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Benzylsulfanyl benzo-heterocycle amides and hydrazones as new agents against drug-susceptible and resistant *Mycobacterium tuberculosis*^{†‡§}

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A series of benzylsulfanyl benzo-heterocycle amides and hydrazones were synthesized and evaluated for anti-tubercular activities. The isonicotinyl hydrazone derivatives **12d**, **12e** and **12f** exhibited good anti-tubercular activity against *Mycobacterium tuberculosis* H₃₇Rv (ATCC #27294) with MIC values of 0.23, 0.24 and 0.24 μM, respectively, and were also active against SDR-TB, MDR-TB and XDR-TB. More importantly, compound **12e** also showed low cytotoxicity and good metabolic stability, and could significantly reduce the mycobacterial burden in a mouse model infected with autoluminescent H37Ra strain, which may serve as a lead compound for further development.

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1. Introduction

Tuberculosis (TB), a highly contagious air-borne disease caused by *Mycobacterium tuberculosis* (*Mtb*), has emerged with multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains and with acquired immune deficiency syndrome (AIDS) in recent years.¹ The World Health Organization (WHO) 2016 “Global Tuberculosis Report” estimated that nearly 1.8 million people died from TB and 10.4 million new TB cases were notified to national authorities in 2015.² In spite of the increasing worldwide incidence of TB, only bedaquiline (SIRTURO®) and delamanid were conditionally approved by the FDA in 2012 (ref. 3 and 4) and EMEA in 2014 for the treatment of MDR-TB. However, bedaquiline exhibited serious adverse effects, such as cardiac arrhythmias, and resulted in higher death rates than the placebo group in a clinical investigation,⁵ which limited its wide application in

clinical practice. Therefore, there is an imperative need to develop novel anti-tubercular drugs that can be equally effective against *Mtb* and MDR-TB without any toxic side effects, and can also reduce the duration of therapy.

To pursue this goal, our research efforts were directed to discovering new chemical classes of anti-tubercular agents. It was indicated that the benzylsulfanyl derivatives of benzoxazole/benzothiazole/benzimidazole have significant antimycobacterial activity.^{6,7} Additionally, some scientists used pyrazinecarboxamide derivatives and hydrazones of isoniazid as anti-tubercular pharmacophores to reduce the toxicity of isoniazid.^{8,9} Following these medicinal chemistry advances, two novel classes of benzylsulfanyl benzo-heterocycle

Fig. 1 The design of anti-tubercular compounds by molecular hybridization.

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amides **7a–7f** and hydrazones **12a–12f** were designed by molecular hybridization between pyridyl amide or hydrazone and a benzyldisulfanyl benzo-heterocycle, respectively (Fig. 1). Some of them showed good anti-tubercular activity against H37Rv, single-drug resistant strains (SDR-TB), MDR-TB and XDR-TB *in vitro*. Here, we report the synthesis and evaluation of benzyldisulfanyl benzo-heterocycle amide and hydrazone derivatives as novel anti-tubercular agents.

2. Results and discussion

Two series of benzyldisulfanyl benzo-heterocycle amide and hydrazone derivatives are shown in Table 1, and the synthetic procedures used for their preparation are demonstrated in Schemes 1 and 2. In Scheme 1, the synthesis of compounds **7a–7f** started from preparation of 4-(bromomethyl) benzonitrile **2** by bromination of α -H in 4-methylbenzonitrile **1** using KBrO₃/NaHSO₃ as a bromination agent under incandescent light illumination. Mercaptomethyl benzonitrile benzo-heterocycle derivatives **4a–4c** were prepared by nucleophilic substitution reaction in *N,N*-dimethylformamide (DMF) in the presence of potassium carbonate at 20 °C or sodium methoxide at room temperature. Reduction of **4a–4c** with lithium aluminium hydride in dry THF produced the mercaptomethyl aniline benzo-heterocycle derivatives **5a–5c**. Condensation derivatives **5a–5c** with pyridine carboxylic acid **6** produced the desired amide analogues **7a–7f**, respectively.

The benzyldisulfanyl benzo-heterocycle hydrazone derivatives **12a–12f** were synthesized as shown in Scheme 2. Selective single nucleophilic substitution of commercially available 1,4-bis(chloromethyl)benzene **8** with thiols **3a–3c** in the presence of methanol solutions of sodium hydroxide led to the formation of derivatives **9a–9c**. Then, mercaptomethyl benzaldehyde benzo-heterocycle derivatives **10a–10c** were obtained by the Sommelet reaction with intermediates **9a–9c**. The desired hydrazone deriv-

Scheme 1 Reagents: (a) KBrO₃, NaHSO₃, illumination, 50–55 °C; (b) K₂CO₃, DMF, 20 °C or Na, CH₃OH, DMF; (c) LiAlH₄, dry THF, N₂, 0 °C; (d) i) SOCl₂, ref; ii) CH₂Cl₂, NEt₃, rt or 47 °C.

atives **12a–12f** were prepared by the condensation of hydrazides **11** in ethanol solution catalyzed by acetic acid.

All newly synthesized compounds were evaluated for their *in vitro* anti-tubercular activity against the *Mtb* strain H37Rv (ATCC # 27294) in Middlebrook 7H12 using the Microplate Alamar Blue Assay (MABA),^{10,11} the results of which are summarized in Table 1. For the sake of comparison, the MIC values of positive drugs (isoniazid (INH), rifampicin (RIF), moxifloxacin (Mox), streptomycin (SM) and PA-824) were also included.

Among these compounds, the isonicotinyl hydrazone derivatives **12d**, **12e** and **12f** exhibited significant activity against *Mtb* H37Rv with MIC values of 0.23 μ M, 0.24 μ M and 0.24 μ M, respectively – better than INH, Mox, SM and PA-824 (Table 1) but less potent than RIF. Meanwhile, most of the benzyldisulfanyl benzazole amide compounds exhibited poor antimycobacterial activity, with MIC values of 0.9–128 μ M. Only one amide derivative **7e** exhibited moderate antimycobacterial activity with a MIC value of 0.9 μ M, which is equivalent to that of isoniazid. From the results, it was concluded that the activities of hydrazone derivatives are better than those of amide derivatives, which suggested that the isonicotinic moiety may serve as a key anti-tubercular pharmacophore. For the benzyldisulfanyl benzo-heterocycle moiety, the preliminary SAR suggested that the benzoxazole

Table 1 *In vitro* anti-tubercular activity of title compounds against H37Rv using the MABA method

Compds	L	X	MIC (μ M)
7a	<i>m</i> -CONCH ₂	NH	60.3
7b	<i>m</i> -CONCH ₂	O	58.2
7c	<i>m</i> -CONCH ₂	S	>128
7d	<i>t</i> -CONCH ₂	NH	120.5
7e	<i>t</i> -CONCH ₂	O	0.9
7f	<i>t</i> -CONCH ₂	S	>128
12a	<i>m</i> -CONHN=CH	NH	114.4
12b	<i>m</i> -CONHN=CH	O	24.8
12c	<i>m</i> -CONHN=CH	S	>128
12d	<i>t</i> -CONHN=CH	NH	0.23
12e	<i>t</i> -CONHN=CH	O	0.24
12f	<i>t</i> -CONHN=CH	S	0.24
INH	—	—	0.81
RIF	—	—	0.08
Mox	—	—	0.5
SM	—	—	0.48
PA-824	—	—	0.44

Scheme 2 Reagents: (a) NaOH, H₂O, CH₃OH, 0–20 °C; (b) hexamethylenamine, 60% C₂H₅OH; (c) CH₃COOH, C₂H₅OH, reflux.

Table 2 The inhibitory activities against DS, MDR and XDR clinical strains of *Mtb*

Compds	MIC (μM)		
	960 (DS-TB)	330 (MDR-TB)	431 (XDR-TB)
7e	16.0	2.0	16.0
12d	0.2	6.4	>12.8
12e	0.2	6.4	3.2
12f	0.2	6.4	>12.8
Ftivazide	0.25	16	32
INH	0.5	8.0	64
RIF	0.07	>19	>19

ring had a positive effect on the anti-tubercular activity compared to benzimidazole and benzothiazole (Table 1).

Then, the four most active compounds (7e, 12d, 12e and 12f) were evaluated against drug susceptible (DS), MDR and XDR clinical strains of *Mtb* (Table 2). The amide compound 7e was only potent against MDR-TB with a MIC value of 2.0 μM . The hydrazones 12d, 12e and 12f exhibited good activities against DS-TB with MIC values comparable to the standard H37Rv strain, and were also potent against MDR-TB with the same MIC values of 6.4 μM , which are better than those of the three control drugs. More importantly, the hydrazone 12e containing benzoxazole was 10- and 20-fold more active against XDR-TB than ftivazide and isoniazid, respectively. This study suggested that this class of compounds may serve as lead compounds for the treatment of clinical drug-resistant *Mtb*.

Encouraged by the results that the three hydrazones (12d, 12e and 12f) gave sub-micromolar MIC values against the H37Rv *Mtb* strain and exhibited potent anti-tubercular activities against MDR-TB, we further screened compounds 12d, 12e and 12f against a panel of SDR-TB strains along with the control drug ftivazide. The three potent compounds exhibited excellent activities against a panel of ATCC SDR-TB comparable to the clinical anti-tubercular drug ftivazide (Table 3).

Moreover, the *in vitro* Vero cell toxicity of the three compounds was determined. The cytotoxicity results were presented as percentage cell viability in Table 4. All the three derivatives 12d, 12e and 12f were not cytotoxic since they did not kill more than 10% of the cells at the maximum concentration tested. It is noteworthy that 12d and 12e are less cytotoxic than the control drug ftivazide.

Further, the *in vitro* metabolic stability of the three compounds was evaluated in human liver microsomes (HLM).

Table 3 The MIC results ($\mu\text{g mL}^{-1}$) against the single resistant *Mtb* strains

SDR-TB	MIC ($\mu\text{g mL}^{-1}$)			
	12d	12e	12f	Ftivazide
ATCC 35837 ethambutol	0.156	0.156	0.312	0.312
ATCC 35830 ethionamide	1.25	1.25	1.25	1.25
ATCC 35827 kanamycin	0.156	0.156	0.312	0.156
ATCC 35821 <i>para</i> -aminosalicylic acid	0.312	0.312	0.625	0.312
ATCC 35820 streptomycin	0.156	0.156	0.312	0.312
H37Rv rifampicin	0.312	0.312	0.625	0.312

Table 4 The toxicity test results against Vero cells

Concentration ($\mu\text{g mL}^{-1}$)	% viability			
	12d	12e	12f	Ftivazide
0 (0.625% DMSO)	100	100	100	100
0.01	119	127	97	95
0.02	116	127	103	105
0.039	115	127	99	11
0.078	116	119	99	114
0.156	115	122	97	96
0.312	111	120	98	103
0.625	112	118	94	100
1.25	119	123	103	105
2.5	114	123	101	110
5	113	123	103	100
10	107	130	96	95
20	107	109	91	90

Table 5 *In vitro* metabolic stability of compounds in human liver microsomes

Compds (10 μM)	Time point (min)	Compounds remaining ^a (%)
12d	15	76.5 $\pm$ 1.4
	30	62.0 $\pm$ 2.4
	60	44.6 $\pm$ 4.7
12e	15	90.1 $\pm$ 2.4
	30	76.5 $\pm$ 3.5
	60	66.6 $\pm$ 2.8
12f	15	76.3 $\pm$ 4.0
	30	57.2 $\pm$ 2.5
	60	34.0 $\pm$ 2.8

^a % of test article remaining at $T = 0$ min is 100%.

The amounts of test compounds remaining at 15, 30 and 60 minutes are summarized in Table 5. The data suggested that all of the compounds showed some metabolism following 60 minutes of incubation with HLM, ranked in the order 12f > 12d > 12e from the most metabolized to the least metabolized. This study provided the basis for *in vivo* evaluation.

Fig. 2 Compound 12e sustainably inhibits the growth of *Mtb* H37Ra following 6 consecutive days of administration. Days post initial treatment (x-axis) are plotted against the corresponding RL Udayn/RL Uday0 ratio (y-axis). Blue: Vehicle (CMC-Na); red: RIF 10 mg kg⁻¹ qd; green: 12e 3.1 mg kg⁻¹ qd.

The anti-tubercular activity of compound **12e** was further evaluated *in vivo* using a cost-efficient mouse model infected with the selected marker-free autoluminescent *Mtb* strain H37Ra.¹² As shown in Fig. 2, compound **12e** exhibited sustained anti-tubercular activity against *Mtb* H37Ra *in vivo* for 6 consecutive days. The activity of compound **12e** with a 3.1 mg kg⁻¹ per day dose is comparable to that of the positive drug RIF with a 10 mg kg⁻¹ per day dose. These results strongly suggest the potential of compound **12e** to serve as a lead compound for further anti-tubercular drug discovery.

3. Conclusions

Two novel classes of benzylsulfanyl benzo-heterocycle amides **7a–7f** and hydrazones **12a–12f** have been designed, synthesized and evaluated for their anti-tubercular activities. The isonicotinyl hydrazone derivatives **12d**, **12e** and **12f** exhibited significant activities against the *Mtb* strain H37Rv with sub-micromolar MIC values, which were better than those of INH, Mox, SM and PA-824. Importantly, these three compounds were also active against the resistant strains (SDR-TB, MDR-TB and XDR-TB) and exhibited low toxicity. Further metabolic stability and *in vivo* studies indicated that compound **12e** significantly reduced the mycobacterial burden in an H37Ra-infected mouse model, suggesting that it can serve as a new lead for further anti-tubercular drug discovery.

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Abbreviations

TB	Tuberculosis
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
MDR-TB	Multi-drug resistant tuberculosis
XDR-TB	Extensively drug-resistant tuberculosis
AIDS	Acquired immune deficiency syndrome
WHO	World Health Organization
SDR-TB	Single-drug resistant tuberculosis
DMF	<i>N,N</i> -Dimethylformamide
MABA	Microplate Alamar Blue Assay
INH	Isoniazid

RIF	Rifampicin
Mox	Moxifloxacin
SM	Streptomycin
HLM	Human liver microsomes

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References

- 1 A. Koul, E. Arnoult, N. Lounis, J. Guillemont and K. Andries, *Nature*, 2011, **469**, 483–490.
- 2 World Health Organization, *Global Tuberculosis Control WHO Report 2016*, WHO/HTM/TB/2016, 10, 2016.
- 3 K. Andries, P. Verhasselt, J. Guillemont, H. W. Gohlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis and V. Jarlier, *Science*, 2005, **307**, 223–227.
- 4 J. Cohen, *Science*, 2013, **339**, 130.
- 5 A. H. Diacon, A. Pym, M. P. Grobusch, J. M. de los Rios, E. Gotuzzo, I. Vasilyeva, V. Leimane, K. Andries, N. Bakare, T. DeMarez, M. Haxaire-Theeuwes, N. Lounis, P. Meyvisch, E. De Paepe, R. P. G. van Heeswijk and B. Dannemann, *N. Engl. J. Med.*, 2014, **371**, 723–732.
- 6 V. Klimešová, J. Kočí, K. Waisser, J. Kaustová and U. Möllmann, *Eur. J. Med. Chem.*, 2009, **44**, 2286–2293.
- 7 R. S. Keri, M. R. Patil, S. A. Patil and S. A. Budagumpi, *Eur. J. Med. Chem.*, 2015, **89**, 207–251.
- 8 S. A. Carvalho, E. F. da Silva, M. V. de Souza, M. C. Lourenço and F. R. Vicente, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 538–541.
- 9 F. Martins, S. Santos, C. Ventura, R. Elvas-Leitão, L. Santos, S. Vitorino, M. Reis, V. Miranda, H. F. Correia, J. Aires-de-Sousa, V. Kovalishyn, D. A. Latino, J. Ramos and M. Viveiros, *Eur. J. Med. Chem.*, 2014, **23**, 119–138.
- 10 L. Collins and S. G. Franzblau, *Antimicrob. Agents Chemother.*, 1997, **41**, 1004–1009.
- 11 K. Falzari, Z. Zhu, D. Pan, H. Liu, P. Hongmanee and S. G. Franzblau, *Antimicrob. Agents Chemother.*, 2005, **49**, 1447–1454.
- 12 J. Tang, B. X. Wang, T. Wu, J. T. Wan, Z. C. Tu, M. Njire, B. J. Wan, S. G. Franzblau, T. Y. Zhang, X. Y. Lu and K. Ding, *ACS Med. Chem. Lett.*, 2015, **6**, 814–818.