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Communication

Benzofuran-isatin-hydroxylimine/thiosemicarbazide hybrids: Design, synthesis and *in vitro* anti-mycobacterial activity evaluation

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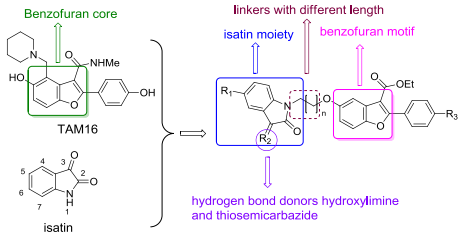
Graphical abstract



A series of novel benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids were designed, synthesized and evaluated for their *in vitro* anti-TB activities against drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates as well as cytotoxicity. All benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids exhibited considerable *in vitro* anti-mycobacterial activities against the tested three MTB strains, and all of them also showed acceptable cytotoxicity. The most active hybrid **7f** was >4.8 and >51 folds more potent than the first line anti-TB agents RIF and INH against both drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates, respectively. The results demonstrated the potential utility of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids as anti-TB agents.

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ABSTRACT

A series of novel benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**–**l** tethered through propylene, butylene and pentylene were designed, synthesized and evaluated for their *in vitro* anti-TB activities against drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates as well as cytotoxicity. All benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids exhibited considerable *in vitro* anti-mycobacterial activities against the tested three MTB strains, and all hybrids (MIC: <0.06−0.062 μg/mL) except **7i** were more potent than the first-line anti-TB agents INH and RIF (MIC: 0.078 μg/mL), and nine of them **7a**, **7b**, **7e**–**h** and **7j**–**l** (MIC: <0.06 μg/mL) were comparable to the parent compound TAM16 (MIC: <0.06 μg/mL). The most active hybrid **7f** (MIC: <0.06, 0.22 and 0.86 μg/mL, respectively) was >4.8 folds more potent than RIF and INH against both drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates. The results demonstrated the potential utility of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids as anti-TB agents.

Tuberculosis (TB), mainly caused by the bacterium *Mycobacterium tuberculosis* (MTB), is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS) [1]. Approximately 5%−10% of the estimated 1.7 billion people infected with MTB will develop TB disease during their lifetime, and about 90% of cases occur among adults [2]. The latest World Health Organization (WHO) global TB report has estimated that around 10 million people including 5.8 million men, 3.2 million women and 1.0 million children developed TB disease in 2017, and 1.3 million deaths occurred in the same year [1].

There are several reasons for the incremental prevalence of TB, and the widely spread of HIV/TB co-infection and drug-resistant TB (DR-TB, especially multi-drug resistant TB (MDR-TB)) are the two major reasons [3,4]. TB is one of the most common opportunistic infection among HIV-positive people, and is the leading cause of death among HIV/TB co-infection patients, which resulted in 300,000 deaths in 2017 [1]. Globally in 2017, there were an estimated 558,000 new cases of rifampicin (RIF)-resistant TB (RR-TB), and 82% of

them were MDR-TB patients. The latest treatment outcome data show treatment success rates of 82% for TB, 77% for HIV-associated TB, 55% for MDR/RR-TB and 34% for extensively drug-resistant TB (XDR-TB) [1]. Specific targets set in the End TB Strategy include a 95% reduction in TB deaths and a 90% reduction in TB incidence by 2035, compared with levels in 2015. The most immediate milestones, set for 2020, are a 35% reduction in TB deaths and a 20% reduction in the TB incidence rate, compared with levels in 2015 [5,6]. Obviously, novel anti-TB agents effective against both drug-susceptible and DR-TB especially MDR-TB are needed to achieve this goal.

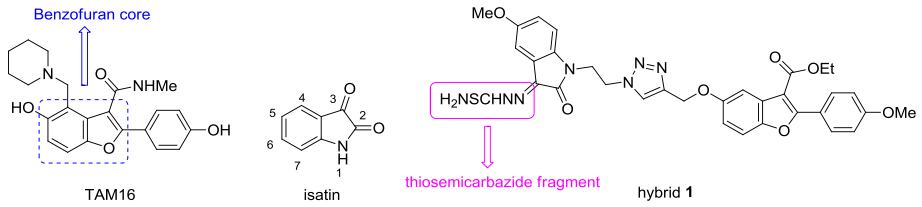


Fig. 1. Chemical structures of TAM16, isatin and 1,2,3-triazole tethered benzofuran-isatin-imine hybrid **1**.

Benzofuran and isatin derivatives possess diverse biological properties [7-10], and some of them which are exemplified by TAM16 (Fig. 1) endow with promising *in vitro* and *in vivo* anti-TB potential [11,12]. TAM16 not only showed broad-spectrum *in vitro* anti-TB activities, but also exhibited excellent *in vivo* physiochemical, toxicological and pharmacological properties [12]. 1,2,3-Triazole tethered benzofuran-isatin-imine hybrids displayed excellent *in vitro* anti-TB activities against both drug-susceptible and MDR-TB strains, and the most active hybrid **1** (MIC: 0.25 and 0.5 μg/mL) was highly active against MTB $H_{37}Rv$ and MDR-TB strains [13], suggesting benzofuran-isatin-imine hybrids are potential anti-TB candidates. The structure-activity relationship (SAR) for isatin-containing hybrids indicated that the linker between the isatin and the other pharmacophores played a pivotal role in exertion of the anti-TB activities [14-19], and introduction of hydrogen bond donors such as hydroxylimine and thiosemicarbazide were favorable to the activity [12,13].

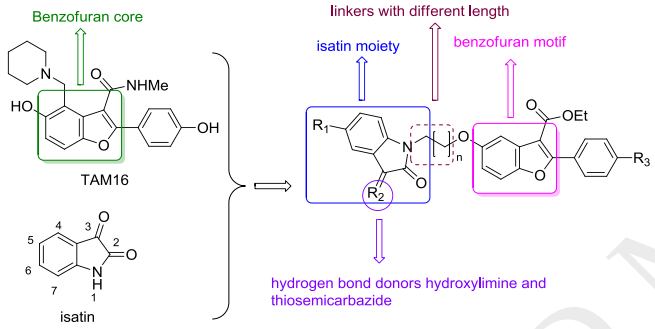
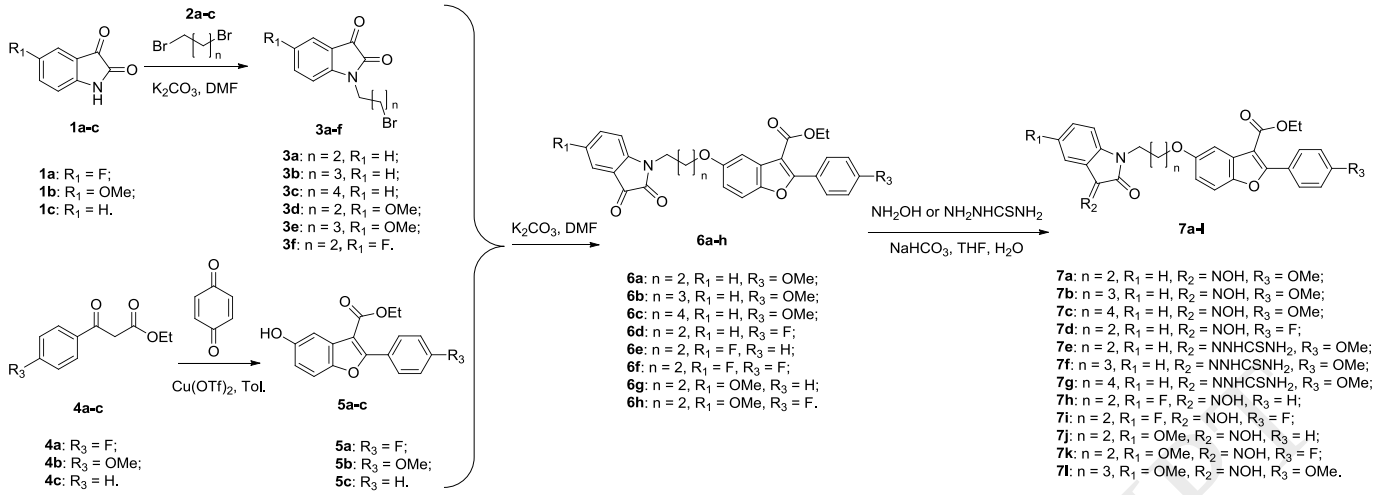


Fig. 2. Design strategy for benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids tethered *via* different linkers.

Based on the above facts and as a continuous program to develop novel anti-TB candidates, a series of novel benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**−**l** tethered *via* propylene, butylene and pentylene were designed, synthesized and evaluated for their anti-mycobacterial activity *in vitro* against both drug-susceptible and MDR-TB strains as well as cytotoxicity in VERO cells. The design strategy was depicted in Fig. 2.

All of the desired benzofuran-isatin-hydroxylamine/-thiosemicarbazide hybrids **7a**−**l** can be obtained by the synthetic route depicted in Scheme 1. 5-Methoxyisatin/5-fluoroisatin/isatin **1a**−**c** were alkylated with 1,3-dibromopropane, 1,4-dibromobutane, or 1,5-dibromopentane **2a**−**c** generated *N*-(3-bromopropyl/4-bromobutyl/5-bromopentyl)isatin derivatives **3a**−**f** (yield: 63%−81%) [15,16]. Cyclization of ethyl 3-(4-substituted phenyl)-3-oxopropanoate **4a**−**c** and benzoquinone with copper(II) triflate ($Cu(OTf)_2$) as catalyst provided benzofuran intermediates **5a**−**c** [13]. Treatment of isatin derivatives **3a**−**f** and benzofuran intermediate **5a**−**c** with potassium carbonate as base yielded the key intermediates **6a**−**h** (51%−77%). Finally, condensations of **6a**−**h** with hydroxylamine hydrochloride or thiosemicarbazide hydrochloride in the presence of sodium bicarbonate provided benzofuran-isatin-hydroxylamine/-thiosemicarbazide hybrids **7a**−**l** (19%−54%).



Scheme 1. Synthesis of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**–**l**.

The anti-mycobacterial activity of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**–**l**, precursor **6a** along with the first-line anti-TB agents isoniazid (INH) and rifampicin (RIF) against MTB $H_{37}Rv$ strains and cytotoxicity towards VREO cells were investigated (Table 1).

All benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**–**l** and precursor **6a** with MIC values ranging from <0.016 μg/mL to 0.156 μg/mL exhibited great anti-mycobacterial activities against MTB $H_{37}Rv$, and all hybrids except **7i** were more potent than the first-line anti-TB agents INH and RIF (MIC: 0.078 μg/mL), and nine of them **7a**, **7b**, **7e**–**h** and **7j**–**l** and precursor **6a** (MIC: <0.06 μg/mL) were comparable to the parent compound TAM16 (MIC: <0.06 μg/mL). The SAR revealed that for benzofuran-isatin-hydroxylimine hybrids, shorter linker between isatin and benzofuran was more favorable to the activity. In general, hybrids with thiosemicarbazide fragment were more potent than the corresponding hydroxylamine analoges, and introduction of -F at *para*-position of phenyl position (R_3) was detrimental to the activity.

All hybrids also displayed acceptable toxicological profiles with CC_{50} ranging from 8 μg/mL to 128 μg/mL. The structure-cytotoxicity relationship study indicated that compared with ketone, introduction of either hydroxylimine or thiosemicarbazide at C-3 position of isatin motif could increase the cytotoxicity as evidenced by ketone **6a** (CC_{50}: 512 μg/mL) was less toxic than hybrids **7a** (CC_{50}: 128 μg/mL) and **7e** (CC_{50}: 128 μg/mL), and the relative contribution order was ketone > hydroxylimine > thiosemicarbazide. Introduction of -F at C-5 position of isatin and *para*-position of phenyl position resulted in the increment of cytotoxicity. In general, hybrids with longer linkers also showed higher cytotoxicity.

Table 1
In vitro anti-mycobacterial activity and cytotoxicity of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**–**l**.

Compounds	MIC (μg/mL)	CC_{50} (μg/mL)	SI
6a	<0.016	512	>32000
7a	<0.016	128	>8000
7b	<0.016	32	>2000
7c	0.062	64	1032
7d	0.031	128	4129
7e	<0.016	128	>8000
7f	<0.016	8	>500
7g	<0.016	16	>1000
7h	<0.016	16	>1000
7i	0.156	32	205
7j	<0.016	128	>8000
7k	<0.016	64	>4000
7l	<0.016	64	>4000
TAM16	<0.016	>1024	>64000
INH	0.078	256	3282
RIF	0.078	512	6564

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

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 hybrids possessed high SI values (SI: ≥205), suggesting that this kind of hybrids hold excellent selectivity profiles.

Encouraged by their strong potency against the drug sensitive MTB H37Rv strain, the most active nine benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**, **7b**, **7e**–**h** and **7j**–**l** and precursor **6a** were selected for further evaluation for *in vitro* activity against two clinical MDR-TB isolates (resistant to INH and RIF). The *in vitro* activity of selected compounds against MDR-TB isolates was listed in Table 2.

Table 2
In vitro activity of selected hybrids against MDR-TB isolates.

Compounds	MIC (μg/mL)	
	MDR-TB-1	MDR-TB-2
6a	2.47	5.92
7a	1.59	1.83
7b	1.94	2.22
7e	0.90	0.94
7f	0.22	0.86
7g	1.41	1.83
7h	2.84	4.26
7j	2.26	3.89
7k	3.24	2.92
7l	12.96	14.18
TAM16	<0.016	<0.016
INH	>40	>40
RIF	>40	>40

All of the selected nine benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids **7a**, **7b**, **7e**–**h** and **7j**–**l** and precursor **6a** demonstrated considerable activity against the tested two clinical MDR-TB isolates with MIC values ranging from 0.22 μg/mL to 14.18 μg/mL. All hybrids were more potent than RIF and INH (MIC: >40 μg/mL), but were less active than TAM16 (MIC: <0.016 μg/mL) against the two clinical MDR-TB isolates. The SAR indicated that benzofuran-isatin-thiosemicarbazide hybrids were more active than the corresponding hydroxylimine analogs. Hybrid **7f** (MIC: 0.22 and 0.86 μg/mL, respectively) was found to be most active against the tested two clinical MDR-TB isolates, and it was >51-fold more potent than RIF and INH (MIC: >40 μg/mL).

In conclusion, a series of novel benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids were designed, synthesized and evaluated for their *in vitro* anti-TB activities against drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates as well as cytotoxicity. All benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids exhibited considerable *in vitro* anti-mycobacterial activities against the tested three MTB strains, and all of them also showed acceptable cytotoxicity. The most active hybrid **7f** was >4.8 and >51 folds more potent than the first line anti-TB agents RIF and INH against both drug-sensitive MTB $H_{37}Rv$ and MDR-TB isolates, respectively. The results demonstrated the potential utility of benzofuran-isatin-hydroxylimine/-thiosemicarbazide hybrids as anti-TB agents.

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