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FULL PAPER

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Synthesis and biological evaluation of some new tricyclic pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine derivatives as potential antitubercular agents

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Abstract

A series of new tricyclic pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidines **8a–l** were synthesized and characterized by IR, NMR (^{1}H and ^{13}C), and mass spectral analysis. The newly synthesized compounds **8a–l** were inspected for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* (MTB) $H_{37}Ra$ using an established XTT reduction menadione assay (XRMA). The title compounds exhibited minimum inhibitory concentrations (MIC_{90}) ranging from 0.09 to >30 μg/mL. Five compounds (**8c**, **8i–l**) were further confirmed for their dose-dependent effect against MTB. These compounds were evaluated in the THP-1 infection model, where **8i** (MIC_{90} = 0.35 μg/mL), **8j** (MIC_{90} = 1.17 μg/mL), **8k** (MIC_{90} = 2.38 μg/mL), and **8l** (MIC_{90} = 1.17 μg/mL) demonstrated significant antitubercular activity. All the *ex vivo* active compounds showed insignificant cytotoxicity against the human cancer cell lines, HeLa, MCF-7, and THP-1. Inactivity of all these compounds against Gram positive and Gram negative bacteria indicates their specificity. Molecular docking studies in the active site of the sterol 14alpha-demethylase (CYP51) enzyme revealed a similar binding mode to the native ligand in the crystal structure, thereby helping to understand the ligand–protein interactions and to establish a structural basis for inhibition of MTB. The results suggest novel pharmacophores as selective and specific inhibitors against MTB that can be explored further to synthesize lead compounds against tuberculosis. In summary, the results clearly indicate the identification of some novel, selective, and specific inhibitors against MTB that can be explored further for potential antitubercular drugs.

KEYWORDS
antituberculosis, cytotoxicity, molecular docking, tricyclic pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine

1 | INTRODUCTION

Among widespread infectious diseases, tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is the primary health problem.[1] One-third of the world's population is infected with latent TB, which is at a risk of developing active TB disease. Despite the availability of several first and second line drugs, TB remains the top 10 death causes worldwide with leading in death counter among the infectious diseases

well above HIV/AIDS.[2] The current preferred treatment strategy for active drug-sensitive TB disease is a minimum of 6 months of therapy with isoniazid, rifampicin, pyrazinamide, ethambutol during the first 2 months (the intensive phase of treatment), followed by isoniazid (INH) and rifampicin (RIF) for 4 months (the continuation phase). However, use of these drugs over longer duration results in the emergence of resistant strains. The treatment for resistant strains has to be switched to second or third line drugs; further exploding the cost of the treatment. Thus, their arises the need for the development of potent and cost-effective drug scaffolds for antitubercular.

In the literature, tetrazole[3] and pyrrolo[2,3-*d*]pyrimidine[4] are well-explored scaffold and also crucial constituent for various drug molecules. Moreover, shows widespread activity such as antimicrobial,[5,6] antifungal,[7,8] antimalarial,[9,10] anticancer,[11,12] antihypertensive,[13] and antituberculosis.[14–18] Some tetrazole and pyrimidine contain a moiety which shows antituberculosis activity as depicted in Figure 1.

Diversity in the scaffold is a key ongoing exercise for the drug design and development to achieve better affinity and efficacy to the molecules. The present investigation deals with the combination of both scaffolds tetrazole and pyrrolo[2,3-*d*]pyrimidine to get the synergetic effect. Tricyclic pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine core moiety also shows activity such as anticancer, antimalarial, and antihypertensive.[19,20] All the newly synthesized tricyclic 7*H*-pyrrolo [3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (**7**) and its derivatives were screened for antituberculosis and antibacterial activities along with docking study.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Synthesis of pyrrolo[2,3-*d*]pyrimidines plenty permutation and combination have been published depending upon the target molecules.[21] We chalked out a synthetic pathway for our target molecules which is illustrated in Scheme 1.

Synthesis of 4-chloropyrrolo[2,3-*d*]pyrimidine was reported in several literatures.[22–24] Based on literature condensation of ethyl cyanoacetate with 2-bromo-1,1-diethoxyethane gave desired ethyl 2-cyano-4,4-diethoxy butanoate (**3**). Cyclization of compound **3** by using thiourea produced 6-amino-5-(2,2-diethoxyethyl)-2-mercaptopyrimidine-4-ol were obtained. Further desulfurization by using Raney nickel produced 6-amino-5-(2,2-diethoxyethyl)pyrimidin-4-ol (**4**).

To avoid some lacuna of reported synthetic route, i.e., desulfurization stage and get directly pyrimidine ring compound (**4**) from compound **3**, cyclization has been performed with formamidine acetate instead of thiourea. 6-Amino-5-(2,2-diethoxyethyl)pyrimidin-4-ol (**4**) formation was confirmed by spectral data. IR spectrum shows two absorption bands at 3387 and 3337 cm^{-1} due to primary amine ($—NH_2$) and 3148 cm^{-1} due to phenolic —OH. The ^1H NMR spectrum of compound **4** showed δ 7.69 (s) is due to pyrimidine ring aromatic proton, 6.06 (s) and 11.47(s) D_2O exchangeable proton of $—NH_2$ and —OH, respectively. 1.05 (m), 3.37 (q), and 3.56 (q) show 2 O-CH_2-CH_3 protons. 2.49 (t) and 4.53 (dd) belong to pyrimidine ring attached CH_2 and CH, respectively.

Compound **4** was treated with aqueous hydrochloric acid solution (5 N) to get 4-hydroxypyrrolo[2,3-*d*]pyrimidine (**5**). The ^1H NMR spectrum showed δ 6.47 (d), 7.03 (d), and 7.83 (s) aromatic ring proton. Besides, two singlets were observed at δ 11.78 and 11.86 with D_2O exchangeable due to —OH and —NH. Chlorination of compound **5** with phosphorus oxychloride produced 4-chloropyrrolo[2,3-*d*]pyrimidine (**6**). This was further treated with sodium azide in DMF solvent to get our core moiety (**7**). This tricyclic compound was confirmed and characterized by IR, mass, and NMR. All experimental procedure and spectral details of compounds were in full agreement with the proposed structure.

Here, while compound **6** reacts with sodium azide two possibilities will be occurred. Either tetrazole ring formation or nucleophilic displacement to afford 4-azido derivative.[25] Literature evidence suggests tetrazole ring formation can be confirmed by IR spectrum of compound **7**. It showed no absorbance band in the 2100 cm^{-1} region which exclude the possibility 4-azido derivative[26] further alkyl, benzyl halide, and sulfonyl substitution on tricyclic compound (**7**) produced derivatives (**8a–l**) with moderate to better yield (Table 1). Spectral data are incorporated in the Supporting Information.

2.2 | Biological evaluation

All the newly synthesized compounds (**8a–l**) were tested against *M. tuberculosis* $H_{37}Ra$ (ATCC 25177) at two time points, i.e., days 8 and 12 using an established XTT reduction menadione assay (XRMA). Among the synthesized compounds, **8c**, **8i–8l** displayed excellent activity toward *M. tuberculosis* $H_{37}Ra$ with MIC_{90} value less than 6 μg/mL. According to the structure–activity relationships of antituberculosis compounds **8j** (MIC: 5.63), **8k** (MIC: 2.13), and **8l** (MIC: 2.51) greatly influence on introduction of sulfonyl amide group contain methyl, phenyl, and 4-methyl phenyl, respectively. Other attempts to introduce aliphatic and aromatic compounds without sulfonyl group failed to produce active compound except **8c** and **8i**, which contain aliphatic propyl and aromatic biphenyl ring with MIC values 28.11 and 0.33 μg/mL, respectively. The results of the screening are tabulated in Table 2.

The synthesized compounds **8c** and **8i–l** were further tested against mycobacteria within THP-1 host macrophages (Table 3). Compounds exhibiting MIC_{90} less than 5 μg/mL was considered as a potent compound. *Ex vivo* studies against *M. tuberculosis* revealed the strong anti-tubercular activity of **8i–l**. Moreover, all the compounds showed time-dependent activity when measured at two time points, i.e., days 8 and 12 (Table 3). Among derivatives, **8i** showed significant activity with MIC_{90} 0.76 and 0.35 μg/mL, respectively, at days 8 and 12. However, the activity is not profound as that of rifampicin. Experimental details have been presented in the Supporting Information.

The active compounds (**8i–l**) from *ex vivo* infection model were evaluated for cytotoxicity on three human cancer cell lines THP-1 (acute monocytic leukemia), HeLa (human epithelial cervical cancer),

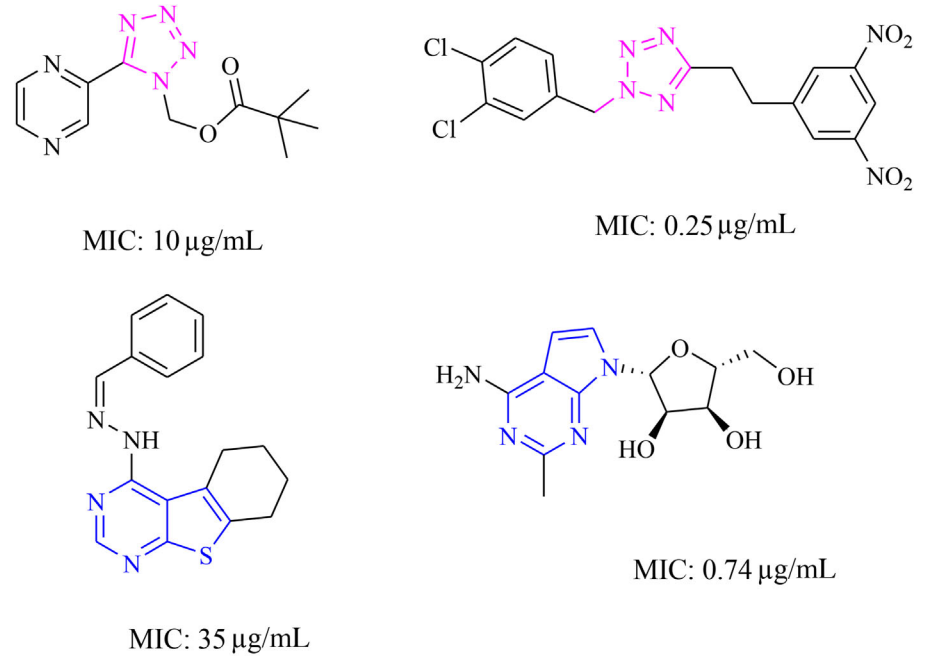
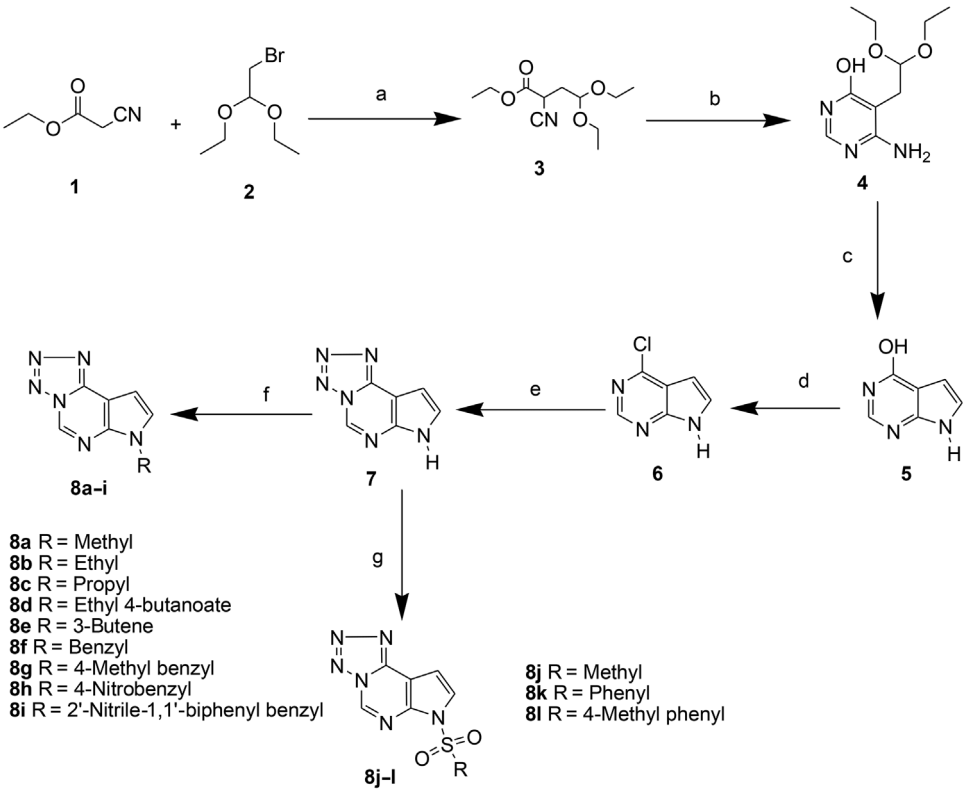


FIGURE 1 Antituberculosis active compounds contain tetrazole and pyrimidine skeleton



SCHEME 1 Synthetic route for the preparation of title compounds **8a–l**. Reagents and conditions: (a) K_2CO_3, DMF, 145–150°C, 78%; (b) formamidine acetate, NaOEt, EtOH, 76–78°C, 85%; (c) 5 N HCl, H_2O, 25–30°C, 90%; (d) $POCl_3$, 100–105°C, 95%; (e) NaN_3, DMF, 25–30°C, 85%; (f) alkyl/benzyl halide, K_2CO_3, DMF, 25–30°C, 82–92%; (g) alkyl/benzyl sulfonyl chloride, aq. NaOH, acetone, 25–30°C, 70–75%

TABLE 1 Physical data of tricyclic pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine derivatives (**8a–l**)

Entry	Compound	R	Yields (%)	m.p. (°C)
1	**8a**	Methyl	88	154–156
2	**8b**	Ethyl	90	146–148
3	**8c**	Propyl	92	134–136
4	**8d**	Ethyl-4-butanoate	85	138–141
5	**8e**	3-Butene	82	140–142
6	**8f**	Benzyl	86	175–177
7	**8g**	4-Methylbenzyl	80	182–184
8	**8h**	4-Nitrobenzyl	82	188–190
9	**8i**	2′-Nitrile-1,1′-biiphenyl benzyl	92	179–181
10	**8j**	Methyl sulfonyl	75	156–158
11	**8k**	Phenyl sulfonyl	72	169–172
12	**8l**	4-Methylphenyl sulfonyl	70	150–152

MCF-7 (human mammary epithelial) using MTT assay and IC_{50} values were determined. The assay measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide by mitochondrial succinate dehydrogenase. The cytotoxic effect of these compounds was checked to determine the growth inhibition (GI_{50} and GI_{90}) (Table 4). It has been noticed that all the active derivatives (**8i–l**) showed GI_{90} values >100 µg/mL in all the cancer cell lines.

Further, the selectivity of the compounds **8i–l** toward human cell lines against MTB was determined via their selectivity index (SI) (Table 5). SI is a significant parameter used to calculate the efficiency of antimicrobial agents. It is a ratio between IC_{50} (cytotoxicity) and MIC_{90} (anti-tubercular activity) value. According to drug susceptibility study of TB, compounds that exhibited SI values >10 were considered to be nontoxic and effective. It was found that **8i–l** have a SI of ≥10 in MTB when compared to all the three cell lines. Importantly, **8i** possessed a more favorable selectivity index (SI > 100) against all three cell lines.

Compounds **8a–l** were further screened against (Gram +ve *Bacillus subtilis* and *Staphylococcus aureus*, Gram −ve *Escherichia coli* and *Pseudomonas aeruginosa*) at 3 µg/mL concentration, to assess their selectivity toward MTB. The antimicrobial activity is summarized in Supporting Information Table S1. None of the compound showed significant activity toward any of the screened bacterial strain.

2.3 | Molecular docking study

Besides, theoretical predictions of molecular docking studies were found to be in harmony with the *in vitro* antitubercular data. To gain an insight into binding mode and the thermodynamic interactions that govern the binding of the most active pyrrolo[3,2-*e*]tetrazolo-[1,5-*c*]pyrimidine derivatives can be utilized for further screening (Supporting Information).

TABLE 2 *In vitro* antitubercular activity of compounds against tuberculosis $H_{37}Ra$

	Day 8		Day 12	
Entry	IC_{50} (µg/mL)	MIC_{90} (µg/mL)	IC_{50} (µg/mL)	MIC_{90} (µg/mL)
8a	6.93	>30	2.12	>30
8b	12.87	>30	20.09	>30
8c	7.11	28.11	0.15	9.12
8d	17.29	>30	26.06	>30
8e	14.3	>30	7.07	>30
8f	>30	>30	>30	>30
8g	1.73	>30	6.17	>30
8h	>30	>30	>30	>30
8i	0.09	0.33	0.12	0.72
8j	0.83	5.63	0.71	4.74
8k	0.37	2.13	0.74	2.07
8l	1.08	2.51	0.43	1.2
Rifampicin	0.020	0.42	0.0019	0.80

TABLE 3 *Ex vivo* antitubercular activity of selected compounds at days 8 and 12

	Day 8		Day 12	
Entry	IC_{50} (μg/mL)	MIC_{90} (μg/mL)	IC_{50} (μg/mL)	MIC_{90} (μg/mL)
8c	6.30	26.58	0.59	25.51
8i	0.03	0.76	0.10	0.35
8j	0.40	3.50	0.21	1.17
8k	0.27	3.84	0.77	2.38
8l	0.12	4.68	0.40	1.17
Rifampicin	0.020	0.53	0.0019	0.80

3 | CONCLUSION

In summary, a series of new pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine and its derivatives contain alkyl, benzyl, and sulfonyl moieties. These compounds were characterized and screened for antituberculosis activity. Among all tested compounds **8i**–**l** demonstrated significant inhibition against MTB $H_{37}Ra$. SI values for these compounds were ≥10 against all the cell lines. These results are encouraging to better define and optimize for further screening.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All reagents, solvents, and raw materials are commercially available and were used without further purification. Melting points were determined by the open capillary method and are uncorrected. IR spectra (neat in cm^{-1}) were recorded using Perkin Elmer Spectrum-100 analyzer. NMR (^1H and ^{13}C) spectra were recorded in $CDCl_3$ and DMSO using a JEOL 400 MHz FT NMR spectrometer; the chemical shifts are reported in ppm relative to TMS. The following abbreviations were used for spin multiplicity: s = singlet, d = doublet, t = triplet, dd = double doublet, m = multiplet, brs = broad singlet. Chemical shifts for ^{13}C NMR are reported in ppm relative to the solvent peak. Mass spectrometry was carried out using an Agilent LC/MSD Trap 1100 series. The reaction monitoring and purity of the compounds were checked by thin layer chromatography (TLC) by using silica gel coated aluminum sheets (Silica Gel 60 F254).

All the chemicals such as sodium salt XTT and MTT, DMSO, ampicillin, and rifampicin were purchased from Sigma–Aldrich, USA. Dubos medium was purchased from DIFCO, USA. Synthesized compounds were dissolved in DMSO and it was used as a stock solution (10 mg/mL) for further biological testing.

The InChI codes of some of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | Synthesis of ethyl 2-cyano-4,4-diethoxybutanoate (3)

A solution of ethyl 2-cyanoacetate (10 g, 88 mmol), 2-bromo-1,1-diethoxyethane (17.5 g, 88 mmol) were dissolved into DMF (50 mL) and anhydrous K_2CO_3 (24.4 g, 132 mmol) under nitrogen atmosphere. After addition, the reaction mixture was maintained at 145–150°C for 5–6 h. The reaction mixture was monitored by TLC. After completion of the reaction, mixture was cooled at ambient temperature and diluted with water (100 mL). The mixture was then extracted with heptane (3 × 40 mL), washed with water (50 mL) and brine solution (50 mL). The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The organic residue was purified by column chromatography on silica gel (using 10% ethyl acetate in hexane as eluting solvents) to afford pale yellow oil of **3** (15.8 g, 78%). IR (film): υ 2190 (—CN), 1775 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.21 (m, J = 7.1 Hz, $2CH_3$), 1.31 (t, J = 7.2 Hz, CH_3), 2.24 (m, CH_2), 3.51 (m, —OCH_2—CH_3), 3.70 (m, CH, and —OCH_2—CH_3), 4.26 (q, J = 7.1 Hz, —OCH_2—CH_3), 4.69 (t, J = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 15.3, 33.7, 62.8, 62.9, 100.1, 116.5, 166; MS (EI): *m/z* calcd. for [$C_{11}H_{19}NO_4$+H]: 230.13. Found: 230.13.

TABLE 4 Growth inhibition of concentration of selective actives on of cell line

	THP-1		HeLa		MCF-7	
Code	GI_{50} (μg/mL)	GI_{90} (μg/mL)	GI_{50} (μg/mL)	GI_{90} (μg/mL)	GI_{50} (μg/mL)	GI_{90} (μg/mL)
8i	>100	>100	>100	>100	>100	>100
8j	>100	>100	>100	>100	>100	>100
8k	>100	>100	>100	>100	>100	>100
8l	>100	>100	>100	>100	>100	>100
Rifampicin	>100	>100	>100	>100	>100	>100

TABLE 5 Selectivity index of active analogs against *Mycobacterium tuberculosis* $H_{37}Ra$ at day 12

Code	THP-1	HeLa	MCF-7
8c	3.92	3.92	3.92
8i	285.71	285.71	285.71
8j	85.47	85.47	85.47
8k	42.01	42.01	42.01
8l	85.47	85.47	85.47

4.1.3 | Synthesis of 6-amino-5-(2,2-diethoxy-ethyl) pyrimidin-4-ol (4)

A solution of ethyl 2-cyano-4, 4-diethoxy butanoate (10 g, 44 mmol) in 50 mL of ethanol and sodium ethoxide (2.3 g, 44 mmol) was added in portion wise under an inert atmosphere at ambient temperature. Freshly prepared solution of formamidine acetate in ethanol (5.7 g, 54 mmol in 50 mL) was added in dropwise manner into the reaction mass and the mixture was refluxed at 78°C for 5 h. The reaction mixture was gradually cooled at 25–30°C, then further cooled at 5–10°C and the pH was adjusted at 7 using 5 N HCl (5 mL). Then, the reaction mixture was stirred at 5–10°C for an hour, filtered and washed with water (50 mL), and dried *in vacuo* to afford compound **4** brown solid (8.4 g, 85%), m.p. 193–195°C. IR (film) υ: 3387 (NH_2), 3148 (OH), 1662 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, $2CH_3$), 2.49 (t, —CH_2), 3.37 (q, —OCH_2—CH_3), 3.56 (q, —OCH_2—CH_3), 4.53 (m, CH), 6.06 (s, NH_2), 7.69 (s, Ar-H), 11.47 (s, OH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.31, 28.74, 61.36, 93.16, 101.85, 146.97, 161.44, 161.73; MS (EI): *m/z* calcd. for [$C_{10}H_{17}N_3O_3$−H]: 226.13. Found: 226.

4.1.4 | Synthesis of 4-hydroxypyrrolo[2,3-*d*]-pyrimidine (5)

A mixture of 6-amino-5-(2, 2-diethoxy-ethyl)pyrimidin-4-ol (**4**) (10 g, 44 mmol) was added 50 mL of 5 N HCl, then reaction mass stirred at ambient temperature for 4 h. The reaction mixture was monitored by TLC. After completion of the reaction, mixture was filtered, washed with water (50 mL), and dried under vacuum to obtain compound **5** off white solid (5.3 g, 90%), m.p. 242–245°C. IR (film) υ: 3094, 3059 (OH), 1663, 1574 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, NH), 11.78 (s, OH), 7.83 (s, 1H), 7.03 (d, *J* = 3.4 Hz, 1H), 6.47 (d, *J* = 3.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.98, 107.66, 120.36, 143.23, 148.08, 158.49; MS (EI): *m/z* calcd. for [$C_6H_5N_3O$+H]: 136.12. Found: 136.1.

4.1.5 | Synthesis of 4-chloropyrrolo[2,3-*d*]pyrimidine (6)

A mixture of 4-hydroxy pyrrolo[2,3-*d*]pyrimidine (**5**) (10 g, 74 mmol) in $POCl_3$ (30 mL) was refluxed for an hour. The reaction mixture was monitored by TLC. After completion of the reaction, mixture was quenched into ice water then precipitation observed. The obtained precipitation was filtered, washed with excess of water, and dried under vacuum. The residue purified by recrystallization using toluene gave compound **6** white solid (10.2 g, 90%), m.p. 154–158°C. IR (film) υ: 3120, 2966 (NH), 1600, 1557 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.59 (d, *J* = 3.4 Hz, 1H), 7.69 (d, *J* = 3.4 Hz, 1H), 8.58 (s, 1H), 12.57 (s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 99.35, 117.13, 128.63, 150.83, 152.16, 152.31; MS (EI): *m/z* calcd. for $C_6H_4ClN_3$+H]: 154.0. Found: 154 $(M+H)^+$, 156 $(M+2+H)^+$.

4.1.6 | Synthesis of 7*H*-pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]-pyrimidine (7)

A mixture of 4-chloropyrrolo[2,3-*d*]pyrimidine (**6**) (15 g, 100 mmol), and sodium azide (8 g, 125 mmol) in DMF (75 mL) was stirred at ambient temperature for 12 h. The reaction mixture was monitored by TLC. After completion of the reaction, mixture was filtered, washed with water (75 mL), and dried under vacuum to afford compound **7** pale yellow solid (14.2 g, 90%), m.p. 200–203°C. IR (film) υ: 3135, 3071 (NH), 1628 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.05 (d, *J* = 3.3 Hz, Ar-H), 7.67 (d, *J* = 3.3 Hz, Ar-H), 9.83 (s, Ar-H), 12.95 (s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.34, 101.80, 125.80, 132.81, 142.05, 146.35; MS (EI): *m/z* calcd. for [$C_6H_4N_6$+H]: 161.05. Found: 161.

4.1.7 | General procedure for the synthesis of compounds 8a–i

To a solution of 7*H*-pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (**7**) (12 mmol), potassium carbonate (18 mmol) and appropriate alkyl and aryl halide (14 mmol) were added DMF (10 mL) at ambient temperature. The reaction mixture was monitored by TLC. After completion of the reaction, mixture was quenched into water (50 mL) and precipitation obtained. The residue was filtered, washed with water, and dried under vacuum to afford compounds **8a–i** (82–92%).

7-Methyl pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8a)

Off white solid, yield: 88%, m.p. 154–156°C. IR (film) υ: 3115, 2979, 1635, 1487, 1352 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.98 (s, CH_3), 7.05 (d, *J* = 3.4 Hz, Ar-H), 7.73 (d, *J* = 3.4 Hz, Ar-H), 9.88 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.19, 100.28, 101.80, 129.68, 132.81, 141.28, 146.22; MS (EI): *m/z* calcd. for [$C_7H_6N_6$+H]: 175.07. Found: 175.0.

7-Ethyl pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8b)

Pale yellow solid, yield: 90%, m.p. 146–148°C. IR (film) υ: 3103, 3057, 1633, 1487 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.45 (t, *J* = 7.2 Hz, CH_3), 4.42 (q, *J* = 7.2 Hz, N-CH_2-CH_3), 7.07 (d, *J* = 3.4 Hz, Ar-H), 7.80 (d, *J* = 3.4 Hz, Ar-H), 9.88 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 16.51, 41.08, 100.18, 102.62, 128.97, 133.50, 141.40, 146.97; MS (EI): *m/z* calcd. for [$C_8H_8N_6$+H]: 189.08. Found: 189.

7-Propyl pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8c)

Pale yellow solid, yield: 90%, m.p. 134–136°C. IR (film) υ: 3078, 1717, 1630, 1461 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, *J* = 7.1 Hz, CH_3), 1.86 (m, —CH_2—CH_3), 4.35 (t, *J* = 7.1 Hz, N—CH_2—CH_2), 7.07 (d, *J* = 3.4 Hz, Ar-H), 7.78 (d, *J* = 3.4 Hz, Ar-H), 9.87 (s, Ar-H); ^{13}C NMR

(100 MHz, DMSO-d_6): δ 10.88, 23.39, 46.80, 100.30, 101.76, 128.73, 132.69, 140.97, 146.19; MS (EI): *m/z* calcd. for [$C_9H_{10}N_6$+H]: 203.1. Found: 203.

7-(Ethyl 4-butanoate)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8d)

White solid, yield: 85%, m.p. 138–141°C. IR (film) υ: 1713, 1630, 1486, 1410 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.12 (t, *J* = 7.1 Hz, CH_3), 2.15–2.08 (m, —CH_2—CH_2), 2.30 (t, *J* = 7.3 Hz, —CO—CH_2), 3.99 (q, *J* = 7.1 Hz, —OCH_2—CH_3), 4.43 (t, *J* = 6.8 Hz, N—CH_2—CH_2), 7.09 (d, *J* = 3.4 Hz, Ar-H), 7.77 (d, *J* = 3.4 Hz, Ar-H), 9.89 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.95, 25.45, 30.53, 44.51, 59.86, 100.54, 101.89, 128.67, 132.77, 141.03, 146.18, 172.01; *m/z* calcd. for [$C_{12}H_{14}N_6O_2$+H]: 275.12. Found: 275.

7-(3-Butene)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8e)

White solid, yield: 82%, m.p. 140–142°C. IR (film) υ: 3107, 3054, 1637, 1534, 1489 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.60 (q, *J* = 6.8 Hz, CH_2—CH═CH_2), 4.45 (t, *J* = 7.0 Hz, CH═CH_2), 4.96 (m, N—CH_2—CH_2), 5.82–5.72 (m, —CH_2—$CHCH_2$), 7.04 (d, *J* = 3.4 Hz, Ar-H), 7.74 (d, *J* = 3.4 Hz, Ar-H), 9.83 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.45, 44.79, 100.54, 101.97, 117.70, 129.01, 132.84, 134.76, 141.15, 146.38; MS (EI): *m/z* calcd. for [$C_{10}H_{10}N_6$+H]: 215.0. Found: 214.7.

7-(Benzyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8f)

Brown solid, yield: 86%, m.p. 175–177°C. IR (film) υ: 3127, 3054, 1633, 1534, 1513 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.63 (s, N—CH_2), 7.12 (d, *J* = 3.2 Hz, Ar-H), 7.25–7.35 (m, Ar-5H), 7.85 (d, *J* = 3.4 Hz, Ar-H), 9.90 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 48.55, 100.85, 102.04, 127.30, 127.66, 128.57, 128.82, 133.10, 137.21, 140.97, 146.20; MS (EI): *m/z* calcd. for [$C_{13}H_{10}N_6$+H]: 251.1. Found: 251.

7-(4-Methylbenzyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8g)

Yellow solid, yield: 86%, m.p. 182–184°C. IR (film) υ: 3108, 3055, 1637, 1486, 1260 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, CH_3), 5.57 (s, N—CH_2), 7.12 (m, Ar-3H), 7.20 (d, *J* = 8.0 Hz, Ar-2H), 7.83 (d, *J* = 3.4 Hz, Ar-H), 9.90 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.57, 48.34, 100.80, 102.02, 127.38, 128.78, 129.11, 133.09, 134.23, 136.98, 140.94, 146.21; MS (EI): *m/z* calcd. for [$C_{14}H_{12}N_6$+H]: 265.0. Found: 264.8.

7-(4-Nitrobenzyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8h)

Yellow solid, yield: 86%, m.p. 188–190°C. IR (film) υ: 3108, 3055, 1637, 1513, 1251, 1074 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.57 (s, N—CH_2), 7.12 (m, Ar-3H), 7.71 (d, *J* = 8.0 Hz, Ar-2H), 7.83 (d, *J* = 3.4 Hz, Ar-H), 9.90 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 48.34, 100.80, 102.02, 121.38, 128.78, 129.11, 133.09, 134.23, 140.94, 146.21, 162.1; MS (EI): *m/z* calcd. for [$C_{13}H_{10}N_7O_2$+H]: 296.1. Found: 296.

7-(2′-Nitrile-1,1′-biphenylbenzyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]-pyrimidine (8i)

White solid, yield: 86%, m.p. 179–181°C. IR (film) υ: 3108, 3055 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.73 (s, N—CH_2), 7.16 (d, *J* = 3.4 Hz, Ar-H), 7.43 (d, Ar-2H), 7.56 (m, Ar-5H), 7.77 (d, *J* = 7.8 Hz, Ar-H), 7.92 (d, *J* = 5.1 Hz, Ar-H), 7.94 (s, Ar-H), 9.92 (s, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 48.23, 101.06, 102.21, 110.07, 118.47, 127.58, 128.25, 129.03, 130.05, 133.35, 133.51, 133.81, 137.22, 137.93, 141.12, 143.96, 146.30; MS (EI): *m/z* calcd. for [$C_{20}H_{13}N_7$+H]: 352.1. Found: 351.9.

4.1.8 | General procedure for the preparation of compounds 8j–l

To a mixture of 4-chloropyrrolo[2,3-*d*]pyrimidine (12 mmol) and appropriate sulfonyl chloride (15 mmol) in acetone (10 mL) was added a solution of aqueous sodium hydroxide (50%) (12 mmol). The reaction mixture was monitored by TLC. After completion of the reaction, mixture was precipitated then filtered, washed with mixture of acetone/water 1:1 (5 mL), and dried under vacuum to afford compounds **8j–l** (70–75%).

7-(Methylsulfonyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]-pyrimidine (8j)

Brown color solid, yield: 75%, m.p. 156–158°C. IR (film) υ: 3120, 2966, 1353, 1262 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.98 (s, N—CH_3), 7.05 (d, *J* = 3.4 Hz, Ar-H), 7.73 (d, *J* = 3.4 Hz, Ar-H), 9.88 (s, Ar-H) ppm, ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.19, 100.28, 101.80, 129.68, 132.81, 141.28, 146.22; MS (EI): *m/z* calcd. for [$C_7H_6N_6O_2S$+H]: 239.0. Found: 238.9.

7-(Phenylsulfonyl)pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (8k)

Brown color solid, yield: 72%, m.p. 169–172°C. IR (film) υ: 3120, 2966, 1557, 1353, 1262 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.40 (d, *J* = 3.7 Hz, Ar-H), 7.69 (t, *J* = 7.8 Hz, Ar-2H), 7.80 (t, *J* = 7.6 Hz, Ar-H), 8.20 (m, Ar-H), 10.05 (s, Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 104.64, 106.64, 127.07, 128.80, 129.96, 135.48, 135.64, 136.80, 140.64, 145.88; MS (EI): *m/z* calcd. for [$C_{12}H_8N_6O_2S$+H]: 301.04. Found: 301.

7-[(4-Methylphenyl)sulfonyl]pyrrolo[3,2-*e*]tetrazolo[1,5-*c*]-pyrimidine (8l)

Dark brown color solid, yield: 70%, m.p. 150–152°C. IR (film) υ: 3120, 2966, 1557 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, CH_3), 7.12 (m, Ar-3H), 7.20 (d, *J* = 8.0 Hz, Ar-2H), 7.83 (d, *J* = 3.4 Hz, Ar-H), 9.90 (s, Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.57, 100.80, 102.02, 127.38, 128.78, 129.11, 133.09, 134.23, 136.98, 140.94, 146.21; MS (EI): *m/z* calcd. for [$C_{13}H_{10}N_6O_2S$+H]: 315.06. Found: 315.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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