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Synthesis and biological evaluation of 2-thiopyrimidine derivatives

Sham M. Sondhi,[a,*] Rajendra N. Goyal,[a] Anand M. Lahoti,[a] Nirupma Singh,[a] Rakesh Shukla[b] and Ram Raghubir[b]

[a]*Department of Chemistry, Indian Institute of Technology Roorkee (IITR), Roorkee 247 667, India*
[b]*Department of Pharmacology, Central Drug Research Institute (CDRI), Lucknow 226 001, India*

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Abstract—Various 2-thiopyrimidine derivatives have been synthesized by an efficient, one-pot reaction of functionalized amines with either 4-isothiocyanato-4-methyl-2-pentanone or 3-isothiocyanatobutanal. All the synthesized compounds were fully characterized by elemental analysis (CHN), FT-IR, ^{1}H NMR, and mass spectral data. One of the compounds, 7,7,8a-trimethyl-hexahydro-thiazolo[3,2-*c*]pyrimidine-5-thione (**17**) showed good anti-inflammatory (37.4% at 100 mg/kg p.o.) and analgesic activity (75% at 100 mg/kg p.o.). 7-(1-Mercapto-3,3,4a-trimethyl-4,4a,5,9b-tetrahydro-3*H*-pyrido[4,3-*b*]indol-7-yl)-3,3,4a-trimethyl-3,4,4a,5-tetrahydrobenzo[4,5]-imidazo[1,2-*c*]pyrimidine-1-thiol (**3**) showed moderate activity against CDK-1 (IC_{50} = 5 μM). The other compounds showed moderate anti-inflammatory (5–20%), analgesic (25–75%) and protein kinase (CDK-5, GSK-3) inhibitory activities (IC_{50} > 10 μM).
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1. Introduction

Pyrimidine derivatives have been very well known in medicinal chemistry for their therapeutic applications.[1] One possible reason for their activity is presence of a pyrimidine base in thymine, cytosine and uracil, which are essential building blocks of nucleic acids,[2] DNA and RNA. One important class of pyrimidine is 2-thiopyrimidine (2-TP) and its derivatives, which are also well known as 2-mercaptopyrimidine compounds.[3] In 2-TP ring sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base.[4] Considering this assumption 2-TPs have attracted substantial interest of synthetic-biochemists.[5] The European patent[6] revealed the application of 2-TP derivatives in preparation of cardiotonic drugs. Studies by Pathak et al. evaluated primary activity of 2-TP derivatives against Mycobacterium tuberculosis[7] (Mtb). 2-TPs also serve as important precursors for asymmetric synthesis of allylic sulfides/sulfonates.[8] Thus synthesis,[9] biological[10] as well as analytical studies[11] of 2-TP derivatives have been topics of interest for chemists.

Recently, two PCT international applications[12] revealed that, 2-TP derivatives possess potent activity against inflammation and immune disorders. Thus, search for novel, potent and selective 2-TP derivatives is desirable in order to substitute drugs having major side effects such as peptic-ulcer formation and gastro-intestinal damage.[13] During the past few years our laboratory has been actively involved in the synthesis of a variety of 2-TP derivatives[14] by a simple but efficient, one-pot reaction of functionalized amines with the well-known precursor β-isothiocyanatoketone.[15] Various mono-, di-, tri- and tetra-cyclic, di/tetrahydro-2-TP derivatives have been synthesized and evaluated for anti-inflammatory and analgesic activity (both in vivo and in vitro).[16] Some of them have shown moderate to good activity for inflammation.[17] Recently we have extended our scope of research to in vitro screening of 2-TP derivatives for kinase (CDK-1, CDK-5, and GSK-3) inhibition.[18] In continuation of our search for potent bioactive molecules, it was considered worthwhile to synthesize a variety of di/tetrahydro 2-TP derivatives. The synthesis[19] and biological studies of various 2-TP derivatives have been discussed in this paper.

2. Chemistry

Various 2-thiopyrimidine derivatives (**3**, **4**, **6**, **7a**, **9a**–**c**, **11a**,**b**, **13a**,**b**, **15**, **17**, **19**, and **21)** have been synthesized

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* Corresponding author. Tel.: +91 1332 285811; fax: +91 1332 273560; e-mail: sondifcy@iitr.ernet.in

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in reasonable yields by the one-pot reaction of either 4-isothiocyanato-4-methyl-2-pentanone[20] (**2a**) or 3-isothiocyanatobutanal[20] (**2b**) with commercially available functionalized amines (**1a**,**b**, **5a**,**b**, **8**, **10**, **12**, **14**, **16**, **18**, and **20**) in absolute methanol. Temperature and pH conditions play an important role in these reactions.

3. Results and discussion

Two moles of 4-isothiocyanato-4-methyl-2-pentanone (**2a**), on condensation with 3,3′-diaminobenzidine (**1a**) in absolute methanol (pH ~ 4–5) under refluxing conditions gave tetrahydro 2-thiopyrimidobenzimidazole derivative **3** with 77% yield (Scheme 1). The structure of **3** was fully supported by elemental analysis, FT-IR, ^{1}H NMR and mass spectral studies. Infra-Red spectra of **3** showed characteristic absorption bands near 1201 and 3150 cm^{-1} for the stretching frequency of C=S and N–H group, respectively. ^{1}H NMR spectra of **3** in $CDCl_3$ + 2 drops of DMSO-d_6, clearly showed two closely packed doublets (δ 2.24 and 2.55 with J_{ab} = 13.5 Hz each) for the anisotropic protons (2× –$H_aCH_bC^*CH_3$). This pattern can be observed only when both (*para* and *meta*) amino groups of the same benzene ring of **1a** are involved in the cyclization with 1 mol of **2a** resulting in pyrimidobenzimidazole ring structure. The other signals observed in the NMR of **3** were as follows; geminal dimethyl groups as two closely packed singlet at δ 1.34 (2× 3H) and 1.38 (2× 3H), protons of methyl groups present on asymmetric carbon atoms (C*) as singlet at δ 1.52 (2× 3H), aromatic protons in the region δ 6.62–7.05 (2× 3H). On D_2O exchange broad peaks for labile protons of –NH groups disappeared from the original position of δ 8.15 (2× –HN–), 8.65 (–HN–C=S), and 9.03 (–HN–C=S), which further supports the structure assigned for **3**. Mass spectra (EI-MS) of **3** gave molecular ion peak at *m*/*z* 492 (15%) (calcd molar mass for $C_{26}H_{32}N_6S_2$; 492.213). Reaction of benzidine (**1b**) with **2a** in absolute methanol at rt as well as under refluxing condition (pH ~ 4–5) gave **4** with 60% yield. The ^{1}H NMR of **4** showed a sharp singlet at δ 4.85 for olefin protons. This singlet is characteristic in order to distinguish dihydro-2-TP (**3**) from tetrahydro-2-TP (**4**) derivatives. Compound **4** is supposed to be symmetrical since a singlet at δ 4.85, was integrated for two identical olefinic protons on either sides of biphenyl ring.

General mechanism[21] for condensation of the functionalized amine with β-keto/aldo-isothiocynate, is presented in Figure 1. At an elevated temperature the initial attack of *para* –NH_2 group of compound **1a** on the isothiocyanate group of **2a** can generate an intermediate species **(i)**, which has an unreacted ketone (or aldehyde) group. This reactive intermediate exists in a favorable cyclic six-member structure **(i)** due to the presence of geminal dimethyl groups. Furthermore intramolecular nucleophilic attack of the –NH group over the

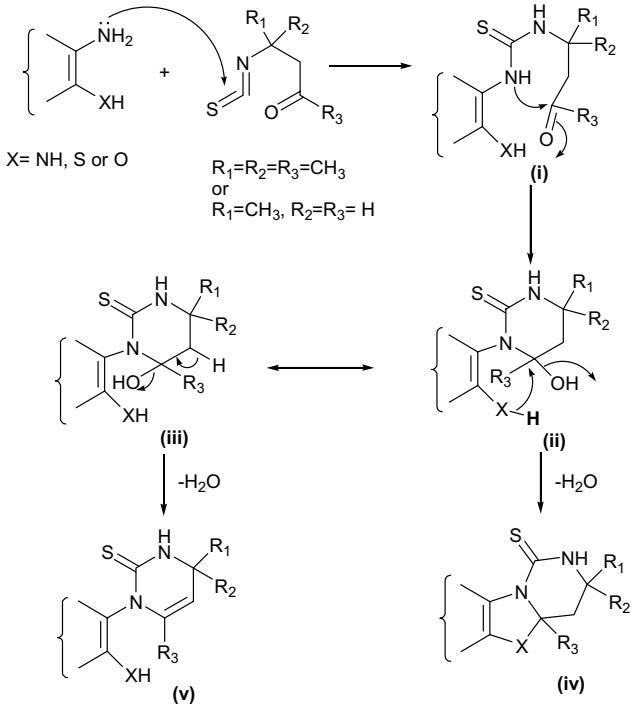
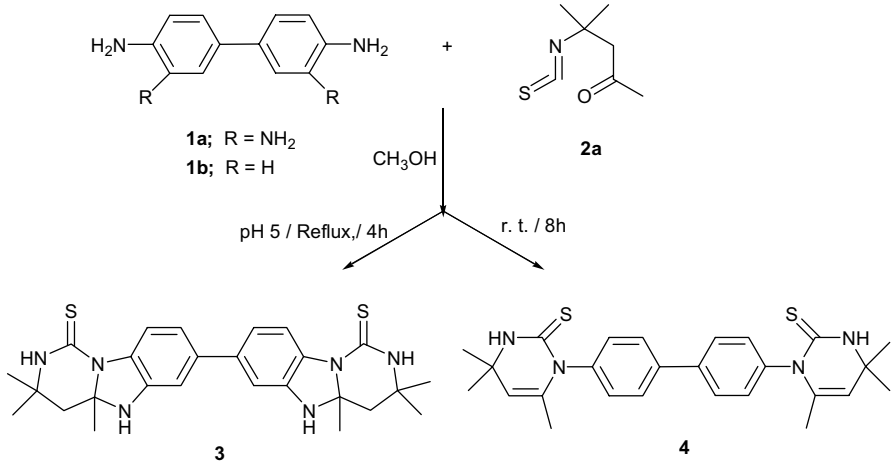


Figure 1.

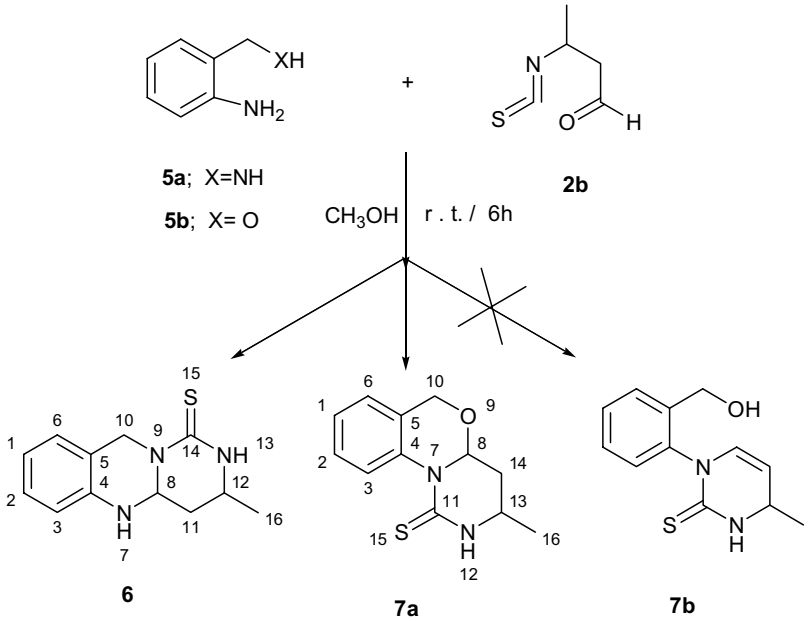


Scheme 1.

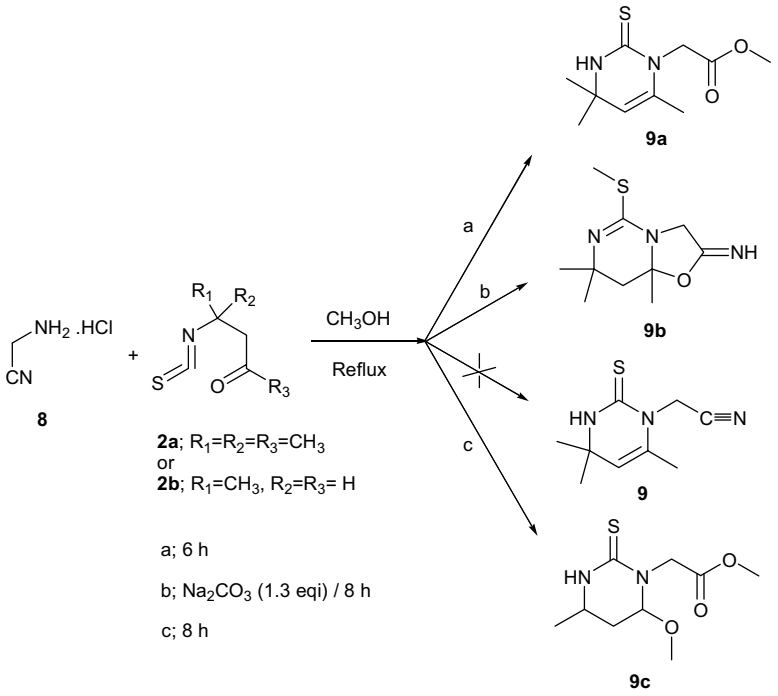
neighboring ketone (or aldehyde) group can yield a alcohol (**ii**). Finally *meta* $-NH_2$ group of **1a** attacks over the reactive species (**ii**), to yield a 2-thiopyrimidobezimidazole ring (**iv**) by elimination of the water molecule. It was expected that the loss of the water molecule in such a reaction was accelerated in the presence of the protic catalyst (like H_2SO_4). An exact similar mechanism was expected on the other side of biphenyl ring of **1a** to yield **3**. Synthesis of various pyrimidobezimidazole ring compounds by a similar mechanism has been well described in the literature.[22] At rt an intermediate similar to **iii** was formed by an intra-molecular nucleophilic attack of *para* $-NH_2$ group of **1b** over **2a**. This intermediate species (**iii**) rapidly looses a water molecule to give a 2-thiopyrimidine ring (**v**) on either sides of biphenyl ring. The mechanism in Figure 1 suggest only probable sequence of steps involved in the formation of tetrahydro-2-thiopyrimidine (**iv**) and dihydro-2-thiopyrimidine (**v**) analogues.

Scheme 2 shows formation of 1,4-dihydro-2-thiopyrimidine derivatives **6** and **7a** by rt stirring of 3-isothiocyanatobutanal (**2b**) with 2-aminobenzylamine (**5a**) and 2-aminobenzyl-alkanol (**5b**), respectively. The mechanism followed in the synthesis of **6** was very similar to that explained for **iv** in Figure 1. It is interesting to note that, the reactive aldehyde group of **2b** is supposed to participate only in the second step and this behavior of intramolecular addition of various diamines with **2b** has been reported in our earlier communication.[16] IR analysis of **6** showed characteristic bands at 3339.7, 3222.6 (N–H stretch), 1559.3, 1489.6 (C=C) and 1210.3 cm^{-1}(C=S) and its ^1H NMR spectra in $CDCl_3$ showed a characteristic septet δ 3.92, integrating for a single proton at $^*C_{12}$. The *J* values (4 and 6.6 Hz) for the proton (at $^*C_{12}$) indicated, its coupling with methyl hydrogen and hydrogen atoms at C_{11}. The different peaks observed in the NMR revealed that compound **6** looks like a stereoisomer, however we were unable to separate its different isomers. The EI-MS spectra of **6** showed a molecular ion peak at *m/z* 233, 40% (calcd mass for $C_{12}H_{15}N_3S$ 233.099) and the fragmented cations observed in the mass spectra were presented in the Experimental section. The structure assigned for **7a** was also fully in agreement with the observed spectral data. Formation of **7b** by the reaction of **5b** with **2b** was also expected[23] but when the reaction was carried out in absolute methanol under refluxing condition with/without adjusting pH ~ 4–5, in TLC only a single spot was observed (after 6 h) at R_f 0.6 ($CHCl_3$–CH_3OH, 9.5:0.5) and its characterization further confirmed it as **7a** (20%) rather than **7b**.

Reaction of the hydrochloride salt of aminoacetonitrile (**8**) with **2a** and **2b** under different experimental conditions is presented in Scheme 3. Reaction of **8** with **2a**, after a 6 h reflux gave a white solid.[24] Its purification and characterization allowed its identification as **9a** rather than **9**. The IR (KBr) spectra of **9a** showed no peaks in the region 2225–2260 cm^{-1} (C≡N stretching), however sharp peaks at 1735.9 and 1212.2 cm^{-1}(C=O and C=S stretching, respectively) were observed. This suggested that the cyanide group of **8** underwent hydrolysis in presence of an acid catalyst (HCl) and finally a methyl ester derivative **9a** was formed in presence of alcohol (methanol). The ^1H NMR of **9a** showed a characteristic signal singlet δ 3.78 (H_3CO–C=O) and 4.79 (H_2C–COOMe) further confirms the presence of methyl ester in **9a**. The FAB-MS spectra for **9a** showed a base MH^+ peak *m/z* 229 (calcd mass for $C_{10}H_{16}N_2SO_2$ 228.093) and also a peak at *m/z* 213 (40%) for a stable (4,4,6-trimethyl-2-thioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetyl cation. For the synthesis of **9**, it was considered necessary to quench the acid formed during the reaction of **8** with **2a**. Accidentally when we used 1.3 equiv (with respect to HCl) of sodium carbonate in the



Scheme 2.



Scheme 3.

reaction, **9b** was isolated. The IR analysis of **9b** neither showed C≡N nor C═O stretching vibrations, however a peak at 1191.5 cm^{-1} was clearly observed. The ^1H NMR of **9b** showed a characteristic double doublet for anisotropic protons with $J = 18.0$ and 17.8 Hz, but no characteristic singlet was observed for the olefin proton. Thus it was established that a tetrahydro-2-thiopyrimidine derivative **9b** was formed during the reaction. This could be possible only when tertiary –OH group (rather than elimination of water) attacks over the neighboring cyanide group to give a five member ring condensed with 2-thiopyrimidine ring. A characteristic singlet observed at δ 3.16 ppm was attributed to S–CH_3 protons and this S-methylation is due to the formation of methyl carbonium ion, in presence of excess base (Na_2CO_3). Formation of S-methylated derivative of 2-TP have been well reported during acidic/basic reaction conditions.[17] The structure assigned for **9b** was fully supported by its FAB-MS spectra. Reaction of **8** with **2b** in refluxing methanol gave **9c** in very low yield (10%). In the formation of **9c** it was expected that the secondary alcohol generated during the formation of tetrahydro-2-thiopyrimidine ring underwent O-methylation[25] (instead of elimination of a water molecule) due to an acid catalyst (HCl) and –C≡N group underwent hydrolysis and esterification (in presence of HCl) to yield **9c**. ^1H NMR spectra of **9c** in $CDCl_3$+2 drops of DMSO-d_6 showed a characteristic singlet δ 3.37 (O–CH_3) with a multiplet in the region δ 1.69 and 2.11 ppm, which supports presence of two asymmetric centers in the vicinity of –CH_2.

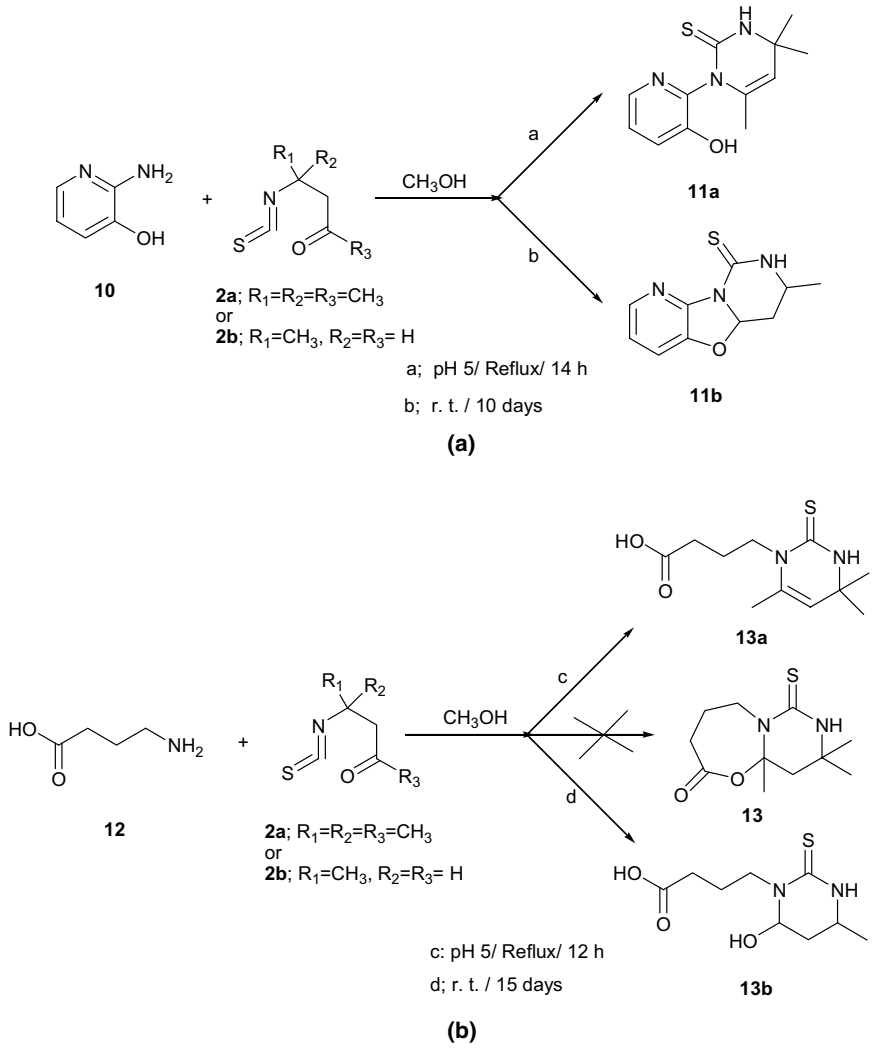
2-Amino-3-hydroxy-pyridine (**10**) on reaction with isothiocyanate **2a** and **2b** gave a 1-(3-hydroxypyridin-2-yl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1*H*)-thione (**11a**) and 2-methyl-1,2,3,9a-tetrahydro-9-oxa-3,4a,5-triaza-fluorene-4-thione (**11b**), respectively (Scheme 4a). Formation of **11a** from **10** was concluded in the presence of an acid catalyst (pH 4–5) in refluxing methanol. Reaction of **10** with **2b** at rt (10 days) gave **11b** and its characterization confirmed it as tetrahydro-2-thiopyrimidine derivative (**iv**) rather than dihydro-2-TP derivative (**v**).

Reaction of 4-aminobutyric acid (**12**) with **2a** was expected to give **13** (Scheme 4b) however **13a** was isolated under the investigational conditions. A compound **13b** was obtained by a reaction of **12** with **2b** at rt after an interval of 15 days. The typical nature of the dehydration of alcohol (**ii**) (Fig. 1) was not seen in **13b**, it may be due to the stability of the secondary alcohol at rt. Full spectral data for **13a** and **13b** was presented in the Experimental section.

Compound **17** (Scheme 5) was formed in 59% yield by a reaction of cysteaminiumchloride (**16**) with **2a** in presence of Na_2CO_3 in refluxing methanol. Compounds **15**, **19**, and **21** were isolated in reasonable yields (~60%) by stirring of 4-aminobenzonitrile (**14**), 2,2,6,6-tetraethyl-piperidin-4-ylamine (**18**) and dapsone (**20**), respectively, with **2a**, in absolute methanol at rt. Compound **21** was separated out of the reaction mixture immediately after 2 h. This typical tendency of dihydro-2-TP derivative to separate out the reaction mixture is well documented in the literature.[26]

3.1. Pharmacology

The compounds **3**, **4**, **6**, **7a**, **9a**–**c**, **11a**,**b**, **13a**,**b**, **15**, **17**, **19**, and **21** have been tested for (i) anti-inflammatory activity in the carrageenin-induced paw oedema model at 100 and 50 mg/kg p.o. (ii) Analgesic activity in the



Scheme 4.

phenylquinone writhing assay at 100 and 50 mg/kg p.o. and (iii) cyclin-dependent kinase (CDK-1 and 5) and glycogen synthase kinase (GSK-3) inhibitory activity. Results of these biological activities are summarized in Table 1.

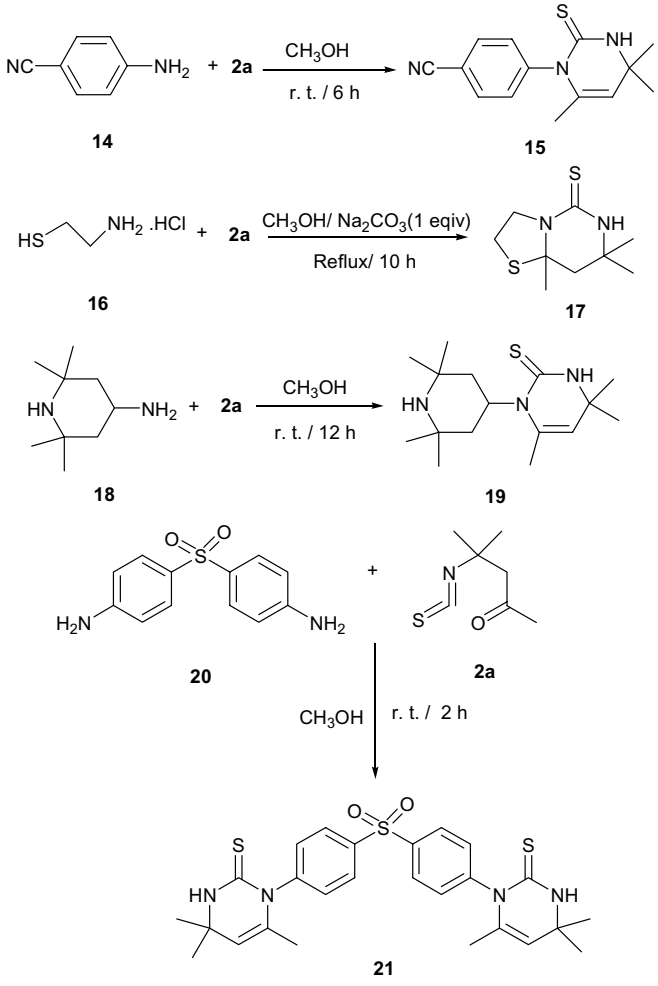
It is clear from Table 1, compound **3** is having some activity against CDK-1 and CDK-5 and the IC_{50} values were 5 and 25 μM, respectively. The other compounds showed moderate activities against CDK-5 and GSK-3 with $IC_{50} > 10$ μM. Compound **17** is having good anti-inflammatory (37.4% at 100 mg/kg p.o.) and analgesic activities (75% at 100 mg/kg p.o.) compared to the standard drug Ibuprofen. Compounds **4**, **7a**, **9a**, **9b**, **11a**, **13a**, **15**, **19**, and **21** showed moderate to good anti-inflammatory activity. Compounds **7a**, **11a** also showed good analgesic activity. Other compounds showed moderate analgesic activity.

4. Conclusions

Various 2-TP derivatives (**3**, **4**, **6**, **7a**, **9a**–**c**, **11a**,**b**, **13a**,**b**, **15**, **17**, **19**, and **21**) have been synthesized by a simple, efficient, one-pot reaction. All these compounds have been synthesized in reasonable yield. Screening of these compounds for anti-inflammatory, analgesic, and protein kinase (CDK-1, CDK-5, and GSK-3) inhibitory activities revealed that compound **3** showed some activity against CDK-1 and compound **17** possess good anti-inflammatory as well as analgesic activities.

5. Experimental

Melting points were uncorrected and obtained on a capillary apparatus. Infra-Red spectra were recorded using a Perkin Elmer 1600 FT Spectrophotometer and only characteristic peaks are reported. ^{1}H NMR spectra were measured in appropriate deuteriated solvent ($CDCl_3$, DMSO-d_6, D_2O) with TMS as internal standard by using Bruker AC-300F, NMR Spectrometer (300 MHz). Chemical shifts (δ) are reported in parts per million (ppm) of the applied field and coupling constants (J) are expressed in Hertz (Hz). The EI-MS spectra were recorded on VG-70-S mass spectrometer. FAB-MS spectra were reordered on Jeol SX-102 (FAB) mass spectrometer. Elemental analyses were



Scheme 5.

Table 1. Anti-inflammatory, analgesic, and kinase (CDK-1, CDK-5 and GSK-3) inhibitory activity data of various 2-TP derivatives

Compound	Anti-inflammatory (%)		Analgesic (%)		Protein kinase IC_{50} (μM)		
	100 mg/kg p.o.	50 mg/kg p.o.	100 mg/kg p.o.	50 mg/kg p.o.	CDK-1	CDK-5	GSK-3
3	0.0	—	25.0	—	**5.0**	25.0	>100
4	11.2	—	25.0	0.0	—	>10	>10
6	0.0	—	50.0	25.0	—	>10	>10
7a	14.7	—	75.0	50.0	—	>10	>10
9a	11.2	—	25.0	—	—	>10	>10
9b	10.3	—	50.0	25.0	—	>10	>10
9c	0.0	—	0.0	—	—	>10	>10
11a	5.2	—	75.0	50.0	—	>10	>10
11b	—	—	—	—	—	>10	>10
13a	16.9	—	50.0	25.0	—	>10	>10
13b	—	18.3[a]	—	10.0	—	>10	>10
15	23.7	—	50.0	25.0	—	>10	>10
17	**37.4**	—	**75.0**	**50.0**	—	>10	>10
19	—	**19.4**[a]	—	20.0	—	>10	>10
21	—	18.7[a]	—	20.0	—	>10	>10
Ibuprofen	56.0	—	100	75.0	—	—	—
Phenylbutazone	58.4	36.4	—	—	—	—	—

[a] ALD_{50} = > 1000 mg/kg p.o.

performed using a Vario EL III elementar analyzer. Starting materials were procured from Aldrich (USA) and Spectrochem® (India) and were used as received. Solvents were purified by standard procedures. All the

reactions were monitored over silica gel-G (Merck) TLC plates and spots were visualized by iodine vapors or by irradiation with ultraviolet light (254 nm). Silica gel (60–120 mesh) used for column chromatography was obtained from Qualigens® (India).

5.1. Chemistry

5.1.1. General procedure for rt reaction of 2a. Functionalized amine (2 mmol) was dissolved in absolute methanol (15 mL). To this was added 4-isothiocyanato-4-methyl-2-pentanone (**2a**) (0.314 g, 2 mmol).[27] The resulting solution was stirred at rt for an appropriate time and the reaction was monitored at different time intervals. After completion of the reaction, methanol was allowed to evaporate at rt in a beaker and the resulting sticky mass was crystallized by using a appropriate solvent or it was subjected to column chromatography.

5.1.2. General procedure for pH adjusted reaction of 2a. Functionalized amine (2 mmol) was dissolved in absolute methanol (25 mL) by heating and cooled to rt. To this was added 4-isothiocyanato-4-methyl-2-pentanone (**2a**) (0.314 g, 2 mmol).[27] The pH of the resulting solution was adjusted to ~4–5 by adding few drops of 10% sulfuric acid in methanol. If any turbidity (salt) was observed it was filtered off and the clear solution was kept for reflux for an appropriate time. Reaction was monitored at different time intervals and after completion, methanol was distilled off under reduced pressure. The resulting mixture was washed with 10% sodium bicarbonate solution[28] (2 × 5 mL) and further with cold water (2 × 5 mL) and then crystallized or subjected to column chromatography.

5.1.3. General procedure for rt reaction of 2b. Functionalized amine (2 mmol) was dissolved in absolute methanol (15 mL). 3-Isothiocyanatobutanal (**2b**) (0.258 g, 2 mmol) was also dissolved separately in absolute methanol (10 mL). Any insoluble polymeric material was filtered off and a clear solution was added to the amine solution. The resulting mixture was stirred at rt. The reaction was monitored at different time intervals and after completion, the contents were transferred to a beaker and methanol was allowed to evaporate at rt. The resulting sticky mass was crystallized or subjected to column chromatography.

5.1.4. General procedure for Na_2CO_3 added reactions. Functionalized amine hydrochloride (2 mmol) was dissolved in absolute methanol (25 mL). The insoluble material was filtered off and to the clear solution 4-isothiocyanato-4-methyl-2-pentanone (**2a**) (0.314 g, 2 mmol) was added. To this reaction mixture a fine powder of sodium carbonate (0.106 g, 1 mmol) was added and kept for reflux with constant stirring. The reaction was monitored at different time intervals and after completion, methanol was distilled off under reduced pressure. The resulting solid was washed with cold water (2 × 5 mL) and then crystallized or subjected to column chromatography.

5.1.4.1. 7-(1-Mercapto-3,3,4a-trimethyl-4,4a,5,9b-tetrahydro-3*H*-pyrido[4,3-*b*]indol-7-yl)-3,3,4a-trimethyl-3,4,4a,5-tetrahydro-benzo[4,5]imidazo[1,2-*c*]pyrimidine-1-thiol (3). Solvent of crystallization, methanol; Violet solid, mp 213–215 °C (0.760 g, 77%); IR (KBr ν cm^{-1}) 3202.3 (N–H), 1613.1, 1494.5 (C=C), 1201.1 (C=S); ^1H NMR ($CDCl_3$+DMSO-d_6 δ); 1.34 (s, 6H, 2× $-CH_3$), 1.38 (s, 6H, 2× $-CH_3$), 1.52 (s, 6H, 2× $-CH_3$), 2.24 (d, 2H, J = 13.5 Hz, $-CH_2$), 2.55 (d, 2H, J = 13.47 Hz, $-CH_2$), 6.30 (d, 2H, J = 8.1 Hz, Ar–H), 6.62 (d, 1H, J = 7.9 Hz, Ar–H), 6.80 (d+s, 2H, Ar–H), 7.05 (q, 1H, Ar–H), 8.15 (d, 2H, exchanges with D_2O, 2× –HN–), 8.65 (d, 1H, exchanges with D_2O, –HN–C=S), 9.03 (s, 1H, exchanges with D_2O, –HN–C=S); EI-MS m/z (% abundance): 492 (M^+, 15%), 460 (M^+-2CH_4, 12.1%), 232 ( , 42.3%), 188 ( , 100%); Anal. Calcd for $C_{26}H_{32}N_6S_2$: C, 63.38; H, 6.55; N, 17.06. Found: C, 63.43; H, 6.60; N, 17.00.

5.1.4.2. 4,4′-Bis(4,4,6-trimethyl-3,4-dihydro-1*H*-pyrimidine-2-thioxo) biphenyl (4). Solvent of elution, EtOAc–hexane (4:6); White solid, mp 273–275 °C (0.552 g, 60%); IR (KBr ν cm^{-1}) 3253.3 (N–H), 1643.1, 1514.6 (C=C), 1201.9 (C=S); ^1H NMR (DMSO-d_6 δ); 1.37 (s, 12H, 4× $-CH_3$), 1.51 (s, 6H, 2× $-CH_3$), 4.86 (s, 2× 1H, olefinic), 7.27 (d, 4H, J = 8.1 Hz, Ar–H), 7.67 (d, 4H, J = 8.2 Hz, Ar–H), 8.41 (br s, 2H, exchanges with D_2O, 2× –HN–C=S); FAB-MS m/z (% abundance): 463 (MH^+, 20%), 431 (MH^+-2CH_4, 50%), 415 (m/z 431 $-CH_4$, 40%); Anal. Calcd for $C_{26}H_{30}N_4S_2$: C, 67.50; H, 6.54; N, 12.11. Found: C, 67.41; H, 6.45; N, 12.18.

5.1.4.3. 3-Methyl-2,3,4,4a,9,10-hexahydro-2,9a,10-triaza-anthracene-1-thione (6). Solvent of crystallization, methanol; White solid, mp 180–182 °C (0.115 g, 25%); IR (KBr ν cm^{-1}): 3222.3 (N–H), 1559, 1489 (C=C), 1215.1 (C=S); ^1H NMR ($CDCl_3$ + DMSO-d_6 δ) 1.20–1.27 (dd, 3H, J = 6.5 and 6.6 Hz, $-CH_3$), 1.73–1.81 (dd, 2H, J = 3.8 and 6.6 Hz, H_2C_{11}), 3.92 (septet, 1H, J = 4 and 6.6 Hz, H^*C_{12}), 4.27 (m, 1H, HC_8), 4.60–4.67 (m, 2H, H_2C_{10}), 6.57–7.10 (m, 4H, Ar–H) 5.46 and 7.33 (br s, 2× 1H, exchanges with D_2O, HN_7 and HN_{13}); EI-MS m/z (% abundance): 233 (M^+, 45%), 232 (M^+-H, 3.8%), 200 (M^+-SH, 15%), 132 ( , 27%), 105 ( , 100%); Anal. Calcd for $C_{12}H_{15}N_3S$: C, 61.77; H, 6.48; N, 18.01. Found: C, 61.82; H, 6.55; N, 17.97.

5.1.4.4. 2-Methyl-1,2,3,10a-tetrahydro-9*H*-10-oxa-3,4a-diaza-phenanthrene-4-thione (7a). Solvent of elution, EtOAc–$CHCl_3$ (1:9); White solid, mp 193–195 °C (0.210 g, 45%); IR (KBr ν cm^{-1}) 3184.0 (N–H), 1525.9, 1439.4 (C=C), 1212.2 (C=S); ^1H NMR ($CDCl_3$ δ) 1.28–1.31 (d, J = 6.3 Hz, $-CH_3$) 1.94–2.04 (m, 1H, J = 2.0, 3.5, 4.0, 6.0 Hz, HC_{14}), 2.19–2.26 (m, 1H, J = 2.0, 3.5, 4.0, 6.0 Hz, HC_{14}), 3.83–3.91 (m, 1H, J = 2.4, 4.0, 6.5 Hz, HC_{13}), 4.91–4.93 (t, 1H, J = 3.3 Hz, HC_8), 5.06 (s, 2H, H_2C_{10}), 6.8 (br s, 1H, exchanges with D_2O, –HN–C=S), 7.01–7.04 (d, 1H, J = 6.0 Hz, Ar–H), 7.18–7.30 (dd, 2H, J = 6.0 and

6.1 Hz, Ar–H), 8.21–8.23 (d, 1H, J = 6.0 Hz, Ar–H); EI-MS m/z (% abundance): 234 (M^+, 100%), 201 (M^+-SH, 30%), 175 (M^+-HSCN, 10%), 147 ( , 20%), 133 ( , 60%); Anal. Calcd for $C_{12}H_{14}N_2SO$: C, 61.51; H, 6.02; N, 11.96. Found: C, 61.55; H, 5.95; N, 12.01.

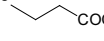
5.1.4.5. (4,4,6-Trimethyl-2-thioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetic acid methyl ester (9a). Solvent of crystallization, methanol; White solid, mp 158–160 °C (0.185 g, 47%); IR (KBr ν cm^{-1}) 3198.5 (N–H), 1735.9 (C=O), 1694.6 (C=N), 1540.3 (C=C), 1222.2 (C=S); ^1H NMR ($CDCl_3$ δ) 1.31 (2s, 6H, 2× $-CH_3$), 1.87 (d, 3H, J = 1.2 Hz, $-CH_3$), 3.78 (s, 3H, $H_3OC-C=O$), 4.79 (d, 1H, J = 1.1 Hz, olefinic), 4.85 (s, 2H, H_2C-N-), 6.95 (br s, 1H, exchanges with D_2O, HN–C=S); FAB-MS m/z (% abundance): 229 (MH^+, 100%), 213 (M^+-CH_3, 40%), 195 (M^+-SH, 10%); Anal. Calcd for $C_{10}H_{16}N_2O_2S$: C, 52.61; H, 7.06; N, 12.27. Found: C, 52.55; H, 7.10; N, 12.30.

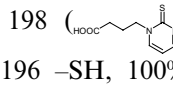
5.1.4.6. 7,7,8a-Trimethyl-5-methylsulfanyl-8,8a-dihydro-7*H*-oxazolo[3,2-*c*]pyrimidin-2-ylideneamine (9b). Solvent of elution, EtOAc–$CHCl_3$ (5:5); Green solid, mp 175–177 °C (0.136 g, 30%); IR (KBr ν cm^{-1}) 3295.7 (N–H), 1634.0 (C=N), 1191.5 (C–S); ^1H NMR ($CDCl_3$ δ): 1.35 (s, 3H, $-CH_3$), 1.44 (s, 3H, $-CH_3$), 1.66 (s, 3H, $-CH_3$), 1.85 (d, 1H, J_{ab} = 18.0 Hz, $H_aCH_b-C^*$), 2.26 (d, 1H, J_{ba} = 18.0 Hz, $H_bCH_a-C^*$), 3.16 (s, 3H, H_3C-S-), 4.55 (d, 1H, J_{cd} = 17.4 Hz, H_cCH_d-N), 5.18 (d, 1H, J_{dc} = 17.7 Hz, H_dCH_c-N-), 8.14 (br s, 1H, exchanges with D_2O, HN–C=S); FAB-MS m/z (% abundance): 228 (MH^+, 100%), 212 (M^+-CH_3, 10%), 113 ( , 80%); Anal. Calcd for $C_{10}H_{17}N_3OS$: C, 52.84; H, 7.54; N, 18.48. Found: C, 52.90; H, 7.60; N, 18.45.

5.1.4.7. (6-Methoxy-4-methyl-2-thioxo-tetrahydropyrimidin-1-yl)-acetic acid methyl ester (9c). Solvent of elution, EtOAc–$CHCl_3$ (2:8); White solid, mp 198–200 °C (0.045 g, 10%); IR (KBr ν cm^{-1}): 3143.7 (N–H), 1728.8 (C=O), 1227.1 (C=S), 1136.6 (C–O); ^1H NMR ($CDCl_3$ δ): 1.26 (q, 3H, J = 3.2 and 6.6 Hz, $-CH_3$), 1.69 (m, 1H, $-H_aCH_b-C^*CH_3$), 2.11 (m, 1H, $-H_bCH_a-C^*CH_3$), 2.60 (t, 1H, J = 6.5 Hz, HC^*CH_3N), 3.37 (d, 3H, $-OCH_3$), 3.75 (s, 3H, $H_3CO-C=O$), 4.04 (d, 1H, J_{ab} = 17.6 Hz, $H_aCH_b-COOCH_3$), 4.56 (t, 1H, $HC-OCH_3$), 5.38 (d, 1H, J_{ba} = 17.5 Hz, $H_bCH_a-COOCH_3$), 7.23 (br s, 1H, exchanges with D_2O, HN–C=S); FAB-MS m/z (% abundance): 232.66 (MH^+, 15%); Anal. Calcd for $C_9H_{16}N_3O_3S$: C, 46.53; H, 6.94; N, 12.06. Found: C, 46.49; H, 6.90; N, 12.12.

5.1.4.8. 1-(3-Hydroxy-pyridin-2-yl)-4,4,6-trimethyl-3,4-dihydro-1*H*-pyrimidine-2-thione (11a). Solvent of crystallization, methanol; Yellow solid, mp 175–177 °C (0.336 g, 67%); IR (KBr ν cm^{-1}): 3115.3 (N–H), 1643.6 (C=N), 1542.8, 1515.1 (C=C), 1203.6 (C=S); ^1H NMR ($CDCl_3$ + DMSO-d_6 δ): 1.77 (2s, 6H, 2× $-CH_3$), 2.00 (s, 3H, $-CH_3$), 4.65 (s, 1H, olefinic), 6.98–7.03 (t, 1H, Ar, J = 7.0 and 8.0 Hz), 7.36–7.38 (d, 1H, Ar, J = 8.0 Hz), 7.64–7.66 (d, 1H, Ar, J = 7.0 Hz), 9.83 (br s, 1H, –HN–C=S, exchanges with D_2O), 12.05 (very br s, 1H, OH-Ar, exchanges with D_2O); EI-MS m/z (% abundance): Does not show M^+ ion peak but 190 (M^+-HSCN, 13.7%), 175 ( , 100%); Anal. Calcd for $C_{12}H_{15}N_3OS$: C, 57.81; H, 6.06; N, 16.85. Found: C, 57.88; H, 6.10; N, 16.79.

5.1.4.9. 2-Methyl-1,2,3,9a-tetrahydro-9-oxa-3,4a,5-triaza-fluorene-4-thione (11b). Solvent of crystallization, methanol; Pale yellow solid, mp 180–182 °C (0.050 g, 10%); IR (KBr ν cm^{-1}): 3216.7 (N–H), 1653.0, 1557.9 (C=C), 1208.7 (C=S); ^1H NMR ($CDCl_3$ + DMSO-d_6 δ): 1.39–1.41 (d, J = 6.0 Hz, $-CH_3$), 1.92–2.05 (dd, J = 3.8 and 12.0 Hz, 1H, $H_aCH_b-HC^*CH_3$), 2.61–2.67 (2t, 1H, J = 3.9 and 12.0 Hz each, $H_bCH_a-HC^*CH_3$), 3.75–3.79 (t, 1H, J = 6.0 Hz, HC*CH3), 5.98–6.03 (dd, 1H, J = 3.7 and 3.8 Hz, O–C*H–N), 6.93–6.96 (dd, 1H, J = 5.1 and 6.9 Hz, Ar–H), 7.07–7.09 (d, 1H, J = 6.7 Hz, Ar–H), 7.92 (br s, 1H, exchanges with D_2O, HN–C=S), 8.03–8.05 (d, 1H, J = 5.1 Hz, Ar–H); FAB-MS m/z (% abundance): 222 (MH^+, 32%), 221 (M^+, 10%), 206 (M^+-CH_3, 5%); Anal. Calcd for $C_{10}H_{11}N_3OS$: C, 54.28; H, 5.01; N, 18.99. Found: C, 54.15; H, 5.10; N, 19.01.

5.1.4.10. 4-(4,4,6-Trimethyl-2-thioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-butyricacid (13a). Solvent of crystallization, methanol; White solid, mp 208–210 °C (0.330 g, 68%); IR (KBr ν cm^{-1}): 3259.0 (N–H), 1706.8 (C=O), 1643.9, 1538.3 (C=C), 1205.3 (C=S); ^1H NMR ($CDCl_3$+DMSO-d_6 δ): 1.22–1.23 (2s, 6H, 2× $-CH_3$), 1.89–2.03 (s+m, 5H, $-CH_3$+$-CH_2$), 2.31–2.38 (dd, 2H, J = 6.0 Hz each, $-CH_2$), 4.23–4.29 (m, 2H, $-CH_2$), 4.68 (s, 1H, olefinic), 7.62 (br s, 1H, exchanges with D_2O, HN–C=S), 12.05 (very br s, 1H, exchanges with D_2O, –COOH); EI-MS m/z (% abundance): 242 (M^+, 48%), 227 (M^+-CH_3, 61.8%), 209 (M^+-SH, 2.5%), 198 (M^+-CO_2, 1.5%), 183 (M^+-HSCN, 5.0%), 156 ( , 6.0%), 141 ( , 100%), 87 ( , 11.6%); Anal. Calcd for $C_{11}H_{18}N_2O_2S$: C, 54.52; H, 7.49; N, 11.56. Found: C, 54.48; H, 7.54; N, 11.60.

5.1.4.11. 4-(6-Hydroxy-4-methyl-2-thioxo-tetrahydropyrimidin-1-yl)-butyric acid (13b). Solvent of crystallization, methanol; White solid, mp 140–142 °C (0.077 g, 18%); IR (KBr ν cm^{-1}): 3271.0 (O–H), 1702.2 (C=O), 1200.0 (C=S); ^1H NMR ($CDCl_3$+DMSO-d_6+D_2O, δ): 1.22–1.24 (d, 3H, J = 6.0 Hz, $-CH_3$), 2.08–2.14 (m, 2H, $-CH_2$), 2.29–2.34 (dd, 2H, J = 2.0 Hz each, H_2C-C^*OH), 3.34–3.38 (q, 1H, J = 6.0 Hz, $HC-CH_3$), 3.50–3.63 (m, 2H, $-CH_2$), 4.23–4.29 (m, 2H, $-CH_2$), 4.54–4.55 (d, 1H, J = 1.8 Hz, HC*N–OH); EI-MS m/z (% abundance): 232 (M^+, 0.7%), 214 (M^+-H_2O, 2.4%), 199 (M^+-SH, 0.4%), 198 ( , 3.3%), 196 ( , 52%), 163 (m/z 196 –SH, 100%); Anal.

Calcd for $C_9H_{16}N_2O_3S$: C, 46.53; H, 6.94; N, 12.06. Found: C, 46.48; H, 7.02; N, 12.08.

5.1.4.12. 4-(4,4,6-Trimethyl-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)benzonitrile[29] (15). Solvent of elution, EtOAc–hexane (2:8); White solid, mp 190–192 °C (0.295 g, 57%); IR (KBr ν cm^{-1}): 3437.3, 3169.6 (N–H), 2229.3 (C≡N), 1683.6, 1522.3 (C=C), 1213.8 (C=S); ^1H NMR ($CDCl_3$ δ): 1.39 (s, 6H, 2× –CH_3), 1.52 (s, 3H, –CH_3), 4.69 (s, 1H, olefinic), 6.67 (br s, 1H, exchanges with D_2O, –HN–C=S), 7.36 (d, 2H, J = 6.0 Hz, Ar–H), 7.73 (d, 2H, J = 6.0 Hz, Ar–H); EI-MS *m/z* (% abundance): 257 (M^+, 27%), 242 (M^+-CH_3, 100%), 198 (M^+-HSCN, 15%); Anal. Calcd for $C_{14}H_{15}N_3S$: C, 65.34; H, 5.87; N, 16.33. Found: C, 65.30; H, 5.92; N, 16.35.

5.1.4.13. 7,7,8a-Trimethyl-hexahydro-thiazolo[3,2-*c*]-pyrimidine-5-thione (17). Solvent of crystallization, methanol; Yellow solid, mp 170–172 °C (0.254 g, 59%); IR (KBr ν cm^{-1}): 3191.8 (N–H), 1270.8 (C=S); ^1H NMR ($CDCl_3$ δ): 1.34 and 1.37 (2s, 6H, 2× –CH_3), 1.76 (s, 3H, –CH_3), 2.31 (s, 2H, H_2C–), 3.11–3.15 (t, 2H, J = 6.6 Hz, H_2C–N), 4.00–4.09 (p, 1H, J = 6.0 and 6.6 Hz, H_aH_bC–S–), 4.88–4.96 (p, 1H, J = 6.0 and 6.6 Hz, H_bH_aC–S–), 6.46 (br s, 1H, exchanges with D_2O, HN–C=S); FAB-MS *m/z* (% abundance): 217 (MH^+, 100%), 183 (M^+-SH, 10%), 100 ( , 13%); Anal. Calcd for $C_9H_{16}N_2S_2$: C, 49.96; H, 7.45; N, 12.95. Found: C, 46.85; H, 7.51; N, 13.01.

5.1.4.14. 4,4,6-Trimethyl-1-(2,2,6,6-tetramethyl-piperidin-4-yl)-3,4-dihydro-1*H*-pyrimidine-2-thione (19). Solvent of crystallization, methanol; Pale yellow solid, mp 195–197 °C (0.390 g, 53%); IR (KBr ν cm^{-1}): 3357.3 (N–H), 1692.1 (C=N), 1538.2 (C=C), 1220.0 (C=S); ^1H NMR ($CDCl_3$ δ): 1.44–1.49 (2s, 21H, 7× –CH_3), 2.04 (m, 2H, –CH_2), 2.12 (m, 2H, –CH_2), 3.35 (m, 1H, –HC–N), 4.74 (d, 1H, J = 5.9 Hz, olefinic), 7.03 (s, 1H, exchanges with D_2O, –HN), 7.31 (br s, 1H, exchanges with D_2O, –HN–C=S); EI-MS *m/z* (% abundance): Does not show M^+ ion peak, 263 ($M^+-2\times CH_4$, 100%), 248 (M^+-SH, –CH_3, 25%), 204 (*m/z* 263 –HSCN, 39%) 107 ( , 35.4%); Anal. Calcd for $C_{16}H_{29}N_3S$: C, 65.04; H, 9.89; N, 14.22. Found: C, 65.13; H, 9.94; N, 14.25.

5.1.4.15. 4,4′-Bis(4,4,6-trimethyl-3,4-dihydro-1*H*-pyrimidine-2-thioxo) biphenyl sulfone (21). Solvent of elution, EtOAc–$CHCl_3$ (3:7); White solid, mp 228–230 °C (0.496 g, 55%); IR (KBr ν cm^{-1}): 3265.0 (N–H), 1691.7, 1649.3, 1595.1, 1532.3 (C=C), 1318.7 (O=S=O), 1210.9 (C=S); ^1H NMR ($CDCl_3$+DMSO-d_6 δ): 1.35–1.37 (2s, 12H, 4× –CH_3), 1.45 (s, 6H, 2× –CH_3), 4.85 (s, 2× 1H, olefinic), 6.69 (d, 2H, J = 8.7 Hz, Ar–H), 7.32 (d, 2H, J = 8.4 Hz, Ar–H), 7.64 (d, 2H, J = 7.8 Hz, Ar–H), 7.9 (d, 2H, J = 7.6 Hz, Ar–H), 8.00 (br s, 2H, exchanges with D_2O, 2× –HN–C=S); FAB-MS *m/z* (% abundance): 563 (MH^+, 30%); Anal. Calcd for $C_{26}H_{30}N_4O_2S_3$: C, 59.29; H, 5.74; N, 10.64. Found: C, 59.34; H, 5.70; N, 10.66.

5.2. Pharmacology

5.2.1. Anti-inflammatory activity assay.[30] Anti-inflammatory activity screening was carried out using carrageenin-induced paw oedema in albino rats. Oedema in one of the hind paws was induced by injection of carrageenin solution (0.1 mL of 1%) into planter aponeurosis. The volume of the paw was plethysmographyically measured immediately and also 3 h after the injection of the irritant. The difference in the volume gave the amount of the oedema developed. Percent inhibition of the oedema between the control group and the compound treated groups was calculated and compared with the group receiving a standard drug.

5.2.2. Analgesic activity assay.[31] Analgesia was measured by the writhing assay using Swiss mice (15–20 g). Female mice were screened for writhing on day one, by injecting intraperitonially 0.2 cm^3 of aqueous solution of phenylquinone. They were kept on a flat surface and the numbers of writhes of each mouse was recorded for 20 min. The mice showing significance (>10) were sorted out and used for analgesic assay on the following day. The mice consisting of 5 in each group and showing significant writhing were given orally a 50 or 100 mg kg^{-1} p.o. dose of the test compounds 15 min prior to phenylquinone challenge. Writhing was again recorded for each mouse in a group and a percentage protection was calculated by using the following formula:

$$\text{Protection} = 100 - [\{(\text{No. writhing for treated mice})/(\text{No. of writhing for untreated-mice})\} \times 100]$$

This was taken as percent analgesia response and was averaged in each group of mice. Percent of animals exhibiting analgesic was determined with each dose.

5.2.3. Kinase inhibition activity

5.2.3.1. Biochemical reagents. Sodium orthovanadate, EGTA, EDTA, Mops, β-glycerophosphate, phenylphosphate, sodium fluoride (NaF), dithiothreitol (DTT), glutathione-agarose, glutathione, bovine serum albumin (BSA), nitrophenylphosphate, leupeptin, aprotinin, pepstatin, soybean trypsin inhibitor, benzamidine, histone H1(type III-S) were obtained from Sigma. The [γ-^{32}P]-ATP (PB 168) was obtained from Amersham. The GS-1 peptide (YRRAAVPPSPSLSRHSSPHQSpEDEEE) was synthesized by Peptide synthesis unit, Institute of Biomolecular Sciences, University of Southampton, UK.

5.2.3.2. Buffers. Homogenization of buffer: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenyl-phosphate, 25 mM Mops (pH 7.2), 15 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM NaF, 1 mM phenylphosphate, 10 μg leupeptin/mL, 10 μg aprotinin/mL, 10 μg soybean trypsin inhibitor/mL and 100 μM benzamidine. Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl (pH 7.5), 50 μg

heparin/mL. Buffer C: homogenization buffer but 5 mM EGTA, no NaF and no protease inhibitors. Tris-Buffered-Salin-Tween-20 (TBST): 50 mM Tris (pH 7.4), 150 mM NaCl, 0.1% Tween-20. Hypotonic Lysis Buffer (HLB): 50 mM Tris–HCl (pH 7.4), 120 mM NaCl, 10% glycerol, 1% Nonidet-P40, 5 mM DTT, 1 mM EGTA, 20 mM NaF, 1 mM orthovanadate, 5 μM microcystin, 100 μg/mL each of leupeptin, aprotinin and pepstain.

5.2.3.3. Kinase preparations and assays. Kinases activities were assayed in Buffer A or C (unless otherwise stated), at 30 °C, at final ATP concentration of 15 μM. Blank values were subtracted and activities calculated as *p* moles of phosphate were incorporated for a 10 min incubation. The activities are usually expressed in percentage of the maximal activity (in absence of the inhibitors). Controls were performed with appropriate dilutions of dimethylsulfoxide. GSK-3 was purified from porcine brain. It was assayed, following a 1/100 dilution in 1 mg BSA/mL 10 mM DTT, with 5 μL, 40 μM GS-1 peptide as a substrate, in buffer A, in presence of 15 μM [γ-^{32}P] ATP (3000 Ci/mM; 1 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid per liter of water. The wet filters were counted in presence of 1 mL ACS (Amersham) scintillation fluid. CDK-1/ cyclin B was extracted in a homogenization buffer from M phase starfish (*Marthasterias glacialis*) oocytes and purified by affinity chromatography on P9^{CKShs1} sepharose beads, from which it was eluted by free P9^{CKShs1} as reported in the literature.[32] The kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in presence of 15 μM [γ-^{32}P]-ATP (3000 Ci/mmol; mCi/mL) in a final volume of 30 μL. After 10 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto P81 phosphocellulose papers and treated as described above. CDK-5/ p25 was reconstituted[33] by mixing equal amounts of recombinant mammalian CDK-5 and p25 expressed in *E. coli* as GST (Glutathione-S-transferase) fusion-protein and purified by affinity chromatography on glutathione-agarose.[34] The activity of p25 was assayed in buffer C as described for CDK-1/cyclin B.

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References and notes

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