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A green expedient synthesis of pyridopyrimidine-2-thiones and their antitubercular activity

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ABSTRACT

The pseudo four-component domino reactions of *N*-substituted-4-piperidones, substituted aromatic aldehydes and thiourea in the presence of solid sodium ethoxide under solvent-free conditions afforded pyridopyrimidine-2-thiones in almost quantitative yields by simply grinding for 1–2 min. at ambient temperature. The synthesized compounds were screened for their in vitro activity against *Mycobacterium tuberculosis* H37Rv. Among them, (*E*)-6-benzyl-8-(2,4-dichlorobenzylidene)-4-(2,4-dichlorophenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-*d*]pyrimidine-2(1*H*)-thione (MIC 2.8 μM) displays the maximum activity, being 2.7 and 1.7 times more active than the first line antitubercular drugs ethambutol and ciprofloxacin, respectively, and less active than rifampicin and isoniazid, by 28 and 7 times, respectively.

Pyrimidinone sub-structure is prevalent in many natural/synthetic biologically active compounds, which find applications in pharmaceutical and biochemical arena.[1] Dihydropyrimidinethiones (DHPMs) or 2-thiopyrimidines (2-TP)[2] find applications as antihypertensive (SQ 32926) (**I**),[3] α_{1a}-adrenergic receptor antagonists,[4] antibacterial, antiinflammatory and antitumour agents.[5] In 2-TP, the sulfur atom serves as an interesting replacement for the oxygen atom bonded to C-2 in uridine base.[6] DHPMs can be converted into either 1,3-diamines or guanidines, which constitute the core structural elements commonly present in natural products (Fig. 1), exemplified by the polycyclic marine alkaloids such as crambines (**II**), ptilomycalin (**III**) and batzelladine.[7] Ptilomycalin (**III**) displays significant activity against a series of cancer cell lines and DNA polymerase activity of the reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT).[8] Batzelladines A and B (**IV** and **V**) are the first low molecular weight natural products reported in the literature to inhibit the binding of HIV-gp120 to the CD4 cell surface receptor protein on T-cells.[9] Monastrol (**VI**), with the pyrimidine-2-thione motif, specifically inhibits the motility of the mitotic kinesin Eg5.[10]

Pyridopyrimidine analogues constitute a novel class of adenosine kinase inhibitors,[11] besides being the most highly potent and selective antagonists of cholecystokinin receptor subtype-1 (CCK1R),[12] tyrosine kinase inhibitors,[13] diuretics,[14] antiviral[15a] and antitumour agents.[15b,c] Structure–activity studies disclose that anticancer activity of pyrimidinones and pyrimidinethiones is ascribable to the presence of (i) nitrogen heterocyclic ring and (ii) thione functionality, which generally enhance the activity. This has kindled substantial interest of researches in synthesis of new pyrimidinones and pyrimidinethiones.

Tuberculosis (TB) is the leading cause of infectious disease mortality in the world. According to World Health Organization report (WHO),[16] in 2008, there were an estimated 8.9–9.9 million incident cases of TB, 9.6–13.3 million prevalent cases of TB, 1.1–1.7 million deaths from TB among HIV-negative people and an additional 0.45–0.62 million TB deaths among HIV-positive people. Further, HIV-infected patients have an elevated risk of primary or reactivated tuberculosis which may enhance HIV replication and the risk of death. The WHO has estimated that, if the present trend continues, TB could claim more than 30 million lives between 2000 and 2020.[17] In the last 50 years, only a few drugs have been approved by the Food and Drug Administration to treat TB. This discloses the difficulties associated with the discovery and clinical testing of new candidates and the absence of pharmaceutical industry research in this area. Hence, the discovery of fast-acting new drugs to effectively cure TB is imperative. As continuation of our research programme embarked on to discover potent antitubercular candidates by 1,3-dipolar cycloaddition[18] and multi-component or domino reactions,[19] we now report an atom economic, four-component, green synthesis of pyrido[4,3-*d*]pyrimidine-2-thiones and their antimycobacterial activities.

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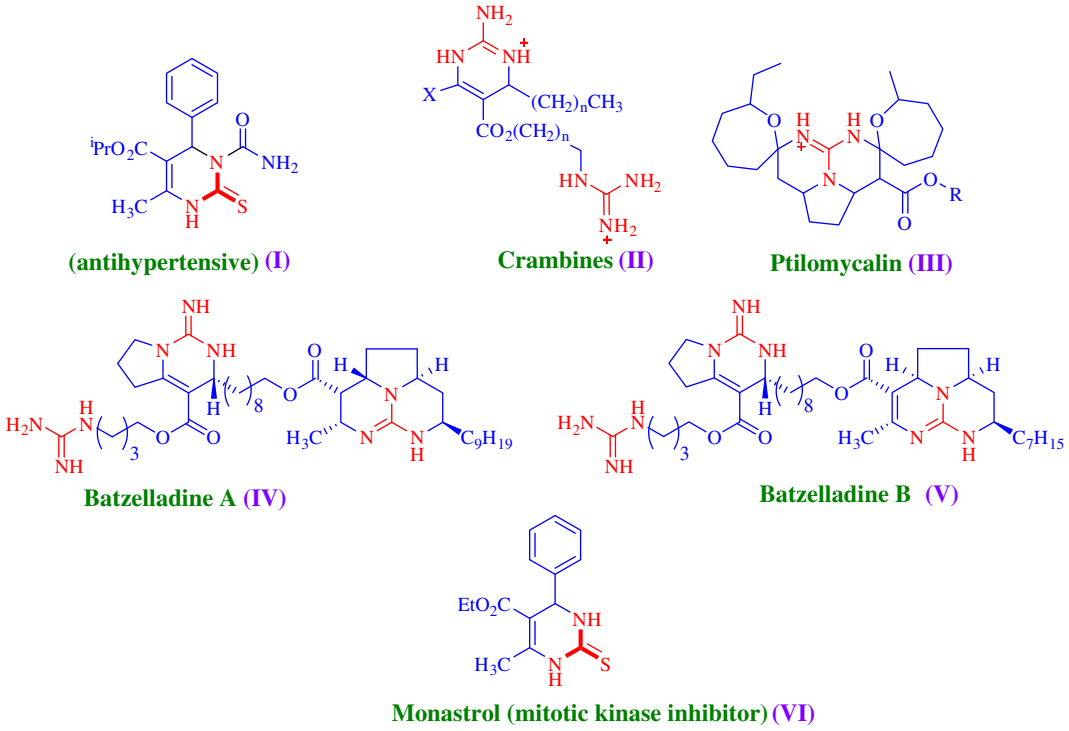
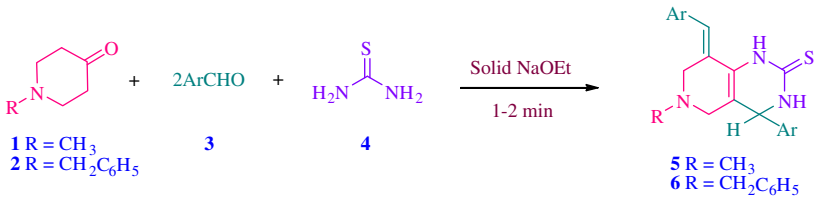


Figure 1. Biologically important compounds containing DHPM and related structural units.



Scheme 1. Synthesis of pyridopyrimidine-2-thiones **5** and **6**.

Multi-component reactions (MCRs) constitute a highly valuable synthetic tool for the construction of polyfunctionalized heterocyclic compounds required for drug discovery programmes,[20] as ~60% of drug candidates either in pipeline or currently in market are heterocycles. They provide convergent, atom economic and eco-friendly synthesis of molecules of high levels of complexity and diversity enabling the assembly of three or more simple and flexible building blocks in practical one-pot operations,[21] besides being amenable for combinatorial library generation.

In the present investigation, pseudo four-component domino reactions of N-substituted-4-piperidones (**1** and **2**), aromatic aldehydes (**3**) and thiourea (**4**) under solvent-free conditions at ambient temperature afforded the pyrimidine-2-thiones **5** and **6** in almost quantitative yields (93–98%; Scheme 1). In a typical experiment, a mixture of **1** or **2** (1 mmol), aromatic aldehydes (2 mmol), thiourea (1 mmol) and solid sodium ethoxide (3 mmol) was ground well uniformly in a semimicro boiling tube at ambient temperature for about 1–2 min during which period the reaction went to completion (TLC). Then water was added to the mixture, the product was filtered and dried in vacuo.[22]

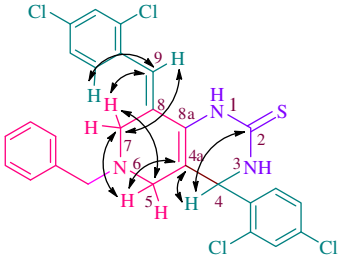


Figure 2. Selected HMBCs of **6k**.

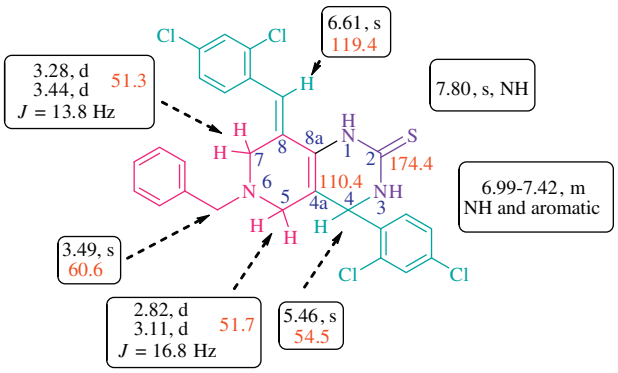
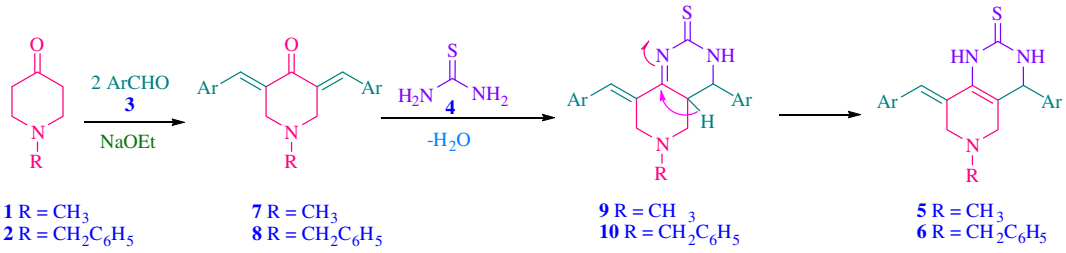


Figure 3. ^{1}H and ^{13}C chemical shifts of **6k**.



Scheme 2. Mechanistic proposal for the formation of pyridopyrimidine-2-thiones **5** and **6**.

It is pertinent to note that in a previous study El-Subbagh et al.[15a] reported the formation of pyrido[4,3-*d*]pyrimidine-2-thiones (**5d**, **5g** and **5s**), respectively, in 80%, 91% and 83% yields from the reaction of equimolar amounts of 1-methyl-3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones **7** with thiourea **4** with two molar equivalents of sodium in *n*-butanol under reflux for 10 h. Rostom et al.[15b] reported **5c** in 27% yield from the reaction of equimolar amounts of **7**, thiourea and sodium hydroxide in ethanol. Consequently, the present solvent-free expedient protocol, requiring only water to work up the product, completed in 1–2 min affording an excellent yield of the product (93–98%), is clearly more advantageous than the literature methods in terms of yield, reaction time and eco-friendliness. It is pertinent to note that similar pseudo four-component synthesis has been reported only for pyrimidinones fused to carboyclic ring bearing arylidene function in lower yields (50–92%) and longer reaction times (3–10 h) from the reaction of cyclopentanone with aromatic aldehydes and urea/thiourea in the presence of $YbCl_3$.[15g] In another related study, the above reactants in presence of TMSCl[15h] in DMF–CH_3CN mixture displayed aryl substituent-mediated product selectivity with inconsistent yields of the bicyclic pyrimidinone for the range of substituents studied.

Structural elucidation of the pyridopyrimidine-2-thiones was accomplished from 1D and 2D NMR spectroscopic data as described for **6k** as a representative case. The ^{1}H NMR spectrum of **6k** has a singlet at 5.46 ppm readily assignable to H-4. The H-4 shows H,H-COSY correlations with the doublets at 2.82 and 3.11 ppm (J = 16.8 Hz) enabling their assignment to H-5a and H-5b. The other doublets at 3.28 and 3.44 ppm (J = 13.8 Hz) can be readily assigned to H-7a and H-7b. The 1H singlet at 6.61 ppm is due to H-9 and the 2H singlet at 3.49 ppm is due to CH_2 of N-CH_2Ph. One of the NH hydrogens of the pyrimidine ring appears as a singlet at 7.80 ppm, while the other merges with the signal of the aromatic hydrogens in the region 6.99–7.42 ppm. The assignment of signals of carbon bearing hydrogens has been done from the chemical shifts of hydrogens and C,H-COSY correlations. The above assignments are also supported by the HMBCs (Fig. 2). The ^{1}H and ^{13}C chemical shifts of **6k** are depicted in Figure 3.

The pyridopyrimidine-2-thiones **5** and **6** are presumably formed by multi-step domino reactions (Scheme 2), triggered by the initial condensation of N-substituted-4-piperidinones and 2 mol of aromatic aldehydes sequentially affording N-substituted-3,5-bis[(*E*)-1-arylmethylidene]-4-piperidinones **7** and **8**. These intermediates **7** and **8** react with thiourea furnishing **5** and **6** via Michael addition–condensation–tautormerization domino sequence.

All the thirty six compounds were screened for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (MTB) by agar dilution method for the determination of MIC in duplicate. The MIC (μM) values of the synthesized compounds along with the standard drugs for comparison are presented in Table 1. Twenty compounds, **5j–5o, 5q**, **5r**, **6a–6c**, **6f**, **6i–6p**, have MIC ranging from 2.8 to 49.1 μM which effectively inhibit MTB. Among them, four compounds, viz **6k**, **6n**, **5q**, **6m** are found to be 2.7, 2.2, 1.4 and 1.1 times, respectively, more active than ethambutol (MIC = 7.6 μM). Two compounds **6k** and **6n**, respectively, are 1.7 and 1.4 times as active as the first line antitubercular drug, ciprofloxacin (MIC = 4.7 μM). When compared to the MIC values of standard drugs, rifampicin (MIC: 0.1 μM) and isoniazid (MIC: 0.4 μM), all the compounds are less active against MTB.

From the viewpoint of structure–activity, the following trends emerge (Table 1 and Chart 1):

(i) In general, the *N*-benzylpyridopyrimidine-2-thiones **6** show better activity than their *N*-methyl analogs **5** suggesting that lipophilicity could be an important factor for activity.

(ii) In both the series **5** and **6,** compounds with aryl ring bearing halogens exhibit enhanced activity, the order of activity in series **5** being: 4-F < 2-Cl = 4-Cl < 3-Br = 4-Br < 3-F < 2,4-Cl_2 < 2,6-Cl_2 and in series **6** the order of activity is found to be:4-$(CH_3)_2N$ < 1-naphthyl < 2,5-$(CH_3O)_2$ = 3,4-$(CH_3O)_2$ < 3,4,5-$(CH_3O)_3$ < 2-Cl = 4-Cl < 4-$(CH_3)_2CH$ < 2-Br < 3-F < 4-F < 2,4-Cl_2.

Table 1
Yield and MIC[a] (μM) values of pyridopyrimidine-2-thiones **5** and **6** against MTB

Entry	Compd		Ar	Yield (%)		MIC (μM)	
	5	**6**		**5**	**6**	**5**	**6**
1	**a**	**a**	4-$(CH_3)_2NC_6H_4$	98	97	57.7	49.1
2	**b**	**b**	2,5-$(CH_3O)_2C_6H_3$	93	94	53.5	46.0
3	**c**	**c**	3,4-$(CH_3O)_2C_6H_3$	94 (27)[b]	96	53.5	46.0
4	**d**	**d**	4-$CH_3OC_6H_4$	94 (80)[c]	97	61.4	51.7
5	**e**	**e**	2-$CH_3C_6H_4$	98	96	66.6	55.4
6	**f**	**f**	4-$(CH_3)_2CHC_6H_4$	96	95	57.9	24.6
7	**g**	**g**	4-$CH_3C_6H_4$	96 (91)[c]	97	66.6	55.4
8	**h**	**h**	C_6H_5	95 (96)[d]	96 (49)[d]	71.9	59.0
9	**i**	**i**	1-Naphthyl	95	97	55.9	47.7
10	**j**	**j**	2-ClC_6H_4	97	98	30.0	25.4
11	**k**	**k**	2,4-$Cl_2C_6H_3$	96	98	12.9	**2.8**
12	**l**	**l**	4-ClC_6H_4	96 (87)[d]	97	30.0	25.4
13	**m**	**m**	3-FC_6H_4	97	95	16.3	**6.8**
14	**n**	**n**	4-FC_6H_4	95	96	32.6	**3.4**
15	**o**	--	3-BrC_6H_4	96	--[e]	24.7	--
16	--	**o**	2-BrC_6H_4	--[e]	97	--	21.5
17	**p**	--	2-$CH_3OC_6H_4$	97	--[e]	61.4	--
18	--	**p**	3,4,5-$(CH_3O)_3C_6H_2$	--[e]	98	--	41.4
19	**q**	--	2,6-$Cl_2C_6H_3$	98	--[e]	**5.6**	--
20	**r**	--	4-BrC_6H_4	95	--[e]	24.7	--
21	**s**	--	2-Thienyl	95 (83)[c]	--[e]	69.5	--
22	**t**	--	2-Naphthyl	96	--[e]	55.9	--
			Rifampicin			0.1	
			Isoniazid			0.4	
			Ciprofloxacin			4.7	
			Ethambutol			7.6	

[a] The MIC is defined as the minimum concentration of the compound required to inhibit 99% of bacterial growth.
[b] Ref. 15b.
[c] Ref. 15a.
[d] Ref. 15f.
[e] These reactions did not proceed.

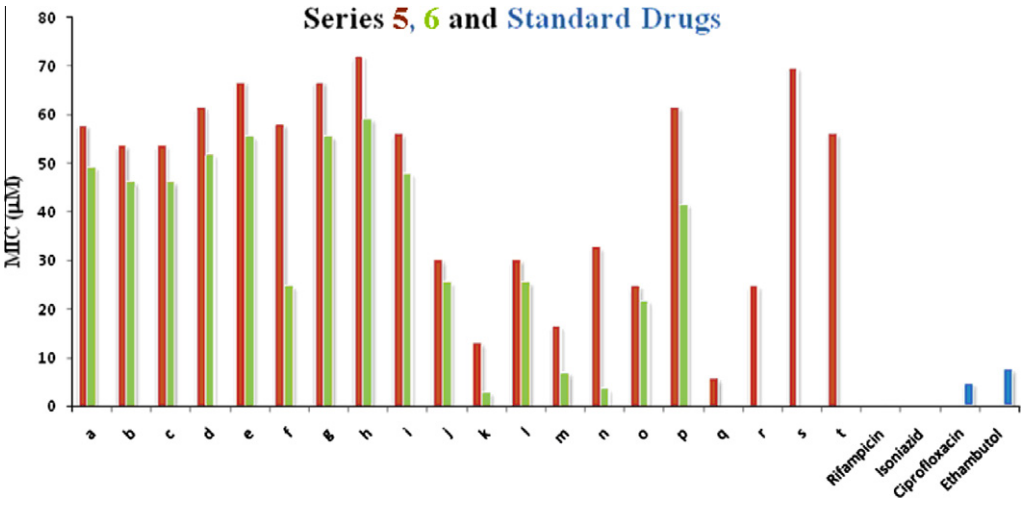


Chart 1. Comparison of MIC (μM) values of series **5**, **6** and standard drugs.

In conclusion, the present investigation describes a one pot, pseudo four-component, green synthesis of several pyridopyrimidinethiones from the reaction of *N*-methyl/benzyl-4-piperidinones (**1**/**2**), substituted aromatic aldehydes and thiourea in presence of solid sodium ethoxide at ambient temperature under solvent-free conditions. The method described is far more advantageous than the literature procedures with respect to convergence, eco-friendliness, yield and reaction time. The only solvent required for this synthesis is water to wash the product. The compounds, **6k** and **6n**, display significant in vitro antimycobacterial activity against *M. tuberculosis* H37Rv.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.045.

References and notes

1. (a) Atwal, K. S.; Rovnyak, G. C.; O'Reilly, B. C.; Schwartz, J. *J. Org. Chem.* **1989**, *54*, 5898; (b) Kappe, C. O.; Fabian, W. M. F.; Semones, M. A. *Tetrahedron* **1997**, *53*, 2803.
2. (a) Mathes, R. A. *J. Am. Chem. Soc.* **1953**, *75*, 1747; (b) Chung, H.-A.; Kweon, D.-H.; Kang, Y.-J.; Park, J.-W.; Yoon, Y.-J. J. *Heterocycl. Chem.* **1999**, *36*, 905.
3. (a) Rovnyak, G. C. et al *J. Med. Chem.* **1992**, *35*, 3254; (b) Alajarin, R. et al *Bioorg. Med. Chem.* **1994**, *2*, 323; (c) Schleifer, K.-J. *J. Med. Chem.* **1999**, *42*, 2204.
4. (a) Nagarathnam, D.; Miao, S. W.; Lagu, B.; Chiu, G.; Fang, J.; Murali Dhar, T. G.; Zhang, J.; Tyagarajan, S.; Marzabadi, M. R.; Zhang, F.; Wong, W. C.; Sun, W.; Tian, D.; Wetzel, J. M.; Forray, C.; Chang, R. S. L.; Broten, T. P.; Ransom, R. W.; Schorn, T. W.; Chen, T. B.; O'Malley, S.; Kling, P.; Schneck, K.; Bendesky, R.; Harrel, C. M.; Vyas, K. P.; Gluchowski, C. *J. Med. Chem.* **1999**, *42*, 4764; (b) Murali Dhar, T. G.; Nagarathnam, D.; Marzabadi, M. R.; Lagu, B.; Wong, W. C.; Chiu, G.; Tyagarajan, S.; Miao, S. W.; Zhang, F.; Sun, W.; Tian, D.; Shen, Q.; Zhang, J.; Wetzel, J. M.; Forray, C.; Chang, R. S. L.; Broten, T. P.; Schorn, T. W.; Chen, T. B.; O'Malley, S.; Ransom, R.; Schneck, K.; Bendesky, R.; Harrel, C. M.; Vyas, K. P.; Zhang, K.; Gilbert, J.; Pettibone, D. J.; Patane, M. A.; Bock, M. G.; Freidinger, R. M.; Gluchowski, C. *J. Med. Chem.* **1999**, *42*, 4778; (c) Barrow, J. C.; Glass, K. L.; Selnick, H. G.; Freidinger, M. R.; Chang, R. S. L.; O'Malley, S. S.; Woyden, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1917.
5. Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937.
6. (a) Sierzputowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Kuo, K.; Gehrke, C.; Agris, P. F. *J. Am. Chem. Soc.* **1987**, *109*, 7171; (b) Sochacka, E.; Fratczak, I. *Tetrahedron Lett.* **2004**, *45*, 6729.
7. (a) Heys, L.; Moore, C. G.; Murphy, P. *J. Chem. Soc. Rev.* **2000**, *29*, 57; (b) Berlinck, R. G. S.; Burtoloso, A. C. B.; Kossuga, M. H. *Nat. Prod. Rep.* **2008**, *25*, 919.
8. Black, G. P.; Coles, S. J.; Hizi, A.; Howard-Jones, A. G.; Hursthouse, M. B.; McGown, A. T.; Loya, S.; Moore, C. G.; Murphy, P. J.; Smith, N. K.; Walshe, N. D. A. *Tetrahedron Lett.* **2001**, *42*, 3377.
9. (a) Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; Brosse, C. D.; Mai, S.; Truneh, A.; Carte, B. *J. Org. Chem.* **1995**, *60*, 1182; (b) Olszewski, A.; Weiss, G. A. *J. Am. Chem. Soc.* **2005**, *127*, 121; (c) Snider, B. B.; Chen, J.; Patil, A. D.; Freyer, A. J. *Tetrahedron Lett.* **1996**, *37*, 6977.
10. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971.
11. Lee, C. H.; Jiang, M.; Cowart, M.; Gfesser, G.; Perner, R.; Kim, K. H.; Gu, Y. G.; Williams, M. W.; Michael, F.; Jarvis, M. F.; Kowaluk, E. A.; Stewart, A. O.; Shripad, S.; Bhagwat, S. S. *J. Med. Chem.* **2001**, *44*, 2133.
12. Martinez, M. M.; Bartolomé-Nebreda, J. M.; Gómez-Monterrey, I.; González-Muñiz, R.; García-López, M. T.; Ballaz, S.; Barber, A.; Fortuño, A.; Río, J. D.; Herranz, R. *J. Med. Chem.* **1997**, *40*, 3402.
13. Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Showalter, H. D. H.; Roberts, B. J.; Vincent, P. W.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 3915.
14. Parish, H. A., Jr.; Gilliom, R. D.; Purcell, W. P.; Browne, R. K.; Spirk, R. F.; Harold, D.; White, H. D. *J. Med. Chem.* **1982**, *25*, 98.
15. (a) El-Subbagh, H.; Abu-Zaid, S. M.; Mahran, M. A.; Badria, F. A.; Al-Obaid, A. M. *J. Med. Chem.* **2000**, *43*, 2915; (b) Rostom, S. A. F.; Hassan, G. S.; El-Subbagh, H. I. *Arch. Der. Pharm. (Weinheim)* **2009**, *342*, 584; (c) Mahran, M. A. *Alex. J. Pharm. Sci.* **2001**, *15*, 149; (d) Buy, O. D.; Gella, I. M.; Orlov, V. D. *V. N. Karazina* **2003**, *596*, 52; (e) Neszmélyi, A.; Lóránd, T. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1559; (f) Lorand, T.; Deli, J.; Szabo, D.; Foldesi, A.; Zschunke, A. *Pharmazie* **1985**, *40*, 536; (g) Zhang, H.; Zhou, Z.; Yao, Z.; Xu, F.; Shen, Q. *Tetrahedron Lett.* **2009**, *50*, 1622; (h) Zhu, Y.; Huang, S.; Pan, Y. *Eur. J. Org. Chem.* **2005**, *6*, 2354.
16. http://www.who.int/tb/publications/global_report/2009/update/en/index.html.
17. Dye, C.; Scheele, S.; Dolin, P.; Pathania, V.; Raviglione, M. C. *J. Am. Med. Assoc.* **1999**, *282*, 677.
18. (a) Kumar, R. R.; Perumal, S.; Senthilkumar, P.; Yogeeswari, P.; Sriram, D. *J. Med. Chem.* **2008**, *51*, 5731; (b) Kumar, R. R.; Perumal, S.; Senthilkumar, P.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2009**, *44*, 3821; (c) Kumar, R. R.; Perumal, S.; Manju, S. C.; Bhatt, P.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3461; (d) Kumar, R. S.; Perumal, S.; Shetty, K. A.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 124; (e) Karthikeyan, S. V.; Perumal, S.; Shetty, K. A.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3006; (f) Karthikeyan, S. V.; Bala, B. D.; Raja, V. P. A.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 350; (g) Kumar, R. S.; Rajeh, S. M.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Tetrahedron: Asymmtry* **2010**, *21*, 1315.
19. (a) Indumathi, S.; Perumal, S.; Banerjee, D.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2009**, *44*, 4978; (b) Balamurugan, K.; Perumal, S.; Reddy, A. S. K.; Yogeeswari, P.; Sriram, D. *Tetrahedron Lett.* **2009**, *50*, 6191; (c) Kumar, R. S.; Rajeh, S. M.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 411; (d) Kumar, R. S.; Rajeh, S. M.; Perumal, S.; Banerjee, D.; Yogeeswari, P.; Sriram, D. *Chem. Pharm. Bull.* **2010**, *58*, 602.
20. (a) Shen, Z.-L.; Xu, X.-P.; Ji, S.-J. *J. Org. Chem.* **2010**, *75*, 1162; (b) Indumathi, S.; Perumal, S.; Manendez, J. C. *J. Org. Chem.* **2010**, *75*, 472; (c) Shaabani, A.; Seyyedhamzeh, M.; Maleki, A.; Behnam, A.; Rezazadeh, F. *Tetrahedron Lett.* **2009**, *50*, 2911; (d) Liu, Y.; Qian, J.; Lou, S.; Zhu, J.; Xu, Z. *J. Org. Chem.* **2010**, *75*, 1309.

21. Zhu, Y.; Huang, S.; Wan, J.; Yan, L.; Pan, Y.; Wu, A. *Org. Lett.* **2006**, *8*, 2599.

22. *General procedure for the synthesis of pyrido[4,3-d]pyrimidine-2-thiones*. A mixture of N-substituted-4-piperidinone (1 mmol), aromatic aldehyde (2 mmol), thiourea (1 mmol) and sodium ethoxide (3 mmol) were ground well in a semimicro boiling tube at ambient temperature for about 1–2 min. After completion of the reaction as evident from TLC, water (50 mL) was added to the mixture and the product was filtered and dried in vacuo.
(E)-6-Benzyl-8-(2,4-dichlorobenzylidene)-4-(2,4-dichlorophenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione **6k**. Isolated as pale yellow solid. (0.487 g, 98%). Mp = 142 °C; IR (KBr): 852, 1285, 1473, 1560, 3388 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 2.82 (d, 1H, *J* = 16.8 Hz, H-5a), 3.11 (d, 1H, *J* = 16.8 Hz, H-5b), 3.28 (d, 1H, *J* = 13.8 Hz, H-7a), 3.44 (d, 1H, *J* = 13.8 Hz, H-7b), 3.49 (s, 2H, CH_2Ph), 5.46 (s, 1H, H-4), 6.61 (s, 1H, H-9), 6.99–7.42 (m, 12H, NH and aromatic), 7.79 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 51.3, 51.7, 54.5, 60.5, 110.4, 119.3, 126.6, 126.7, 127.4, 127.8, 128.2, 128.4, 128.9, 129.4, 129.7, 130.3, 131.1, 132.0, 133.2, 134.1, 134.6, 135.3, 135.9, 136.7, 174.4. Anal. calcd for $C_{27}H_{21}Cl_4N_3S$: C, 57.77; H, 3.77; N, 7.49. Found: C, 57.87; H, 3.86; N, 7.38%.
(E)-8-(2-Chlorobenzylidene)-4-(2-chlorophenyl)-6-methyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione **5j**. Isolated as white solid. (0.357 g, 97%). Mp = 145 °C; IR (KBr): 759, 1035, 1195, 1479, 1571, 3376 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 2.23 (s, 3H, N-CH_3), 2.75 (d, 1H, *J* = 16.7 Hz, H-5a), 3.05 (d, 1H, *J* = 16.7 Hz, H-5b), 3.22 (d, 1H, *J* = 13.4 Hz, H-7a), 3.35 (d, 1H, *J* = 13.4 Hz, H-7b), 5.55 (s, 1H, H-4), 6.85 (s, 1H, H-9), 7.15–7.49 (m, 8H, aromatic), 8.04 (br s, 1H, NH), 8.39 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 44.4, 53.7, 54.4, 110.6, 119.8, 125.8, 126.1, 127.5, 127.7, 128.6, 129.2, 129.3, 129.4, 130.3, 131.9, 133.5, 133.7, 137.8, 172.7. Anal. calcd for $C_{21}H_{19}Cl_2N_3S$: C, 60.58; H, 4.60; N, 10.09. Found: C, 60.65; H, 4.51; N, 10.17%.