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Design, synthesis and *in vitro* anti-mycobacterial activities of homonuclear and heteronuclear bis-isatin derivatives

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ABSTRACT

A series of novel homonuclear and heteronuclear bis-isatin derivatives tethered through ethylene were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against MTB H37Rv and MDR-TB. All hybrids exhibited potential anti-mycobacterial activities against MTB H37Rv and MDR-TB with MIC ranging from 16 to 256 μg/mL. In particular, the heteronuclear bis-isatin **4i** (MIC: 25 and 16 μg/mL) was most active against MTB H37Rv and MDR-TB strains, and could act as a lead for further optimization.

1. Introduction

According to the latest World Health Organization (WHO) report, tuberculosis (TB) is the ninth leading cause of death throughout the world and the leading cause from a single infectious agent, ranking above HIV/AIDS [1]. There was around 10.4 million people fell ill with TB in the year 2016, resulting 1.67 million deaths. The new virulent forms of *Mycobacterium tuberculosis* (MTB) such as drug-resistant TB (DR-TB) and multidrug-resistant TB (MDR-TB) has already increased up to alarming level in the recent decades [2,3]. In 2016, there were 600,000 new cases with resistance to rifampicin (**RIF**), of which 490,000 had MDR-TB [1]. Although several drugs such as ciprofloxacin, amikacin, cycloserine and ethionamide have been approved as the second-line anti-TB agents for the treatment of TB infected patients, these agents are less effective and more toxic generally [4,5]. Therefore, it's imperative to develop novel anti-TB agents.

Isatin (1*H*-indole-2,3-dione, Fig. 1), found in many plants, such as *Isatis tinctoria*, *Calanthe discolor* and in *Couroupita guianensis*, is a versatile structure for chemical modification. Its derivatives exhibited a varied of biological properties such as antibacterial [6,7], anticancer [8], antimalarial [9] and anti-TB activities [10–14]. Moreover, some isatin-based drugs such as sunitinib and nintedanib have been approved for clinical use for the treatment of various diseases. The broad spectrum of biological activities combined with a wide range of structural modifications makes isatin an important prototype in drug development.

Dimer means a chemical structure formed from two similar or the same sub-units, and dimers have caused great interests in medicinal chemistry since they usually exhibited some unique properties such as enhanced biological activities when compared with the corresponding monomeric compounds [15]. It is worth to notice that some dimers such as dicoumarol (Fig. 1) has already used in clinical practice, demonstrating their potential in the development of new drugs [16].

Isatin dimers demonstrated endow with diverse biological profiles including anti-TB activity [17–19]. Our previous demonstrated that some propylene-tethered homonuclear isatin dimers displayed considerable anti-TB activity against both MTB H37Rv and MDR-TB, and the linkers of the two isatin motifs were crucial for the anti-TB activity of isatin derivatives [19]. Besides, our results also indicated that the ethylene linker is better than propylene linker [20–23].

Based on the above research results and as a continuous research program, a set of novel homonuclear and heteronuclear bis-isatin derivatives tethered through ethylene was designed, synthesized and assessed for their *in vitro* anti-mycobacterial activities against MTB H37Rv and MDR-TB in this study. To the best of our knowledge, this is the first attempt in making heteronuclear bis-isatin derivatives as potential anti-TB agents.

2. Results and discussion

Detailed synthetic route for homonuclear and heteronuclear bis-isatin derivatives **4a–i** was depicted in Scheme 1. Isatin was alkylated with 1,2-dibromoethane in the presence of potassium carbonate to provide the corresponding *N*-(2-bromoethyl)isatin **2** (yield: 49%) by

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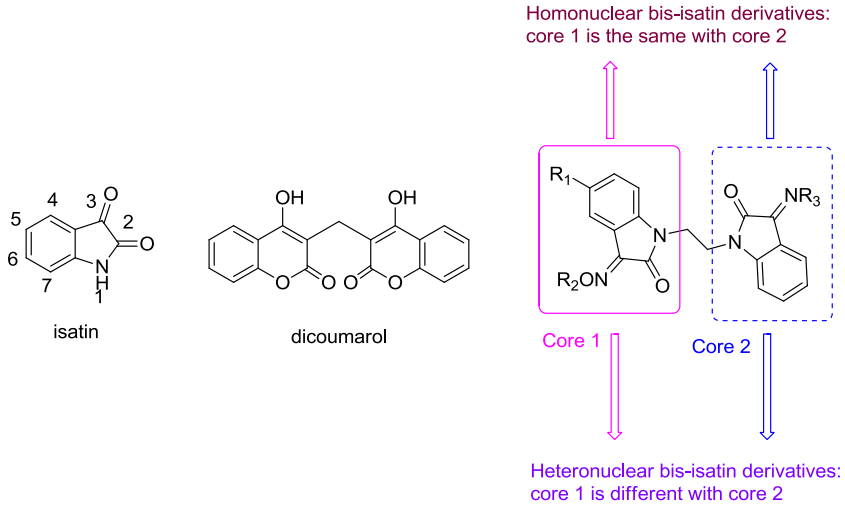
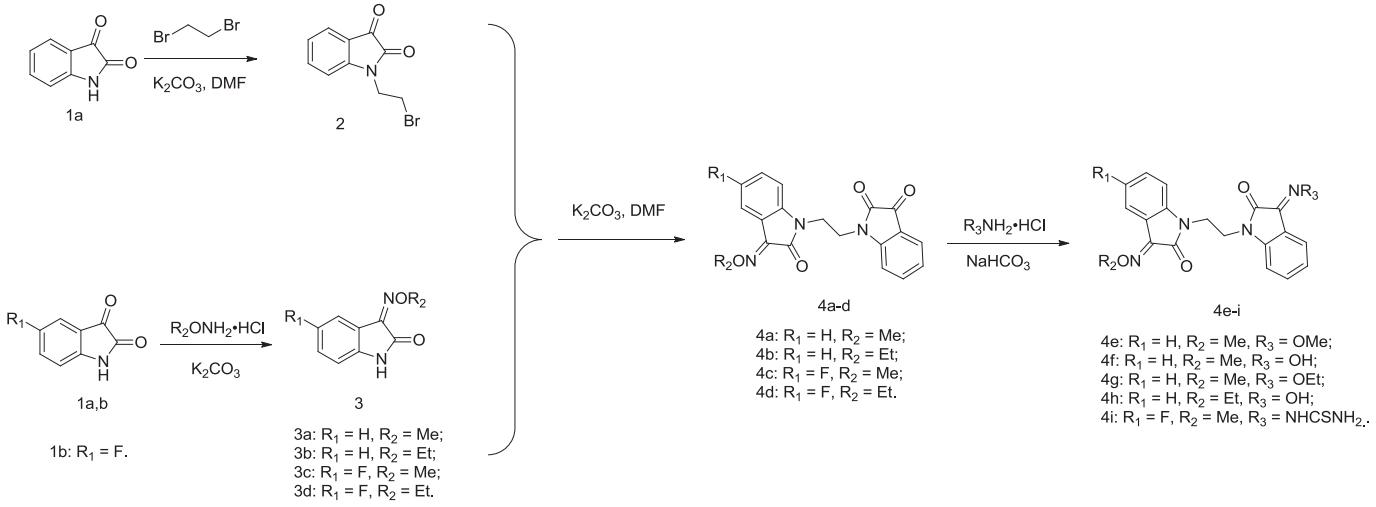


Fig. 1. Chemical structures of isatin, dicoumarol, homonuclear and heteronuclear bis-isatin derivatives.



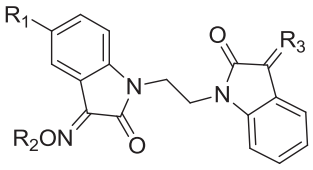
Scheme 1. Synthesis of bis-isatin derivatives **4a–i**.

literature methods [20,21]. The intermediates **3a–d** (yield: 57–69%) were obtained by reacting C-5 substituted isatins with methoxyamine or ethoxyamine hydrochloride in presence of potassium carbonate [22]. The precursors **2** and **3a–d** were utilized for the synthesis of desired heteronuclear bis-isatin derivatives **4a–d** (yield: 47–64%) with potassium carbonate as base [23]. Finally, condensations of targets **4a-c** with hydroxylamine or methoxyamine or ethoxyamine hydrochloride in the presence of sodium bicarbonate provided targets **4e–i** (23–79%) [19].

All homonuclear and heteronuclear bis-isatin derivatives **4a–i** were evaluated for their *in vitro* anti-mycobacterial activities against MTB H37Rv and MDR-TB strains (see Table 1). The MDR-TB strain was resistant to isoniazid (**INH**), rifampicin (**RIF**) and ethambutol (**EMB**). The minimum inhibitory concentration (MIC) is defined as the lowest concentration that inhibits the visible bacterial growth.

The results showed that all homonuclear and heteronuclear bis-isatin derivatives exhibited considerable anti-mycobacterial activities against MTB H37Rv and MDR-TB strains with MIC ranging from 16 to 256 μg/mL. The structure-activity relationship (SAR) revealed that substituents at C-3 and C-5 positions of isatin motifs have great influence on the activity: 1) for C-3 position, in general, the mono-substituted bis-isatin derivatives were more active than the bis-substituted and unsubstituted analogs, and the relative contribution of the substituents to the activity was as follows:

Table 1
Structures and anti-mycobacterial activities of hybrids **4a–i**.



Compd.	R_1	R_2	R_3	MIC (μg/mL)	
				MTB $H_{37}Rv$	MDR-TB
4a	H	NOMe	O	100	64
4b	H	NOEt	O	100	128
4c	F	NOMe	O	100	32
4d	F	NOEt	O	100	64
4e	H	NOMe	NOMe	100	128
4f	H	NOEt	NOH	200	256
4g	H	NOMe	NOEt	100	256
4h	H	NOEt	NOH	200	256
4i	F	NOMe	$NNHCSNH_2$	25	16
INH	–	–	–	0.05	> 128
RIF	–	–	–	0.39	64

$NNHCSNH_2$ > O > NOMe > NOEt > NOH; 2) introduction of electron-withdrawing group -F at C-5 position of isatin moiety was preferred. Interestingly, the heteronuclear bis-isatin derivative **4a** was more potent than the corresponding homonuclear analog **4e**, while heteronuclear bis-isatin **4g** was less active than **4e**.

It is worth to notice that the resistance index (RI) for almost all homonuclear and heteronuclear bis-isatin derivatives was around 1, and four of them was < 1, indicating they may bear novel action mechanism.

Among the synthesized homonuclear and heteronuclear bis-isatin derivatives, the heteronuclear bis-isatin **4e** (MIC: 25 and 16 μg/mL) was most active against MTB H37Rv and MDR-TB strains. It was 4 and > 8 folds more potent than the first-line anti-TB agents **RIF** (MIC: 64 μg/mL) and **INH** (MIC: > 128 μg/mL) against MDR-TB, and has the potential for further investigations.

In conclusion, a new class of novel homonuclear and heteronuclear bis-isatin derivatives tethered through ethylene and propylene was designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against MTB H37Rv and MDR-TB in this paper. The most active heteronuclear bis-isatin **4e** exhibited considerable activity against MDR-TB, and could act as a starting point for further optimization. The SAR was discussed, and $NNHCSNH_2$ at C-3 position as well as -F at C-5 position favored the activity. The enriched SAR may pave the way for further development of this kind of bis-isatin derivatives especially heteronuclear bis-isatin derivatives.

3. Experimental section

3.1. The general procedure for preparing targets *4a–i*

To a mixture of C-5 substituted isatins (10 mmol) and potassium carbonate (30 mmol), in water (10 mL) and THF (50 mL), methoxyamine or ethoxyamine hydrochloride (15 mmol) was added. The mixture was stirred at 50 °C for 12 h. After cooling to room temperature, the mixture was extracted with EA (100 mL*2). The combined organic layers were washed with H_2O (100 mL*2) and brine (100 mL) in sequence, and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give crude intermediates **3a-d** which were used directly without further purification.

To a mixture of intermediates **3a-d** (5 mmol) and potassium carbonate (20 mmol) in DMF (50 mL), intermediate **2** was added. The mixture was stirred at room temperature overnight. TLC (PE:EA = 1:1) indicated the reaction completed. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by silica gel chromatography eluted with PE:EA = 3:1 to give the desired products **4a-d**.

The mixture of **4a–c** (1 mmol), potassium carbonate (3 mmol), and methoxyamine or ethoxyamine hydrochloride (1.4 mmol) in water (10 mL) and THF (50 mL) was stirred at 50 °C for 12 h. After cooling to room temperature, the mixture was extracted with EA (100 mL*2). The combined organic layers were washed with H_2O (100 mL*2) and brine (100 mL) in sequence, and dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give a residue which was purified by silica gel chromatography eluted with PE:EA = 2:1 to give the desired products **4e-i**.

3.1.1. 1-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)indoline-2,3-dione (*4a*)

Yellow solid, yield: 51%. Mp: 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.95–4.09 (4H, m, $-CH_2CH_2-$), 4.17, 4.20 (3H, s, NOMe), 7.05–7.16 (4H, m, Ar−H), 7.42 (1H, t, Ar−H), 7.53 (1H, d, Ar−H), 7.63 (1H, t, Ar−H), 7.84 (1H, d, Ar−H). ESI-MS *m/z*: 350 $[M + H]^+$.

3.1.2. 1-(2-(3-(ethoxyimino)-2-oxoindolin-1-yl)ethyl)indoline-2,3-dione (*4b*)

Yellow solid, yield: 56%. Mp: 128–130 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (3H, t, $NOCH_2\underline{CH_3}$), 3.98–4.04 (4H, m, $-CH_2CH_2-$), 4.41 (2H, q, $NO\underline{CH_2}CH_3$), 7.08–7.15 (4H, m, Ar−H), 7.41 (1H, t, Ar−H), 7.53 (1H, d, Ar−H), 7.62 (1H, t, Ar−H), 7.86 (1H, d, Ar−H). ESI-MS *m/z*: 364 $[M + H]^+$.

3.1.3. 1-(2-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)indoline-2,3-dione (*4c*)

Yellow solid, yield: 67%. Mp: 177–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.97–4.18 (4H, m, $-CH_2CH_2-$), 4.21, 4.22 (3H, s, NOMe), 7.12–7.16 (3H, m, Ar−H), 7.26–7.31 (1H, m, Ar−H), 7.63 (1H, d, Ar−H), 7.64–7.66 (2H, m, Ar−H). ESI-MS *m/z*: 368 $[M + H]^+$.

3.1.4. 1-(2-(3-(ethoxyimino)-5-fluoro-2-oxoindolin-1-yl)ethyl)indoline-2,3-dione (*4d*)

Yellow solid, yield: 44%. Mp: 136–138 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (3H, t, $NOCH_2\underline{CH_3}$), 3.93–4.02 (4H, m, $-CH_2CH_2-$), 4.48 (2H, q, $NO\underline{CH_2}CH_3$), 7.07–7.37 (4H, m, Ar−H), 7.41–7.65 (3H, m, Ar−H). ESI-MS *m/z*: 382 $[M + H]^+$.

3.1.5. 1,1′-(ethane-1,2-diyl)bis(3-(methoxyimino)indolin-2-one) (*4e*)

Yellow solid, yield: 71%. Mp: 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96–4.00 (4H, m, $-CH_2CH_2-$), 4.09, 4.17 (6H, s, 2 × NOMe), 6.97–7.09 (4H, m, Ar−H), 7.40–7.46 (3H, m, Ar−H), 7.84 (1H, d, Ar−H). ESI-MS *m/z*: 379 $[M + H]^+$.

3.1.6. 3-(hydroxyimino)-1-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethyl) indolin-2-one (*4f*)

Yellow solid, yield: 79%. Mp: 193–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98–4.00 (4H, m, $-CH_2CH_2-$), 4.09, 4.17 (3H, s, NOMe), 7.02–7.08 (4H, m, Ar−H), 7.37–7.39 (2H, m, Ar−H), 7.84 (1H, d, Ar−H), 7.94 (1H, d, Ar−H), 13.36 (1H, s, NOH). ESI-MS *m/z*: 365 $[M + H]^+$.

3.1.7. 3-(ethoxyimino)-1-(2-(3-(methoxyimino)-2-oxoindolin-1-yl)ethyl) indolin-2-one (*4g*)

Yellow solid, yield: 45%. Mp: 133–135 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (3H, t, $NOCH_2\underline{CH_3}$), 3.96–4.00 (4H, m, $-CH_2CH_2-$), 4.09, 4.17 (3H, s, NOMe), 4.42 (2H, q, $NO\underline{CH_2}CH_3$), 7.03–7.08 (4H, m, Ar−H), 7.40–7.46 (3H, m, Ar−H), 7.85 (1H, t, Ar−H). ESI-MS *m/z*: 393 $[M + H]^+$.

3.1.8. 3-(ethoxyimino)-1-(2-(3-(hydroxyimino)-2-oxoindolin-1-yl)ethyl) indolin-2-one (*4h*)

Yellow solid, yield: 57%. Mp: 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (3H, t, $NOCH_2\underline{CH_3}$), 3.98–4.02 (4H, m, $-CH_2CH_2-$), 4.46 (2H, q, $NO\underline{CH_2}CH_3$), 7.02–7.12 (4H, m, Ar−H), 7.40–7.43 (2H, m, Ar−H), 7.81 (1H, d, Ar−H), 7.92 (1H, d, Ar−H), 13.42 (1H, s, NOH). ESI-MS *m/z*: 379 $[M + H]^+$.

3.1.9. 2-(1-(2-(5-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)ethyl)-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (*4i*)

Yellow solid, yield: 23%. Mp: 206–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.99–4.03 (4H, m, $-CH_2CH_2-$), 4.18 (3H, s, NOMe), 7.03 (1H, d, Ar−H), 7.12–7.16 (2H, m, Ar−H), 7.28–7.34 (2H, m, Ar−H), 7.64–7.68 (2H, m, Ar−H), 8.75, 9.09 (1H, s, $NNHCS\underline{NH_2}$), 12.18 (1H, s, $N\underline{NH}CSNH_2$). ESI-MS *m/z*: 441 $[M + H]^+$.

3.2. MIC determination

The bis-isatin derivatives **4a–i** along with the references **RIF** and **INH** were evaluated for their *in vitro* activities against MTB H37Rv and MDR-TB *via* rapid direct susceptibility test technique [11,12]. The wells of a sterile 48-well plate were filled with 100 mL two-fold diluted tested compounds and 100 mL MTB H37Rv or MDR-TB suspension containing 4×10^{-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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2018.03.018.

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