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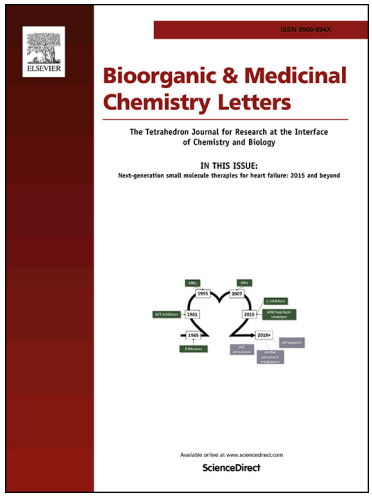

Ciprofloxacin-1,2,3-triazole-isatin hybrids tethered *via* amide: Design, synthesis, and *in vitro* anti-mycobacterial activity evaluation

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Ciprofloxacin-1,2,3-triazole-isatin hybrids tethered *via* amide: Design, synthesis, and *in vitro* anti-mycobacterial activity evaluation

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Abstract: The purpose of this study was to prepare various novel amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l**, and evaluate their *in vitro* anti-mycobacterial activity as well as cytotoxicity in VERO cells. The synthesized hybrids showed considerable *in vitro* activity against both MTB $H_{37}Rv$ and MDR-MTB with MIC of 0.12 to 32 μg/mL, and acceptable cytotoxicity in VERO cells (CC_{50}: 8.0->128.0 μg/mL). In particular, the most active hybrid **7a** ($MIC_{MTB\ H37Rv}$: 0.5 μg/mL and $MIC_{MDR\text{-}MTB}$: 0.12 μg/mL) had the activity in the same level with the first-line anti-tubercular agents isoniazid (MIC: 0.12 μg/mL) and rifampicin (MIC: 0.25 μg/mL), and it was 2-fold more active than the parent ciprofloxacin (MIC: 1.0 μg/mL) against MTB $H_{37}Rv$, and ≥16 folds more potent than ciprofloxacin (MIC: 2.0 μg/mL), isoniazid (MIC: >64 μg/mL) and rifampicin (MIC: >64 μg/mL) against MDR-MTB. Moreover, hybrid **7a** (CC_{50}: 16.0 μg/mL) also displayed considerable cytotoxicity towards VERO cells. Thus, hybrid **7a** could act as a platform for further investigations.

Keywords: ciprofloxacin; isatin; 1,2,3-triazole; anti-mycobacterial; anti-tubercular; drug-resistant

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), mainly occurs in the lungs[1,2]. TB is widespread (one-third of the world population infected with MTB, and 5-10% of them will develop TB disease during their lifetime)[3], infectious (one contagious patient can infect 10-20 contacts)[4], and of high mortality (over 1 million death annually)[3], which has serious influence on the global healthcare system. The widespread of drug-resistant TB (DR-TB, especially multi-drug resistant TB/MDR-TB) and HIV/TB co-infection (TB is the leading cause of death among HIV/TB co-infection patients) are main reasons for the incremental prevalence of TB[5,6]. It has been estimated by The World Health Organization (WHO) that 558,000 new cases of rifampicin-resistant TB (RR-TB) occurred in 2017, of which 82% were MDR-TB patients [3]. Around 3.5% of new TB cases and 18% of previously treated cases had MDR-TB or RR-TB. However, only a handful of drug candidates are under clinical trials currently, and a few new clinically approved anti-TB drugs have emerged during the past 30 years[7]. Therefore, it is urgent to develop novel anti-TB drugs with great potency against both drug-sensitive TB and DR-TB especially MDR-TB.

Ciprofloxacin, the third-generation quinolone antibiotic, has already used as the second-line anti-TB agent to treat the MDR-TB[8]. However, the emergency of extensively drug-resistant TB (XDR-TB) and totally drug-resistant TB (TDR-TB) make ciprofloxacin less and less effective[9,10]. Isatin structural scaffolds and ciprofloxacin could act on DNA gyrase, so hybridization of isatin with ciprofloxacin has the potential to enhance the inhibitory activity against DNA gyrase, and consequently improve the anti-TB activity[11,12]. Some ciprofloxacin-1,2,3-triazole-isatin hybrids showed excellent potency against both drug-sensitive MTB and MDR-MTB[13,14], and the structure-activity relationship (SAR) revealed that the linker between ciprofloxacin and 1,2,3-triazole has great influence on the activity, and the most common linker was alkyl with different length[15-18]. Moreover, *N*-acylated ciprofloxacin derivatives showed enhanced biological activity when compared with the parent ciprofloxacin, so amide may be preeminent linker[19-23].

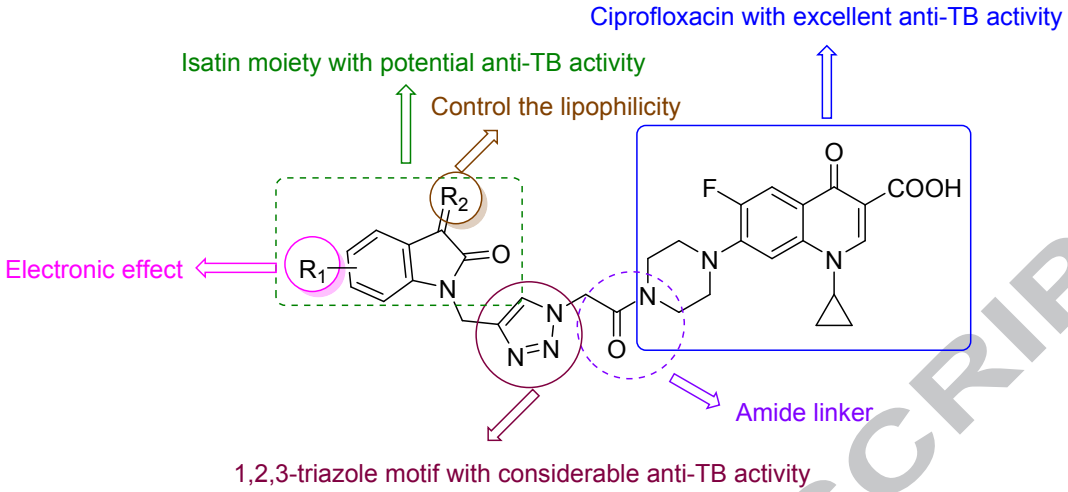
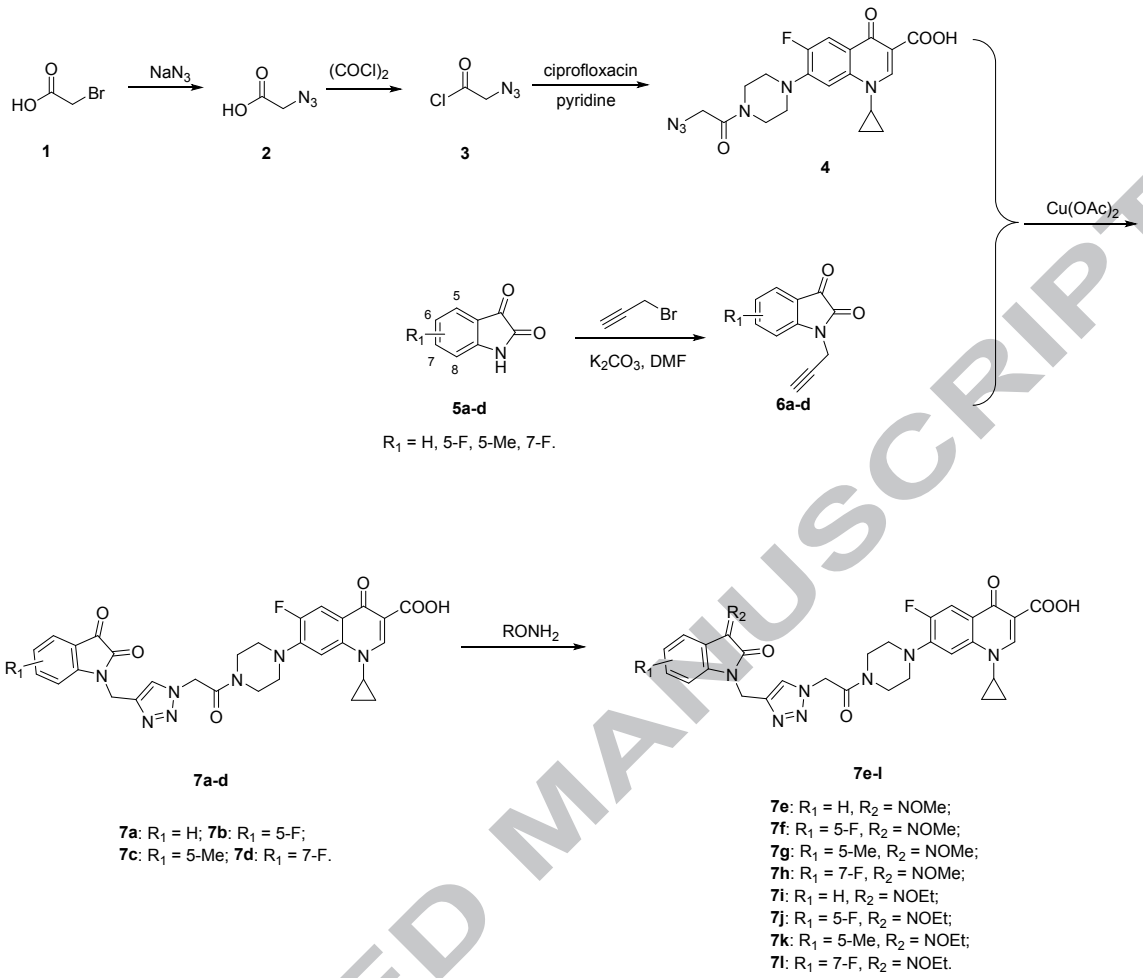


Figure 1 Design strategy for amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids

Based on the above facts, a class of novel amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids were designed, synthesized, and the *in vitro* anti-mycobacterial activity against both drug-susceptible MTB $H_{37}Rv$ and MDR-MTB strains as well as cytotoxicity in VERO cells were evaluated to search novel anti-TB candidates in this paper. The design strategy was depicted in **Figure 1**.

The novel amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l** could be achieved by the synthetic route depicted in **Scheme 1**. The 2-azidoacetic acid **2** was obtained by the treatment of 2-bromoacetic acid **1** with sodium azide in H_2O, and then 2-azidoacetic acid **2** reacted with oxalyl chloride with DMF as catalyst to give 2-azidoacetyl chloride **3**[24]. Condensation of ciprofloxacin with 2-azidoacetyl chloride **3** with pyridine as base yielded the amide **4**. Isatin/5-methylisatin/5-fluoroisatin/7-fluoroisatin **5a-d** were alkylated with propargyl bromide and generated isatin intermediates **6a-d**[25,26]. Cyclization of amide **4** and isatin intermediates **6a-d** with $Cu(OAc)_2$ as catalyst in DMF provided the desired amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-d**[27]. Finally, condensations of **7a-d** with methoxylamine hydrochloride or ethoxylamine hydrochloride in the presence of sodium bicarbonate afforded the rest ciprofloxacin-1,2,3-triazole-isatin hybrids **7e-l**.



Scheme 1 Synthesis of amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l**

The anti-mycobacterial activity of the desired amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l** against MTB $H_{37}Rv$ and MDR-MTB (resistant to isoniazid and rifampicin) strains and cytotoxicity towards VREO cells were investigated, and the results were presented in **Table 1**.

Table 1 *In vitro* anti-mycobacterial activity and cytotoxicity of amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l**

Compounds	MIC (μg/mL)		CC_{50} (μg/mL)	SI
	MTB $H_{37}Rv$	MDR-MTB		

7a	0.5	0.12	16.0	32.0
7b	1.0	1.0	32.0	32.0
7c	1.0	2.0	16.0	16.0
7d	4.0	2.0	8.0	2.0
7e	8.0	8.0	128.0	16.0
7f	8.0	16.0	64.0	8.0
7g	32.0	8.0	8.0	0.25
7h	32.0	32.0	32.0	1.0
7i	32.0	16.0	64.0	2.0
7j	16.0	16.0	8.0	0.5
7k	32.0	32.0	32.0	1.0
7l	32.0	32.0	16.0	0.5
Ciprofloxacin	1.0	2.0	128.0	128.0
Isoniazid	0.12	>64	64.0	512.0
Rifampicin	0.25	>64	128.0	512.0

[a]SI: selectivity index, $CC_{50}/MIC_{MTB\ H37Rv}$

From **Table 1**, it can be concluded that all the synthesized hybrids **7a-l** were active against both drug-sensitive MTB $H_{37}Rv$ and MDR-MTB with MIC values in a range of 0.12 to 32 μg/mL. The SAR indicated that introduction of alkyloxime onto C-3 position of isatin moiety was detrimental to the activity. Compared with unsubstituted

analogs, hybrids with either electron-withdrawing (fluoro and chloro) or electron-donating group (methyl) could reduce the activity. The potency of the synthesized hybrids against MDR-MTB were higher than that against drug-sensitive MTB $H_{37}Rv$, demonstrating that this kind of hybrids might have novel mechanism on MDR-MTB. In particular, conjugate **7a** ($MIC_{MTB\ H37Rv}$: 0.5 μg/mL and $MIC_{MDR\text{-}TB}$: 0.12 μg/mL) which was found to be the most active compound against MTB $H_{37}Rv$ and MDR-MTB strains, has the same level activity with the first-line anti-TB agents isoniazid (MIC: 0.12 μg/mL) and rifampicin (MIC: 0.25 μg/mL), and it was 2-fold more active than the parent ciprofloxacin (MIC: 1.0 μg/mL) against MTB $H_{37}Rv$. Moreover, hybrid **7a** was ≥16 folds more potent than ciprofloxacin (MIC: 2.0 μg/mL), isoniazid (MIC: >64 μg/mL) and rifampicin (MIC: >64 μg/mL) against MDR-MTB. The above results demonstrated that hybrid **7a** has the potential to treat both drug-sensitive MTB and MDR-MTB infections.

All hybrids also displayed acceptable toxicological properties with CC_{50} ranging from 8.0 to 128.0 μg/mL, but the cytotoxicity of all the hybrids except **7e** was higher than the parent ciprofloxacin (CC_{50}: 128.0 μg/mL). The structure-cytotoxicity relationship study indicated that hybrids with alkyloxime at C-3 position of isatin moiety had lower cytotoxicity compared to the corresponding ketones, and hybrids with substituents at C-5 position of isatin motif were much more toxic than the unsubstituted analogs. In particular, the most active hybrid **7a** (CC_{50}: 16.0 μg/mL) displayed moderate cytotoxicity against VERO cells, and the selective index (SI) was 32.0, worth to be further investigated.

In summary, a series of amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l**, were designed, synthesized, and the *in vitro* anti-mycobacterial activity as well as cytotoxicity in VERO cells were evaluated in this study. All synthesized hybrids showed promising *in vitro* activity against both drug-sensitive and MDR MTB strains, and acceptable cytotoxicity in VERO cells. Among them, the most active hybrid **7a** was no inferior to the first-line anti-TB agents isoniazid, rifampicin, and the parent ciprofloxacin against the tested strains *in vitro*. The enriched SAR may help to identify new chemical entities as potent anti-TB agents.

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1. The novel hybrids **7a-l** showed considerable antimycobacterial activity;
2. Hybrid **7a** was no inferior to the first-line anti-TB;
3. The SAR and structure-cytotoxicity relationship were discussed.

Graphical Abstract

Ciprofloxacin-1,2,3-triazole-isatin hybrids tethered *via* amide: Design, synthesis, and *in vitro* anti-mycobacterial activity evaluation

Rongxing Chen[a,1], Hao Zhang[b,1], Tianwei Ma[a], Huarui Xue[a], Zhong Miao[a], Liyan Chen[a], Xiangkui Shi[a,*]

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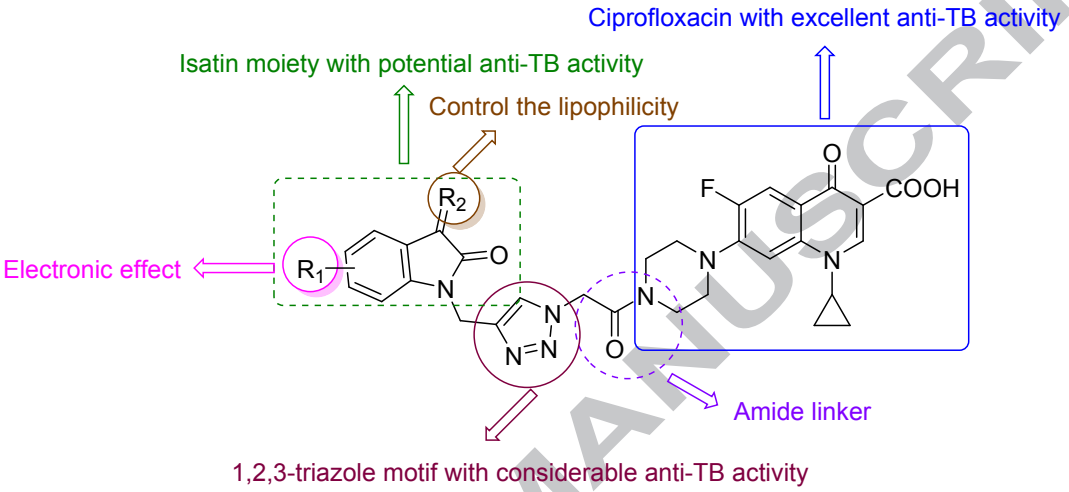

The purpose of this study was to prepare various novel amide tethered ciprofloxacin-1,2,3-triazole-isatin hybrids **7a-l**, and evaluate their *in vitro* and *in vivo* anti-mycobacterial activity as well as cytotoxicity in VERO cells. Among them, the most active hybrid **7a** was no inferior to the first-line anti-TB agents isoniazid, rifampicin, and the parent ciprofloxacin against the tested strains *in vitro*. The enriched structure-activity relationship may help global efforts for identification of new chemical entities as potent anti-TB agents.