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Discovery of New Isoniazid Derivatives As Anti-tubercular Agents: *In silico* Studies, Synthesis, and *In vitro* Activity Evaluation

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ABSTRACT

This research aimed to discover novel isoniazid (INH) derivatives as anti-tubercular (anti-TB) agents. The chemical structures of isoniazid-based pyridazinone (IBP) derivatives were designed, and their toxicity and pharmacokinetic properties were predicted using the ProTox II and Swiss-ADME databases. The molecular docking of non-toxic IBPs was also performed concerning INH, pyrazinamide (PYZ), ethionamide (ETH), macozinone (MCZ), and BTZ043 utilizing DprE1 enzyme's proteins (PDB IDs: 4F4Q, 4NCR and 6HEZ). Based on the in silico study results, IBP19, IBP21, IBP22, and IBP29 were selected for their synthesis, and the spectral analysis confirmed their chemical structures. *In vitro*, anti-TB activity against Mtb H37Rv strain and MTT assay (against HepG2 and Vero cell lines) of IBP19, IBP21, IBP22, and IBP29 were also carried out. A total of eleven non-toxic IBPs were identified with promising pharmacokinetic parameters. The docking score (DS in kcal/mol against 6HEZ protein) of IBP19 (-9.52), IBP21 (-8.78), IBP22 (-9.07), and IBP29 (-9.99) was better than MCZ (-8.76) and BTZ043 (-8.56) revealing their DprE1 enzyme inhibitory action. The *in vitro* anti-TB activity evaluation (MIC values) confirmed that IBP19 (1.562 µg/ml), IBP21 (1.562 µg/ml), IBP22 (1.562 µg/mL), and IBP29 (1.562 µg/mL) had almost double potency than INH (3.125 µg/mL), and PYZ (3.125 µg/mL). IBP19, IBP21, IBP22, and IBP29 also displayed a CC_{50} value of > 300 µg/mL against HCL and VCL cell lines. This effect was better than INH (> 200 µg/mL), ETH (> 150 µg/mL), and PYZ (> 200 µg/mL). Accordingly, IBP19, IBP21, IBP22, and IBP29 provide a new template for developing safe and effective novel DprE1 inhibitors.

Keywords: Isoniazid, Pyridazinone, Tuberculosis, DprE1 enzyme, *In silico* studies, Synthesis, Anti-tubercular agents.

INTRODUCTION

Mycobacterium tuberculosis (Mtb), which causes contagious tuberculosis (TB), has evolved into a global health crisis[1,2]. According to WHO's global TB report of 2022, around 10.6 million individuals were diagnosed with TB in 2021, cases of drug-resistant TB have increased due to the COVID-19 pandemic, and untreated TB has about a 50% mortality rate[3]. TB cure needs long-term treatment (about 4-6 months) with anti-TB drugs like isoniazid (INH), pyrazinamide (PYZ), rifampin, ethambutol, and ethionamide (ETH). The chronic use of most existing treatments causes patient non-compliance, leading to drug-related toxicity (hepatotoxicity) and the development of drug resistance[4,5]. Accordingly, scientists are striving to identify new drug targets (DprE1 and MmpL3) and chemical templates for developing better, safer, and more effective TB drugs with shorter treatment duration[1,4-7]. DprE1 enzyme is an important drug target to combat drug resistance issues[1,2]. DprE1 enzyme is absent in humans, but it is essential for the cell wall development of Mtb. The periplasmic location of DprE1 is another advantage for anti-TB drug development. These features of the DprE1 enzyme imply that DprE1 inhibitors have high selectivity for Mtb, may have negligible or small side effects on the human body, and will benefit drug-resistant TB patients who need long-term treatment. Macozinone (MCZ), BTZ043, OPC-167832, and TBA-7371 are validated DprE1 inhibitors in clinical trials[1,4-7].

Recently, scientists have been modifying the chemical structure of INH to create new analogs with improved safety and efficacy[8,9]. It is proposed that the metabolic protection of the hydrazine unit of INH with a lipophilic moiety can improve its clinical benefits[9]. Pyridazinone is a six-membered diazine of pharmaceutical importance, and many pyridazinone-based drugs are in clinical use to treat various diseases[10-12]. Recent studies have also demonstrated pyridazinone ring-based compounds as promising anti-TB agents[8,9,11,14-17]. Additionally, the isoniazid-based pyridazinone (IBP) derivatives can easily be obtained simply by reacting the hydrazine unit of INH and 4-oxobutanoic acids derivatives[18]. Accordingly, this research was planned to discover novel IBP derivatives as DprE1 inhibitors possessing enhanced anti-TB activity and promising safety profiles by protecting the hydrazine unit of INH.

MATERIALS AND METHODS

General

The analytical grade chemicals/reagents used in the following methods were obtained from Sigma Aldrich (USA). The data of the melting points (Gallenkamp apparatus), FTIR (Shimadzu 440 spectrophotometer), ^{13}C-NMR and ^{1}H-NMR (Varian Gemini 125/500 MHz spectrophotometer), and mass spectra (70 eV GCMS/QP 1000 Ex mass spectrophotometer) were utilized to acquire the spectral information of the synthesized Isoniazid-based pyridazinones (IBP) (Scheme 1). The name of the software used in this research work is mentioned in the relevant parts.

Design of the compounds

The chemical structures of thirty-three IBPs were designed with ChemDraw (version 21) software based on the reaction between isoniazid and 4-oxobutanoic acid derivatives reported in the United States Patent Number US4052395A (Figure 1)[19].

Prediction of the toxicity

The toxicity properties of the designed IBPs were evaluated using the ProTox-II server[20]. ChemDraw was used to make the Mol Files of the chemical structures of IBPs. The notepad was employed to read and copy IBP's Mol files. The copied content was then pasted into the ProTox-II database, the start button was pressed, and the toxicity data of IBPs was recorded (Table 1).

Prediction of the pharmacokinetic parameters

Swiss-ADME software was utilized to predict IBP's pharmacokinetic parameters[20,21]. The chemical structures of IBPs as Mole Files were imported to the Swiss-ADME software and the run button was pressed to get the pharmacokinetic data (Table 2). The following equation was used to calculate the % oral absorption of the IBPs[22], wherein TPSA is the topological surface area.

$$\%Absorption=109-(0.345 \times TPSA)$$

Molecular docking

This study was done on Molecular Operating Environment software (MOE) (2019.0102 version, Chemical Computing Group Inc., Canada). Three DprE1 enzyme proteins (PDB IDs: 4F4Q, 4NCR, and 6HEZ) were employed for the docking study of IBPs, INH, ETH, PYZ, BTZ043, and MCZ[2]. The DprE1 proteins (PDB IDs: 4F4Q, 4NCR, and 6HEZ) were imported separately in the software, purified by pressing the Quickprep button and saved

in the specified folder. The MDB files of the IBPs were also created and saved in the specified folder. A single purified protein (4F4Q, 4NCR, or 6HEZ) was imported into the software, and the MDB-Files (IBPs, INH, ETH, PYZ, BTZ043 and MCZ) were docked by pressing the dock button. The docking score (DS in kcal/mol) and the root mean square deviation (RMSD) of IBPs, INH, ETH, PYZ, BTZ043, and MCZ were recorded (Table 3).

Synthesis of IBPs

A solution of INH (0.01 mole) and 4-(5-chloro-2-hydroxy-4-methylphenyl)-4-oxobutanoic acid (0.01 mole) in ethanol (50 mL) was refluxed for four hours. A solid was formed during the reaction, which was filtered hot with a Whatman filter paper to get IBP19. The product was recrystallized by ethanol. This procedure was also employed to prepare IBP21, IBP22, and IBP29 using the appropriate 4-oxobutanoic acid derivative (Scheme 1). The characterization data of IBP19, IBP21, IBP22, and IBP29 is provided in Table 4.

Anti-TB activity

The Microplate Alamar Blue Assay (MABA) technique was employed to perform this experiment utilizing Mtb H37Rv strain[2,23,24]. Resazurin (blue) is used in this assay, and its color shifts from blue to pink when bacteria multiply. Each chemical and reagent used in this experiment was prepared following the published protocol[24]. The sterile DMSO was employed to make different dilutions (50, 25, 12.5, 6.25, 3.125, 1.562, and 0.781 µg/mL) of IBP19, IBP21, IBP22, IBP29, INH, ETH, and PYZ. The MIC of each compound was determined by reading the microplate at 530 (excitation) and 590nm (emission) (Table 5).

MTT-assay

The MTT test was used to determine the toxicity profile of IBP19, IBP21, IBP22, IBP29, INH, ETH, and PYZ concerning HepG2 cell (HCL) and Vero cell (VCL) lines[13,14]. The assay relies on the ability of HCL and VCL to produce a dehydrogenase enzyme that transforms MTT into formazan crystals. Formazan (purple) color intensity is assessed colorimetrically, and cell viability is determined. The previously published procedure was followed to prepare chemicals and different concentrations of test/standard compounds (300, 250, 200, 150, 100, and 50 µg/mL) for this experiment, and the calculation of CC^{50} values (minimum concentration required for 50% cell death) and selectivity index ($SI=CC_{50}/MIC$) (Table 5)[2].

Statistical analysis

SPSS was used for the statistical analysis of the experimental data (version 20, Chicago, IL, USA). The results are considered statistically significant if the p-value (N=3; Mean±SD) is less than 0.05.

RESULTS

The chemical structures of thirty-three IBPs were designed based on the reaction between isoniazid and 4-oxobutanoic acid derivatives (Figure 1)[19].

The toxicity of the IBPs, INH, ETH, PYZ, MCZ, and BTZ043 was predicted by ProTox II software 20 (Table 1).

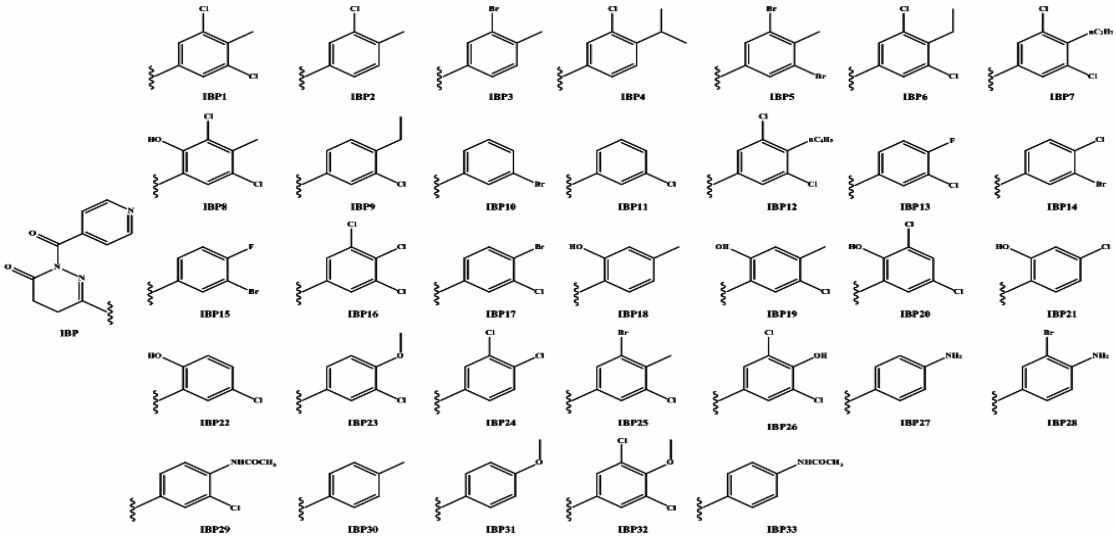


Fig. 1. Chemical structures of designed IBPs

Table 1: Predicted toxicity data of IBPs

Compound	LD_{50} (mg/kg)	Toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
INH	133	3	Yes	Yes	No	No	No
ETH	1000	4	Yes	No	No	No	No
PYZ	1000	4	Yes	No	No	No	No
MCZ	1000	4	No	Yes	Yes	Yes	No
BTZ043	1000	4	No	Yes	Yes	Yes	No
IBP4	500	4	No	No	No	No	No
IBP16	500	4	No	No	No	No	No
IBP19	500	4	No	No	No	No	No
IBP20	500	4	No	No	No	No	No
IBP21	500	4	No	No	No	No	No
IBP22	500	4	No	No	No	No	No
IBP23	500	4	No	No	No	No	No
IBP24	500	4	No	No	No	No	No
IBP26	500	4	No	No	No	No	No
IBP29	1000	4	No	No	No	No	No
IBP32	500	4	No	No	No	No	No

The LD_{50} of the IBPs ranged from 500-1000 mg/kg, and their toxicity class was 4. Notably, the predicted LD_{50} of INH (133 mg/kg) was less than the predicted toxicity of IBP's LD_{50}. Twenty-two IBPs showed toxic properties (hepatotoxicity + carcinogenicity = 1; immunotoxicity + carcinogenicity = 1; immunotoxicity=1; carcinogenicity=19). Eleven IBPs demonstrated non-toxic properties (Table 1). The study also revealed the toxicity of INH (hepatotoxicity+carcinogenicity), ETH (hepatotoxicity), PYZ (hepatotoxicity), and MCZ (carcinogenicity, immunotoxicity, and mutagenicity) and BTZ043 (carcinogenicity, immunotoxicity, and mutagenicity). The eleven compounds (IBP4, IBP16, IBP19, IBP20, IBP21, IBP22, IBP23, IBP24, IBP26, IBP29, and IBP32) were selected for their Swiss-ADME analysis (Table 2) and molecular docking studies (Table 3)[20,21].

Table 2: The physicochemical properties, pharmacokinetics, drug-likeness, and lead-likeliness properties data of IBPs

			Pharmacokinetic parameters									
Compounds	TPSA	Log ($P_{o/w}$)	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Drug-likeness (Lipinski)	Calculated ora l% Absorption*
INH	68.01	-0.35	High	No	No	No	No	No	No	No	Yes	85.53
ETH	71.00	1.47	High	No	No	No	No	No	No	No	Yes	84.50
PYZ	68.87	-0.37	High	No	No	No	No	No	No	No	Yes	85.30
MCZ	110.50	3.86	High	No	No	No	Yes	Yes	No	Yes	Yes	70.87
BTZ043	125.72	2.87	High	No	Yes	No	Yes	Yes	No	No	Yes	65.62
IBP4	62.63	3.32	High	Yes	No	Yes	Yes	Yes	No	No	Yes	87.39
IBP16	62.63	3.36	High	Yes	No	Yes	Yes	Yes	No	No	Yes	87.39
IBP19	82.86	2.30	High	No	No	Yes	No	No	No	No	Yes	80.41
IBP20	82.86	2.50	High	No	No	Yes	Yes	Yes	No	No	Yes	80.41
IBP21	82.86	2.02	High	No	No	Yes	No	No	No	No	Yes	80.41
IBP22	82.86	1.97	High	No	No	No	No	No	No	No	Yes	80.41
IBP23	71.86	2.36	High	Yes	No	Yes	Yes	Yes	No	No	Yes	84.20
IBP24	62.63	2.89	High	Yes	No	Yes	Yes	Yes	No	No	Yes	87.39
IBP26	82.86	2.51	High	No	No	Yes	Yes	Yes	No	No	Yes	80.41
IBP29	91.73	2.01	High	No	No	No	No	No	No	No	Yes	77.35
IBP32	71.86	2.92	High	Yes	No	Yes	Yes	Yes	No	Yes	Yes	84.20

The predicted oral absorption of the IBPs was high (77.3%-87.39%) as compared to MCZ (70.87%) and BTZ043 (65.62%). INH, ETH, PYZ, and the IBPs were not predicted as enzyme inhibitors or as a substrate of P-gp. MCZ and BTZ043 showed inhibitory potential for CYP2C19 and CYP2C9.

BTZ043 was also predicted as a substrate of P-gp. IBP4 and IBP16 displayed the ability to cross the blood-brain barrier (BBB). None of the IBPs was a substrate of P-gp. Additionally, none of them showed a propensity to inhibit CYP2D6 and CYP3A4. All IBPs passed Lipinski's rule of drug-likeliness.

Table 3: Molecular docking data of selected IBPs

Compounds	4F4Q			4NCR			6HEZ		
	DS	%Inhibition	RMSD	DS	%Inhibition	RMSD	DS	%Inhibition	RMSD
IBP4	-6.85	82.43	0.92	-6.88	87.64	1.18	-7.78	88.81	1.10
IBP16	-6.38	76.77	0.76	-6.30	80.25	0.87	-7.58	86.52	1.16
IBP19	-8.68	104.45	1.31	-7.93	101.01	1.15	-9.52	108.67	1.01
IBP20	-6.51	78.33	1.30	-5.98	76.17	1.40	-7.34	83.78	1.49
IBP21	-8.48	102.04	1.35	-8.10	103.18	1.51	-8.78	100.22	0.87
IBP22	-8.63	103.85	1.44	-8.19	104.33	1.37	-9.07	103.53	1.33
IBP23	-6.24	75.09	1.45	-6.07	77.32	0.95	-8.04	91.78	1.27
IBP24	-6.48	77.97	1.39	-6.21	79.10	1.01	-7.48	85.38	1.31
IBP26	-6.71	80.74	0.81	-6.28	80.0	1.00	-7.54	86.07	0.75
IBP29	-8.99	108.18	1.44	-8.64	110.06	1.39	-9.99	114.04	1.40
IBP32	-7.10	85.43	1.27	-7.41	94.39	0.99	-8.12	92.69	1.43
INH	-4.99	60.04	0.97	-4.51	57.45	1.42	-4.86	55.47	1.00
ETH	-5.46	65.70	0.99	-5.91	75.28	1.33	-5.77	65.86	0.60
PYZ	-4.36	52.46	1.28	-5.01	63.82	1.13	-5.10	58.21	0.67
BTZ043	-8.16	98.19	1.27	-7.62	97.07	1.14	-8.56	97.71	1.09
MCZ	-8.31	100	1.33	-7.85	100	1.41	-8.76	100	1.14

The RMSD data of all IBPs was<1.5, indicating a closer binding to the DprE1 protein 2. In all cases, the DS of the IBP4, IBP16, IBP20, IBP23, IBP24, and IBP26 were less than BTZ043 and MCZ and displayed low inhibitory potential for the DprE1 enzyme. IBP32 displayed good inhibition of the DprE1 enzyme (85.43%-94.39%) but less than BTZ043 and MCZ (Table 3) (Fig. 2a, Fig. 2b, Fig. 2c, Fig. 2d, Fig. 2e and Figure 2f).

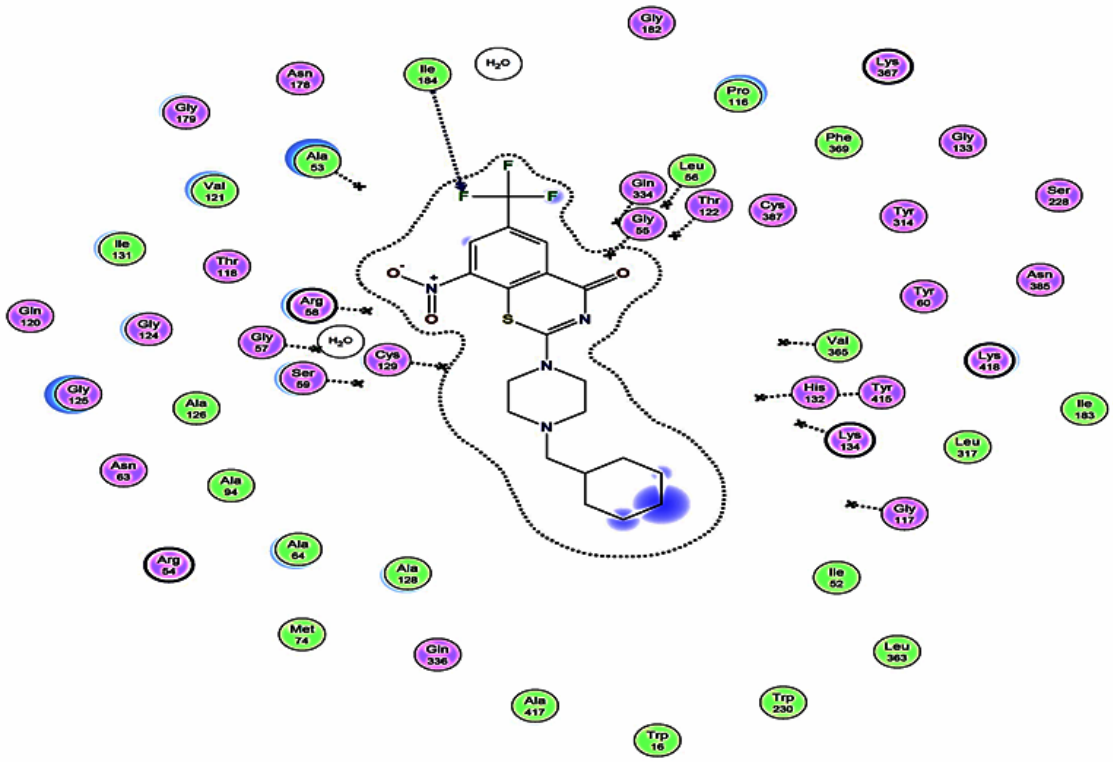


Fig. 2a. Interaction of MCZ with 6HEZ protein of DprE1

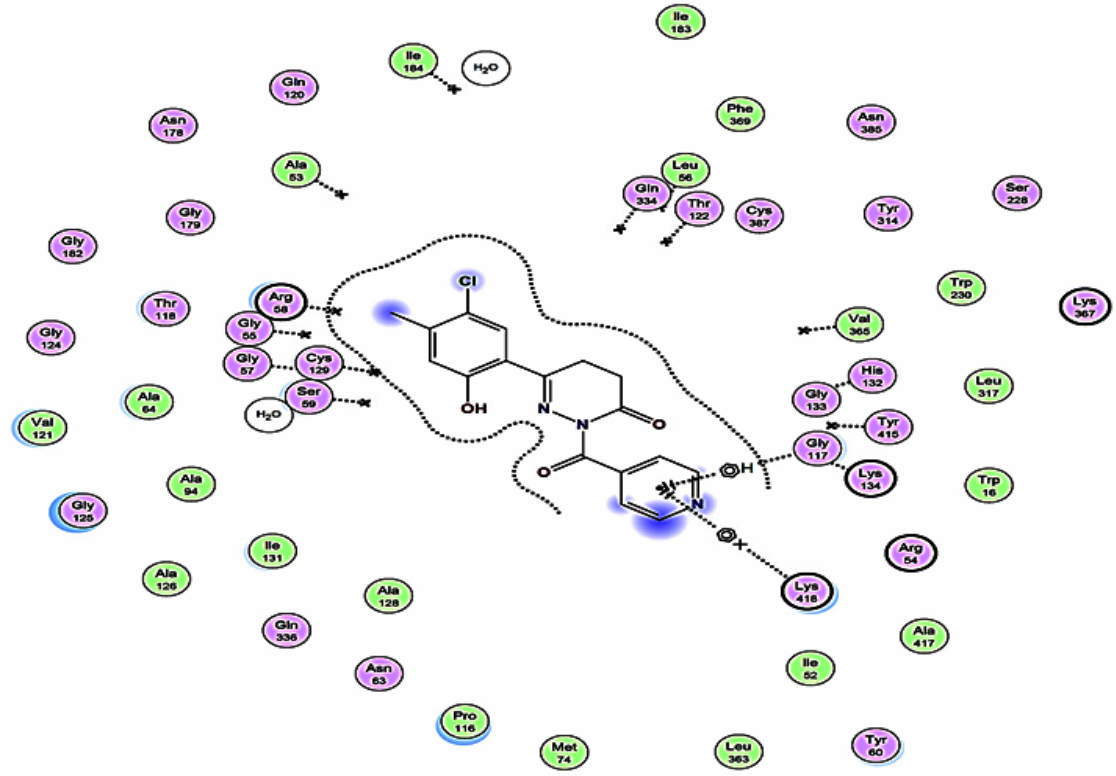


Fig. 2b. Interaction of BTZ043 with 6HEZ protein of DprE1

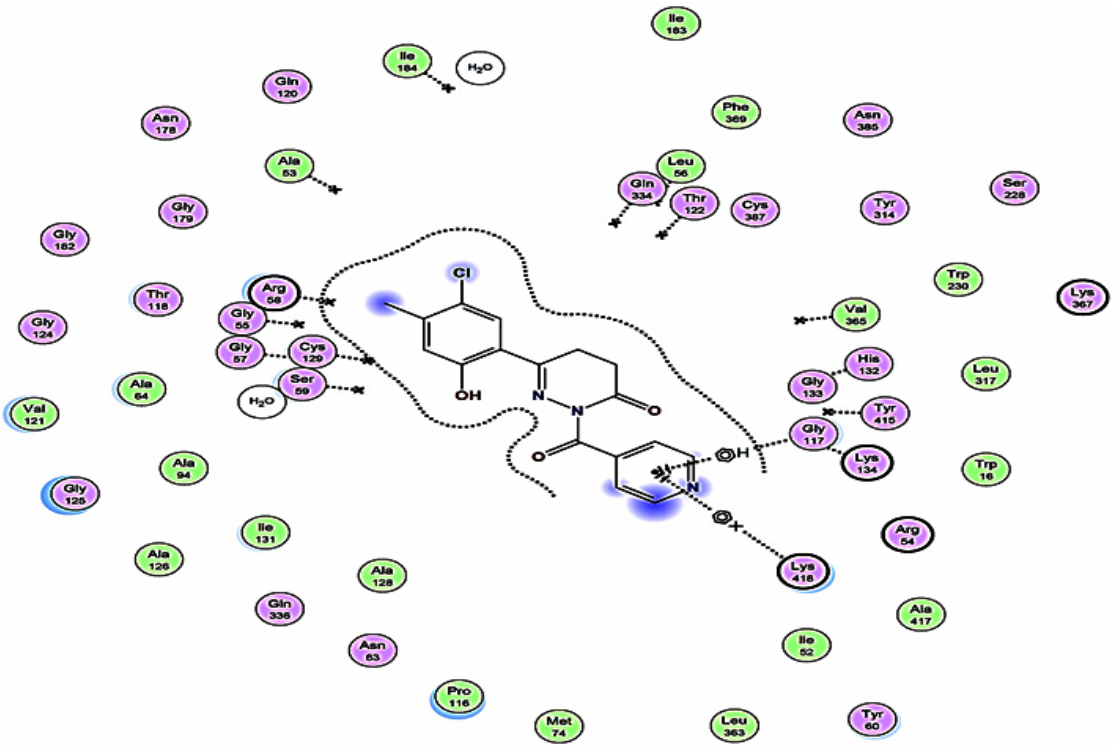


Fig. 2c. Interaction of IBP19 with 6HEZ protein of DprE1

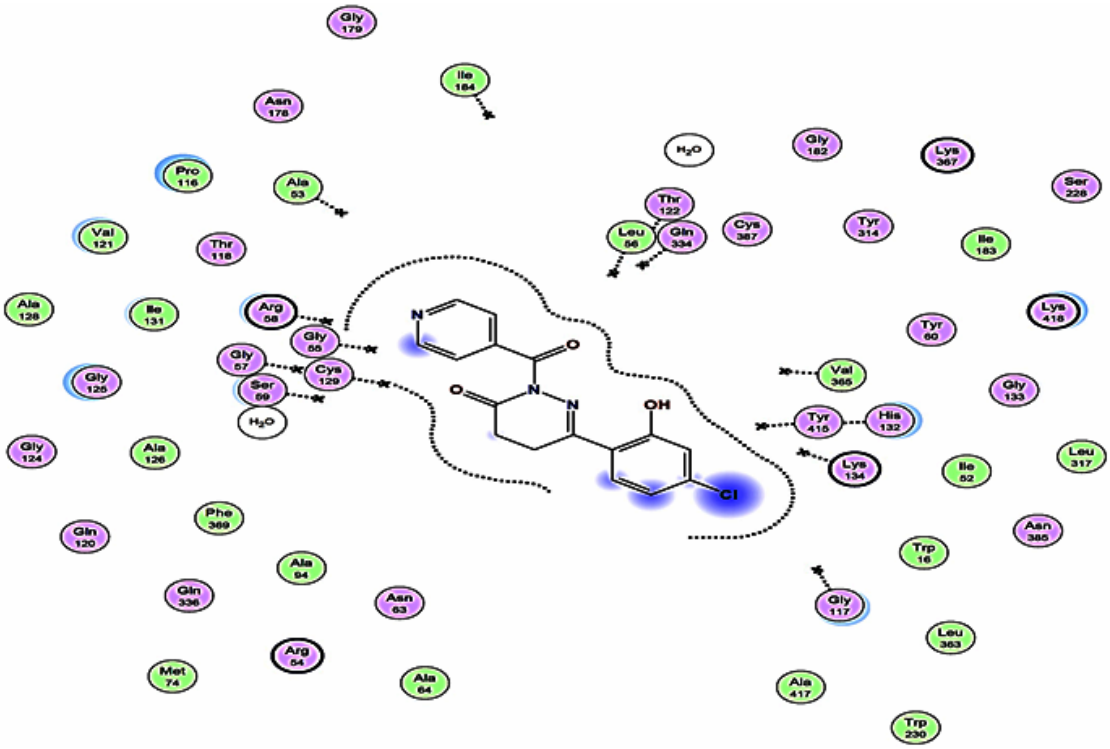


Fig. 2d. Interaction of IBP21 with 6HEZ protein of DprE1

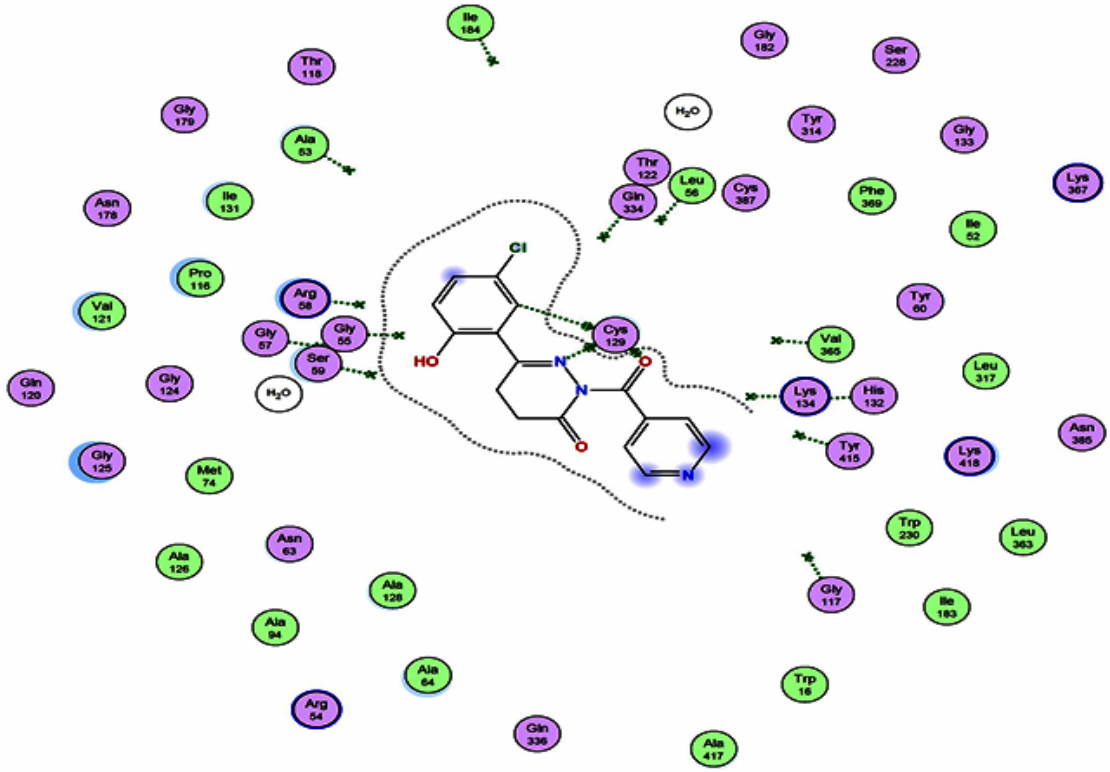


Fig. 2e. Interaction of IBP22 with 6HEZ protein of DprE1

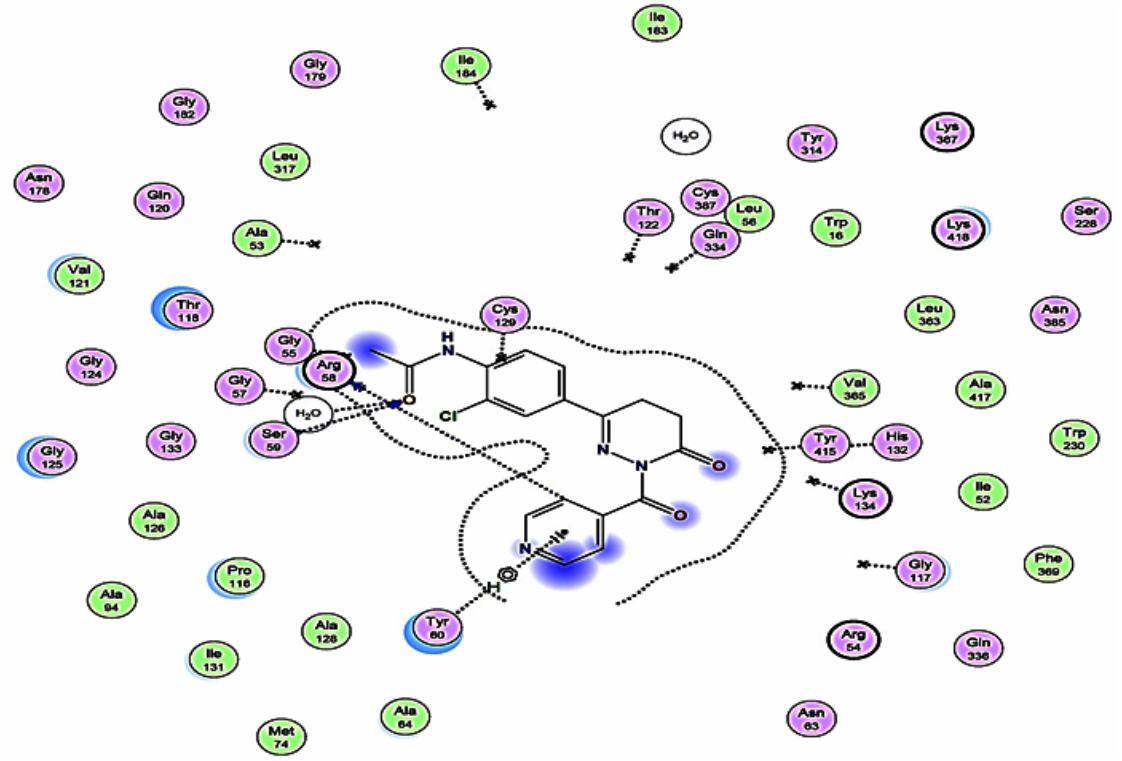


Fig. 2f. Interaction of IBP29 with 6HEZ protein of DprE1

The main interacting amino acids of MCZ (Fig. 2a) and BTZ043 (Fig. 2b) were Ile184, Ala53, Arg58, Gly57, Cys129, Ser59, Gly117, Lys134, His132, Tyr415, Val365, Thr122, Leu56, Gln334, Gly55, Arg54, Lys367, and Lys418 of 6HEZ protein. Interestingly, IBP19 (Fig. 2c), IBP21 (Fig. 2d), IBP22 (Fig. 2e), and IBP29 (Fig. 2f) also interacted with the same amino acids in addition to other amino acids of the 6HEZ protein.

IBP19, IBP21, IBP22, and IBP29 revealed better DprE1 inhibition than BTZ043 and MCZ (Table 3), non-toxic properties (Table 1), and promising pharmacokinetic parameters (Table 2). The bioavailability radars of IBP19, IBP21, IBP22, and IBP29 also suggest good bioavailability comparable to the bioavailability radars of INH, PYZ, and ETH (Fig. 3). In Fig. 3, the pink zone area refers to an area of acceptable bioavailability, whereas the red line indicates the physicochemical properties affecting the bioavailability of the compounds. The compound is bioavailable if the red line remains in the pink zone. Based on the above facts, IBP19, IBP21, IBP22, and IBP29 were selected for their synthesis (Scheme 1).

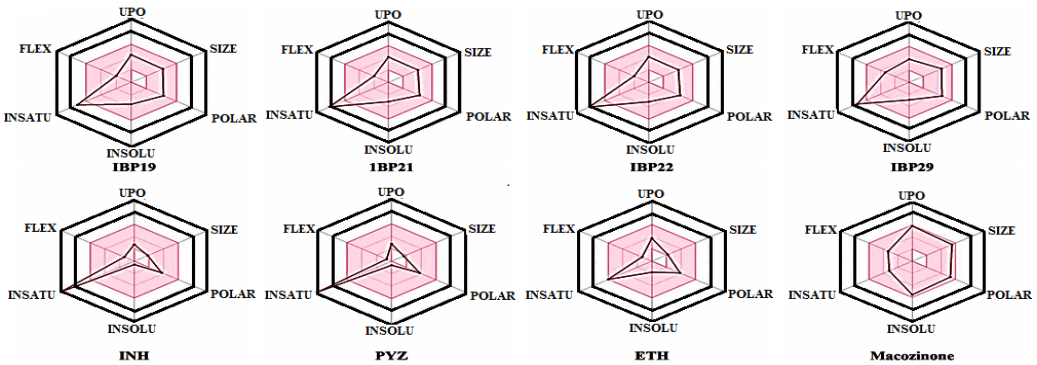
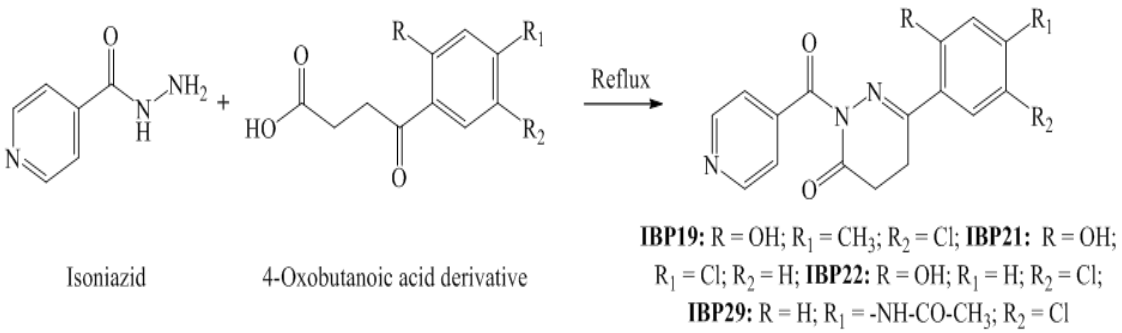


Fig. 3. The bioavailability radar of IBP19, IBP21, IBP22, IBP29, MCZ and BTZ043



Scheme 1. Synthesis of IBP19, IBP21, IBP22 and IBP29

The spectral data established and confirmed the designed structure of IBP19, IBP21, IBP22, and IBP29 (Table 4).

After establishing the chemical structures of IBP19, IBP21, IBP22, and IBP29, their anti-TB activity (MIC in µg/ml) was identified against Mtb H37Rv in addition to their MTT toxicity assay against HCL and VCL cell lines (Table 5) (Figure 4)[2].

Table 4: Spectral analysis data IBP19, IBP21, IBP22 and IBP29

Compound (MF; MW; m.p.; R_f values*; FTIR in KBr, ν in cm^{-1})	^{1}H-NMR(DMSO-d_6, 500 MHz, δ in ppm)	^{13}C-NMR(DMSO-d_6, 125 MHz, δ in ppm)	Mass (m/z)
IBP19 ($C_{17}H_{14}ClN_3O_3$; 343; 155-157°C; 0.78; 3421 (OH),1718 and 1711 (C=O), 1562 (C=N), 1531 (C=C))	2.33 (s, 3H, CH3), 2.40 (t, 2H, C4-methylene), 2.88 (t, 2H, C5-methylene), 6.70 (s, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.78 (dd, 2H, Ar-H), 8.73 (dd, 2H, Ar-H), 10,25 (s, IH, OH)	19.1 (CH3), 23.3 (C5-pyridazinone), 31.2 (C4-pyridazinone), 116.1, 118.8, 120.6 (2C), 123.4, 129.4, 139.7, 143.1, 145.4, 148.6 (2C), 158.0 (C-OH), 169.1 (C=O, pyridazinone), 169.5 (C=O)	343 (M^+, 100.0%), 344 (M++1), 345 (M++2), 237, 202, 141, 106
IBP21 ($C_{16}H_{12}ClN_3O_3$; 329; 166-168°C; 0.75; 3422 (OH),1712 and 1721 (C=O), 1564 (C=N), 1532 (C=C))	2.33 (t, 2H, C4-methylene), 2.89 (t, 2H, C5-methylene), 7.05 (s, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 7.42 (d, 1H, Ar-H), 7.76 (dd, 2H, Ar-H), 8.73 (dd, 2H, Ar-H), 10.23 (s, 1H, OH)	23.3 (C5-pyridazinone), 31.2 (C4-pyridazinone), 115.7 (2C), 120.0, 120.6 (2C), 131.0, 138.1, 139.7, 145.4, 148.6 (2C), 161.4 (C-OH), 169.1 (C=O, pyridazinone), 169.4 (C=O)	329 (M^+, 100.0%), 330 (M^++1),331 (M^++2), 223, 202, 127, 106
IBP22 ($C_{16}H_{12}ClN_3O_3$; 329; 163-165oC; 0.82; 3422 (OH),1712 and 1720 (C=O), 1565 (C=N), 1533 (C=C))	2.41 (t, 2H, C4-methylene), 2.90 (t, 2H, C5-methylene), 6.93 (d, 1H, Ar-H), 7.30 (d, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.76 (dd, 2H, Ar-H), 8.71 (dd, 2H, Ar-H), 11.05 (s, 1H, OH)	23.3 (C5-pyridazinone), 31.2 (C4-pyridazinone), 117.5, 119.1, 120.6 (2C), 126.0, 129.5, 132.5, 139.7, 145.4, 148.6 (2C), 159.5 (C-OH), 169.1 (C=O, pyridazinone), 169.4 (C=O)	329 (M^+, 100.0%)330 (M^++1),331 (M^++2), 223, 202, 127, 106
IBP29 ($C_{18}H_{15}ClN_4O_3$; 370; 148-150°C; 0.84; 3264 (NH),1721, 1712, and 1701 (C=O), 1563 (C=N), 1530 (C=C))	2.05 (s, 3H, CH3), 2.40 (t, 2H, C4-methylene), 2.91 (t, 2H, C5-methylene), 7.68 (dd, 2H, Ar-H), 7.77 (dd, 2H, Ar-H), 7.98 (s, 1H, Ar-H), 8.73 (dd, 2H, Ar-H), 9.32 (s, 1H, NH)	23.0 (CH3), 23.3 (C5-pyridazinone), 31.2 (C4-pyridazinone), 120.6 (2C), 122.0, 126.4, 128.3, 129.5, 130.0, 135.8, 139.7, 146.8, 148.6 (2C), 167.8 (CO, acetamide), 169.1 (C=O, pyridazinone), 169.4 (C=O)	370 (M^+, 100.0%), 371 (M^++1), 372 (M^++2), 264, 202, 168, 106

*Rf values in a benzene and acetone mixture (9:1).

Table 5: *In vitro* activity evaluation data of IBP19, IBP21, IBP22, and IBP29

Compound	MIC(µg/mL) against Mtb H37Rv*	MTT assay data(CC_{50} in µg/mL)		Selectivity Index	Comparative anti-TB activity
		HCL	VCL		
IBP19	1.562±0.0	> 300	> 300	> 192	200%
IBP21	1.562±0.0	> 300	> 300	> 192	200%
IBP22	1.562±0.0	> 300	> 300	> 192	200%
IBP29	1.562±0.0	> 300	> 300	> 192	200%
INH	3.125±0.0	> 200	> 200	> 64	100%
ETH	6.25±0.0	> 150	> 150	> 24	50%
PYZ	3.125±0.0	> 200	> 200	> 64	100%

*p<0.05.

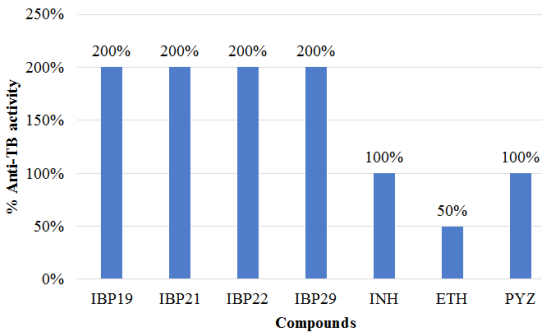


Fig. 4. Comparative anti-TB activity of IBP19, IBP21, IBP22, IBP29, INH, ETH and PYZ

DISCUSSION

This research intended to provide safe and effective IPBs for the treatment of TB. The chemical structures of thirty-three IPBs were designed (Fig. 1), and their toxicity (Table 1), pharmacokinetic parameters (Table 2), and docking studies (Table 3) were performed by *in silico* methods. A total of eleven non-toxic IBPs (IBP4, IBP16, IBP19, IBP20, IBP21, IBP22, IBP23, IBP24, IBP26, IBP29, and IBP32) were identified with promising pharmacokinetic parameters (Table 1 and Table 2). The docking study revealed IBP19, IBP21, IBP22, and IBP29 as better inhibitors of the DprE1 enzyme than MCZ and BTZ043 (Table 3). It is interesting to note that MCZ, BTZ043, IBP19, IBP21, IBP22, and IBP29 interacted with the similar amino acids of the 6HEZ protein (Fig. 2a, Fig. 2b, Fig. 2c, Fig. 2d, Fig. 2e, and Fig. 2f). These findings imply that MCZ, BTZ043, IBP19, IBP21, IBP22, and IBP29 bind at the same pocket of the DprE1 enzyme as its inhibitors. The bioavailability radars of IBP19, IBP21, IBP22, and IBP29 were comparable to those of INH, PYZ, and ETH (Fig. 3). Based on the in silico study results, IBP19, IBP21, IBP22, and IBP29 were synthesized (Scheme 1) and evaluated for their anti-TB activity. The spectral data established and confirmed the designed chemical structure of IBP19, IBP21, IBP22, and IBP29 (Table 4). These compounds exhibited characteristic peaks for the two carbonyl groups (INH and pyridazinone ring), two methylene groups of the pyridazinone ring, and the characteristic peaks for the substituents of the phenyl ring (-OH and -NH- groups). The in vitro anti-TB activity evaluation confirmed that IBP19, IBP21, IBP22, and IBP29 had almost double potency than INH and PYZ (Table 5 and Figure 4). IBP19, IBP21, IBP22, and IBP29 also displayed a CC_{50} value of >300 µg/mL against HCL and VCL cell lines. This effect was better than INH (>200 µg/mL), ETH (>150 µg/mL), and PYZ (>200 µg/mL) (Table 5).

The structure-activity relationship of IBPs indicates that a hydrophilic group at the phenyl ring (OH and acetamide) provides IBPs with a promising safety profile. Introducing a lipophilic group may provide a potent DprE1 inhibitor, and a lipophilic compound is also needed to treat brain TB 1. An increase in the Log P (lipophilicity indicator) may provide toxic compounds and inhibitory effects of the metabolizing enzymes (Table 2). Therefore, designing a non-toxic lipophilic IBP with a high binding affinity (DS) for the DprE1 is challenging but feasible. This task can be achieved by modifying the pyridazinone ring and its substituents[10-12]. TB treatment is lengthy, leading to side effects, drug interaction, and drug-resistance issues with the existing anti-TB drugs 1,25. Accordingly, IBP19, IBP21, IBP22, and IBP29 are advantageous compounds concerning their non-toxic nature and absence of inhibitory effects on metabolizing enzymes (Table 2). The IBPs provide a new template for developing safe and effective novel DprE1 inhibitors for treating TB.

CONCLUSION

Four compounds (IBP19, IBP21, IBP22, and IBP29) have been identified as non-toxic and potent inhibitors of the DprE1 enzyme possessing promising pharmacokinetic profiles. These IBPs were more potent than INH, PYZ, and ETH against Mtb and showed no propensity for drug interactions. However, further *in vitro* and *in vivo* studies are required to affirm the safety and efficacy of IBP19, IBP21, IBP22, and IBP29. Many modifications in the designed IBPs are possible to get improved and potent DprE1 inhibitors to combat drug-resistant TB. Accordingly, IBP19, IBP21, IBP22, and IBP29 provide a new template for developing safe and effective novel DprE1 inhibitors.

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Conflict of interest

No conflict of interest is associated with this work.

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