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Design, synthesis, antitubercular and antibacterial activities of pyrrolo[3,2-b]pyridine-3-carboxamide linked 2-methoxypyridine derivatives and *in silico* docking studies

Srinu Bodige[a], Parameshwar Ravula[b], Kali Charan Gulipalli[a], Srinivas Endoori[a], Purna Koteswara Rao Cherukumalli[a], Narendra Sharath Chandra JN[c], and Nareshvarma Seelam[a]

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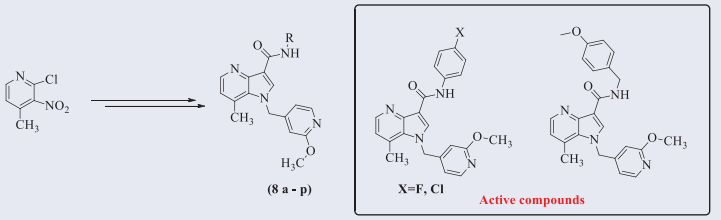
ABSTRACT

A novel series pyrrolo[3,2-b]pyridine-3-carboxamide linked 2-methoxypyridine derivatives have been designed, synthesized and confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, MS, and elemental analysis. The synthesized compounds were screened for their antitubercular activity using microplate alamar blue assay method and antibacterial activity. Among the tested compounds, 4- fluorophenyl (**8m),** 4-chlorophenyl **(8n)** and 4-methoxyphenyl **(8i)** showed potent anti-TB activity (3.12 μg/mL) in comparison with reference drug, Pyrazinamide ((3.12 μg/mL). In addition, all compounds were docked into DprE1 (PDB code: 4KW5) to explore their binding interactions at the active site. The compounds exhibited essential key interactions as that of reported DprE1 inhibitors and hence, the synthesized compounds may be considered as molecular scaffolds for antitubercular activity. Compounds, 4-chlorophenyl **(8n)** and 4-flurophenyl (**8m**) showed significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* strains. *In silico* prediction of toxicities, druglikeness and drug score profiles of the tested compounds are promising.

GRAPHICAL ABSTRACT



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KEYWORDS

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Introduction

Tuberculosis (TB) is one of the most widespread infectious disease which is caused by *Mycobacterium tuberculosis*. It is important to note that despite having an effective treatment, about 1.4 million deaths and 10.9 million clinical cases in 2015.[1] However, extensive duration of therapy and the emergence of multidrug resistant tuberculosis (MDR-TB) have created an utmost need to identify more selective new anti-TB drugs for efficient treatment.

Mycobacterium tuberculosis has a complex cell wall arrangement implicated in multiple functions related to pathogenesis and cellular physiology. Disruption of its cell wall synthesis prevents the multiplication and growth of the organism. Targeting the cell wall synthesis has an important approach in drug design. Decaprenyl phosphoryl-b-D-ribose 20-epimerase (DprE1) is involved in the conversion of decaprenylphosphoryl-β-Dribose (DPR) to decaprenylphosphoryl-β-D-arabinofuranose (DPA), an essential precursor for cell wall synthesis and survival, hence, this enzyme considered as potential target for tuberculosis.

In recent years, 1,4-azaindoles reported as DprE1 inhibitors[2,3] and in addition, their derivatives have been paid more attention because of their diverse pharmacological activities such as antitubercular,[4] antibacterial,[5] anthelmintic,[6] and anticancer.[7,8] On the other hand, pyridine is found to be a core structure in antitubercular agent, Isonizide.[9]

In view of the aforesaid facts, and in continuation of our research on 1,4-azaindoles, in the present study, it is planned to design target compounds by incorporating pyridine nucleus in to 1,4-azaindole motif with a view to produce hybrid molecules (Fig. 1). Further, various alicyclic, aliphatic and aromatic amide linkages are introduced at 3rd position of azaindole for structure activity relationship studies. The designed compounds were synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR (for **8b, 8c, 8l** and, **8m**), MS and elemental analysis. In addition, all the synthesized compounds were evaluated for their anti-TB activity against *Mycobacterium tuberculosis* using a microplate alamar blue assay (MABA) method and antibacterial activity. Furthermore, molecular docking study was performed for synthesized compounds against DprE1 to explore their binding interactions at the active site. Osiris software was used for prediction of physiochemical properties, which includes bioavailability, lipophilicity, druglikeness, drug score, mutagenicity, tumorigenicity, irritancy and C Log P of the novel pyrrolo [3,2-b]pyridine-3-carboxamide linked 2-methoxypyridine derivatives.

Results and discussion

According to Scheme 1, 2-chloro-4-methyl-3-nitropyridine **(1)** was reacted with ethyl 2-cyanoacetate **(2)** in presence of t-BuOK to get the ethyl 2-cyano-2-(4-methyl-3-nitropyridin-2-yl) acetate **(3)**. The appearance of a broad singlet at δ 14.41 in ^{1}H NMR (DMSO-d_6) and δ 168.5, δ 138.9 in ^{13}C NMR (DMSO-d_6) showed this to be completely in the vinylogous urethanes form **3a** at room temperature.[10] Further, reduction of nitro group followed by intramolecular cyclization was done in Pd/C in EtOH to afford the 4-azaindole ester derivative **(4)**.[11] ^{1}H NMR spectrum of compound **(4)** in (DMSO-d_6)

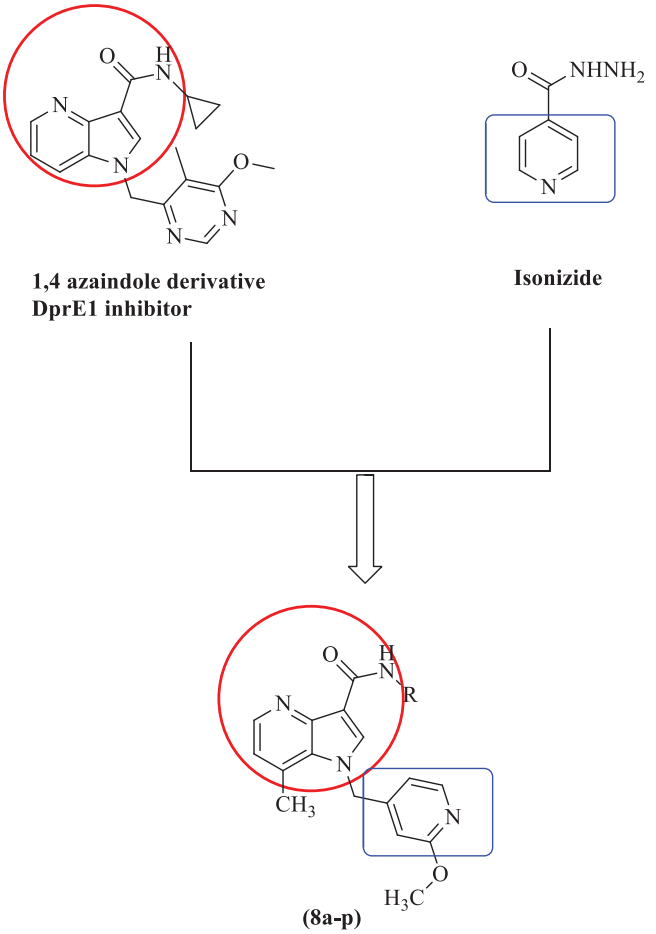
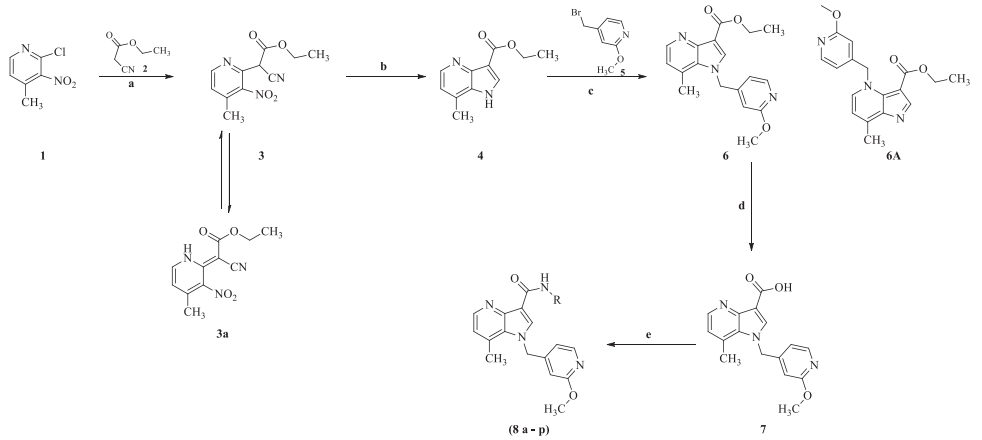


Figure 1. A design strategy of pyrrolo[3,2-b]pyridine-3-carboxamide linked 2-methoxypyridine derivatives from the reference drug isonizide and 1,4-azaindole derivatives.

showed singlet at δ 8.26 corresponding to an aromatic pyrrole proton, one broad singlet at δ 12.22 which corresponds to NH proton in pyrrole ring, two doublets at δ 8.34 and 7.03 corresponding to pyridine protons which confirmed the structures. ^{13}C NMR spectra of compound showed aromatic pyrrole carbon peaks in the range of δ 118.3–107.1 and in addition *m/z* 205.1 $[M+H]^+$ in the mass spectrum confirmed compound **(4)**.

Compound (**4**) was reacted with 4-(bromomethyl)-2-methoxypyridine **(5)** in the presence of K_2CO_3 to get compound **(6)** as major isomer with 65% yield and regioisomer (6 A) as minor compound with 24% yield. The crude product was purified by column chromatography (Combiflash, 0–10% CH_3OH/CH_2Cl_2) to afford compound **(6)** which was confirmed by the disappearance of NH proton at δ 12.22 and the appearance of singlet at δ 5.75 which corresponds to CH_2 protons of 2-methoxypyridine group and the aromatic protons appeared doublet at δ 8.10, 6.53 and singlet at δ 6.17 corresponding to 2-methoxypyridine protons and singlet at δ 8.47 corresponding to an aromatic pyrrole proton. The structures were further confirmed by 2D NOESY. A strong nOe cross peak between the CH_3 (methyl protons of the pyridine ring) and N–CH_2 and also



Scheme 1. Synthesis of title compounds. Reagents and conditions (a) **2** (1.7 equiv), t-BuOK (1.6 equiv), IPA, reflux, 6 h, 74% (b) Pd/C, EtOH, 40°C, 24 h, 80% (c) **5** (1.0 equiv), K_2CO_3 (1.2 equiv), DMF, rt, 16 h, 65% (d) LiOH.H_2O (1.5 equiv), MeOH: THF (1:1), rt, 4 h, 86% (e) R-NH_2 (1.2 equiv), HATU (1.5 equiv), DIPEA (2.0 equiv), DMF, rt, 16 h.

cross peak between aromatic proton in pyrrole ring and N–CH_2 was clearly observed in (**6**) confirming the alkylation on pyrrole NH. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound (**6**) showed aromatic carbons in the range of δ 113.9–164.0. In addition, the mass spectrum of compound (**6**) showed $[M+H]^+$ peak at *m/z* 326.2 which confirmed structure.

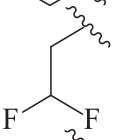
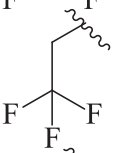
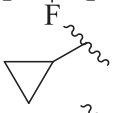
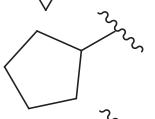
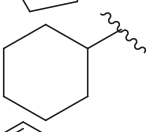
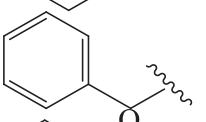
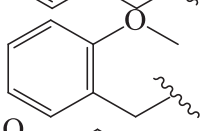
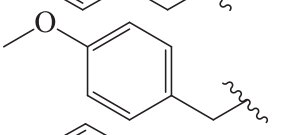
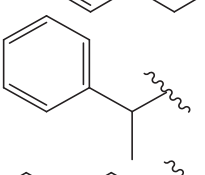
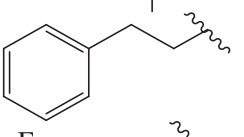
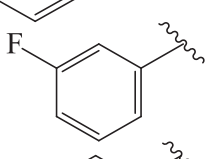
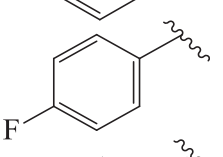
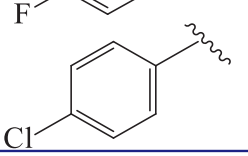
Compound (**6**) was hydrolyzed with LiOH.H_2O to afford the acid compound (**7**). The compound (**7**) was confirmed by the disappearance of ethyl ester protons at δ 4.29 and 1.32 in the ^{1}H NMR spectrum. Moreover, the mass spectrum of compound (**7**) showed $[M+H]^+$ peak at *m/z* 298.0 clearly indicated the formation compound.

The resultant product (**7**) was made to react with different amines in HATU/DIPEA condition to afford the corresponding amide derivatives **(8a–p)** with good % yield (Table 1). The appearance of signal around δ 8.90 due to NH proton in the ^{1}H NMR spectra of compounds **(8a–p)** clearly indicated the formation of proposed structures. Further support of FT-IR, ^{13}C NMR, mass and elemental analysis also confirmed the structures. Compounds **8b, 8c, 8l** and, **8m** were also confirmed by ^{19}F -NMR analysis. The physical and spectral data of compounds were given under experimental section.

Antitubercular activity

The synthesized compounds **(8a–p)** were evaluated for anti-TB activity against *Mycobacterium tuberculosis* using MABA method and their minimum inhibitory concentration (MIC) values in (µg/mL) were presented in Table 2. Among the tested compounds, compounds with aryl moiety (C-4 substitution) showed potent activity against *Mycobacterium tuberculosis* in comparison with reference drug, Pyrazinamide (Fig. 2). Compounds 4-fluorophenyl **(8m),** 4-chlorophenyl **(8n)** and 4-methoxyphenyl **(8i)** showed potent anti-TB activity (3.12 µg/mL) which is equal to activity of standard drug, Pyrazinamide (3.12 µg/mL). The aryl ring, when replaced with saturated cyclic rings (**8d, 8e,** and **8f**), led to the reduction in anti-TB activity while the remaining

Table 1. Protocol for the synthesis of the title compounds.

Entry	R	Yield (%)
8a		83
8b		81
8c		74
8d		65
8e		89
8f		82
8g		85
8h		85
8i		83
8j		81
8k		83
8l		68
8m		76
8n		77

(*continued*)

Table 1. Continued.

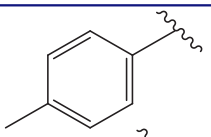
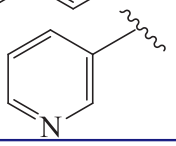
Entry	R	Yield (%)
8o		82
8p		83

Table 2. Antitubercular activity of synthesized compounds (**8a–p**).

Compound	MIC (μg/mL)
8a	12.5
8b	6.25
8c	6.25
8d	>25.0
8e	12.5
8f	6.25
8g	12.5
8h	6.25
8i	3.12
8j	12.5
8k	12.5
8l	12.5
8m	3.12
8n	3.12
8o	6.25
8p	12.5
Pyrazinamide	3.12

compounds displayed moderate to less activity. Compound (**8a)** bearing ethyl side chain showed less anti-TB activity with MIC value of 12.5 μg/mL when compared to compounds **(8b)** and (**8c)** with di and trifluoroethyl side chains respectively (MIC = 12.5 μg/mL).

Antibacterial activity

Further, the synthesized compounds were evaluated for their antibacterial activity using Agar well diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) strains. The obtained screening data are shown in Table 3

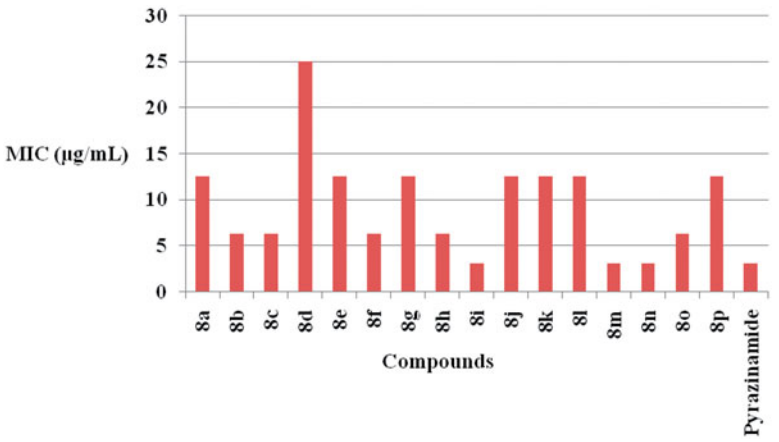


Figure 2. Antitubercular activity of synthesized compounds (**8a-p**).

Table 3. Antibacterial activity of synthesized compounds (**8a–p**).

	Zone of inhibition (mm)			
	Escherichia coli Gram-negative) (Conc. µg/mL)		*Staphylococcus aureus* (Gram-positive) (Conc. µg/mL)	
Compound	100	200	100	200
8a	14	16	15	18
8b	15	18	17	20
8c	17	19	19	22
8d	10	14	12	14
8e	12	15	15	18
8f	15	18	16	19
8g	17	19	14	16
8h	15	18	15	18
8i	18	20	17	20
8j	15	17	15	17
8k	14	18	14	16
8l	18	19	15	18
8m	22	26	20	27
8n	25	28	23	28
8o	14	18	16	19
8p	15	18	16	20
Chloramphenicol	27	30	25	29

indicated that the compounds (**8n)** and (**8m)** bearing 4-chlorophenyl and 4-flurophenyl moieties respectively showed significant antibacterial activity. The introduction of saturated cyclic rings (**8d**, **8e,** and **8f)** showed less activity than fluorine side chain bearing compounds (**8a**, **8b,** and **8c)**. Remaining compounds were found to possess moderate activity against gram + ve and gram-ve strains when compared with standard drug Chloramphenicol.

Assessment of lipophilicity, drug likeness, drug score profiles and toxicity risks

The Osiris Explorer was utilized for anticipating the overall toxicity of the synthesized compounds. The prediction process depends on a set of predetermined data of

Table 4. Computationally predicted lipophilicity, solubility, druglikeness, drug score profiles, and toxicity risks of the synthesized compounds.

Compound	C Log P[a]	Log S[b]	Druglikeness	Drug score	Molecular weight	Toxicity risks[c]
8a	2.13	−2.09	2.15	0.80	324	Negative
8b	1.66	−2.56	−1.78	0.50	360	Negative
8c	2.61	−2.83	−5.98	0.41	378	Negative
8d	2.33	−2.56	2.42	0.84	336	Negative
8e	2.96	−3.10	−0.29	0.58	364	Negative
8f	3.28	−3.37	−2.61	0.41	378	Negative
8g	3.06	−3.11	1.35	0.71	386	Negative
8h	2.96	−3.13	1.59	0.70	416	Negative
8i	2.96	−3.13	2.50	0.73	416	Negative
8j	3.52	−3.19	2.77	0.72	400	Negative
8k	3.41	−3.23	2.06	0.71	400	Negative
8l	3.34	−3.61	−1.32	0.36	390	Negative
8m	3.34	−3.61	0.40	0.59	390	Negative
8n	3.89	−4.03	2.62	0.64	406	Negative
8o	3.59	−3.64	0.21	0.34	386	Negative
8p	2.20	−2.50	1.48	0.62	373	Negative

[a]Calculated lipophilicity.
[b]solubility parameter.
[c]mutagenicity, tumorigenicity, irritancy, and reproductive effects.

structural fragments that offers ascend to toxicity alerts in case they are encountered in the structure. All the synthesized compounds exhibited low *in silico* toxicity risks as given in Table 4. As indicated by the Osiris program, the orally administered molecules are considered to be drug candidates, if it fulfills the accompanying criteria: (a) C Log P must not be more than 5; (b) Log S values more than −5; (c) molecular weight under 500. Compounds violating any one of these rules are expected to have bioavailability problems. The results showed (Table 4) that the hydrophilicity (C Log P) of all the synthesized compounds was less than 5.0, Log S values were observed more than −5 with the molecular weight under 500.

Further, the program also determines compound druglikeness based on topological descriptors, fingerprints on MDL structure keys, C Log P and molecular weights. The positive value states that drug molecules contain fragments which are frequently present in commercial drugs. The drug score combines druglikeness, Log S, C Log P, molecular weight and toxicity risks that might be utilized to judge the virtual compounds for their overall potential to qualify as a possible drug. A value around 0.5 makes synthesized compounds as a promising lead for the development of safe and efficient drug. Prediction of druglikeness and drug score for the target compounds are given in Table 4. Interestingly, all the derivatives exhibited good values of druglikeness and drug score. This information recommends that synthesized molecules could be considered as drug candidates.

Molecular docking

In the present study, the synthesized compounds were docked into DprE1 (PDB code: 4KW5) to explore their docking interactions at the active site. Molecular docking revealed that prepared compounds **(8a–p)** interacted with amino acid residues such LYS 418, GLY 117, LYS 134 and SER 228 which are also found to interact with reported

Table 5. Docking interaction patterns of synthesized compounds **(8a-p)** with active site amino acids of DprE1 in molecular docking studies.

Compd	Active site amino acid interactions	Percentage binding (%)	Distance between ligand and active site amino acid in Å	Type of interaction
8a	HIS 132			Stacking
	LYS 418, GLY 117, VAL 365	–	–	Hydrophobic
8b	LYS 418	49.2	2.73	Hydrogen
	TYR 314, GLY 117, VAL 365, TRP 230	–	–	Hydrophobic
8c	GLY 117	38.4	2.55	Hydrogen
	TRP 230	–	–	Stacking
	LYS 418, THR 314, VAL 365	–	–	Hydrophobic
8d	LYS 134	46.9	2.67	Hydrogen
	GLY 117, VAL 365, TRP 230, TYR 314, SER 245	–	–	Hydrophobic
8e	SER 228	83.0	2.53	Hydrogen
	LYS 418	45.0	2.54	Hydrogen
	GLY 117, VAL 365, TRP 230, TYR 314, HIS 132	–	–	Hydrophobic
8f	LYS 418	52.9	2.61	Hydrogen
	GLY 117, VAL 365, TRP 230, TYR 314, SER 228	–	–	Hydrophobic
8g	HIS 132			Stacking
	GLY 117, VAL 365, LYS 418, ILE 131	–	–	Hydrophobic
8h	HIS 132			Stacking
	GLY 117, VAL 365, LYS 418, TYR 60, LEU 363	–	–	Hydrophobic
8i	HIS 132	39.5	2.74	Hydrogen
	GLY 117, VAL 365, LYS 418, THR 118, ILE 131	–	–	Hydrophobic
8j	SER 228	50.6	2.58	Hydrogen
	LYS 134	–	–	Stacking
	TRP 230	–	–	Stacking
	GLY 117, VAL 365, TYR 314, HIS 132	–	–	Hydrophobic
8k	SER 228	30.0	2.49	Hydrogen
	LYS 418	–	–	Stacking
	GLY 117, VAL 365, TYR 314, TRP 230	–	–	Hydrophobic
8l	LYS 418	10.1	3.39	Hydrogen
	LYS 418	46.8	2.49	Hydrogen
	GLY 117, VAL 365, TYR 314, TRP 230	–	–	Hydrophobic
8m	LYS 418	41.5	2.46	Hydrogen
	GLY 117, VAL 365, TYR 314, TRP 230	–	–	Hydrophobic
8n	SER 228	22.0	2.51	Hydrogen
	LYS 418	–	–	Stacking
	GLY 117, VAL 365, TYR 314, TRP 230, HIS 132	–	–	Hydrophobic
8o	LYS 418	34.9	2.64	Hydrogen
	SER	85.0	2.65	Hydrogen
	GLY 117, VAL 365, CYS 387, TRP 230	–	–	Hydrophobic
8p	LYS 418	16.2	3.20	Hydrogen
	LYS 868	51.6	2.56	Hydrogen
	GLY 117, VAL 365, TYR 314, TRP 230	–	–	Hydrophobic

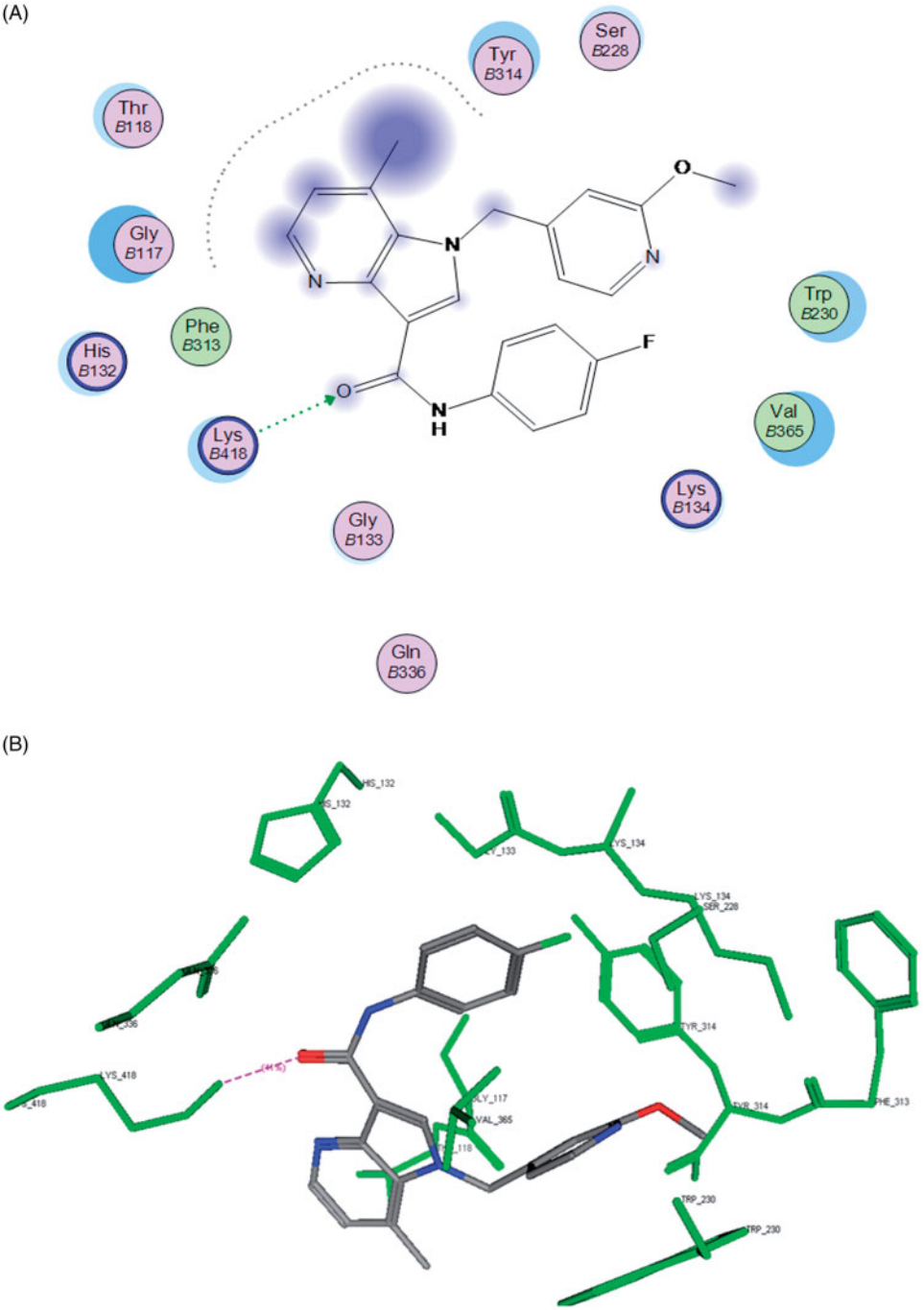


Figure 3. (A) Two-dimensional representation of the interacting mode of **8 m** with DprE1. (B) Three-dimensional structural model of compound **8 m** into DprE1.

DprE1 inhibitors.[12–14] Compound **8m** showed effective hydrogen bond interaction with LYS 418 of 41.5% binding at a distance of 2.46 Å. Moreover, the compound was surrounded by GLY 117, VAL 365, TYR 314 and TRP 230. These interactions

underscore the importance of inhibitory capacity against DprE1. Data pertaining to the docking interaction of compounds **(8a–p)** with amino acids on DprE1active site was displayed in Table 5 indicated that all the compounds have shown essential key interactions as that of reported DprE1 inhibitors and hence, the synthesized compounds could be considered as promising inhibitors for tuberculosis. The two-dimensional and three-dimensional representations of compound **8m** were shown in Figure 3.

Conclusion

A novel series pyrrolo[3,2-b]pyridine-3-carboxamide linked 2-methoxypyridine derivatives have been designed, synthesized and characterized. The synthesized compounds were screened for their antitubercular and antibacterial activities. Among the tested compounds, 4-fluorophenyl **(8m),** 4-chlorophenyl **(8n)** and 4-methoxyphenyl **(8i)** showed potent anti-TB activity (3.12 µg/mL) in comparison with reference drug, Pyrazinamide (3.12 µg/mL). Moreover, the compounds exhibited essential docking interactions as that of reported DprE1 inhibitors and hence, the synthesized compounds may be considered as molecular scaffolds for antitubercular activity. Compounds, 4-chlorophenyl **(8n)** and 4-flurophenyl (**8m**) showed significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* strains. *In silico* prediction of toxicities, druglikeness and drug score profiles of the tested compounds are promising.

Experimental section

Chemistry

All chemicals were purchased from Sigma–Aldrich, Merck and Combi blocks, and were used without further purification. Melting points (m.p.) were uncorrected and determined in one end open capillary tubes using Guna Digital Melting Point apparatus. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. FT-IR spectra were recorded on a Bruker FT-IR spectrometer. ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer operating at 400 MHz for ^{1}H, ^{19}F and 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS). Mass spectra were recorded on Agilent technologies mass spectrometer.

Ethyl 2-cyano-2-(4-methyl-3-nitropyridin-2-yl)acetate (3)

To a stirred solution of potassium t-butoxide (10.4 g, 93.0 mmol) in t-butanol (90.0 mL) and isopropanol (150 mL) was added ethyl cyanoacetate (11.1 g, 98.8 mmol). After 5 min, 2-chloro-4-methyl-3-nitropyridine **1** (10.0 g, 58.1 mmol) was added and the mixture heated to reflux for 6 h. The dark red solution was cooled to room temperature and solvents removed under high vacuum. The residue was washed with 1 M hydrochloric acid, water and recrystallized twice from methanol to give the title compound as orange color solid, Yield, 10.7 g, 74%.

M.p. 135–137 °C, FT-IR (neat) ν_{max} cm^{-1} 3054, 2224, 1641, 1562, 1330, 1200. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 14.41 (br s, 1H), 8.23 (d, 1H, J = 6.4 Hz), 7.00 (d, 1H, J = 6.4 Hz), 4.20 (q, 2H, J = 7.2 Hz), 2.30 (s, 3H), 1.25 (t, 3H, J = 7.2 Hz). ^{13}C NMR

(100 MHz, DMSO-d_6) δ 168.8, 146.5, 144.6, 139.6, 138.9, 115.7, 115.4, 60.10, 58.51, 17.80, 14.33.MS (MM) *m/z* 250.0 $[M+H]^+$; Anal. Calcd. $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 16.86; Found: C, 53.10; H, 4.36; N, 16.94.

Ethyl 7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylate (4)

To a solution of ethyl 2-cyano-2-(4-methyl-3-nitropyridin-2-yl) acetate **3** (10.0 g, 40.1 mmol) in EtOH (100 mL) was added 5% Pd/C (0.32 g) and the mixture was stirred under 100 psi hydrogen atmosphere at 40 °C for 24 h. The Pd/C was removed by through celite pad and the filtrate was concentrated to give. The crude was purified by column chromatography on silica gel (Combiflash, 0 to 5% CH_3OH/CH_2Cl_2) afford the desired product as a white solid, Yield, 6.60 g, 80%.

M.p. 241–243 °C, FT-IR (neat) ν_{max} cm^{-1} 3423, 3187, 3036, 1677, 1578, 1071. ^1H NMR (400 MHz, DMSO-d_6): δ 12.22 (br s, 1H), 8.34 (d, 1H, J = 4.4 Hz), 8.26 (s, 1H), 7.03 (d, 1H, J = 4.8 Hz), 4.27 (q, 2H, J = 7.2 Hz), 2.51 (s, 3H), 1.31 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.2, 144.4, 142.7, 134.8, 130.2, 129.3, 118.3, 107.1, 58.85, 16.07, 14.53.MS (MM) *m/z* 205.1 $[M+H]^+$; Anal. Calcd. $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 15.67; Found: C, 64.60; H, 5.80; N, 15.77.

Ethyl 1-((2-methoxypyridin-4-yl) methyl)-7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylate (6)

To a solution of ethyl 7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylate **4** (6.00 g, 29.4 mmol) in DMF (60.0 mL) was added K_2CO_3 (4.87 g, 35.2 mmol) under nitrogen atm. and the reaction mixture was stirred at rt for 5 min. then 4-(bromomethyl)-2-methoxypyridine **5** (5.90 g, 29.4 mmol) was added in one portion and the reaction was stirred at rt overnight. The reaction mixture was diluted with water and extracted with EtOAC (3 × 40.0 mL). The combined organic layers were washed with H_2O (50.0 mL), brine solution (50.0 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (Combiflash, 0 to 10% CH_3OH/CH_2Cl_2) to afford the title compound, ***ethyl 1-((2-methoxypyridin-4-yl)methyl)-7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylate (6)***, Yield, 6.20 g, 65%. M.p. 120–122 °C, FT-IR (neat) ν_{max} cm^{-1} 3180, 1654, 1602, 1210. ^1H NMR (400 MHz, DMSO-d_6): δ 8.47 (s, 1H), 8.36 (d, 1H, J = 4.8 Hz), 8.10 (d, 1H, J = 5.2 Hz), 6.97 (d, 1H, J = 4.4 Hz), 6.54 (d, 1H, J = 4.8 Hz), 6.17 (s, 1H), 5.75 (s, 2H), 4.29 (q, 2H, J = 7.2 Hz), 3.78 (s, 3H), 2.39 (s, 3H), 1.32 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 162.6, 151.1, 147.6, 144.9, 143.8, 140.0, 130.4, 128.6, 120.3, 113.9, 106.8, 106.4, 59.11, 53.14, 51.06, 17.74, 14.50.MS (MM) *m/z* 326.2 $[M+H]^+$; Anal. Calcd. $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91; Found: C, 66.55; H, 5.78; N, 12.80. And region isomer ***ethyl 4-((2-methoxypyridin-4-yl)methyl)-7-methyl-4H-pyrrolo[3,2-b]pyridine-3-carboxylate (6 A)***, Yield, 2.28 g, 24%. M.p. 121–123 °C, FT-IR (neat) ν_{max} cm^{-1} 3180, 1654, 1602, 1210. ^1H NMR (400 MHz, DMSO-d_6): δ 8.95 (s, 1H), 8.59 (d, 1H, J = 6.0 Hz), 8.14 (d, 1H, J = 5.2 Hz), 7.61 (d, 1H, J = 6.0 Hz), 6.68 (d, 1H, J = 5.2 Hz), 6.37 (s, 1H), 5.95 (s, 2H), 4.29 (q, 2H, J = 7.2 Hz), 3.81 (s, 3H), 2.66 (s, 3 H), 1.31 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 163.7, 150.9, 147.2, 143.8, 143.3, 138.7, 130.9, 128.1, 119.5, 113.6,

106.1, 59.26, 52.78, 50.56, 17.41, 14.78.MS (MM) *m/z* 326.2 $[M+H]^+$; Anal. Calcd. $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91; Found: C, 66.56; H, 5.80; N, 12.81.

1-((2-methoxypyridin-4-yl)methyl)-7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylic acid (7)

To a solution of ethyl 1-((2-methoxypyridin-4-yl)methyl)-7-methyl-1H-pyrrolo[3,2-b]pyridine-3-carboxylate **6** (6.00 g, 18.4 mmol) in MeOH:THF (1:1, 50.0 mL) was added $LiOH.H_2O$ (1.16 g, 27.6 mmol) in H_2O (5.00 mL) and the reaction was stirred at rt for 4 h. The reaction mixture was concentrated and the crude mixture was washed with EtOAc (2 × 20.0 mL). Aqueous layer P^H was adjusted to 3 with 2 N HCl and extracted with EtOAC (3 × 40.0 mL). The combined organic layers were washed with H_2O (50.0 mL), brine solution (50.0 mL), dried over Na_2SO_4 and concentrated to afford the title compound as white solid, Yield, 4.70 g, 86%.

M.p. 258–260 °C, FT-IR (neat) ν_{max} cm^{-1} 3450, 3052, 1713, 1597, 1504, 1205. ^1H NMR (400 MHz, DMSO-d_6): δ 8.31 (s, 1H), 8.26 (d, 1H, J = 4.4 Hz), 8.11 (d, 1H, J = 5.2 Hz), 6.98 (d, 1H, J = 4.8 Hz), 6.56 (d, 1H, J = 5.2 Hz), 6.18 (s, 1H), 5.73 (s, 2H), 3.78 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 164.0, 151.3, 147.6, 144.1, 143.7, 139.0, 131.3, 128.5, 119.9, 113.9, 106.5, 53.15, 50.93, 17.77.MS (MM) *m/z* 298.0 $[M+H]^+$; Anal. Calcd. $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13; Found: C, 64.74; H, 5.18; N, 14.04.

General procedure for the synthesis of title compounds (8a–8p)

To a solution of **7** (0.84 mmol) in DMF (2.00 mL), HATU (1.26 mmol) and DIPEA (1.68 mmol) were added at room temperature and stirred for 15 min. Then, appropriate amine (1.00 mmol) was added and stirred at room temperature for 16 h. The reaction mixture was diluted with water (20 mL) and extracted with EtOAC (2 × 15.0 mL). The combined organic layers were washed with H_2O (15 mL), brine solution (15 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (methanol/dichloromethane) to afford the title compounds (**8a–p**).

Antitubercular activity

The antitubercular activity of compounds was assessed against Mycobacterium tuberculosis using MABA method. This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 μl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 μl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100–0.2 μg/ml. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 μl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue

color in the well was interpreted as no bacterial growth, and pink color was scored as growth.[15]

Antibacterial activity

Antibacterial activity of all the synthesized compounds was screened by Agar well diffusion method, as recommended by the national committee for clinical laboratory standards against Gram-positive bacteria such as *Staphylococcus aureus* (NCIM 2079) and Gram-negative bacteria *Escherichia coli* (NCIM 2068) at two concentrations 100 μg/mL and 200 μg/mL. The obtained results were compared with standard drug Chloramphenicol. The fresh culture of bacteria is obtained by inoculating bacteria into nutrient broth media and incubated at 37 °C for 24 hr. This culture mixed with nutrient agar media was poured into petridishes under aseptic conditions. After solidification of media, bores are made by using sterile cork borer (8 mm diameter). Into these cups, standard drug and synthesized drugs are introduced; the plates were placed in the refrigerator at 100 °C for proper diffusion of drugs into the media. After 2 hr, the Petri plates were transferred to an incubator and maintained at 37 ± 2 °C for 24 hr. After the incubation period, the Petri plates were observed for the zone of inhibition. The results are evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug.[16,17]

Docking studies

Docking was performed on windows 2007 using MOE 2008.10 version. DprE1 enzyme was imported from the protein data bank (PDB ID: 4KW5) and the receptor was visualized using sequence option and non interacting water molecules were deleted along with the previously bound ligand. The partial charge of protein was adjusted, using the force field method AMBER 99. Later, the protein was subjected to 3 D protonation at cut off 12.0, and further hydrogen was added according to standard geometry and the enzyme was energy minimized using force field MMFF94x at 0.01 KJ mole gradients. The ligand preparation was done by drawing the structure of ligands by using a builder module and adjusting the partial charges using Hamilton MMFF94 force field method and subsequently 3 D protonation and hydrogen addition was performed according to standard geometry. Ligands were energy minimized at cut off 12 using MMFF94x force field at 0.01KJ mole gradient. Docking was performed using the option simulation followed by dock on selected active site amino acids using sequence option, and further docked with setting options such as receptor and solvent, selected residues, alpha triangle, affinity DG, force field refinement and best 10 poses. After obtaining docking results out of the 10 best posed resulted for each chemical structure, the best pose was retained. The resultant best pose score values in the series were used for analysis of docking and interaction.[18,19]

Assessment of lipophilicity, druglikeness, drug score profiles and toxicity risks

Shredding of each molecule at every rotatable bond gave rise to a set of core fragments. These in turn were used to reform all possible bigger fragments which could be the

substructure of the original molecule. Afterward, a search process of substructure determined the occurrence frequency of every one of the fragment (core and constructed fragments) within all traded drugs of 3300 as well as 15,000 commercially available chemicals (Fluka) to predict lipophilicity, druglikeness, drug score profiles and toxicity risks.[20,21]

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Disclosure statement

No potential conflict of interest was reported by the authors.

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