Regular Article

Synthesis, Antimycobacterial Evaluation and Docking Studies of Some 7-Methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones

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Two series of 3-substituted-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5] thieno[2,3-*d*]pyrimidin-4(3*H*)-one (6a–k) and 3-substituted-7,2-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (7a–k) derivatives were synthesized and characterized using spectral data *i.e.*, IR, ^{1}H-, ^{13}C-NMR, Mass and CHN elemental analyses. The synthesized compounds were evaluated for antibacterial activity against each of two strains of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria and antimycobacterial activity screened against two strains *i.e.*, *Mycobacterium tuberculosis* (MTB) H37Rv and an isoniazid-resistant clinical sample. Further to validate potentiality of our design was analyzed using molecular docking studies by taking crystal structure of MTB pantothenate synthetase (MTB-PS) (PDB: 3IVX). In this study, some compounds 6k (Minimum Inhibitory Concentration (MIC): MIC-22 μM), 7d (MTB: MIC-22 μM) and 7k (MTB: MIC-11 μM) showed potential antibacterial and antimycobacterial activities.

Key words antimycobacterial activity; isoniazid; *Mycobacterium tuberculosis* H37Rv; pantothenate synthetase; 5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno(2,3-*d*)pyrimidine-4(3*H*)-one

Tuberculosis (TB) is considered as a serious public health threat worldwide, as current therapy is limited due to the emergence of multi-drug resistance (MDR) and extensively drug resistance (XRD), regimen therapy, and severe side effects of existing drugs and long duration of therapy.[1,2] Moreover, TB is more widespread due to its coincidence with AIDS and Human Immunodeficiency Virus (HIV) co-infection.[3] Development of drug resistance (MDR and XDR) to widely used first line drugs is an obstacle in the treatment and control programs of this disease. Resistance to isoniazid (INH) is more predominant in MDR TB as about 30% of clinical isolates found to be resistant to it, worldwide. Several decades of extensive research resulted in identification and development of only few drugs such as bedaquiline and delamanide, which were approved as drugs of choice in drug resistant (MDR and XDR) TB. Several other novel drug candidates for drug resistant TB such as oxazolidinones (linezolid), nitroimidazoles (PA-824) and ethylenediamines (SQ-109) *etc.*, are currently under clinical development.[4] There is an urgent need of shorter and simple regimens, effective, safe and well tolerated drugs for drug-resistant and drug-susceptible TB.

In view of development of hetero-fused thieno[2,3-*d*]-pyrimidine-4(3*H*)-one analogues various recent reports prompted us to continue our studies by retaining 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine as basic nucleus.[5] It was a common feature in some reported *Mycobacterium tuberculosis* Pantothenate synthetase (MTB-PS) inhibitors such as 6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (A, SID 92104095; IC_{50}=3.3 μg/mL),[6] 6-acetyl-4,5,6,7-tetrahydro thieno[2,3-*c*]pyridine-3-carboxamide (B, SID 92097880; IC_{50}=0.5 μg/mL))[6] derivatives, as described earlier.[5] In addition, Rashmi *et al.* reported potentiality of some thieno[2,3-*d*]pyrimidines (C) on *Mycobacterium*.[7] Narayana *et al.* reported the antibacterial activity of some 4,5,6,7-tetrahydro[1]benzothieno[2,3-*d*]-pyridimidine-4(3*H*)-ones (D).[8] These studies proved the essentiality of 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (A, B) nucleus and arylidineamino side chain at 3rd or 4th position in C and D in their antibacterial and antimycobacterial activity (Fig. 1). In the design of molecules, both the structural features were adopted. The 3-amino-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one was considered as a key structure for the synthesis. The bulky (benzyl) group present in the previous series[5] was substituted with a small (methyl) group at 7th position of 5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidine nucleus. Aurelio *et al.* also reported the allosteric modulation effects of 2-amino-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines on A_1 adenosine receptor.[9] Modifications at 7th position with small and medium pharmacophores such as *N*-methyl, *N*-benzyl and *N*-ethoxycarbonyl resulted differences in their biological activity. As per their studies, it was due to increase in basicity with *N*-methyl substitution. Although, the structure activity relationship was described for A_1 adenosine receptor modulation, the significance of 7th substitution was considered in this study and attempted with these modifications perhaps it could show a better antimycobacterial activity. In

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this direction our studies are continued on the development of novel hetero-fused thieno[2,3-*d*]pyrimidine analogues against drug-resistant TB (INH-resistant TB).[5] Two series of molecules 7-methyl-3-(substituted-aryledineamino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**6a**–**k**) and 2,7-dimethyl-3-(substituted-aryledineamino)-2-methyl-5,6,7,8-tetrahydropyrido [4′,3′:4,5]thieno[2,3-*d*]pyrim idin-4(3*H*)-ones (**7a**–**k**) have been synthesized and evaluated for their antitubercular activity. Initial antibacterial screening was performed against each of two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus)* and Gram-negative (*Escherichia coli* and *Klebsiella pneumonia)* bacteria. The antimycobacterial activity was conducted for selected compounds based on results obtained in initial antibacterial screening. *Mycobacterium tuberculosis* (MTB) H37Rv and an INH-resistant clinical sample were used as standard and test organisms.

Further, *in silico* binding interactions of these analogues were studied using target protein MTB-PS (PDB: 3IVX) with the help of Molegro Virtual Docker (MVD) software. These investigations indicated that some of the compounds expressed promising *in vitro* antibacterial and antimycobacterial activities against the test strains.

Results and Discussion

Chemistry The synthesis of target compounds 7-methyl-3-(substituted-aryledineamino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**6a**–**k**) and 2,7-dimethyl-3-(substituted-aryledineamino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a**–**k**) was achieved as shown in Chart 1.

The requisite key starting material **3** was synthesized by Gewald Reaction (GR) using *N*-methyl-4-piperidone (**1**), elemental sulphur and ethylcyanoacetate (**2**) by following a reported procedure[9,10] which gave the product in 85% of yield. The compound **3** was converted to iminoester derivatives by heating under reflux in triethylorthoformate and triethylorthoacetate, which gave thick brown oily products **4a** and **4b**, respectively. Compounds **4a** and **4b** were treated with hydrazine hydrate[5,11–13] to get their cyclized products **5a** and **5b**. In IR spectrum compound **5b** showed two characteristic signals at 3450 cm^{-1} and 3300 cm^{-1} assignable to N–H stretching for 3-NH_2 group and another signal at 1670 cm^{-1} assignable to C=O stretching of carbonyl group of pyrimidine ring. The ^{1}H-NMR spectrum of compound **5b** showed a singlet at δ=2.20 ppm which could be assignable to methyl (2-CH_3) group of pyrimidinone ring. The presence of another broad singlet at the δ=5.13 ppm could be assignable to primary amino functionality.

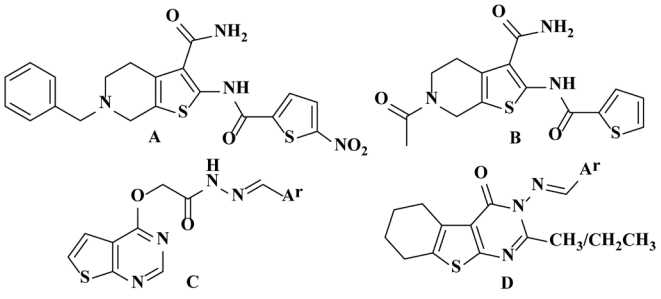
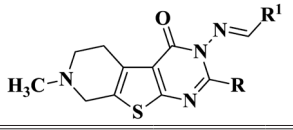
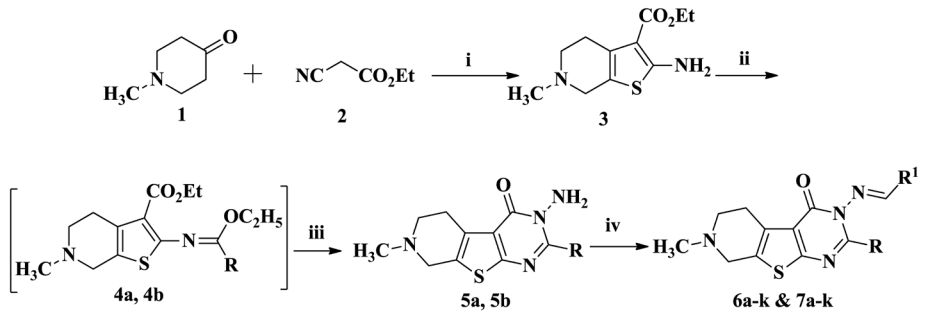


Fig. 1. General Structures of Some Potent Antibacterial Agents (A, B: MTB-PS Inhibitors)

Table 1. Physical Data of Synthesized Compounds **6a**–**k** and **7a**–**k**



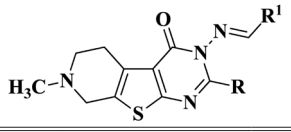
Comp. Code	R	R^1	Mol. Wt. (M)	Melting range (°C)	Yield (%)
6a	H	-C_6H_5	324	164–166	87
6b	H	4-ClC_6H_4	358	196–198	78
6c	H	4-FC_6H_4	343	180–182	64
6d	H	4-CNC_6H_4	350	236–238	67
6e	H	4-$OCH_3C_6H_4$	354	168–170	80
6f	H	4-OHC_6H_4	340	220–222	65
6g	H	3,4,5-$(OCH_3)_3C_6H_2$	414	192–194	67
6h	H	2-$NO_2C_6H_4$	369	159–161	72
6i	H	Thiophene-2-yl	330	120–122	66
6j	H	Furan-2-yl	314	156–158	80
6k	H	2,6-$Cl_2C_6H_3$	392	190–192	82
7a	CH_3	-C_6H_5	338	185–187	85
7b	CH_3	4-ClC_6H_4	372	153–155	71
7c	CH_3	4-FC_6H_4	355	230–232	65
7d	CH_3	4-CNC_6H_4	363	200–202	68
7e	CH_3	4-$OCH_3C_6H_4$	368	173–175	82
7f	CH_3	4-OHC_6H_4	354	218–220	76
7g	CH_3	3,4,5-$(OCH_3)_3C_6H_2$	428	198–200	70
7h	CH_3	2-$NO_2C_6H_4$	383	154–156	78
7i	CH_3	Thiophene-2-yl	312	178–180	70
7j	CH_3	Furan-2-yl	328	172–174	79
7k	CH_3	2,6-$Cl_2C_6H_3$	406	181–183	80



i) S_8, morpholine, ethanol, reflux, 1–2 h; ii) Triethylorthoformate/triethylorthoacetate, reflux, 2–4 h; iii) $NH_2NH_2H_2O$, ethanol, stir, 1–2 h; iv) R^1CHO, glacial CH_3COOH, ethanol, reflux, 12 h.

Chart 1. General Chart for Synthesis of Compounds **6a**–**k** and **7a**–**k**

Table 2. Antibacterial Activity (MIC) Data of Test Compounds **6a**–**k** and **7a**–**k**



Comp. Code	R	R^1	MIC (μM)			
			S. aureus MTCC 96	*B. subtilis* MTCC 441	*E. coli* MTCC 443	*K. pneumoniae* MTCC 109
6a	H	-C_6H_5	NA	NA	NA	NA
6b	H	4-ClC_6H_4	43.64	43.64	21.82	87.29
6c	H	4-FC_6H_4	46	46	23	46
6d	H	4-CNC_6H_4	48	48	24	48
6e	H	4-$OCH_3C_6H_4$	44.1	88.2	44.1	NA
6f	H	4-OHC_6H_4	NA	NA	NA	NA
6g	H	3,4,5-$(OCH_3)_3C_6H_2$	NA	NA	NA	NA
6h	H	2-$NO_2C_6H_4$	84	84	84	84
6i	H	Thiophene-2-yl	48	48	24	48
6j	H	Furan-2-yl	98	98	98	<98
6k	H	2,6-$Cl_2C_6H_3$	22	22	11	22
7a	CH_3	-C_6H_5	NA	NA	NA	NA
7b	CH_3	4-ClC_6H_4	44	44	22	44
7c	CH_3	4-FC_6H_4	46	46	23	46
7d	CH_3	4-CNC_6H_4	24	24	12	24
7e	CH_3	4-$OCH_3C_6H_4$	44	44	22	44
7f	CH_3	4-OHC_6H_4	NA	NA	NA	NA
7g	CH_3	3,4,5-$(OCH_3)_3C_6H_2$	NA	NA	NA	NA
7h	CH_3	2-$NO_2C_6H_4$	162	162	162	162
7i	CH_3	Thiophene-2-yl	28	28	28	28
7j	CH_3	Furan-2-yl	50	50	50	50
7k	CH_3	2,6-$Cl_2C_6H_3$	21	21	10	21
Std	—	—	<1	<1	<1	<1
Ctrl	—	—	NA	NA	NA	NA

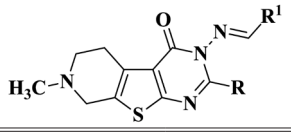
Std.=Streptomycin; Ctrl=*N*,*N*-dimethylformamide (DMF) in water (25% v/v); NA=Not active.

In the last step different arylidineamino analogues **6a**–**k** and **7a**–**k** were prepared by condensation of **5a** and **5b** with differently substituted aromatic aldehydes following some reported protocols.[5,7,8,14] The IR spectra of compound **6a**–**k** and **7a**–**k** showed characteristic signals of C=O stretching and C=N stretching (-imine). The loss of strong N–H stretching signals of 3-NH_2 group was noticeable which revealed the formation of imine. The ^{1}H-NMR spectra for compounds **6a**–**k** showed characteristic singlet at δ=9.4–10.2 ppm could be assignable for single proton of imine (–N=CH) and another sharp singlet at δ=8.2–8.3 ppm could be assignable to single proton (2-H) of pyrimidinone ring. Compounds **7a**–**k** showed singlet at δ=9.2–10.2 ppm could be assignable for single proton of imine (–N=CH) and another singlet at δ=2.59–2.63 ppm could be assignable for methyl (2-CH_3) protons of pyrimidinone ring. The mass spectra (Electron Spray Ionization (ESI) and Electron Impact (EI) mode) showed the molecular ion (M^+, M^++1, M^++2) peaks for all the compounds. The halogenated compounds showed the isotopic peaks (M^++1 and M^++2), which further confirms the presence of chlorine (Cl) as a substituent, in compounds **6b**, **6k**, **7b** and **7k**. The physical data of all the final compounds was shown in Table 1.

Antibacterial Activity All the newly synthesized compounds **6a**–**k** and **7a**–**k** were assessed for their *in vitro* antibacterial activity against *S. aureus* (MTCC-96), *B. subtilis* (MTCC-441), *E. coli* (MTCC-443) and *K. pneumoniae* (MTCC-109) using streptomycin as a reference. Broth micro dilution method[15,16] was employed for determination of minimum inhibitory concentration (MIC) for all test compounds. The MIC (μM) data of all the compounds were tabulated in Table 2.

Among all the synthesized compounds, **6b**, **6c**, **6d**, **6i**, **6k**, **7b**, **7c**, **7d** and **7k** exhibited good activity with MIC in the range of 11–24 μg/mL against *E. coli* and rest of the compounds showed moderate to less activity. The structure activity relationship reveals that presence of thieno(2,3-*d*)-pyrimidine-4-one ring with 3-arylidineamino side chain skeleton is crucial for biological activity. In general, the arylidineamino side chain bearing substituted phenyl and heterocyclic groups showed more activity over unsubstituted analogues. Phenyl ring substituted with electron withdrawing groups such as chloro (4-Cl: **6b**, **7b**; 2,6-dichloro: **6k**, **7k**), flouro (4-F: **6c**, **7c**) (excepting 4-methoxy: **6e**, **7e**) and also with thiophene (**6i**, **7i**) system exhibited better activity when compared to electron donating groups such as hydroxyl (4-OH: **6f**, **7f**), 3, 4, 6-trimethoxyl (**6g**, **7g**). However nitro (2-NO_2) containing compounds (**6h**, **7h**) showed less activity. Compounds possessing 2,6-dichloro (**6k**, **7k**) substitution on phenyl ring exhibited potent activity on all the test organisms (Gram-positive and Gram-negative bacteria) used. Variations in the phenyl substitution might have altered the lipophilic character of molecules which is an important feature for permeability across the

Table 3. Antimycobacterial Activity (*in Vitro*) of Test Compounds



Comp. Code	R	R^1	MIC (μM)			
			MTB H37Rv		Clinical sample of MTB	
			14 d	21 d	14 d	21 d
6b	H	4-ClC_6H_4	46	46	46	46
6c	H	4-FC_6H_4	88	88	88	88
6d	H	4-CNC_6H_4	180	180	180	180
6k	H	2,6-$Cl_2C_6H_3$	22	22	22	22
7b	CH_3	4-ClC_6H_4	24	24	24	24
7c	CH_3	4-FC_6H_4	25	25	25	25
7d	CH_3	4-CNC_6H_4	22	22	22	22
7e	CH_3	4-$OCH_3C_6H_4$	84	84	84	84
7i	CH_3	Thiophene-2-yl	98	98	98	98
7k	CH_3	2,6-$Cl_2C_6H_3$	11	11	11	11
Std[1]	—	—	0.1	0.1	4	4
Std[2]	—	—	2	2	2	2
Std[3]	—	—	4	4	4	4
Std[4]	—	—	0.09	0.09	2	2
Ctrl	—	—	NA	NA	NA	NA

Std[1]=INH; Std[2]=Streptomycin; Std[3]=Ethambutol; Std[4]=Rifampicin; Ctrl=DMF in water (25% v/v); NA=Not active.

Table 4. H-Bond Interactions of Biologically Active Ligands **6k**, **7d** and **7k**

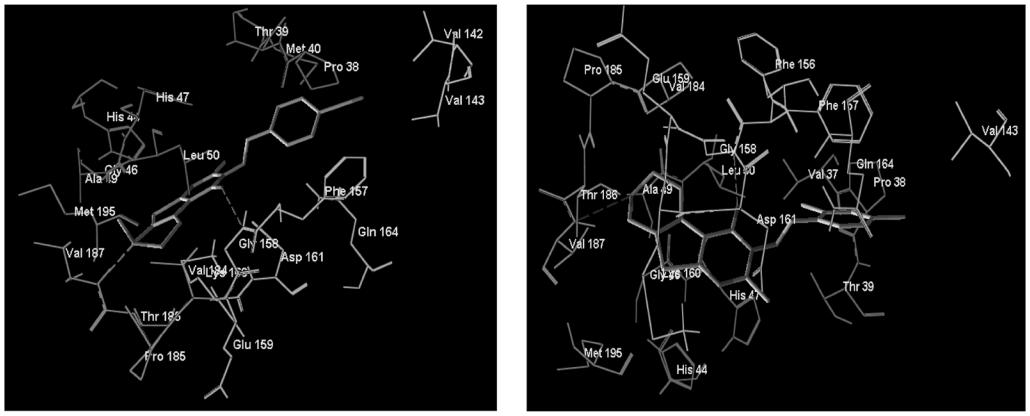
Comp code	Residue	Atom ID	PDB atom name	Ligand name	Distance
6k	Gly 158	1163	N (d)	O (a)	2.93
	Val 187	1397	N (d)	N (a)	2.70
	Thr 186	1386	O (d+a)	N (a+d)	3.14
7d	Gly 158	1163	N (d)	O (a)	1.65
	Val 187	1397	N (d)	N (a)	3.09
7k	Gly 158	1163	N (d)	O (a)	2.60
	Val 187	1397	N (d)	N (a)	3.11

bacterial cell membrane. The 4-hydroxyl (activating group) substituted compounds (**6f** and **7f**) were found to be less potent might be due to their poor lipophilicity. On overall assessment most of the compounds showed activity against *E. coli* (Gram-negative bacteria). Methyl substitution at 2nd position of pyrimidine ring relatively enhanced the biological activity.

Antimycobacterial Activity Few compounds randomly selected for antimycobacterial studies by considering the preliminary antibacterial activity data. The MTB H37Rv and an INH-resistant clinical sample were taken as reference and test organisms, respectively. The MIC was determined on Middlebrook 7H9 medium supplemented with OADC using broth micro dilution method with the help of four well known drugs (INH, Streptomycin, Rifampicin and Ethambutol) as reference.[17] The test samples were prepared by serial dilution method in different strengths in DMF in water (25% v/v) as diluent. Among all the tested compounds **7k**, bearing 2, 6-dichloro substitution on arylidineamino side chain showed greater activity with MIC-11 μM against MTB H37Rv and also clinical sample (INH-resistant). Other compounds bearing 2,6-dichloro (**6k**), 4-Cl (**7b**), 4-F (**7c**) and 4-CN (**7d**) showed activity in the MIC range of 22–25 μM on both the strains. The additional chloro (Cl) group at 2nd position of phenyl ring in **7k**, **6k** instead monochloro (4-Cl) substitution in **6b**, **7b** enhanced the biological activity. Compounds, **6c**, **6d**, **7e** and **7i** exhibited poor in potency and their MIC range was observed at $\geq$46 μM. The antimycobacterial activity (MIC in μM) data is tabulated in Table 3.

Molecular Modeling The molecular docking studies were performed on target protein MTB-PS, which has been proven to be valid drug target for rational design of new analogues for TB by several reports.[18,19] The crystal structure of MTB-PS was retrieved from protein data bank ((PDB: 3IVX) (http://www.rcsb.org/pdb/explore.do?structure Id=3ivx). Ligands possessing 4-flouro (**6c**), 4-hydroxy (**6f**), 2-nitro (**6h**), 4-methoxy (**7e**) and 2,4-dichloro (**6k**, **7k**) substitution on phenyl ring of arylidineamino side chain showed best docking scores, *i.e.*, −118.43, −118.42, −122.87, −121.03, −120.68 and −115.63, respectively.

The carbonyl (C=O, H-bond acceptor) group at 4th position in pyrimidine ring showed strong hydrogen bond interaction with Gly 158 (H-bond donor) amino acid residue in all (**6a**–**k** and **7a**–**k**) the ligands. Another H-bond interaction was observed at nitrogen (7-N) atom of the 5,6,7,8-tetrahydrothieno[2,3-*d*]pyridine ring with Val 187 amino acid residue (Table 4).



A B

Fig. 2. Snapshots of the Typical Binding Interactions of Ligands **7b** (A) and **7k** (B) with Active Site of MTB-PS, Hydrogen Bond Interactions of C=O Group with Residue of Gly 158 and Nitrogen (7-N) with Val 187

Additionally, the steric interactions observed for all the molecules with amino acid residues such as Leu 50, Val 184, Pro 185, Gln164 and Pro 38. All these molecules **6a**–**k** and **7a**–**k** lack additional π–π stacking and van der Waals interactions due to presence of *N*-methyl group at 7th position, hence they showed less potency compared to *N*-benzyl analogues.[5] The binding mode and orientation pattern at active site were analyzed for all molecules. The docking poses of the experimentally more potent molecules **7b** and **7k** were showed in Fig. 2.

Conclusion

In conclusion this study deals with the design and synthesis of novel analogues of 7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones. Among all the compounds **6a**, **6b**, **6c**, **6d**, **6k**, **7b**, **7c**, **7d**, **7e** and **7k** showed promising antibacterial activity against *E. coli* in the MIC range 10–24 μM and compounds **6k**, **7b**, **7c**, **7d** and **7k** further showed antimycobacterial activity in the MIC range 10–25 μM against MTB H37Rv and an INH-resistant clinical sample. Among these compound **7k** was found to be more potent (MIC-11 μM). Further the molecular docking studies helped in knowing the binding modes of core structure with active sites and the docking scores obtained were correlated with the MIC values (μM) of most of the compounds. Further investigations are in progress on enzyme inhibitory activity, cytotoxicity and pharmacokinetic properties to validate these results.

Experimental

Chemistry Melting points of all the compounds were determined in open capillaries on melting point apparatus (Biotechniques India-BTI-34), and are uncorrected. Purity and homogeneity was verified by pre-coated TLC plates (E. Merck silica gel 60 F_{254}). IR spectra were recorded using KBr pellet method on Bruker spectrophotometer and Perkin-Elmer FTIR 240-C (υ_{max} in cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR was recorded on a Bruker spectrometer 500 MHz and 400 MHz respectively using tetramethylsilane (TMS) as internal standard, Central Instrumentation Laboratory, Indian Institute of Chemical Technology, Hyderabad, India. The chemical shifts (δ) are reported in part per million (ppm) relative to TMS using $CDCl_3$ as solvent. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), brs (broad singlet), dd (double doublet) and m (multiplet). MS were recorded on ESI and EI mode using QSTARXL hybrid MS/MS system (Applied Bio-systems, U.S.A.), Central Instrumentation Laboratory, Indian Institute of Chemical Technology, Hyderabad, India. Elemental analysis was carried out using vario MICRO CUBE Elementar (2mgChem80s method), Kakatiya University, Warangal. All the chemicals used in the synthesis were procured from Aldrich Company Ltd. (Bengaluru, India) and Himedia Chemicals (Mumbai, India). All the solvents used were purchased from SD Fine Chemicals Ltd. (Mumbai, India) and were employed without further purification.

Ethyl-2-amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]-pyridine-3-carboxylate (3) *N*-Methyl-4-piperidone (**1**) (5 g, 26.42 mmol), ethyl cyanoacetate (**2**) (3.09 mL, 29.06 mmol) and sulphur (1.02 g, 31.70 mmol) were suspended in ethanol (55 mL). Morpholine (4.62 mL, 52.84 mmol) was added and the mixture was heated under reflux gently with stirring for 2 h. The cooled solution was diluted with water and extracted with dichloromethane (3×50 mL). The combined organic layers were washed with water, then brine, dried ($MgSO_4$), filtered and concentrated. The resultant residue was triturated with hot methanol (20 mL) and washed with ice cold methanol to afford the desired product as an off white powder.

Yield, 7.1 g (85%). mp 110–111°C. IR (KBr) cm^{-1}: 3344, 3254 (N–H, *str*), 2986, 2878 (C–H, *str*), 1685 (C=O, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.03 (2H, brs, 2-NH_2), 4.27 (2H, q, J=7.2 Hz, CH_2 of carboxylate), 3.39 (2H, s, 7-CH_2), 2.85 (2H, t, J=5.7 Hz, 5-CH_2), 2.68 (2H, t, J=5.8 Hz, 4-CH_2), 2.45 (3H, s, 7-NCH_3), 1.34 (3H, t, J=7.2 Hz, –CH_3 of carboxylate). ESI-MS (*m*/*z*; %): 241 (M^++1; 100).

3-Amino-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5a) A solution of **3** (1·10 g, 57 mmol) in triethylorthoformate (5 mL) was heated under reflux for 2–4 h. Excess triethylorthoformate was removed *in vacuo*. The residue was treated with ethyl acetate in hexane (20% v/v) and dried to obtain light brown oily product (**4a**). It was used directly in next step without purification. A mixture of **4a** and hydrazine hydrate (1 mL) in 10 mL of absolute ethanol was stirred at room temperature for 1–2 h. The separated fine solid was filtered, washed with ethanol and purified by recrystallization from ethanol to afford analytically pure pale yellow granules.

Yield, 0.76 g (71%). mp 194–196°C. IR (KBr) cm^{-1}: 3450, 3300 (N–H, *str*), 2910 (C–H, *str*), 1670 (C=O, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (3H, s, 7-NCH_3), 2.80 (2H, t, J=6 Hz, 5-CH_2), 3.20 (2H, t, J=6 Hz, 6-CH_2), 3.67 (2H, s, 8-CH_2), 5.13 (2H, brs, 3-NH_2), 8.20 (1H, s, 2-H pyrimidinone). ESI-MS (*m*/*z*; %): 236 (M^++1; 100).

3-Amino-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5b) A solution of **3** (1·10 g, 57 mmol) in triethylorthoacetate (5 mL) was heated under reflux for 2–4 h. Excess triethyorthoacetate was removed *in vacuo*. The residue was treated with ethyl acetate in hexane (20% v/v) and dried to obtain light brown oily product (**4b**). It was used directly in next step without purification. A mixture of **4b** and hydrazine hydrate (1 mL) in 10 mL of absolute ethanol was stirred at room temperature for 1–2 h. The separated fine solid was filtered, washed with ethanol and purified by recrystallization from ethanol to afford analytically pure pale yellow granules.

Yield, 0.84 g (74%). mp 200–202°C. IR (KBr) cm^{-1}: 3450, 3300 (N–H, *str*), 2910 (C–H, *str*), 1670 (C=O, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.20 (3H, s, 2-CH_3 of pyrimidinone), 2.50 (3H, s, 7-NCH_3), 2.80 (2H, t, J=5.82 Hz, 5-CH_2), 3.20 (2H, t, J=5.73 Hz, 6-CH_2), 3.67 (2H, s, 8-CH_2), 5.13 (2H, brs, 3-NH_2). ESI-MS (*m*/*z*; %): 250 (M^++1; 85).

7-Methyl-3-(substituted-aryledineamino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (6a–k)

General Method

A mixture of **5a** (0.001 M) and appropriate aldehyde (0.001 M) in catalytic amount of glacial acetic acid in 10 mL of absolute ethanol was heated under reflux for 2–6 h. On cooling, the separated solid was filtered, washed with cold ethanol and recrystallized from acetic acid: ethanol to afford desired product.

3-(Benzylideneamino)-7-methyl-5,6,7,8-tetrahydropyrido [4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4 (3*H*)-one (**6a**) [El-Kashef *et al.*].[11)]

3-((4-Chlorobenzylidene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6b**) [El-Kashef *et al.*].[11)]

3-((4-Fluorobenzylidene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6c**)

Yield, 0.22 g (64%). mp 180–182°C. IR (KBr) cm^{-1}: 3069, 3031 (aromatic C–H, *str*), 2919 (aliphatic C–H, *str*), 1672 (C=O, *str*), 1601 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.59 (3H, s, 7-CH_3), 2.79 (2H, t, J=5.88 Hz, 5-CH_2), 3.18 (2H, t, J=5.94 Hz, 6-CH_2), 3.67 (2H, s, 8-CH_2), 7.18 (2H, t, J=8.62 Hz, 3′,5′-Ar-H), 7.85 (2H, dd, J=5.4, 5.4 Hz, 2′,6′-Ar-H), 8.24 (1H, s, 2-H of pyrimidinone), 9.53 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.12, 45.48, 51.89, 53.76, 116.34–116.34 (*d*), 122.92, 129.31–129.34 (*d*), 130.13, 130.71–130.80 (*d*), 132.07, 145.57, 156.37, 160.86, 162.43, 164.01, 166.53. ESI-MS (*m*/*z*; %): 343 (M^++1; 100). *Anal.* Calcd for $C_{17}H_{15}FN_4OS$: C, 59.63; H, 4.42; N, 16.36. Found: C, 58.92; H, 4.46; N, 16.88.

4-(((7-Methyl-4-oxo-5,6,7,8-tetrahydropyrido[4′,3′:4,5]-thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)imi no)methyl)benzonitrile (**6d**)

Yield, 0.23 g (67%). mp 236–238°C. IR (KBr) cm^{-1}: 3099 (aromatic C–H, *str*), 2955 (aliphatic C–H, *str*), 2229 (CN, *str*), 1674 (C=O, *str*), 1545 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.56 (3H, s, 7-NCH_3), 2.79 (2H, t, J=5.12 Hz, 5-CH_2), 3.18 (2H, t, J=5.22 Hz, 6-CH_2), 3.66 (2H, s, 8-CH_2), 7.78 (2H, d, J=8.86 Hz, 3′,5′-Ar-H), 7.94 (2H, d, J=8.84 Hz, 2′,6′-Ar-H), 8.28 (1H, s, 2-H of pyrimidinone), 9.92 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.12, 45.47, 51.84, 53.73, 115.24, 118.08, 122.87, 128.76, 130.23, 132.51, 132.61, 137.44, 146.02, 156.76, 159.70, 160.69. ESI-MS (*m*/*z*; %): 350 (M^++1; 100). *Anal.* Calcd for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04. Found: C, 61.58; H, 4.76; N, 19.82.

3-((4-Methoxybenzylidene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6e**) [El-Kashef *et al.*].[11)]

3-((4-Hydroxybenzylidene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6f**) [El-Kashef *et al.*].[11)]

7-Methyl-3-((3,4,5-trimethoxybenzylidene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6g**)

Yield, 0.28 g (67%). mp 192–194°C. IR (KBr) cm^{-1}: 3059 (aromatic C–H, *str*), 2937, 2776 (aliphatic C–H, *str*), 1672 (C=O, *str*), 1576 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.52 (3H, s, 7-NCH_3), 2.81 (2H, t, J=5.86 Hz, 5-CH_2), 3.22 (2H, t, J=5.89 Hz, 6-CH_2), 3.69 (2H, s, 8-CH_2), 3.94 (6H, s, 3′,5′-Ar-OCH_3), 3.96 (3H, s, 4′-Ar-OCH_3), 7.12 (2H, s, 2′,6′-Ar-H), 8.26 (1H, s, 2-H of pyrimidinone), 9.44 (1H, s, N=CH). ESI-MS (*m*/*z*; %): 415 (M^++1; 100). *Anal.* Calcd for $C_{20}H_{22}N_4O_4S$: C, 57.96; H, 5.35; N, 13.52. Found: C, 57.92; H, 5.46; N, 13.68.

7-Methyl-3-((2-nitrobenzylidene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6h**)

Yield, 0.27 g (72%). mp 159–161°C. IR (KBr) cm^{-1}: 3019 (aromatic C–H, *str*), 2932, 2774 (aliphatic C–H, *str*), 1686 (C=O, *str*), 1524 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (3H, s, 7-NCH_3), 2.79 (2H, t, J=5.88 Hz, 5-CH_2), 3.18 (2H, t, J=5.88 Hz, 6-CH_2), 3.67 (2H, s, 8-CH_2), 7.72 (1H, dt, J=8.02 Hz, 4′-Ar-H), 7.78 (1H, dt, J=8.02 Hz, 5′-Ar-H), 8.18 (2H, dd, J=8.12, 1.52 Hz, 3′,6′-Ar-H), 8.28 (1H, s, 2-H of pyrimidinone),10.18 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.04, 45.43, 51.84, 53.72, 122.94, 124.86, 128.49, 129.42, 130.36, 131.97, 132.28, 133.73, 145.57, 148.86, 156.20, 159.57, 160.75. ESI-MS (*m*/*z*; %): 370 (M^++1; 100). *Anal.* Calcd for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96. Found: C, 55.42; H, 4.46; N, 18.88.

7-Methyl-3-((thiophen-2-ylmethylene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6i**)

Yield, 0.22 g (66%). mp 120–122°C. IR (KBr) cm^{-1}: 3051 (aromatic C–H, *str*), 2938, 2785 (aliphatic C–H, *str*), 1683 (C=O, *str*), 1581 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (3H, s, 7-NCH_3), 2.81 (2H, t, J=5.88 Hz, 5-CH_2), 3.19 (2H, t, J=5.79 Hz, 6-CH_2), 3.68 (2H, s, 8-CH_2), 7.26 (1H, dt, J=5, 1.12 Hz, 4′-Ar-H of thiophene), 7.54 (1H, dd, J=3.88, 1.53 Hz, 3′-Ar-H of thiophene), 7.58 (1H, dd, J=5.01, 3.12 Hz, 5′-Ar-H of thiophene), 8.25 (1H, s, 2-H of pyrimidinone), 9.78 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 25.80, 49.87, 51.64, 62.01, 112.54, 118.74, 127.36, 128.44, 129.14, 130.28, 137.96, 146.90, 153.58, 155.08, 161.19. ESI-MS (*m*/*z*; %): 331 (M^++1; 100). *Anal.* Calcd for $C_{15}H_{14}N_4OS_2$: C, 54.52; H, 4.27; N, 16.96. Found: C, 54.82; H, 4.46; N, 16.88.

3-((Furan-2-ylmethylene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6j**)

Yield, 0.25 g (80%). mp 156–158°C. IR (KBr) cm^{-1}: 3104

(aromatic C–H, *str*), 2920, 2841 (aliphatic C–H, *str*), 1662 (C=O, *str*), 1539 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.54 (3H, s, 7-NCH_3), 2.82 (2H, t, J=5.82 Hz, 5-CH_2), 3.20 (2H, t, J=5.83 Hz, 6-CH_2), 3.68 (2H, s, 8-CH_2), 6.58 (1H, dd, J=3.48, 1.76 Hz, 4′-Ar-H of furan), 7.02 (1H, d, J=3.22 Hz, 5′-Ar-H of furan), 7.69 (1H, d, J=1.55 Hz, 3′-Ar-H of furan), 8.29 (1H, s, 2-H of pyrimidinone), 9.54 (1H, s, N=CH). EI-MS (*m/z*; %): 314 (M^+; 62), 315 (M^++1; 12). *Anal.* Calcd for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82. Found: C, 57.72; H, 4.46; N, 16.18.

3-((2,4-Dichlorobenzylidene)amino)-7-methyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**6k**)

Yield, 0.32 g (82%). mp 190–192°C. IR (KBr) cm^{-1}: 3083 (aromatic C–H, *str*), 2938, 2783 (aliphatic C–H, *str*), 1681(C=O, *str*), 1582 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (3H, s, 7-NCH_3), 2.79 (2H, t, J=5.98 Hz, 5-CH_2), 3.19 (2H, t, J=5.56 Hz, 6-CH_2), 3.71 (2H, s, 8-CH_2), 7.34 (1H, dd, J=1.6, 2.5 Hz, 5′-Ar-H), 7.47 (1H, d, J=2 Hz, 3′-Ar-H), 8.13 (1H, d, J=8.2 Hz, 6′-Ar-H), 8.23 (1H, s, 2-H of pyrimidinone), 10.09 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.14, 45.44, 51.84, 53.73, 122.93, 127.74, 128.45, 129.60, 129.98, 130.30, 132.17, 136.80, 138.56, 145.80, 156.40, 158.37, 160.67. ESI-MS (*m/z*; %): 394 (M^++2; 35), 392 (M^+; 100). *Anal.* Calcd for $C_{17}H_{14}Cl2N_4OS$: C, 51.92; H, 3.59; N, 14.25. Found: C, 51.72; H, 3.46; N, 14.48.

2,7-Dimethyl-3-(substituted-aryledineamino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (7a–k)

General Method

A mixture of **5b** (0.001 M) and appropriate aldehyde (0.001 M)) in catalytic amount of glacial acetic acid in 10 mL of absolute ethanol was heated under reflux for about 2–6 h. On cooling, the separated solid was filtered, washed with cold ethanol and recrystallized from acetic acid: ethanol to afford desired product.

3-(Benzylideneamino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7a**)

Yield, 0.29 g (85%). mp 185–187°C. IR (KBr) cm^{-1}: 3066 (aromatic C–H, *str*), 2957 (aliphatic C–H, *str*), 1640 (C=O, *str*), 1547 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.53 (3H, s, 7-NCH_3), 2.62 (3H, s, 2-CH_3 of pyrimidinone), 2.80 (2H, t, J=5.83 Hz, 5-CH_2), 3.16 (2H, t, J=5.81 Hz, 6-CH_2), 3.65 (2H, s, 8-CH_2), 7.51 (2H, t, J=7.38 Hz, 3′,5′-Ar-H), 7.56 (1H, dt, J=2.67, 1.85 Hz, 4′-Ar-H), 7.90 (2H, d, J=7.07 Hz, 2′,6′-Ar-H), 8.94 (1H, s, N=CH). EI-MS (*m/z*; %): 338 (M^+; 31), 339 (M^++1; 6). *Anal.* Calcd for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 63.06; H, 4.66; N, 17.16.

3-((4-Chlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7b**)

Yield, 0.26 g (71%). mp 153–155°C. IR (KBr) cm^{-1}: 3069 (aromatic C–H, *str*), 2919 (aliphatic C–H, *str*), 1672 (C=O, *str*), 1546 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (3H, s, 7-NCH_3), 2.61 (3H, s, 2-CH_3 of pyrimidinone), 2.84 (2H, t, J=5.88 Hz, 5-CH_2), 3.18 (2H, t, J=5.91 Hz, 6-CH_2), 3.68 (2H, s, 8-CH_2), 7.39 (2H, d, J=7.89 Hz, 3′,5′-Ar-H), 7.50 (2H, d, J=7.98 Hz, 2′,6′-Ar-H), 10.42 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.52, 26.10, 45.45, 51.87, 53.74, 122.91, 129.29, 129.72, 130.15, 131.62, 132.12, 138.41, 145.68, 156.46, 160.81, 161.98. ESI-MS (*m/z*; %): 374 (M^++2; 33), 372 (M^+; 100). *Anal.* Calcd for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61. Found: C, 56.52; H, 4.16; N, 15.16.

3-((4-Fluorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7c**)

Yield, 0.23 g (65%). mp 230–232°C. IR (KBr) cm^{-1}: 3069 (aromatic C–H, *str*), 2919 (aliphatic C–H, *str*), 1672 (C=O, *str*), 1601 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (3H, s, 7-CH_3), 2.59 (3H, s, 2-CH_3 of pyrimidinone), 2.77 (2H, t, J=6 Hz, 5-CH_2), 3.13 (2H, t, J=6 Hz, 6-CH_2), 3.63 (2H, s, 8-CH_2), 7.18 (2H, t, J=8.8 Hz, 3′,5′-ArH), 7.90 (2H, dd, J=5.6, 5.6 Hz, 2′,6′-Ar-H), 8.91 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.52, 25.97, 45.50, 51.94, 53.73, 116.14–116.36 (*d*), 120.85, 128.90–128.93 (*d*), 129.87, 130.24, 131.00–131.09 (*d*), 153.99, 155.51, 161.19, 164.19, 165.76, 166.72. EI-MS (*m/z*; %): 356 (M^+; 38), 234 (M^+-$C_7H_4Cl_2N$; 45), 192 (M^+-$C_9H_4Cl_2NO$; 100). *Anal.* Calcd for $C_{17}H_{15}FN_4OS$: C, 59.63; H, 4.42; N, 16.36. Found: C, 59.22; H, 4.86; N, 16.06.

4-(((2,7-Dimethyl-4-oxo-5,6,7,8-tetrahydropyrido[4′,3′:4,5]-thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)imino)methyl)benzonitrile (**7d**)

Yield, 0.25 g (68%). mp 200–202°C. IR (KBr) cm^{-1}: 3050 (aromatic C–H, *str*), 2981 (aliphatic C–H, *str*), 2234 (CN, *str*), 1676 (C=O, *str*), 1550 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.52 (3H, s, 7-NCH_3), 2.63 (3H, s, 2-CH_3 of pyrimidinone), 2.79 (2H, t, J=5.22 Hz, 5-CH_2), 3.14 (2H, t, J=5.54 Hz, 6-CH_2), 3.64 (2H, s, 8-CH_2), 7.79 (2H, d, J=8.82 Hz, 3′,5′-Ar-H), 8.01 (2H, d, J=8.89 Hz, 2′,6′-Ar-H), 9.22 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.64, 25.98, 45.48, 51.88, 53.69, 115.56, 118.02, 120.87, 129.02, 129.96, 130.68, 132.63, 136.97, 153.66, 155.65, 161.10, 163.32. ESI-MS (*m/z*; %): 364 (M^++1; 100). *Anal.* Calcd for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27. Found: C, 62.42; H, 4.86; N, 19.66.

3-((4-Methoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7e**)

Yield, 0.31 g (82%). mp 173–175°C. IR (KBr) cm^{-1}: 3095 (aromatic C–H, *str*), 2921 (aliphatic C–H, *str*), 1684 (C=O, *str*) 1567 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.53 (3H, s, 7-NCH_3), 2.60 (3H, s, 2-CH_3 of pyrimidinone), 2.80 (2H, t, J=5.82 Hz, 5-CH_2), 3.16 (2H, t, J=5.79 Hz, 6-CH_2), 3.66 (2H, s, 8-CH_2), 3.91 (3H, s, Ar-OCH_3), 7.01 (2H, d, J=8.81 Hz, 3′,5′-Ar-H), 7.86 (2H, d, J=8.80 Hz, 2′,6′-Ar-H), 8.75 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.66, 26.10, 45.46, 51.91, 53.76, 55.44, 114.43, 122.92, 125.47, 130.07, 130.57, 131.76, 145.28, 156.19, 160.93, 163.12, 164.29. EI-MS (*m/z*; %): 368 (M^+ ; 18), 192 (M^+-$C_{10}H_9O_2N$; 15), 57 (M^+-$C_{16}H_{13}N_3O_2S$; 100). *Anal.* Calcd for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21. Found: C, 61.58; H, 5.12; N, 15.06.

3-((4-Hydroxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7f**)

Yield, 0.27 g (76%). mp 218–220°C. IR (KBr) cm^{-1}: 3250 (aromatic C–H, *str*), 2949 (aliphatic C–H, *str*), 1647 (C=O, *str*), 1546 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.41 (3H, s, 7-NCH_3), 2.48 (3H, s, 2-CH_3 of pyrimidinone), 2.71 (2H, t, J=5.86 Hz, 5-CH_2), 2.97 (2H, t, J=5.80 Hz, 6-CH_2), 3.59 (2H, s, 8-CH_2), 6.98 (2H, d, J=5.14 Hz, 3′,5′-Ar-H), 7.84 (2H, d, J=8.32 Hz, 2′,6′-Ar-H), 8.67 (1H, s, N=CH), 10.47 (1H, s, Ar-OH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.51, 26.13, 45.48, 51.90, 53.77, 122.91, 129.32, 129.76, 130.18, 131.64, 132.15, 138.45, 145.70, 156.49, 160.85, 162.03. ESI-MS (*m/z*; %): 355 (M^++1; 100). *Anal.* Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81. Found: C, 61.38; H, 4.96; N, 16.12.

2,7-Dimethyl-3-((3,4,5-trimethoxybenzylidene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7g**)

Yield, 0.3 g (70%). mp 198–200°C. IR (KBr) cm^{-1}: 3059 (aromatic C–H, *str*), 2937 (aliphatic C–H, *str*), 1672 (C=O, *str*), 1576 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.53 (3H, s, 7-NCH_3), 2.62 (3H, s, 2-CH_3 of pyrimidinone), 2.80 (2H, t, J=5.81 Hz, 5-CH_2), 3.16 (2H, t, J=5.76 Hz, 6-CH_2), 3.66 (2H, s, 8-CH_2), 3.94 (6H, s, 3′,5′-Ar-OCH_3), 3.96 (3H, s, 4′-Ar-OCH_3), 7.16 (2H, s, 2′,6′-Ar-H), 8.77 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.48, 25.97, 45.47, 51.93, 53.71, 56.29, 60.99, 106.28, 120.81, 127.60, 129.85, 130.15, 142.29, 153.36, 153.60, 155.49, 161.21, 167.28. ESI-MS (*m/z*; %): 429 (M^++1; 100). *Anal.* Calcd for $C_{21}H_{24}N_4O_4S$: C, 58.86; H, 5.65; N, 13.07. Found: C, 58.58; H, 5.26; N, 13.12.

2,7-Dimethyl-3-((2-nitrobenzylidene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7h**)

Yield, 0.3 g (78%). mp 154–156°C. IR (KBr) cm^{-1}: 3019 (aromatic C–H, *str*), 2932 (aliphatic C–H, *str*), 1686 (C=O, *str*), 1524 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (3H, s, 7-NCH_3), 2.62 (3H, s, 2-CH_3 of pyrimidinone), 2.79 (2H, t, J=6 Hz, 5-CH_2), 3.24 (2H, t, J=6 Hz, 6-CH_2), 3.67 (2H, s, 8-CH_2), 7.66 (1H, dt, J=8.12 Hz, 4′-Ar-H), 7.80 (1H, dt, J=8.12 Hz, 5′-Ar-H), 8.20 (2H, dd, J=8.34, 1.46 Hz, 3′,6′-Ar-H), 9.44 (1H, s, N=CH). ESI-MS (*m/z*; %): 384 (M^++1; 26), 340 ($M^+-C_2H_3O$; 100). *Anal.* Calcd for $C_{18}H_{17}N_5O_3S$: C, 56.38; H, 4.47; N, 18.27. Found: C, 56.48; H, 4.91; N, 17.98.

2,7-Dimethyl-3-((thiophen-2-ylmethylene)amino)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7i**)

Yield, 0.24 g (70%). mp 178–180°C. IR (KBr) cm^{-1}: 3051 (aromatic C–H, *str*), 2938, 2785 (aliphatic C–H, *str*), 1683 (C=O, *str*), 1581 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.53 (3H, s, 7-NCH_3), 2.62 (3H, s, 2-CH_3 of pyrimidinone), 2.80 (2H, t, J=5.82 Hz, 5-CH_2), 3.16 (2H, t, J=5.80 Hz, 6-CH_2), 3.65 (2H, s, 8-CH_2), 7.18 (1H, dd, J=5.00, 3.70 Hz, 4′-Ar-H), 7.58 (1H, dd, J=3.67, 1.01 Hz, 5′-Ar-H), 7.61 (1H, td, J=4.97, 0.90 Hz, 3′-Ar-H), 9.13 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.51, 25.76, 49.83, 51.59, 61.96, 112.49, 118.66, 127.33, 128.40, 129.10, 130.25, 137.92, 146.85, 153.55, 155.02, 155.53, 161.16. ESI-MS (*m/z*; %): 315 (M^+−CO; 92), 344.9 (M^++1; 45). *Anal.* Calcd for $C_{16}H_{16}N_4OS_2$: C, 55.79; H, 4.68; N, 16.27. Found: C, 55.88; H, 4.61; N, 16.32.

3-((Furan-2-ylmethylene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7j**)

Yield, 0.26 g (79%). mp 172–174°C. IR (KBr) cm^{-1}: 3123 (aromatic C–H, *str*), 2936, 2803 (aliphatic C–H, *str*), 1671 (C=O, *str*), 1557 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (3H, s, 7-NCH_3), 2.60 (3H, s, 2-CH_3 of pyrimidinone), 2.77 (2H, t, J=5.98 Hz, 5-CH_2), 3.13 (2H, t, J=5.61 Hz, 6-CH_2), 3.62 (2H, s, 8-CH_2), 6.60 (1H, t, J=1.22 Hz, 4′-Ar-H of furan), 7.26 (1H, d, J=3.22 Hz, 3′-Ar-H of furan), 7.68 (1H, d, J=3.22 Hz, 5′-Ar-H of furan), 8.77 (1H, s, N=CH). ESI-MS (*m/z*; %): 329 (M^++1; 100). *Anal.* Calcd for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06. Found: C, 58.48; H, 4.61; N, 16.72.

3-((2,4-Dichlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**7k**)

Yield, 0.32 g (80%). mp 181–183°C. IR (KBr) cm^{-1}: 3083 (aromatic C–H, *str*), 2938 (aliphatic C–H, *str*), 1681 (C=O, *str*), 1582 (C=N, *str*). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.54 (3H, s, 7-NCH_3), 2.63 (3H, s, 2-CH_3 of pyrimidinone), 2.80 (2H, t, J=5.79 Hz, 5-CH_2), 3.17 (2H, t, J=5.82 Hz, 6-CH_2), 3.66 (2H, s, 8-CH_2), 7.39 (1H, dd, J=8.51, 1.50 Hz, 5′-Ar-H), 7.51 (1H, d, J=1.99 Hz, 3′-Ar-H), 8.17 (1H, d, J=8.48 Hz, 6′-Ar-H), 9.42 (1H, s, N=CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 22.51, 26.14, 45.44, 51.84, 53.73, 122.93, 127.74, 128.45, 129.60, 129.98, 130.30, 132.17, 136.80, 138.56, 145.80, 156.40, 158.37, 166.67. EI-MS (*m/z*; %): 408 (M^++2, 14), 406 (M^+; 20), 234 ($M^+-C_7H_4Cl_2N$; 45), 192 ($M^+-C_9H_4Cl_2NO$; 100). *Anal.* Calcd for $C_{18}H_{16}Cl_2N_4OS$: C, 53.08; H, 3.96; N, 13.76. Found: C, 52.86; H, 3.67; N, 14.52.

Antibacterial Activity The synthesized final compounds were screened for their *in vitro* antibacterial activity against mentioned strains of Gram-positive and Gram-negative bacteria. The MIC values were determined by employing similar techniques of our studies,[5] and the results were compared with standard (streptomycin). The MIC values were expressed in micro moles (μM) and the results were listed in Table 1.

Antimycobacterial Activity The MTB H37Rv and an INH-resistant clinical sample of *Mycobacteria* were used for screening and the MIC values were determined using a broth micro dilution method as described in our previous protocols.[5] The stock solution of all compounds was prepared in the concentration of 1 mg/mL dissolved in DMF in water (25% v/v). About ten test concentrations ranging from 1000–1 μg/mL were used to assess the efficacy of each compound.

Molecular Docking The molecular docking studies were performed using Molegro Virtual Docker (MVD) tools24 on targeted protein MTB-PS (PDB: 3IVX).

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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