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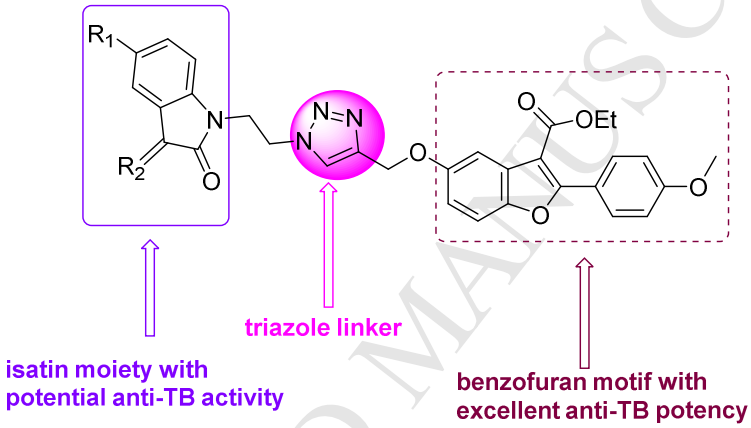

A series of novel benzofuran-isatin hybrids were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities as well as cytotoxicity. Preliminary results indicated that all hybrids with acceptable cytotoxicity in VERO cells exhibited considerable anti-mycobacterial activities against MTB H_{37}Rv and MDR-TB strains. It is worthy to note that hybrid **8f** with no cytotoxicity towards VERO cells, was found to be the most active compound against MTB H_{37}Rv and MDR-TB. Comparing to the first-line anti-TB agents (rifampicin and isoniazid), hybrid **8f** has shown over two magnitude more active against MDR-TB. However, both the metabolic stability and *in vivo* pharmacokinetic profiles of **8f** were inferior to inhibitor **TAM16**. Further modification based on hybrid **8f** is needed to improve metabolic stability and pharmacokinetic profiles.

Design, synthesis and anti-mycobacterial activity evaluation of benzofuran-isatin hybrids

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Abstract: Herein we report the design and synthesis of a series of novel benzofuran-isatin hybrids, and *in vitro* evaluation of their anti-mycobacterial activity against both drug-susceptible and multi-drug resistant (MDR) *Mycobacterium tuberculosis* (MTB) strains. In parallel, cytotoxicity of these hybrids was also tested in VERO cells. Preliminary results indicated that all hybrids with acceptable cytotoxicity in VERO cells (CC_{50}: 128->1,024 μg/mL) exhibited considerable anti-mycobacterial activities against MTB $H_{37}Rv$ and MDR-TB with MIC ranging from 0.25 to 8 μg/mL. It is worth noting that hybrid **8f** with no cytotoxicity towards VERO cells (CC_{50}: >1,024 μg/mL) was found to be the most active compound (MIC: 0.25 and 0.5 μg/mL) against MTB $H_{37}Rv$ and MDR-TB strains. Comparing to the first-line anti-*tuberculosis* agents rifampicin and isoniazid, hybrid **8f** has shown over two magnitude more active against MDR-TB. The hybrid **8f** was further evaluated for its metabolic stability and *in vivo* pharmacokinetic profiles, with the results showing that hybrid **8f** exhibited lower metabolic stability compared to inhibitor **TAM16**. Further modification based on hybrid **8f** is needed to improve the metabolic stability and pharmacokinetic profiles.

Keywords: benzofuran-isatin hybrids; anti-mycobacterial; anti-tubercular; multi-drug resistant; structure-activity relationship

1. Introduction

Tuberculosis (TB) is an infectious disease predominantly caused by the bacterium *Mycobacterium tuberculosis* (MTB), which mainly makes the lung infection [1].

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According to the reports, TB has been the ninth leading cause of death around the world, ranking above HIV/AIDS [1,2]. The latest global TB report from the World Health Organization (WHO) showed that approximately one-third of the population worldwide infected MTB, with around 10.4 million incident cases and 1.67 million deaths in the year 2016[1]. Drug-resistant TB (DR-TB), especially multi-drug resistant TB (MDR-TB), is a persistent problem for the treatment of the global TB epidemic [3,4]. It is estimated 600,000 incident cases of the first-line anti-TB agent rifampicin (**RIF**)-resistant TB happened in 2016, of which 490,000 cases were MDR-TB [1]. Therefore, it is urgent to develop novel agents to treat both drug-susceptible and MDR-TB.

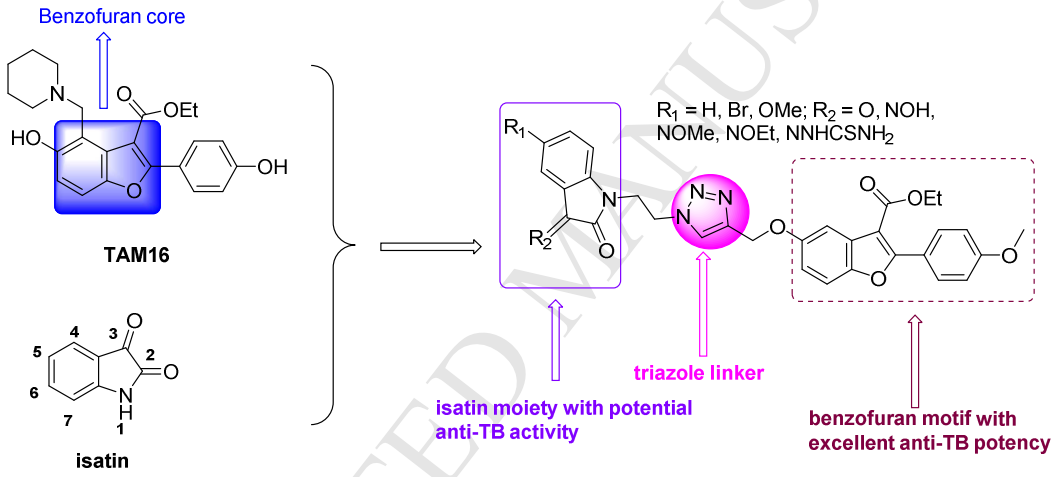


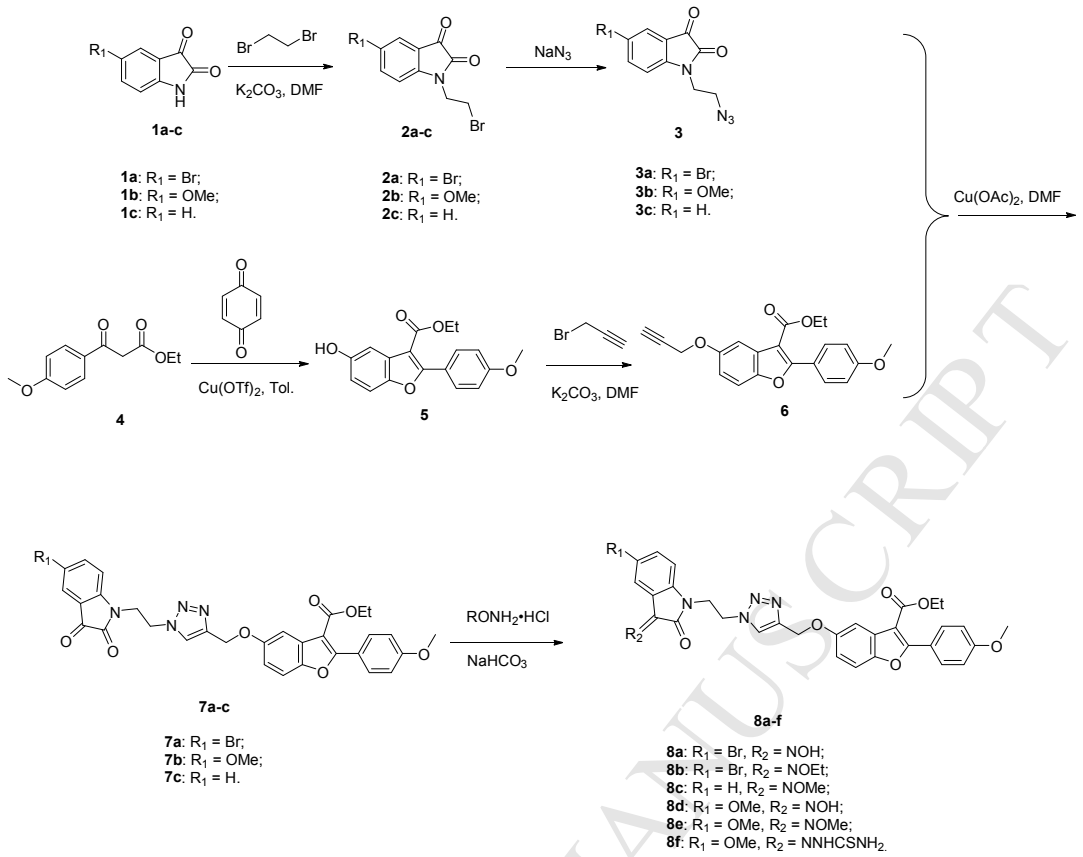
Figure 1 Schematic showing the design strategy for the novel benzofuran-isatin hybrids

Benzofuran-based derivatives have been attracted great interest in medicinal chemistry, due to their various biological properties, such as antibacterial [5,6], anticancer [7], antimalarial [8] and anti-TB activities [9-13]. The benzofuran-based lead compound **TAM16** (**Figure 1**) showed therapeutic potential in MDR-TB patients. **TAM16** could inhibit the C-terminal thioesterase domain activity of polyketide synthase 13, and display great activity against 38 clinical MTB strains, including MDR-TB *in vitro*. Notably, resistant mutants emerged at frequencies two magnitude lower than that for the first-line anti-TB agent isoniazid (**INH**) [13]. *In vivo*, **TAM16** also exhibited excellent physiochemical, toxicological and pharmacological properties, with the similar treatment efficiency as **INH** in multiple TB infected mouse models.

Moreover, the combined application of **TAM16** and **RIF** in the chronic infection model was as effective as the gold-standard combination of **INH** and **RIF** at reducing the bacterial load, but more active than **RIF** alone. Meanwhile, isatin (indoline-2,3-dione) derivatives (**Figure 1**) has shown potential anti-TB activities according to the previous reports [14-18]. In this case, the combination of **TAM16** and isatin together lead to novel hybrids that may possess higher anti-TB activity than themselves alone. Herein we report the design and synthesis of various novel triazole-tethered benzofuran-isatin hybrids, *in vitro* evaluation of anti-mycobacterial activities, and cytotoxicity test in VERO cells as well. In this study, both drug-sensitive and drug-resistant MTB strains are expected to be effectively treated with the novel hybrids. Preliminary structure-activity relationship (SAR) studies are also performed to facilitate the further development of these hybrids.

2 Results and discussion

The desired benzofuran-isatin hybrids **7a-c** and **8a-f** were synthesized following the methods described in **Scheme 1**. Isatin/5-methoxyisatin/5-bromoisatin alkylated with 1,2-dibromoethane, producing the *N*-(2-bromoethyl)isatin derivatives **2a-c** with yields of 49-77% [15,16]. Subsequently, treatment of *N*-(2-bromoethyl)isatins **2a-c** with sodium azide at 60 °C provided the desired azido precursors **3a-c** (yield: 57-78%). The benzofuran intermediate **5** was synthesized *via* copper-catalyzed cyclization of ethyl 3-(4-methoxyphenyl)-3-oxopropanoate **4** and benzoquinone, and the propargyl benzofuran intermediate **6** was obtained by treatment of intermediate **5** with propargyl bromide according to the method reported in the literature [12, 15]. The precursors **3a-c** and **6** were utilized for the synthesis of desired 1*H*-1,2,3-triazole-tethered benzofuran-isatin hybrids **7a-c** *via* copper-promoted azide-alkyne cycloaddition reaction in the presence of $Cu(OAc)_2$ (yield: 38-47%) [15]. Finally, condensation of **7a-c** with the corresponding amine hydrochlorides in the presence of sodium bicarbonate yielded target compounds **8a-f** (21-72%)



Scheme 1 Synthesis of benzofuran-isatin hybrids **7a-c** and **8a-f**

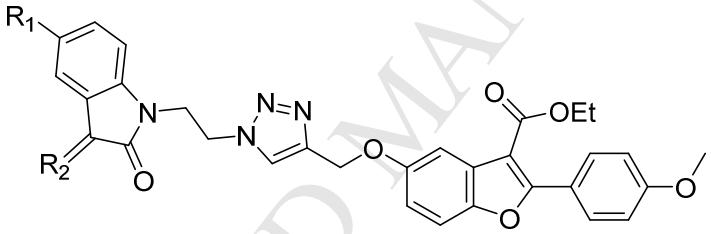
The anti-mycobacterial activities of benzofuran-isatin hybrids **7a,b** and **8a-f** against MTB $H_{37}Rv$ and MDR-TB (resistant to ethambutol, **INH** and **RIF**) strains were investigated. The minimum inhibitory concentration (MIC) is defined as the lowest concentration that inhibits the visible bacterial growth. The MIC values for the synthesized hybrids, together with **TAM16**, **RIF** and **INH**, were measured (**Table 1**). All hybrids exhibited promising anti-mycobacterial activities against MTB $H_{37}Rv$ and MDR-TB strains, with MIC ranging from 0.25 to 8 μg/mL. However, all hybrids were less active than the parent compound **TAM16** (MIC: <0.06 μg/mL), indicating that benzofuran backbone is crucial for the binding with polyketide synthase 13.

The SAR results indicated that substitutents on both C-3 and C-5 position of isatin moiety significantly influenced their anti-mycobacterial activities. Hybrids with electron-donating -OMe group at C-5 position were found more active than the corresponding unsubstituted or -Br substituted hybrids. At C-3 position, hydrogen-bond donor such as hydrazinecarbothioamide or oximine could also enhance the anti-mycobacterial activity. The relative contribution of the substituents at C-3

position to the activity in the hybrids was: $NNHCSNH_2$ > NOH > NOMe > NOEt > O.

Among the synthesized benzofuran-isatin hybrids, compound **8f** ($MIC_{MTB\ H37Rv}$: 0.25 μg/mL and $MIC_{MDR\text{-}TB}$: 0.5 μg/mL) was the most active against MTB $H_{37}Rv$ and MDR-TB strains, with 128 and over 256 folds more effective than the **RIF** (MIC: 64 μg/mL) and **INH** (MIC: >128 μg/mL) against MDR-TB, and 2 times more active than **RIF** (MIC: 0.5 μg/mL) against MTB $H_{37}Rv$. It is worth mentioning that the values of the resistance index (RI, $MIC_{(MDR\text{-}TB)}/MIC_{(MTB\ H37Rv)}$) for the benzofuran-isatin hybrids were around 1, suggesting that these hybrids may have novel mechanism of action against TB.

Table 1 Structure, anti-mycobacterial activity and cytotoxicity of benzofuran-isatin hybrids **7a,b** and **8a-f**



Compounds	R_1	R_2	MIC (μg/mL)		CC_{50} (μM)	SI[a]
			MTB $H_{37}Rv$	MDR-TB		
7a	Br	O	4	8	512	128
7b	OMe	O	2	2	128	64
8a	Br	NOH	1	0.5	512	512
8b	Br	NOEt	2	4	256	128
8c	H	NOMe	1	2	>1,024	>1,024
8d	OMe	NOH	0.5	1	>1,024	>2,048
8e	OMe	NOMe	1	0.5	>1,024	>1,024

8f	OMe	$NNHCSNH_2$	0.25	0.5	>1,024	>4,096
TAM16	-	-	<0.06	<0.06	>1,024	>16,384
INH	-	-	0.06	>128	256	4,096
RIF	-	-	0.5	64	512	1,024

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

Cytotoxicity (CC_{50}) of hybrids **7a,b** and **8a-f** was sequentially tested in VERO cells, (green monkey kidney epithelial cells) [16]. All hybrids displayed excellent toxicological profiles with CC_{50} ranging from 128 to >1,024 μg/mL. The structure-cytotoxicity relationship study indicated that substitution of isatin moiety on C-3 position for hybrids **8a-f** generally reduced the cytotoxicity compared to the corresponding ketones, **7a,b**. Interestingly, the most active hybrid **8f** (CC_{50}: >1,024 μg/mL) showed no discernible cytotoxicity against VERO cells.

To evaluate the selectivity profiles of these hybrids, the selectivity index (SI) values from the ratio of $CC_{50}/MIC_{MTB\ H37Rv}$ were calculated (**Table 1)**. All benzofuran-isatin hybrids possessed moderate to high SI values, and half of the derivatives have SI values even greater than 1,024. The SI of the most active hybrid **8f** was over 4,096, higher than **RIF** and **INH** (SI values: 1,024 and 4,096, respectively), suggesting that hybrid **8f** holds a profile of excellent selectivity.

Table 2 The metabolic stability and *in vivo* pharmacokinetic values of hybrid **8f** and **TAM16** in mice

Compd.	Metabolic stability ($t_{1/2}$, min)	Pharmacokinetics (p.o.)			
		C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)
8f	28.9	389	4.26	1.24	2,564
TAM16	>60	5,064	9.88	3.46	39,329

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

C_{max}: the peak concentration;

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

AUC: the area under *the* curve.

Hybrid **8f** was selected for the further investigation on metabolic stability and *in vivo* pharmacokinetics in mice (25 mg/kg, single oral administration) due to its excellent selectivity. The results were listed in **Table 2**. Comparing to **TAM16** ($t_{1/2}$: over 60 min), hybrid **8f** showed much lower metabolic stability ($t_{1/2}$: 28.9 min). Pharmacokinetic investigation displayed that hybrid **8f** was inferior to **TAM16** in term of C_{max} (389 ng/mL *vs* 5,064 ng/mL), $t_{1/2}$ (4.26 h *vs* 9.88 h), T_{max} (1.24 h *vs* 3.46 h) and $AUC_{0\text{-}inf}$ (2,564 ng•h/mL *vs* 39,329 ng•h/mL). Therefore, further modification based on hybrid **8f** is needed to improve its metabolic stability and pharmacokinetic profiles.

3 Conclusions

In summary, a series of novel triazole-tethered benzofuran-isatin hybrids were designed and prepared *via* concise organic synthesis. All synthetic benzofuran-isatin compounds exhibited decent *in vitro* anti-mycobacterial activities against both susceptible and multidrug-resistant MTB strains. These hybrids showed minimal cytotoxicity, and the most active hybrid **8f** had comparable anti-mycobacterial activity to **RIF** and **INH** against both drug-sensitive MTB $H_{37}Rv$ and MDR-TB strains. However, both the metabolic stability and pharmacokinetic profiles for **8f** could be improved through the further modification of its functional groups.

4. Experimental section

4.1. Synthesis

N-(2-azidoethyl)isatins **3a-c** and ethyl 2-(4-methoxyphenyl)-5-(prop-2-yn-1-yloxy)benzofuran-3-carboxylate **6** were prepared *via* the methods reported in the literature [13, 18].

A mixture of N-(2-azidoethyl)isatins **3a-c** (1.0 mmol), ethyl 2-(4-methoxyphenyl)-5-(prop-2-yn-1-yloxy)benzofuran-3-carboxylate **6** (1.0 mmol)

and $Cu(OAc)_2$ (100 mg) in DMF (50 mL) was stirred at room temperature for 48 h under N_2. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with PE: EA=3:1 to give benzofuran-isatin hybrids **7a-c**.

To **7a-c** (1 mmol), sodium bicarbonate (2 mmol), hydroxylamine/ methoxyamine/ethoxyamine hydrochloride (1.8 mmol), water (10 mL) and tetrahydrofuran (THF, 30 mL) were added and the mixture was stirred at 40 ^oC for 12 h. After cooling to room temperature, the mixture was extracted with EA (20 mL×3). The organic phases were washed with water (50 mL×2) and brine (50 mL), and then dried with Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give a residue, which was further purified by silica gel chromatography with PE: EA=3:1 to give benzofuran-isatin hybrids **8a-f**.

4.1.1. ethyl 5-((1-(2-(5-bromo-2,3-dioxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)-3a,7a-dihydrobenzofuran-3-carboxylate (**7a**)

Yellow solid, yield: 38%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, t, J = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.88 (3H, s, OMe), 4.17 (2H, s, -CH_2-), 4.36 (2H, q, J = 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.69 (2H, t, J = 1.2 Hz, -CH_2-), 5.22 (2H, t, J = 1.2 Hz, -CH_2-), 6.84 (1H, d, J = 6.4 Hz, Ar-H), 7.06 (1H, d, J = 7.2 Hz, Ar-H), 7.12 (2H, d, J = 7.2 Hz, Ar-H), 7.58-7.71 (4H, m, Ar-H), 8.00 (1H, d, J = 7.2 Hz, Ar-H), 8.34 (1H, s, triazole-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.612, 162.715, 161.461, 157.336, 155.592, 148.452, 143.663, 143.321, 133.237, 131.917, 131.414, 127.929, 125.515, 123.219, 121.680, 115.307, 114.505, 112.318, 109.487, 107.776, 106.485, 64.947, 62.191, 60.856, 55.876, 47.469, 14.520. ESI-MS m/z: 667 $[M+Na]^+$, 669 $[M+2+Na]^+$.

4.1.2. ethyl 5-((1-(2-(5-methoxy-2,3-dioxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**7b**)

Yellow solid, yield: 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.75 (3H, s, OMe), 3.88 (3H, s, OMe), 4.15 (2H, s, -CH_2-), 4.36 (2H, q, J = 6.0 Hz, $CO_2\underline{CH_2}CH_3$), 4.69 (2H, t, J = 1.2 Hz, -CH_2-), 5.18 (2H, t, J = 1.2 Hz,

-CH_2-), 6.86 (1H, d, *J* = 7.2 Hz, Ar-H), 7.05 (1H, d, *J* = 6.8 Hz, Ar-H), 7.12-7.13 (4H, m, Ar-H), 7.57 (1H, s, Ar-H), 7.61 (1H, d, *J* = 6.8 Hz, Ar-H), 8.01 (1H, d, *J* = 6.8 Hz, Ar-H), 8.34 (1H, s, triazole-H). ESI-MS m/z: 597 $[M+H]^+$, 619 $[M+Na]^+$.

4.1.3. ethyl 5-((1-(2-(5-bromo-3-(hydroxyimino)-2-oxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8a**)

Yellow solid, yield: 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, t, *J* = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.88 (3H, s, OMe), 4.20 (2H, s, -CH_2-), 4.36 (2H, q, *J* = 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.70 (2H, t, *J* = 1.2 Hz, -CH_2-), 5.18 (2H, t, *J* = 1.2 Hz, -CH_2-), 6.81 (1H, d, *J* = 6.8 Hz, Ar-H), 7.06 (1H, d, *J* = 7.2 Hz, Ar-H), 7.12 (2H, d, *J* = 6.8 Hz, Ar-H), 7.44 (1H, d, *J* = 6.8 Hz, Ar-H), 7.58-7.62 (2H, m, Ar-H), 8.00 (1H, d, *J* = 7.2 Hz, Ar-H), 8.06 (1H, s, Ar-H), 8.31 (1H, s, triazole-H), 13.78 (1H, s, NOH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.627, 163.619, 161.461, 158.661, 156.146, 155.606, 148.454, 144.524, 143.379, 131.414, 127.929, 125.719, 124.436, 121.680, 118.275. 114.484, 114.192, 112.311, 111.830, 109.511, 107.768, 106.434, 62.191, 60.856, 56.306, 55.876, 47.440, 14.513. ESI-MS m/z: 660 $[M+H]^+$, 662 $[M+2+H]^+$.

4.1.4. ethyl 5-((1-(2-(5-bromo-3-(ethoxyimino)-2-oxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)me thoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8b**)

Yellow solid, yield: 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36-1.41 (6H, m, $NOCH_2\underline{CH_3}$ and $CO_2CH_2\underline{CH_3}$), 3.89 (3H, s, OMe), 4.16-4.20 (2H, m, -CH_2-), 4.35-4.51 (4H, m, $NO\underline{CH_2}CH_3$ and $CO_2\underline{CH_2}CH_3$), 4.70 (2H, t, *J* = 1.2 Hz, -CH_2-), 5.16 (2H, t, *J* = 1.2 Hz, -CH_2-), 6.82 (1H, d, *J* = 7.2 Hz, Ar-H), 7.06 (1H, d, *J* = 7.2 Hz, Ar-H), 7.13 (2H, d, *J* = 6.8 Hz, Ar-H), 7.44-7.48 (1H, m, Ar-H), 7.51-7.57 (2H, m, Ar-H), 7.85 (1H, d, *J* = 7.2 Hz, Ar-H), 8.00 (2H, d, *J* = 7.2 Hz, Ar-H), 8.30 (1H, s, triazole-H). ESI-MS m/z: 710 $[M+H]^+$, 712 $[M+2+H]^+$.

4.1.5. ethyl 5-((1-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8c**)

Yellow solid, yield: 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, t, *J* = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.85 (3H, s, OMe), 4.15-4.22 (5H, m, -CH_2- and NOMe), 4.36 (2H, q, *J*

= 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.70 (2H, t, *J* = 1.6 Hz, -CH_2-), 5.17 (2H, t, *J* = 1.2 Hz, -CH_2-), 6.92 (1H, d, *J* = 6.8 Hz, Ar-H), 7.06-7.14 (4H, m, Ar-H), 7.35-7.38 (1H, m, Ar-H), 7.56 (1H, s, Ar-H), 7.62 (1H, d, *J* = 6.8 Hz, Ar-H), 7.88 (1H, d, *J* = 6.0 Hz, Ar-H), 8.01 (2H, d, *J* = 6.8 Hz, Ar-H), 8.30 (1H, s, triazole-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ESI-MS m/z: 618 $[M+Na]^+$.

4.1.6. ethyl 5-((1-(2-(3-(hydroxyimino)-5-methoxy-2-oxoindolin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8d**)

Yellow solid, yield: 53%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, t, *J* = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.73 (3H, s, OMe), 3.88 (3H, s, OMe), 4.18 (2H, s, -CH_2-), 4.36 (2H, q, *J* = 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.69 (2H, t, *J* = 1.2 Hz, -CH_2-), 5.18 (2H, t, *J* = 1.2 Hz, -CH_2-), 6.81 (1H, d, *J* = 6.8 Hz, Ar-H), 6.88 (1H, d, *J* = 6.8 Hz, Ar-H), 7.06 (1H, d, *J* = 7.2 Hz, Ar-H), 7.12 (2H, d, *J* = 6.8 Hz, Ar-H), 7.58-7.62 (3H, m, Ar-H), 8.00 (2H, d, *J* = 7.2 Hz, Ar-H), 8.30 (1H, s, triazole-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.612, 162.599, 161.454, 161.345, 156.664, 155.606, 148.446, 143.321, 137.283, 131.414, 127.921, 125.501, 121.695, 118.049, 115.847, 114.498, 114.185, 114.024, 112.303, 110.116, 107.768, 106.463, 65.020, 62.205, 60.858, 56.139, 56.037, 55.876, 47.484, 14.513. ESI-MS m/z: 634 $[M+Na]^+$.

4.1.7. ethyl 5-((1-(2-(5-methoxy-3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8e**)

Yellow solid, yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, t, *J* = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.74 (3H, s, OMe), 3.88 (3H, s, OMe), 4.15-4.22 (5H, m, -CH_2- and NOMe), 4.35 (2H, q, *J* = 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.68 (2H, t, *J* = 4.0 Hz, -CH_2-), 5.18 (2H, t, *J* = 1.2 Hz, -CH_2-), 6.84-6.96 (2H, m, Ar-H), 7.06 (1H, d, *J* = 7.2 Hz, Ar-H), 7.13 (2H, d, *J* = 7.2 Hz, Ar-H), 7.45 (1H, s, Ar-H), 7.57 (1H, s, Ar-H), 7.62 (1H, d, *J* = 7.2 Hz, Ar-H), 8.01 (2H, d, *J* = 7.2 Hz, Ar-H), 8.29 (1H, s, triazole-H). ESI-MS m/z: 648 $[M+H]^+$.

4.1.8. ethyl 5-((1-(2-(3-(2-carbamothioylhydrazono)-5-methoxy-2-oxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**8f**)

Yellow solid, yield: 21%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 5.6 Hz, $CO_2CH_2\underline{CH_3}$), 3.76 (3H, s, OMe), 3.88 (3H, s, OMe), 4.21 (2H, s, -CH_2-), 4.36 (2H, q, J = 5.6 Hz, $CO_2\underline{CH_2}CH_3$), 4.72 (2H, t, J = 1.2 Hz, -CH_2-), 5.16 (2H, t, J = 1.2 Hz, -CH_2-), 6.87 (2H, s, Ar-H), 7.04 (1H, d, J = 7.6 Hz, Ar-H), 7.12 (2H, d, J = 6.8 Hz, Ar-H), 7.42 (1H, s, Ar-H), 7.56-7.65 (2H, m, Ar-H), 7.99 (2H, d, J = 6.4 Hz, Ar-H), 8.32 (1H, s, triazole-H), 8.74, 9.16 (2H, s, NNHCS$\underline{NH_2}$), 12.23 (1H, s, NN$\underline{H}$CSNH_2). ESI-MS m/z: 692 $[M+Na]^+$.

4.2. MIC determination

The *in vitro* activities of benzofuran-isatin derivatives **7a,b** and **8a-f**, along with the references **TAM16**, **RIF** and **INH** against MTB H37Rv and MDR-TB were evaluated *via* rapid direct susceptibility test technique [19,20]. 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.

4.3. Cytotoxicity

The cytotoxicity (CC_{50}) of the synthesized benzofuran-isatin hybrids **7a,b** and **8a-f**, together with the references **TAM16**, **RIF** and **INH** were examined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability assay in a mammalian VERO cells [21,22]. The compounds were dissolved in DMSO with concentrations from 1024 to 1 μg/mL. The VERO cells were maintained in culture medium at 37 ^{o}C under 5% CO_2 atmosphere. Cells were seeded in 96-well plates (1×10^4 cell per well) and allowed to recover for 24 h. 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.

4.4 Pharmacokinetic Profiles determination

SPF female ICR mice (20-25 g) were used in the pharmacokinetic study, and each treatment group had 3 mice which were dosed with hybrid **8f** or **TAM16** suspension at 25 mg/kg (oral administration/p.o.) [23]. Compounds were suspended in 0.5% CMC for p.o., and blood was collected from the jugular vein of each mouse at the following time points: 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after oral administration. 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 determined directly from the experimental data using WinNonlin V6.2.1.

References

[1] World Health Organization. Global Tuberculosis Report 2017, 2017.

[2] Z. Xu, C. Gao, Q. C. Ren, X. F. Song, L. S. Feng, Z. S. Lv. Recent advances of pyrazole-containing derivatives as anti-tubercular agents. *Eur. J. Med. Chem.* 139 (2017) 429-440

[3] Y. Q. Hu, Z. Xu, S. Zhang, X. Wu, J. W. Ding, Z. S. Lv, L. S. Feng. Recent Developments of Coumarin-containing Derivatives and Their Anti-tubercular Activity. *Eur. J. Med. Chem.* 136 (2017) 122-130

[4] S. Zhang, Z. Xu, C. Gao, Q. C. Ren, C. Le, Z. S. Lv, L. S. Feng. Triazole Derivatives and Their Anti-tubercular Activity. *Eur. J. Med. Chem.* 138 (2017) 501-513

[5] X. Jiang, W. Liu, W. Zhang, F. Jiang, Z. Gao, H. Zhuang, L. Fu. Synthesis and antimicrobial evaluation of new benzofuran derivatives. *Eur. J. Med. Chem.* 46 (2011) 3526-3530

[6] J. Liu, F. Jiang, X. Jiang, W. Zhang, J. J. Liu, W. Liu, L. Fu. Synthesis and amtimicrobial evaluation of 3-methanone-6-substituted-benzofuran derivatives. *Eur. J. Med. Chem.* 54 (2012) 879-886

[7] A. Baldisserotto, M. Demurtas, I. Lampronti, D. Moi, G. Balbon, S. Vertuani, S. Manfredini, V. Onnis. Benzofuran hydrazones as potential scaffold in the development of multifunctional drugs: Synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity. *Eur. J. Med. Chem.* 156 (2018) 118-125

[8] R. J. Nevagi, S. N. Dighe, S. N. Dighe. Biological and medicinal significance of benzofuran. *Eur. J. Med. Chem.* 97 (2015) 561-581

[9] K. Manna, Y. K. Agrawal. Design, synthesis and antitubercular evaluation of novel series of 3-benzofuran-5-aryl-1-pyrazolyl-pyridinylmethanone and 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphtyridin analogs. *Eur. J. Med. Chem.* 45 (2010) 3831-3839

[10] H. K. Shamsuzzaman. Bioactive benzofuran derivatives: A review. *Eur. J. Med. Chem.* 97 (2015) 483-504

[11] R. P. Tripathi, A. K. Yadav, A. Ajay, S. S. Bisht, V. Chaturvedi, S. K. Sinha. Application of Huisgen (3+2) cycloaddition reaction: Synthesis of 1-(2,3-dihydrobenzofuran-2-yl)-methyl[1,2,3]-triazoles and their antitubercular evaluations. *Eur. J. Med. Chem.* 45 (2010) 142-148

[12] W. Zhang, S. C. Lun, S. H. Wang, X. W. Jiang, F. Yang, J. Tang, A. L. Manson, A. M. Earl, H. Gunosewoyo, W. R. Bishai, L. F. Yu. Idenfication of novel coumestan derivatives as polyketide synthase 13 inhibitors against Mycobacterium tuberculosis. *J. Med. Chem.* 61 (2018) 791-803

[13] A. Aggarwal, M. K. Parai, N. Shetty, D. Wallis, L. Woolhiser, C. Hastings, N. K. Dutta, S. Galaviz, R. C. Dhakal, R. Shrestha, S. Wakabayashi, C. Walpole, D. Matthews, D. Floyd, P. Scullion, J. Riley, O. Epemolu, S. Norval, T. Snavely, G. T. Robertson, E. J. Rubin, T. R. Loerger, F. A. Sirgel, R. vam der Merwe, P. D. van Helden, P. Keller, E. C. Böttger, P. C. Karakousis, A. J. Lenaerts, J. C. Sacchettini. Development of a Novel Lead that Targets M. tuberculosis Polyketide Synthase 13. *Cell* 170 (2017) 249-259

[14] Z. Xu, S. Zhang, C. Gao, F. Zhao, Z. S. Lv, L. S. Feng. Isatin hybrids and their anti-tuberculosis activity. *Chin. Chem. Lett.* 28 (2017) 159-167

[15] Z. Xu, X. F. Song, Y. Q. Hu, M. Qiang, Z. S. Lv. Azide-alkyne cycloaddition towards 1*H*-1,2,3-triazole-tethered gatifloxacin and isatin conjugates: design, synthesis and *in vitro* anti-mycobacterial evaluation. *Eur. J. Med. Chem.* 138 (2017) 66-71.

[16] Z. Xu, S. Zhang, X. F. Song, M. Qiang, Z. S. Lv. Design, synthesis and *in vitro* anti-mycobacterial evaluation of gatifloxacin-1*H*-1,2,3-triazole-isatin hybrids. *Bioorg. Med. Chem. Lett.* 27 (2017) 3643-3646

[17] Z. Xu, X. F. Song, M. Qiang, Z. S. Lv. 1*H*-1,2,3-triazole-tethered 8-OMe ciprofloxacin and isatin hybrids: design, synthesis and *in vitro* anti-mycobacterial activities. *J. Heterocyclic Chem.* 54 (2017) 3735-3741

[18] Z. Xu, X. F. Song, Y. Q. Hu, M. Qiang, Z. S. Lv. Design, synthesis and *in vitro* anti-mycobacterial activities of 8-OMe ciprofloxacin-1*H*-1,2,3-triazole-isatin-(thio) semicarbazide/oxime hybrids. *J. Heterocyclic Chem.* 55 (2018) 77-82

[19] L. S. Feng, M. L. Liu, S. Zhang, Y. Chai, B. Wang, Y. B. Zhang, K. Lv, Y. Guan, H. Y. Guo, C. L. Xiao. Synthesis and *in vitro* antimycobacterial activity of 8-OCH_3 ciprofloxacin methylene and ethylene isatin derivatives. *Eur. J. Med. Chem.* 46 (2011) 341-348.

[20] L. S. Feng, M. L. Liu, B. Wang, Y. Chai, X. Q. Hao, S. Meng, H. Y. Guo. Synthesis and *in vitro* antimycobacterial activity of balofloxacin ethylene isatin derivatives. *Eur. J. Med. Chem.* 45 (2010) 3407-3412.

[21] J. Renuka, K. I. Reddy, K. Srihari, V. U. Jeankumar, M. Shravan, J. P. Sridevi, P. Yogeeswari, K. S. Babu, D. Sriram. Design, synthesis, biological evaluation of substituted benzofurans as DNA gyraseB inhibitors of *Mycobacterium tuberculosis. Bioorg. Med. Chem. 22 (2014) 4924-4934*

[22] K. I. Reddy, K. Srihari, J. Renuka, K. S. Sree, A. Chuppala, V. U. Jeankumar, J. P. Sridevi, K. S. Babu, P. Yogeeswaria, D. Sriram. An efficient synthesis and

biological screening of benzofuran and benzo[*d*]isothiazole derivatives for *Mycobacterium tuberculosis* DNA GyrB inhibition. *Bioorg. Med. Chem. 22 (2014) 6552-6563*

[23] X. J. Yan, Z. S. Lv, J. Wen, S. J. Zhao, Z. Xu. Synthesis and *in vitro* evaluation of novel substituted isatin-propylene-1*H*-1,2,3-triazole-4-methylene-moxifloxacin hybrids for their anti-mycobacterial activities. *Eur. J. Med. Chem.* 143 (2018) 899-904

A series of novel benzofuran-isatin hybrids were assessed for their *in vitro* anti-mycobacterial activities.

All hybrids exhibited considerable anti-mycobacterial activities.

The activity of hybrid **8f** was no inferior to **RIF** and **INH**, but the metabolic stability and pharmacokinetic profiles still waits for optimization.