$CH_2$ of linker), 6.88 (1H, d, Ar-H), 7.40 (1H, d, Ar-H), 7.71-7.77 (2H, m, Ar-H), 8.04 (1H, s, triazole-H), 8.69 (1H, s, C2-H), 14.95 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.45, 16.05, 41.37, 47.36, 48.09, 50.99, 51.26, 54.21, 57.44, 63.51, 65.25, 107.00, 110.98, 116.34, 121.20, 125.01, 126.90, 126.97, 132.61, 134.61, 139.48, 139.57, 142.56, 142.64, 143.17, 146.20, 150.97, 154.65, 157.14, 162.31, 166.13, 176.78. ESI-MS *m/z*: 693 $[M+H]^+$.

*3.1.2.4. 1-cyclopropyl-6-fluoro-7-(4-((1-(2-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**5h**)*

Light yellow solid, yield: 63%. Mp: 139-141 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 1.02-1.13 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.33-2.35 (2H, m, piperazine-2H), 2.61 (1H, d, piperazine-1H), 2.94 (1H, t, piperazine-1H), 3.25-3.31 (3H, m, piperazine-3H), 3.70 (3H, s, -$OCH_3$), 3.72 (2H, q, -$CH_2$ chain), 4.12-4.19 (6H, m, cyclopropyl-CH, -$CH_2$ of linker and $NOCH_3$), 4.66 (2H, d, -$CH_2$ of linker), 6.90 (1H, d, Ar-H), 7.24 (1H, t, Ar-H), 7.58 (1H, d, Ar-H), 7.73 (1H, d, Ar-H), 8.03 (1H, s, triazole-H), 8.70 (1H, s, C2-H), 14.96 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.44, 16.07, 41.25, 47.31, 48.11, 50.96, 51.25, 54.28, 57.46, 63.47, 65.16, 107.01, 110.57, 114.59, 114.85, 115.67, 119.33, 119.56, 121.16, 124.97, 134.64, 139.46, 140.13, 143.13, 143.20, 146.17, 150.98, 154.64, 157.10, 159.46, 162.50, 166.14, 176.79. ESI-MS *m/z*: 677 $[M+H]^+$.

*3.1.2.5. 1-cyclopropyl-6-fluoro-7-(4-((1-(2-(3-(hydroxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5i**)*

Light yellow solid, yield: 54%. Mp: 188-190 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 1.03-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.30-2.37 (2H, m, piperazine-2H), 2.60 (1H, d, piperazine-1H), 2.95 (1H, t, piperazine-1H), 3.20-3.21 (3H, m,

piperazine-3H), 3.69 (3H, s, -$OCH_3$), 3.72 (2H, q, -$CH_2$ chain), 4.16-4.17 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.67-4.68 (2H, m, -$CH_2$ of linker), 6.84 (1H, d, Ar-H), 6.97 (1H, t, Ar-H), 7.29 (1H, t, Ar-H), 7.73 (1H, d, Ar-H), 7.91 (1H, d, Ar-H), 8.04 (1H, s, triazole-H), 8.70 (1H, s, C2-H), 13.42 (1H, brs, NOH), 14.94 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.40, 9.46, 16.03, 41.28, 47.46, 48.16, 51.03, 51.19, 54.33, 57.42, 63.48, 107.00, 109.05, 115.58, 121.06, 121.16, 122.98, 124.90, 127.27, 132.25, 134.64, 139.60, 143.12, 143.27, 143.66, 146.18, 150.98, 154.67, 157.15, 163.49, 166.16, 176.79. ESI-MS *m/z*: 645 $[M+H]^+$.

*3.1.2.6. 1-cyclopropyl-6-fluoro-7-(4-((1-(2-(3-(hydroxyimino)-5-methyl-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**5j**)*

Light yellow solid, yield: 47%. Mp: 175-177 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.02-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.19 (3H, s, -$CH_3$), 2.28-2.32 (2H, m, piperazine-2H), 2.59 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.17-3.26 (3H, m, piperazine-3H), 3.69 (s, 3H, -$OCH_3$), 3.72 (2H, q, -$CH_2$ chain), 4.13-4.16 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.64-4.67 (2H, m, -$CH_2$ of linker), 6.69 (1H, d, Ar-H), 7.10 (1H, d, Ar-H), 7.70-7.76 (2H, m, Ar-H), 8.00 (1H, s, triazole-H), 8.69 (1H, s, C2-H), 13.70 (1H, brs, NOH), 14.74 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.42, 16.00, 20.89, 41.25, 47.54, 48.19, 51.01, 51.20, 54.34, 57.43, 63.48, 107.05, 108.73, 115.63, 121.21, 124.87, 127.78, 131.86, 132.35, 134.60, 139.47, 140.89, 143.29, 143.77, 146.22, 150.95, 154.66, 157.14, 163.55, 166.14, 176.76. ESI-MS *m/z*: 659 $[M+H]^+$.

*3.1.2.7. 7-(4-((1-(2-(5-chloro-3-(hydroxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**5k**)*

Light yellow solid, yield: 48%. Mp: 194-196 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.00-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.33 (2H, m, piperazine-2H), 2.60 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.27-3.31 (3H, m, piperazine-3H), 3.65-3.80 (5H, m, -$CH_2$ chain and -$OCH_3$), 4.16-4.19 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.65-4.67 (2H, m, -$CH_2$ of linker), 6.83 (1H, d, Ar-H), 7.37 (1H, d, Ar-H), 7.73 (1H, d, Ar-H), 7.87 (1H, t, Ar-H), 8.01 (1H, s, triazole-H), 8.70 (1H, s, C2-H), 13.77 (1H, brs, NOH), 14.98 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.45, 15.99, 41.25, 47.45, 48.13, 50.98, 51.23, 51.77,

54.23, 57.42, 63.53, 107.01, 110.63, 116.65, 121.21, 124.99, 126.50, 126.70, 131.63, 134.63, 139.61, 141.91, 142.91, 143.23, 146.19, 150.99, 154.68, 157.16, 163.15, 165.25, 166.15, 176.80. ESI-MS *m/z*: 679 $[M+H]^+$.

*3.1.2.8. 1-cyclopropyl-6-fluoro-7-(4-((1-(2-(5-fluoro-3-(hydroxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**5l**)*

Light yellow solid, yield: 43%. Mp: 191-192 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.02-1.14 (7H, m, 2×cyclopropyl-$CH_2$ and -$CH_3$), 2.31-2.35 (2H, m, piperazine-2H), 2.60 (1H, d, piperazine-1H), 2.96 (1H, t, piperazine-1H), 3.27-3.30 (3H, m, piperazine-3H), 3.69 (3H, s, -$OCH_3$), 3.72 (2H, q, -$CH_2$ chain), 4.16-4.19 (3H, m, cyclopropyl-CH and -$CH_2$ of linker), 4.65-4.68 (2H, m, -$CH_2$ of linker), 6.84-6.87 (1H, m, Ar-H), 7.18 (1H, t, Ar-H), 7.29 (1H, t, Ar-H), 7.65 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 8.01 (1H, s, triazole-H), 8.70 (1H, s, C2-H), 13.70 (1H, brs, NOH), 14.97 (1H, brs, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 9.39, 16.00, 41.25, 47.40, 48.14, 50.96, 51.21, 54.31, 57.40, 63.48, 107.00, 110.11, 114.09, 115.99, 116.08, 118.31, 118.55, 121.07, 124.96, 134.62, 139.46, 143.24, 143.36, 146.18, 150.96, 154.65, 157.08, 159.44, 163.34, 166.14, 176.77. ESI-MS *m/z*: 663 $[M+H]^+$.

## *3.2.* MIC determination

Conjugates **5a-l** along with **GTFX**, **RIF** and **INH** were evaluated *in vitro* activity against MTB $H_{37}$Rv and MDR-TB *via* rapid direct susceptibility test technique [9]. The compounds along with the references **GTFX**, **RIF** and **INH** were dissolved in dimethyl sulfoxide (DMSO) and two-fold diluted at concentrations from 0.0125 to 200 $\mu$g/mL (for MTB $H_{37}$Rv) or 0.062 to 128 $\mu$g/mL (for MDR-MTB). 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-MTB suspension containing $4\times10^{-3}$ 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

re-determined 7 days later. The MIC is defined as the concentration of the compound required to give complete inhibition of bacterial growth.

### *3.3. Cytotoxicity*

The synthesized conjugates **5a-l** along with the references **GTFX**, **RIF** and **INH** were further examined for toxicity ($CC_{50}$) in a mammalian VERO cell line dissolved in DMSO at concentrations from 1000 to 4 $\mu$g/mL [10]. 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 $1\times10^4$ 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.

## References

[1] World Health Organization, Global Tuberculosis Report 2016 (WHO/HTM/TB/2016.10). World Health Organization, Geneva (2016).

[2] Z. Xu, S. Zhang, C. Gao, F. Zhao, Z. S. Lv, L. S. Feng, *Chin. Chem. Lett.* 2 (2017) 159-167

[3] Y. Q. Hu, S. Zhang, F. Zhao, C. Gao, L. S. Feng, Z. S. Lv, Z. Xu, X. Wu, *Eur. J. Med. Chem.* 133 (2017) 255-267

[4] Y. Q. Hu, Z. Xu, S. Zhang, X. Wu, J. W. Ding, Z. S. Lv, L. S. Feng, *Eur. J. Med. Chem.* 136 (2017) 122-130

[5] J. Crofton, P. Choculet, D. Maher, Guidelines for the Management of Drug-Resistant Tuberculosis WHO/TB/96-210 (Rev.1). World Health Organization, Geneva (1997).

[6] D. Sriram, A. Aubry, P. Yogeeswaria, L. M. Fisher, *Bioorg. Med. Chem. Lett.* 16 (2006) 2982-2985

[7] D. Sriram, P. Yogeeswaria, J. S. Basha, D. R. Radhaet, V. Nagaraja, *Bioorg. Med. Chem.* 13 (2005) 5774-5778.

[8] S. N. Pandeya, D. Srirama, P. Yogeeswari, S. Ananthan, *Chemother*. 47 (2001) 266-269

[9] S. H. Zhao, R. Pine, J. Domagala, K. Drlica, *Antimicrob. Agents Chemother*. 43 (1999) 661-666.

[10] L. S. Feng, M. L. Liu, S. Zhang, *Eur. J. Med. Chem*. 46 (2011) 341-348

[11] L. S. Feng, M. L. Liu, Y. B. Zhang, *Chem. Res. Chin. Univ*. 28 (2012) 61-66

[12] Z. L. Wan, M. L. Liu, L. S. Feng, B. Wang, X. D. Zheng, H. Y. Guo, *Chin. J. Antibiot.* 36 (2011) 37-43.

[13] H. N. Nagesh, K. M. Naidu, D. H. Rao, J. P. Sridevi, D. Sriram, P. Yogeeswari, K. V. G. C. Sekhar, *Bioorg. Med. Chem. Lett.* 23 (2013) 6805-6810.

[14] B. Zhou, Y. He, X. Zhang, J. Xu, Y. Luo, Y. Wang, S. G. Franzblau, Z. Yang, R. J. Chan, Y. Liu, J. Zheng, Z. Y. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* 107 (2010) 4573-4578.

[15] K. D. Thomas, A. V. Adhikari, I. H. Chowdhury, E. Sumesh, N. K. Pal, *Eur. J. Med. Chem.* 46 (2011) 2503-2512

[16] H. N. Lin, C. T. Walsh, *J. Am. Chem. Soc.* 126 (2004) 13998 -14003

[17] J. C. McPherson, R. Royce, B. B. Thomas, J. F. Hartmann, D. Farcasiu, I. Bereczki, E. Röth, S. Tollas, E. Ostorházi, F. Rozgonyi, P. Herczegh, *Eur. J. Med. Chem.* 47 (2012) 615-618

[18] K. Kumar, B. Pradines, M. Madamet, R. Amalvict, N. Benoit, V. Kumar, *Eur. J. Med. Chem.* 87 (2014) 801-804

**5g** was 4-8 times more potent *in vitro* than the references **GTFX** and **RIF** against MTB $H_{37}Rv$. The most potent **5g** and **5h** were 4->512 times more active than the three references (MIC: 1.0->128 $\mu$g/mL) against MDR-TB, and the results warrant further development of the anti-TB properties of triazole-tethered **FQs** isatin conjugates.