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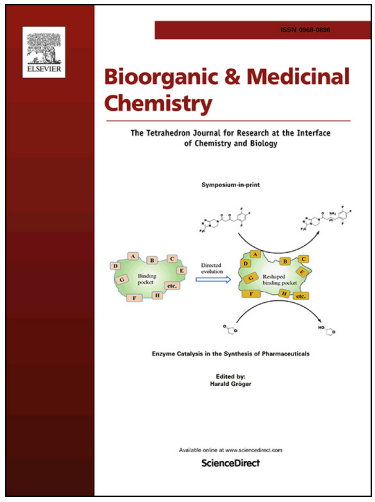

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Benzofuran-isatin hybrids tethered *via* different length alkyl linkers and their *in vitro* anti-mycobacterial activities

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Abstract: A series of novel benzofuran-isatin hybrids **6a-m** tethered through different length alkyl linkers propylene, butylene, pentylene and hexylene were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against both drug-susceptible and multi-drug resistant (MDR) *Mycobacterium tuberculosis* (MTB) and cytotoxicity towards VERO cells. All hybrids with acceptable cytotoxicity in VERO cells (CC_{50}: 64->1,024 μg/mL) also exhibited considerable anti-mycobacterial activities against both drug-susceptible and MDR-MTB strains with MIC in a range of 0.125 to 4 μg/mL. The SAR indicated that the length of the linker played a pivotal role on the activity, and the longer linker could enhance the activity. The most active hybrid **6d** (MIC: 0.125 and 0.125 μg/mL) was comparable to or better than rifampicin (MIC: 0.5 μg/mL) and isoniazid (MIC: 0.06 μg/mL) against MTB $H_{37}Rv$, and was ≥256 folds more potent than rifampicin (MIC: 64 μg/mL) and isoniazid (MIC: >128 μg/mL) against MDR-MTB strain, but was less active than **TAM16** (MIC: <0.06 μg/mL against the tested two strains). The hybrid **6d** also showed low cytotoxicity towards VERO cell (CC_{50}: 128 μg/mL), but it was inferior to **TAM16** in metabolic stability and *in vivo* pharmacokinetic profiles.

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

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1. Introduction

Tuberculosis (TB), predominantly caused by *Mycobacterium tuberculosis* (MTB), is an infectious disease which has been the ninth leading cause of death globally [1,2]. Specific targets set in the End TB Strategy include a 90% reduction in TB deaths and a 80% reduction in TB incidence (new cases per year) by 2030, compared with 2015 [1]. However, the widely spread of drug-resistant TB (DR-TB), especially multi-drug resistant TB (MDR-TB), is one of the major obstacles for the treatment of the global TB epidemic [3,4]. Therefore, it is imperative to develop new agents with great potency against both susceptible and MDR MTB infections.

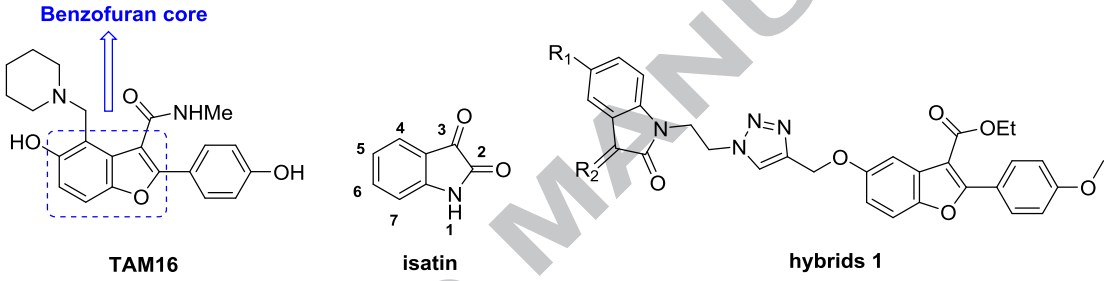


Figure 1 Chemical structures of **TAM16**, isatin and 1,2,3-triazole tethered benzofuran-isatin hybrids **1**

Benzofuran-based derivatives possess various biological properties including anti-TB activity [5-13]. Benzofuran derivative **TAM16** (**Figure 1**) demonstrated promising activity against 38 MTB isolates including MDR-MTB *in vitro*, and exhibited excellent *in vivo* pharmacological properties, indicating its potential for the treatment of TB [13]. Isatin (indoline-2,3-dione) (**Figure 1**) also endowed with promising anti-TB activities [14-17], and incorporation of the pharmacophores of **TAM16** and isatin into one molecular may provide more effective anti-TB candidates [18,19].

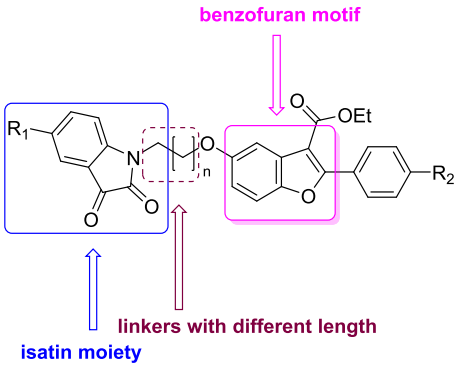
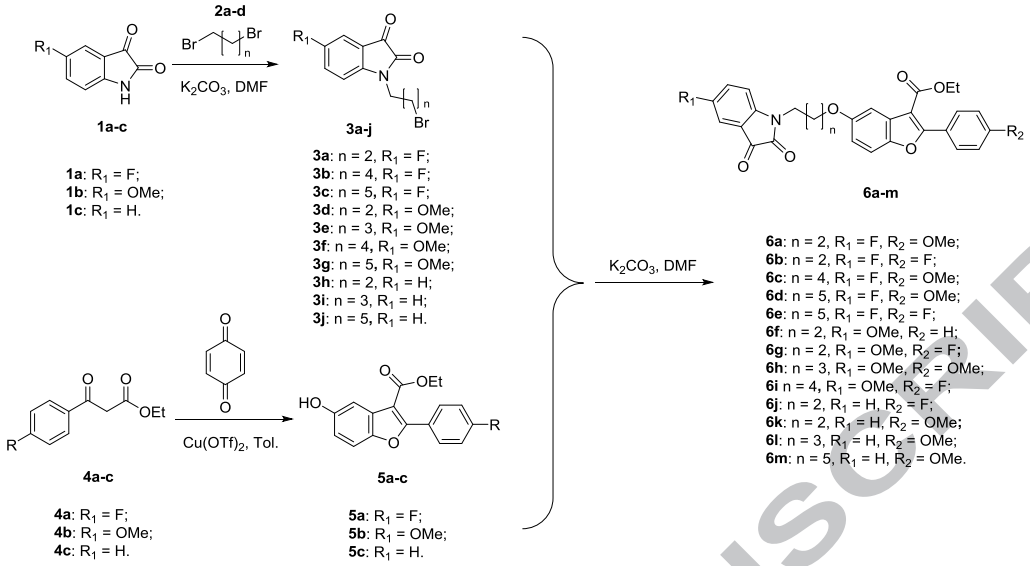


Figure 2 The design strategy for benzofuran-isatin hybrids

Our previous studies indicated the 1,2,3-triazole tethered benzofuran-isatin hybrids **1** possess great potency against both drug-susceptible and MDR MTB strains [20]. In this paper, a series of novel benzofuran-isatin hybrids **6a-m** tethered through different length linkers propylene, butylene, pentylene and hexylene were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against both drug-susceptible and MDR MTB strains. We intend to investigate the influence of the linkers between the benzofuran and isatin motifs on their anti-mycobacterial activities in this study.

2 Results and discussion

All of the desired benzofuran-isatin hybrids **6a-m** were prepared by the synthetic route depicted in **Scheme 1**. Alkylation of 5-substituted isatins **1a-c** with 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane or 1,6-dibromohexane provided *N*-(3-bromopropyl/4-bromobutyl/5-bromopentyl/6-bromohexyl)isatin derivatives **3a-j** (yield: 63-87%) [15,16]. The benzofuran intermediates **5a-c** was obtained by cyclization of ethyl 3-(4-substituted phenyl)-3-oxopropanoates **4a-c** and benzoquinone with copper (II) triflate ($Cu(OTf)_2$) as catalyst [12]. Treatment of isatin derivatives **3a-j** and benzofuran intermediates **5a-c** with potassium carbonate as base generated the desired benzofuran-isatin hybrids **6a-m** (58-83%).



Scheme 1 Synthesis of benzofuran-isatin hybrids **6a-m**

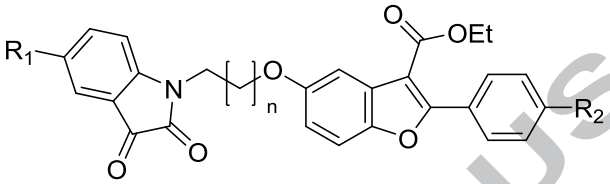
The minimum inhibitory concentration (MIC) of the desired benzofuran-isatin hybrids **6a-m** along with the references **TAM16, RIF** and **INH** against MTB $H_{37}Rv$ and MDR-MTB strains was listed in **Table 1**.

All hybrids **6a-m** tethered *via* propylene, butylene, pentylene and hexylene displayed considerable anti-mycobacterial activities against the tested MTB $H_{37}Rv$ and MDR-MTB strains with MIC in a range of 0.125 to 4 μg/mL, but were less active than **TAM16** (MIC: <0.06 μg/mL). The SAR results suggested that the activity was influenced greatly by the substituents at C-5 position of isatin moiety, and hybrids with electron-withdrawing -F at this position showed higher activity, while electron-donating -OMe reduced the activity when compared with unsubstituted analogs. For R position, electron-donating -OMe was favorable to the activity, while -F decreased the activity. The length of the linker played a pivotal role on the activity, and the longer linker could enhance the activity, and the contribution order was hexylene > pentylene > butylene > propylene.

Among the synthesized benzofuran-isatin hybrids, hybrid **6d** (MIC: 0.125 and 0.125 μg/mL) was found to be the most active compound against MTB $H_{37}Rv$ and MDR-MTB strains, which was comparable to or better than **RIF** (MIC: 0.5 μg/mL) and **INH** (MIC: 0.06 μg/mL) against MTB $H_{37}Rv$, and was ≥512 folds more potent than **RIF** (MIC: 64 μg/mL) and **INH** (MIC: >128 μg/mL) against MDR-MTB strain,

but was less active than **TAM16** (MIC: <0.06 μg/mL) against the tested two strains. The resistance index values (RI, $MIC_{(MDR\text{-}TB)}/MIC_{(MTB\ H37Rv)}$) for a significant part of the synthesized benzofuran-isatin hybrids **6a-m** were around 1, indicating that these hybrids may have novel action mechanism.

Table 1 Structure, anti-mycobacterial activity and cytotoxicity of benzofuran-isatin hybrids **6a-m**



6a-m

Compd.	R_1	R_2	n	MIC (μg/mL)		CC_{50} (μM)	SI[b]
				MTB H_{37}Rv	MDR-MTB[a]		
6a	F	OMe	2	0.5	0.25	256	512
6b	F	F	2	1	1	512	512
6c	F	OMe	4	0.25	0.25	128	512
6d	F	OMe	5	0.125	0.125	128	1,024
6e	F	F	5	0.25	0.25	512	2,048
6f	OMe	H	2	0.25	0.25	256	1,024
6g	OMe	F	2	1	2	128	128
6h	OMe	OMe	3	2	4	64	32
6i	OMe	F	4	0.5	1	128	256
6j	H	F	2	2	1	256	128

6k	H	OMe	2	1	1	64	64
6l	H	OMe	3	0.5	1	>1,024	>2,048
6m	H	OMe	5	0.25	0.125	256	1,024
TAM16	-	-	-	<0.06	<0.06	>1,024	>16,384
INH	-	-	-	0.06	>128	256	4,096
RIF	-	-	-	0.5	64	512	1,024

[a]MDR-MTB: resistant to ethambutol, **INH** and **RIF**

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

All hybrids also showed low cytotoxicity with CC_{50} ranging from 64 to >1,024 μg/mL, and possessed moderate to high selectivity index (SI: $CC_{50}/MIC_{MTB\ H37Rv}$) values (32->2,048). The structure-cytotoxicity relationship study indicated that introduction of -F or -OMe at C-5 position of isatin motif could reduce the cytotoxicity when compared with the unsubstituted analogs. The most active hybrid **6d** (CC_{50}: 128 μg/mL) also showed acceptable cytotoxicity against VERO cells. The SI value of the most active hybrid **6d** was over 1,024, and was comparable to that of **RIF** and **INH** (SI values: 1,024 and 4,096, respectively), demonstrating that hybrid **6d** possess an excellent selectivity profile.

Table 2 The metabolic stability and *in vivo* pharmacokinetic values of hybrid **6d** 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)
6d	31.6	1,218	2.48	1.76	3,682
TAM16	>60	6,830	8.59	3.12	31,756

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

C_{max}: the peak concentration;

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

AUC: the *area under* the *curve*.

The most active hybrid **6d** was selected for the further investigation on metabolic stability and *in vivo* pharmacokinetics in mice (25 mg/kg, single oral administration), and the results were listed in **Table 2**. Compared with the parent **TAM16** ($t_{1/2}$: >60 min), hybrid **6d** showed much lower metabolic stability ($t_{1/2}$: 31.6 min). Pharmacokinetic parameters of hybrid **6d** was also inferior to the parent **TAM16** in terms of C_{max} (1,218 ng/mL *vs* 6,830 ng/mL), $t_{1/2}$ (2.48 h *vs* 8.59 h), T_{max} (1.76 h *vs* 3.12 h) and $AUC_{0\text{-}inf}$ (3,682 ng•h/mL *vs* 3,1756 ng•h/mL). Thus, hybrid **6d** still needs to be further modification.

3 Conclusions

In conclusion, thirteen novel benzofuran-isatin hybrids **6a-m** tethered *via* different length linkers propylene, butylene, pentylene and hexylene were designed, synthesized and evaluated for their *in vitro* anti-mycobacterial activities against MTB $H_{37}Rv$ and MDR-MTB strains as well as cytotoxicity in VERO cells. All hybrids exhibited considerable *in vitro* anti-mycobacterial activities and low cytotoxicity. In spite of the *in vitro* activity, metabolic stability and pharmacokinetic profiles for the most active hybrid **6d** were inferior to **TAM16**, the anti-mycobacterial activity of hybrid **6d** was comparable to or better than the first-line anti-TB agents **RIF** and **INH** against both drug-sensitive MTB $H_{37}Rv$ and MDR-MTB strains. Therefore, hybrid **6d** could act as a starting point for further optimization to improve the activity, metabolic stability and pharmacokinetic profiles.

4. Experimental section

N-(3-bromopropyl/4-bromobutyl/5-bromopentyl/6-bromohexyl)isatin derivatives **3a-j** (yield: 63-87%) [15,16] and ethyl 2-(4-substituted

phenyl)-5-(prop-2-yn-1-yloxy)benzofuran-3-carboxylate **5a-c** were prepared *via* literature methods [12,13].

A mixture of *N*-(3-bromopropyl/4-bromobutyl/5-bromopentyl/6-bromohexyl)isatin derivatives **3a-j** (1.0 mmol), ethyl 2-(4-substituted phenyl)-5-(prop-2-yn-1-yloxy)benzofuran-3-carboxylate **5a-c** (1.0 mmol) and K_2CO_3 (3.0 mmol) in DMF (50 mL) was stirred at room temperature for 48 h. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with PE:EA=1:1 to give the desired benzofuran-isatin hybrids **6a-m**.

4.1. ethyl 5-(3-(5-fluoro-2,3-dioxoindolin-1-yl)propoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6a**)

Yellow solid, yield: 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 4.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.11 (2H, t, J = 4.0 Hz, -CH_2-), 3.85 (3H, s, OCH_3), 3.89 (2H, t, J = 4.0 Hz, -CH_2-), 4.12 (2H, t, J = 4.0 Hz, -CH_2-), 4.33 (2H, q, J = 4.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.94 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.09 (2H, d, J = 4.0 Hz, Ar-H), 7.25-7.28 (1H, m, Ar-H), 7.39 (1H, d, J = 4.0 Hz, Ar-H), 7.45-7.50 (2H, m, Ar-H), 7.57 (1H, d, J = 8.0 Hz, Ar-H), 7.94-7.97 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.32, 163.62, 161.44, 161.26, 158.77, 157.67, 156.02, 148.32, 147.45, 131.41, 127.93, 124.48, 124.24, 121.70, 119.04, 118.96, 114.50, 112.42, 112.31, 111.98, 111.74, 107.69, 105.75, 100.01, 66.23, 60.84, 55.87, 37.35, 27.05, 14.49. ESI-MS m/z: 540 $[M+Na]^+$.

4.2. ethyl 5-(3-(5-fluoro-2,3-dioxoindolin-1-yl)propoxy)-2-(4-fluorophenyl)benzofuran-3-carboxylate (**6b**)

Yellow solid, yield: 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.12 (2H, t, J = 4.0 Hz, -CH_2-), 3.89 (2H, t, J = 4.0 Hz, -CH_2-), 4.12 (2H, t, J = 4.0 Hz, -CH_2-), 4.32 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.98 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.24-7.28 (1H, m, Ar-H), 7.36-7.41 (2H, m, Ar-H), 7.44-7.42 (2H, m, Ar-H), 7.60 (1H, d, J = 12.0 Hz, Ar-H), 8.01-8.04 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.33, 164.89, 163.37, 162.42, 160.04, 158.76, 157.67, 156.12, 148.58, 147.43, 132.36, 132.27, 127.60, 126.01, 125.98, 124.48, 124.24, 119.02, 118.95,

115.91, 115.69, 115.05, 112.53, 112.48, 112.41, 111.97, 111.73, 108.93, 105.73, 66.24, 60.99, 37.34, 27.04, 14.40. ESI-MS m/z: 528 $[M+Na]^+$.

4.3. ethyl 5-((5-(5-fluoro-2,3-dioxoindolin-1-yl)pentyl)oxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6c**)

Yellow solid, yield: 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.51-1.54 (2H, m, -CH_2-), 1.66-1.70 (2H, m, -CH_2-), 1.78-1.82 (2H, m, -CH_2-), 3.68 (2H, t, J = 8.0 Hz, -CH_2-), 3.85 (3H, s, OCH_3), 4.01 (2H, t, J = 8.0 Hz, -CH_2-), 4.32 (2H, q, J = 4.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.96 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.09 (2H, d, J = 8.0 Hz, Ar-H), 7.41-7.45 (2H, m, Ar-H), 7.49-7.57 (2H, m, Ar-H), 7.57 (1H, d, J = 8.0 Hz, Ar-H), 7.94-7.97 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.40, 163.64, 161.43, 161.22, 158.61, 157.69, 156.25, 148.25, 147.42, 131.41, 127.97, 124.59, 124.35, 121.73, 118.89, 114.45, 114.16, 112.62, 112.55, 112.29, 112.03, 111.79, 107.69, 105.67, 68.36, 60.81, 55.87, 28.83, 26.89, 23.31, 14.47. ESI-MS m/z: 568 $[M+Na]^+$.

4.4. ethyl 5-((6-(5-fluoro-2,3-dioxoindolin-1-yl)hexyl)oxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6d**)

Yellow solid, yield: 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.35-1.49 (4H, m, 2×-CH_2-), 1.59-1.66 (2H, m, -CH_2-), 1.70-1.77 (2H, m, -CH_2-), 3.66 (2H, t, J = 8.0 Hz, -CH_2-), 3.85 (3H, s, OCH_3), 4.00 (2H, t, J = 8.0 Hz, -CH_2-), 4.32 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.94 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.07 (2H, d, J = 8.0 Hz, Ar-H), 7.19-7.22 (1H, m, Ar-H), 7.40-7.43 (2H, m, Ar-H), 7.49-7.54 (2H, m, Ar-H), 7.93-7.96 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.38, 163.62, 161.40, 161.17, 160.07, 158.53, 157.67, 156.23, 148.22, 147.42, 131.37, 127.97, 124.56, 124.32, 121.73, 118.85, 114.43, 114.11, 112.54, 112.47, 112.21, 111.99, 111.75, 107.66, 105.64, 68.40, 60.78, 55.83, 49.07, 29.08, 27.13, 26.45, 25.73, 14.44. ESI-MS m/z: 582 $[M+Na]^+$.

4.5. ethyl ethyl 5-((6-(5-fluoro-2,3-dioxoindolin-1-yl)hexyl)oxy)-2-(4-fluorophenyl)benzofuran-3-carboxylate (**6e**)

Yellow solid, yield: 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.39-1.51 (4H, m, 2×-CH_2-), 1.62-1.65 (2H, m, -CH_2-), 1.73-1.77 (2H, m, -CH_2-), 3.68 (2H, t, J = 4.0 Hz, -CH_2-), 4.03 (2H, t, J = 4.0 Hz, -CH_2-), 4.32 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 7.00 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.21-7.24 (2H, m, Ar-H), 7.36-7.54 (5H, m, Ar-H), 7.58 (1H, d, J = 8.0 Hz, Ar-H), 8.01-8.05 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.39, 163.39, 162.41, 160.00, 158.57, 157.68, 156.36, 148.51, 147.45, 132.36, 132.27, 127.65, 126.04, 124.58, 124.34, 115.90, 115.68, 115.06, 112.58, 112.51, 108.94, 105.63, 100.00, 68.45, 60.96, 29.05, 27.13, 26.44, 25.73, 14.39. ESI-MS m/z: 570 $[M+Na]^+$.

4.6. ethyl 5-(3-(5-methoxy-2,3-dioxoindolin-1-yl)propoxy)-2-phenylbenzofuran-3-carboxylate (**6f**)

Yellow solid, yield: 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.24-2.27 (2H, m, -CH_2-), 3.85 (3H, s, OCH_3), 4.00 (2H, t, J = 8.0 Hz, -CH_2-), 4.13 (2H, t, J = 8.0 Hz, -CH_2-), 4.42 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.90 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 6.99-7.02 (3H, m, Ar-H), 7.07-7.11 (1H, m, Ar-H), 7.38 (1H, d, J = 12.0 Hz, Ar-H), 7.50-7.55 (2H, m, Ar-H), 7.59-7.61 (1H, m, Ar-H), 8.00-8.02 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.46, 164.16, 161.77, 161.21, 160.70, 158.39, 155.52, 151.08, 148.70, 138.46, 131.14, 128.22, 125.42, 123.91, 123.71, 122.10, 117.58, 113.89, 113.52, 111.57, 110.22, 109.86, 107.78, 105.94, 65.59, 60.55, 55.40, 37.49, 27.43, 14.35. ESI-MS m/z: 522 $[M+Na]^+$.

4.7. ethyl 5-((5-(5-fluoro-2,3-dioxoindolin-1-yl)pentyl)oxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6g**)

Yellow solid, yield: 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.12 (2H, t, J = 4.0 Hz, -CH_2-), 3.76 (3H, s, OCH_3), 3.87 (2H, t, J = 4.0 Hz, -CH_2-), 4.12 (2H, t, J = 4.0 Hz, -CH_2-), 4.32 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.98 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.14-7.20 (3H, m, Ar-H), 7.37-7.42 (3H, m, Ar-H), 7.60 (1H, d, J = 12.0 Hz, Ar-H), 8.01-8.05 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.20, 163.37, 160.06, 158.73, 156.14, 156.10, 148.60, 145.02, 132.37,

132.28, 127.61, 124.26, 118.55, 115.92, 115.70, 115.06, 112.53, 112.14, 109.66, 105.78, 99.99, 66.30, 60.98, 56.34, 37.28, 27.12, 14.41. ESI-MS m/z: 540 $[M+Na]^+$.

4.8. ethyl 5-(4-(5-methoxy-2,3-dioxoindolin-1-yl)butoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6h**)

Yellow solid, yield: 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.80-1.84 (4H, m, 2×-CH_2-), 3.71-3.76 (5H, m, -CH_2- and OCH_3), 3.85 (3H, s, OCH_3), 4.06 (2H, t, J = 8.0 Hz, -CH_2-), 4.33 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.96 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.08-7.16 (4H, m, Ar-H), 7.22-7.25 (1H, m, Ar-H), 7.42 (1H, d, J = 4.0 Hz, Ar-H), 7.55 (1H, d, J = 8.0 Hz, Ar-H), 7.94-7.97 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.19, 163.63, 161.43, 161.24, 158.62, 156.13, 148.27, 144.92, 131.40, 127.96, 124.28, 121.72, 118.45, 114.46, 114.16, 112.29, 112.23, 109.69, 107.68, 105.79, 68.03, 60.82, 56.33, 55.86, 26.47, 23.96, 14.46. ESI-MS m/z: 566 $[M+Na]^+$.

4.9. ethyl 2-(4-fluorophenyl)-5-((5-(5-methoxy-2,3-dioxoindolin-1-yl)pentyl)oxy)benzofuran-3-carboxylate (**6i**)

Yellow solid, yield: 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.50-1.55 (2H, m, -CH_2-), 1.65-1.71 (2H, m, -CH_2-), 1.76-1.82 (2H, m, -CH_2-), 4.68 (2H, t, J = 8.0 Hz, -CH_2-), 3.85 (3H, s, OCH_3), 4.01 (2H, t, J = 8.0 Hz, -CH_2-), 4.32 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.96 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.08-7.11 (2H, m, Ar-H), 7.22-7.25 (1H, m, Ar-H), 7.41-7.45 (2H, m, Ar-H), 7.49-7.57 (2H, m, Ar-H), 7.94-7.97 (2H, m, Ar-H). ESI-MS m/z: 568 $[M+Na]^+$.

4.10. ethyl 5-(3-(2,3-dioxoindolin-1-yl)propoxy)-2-(4-fluorophenyl)benzofuran-3-carboxylate (**6j**)

Yellow solid, yield: 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.13 (2H, t, J = 8.0 Hz, -CH_2-), 3.89 (2H, t, J = 4.0 Hz, -CH_2-), 4.12 (2H, t, J = 4.0 Hz, -CH_2-), 4.33 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.98 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.11 (1H, t, J = 4.0 Hz, Ar-H), 7.24 (1H, d, J = 8.0 Hz, Ar-H), 7.37-7.42

(3H, m, Ar-H), 7.54-7.64 (3H, m, Ar-H), 8.01-8.05 (2H, m, Ar-H). ESI-MS m/z: 510 $[M+Na]^+$.

4.11. ethyl 5-(3-(2,3-dioxoindolin-1-yl)propoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6k**)

Yellow solid, yield: 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 4.0 Hz, $CO_2CH_2\underline{CH_3}$), 2.13 (2H, t, J = 4.0 Hz, -CH_2-), 3.86 (3H, s, OCH_3), 3.88 (2H, t, J = 4.0 Hz, -CH_2-), 4.12 (2H, t, J = 4.0 Hz, -CH_2-), 4.34 (2H, q, J = 4.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.96 (1H, dd, J = 4.0, 8.0 Hz, Ar-H), 7.08-7.13 (3H, m, Ar-H), 7.24 (1H, d, J = 8.0 Hz, Ar-H), 7.40 (1H, d, J = 8.0 Hz, Ar-H), 7.54-7.63 (3H, m, Ar-H), 7.96 (2H, d, J = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.95, 163.63, 161.44, 161.26, 158.74, 156.03, 151.23, 148.32, 138.52, 131.41, 127.93, 124.87, 123.53, 121.71, 118.09, 114.51, 114.18, 112.31, 111.06, 107.70, 105.77, 66.24, 60.84, 55.87, 37.28, 27.14, 14.51. ESI-MS m/z: 522 $[M+Na]^+$.

4.12. ethyl 5-(4-(2,3-dioxoindolin-1-yl)butoxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6l**)

Yellow solid, yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (3H, t, J = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.86-1.94 (4H, m, 2×-CH_2-), 3.78 (2H, t, J = 8.0 Hz, -CH_2-), 3.84 (3H, s, OCH_3), 4.04 (2H, t, J = 4.0 Hz, -CH_2-), 4.38 (2H, q, J = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.85-6.91 (2H, m, Ar-H), 6.94-6.89 (2H, m, Ar-H), 7.06 (1H, t, J = 8.0 Hz, Ar-H), 7.34 (1H, d, J = 12.0 Hz, Ar-H), 7.48 (1H, d, J = 4.0 Hz, Ar-H), 7.50-7.53 (1H, m, Ar-H), 7.95-7.99 (2H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.51, 164.17, 161.63, 161.17, 158.22, 155.86, 150.86, 148.54, 138.39, 131.11, 128.15, 125.40, 123.67, 122.11, 117.57, 113.94, 113.50, 111.47, 110.22, 107.75, 105.86, 67.72, 60.51, 55.39, 29.91, 26.63, 24.13, 14.35. ESI-MS m/z: 536 $[M+Na]^+$.

4.13. ethyl 5-((6-(2,3-dioxoindolin-1-yl)hexyl)oxy)-2-(4-methoxyphenyl)benzofuran-3-carboxylate (**6m**)

Yellow solid, yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, t, *J* = 8.0 Hz, $CO_2CH_2\underline{CH_3}$), 1.48-1.56 (4H, m, 2×-CH_2-), 1.76-1.87 (4H, m, 2×-CH_2-), 3.76 (2H, t, *J* = 8.0 Hz, -CH_2-), 3.88 (3H, s, OCH_3), 4.05 (2H, t, *J* = 8.0 Hz, -CH_2-), 4.43 (2H, q, *J* = 8.0 Hz, $CO_2\underline{CH_2}CH_3$), 6.91-6.94 (2H, m, Ar-H), 7.02 (2H, d, *J* = 8.0 Hz, Ar-H), 7.12 (1H, t, *J* = 4.0 Hz, Ar-H), 7.40 (1H, d, *J* = 8.0 Hz, Ar-H), 7.53 (1H, d, *J* = 4.0 Hz, Ar-H), 7.57-7.63 (2H, m, Ar-H), 7.99-8.02 (2H, m, Ar-H). ESI-MS m/z: 565 $[M+Na]^+$.

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Graphical Abstract

Benzofuran-isatin hybrids tethered *via* different length alkyl linkers and their *in vitro* anti-mycobacterial activities

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