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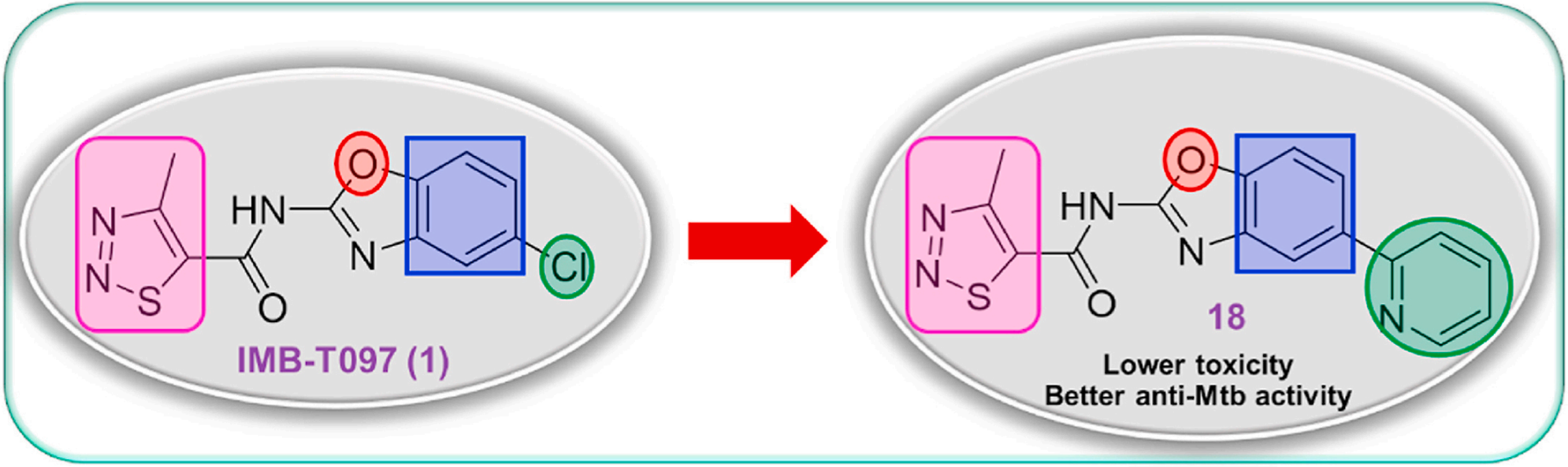
IMB-T097 (1)
18
Lower toxicity
Better anti-Mtb activity

Design, Synthesis, and Evaluation of Substituted 2-acylamide-1,3-benzo[d]zole Analogues as Agents against MDR- and XDR-MTB

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ABSTRACT

N-(5-Chlorobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamideox-amide has been identified as a potent inhibitor of Mtb H37Rv, with a minimum inhibitory concentration (MIC) of 0.42 μM. In this study, a series of substituted 2-acylamide-1,3-zole analogues were designed and synthesized, and their anti-Mtb activities were analyzed. In total, 17 compounds were found to be potent anti-Mtb agents, especially against the MDR- and XDR-MTB strains, with MIC values < 10 μM. These analogues can inhibit both drug-sensitive and drug-resistant Mtb. Four representative compounds were selected for further profiling, and the results indicate that compound **18** is acceptably safe and has favorable pharmacokinetic (PK) properties. In addition, this compound displays potent activity against Gram-positive bacteria, with MIC values in the range of 1.48–11.86 μM. The data obtained herein suggest that promising anti-Mtb candidates may be developed via structural modification, and that further research is needed to explore other compounds.

KEYWORDS

Substituted 2-acylamide-1,3-zole, Anti-tuberculosis agent, MDR-MTB, XDR-MTB

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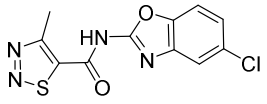
1. Introduction

Tuberculosis (TB), an airborne contagious disease caused by *mycobacterium tuberculosis* (Mtb) bacteria, is considered to be one of the most serious health hazards in the world, and it is ranked as the top infectious killer on a global level [1]. According to the World Health organization (WHO), an estimated 10.0 million people contracted TB worldwide in 2018. Of the infected patients, an estimated 1.45 million die each year, including 1.2 million HIV-negative and 0.25 million HIV-positive patients [2]. The first-line drugs currently used in combination anti-Mtb chemotherapy (rifampin, isoniazid, pyrazinamide, and ethambutol) constitute the most effective weapons against drug-sensitive TB, as they target different physiological phases (replicating and non-replicating [3]) affected by Mtb in human lungs. However, these drugs are limited by the need for prolonged directly observed therapy (DOT) treatment (at least 6 months) and follow-up support [3–5]. Moreover, the emergence of multi-drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of Mtb have limited the effectiveness of the available drugs and exacerbated the TB epidemic [6–8]. The risk of disease reactivation in asymptomatically infected individuals or those harboring latent Mtb constitutes an additional challenge associated with TB, particularly if these individuals are co-infected with HIV, have diabetes, or are subject to anti-tumor necrosis factor therapy [6,9].

Most first-line and second-line anti-Mtb drugs were discovered in the 1950s and 1960s [9]. In fact, only two drugs (bedaquiline and delamanid) were approved by the US Food and Drug Administration (FDA) for MDR-TB treatment during the last four decades. Although many new/repurposed drugs and combination therapies, such as **FS-1**, **PA-824**, **TBA-7371**, linezolid, and nitrazoxanide, are available at the Clinical Trials website, new chemicals that simplify and shorten treatment, target MDR-MTB or XDR-MTB strains, require low dosing frequency and can be co-administered with HIV medications [6,10,11] still need to be developed in order to control the TB epidemic.

Whole-cell-based high-throughput screening (HTS) constitutes a promising method for the synthesis of new anti-Mtb compounds [12–14]. Previously, we had shown that the **IMB-T097** molecule, *N*-(5-chlorobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamideox-amide (Figure 1) prepared using whole-cell-based HTS is a potent inhibitor of Mtb H37Rv, with an MIC of 0.42 μM [15]. Herein, we establish a robust method for the synthesis of drug-like molecules based on the preliminary structure–activity relationship (SAR) analysis of **IMB-T097**. Considering that fused or non-fused 2-aminothiazole is a frequent emergency segment in antibacterial agents [16-20], and that non-fused 2-amino oxazol analogues have weak anti-Mtb H37Rv activity (the data are not shown in this study), only fused oxazole derivatives are explored.



IMB-T097

anti-Mtb H37Rv MIC: 0.42 μM

Figure 1. Hit molecule: **IMB-T097**

2. Chemistry

Figure 2 depicts the systematic structural modifications of **IMB-T097** implemented in this study. To examine the effect of electrical properties and steric hindrance on anti-Mtb activity, a series of derivatives with different R_2 groups was synthesized. Another series of **IMB-T097** analogues was prepared by replacing the 1,2,3-thiadiazole ring with different five- or six-membered rings (R_1), including thiophene, furan, and benzene. Also, the benzoxazole group was replaced with benzothiazole, benzimidazole, or oxazolo[4,5-b]pyridine. Among the synthesized derivatives, compound **6** that has a large hydrocarbon group (*t*-Bu) at C-5 position showed excellent anti-Mtb activity (MIC: ~0.40 μM). Therefore, other compounds comprising large monosubstituted groups or fused cycles at C-4 and/or C-5 positions of the 2-amine benzo[d]oxazol were also explored.

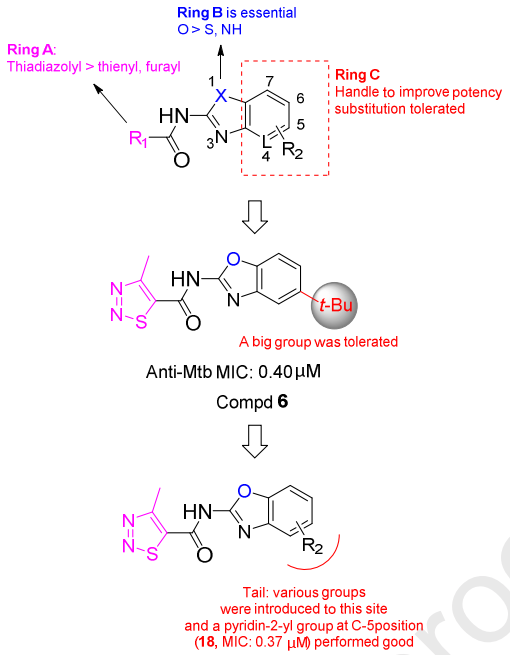
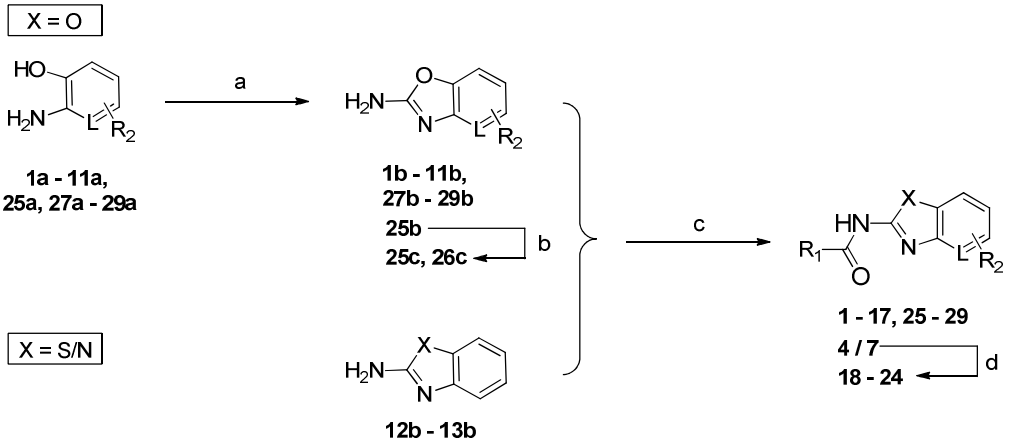


Figure 2. Systematic modification and SAR of **IMB-T097**

The synthetic route used to prepare the target compounds is outlined in Scheme 1. The derivatives in which X is an oxygen atom were prepared by reacting different 2-amino phenols (**1a**–**11a**, **25a**, **27a**–**29a**) with cyanogen bromide in methanol. The resulting 2-amine benzo[d]oxazols were obtained at relatively high yields (75%–93%). Compounds **25c** and **26c** were synthesized via 2-aminobenzo[d]oxazol-4-ol substitution of compound **25b** at C-4 position in the presence of a halide and a base. Benzo[d]thiazol-2-amine **12b** and 1H-benzo[d]imidazol-2-amine **13b** were directly purchased from a commercial supplier. As for the substituted 2-acylamide-1,3-benzo[d]zole analogues (**1**–**17**, **25**–**29**), they were prepared by condensation reaction of carboxylic acid with amide (**1b**–**11b**, **25c**–**26c**, **27b**–**29b**) using the EDCI-HoBt-TEA system and the DMF solvent. The yields of these analogues ranged between 22 and 75%. The compounds comprising an unsaturated cycle at C-4 or C-5 positions (compounds **18**–**24**) were synthesized via the Suzuki-Miyaura cross coupling reaction of 4-Br/5-Br substituted compounds **4** or **7** with the corresponding boric acid. Considering that pyridin-2-ylboronic acid is an unstable agent that cannot be readily used in the laboratory, compound **19** was prepared via the Stille coupling reaction with an alternative agent, 2-(tributylstannyl)pyridine. Finally, compound **24** was obtained with 19% yield by reacting compound **4** with morpholine (Buchwald–Hartwig cross coupling reaction).



1 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-Cl, X = O, L = C
2 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = H, X = O, L = C
3 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-F, X = O, L = C
4 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-Br, X = O, L = C
5 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-CH_3, X = O, L = C
6 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-*t*-Bu, X = O, L = C
7 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 4-Br, X = O, L = C
8 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 6-Cl, X = O, L = C
9 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 6-*t*-Bu, X = O, L = C
10 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-methoxycarbonyl,, X = O, L = C
11 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = H, X = O, L = N
12 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = H, X = S, L = C
13 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = H, X = NH, L = C
14 R_1 = Furanyl-2-yl, R_2 = H, X = O, L = C
15 R_1 = Thienyl-2-yl, R_2 = H, X = O, L = C
16 R_1 = 5-Cl-thienyl-2-yl, R_2 = H, X = O, L = C
17 R_1 = Phenyl, R_2 = H, X = O, L = C
18 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-(pyridin-2-yl), X = O, L = C
19 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 4-(pyridin-2-yl), X = O, L = C
20 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-(pyridin-3-yl), X = O, L = C
21 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-(pyridin-4-yl), X = O, L = C
22 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-((4-methoxycarbonyl)phenyl), X = O, L = C
23 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-(1-methyl-1H-pyrazol-4-yl), X = O, L = C
24 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 5-morpholino, X = O, L = C
25 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 4-(pyridin-2-ylmethoxy), X = O, L = C
26 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = 4-(pyrimidin-2-yloxy), X = O, L = C
27 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = C_{4+5} = phenyl, X = O, L = C
28 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = C_{4+5} = pyridin-2-yl, X = O, L = C
29 R_1 = 4-methyl-1,2,3-thiadiazole-5-yl, R_2 = C_{4+5} = pyridin-5-yl, X = O, L = C

Scheme 1. General scheme of the synthesis of Disubstituted Azole Analogues **1**–**29**. Reagents and conditions: (a) BrCN, MeOH, 0°C – rt, overnight; (b) copper, Cs_2CO_3, DMF, 110°C, 2 h or K_2CO_3, ACN, reflux, 8 h; (c) carboxylic acid, EDCI, HoBt, TEA, DMF, 0°C – rt, 16 h; (d) Suzuki–Miyaura reaction: boronic acid, $Pd(PPh_3)_4$, K_3PO_4, DMF-H_2O, microwave, 150°C, 30 min; Stille reaction: 2-(tributylstannyl)pyridine, $Pd_2(dba)_3$, K_3PO_4, anhydrous DMF, reflux, 5 h; Buchwald-Hartwig reaction: XPhos, $Pd(PPh_3)_4$, *t*-BuOK, DME, 110°C, 2 h.

3. Results and discussion

3.1. *In vitro* anti-Mtb activity and structure activity relationship (SAR)

In vitro analyses of the activities of all synthesized 2-acylamide-1,3-benzo[d]zole analogues against *M. tuberculosis* H37Rv, the MDR strain (FJ05120), and the XDR strain (FJ05195) were conducted in 7H9 medium using the Micro plate Alamar Blue Assay (MABA). The isoniazid (INH), rifampicin (RIF), streptomycin (SM), and ethambutol (EMB) first-line drugs were used as standards (Table 1).

Seventeen of the 29 target compounds prepared herein showed high anti-H37Rv potency, as well as excellent activity against the MDR- and XDR-MTB strains (MIC < 10 μM). In fact, many of these compounds were found to be more effective than the standard drugs. Specifically, strong anti-Mtb activity (MIC < 10 μM) was observed for the compounds having electron-withdrawing or electron-donating groups on the aryl ring (**1**-**10**). Notably, the anti-Mtb activity of the compound comprising a 5-*tert*-butyl substituent (compound **6,** MIC ~0.40 μM) was found to be 16 times less than that of the compound with a 6-*tert*-butyl substituent (compound **9**, MIC 3.16–6.32 μM), which indicates that the exotonier of C-5 is more suitable for holding large groups.

When the benzene ring of compound **2** was replaced with pyridine (compound **11**), the MIC of anti-Mtb activity was increased from 0.48 to 7.66 μM, possibly due to the low cLogP value of **11**. Compared with the oxazole analogues, the thiazole and imidazole compounds (**12** and **13**) showed lower anti-Mtb activity (MIC 28.95–57.90 μM), indicating that oxazole is a more suitable segment in the scaffold. Similarly, the substitution of the 1,2,3-thiadiazole ring with other aromatic rings diminished anti-Mtb activity, especially for compound **17** (MIC 268.63–537.25 μM).

Table 1
In vitro anti-MTB evaluation of all the synthesized compounds and four controls against H37Rv, MDR-MTB (FJ05120), and XDR-MTB (FJ05195) and cell cytotoxicity in HEK-293.

Compd	ClogP[a]	Strain range of anti-MTB MIC (μM)			MTT IC_{50} (μM)
		H37Rv	MDR[b] FJ05120 HR[c]	XDR[c] FJ05195 HRSCO[c]	HEK-293
1	1.60	0.42	1.70	1.70	6.65
2	0.84	0.48	0.48	0.48	19.56
3	1.03	0.45	0.45	1.80	42.03
4	1.75	2.95	1.47	1.47	45.01
5	1.34	1.82	7.29	7.29	21.93
6	2.67	0.40	0.40	0.40	6.73

7	1.75	1.47	0.74	0.74	10.59
8	1.60	0.42	0.42	0.85	7.14
9	2.67	3.16	6.32	6.32	2.42
10	0.89	3.14	1.57	3.14	ND[d]
11	-0.17	7.66	3.83	7.66	15.18
12	1.53	28.95	57.90	57.90	>100
13	0.99	30.85	30.85	30.85	>100
14	1.93	17.53	70.11	35.06	>100
15	2.50	16.38	32.75	32.75	81.94
16	3.25	14.35	14.35	14.35	ND
17	2.75	268.63	537.25	537.25	>100
18	1.45	0.74	0.37	0.37	>100
19	1.45	23.71	23.71	47.43	ND
20	1.24	2.96	1.48	2.96	54.93
21	1.24	1.48	0.74	1.48	ND
22	2.70	1.27	0.63	0.63	ND
23	0.85	1.47	0.73	1.47	ND
24	0.71	11.58	23.16	23.16	18.32
25	1.40	5.44	2.72	5.44	61.82
26	0.49	2.82	5.64	2.82	>100
27	2.84	103.12	103.12	103.12	80.86
28	0.89	102.79	6.42	25.70	ND
29	0.89	102.79	25.70	25.70	59.03
INH		<3.65	7.29	29.17	--
RIF		<0.61	>311.08	>311.08	--
SM		<0.69	<0.69	87.83	--
EMB		<2.45	4.89	39.16	--

[a]ClogP values were calculated using ChemDraw version 12.0, Perkin–Elmer.
[b]MDR-MTB = multidrug resistant Mtb; XDR-MTB = extensively drug resistant Mtb.
[c]Drugs abbreviated as H = isoniazid, R = rifampicin, S = streptomycin, C = capreomycin, O = ofloxacin.
[d]Not determined.

All of the derivatives with substituents at C-4 (compounds **19**, **25**, and **26**) or C-5 (compounds **18**, and **20-24**) presented potent anti-Mtb activity (MIC 0.37–23.16 μM). Except for the compound substituted with an alkyl heterocycle (compound **24**, MIC 11.58–23.16 μM), the C-5-substitued derivatives (compounds **18**, and **20**–**23**) showed particularly high anti-Mtb activity (MIC 0.37–2.96 μM), with pyridin-2-yl substitution (compound **18**) yielding the best effectiveness (MIC 0.37 μM). The obtained results confirm that C-4 and C-5 are capable of accommodating large groups, resulting in broader exposure to the target. Inspired by the effectiveness of the C-4 and C-5 substituted derivatives, three other analogues with fused aromatic rings at these positions (compounds **27-29**) were further synthesized. Unfortunately, these compounds showed relatively low anti-Mtb activity, which is probably due to their rigid characteristics.

3.2. Cell cytotoxicity and acute toxicity

The above 29 compounds were tested for mammalian cell cytotoxicity using human embryonic kidney cells (HEK-293) measured as IC_{50} value as compared to control, and the results are reported in Table 1. The result reflects that compounds **1**–**11 (**except compound **10)** exhibit a certain degree of cytotoxicity (IC_{50}, 2.42–45.01 μM). Meanwhile, the compounds **18**, **20**, **25**, **26** and **29,** which have different aryl groups at C-4 or/and C-5 site, show lower cytotoxicity ($IC_{50} \geq 54.93$ μM).

Considering the potent anti-Mtb activities, compounds **1**, **6**, **18**, and **26**, which represent different design strategies were selected for

further profiling. As shown in Table 2, compounds **1** and **6** yield similar IC_{50} values (6.65 and 6.73 μM, respectively), with the latter showing a higher selectivity index value due to its stronger anti-Mtb activity (MIC of **6** is 4 times less than that of **1**). As for compounds **18** and **26**, they both present low cytotoxicity (IC_{50} > 100 μM) and high selectivity index. *In vivo* acute toxicity study was implemented by recording the number of survivors after an oral single dose in mice of 1000 mg/kg following a 7 days observation. As shown in Table 2, compound **1** shows some oral acute lethal toxicity, while compounds **6** and **18** show very low oral acute lethal toxicity. In general, derivatives **1**, **6**, and **18** are safe for use in mice.

Table 2

The cytotoxicity and acute toxicity of compounds **1**, **6**, **18**, and **26**.

compd	IC_{50} (μM) HEK-293 cell lines	Selectivity Index (SI) [a]	P. O. dose (mg/kg)	no. of animals that survived/ total no. of animals
1	6.65	4	1000	4/6
6	6.73	17	1000	6/6
18	>100	>270	1000	6/6
26	>100	>18	N/A[b]	N/A[b]

[a]Selectivity Index: IC_{50} (μM)/MIC (μM); [b]not tested, since **26** shows poor PK property

3.3. *In vivo* pharmacokinetics (PK)

The *in vivo* pharmacokinetic (PK) profiles of compounds **6**, **18,** and **26** were tested in rats after oral administration of a single 40 mg/kg dose. Blood samples were collected at times ranging between 0 and 8 h post-ingestion. As shown in Table 3, the C_{max}, $AUC_{(0-t)}$, MRT, and T_{max} values of compound **26** are 6.93 ± 1.44 ng/mL, 37.97 ± 10.21 h•ng/mL, 4.12 ± 0.38 h, and 2.83 h, respectively. Comparatively, the PK properties of compound **6** are relatively better, with C_{max} = 6836.55 ± 740.10 ng/mL, AUC(0-t) = 24802.17 ± 2888.66 h•ng/mL, and MRT = 5.26 ± 0.35. However, the best properties were observed for compound **18** whose C_{max}, $AUC_{(0-t)}$, and MRT values are 9048.41 ± 1473.34 ng/mL, 27794.11 ± 4255.06 h•ng/mL, and 5.59 ± 0.12 h, respectively. Since the analyzed blood samples were collected during the limited period of 8 h only, the $T_{1/2}$, Vd/F, and CL/F values of **6** and **18** could not be obtained. Overall, the PK results suggest that substitution at C-5 position favors the PK profiles of the benzoxazole scaffold.

Table 3

Pharmacokinetic parameters of compounds **6**, **18**, and **26** in rats administered with single 40 mg/kg oral doses (Mean ± SD, n = 3).

compd	T_{max} (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (h·ng/mL)	MRT (h)
6	≥8	6836.55 ± 740.10	24802.17 ± 2888.66	5.26 ± 0.35
18	≥8	9048.41 ± 1473.34	27794.11 ± 4255.06	5.59 ± 0.12
26	2.83	6.93 ± 1.44	37.97 ± 10.21	4.12 ± 0.38

3.4. Broad-spectrum panel study

Ten representative bacterial strains (Five Gram-positive: *S. aureus* 29213, *S. aureus* 33591, *S. epidermidis* 12228, *E. faecalis* 29212, and *E. faecium* 700221; and five Gram-negative: *E. coli* 25922, *K. pneumonia* 700603, *K. pneumonia* 7, *P. aeruginosa* 27853, *A. calcoacetious* 19606) were used to study the broad-spectrum panels of compounds **6** and **18**. The results summarized in Table 4 indicate that both compounds exhibit more potent activity against the Gram-positive bacterial strains (MIC 1.48–12.64 μM) than against the Gram-negative ones (MIC > 379.42 μM).

Table 4

Broad-spectrum panels of compounds **6** and **18**.

Species	MIC (μM)	
	6	**18**
*S. aureus*ATCC29213[a]	6.32	5.93
*S. aureus*ATCC33591[b]	6.32	1.48
*S. epidermidis*ATCC12228[c]	6.32	2.96
*E. faecalis*ATCC29212[d]	12.64	11.86

*E. faecium*ATCC700221 [e]	12.64	5.93
E. coli ATCC25922[f]	> 404.58	> 379.42
*K. pneumonia*ATCC700603[g]	> 404.58	> 379.42
K. pneumonia 7[f]	> 404.58	> 379.42
*P. aeruginosa*ATCC27853	> 404.58	> 379.42
*A. calcoacetious*ATCC19606	> 404.58	> 379.42

[a]Methicillin sensitive *Staphylococcus aureus*, MSSA; [b]Methicillin resistant *Staphylococcus aureus*, MRSA; [c]Methicillin sensitive *Staphylococcus epidermidis*, MSSE; [d]Vancomycin susceptible *Enterococcus*, VSE; [e]Vancomycin resistant *Enterococcus*, VRE; [f]Extended-Spectrum β-Lactamases negative, ESBLs (-); [g]Extended-Spectrum β-Lactamases positive, ESBLs (+).

4. Conclusion

A series of substituted 2-acylamide-1,3-benzo[d]zole analogues were designed and synthesized, and their activities against H37Rv, MDR-MTB, and XDR-MTB were assessed. The obtained results indicate that most of the synthesized compounds exhibit strong anti-Mtb activity. The mechanism of this activity is probably novel, considering that the analogues can inhibit both, drug sensitive and drug resistant Mtb. Based on preliminary structure–activity relationship (SAR) assessments, large-group-substitution at positions C-4 and C-5 of **IMB-T097** might lead to higher activity due to greater exposure to the target. Of the 29 compounds prepared herein, four were assessed in terms of their potential to be used as drugs. Of these four compounds, **18** displayed the lowest cytotoxicity and acute toxicity, as well as the best pharmacokinetic (PK) properties. Broad-spectrum panel studies revealed that compound **18** is also a potent anti-Gram-positive agent. In order to develop a promising novel anti-Mtb candidate for TB treatment, especially MDR-TB and XDR-TB, a further SAR exploration of compound **18** and corresponding ADMET research are in progress and the study results will be communicated in due time.

5. Experimental

5.1. General information

All of the reagents and solvents were purchased from commercial suppliers and used without further purification. The reactions were monitored using thin-layer chromatography (TLC) analysis performed on commercial silica-gel plates (GF254), or using LC-MS analysis on an Agilent 1100 HPLC system. ^{1}H NMR (nuclear magnetic resonance) and ^{13}C NMR spectra were recorded in DMSO-d_6/$CDCl_3$/CD_3OD solvent on a Bruker spectrometer (400 MHz/100 MHz, and 600 MHz/150 MHz), using the tetramethylsilane (TMS) peak as an internal reference. ESI-HRMS data were measured on Thermo LTQ XL.

5.2. Synthesis of compounds

5.2.1 *Ethyl 4-methyl-1,2,3-thiadiazole-5-carboxylatete*

Methyl hydrazinecarboxylate (5.40 g, 0.06 mol) was dissolved in 30 mL anhydrous EtOH, and then a solution of ethyl acetoacetate (7.81 g, 0.06 mol) in 20 mL anhydrous EtOH was slowly added. The mixture was stirred and reacted at room temperature for 6 h before evaporating the solvent under reduced pressure. The yellow solid (hydrazone) obtained at the end was used in the next step without further purification.

A solution of the crude hydrazone dissolved in 50 mL dichloromethane was added dropwise to 15 mL of $SOCl_2$ at temperatures < 5°C. The mixture was stirred and reacted overnight at room temperature, and then the solvent and excess $SOCl_2$ were evaporated under reduced pressure. The residue was purified on a silica chromatography column using PE/EA (1/1, v/v) as mobile phase. The yield of the yellow oil (5.37 g) collected at the end was found to be 52%, and based on MS-ESI (mass spectrometry-electron spray ionization) analysis, its *m/z* ratio is 173.06 $[M+H]^+$.

5.2.2 *4-methyl-1,2,3-thiadiazole-5-carboxylic acid*

In a 100 mL flask, 20 mL $NaOH_{(aq)}$ (3 M) were added to 10 mL EtOH and 5.14 g (0.03 mol) ethyl 4-methyl-1,2,3-thiadiazole-5-carboxylatet. The mixture was stirred at room temperature for 3 h, and then EtOH was evaporated and the residue was dispersed in 40 mL of water. The pH of the solution was adjusted to ~3 using HCl (conic.). The precipitated solid was collected and washed with ester to give the title compound (white solid, 3.67 g) at 85% yield without any further purification. ^{1}H NMR (400 MHz, CD_3OD): δ 2.85 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 161.74, 160.48, 140.82, 12.29. MS-ESI (*m/z*): 145.0 $[M+H]^+$.

5.2.3 General Procedure for the Synthesis of benzoxazol-2-amino compounds **(1b–12b, 25b, 27b-29b)**

BrCN (2.03 g, 17.6 mmol, 1.1eq) was slowly added to a solution of 2-aminophenol (16.0 mmol, 1.0 eq) in 30 mL MeOH. The reaction mixture was stirred overnight at room temperature. Excess BrCN was quenched with saturated Na_2CO_3 in a fume hood, until the pH value reached ~7. Then, MeOH was evaporated and EtOAc (30 mL) was added. The organic phase was washed with water (20 mL × 2) and brine (20 mL), and then it was dried over anhydrous Na_2SO_4, filtered, and evaporated to give the title compound without further purification.

*5.2.3.1 5-Chlorobenzo[d]oxazol-2-amine (**1b**).* Pale solid, 91% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61 (brs, 2H), 7.32 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 6.97 (dd, J = 8.0, 2.0 Hz, 1H). MS-ESI (*m/z*): 169.0 $[M+H]^+$.

*5.2.3.2 benzo[d]oxazol-2-amine (**2b**).* White solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.16 (t, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 9.58 (s, 2H). MS-ESI (*m/z*): 135.1 $[M+H]^+$.

*5.2.3.3 5-fluorobenzo[d]oxazol-2-amine (**3b**).* Pale solid, 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.64 (brs, 2H), 6.76–7.31 (m, 3H). MS-ESI (*m/z*): 153.0 $[M+H]^+$.

*5.2.3.4 5-bromobenzo[d]oxazol-2-amine (**4b**).* Beige solid, 87% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61 (brs, 2H), 7.36 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.12 (dd, J = 8.4, 2.0 Hz, 1H). MS-ESI (*m/z*): 213.2 $[M+H]^+$.

*5.2.3.5 5-methylbenzo[d]oxazol-2-amine (**5b**).* Pale solid, 93% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28 (brs, 2H), 7.18 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 2.0 Hz, 1H), 6.77 (dd, J = 8.0, 2.0 Hz, 1H), 2.31 (s, 3H). MS-ESI (*m/z*): 148.9 $[M+H]^+$.

*5.2.3.6 5-(tert-butyl)benzo[d]oxazol-2-amine (**6b**).* Yellow solid, 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.54 (brs, 2H), 7.20 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 2.0 Hz, 1H), 6.65 (dd, J = 8.0, 2.0 Hz, 1H), 1.30 (s, 9H). MS-ESI (*m/z*): 191.1 $[M+H]^+$.

*5.2.3.7 4-bromobenzo[d]oxazol-2-amine (**7b**).* Beige solid, 81% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (brs, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.01 (t, J = 8.0 Hz, 1H). MS-ESI (*m/z*): 213.2 $[M+H]^+$.

*5.2.3.8 6-chlorobenzo[d]oxazol-2-amine (**8b**).* Brown solid, 80% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61 (brs, 2H), 7.50 (s, 1H), 7.13–7.19 (m, 2H). MS-ESI (*m/z*): 169.0 $[M+H]^+$.

*5.2.3.9 6-(tert-butyl)benzo[d]oxazol-2-amine (**9b**).* Pale solid, 75% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (brs, 2H), 7.62 (d, J= 1.2 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H) 1.27 (s, 9H). MS-ESI (*m/z*): 191.2 $[M+H]^+$.

*5.2.3.10 methyl 2-aminobenzo[d]oxazole-5-carboxylate (**10b**).* Slightly pink solid, 80% yield.^{1}H NMR (400 MHz, DMSO-d_6): δ 7.69 (d, J = 1.6 Hz, 2H), 7.61–7.63 (m, 3H), 7.40 (d, J = 8.4 Hz, 1H), 3.80 (s, 3H). MS-ESI (*m/z*): 193.10 $[M+H]^+$.

*5.2.3.11 Oxazolo[4,5-b]pyridin-2-amine (**11b**).* Black solid, 78% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (dd, J = 5.2, 1.4 Hz, 1H), 7.60 (dd, J = 7.8, 1.4 Hz, 1H), 6.90 (dd, J = 7.8, 5.2 Hz, 1H). MS-ESI (*m/z*): 135.1 $[M+H]^+$.

*5.2.3.12 2-aminobenzo[d]oxazol-4-ol (**25b**).* Black solid, 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.23 (brs, 1H), 8.65 (brs, 2H), 8.92–8.95 (m, 2H), 6.72 (m, 1H). MS-ESI (*m/z*): 151.2 $[M+H]^+$.

*5.2.3.13 naphtho[1,2-d]oxazol-2-amine (**27b**).* Brown solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.51–7.56 (m, 2H), 7.46–7.47 (m, 2H), 7.42–7.44 (m, 2H). MS-ESI (*m/z*): 185.1 $[M+H]^+$.

*5.2.3.14 Oxazolo[5,4-h]quinolin-2-amine (**28b**).* Pale solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (dd, J = 8.8 Hz, 1H), 8.94 (dd, J = 8.8 Hz, 1H), 8.12 (brs, 2H), 7.99 (dd, J = 8.0, 4.0 Hz, 1H), 7.88 (dd, J = 8.0, 4.0 Hz, 1H), 7.72–7.82 (m, 1H). MS-ESI (*m/z*): 186.1 $[M+H]^+$.

*5.2.3.15 oxazolo[4,5-f]quinolin-2-amine (**29b**).* Slightly yellow solid, 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (dd, J = 4.0, 1.2 Hz, 1H), 8.47 (dd, J = 8.4, 1.2 Hz, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H),7.59 (brs, 2H), 7.49 (dd, J = 8.4, 4.0 Hz,

1H). MS-ESI (*m/z)*: 186.1 $[M+H]^+$.

5.2.4 *4-(pyridin-2-ylmethoxy)benzo[d]oxazol-2-amine (**25c**)*

2-(Bromomethyl)pyridine hydrobromide (835 mg, 3.3 mmol) and potassium carbonate (911 mg, 6.6 mmol) were added to a solution of 2-aminobenzo[d]oxazol-4-ol **25b** (450 mg, 3.0 mmol) in 15 mL acetonitrile. The mixture was stirred at 70° C for 4 h and the reaction progress was monitored by LCMS. Solid impurities were removed by filtration, and the filtrate was evaporated under vacuum. The product solution was reconstituted in 20 mL DCM, washed with water and brine, and then dried over $MgSO_4$. Subsequently, the solution was loaded on a silica chromatography column and eluted with DCM/MeOH (20/1, v/v) to afford the pure brown solid product **25c** (410 mg, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (brs, 2H), 8.56–8.67 (m, 2H), 8.61 (d, J = 4.8 Hz, 1H), 8.35 (d, J = 2.8 Hz, 1H), 8.22 (m, 1H), 7.89 (m, 1H), 6.59 (t, J = 8.0 Hz, 1H), 5.25 (s, 2H). MS-ESI (*m/z)*:242.0 $[M+H]^+$.

5.2.5 *4-(pyrimidin-2-yloxy)benzo[d]oxazol-2-amine (**26c**)*

A mixture of 2-aminobenzo[d]oxazol-4-ol **25b** (650 mg, 4.33 mmol), 2-iodopyrimidine (893 mg, 4.33 mmol), copper (273 mg, 4.33 mmol), and cesium carbonate (2.11 g, 6.50 mmol) in 10 mL DMF was reacted in a microwave at 100° C for 30 min. Thereafter, the reaction mixture was filtered by cilte and washed with DCM. The filtrate was purified by column chromatography with DCM/MeOH (95/5, v/v) mobile phase. The yellow solid product collected at the end (210 mg) was labeled **26c**, and the reaction yield was determined to be 21%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (d, J = 4.4 Hz, 2H), 7.44 (s, 1H), 7.19 – 7.24 (m, 2H), 6.94 – 6.99 (m, 2H). MS-ESI (*m/z)*: 229.2 $[M+H]^+$.

5.2.6 General Procedure for the Synthesis of compounds **1–17** and **25–29**

TEA (2.0 eq), EDCI (1.5 eq), and HoBt (1.5 eq) were added to a solution of 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (10 mmol, 1.0 eq) in 15 mL DMF kept at 0°C. The mixture was stirred at this temperature for 1 h before adding the amine (1.0 eq). The resulting solution was then stirred and allowed to react for 16 h at room temperature. The progress of the reaction was monitored using LCMS. At the end of the 16 h, the mixture was poured into 30 mL DCM-THF (5/1, v/v) and washed with water (30 mL). The organic phase was collected, and the solvent was slowly evaporated in a rotary evaporator under vacuum. When lots of solid material appeared, the evaporation process was ceased, and the solid was filtered. The crude material was triturated with acetonitrile or methanol to give the desired product with high purity (> 95%).

*5.2.6.1 N-(5-chlorobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**1, IMB-T097**).* Slightly yellow solid, 41% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.33 (brs, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.46 (s, 1H), 7.36 (dd, J = 8.4, 2.4 Hz, 1H), 2.92 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.1, 159.8, 146.6, 142.6, 129.3, 124.3, 113.1, 112.1, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_8N_4O_2ClS$ $[M+H]^+$ 295.00510; found 295.00520.

*5.2.6.2 N-(benzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**2**).* White solid, 71% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.33 (brs, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.36 (dt, J = 7.8, 1.2 Hz, 1H), 7.32 (dt, J = 7.8, 1.2 Hz, 1H), 2.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.3, 159.9, 147.4, 143.3, 129.7, 125.4, 124.5, 113.0, 110.7, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_9O_2N_4S$ $[M+H]^+$ 261.04407; found 261.04517.

*5.2.6.3 N-(5-fluorobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**3**).* White solid, 65% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.34 (brs, 1H), 7.65 (dd, J = 9.0, 4.2 Hz, 1H), 7.26 (brd, J = 6.6 Hz, 1H), 7.17 (ddd, J = 9.0, 9.0, 3.0 Hz, 1H), 2.90 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.2, 160.0, 158.6, 146.8, 140.0, 111.8, 111.7, 111.3, 111.1, 100.9, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_8O_2N_4FS$ $[M+H]^+$ 279.03465; found 279.03418.

*5.2.6.4 N-(5-bromobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**4**).* White solid, 46% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.33 (brs, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 2.4 Hz, 1H), 7.50 (dd, J = 8.4, 2.4 Hz, 1H), 2.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.0, 127.0, 116.9, 112.5, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_6O_2N_4BrS$ $[M-H]^-$ 336.93894; found 336.93886.

*5.2.6.5 4-methyl-N-(5-methylbenzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**5**).* White solid, 56% yield. ^{1}H NMR (600 MHz,

DMSO-d_6): δ 13.29 (brs, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.27 (s, 1H), 7.13 (d, J = 8.4 Hz, 1H), 2.94 (s, 3H), 2.39 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.3, 160.0, 159.8, 147.6, 141.3, 135.1, 129.6, 125.1, 112.8, 110.2, 21.0, 13.5. HRMS-ESI (*m/z*): calculated for $C_{12}H_{11}N_4O_2S$ $[M+H]^+$ 275.05972; found 275.05963.

5.2.6.6 N-(5-(tert-butyl)benzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(6)****.* Slightly brown solid, 70% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.21 (brs, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.36 (dd, J = 9.0, 1.8 Hz, 1H), 2.92 (s, 3H), 1.30 (s, 9H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.2, 160.1, 159.8, 148.5, 147.4, 141.2, 129.4, 121.8, 110.0, 109.5, 34.8, 31.3, 13.5. HRMS-ESI (*m/z*): calculated for $C_{15}H_{17}O_2N_4S$ $[M+H]^+$ 317.10667; found 317.10661.

5.2.6.7 N-(4-bromobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(7)****.* Pale solid, 22% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (dd, J = 8.4, 0.8 Hz, 1H), 7.57 (d, J = 8.4, 0.8 Hz, 1H), 7.27 (t, J = 8.4 Hz, 1H), 2.85 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6): δ 160.5, 155.4, 147.6, 127.8, 125.4, 109.9, 13.4. HRMS-ESI (*m/z*): calculated for $C_{11}H_8O_2N_4BrS$ $[M+H]^+$ 339.9546; found 338.9549.

5.2.6.8 N-(6-chlorobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(8)****.* White solid, 58% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.37 (brs, 1H), 7.84 (d, J = 1.8 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.42 (dd, J = 8.4, 1.8 Hz, 1H), 2.93 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.0, 159.6, 146.9, 144.1, 128.3, 125.5, 114.3, 111.3, 109.1, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_8O_2N_4ClS$ $[M+H]^+$ 295.00510; found 295.00517.

5.2.6.9 N-(6-(tert-butyl)benzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(9)****.* Yellow solid, 36% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.24 (brs, 1H), 7.61 (s, 1H), 7.39 (m, 2H), 2.93 (s, 3H), 1.30 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.5, 156.0, 143.7, 122.3, 112.6, 107.5, 105.8, 34.9, 13.6, 12.2. HRMS-ESI (*m/z*): calculated for $C_{15}H_{17}O_2N_4S$ $[M+H]^+$ 317.10667, found 317.10795.

5.2.6.10 methyl 2-(4-methyl-1,2,3-thiadiazole-5-carboxamido)benzo[d]oxazole-5-carboxylate ***(10)****.* White solid, 28% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.46 (brs, 1H), 7.99 (s, 1H), 7.95 (dd, J = 8.4, 1.8 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 3.88 (s, 3H), 2.94 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.4, 160.1, 126.8, 126.0, 110.9, 52.5, 13.5. HRMS-ESI (*m/z*): calculated for $C_{11}H_9ON_4S_2$ $[M+H]^+$ 277.02123, found 277.02144.

5.2.6.11 4-methyl-N-(oxazolo[4,5-b]pyridin-2-yl)-1,2,3-thiadiazole-5-carboxamide ***(11)****.* Pale solid, 22% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.32 (d, J = 4.2 Hz, 1H), 8.03 (dd, J = 8.4, 1.2 Hz, 1H), 7.33 (dd, J = 8.4, 5.4 Hz, 1H), 2.90 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.0, 159.0, 119.3, 118.0, 13.4. HRMS-ESI (*m/z*): calculated for $C_{10}H_8O_2N_5S$ $[M+H]^+$ 262.03932, found 262.03888.

5.2.6.12 N-(benzo[d]thiazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(12)****.* Pale solid, 46% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.97 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 2.80 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.3, 157.9, 143.7, 139.7, 132.0, 121.7, 118.8, 111.8, 13.2. HRMS-ESI (*m/z*): calculated for $C_{11}H_9O_2N_4S$ $[M+H]^+$ 261.04407, found 261.04502.

5.2.6.13 N-(1H-benzo[d]imidazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide ***(13)****.* Yellow solid, 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.70 (brs, 1H), 7.41 (m, 2H), 7.22 (m, 2H), 2.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.5, 158.8, 152.1, 149.1, 129.0, 123.0, 111.6, 13.4; HRMS-ESI (*m/z*): calculated for $C_{11}H_{10}ON_5S$ $[M+H]^+$ 260.06006; found 260.06141.

5.2.6.14 N-(benzo[d]oxazol-2-yl)furan-2-carboxamide ***(14)****.* White solid, 66% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.02 (brs, 1H), 8.00 (brs, 1H), 7.62 (overlap, 1H), 7.60 (overlap, 2H), 7.33 (dt, J = 7.2, 1.2 Hz, 1H), 7.29 (dt, J = 7.2, 1.2 Hz, 1H), 6.72 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.9, 147.8, 147.3, 145.8, 140.6, 124.7, 123.8, 118.4, 117.0, 112.3, 110.2. HRMS-ESI (*m/z*): calculated for $C_{12}H_9O_3N_2$ $[M+H]^+$ 229.06077; found 229.06061.

5.2.6.15 N-(benzo[d]oxazol-2-yl)thiophene-2-carboxamide ***(15)****.* Slightly yellow solid, 71% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.13 (brs, 1H), 8.15 (brs, 1H), 7.95 (brs, 1H), 7.62 (overlap, 2H), 7.33 (dt, J = 7.2, 1.2 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.23 (brs, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 155.0, 147.7, 137.7, 133.9, 131.4, 128.4, 124.8, 123.8, 118.3, 110.2. HRMS-ESI (*m/z*):

calculated for $C_{12}H_9O_2N_2S$ $[M+H]^+$ 245.03792; found 245.03760.

*5.2.6.16 N-(benzo[d]oxazol-2-yl)-5-chlorothiophene-2-carboxamide (**16**).* White solid, 49% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.74 (brs, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.51 (brs, 1H), 7.33 (dt, J = 7.2, 1.2 Hz, 1H), 7.29 (dt, J = 7.2, 1.2 Hz, 1H), 7.24 (brs, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 131.2, 128.5, 125.0, 124.0, 110.3. HRMS-ESI (*m/z*): calculated for $C_{12}H_8ClO_2N_2S$ $[M+H]^+$ 278.99895, found 278.99908.

*5.2.6.17 N-(benzo[d]oxazol-2-yl)benzamide (**17**).* White solid, 75% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.06 (brs, 1H), 8.05 (overlap, 2H), 7.63 (brt, J = 7.2 Hz, 2H), 7.60 (overlap, 1H), 7.54 (brt, J = 7.2 Hz, 2H), 7.33 (dt, J = 7.2, 1.2 Hz, 1H), 7.30 (dt, J = 7.2, 1.2 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.0, 155.5, 147.8, 140.6, 132.6, 128.5, 124.7, 123.9, 118.3, 110.2. HRMS-ESI (*m/z*): calculated for $C_{14}H_{11}O_2N_2$ $[M+H]^+$ 239.08150; found 239.08170.

*5.2.6.18 4-methyl-N-(4-(pyridin-2-ylmethoxy)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**25**).* Yellow solid, 47% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.45 (ddd, J = 4.8, 1.6, 0.8 Hz, 1H), 7.81 (dt, J = 7.6, 2.0 Hz, 1H), 7.44 (brd, J = 7.6 Hz, 1H), 7.29 (ddd, J = 6.0, 4.8, 0.8 Hz, 1H), 7.14 (dd, J = 8.4, 8.4 Hz, 1H), 7.13 (s, 1H), 6.79 (ddd, J = 8.4, 8.4, 6.0 Hz, 1H), 5.55 (s, 2H), 2.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.7, 159.3, 155.8, 154.8, 149.1, 148.4, 145.8, 144.3, 137.1, 124.6, 122.7, 121.4, 117.4, 112.8, 105.7, 102.0, 48.9, 13.4. HRMS-ESI (*m/z*): calculated for $C_{17}H_{14}O_3N_5S$ $[M+H]^+$ 368.08119, found 368.08169.

*5.2.6.19 4-methyl-N-(4-(pyrimidin-2-yloxy)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**26**).* White solid, 29% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (d, J = 4.8 Hz, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 4.8 Hz, 1H), 7.24 (d, J = 8.0, 0.8 Hz, 1H), 2.83 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 160.3, 160.1, 158.4, 158.0, 129.7, 124.7, 118.4, 117.2, 108.0, 105.8, 13.4. HRMS-ESI (*m/z*): calculated for $C_{15}H_{11}O_3N_6S$ $[M+H]^+$ 355.06079, found 355.06039.

*5.2.6.20 4-methyl-N-(naphtho[1,2-d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**27**).* Black solid, 58% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.69 (dt, J = 8.0, 1.2 Hz, 1H), 7.59 (dt, J = 80, 1.2 Hz, 1H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.0, 130.8, 128.8, 127.2, 125.7, 125.2, 121.9, 110.7, 13.5. HRMS-ESI (*m/z*): calculated for $C_{15}H_{11}O_2N_4S$ $[M+H]^+$ 311.05972, found 311.05972.

*5.2.6.21 4-methyl-N-(oxazolo[5,4-h]quinolin-2-yl)-1,2,3-thiadiazole-5-carboxamide (**28**).* Brown solid, 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.55 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.2 Hz, 2H), 7.64 (s, 1H), 2.90 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.1, 150.9, 137.1, 125.9, 124.7, 121.1, 111.6, 105.7, 13.4. HRMS-ESI (*m/z*): calculated for $C_{14}H_{10}O_2N_5S$ $[M+H]^+$ 312.05497, found 312.05450.

*5.2.6.22 4-methyl-N-(oxazolo[4,5-f]quinolin-2-yl)-1,2,3-thiadiazole-5-carboxamide (**29**).* Yellow solid, 25% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 2H), 7.90 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 6.8 Hz, 1H), 7.50 (s, 1H), 2.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 157.8, 148.6, 145.4, 143.2, 120.5, 119.1, 113.4, 13.3. HRMS-ESI (*m/z*): calculated for $C_{14}H_{10}O_2N_5S$ $[M+H]^+$ 312.05497, found 312.05453.

5.2.7 *4-methyl-N-(5-(pyridin-2-yl)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**18**)*

A mixture of *N*-(5-bromobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide **4** (600 mg, 1.77 mmol) and 2-(butyldipentylstannyl)pyridine (843 mg, 2.12 mmol) was dissolved in 10 mL anhydrous DMF, and then CuI (50 mg, 0.27 mmol), $Pd_2(dba)_3$ (165 mg, 0.18 mmol), and TEA (354 mg, 3.54 mmol) were added. The solution was stirred and reacted at 90°C under nitrogen atmosphere. After 5 h, the reaction mixture was filtered through celite, and the solvent was evaporated under vacuum. The residue was purified by prep-TLC using THF/MeOH (20/1, v/v) as an eluent. The yield of the obtained while solid product **18** (310 mg) was found to be 52%.^{1}H NMR (400 MHz, DMSO-d_6): δ 13.46 (brs, 1H), 8.68 (brd, J = 6.0 Hz, 1H), 8.16 (d, J = 1.2 Hz, 1H), 8.03 (dd, J = 8.4, 1.6 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.90 (td, J = 8.0, 1.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.38 (ddd, J = 8.0, 8.0, 1.6 Hz, 1H), 2.95 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6): δ 160.0, 155.0, 149.6, 137.5, 136.4, 123.2, 122.8, 120.4, 110.8, 105.8, 13.5. HRMS-ESI (*m/z*): calculated for $C_{16}H_{12}O_2N_5S$ $[M+H]^+$ 338.07062, found 338.07050.

5.2.8 *4-methyl-N-(4-(pyridin-2-yl)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**19**).* The method used to synthesize this

compound is the same as that used to prepare **18**. **19**: slightly yellow solid, 41% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38 (ddd, J = 4.8, 2.0, 0.8 Hz, 1H), 7.76 (ddd, J = 8.0, 7.6, 2.0 Hz, 1H), 7.65 (dt, J = 8.0, 0.8 Hz, 1H), 7.60 (brd, J = 8.4 Hz, 2H), 7.50 (dd, J = 8.4, 2.0 Hz, 1H), 7.45 (ddd, J = 7.6, 4.8, 0.8 Hz, 1H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.0, 150.6, 141.5, 139.6, 128.2, 127.1, 123.5, 116.9, 112.6, 13.5. HRMS-ESI (*m/z*): calculated for $C_{16}H_{12}O_2N_5S$ $[M+H]^+$ 338.07062, found 338.07068.

5.2.9 *4-methyl-N-(5-(pyridin-3-yl)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**20**)*

A mixture of *N*-(5-bromobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide **4** (130 mg, 0.38 mmol), pyridin-3-ylboronic acid (230 mg, 1.90 mmol), K_3PO_4 (400 mg, 1.90 mmol), and $Pd(PPh_3)_4$ (43 mg, 0.038 mmol) in 7 mL DMF/H_2O (6/1, v/v) was heated in a microwave at 150°C under nitrogen atmosphere and monitored using LCMS. After 30 min, the solvent was evaporated under vacuum, and the residue was purified by prep-TLC using DCM/THF/MeOH (10/10/1, v/v/v) as an eluent. The yield of the obtained yellow solid product **20** (45 mg) was found to be 35%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (d, J = 1.6 Hz, 1H), 8.52 (dd, J = 4.8, 1.6 Hz, 1H), 8.01 (brd, J = 8.0 Hz, 1H), 7.71 (d, J = 1.6 Hz, 1H), 7.44 (dd, J = 7.6, 4.8 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 8.4, 1.6 Hz, 1H), 2.89 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.8, 163.7, 157.4, 147.8, 147.6, 147.4, 136.4, 134.0, 132.0, 123.8, 120.1, 114.7, 108.8, 105.8, 13. 3. HRMS-ESI (*m/z*): calculated for $C_{16}H_{12}O_2N_5S$ $[M+H]^+$ 338.07062, found 338.07100.

5.2.10 *4-methyl-N-(5-(pyridin-4-yl)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**21**).* The method used to synthesize this compound is the same as that used to prepare **20**. **21**: pale solid, 31% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (d, J = 5.2 Hz, 2H), 7.82 (s, 1H), 7.65 (d, J = 5.2 Hz, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 2.89 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.9, 163.8, 157.4, 152.1, 150.0, 148.1, 147.8, 147.7, 144.6, 132.0, 121.1, 120.0, 114.6, 109.5, 108.8, 13.3. HRMS-ESI (*m/z*): calculated for $C_{16}H_{12}O_2N_5S$ $[M+H]^+$ 338.07062, found 338.07070.

5.2.11 *methyl 4-(2-(4-methyl-1,2,3-thiadiazole-5-carboxamido)benzo[d]oxazol-5-yl)benzoate (**22**).* The method used to synthesize this compound is the same as that used to prepare **20**. **22**: white solid, 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (m, 4H), 7.19 (m, 3H), 3.84 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.2, 163.2, 151.9, 139.6, 135.6, 129.9, 126.7, 125.3, 52.6, 13.5. HRMS-ESI (*m/z*): calculated for $C_{19}H_{15}O_4N_4S$ $[M+H]^+$ 395.08085, found 395.08017.

5.2.12 *4-methyl-N-(5-(1-methyl-1H-pyrazol-4-yl)benzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**23**).* The method used to synthesize this compound is the same as that used to prepare **20**. **23**: yellow solid, 35% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.78 (s, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 7.18 (dd, J = 7.8, 1.8 Hz, 1H), 3.85 (s, 3H), 2.87 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.1, 162.9, 157.2, 152.4, 146.2, 144.1, 135.8, 127.4, 127.3, 122.7, 117.9, 112.4, 108.4, 38.6, 13.2. HRMS-ESI (*m/z*): calculated for $C_{15}H_{13}O_2N_6S$ $[M+H]^+$ 341.08152, found 341.08195.

5.2.13 *4-methyl-N-(5-morpholinobenzo[d]oxazol-2-yl)-1,2,3-thiadiazole-5-carboxamide (**24**)*

XPhos (76mg, 0.16 mmol) and $Pd(PPh_3)_4$ (92 mg, 0.08 mmol) were added to a mixture of N-(5-bromobenzo[d]oxazol-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide **4** (260 mg, 0.77 mmol), morpholine (134 mg, 1.54 mmol), and *t*-BuOK (173mg, 1.54 mmol) in 10 mL anhydrous DME. The resulting solution was reacted at 110°C under nitrogen atmosphere for 2 h and monitored using LCMS. Then, the solvent was evaporated under vacuum and the residue was purified by prep-TLC using DCM/MeOH (20/1, v/v) as eluent. The yellow solid product obtained at the end was labeled **24** (50 mg), and its yield was determined to be 19%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.6 (s, 1H), 3.75 (t, J = 4.8 Hz, 4H), 3.08 (t, J = 4.8 Hz, 4H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.7, 149.6, 112.6, 110.8, 66.0, 49.2, 13.5. HRMS-ESI (*m/z*): calculated for $C_{15}H_{16}O_3N_5S$ $[M+H]^+$ 346.09684, found 346.09750.

5.3. Determination of Anti-Mtb MIC values

The anti-Mtb activities of the synthesized compounds were tested against the standard Mtb strain H37Rv (ATCC 27294), as well as the drug-resistant MDR-FJ05120 and XDR-FJ05195 strains (clinical isolates). The first-line anti-TB drugs isoniazid, rifampicin, streptomycin, and ethambutol were used as positive controls. The MICs of *M. tuberculosis* replication inhibition were determined by Microplate Alamar Blue Assay (MABA). The final concentrations of the investigated compounds varied between 0.125 and 128 mg/L. The bacterial strains (10^6 cfu/mL cell concentration) were cultured with each one of the synthesized compounds (varying concentrations)

at 37°C in Middlebrook 7H9 broth (Difco) supplemented with 0.2% glycerol and 10% oleic acid-alumin-dextrose-citric acid (OADC). The culture was arrested when the mid-log phase of growth was reached. The MIC values were then measured in sterile 96-well plates with a final volume of 100 μL per well. These values correspond to the lowest drug concentrations for which the color of alamar blue reagent remains unchanged [21].

5.4. Cell cytotoxicity

The cytotoxicity of all compounds was examined in HEK-297T cells. The compounds were dissolved in DMSO at a concentration of 10 mM, and then the solutions were diluted with DMEM culture medium containing 5% PBS to varying concentrations between 0.78 and 200 μM. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well, and then they were incubated with the compounds at 37°C. Forty-eight hours later, 20 μL of the Cell Counting Kit-8 (CCK-8) solution was added to each well, and the mixtures were incubated for another 4 h. Finally, the absorbance of each well was measured at 450 nm using a Microplate reader (EnVision, PerkinElmer). The IC_{50} values were calculated based on a concentration-response curve using GraphPad Prism 5.

5.5. Acute Toxicity

SPF BALAC/c female mice purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) weighing between 18 and 22 g were used to evaluate the *in vivo* acute toxicity of compounds **1**, **6**, and **18**. The mice were randomly divided into groups of six, and each group was administered with one of the investigated compounds in 0.5% CMC-Na solution (single 1000 mg/kg dose) by oral gavage. All of the mice were reared in appropriate environments where the temperature was maintained between 20 and 24°C, with mean day and night times. The number of surviving mice was monitored during the 14-day period following compound administration. The animal care and experimental procedures performed herein are in accordance with the regulations of the Institutional Animal Care and Use Committee of the Institute of Medicinal Biotechnology.

5.6. Determination of pharmacokinetic profiles

SPF male SD rats weighing between 180 and 220 g (Shanghai Sippr-BK laboratory animal Co., Ltd.) were fasted overnight in metabolism cages before being administered with one of the investigated compounds. The rats were allowed free access to water, but they were given food only after 4 h of compound administration. Each treatment group consisted of three rats, and all of them were treated with a single dose (40 mg/kg) of the tested compounds suspended in 5% DMSO, 10% Solutol, and 85% deionized water. Blood samples (200 μL) were collected from the jugular veins of rats at 0.25, 0.5, 1, 2, 4, 6, and 8 h after compound administration. The samples were put on ice and centrifuged at 6800 g and 4°C for 6 min in order to obtain the plasmas after 2 h. The protein contents in 20 μL aliquots of the plasma samples were precipitated with 400 μL MeOH containing the internal standard (100 ng/mL). The mixtures were vortexed for 1 min then centrifuged at 18000 g for 7 min. Subsequently, 200 μL of the supernatants were transferred to 96-well plates, and 3 μL aliquots were analyzed by LC-MS/MS. The area under the concentration time curve (AUC), the peak concentration (C_{max}), the time to reach peak concentration (T_{max}), and mean residence time (MRT) of each sample were determined based on the plasma concentration data using WinNonlin.

5.7. Broad spectrum panel analysis

The anti-bacterial activities and MIC values of compounds **6** and **18** were determined for a series of Gram-positive and Gram-negative bacterial pathogens, in accordance with the agar dilution method recommended by the CLSI. The selected strains represent important bacteria, and they include five Gram-positive (*S. aureus* ATCC 29213, *S. aureus* ATCC 33591, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *E. faecium* ATCC 700221) and five Gram-negative strains(*E. coli* ATCC 25922, *K. pneumonia* ATCC700603, *K. pneumonia* 7, *P. aeruginosa* ATCC 27853, *A. calcoacetious* ATCC 19606). Inoculations were adjusted using a multipoint inoculator (Bolney, Sussex, UK) to yield cell concentrations of about 10^4 cfu/spot, and then the cells were incubated at 35°C for 18 h. The MICs of compounds **6** and **18** were taken to be the lowest concentrations at which bacterial growth is inhibited.

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Abbreviations

EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride; HoBt, 1-hydroxybenzotriazole; TEA, triethylamine; DMF, *N*,*N*-dimethylformamide; XPhos, (2-aminobiphenyl)-cyclometalated palladium mesylateprecatalyst complex; DME, 1,2-dimethoxyethane; AUC, area under curve; MRT, mean residence time.

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Highlights

- Twenty-nine 2-acylamide-1,3-benzo[d]zole analogues were synthesized.
- 17 compounds were found to be potent anti-Mtb agents, especially against the MDR- and XDR-MTB strains, with MIC values < 10 μM.
- Compound **18**, with a 5-(pyridin-2-yl) at R_2, show potent anti-Mtb activities, acceptably safe and favorable pharmacokinetic (PK) properties.
- Compound **18** is also a potent anti-Gram-positive agent.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: