Tetrahedron 67 (2011) 5156–5161

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Novel synthesis approach and antiplatelet activity evaluation of 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines

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ARTICLE INFO

Article history:
Received 7 March 2011
Received in revised form 4 May 2011
Accepted 13 May 2011
Available online 19 May 2011

Keywords:
Pyrimidine
Diazotization
Disulfide
Synthesis

ABSTRACT

A new and efficient procedure has been designed for the preparation of 6-alkylamino-2,4-dialkyl(aryl) thiopyrimidines. The first alkylthio group was introduced into the pyrimidine ring by S-alkylation. The introduction of the second one was successfully achieved using the diazotization–alkylthionation method to afford 2,4-dialkyl(aryl)thio-6-chloropyrimidines. Subsequent nucleophilic displacement by the corresponding amines conveniently gave a series of the target compounds. Thus, the two same or different alkylthio groups were easily introduced into the pyrimidine ring through the two different approaches. The human anti-platelet aggregation activity of the newly synthesized compounds is also described.

1. Introduction

Pyrimidines play an essential role in several biological processes and have considerable chemical and pharmacological importance in terms that the pyrimidine ring can be found in nucleoside antibiotics, antibacterial and cardiovascular.[1–6] Pyrimidine derivatives, especially, alkylthio-substituted pyrimidines, such as 2-propylthio-triazolopyrimidines[7] and thienopyrimidines[8] have attracted much attention because of their quite high anti-platelet aggregation activity as inhibitors for P2-receptor family. As related works, there have been active attempts to develop the antagonist of P2Y receptors (which mediate platelet aggregation induced by adenosine diphosphate, ADP) by employing adenine nucleotide derivatives containing two phosphate groups, i.e., adenosine-3′,5′-bisphosphate analogues, as $P2Y_1$ receptor antagonists[9,10] and 4-alkoxyl-2-alkylthio-6-aminopyrimidine derivatives as $P2Y_{12}$ receptor antagonists.[11] The evaluated anti-platelet aggregation ability of a series of the synthesized pyrimidine derivatives proves their potential as lead compounds to develop a new series of $P2Y_{12}$ antagonists.[11] Furthermore, the results appear to suggest the importance of the chemical structure of alkylthio substituents and of the presence of a free amino group for the activity. As for adenine nucleotide analogues against P_{2T} receptor, the effective enhancement of the activity by N-monoalkylation at the 6-position of the adenine moiety has been found out by Ingall et al.[12] Considering such findings on the structure of the antagonist candidates, we achieved one hypothesis that N-monoalkylation of pyrimidine compounds might also increase anti-platelet aggregation activity. Furthermore, to date, there are few report on the synthesis and evaluation of dialkyl(aryl)thio-substituted pyrimidines as platelet aggregation inhibitors. Hence, we designed to introduce another alkylthio group into the pyrimidine ring (Fig. 1). As it is well known, the introduction of alkyl/arylthio groups into the pyrimidine ring is commonly achieved through either the alkylation of thiol groups or the nucleophilic substitution of halogens by alkylmercaptides. However, these

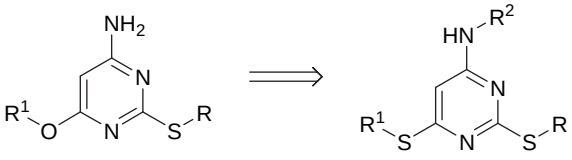

Fig. 1. Structures of 4-alkoxyl-2-alkylthio-6-aminopyrimidines and 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines.

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doi:10.1016/j.tet.2011.05.056

processes must be conducted under harsh conditions, especially in the absence of activating substituents. In the present study, we successfully introduced the two same or different alkyl(aryl)thio groups into the pyrimidine ring with two different approaches, S-alkylation and diazotization–alkylthionation, giving the key intermediate 2,4-dialkyl(aryl)thio-6-chloropyrimidines. Thus far, there has been no report on the application of the diazotization–alkylthionation reaction to aminopyrimidine derivatives. The subsequent nucleophilic displacement of the chloro groups by the corresponding amines affords a series of 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines. Herein, we describe the details of the convenient synthesis and the evaluation results of all the synthesized compounds as human platelet aggregation inhibitors.

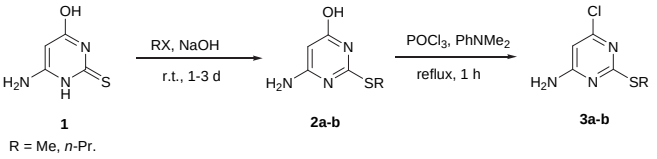
2. Results and discussion

2.1. Chemistry

6-Alkylamino-2,4-dialkyl(aryl)thiopyrimidines have been previously prepared by two methods. One was the treatment of 3-phenyl-l,2,3-triaxolo[4,5-*d*]pyrimidine-5,7-dithione with butyl lithium and an alkylating agent.[13] Though the route was short, the reaction was conducted at a low temperature (−70 °C), resulting in a low yield (30%). Another method was the conversion of 4,6-dihydroxyl-2-mercaptopyrimidine to 6-alkylamino-2,4-dialkylthiopyrimidines via S-alkylation, chlorination, and nucleophilic substitution.[14] However, it was likely to afford the byproduct of 2,4,6-trialkylthiopyrimidines.

During the last few years, our group has been working on the development of new platelet aggregation antagonists with the sulfur-substituted pyrimidine derivatives. In our method described below, 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines can be synthesized in good yields from a commercially available starting material, 4-amino-6-hydroxyl-2-mercaptopyrimidine **1**, under simple and mild reaction conditions.

We first synthesized 2-alkylthio-4-amino-6-chloropyrimidines (**3a**,**b**) from the starting compound **1** by a well-established procedure (Scheme 1).[15–17] The tautomerized thiol group was alkylated under basic conditions in the presence of the appropriate alkyl halide to obtain **2a**,**b**. Then, the hydroxyl group was chlorinated with phosphoryl chloride ($POCl_3$)[18] in combination with dimethylaniline to increase the yield. After the excess $POCl_3$ was removed in vacuo, the residue was poured into the mixture of the cooled concentrated ammonium hydroxide and chloroform. When the residue was dissolved completely, it was extracted with chloroform, concentrated, and then purified by column chromatography to afford 2-alkylthio-4-amino-6-chloropyrimidines (**3a**,**b**).



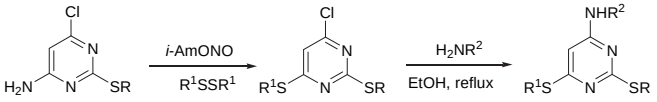
1 **2a-b** **3a-b**

R = Me, *n*-Pr.

Scheme 1. Synthesis of compounds **3** from **1** via S-alkylation and chlorination.

The introduction of dialkyl(aryl)thio groups into the pyrimidine rings was a key step. Initially, we attempted to prepare the designed compounds **5** from **3** via displacement of sodium alkylmercaptides and N-alkylation. However, the N-alkylation of 4-amino-2,6-dialkyl(aryl)thiopyrimidines would give a mixture of two products, monoalkyl and dialkyl products.[19] Thus, we took into account the need to employ an alternative method.

It is already known that primary aromatic amines,[20] such as aniline,[21] pyridine,[20] and guanosine derivatives,[12] can be directly converted into sulfides through diazotization under nonaqueous and neutral conditions. However, the diazotization–alkylthionation reaction of aminopyrimidine derivatives has not been reported in earlier studies. We have found the addition of isoamyl nitrite (*i*-AmONO) into the mixture of 2-alkylthio-4-amino-6-chloropyrimidine derivatives **3** and excess dialkyl(aryl) disulfide at 60 °C yields the corresponding alkyl(aryl)thio derivatives. In the reaction process, the mixed solution became deep brown with the immediate evolution of gas, which lasted until the completion of the reaction. As a result, we have succeeded in transforming the free amino groups of the compounds **3a**,**b** to alkyl(aryl)thio groups, whereby 2,4-dialkyl(aryl)thio-6-chloropyrimidines (**4a**–**e**) were obtained (Scheme 2). However, the mechanism of this reaction presently has not been identified.[21–24]



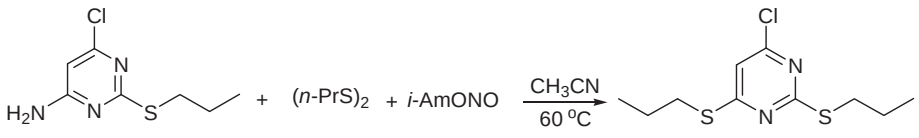
3a-b **4a-e** **5a-i**

R = Me, *n*-Pr; R^1 = *n*-Pr, *i*-Pr, Ph, Bn; R^2 = Bn, CH_2CH_2Ph.

Scheme 2. Preparation of compounds **5** from **3**.

To optimize the synthesis conditions, we selected 4-amino-6-chloro-2-propylthiopyrimidines **3b** and dipropyl disulfide as model substrates, and examined the effects of different amount of the dialkyl(aryl) disulfide in the presence of *i*-AmONO (6.2 equiv of **3b**) in anhydrous acetonitrile at 60 °C. The examination was initially commenced with 10 M equiv of dipropyl disulfide according to the previous literature. However, the yield at the molar ratio of 7:1 (Table 1,

Table 1
Optimizing reaction conditions for synthesis of **4b**



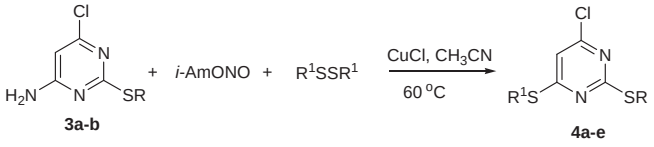
3b **4b**

Entry	Compound **3b** (equiv)	$(n\text{-PrS})_2$ (equiv)	*i*-AmONO (equiv)	Catalyst	Time (h)	Product **4b** (Yield, %)
1	1	10	6.2	No	2	76
2	1	7	6.2	No	2	75
3	1	5	6.2	No	2	65
4	1	1	6.2	No	2.5	46
5	1	7	6.2	CuCl	1	77
6	1	7	6.2	CuCl	2	78
7	1	7	6.2	No	1	55

entry 2) was comparable to that at 10:1, although it became lower with further decrease of the molar ratio (Table 1, entries 3 and 4). That is, the examination results with other molar ratios indicate that the reaction can be sufficiently carried out with 7:1 of dipropyl disulfide to **3**. In the series of examinations, more importantly, we have found that the reaction is definitely accelerated by the addition of CuCl even under the same condition (Table 1, entries 2, 5, 6, and 7), which indicates that the reaction is probably based on a radical mechanism. Isoamyl nitrite would react with **3** under nonaqueous and neutral conditions to immediately release N_2 gas and concomitantly produce the pyrimidinyl radical, which would efficiently be trapped by the surrounding abundant dialkyl(aryl) disulfide.

Under our optimized conditions, we examined the reaction efficiency of dipropyl disulfide, diisopropyl disulfide, diphenyl disulfide, and dibenzyl disulfide with (**3a**,**b**) in the presence of *i*-AmONO, respectively. As can be seen from Table 2, almost all the reactions were efficiently carried out to give **4** in good yields (72–82%). However, the reaction yield on diphenyl disulfide became lower (52%). It is assumed that a p-π conjugative effect in the phenylthio radical would lower its reactivity compared with other alkylthio radicals.

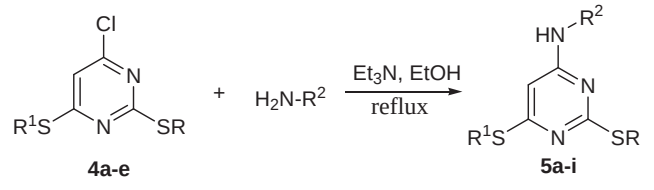
Table 2
The diazotization–alkylthionation reaction of **3**



Entry	Compound **3**	R	R^1	Time (h)	Product **4**	Yield (%)
1	**3a**	Me	*n*-Pr	1	**4a**	81
2	**3b**	*n*-Pr	*n*-Pr	2	**4b**	75
3	**3b**	*n*-Pr	*i*-Pr	2.5	**4c**	72
4	**3b**	*n*-Pr	C_6H_5	2.5	**4d**	52
5	**3b**	*n*-Pr	$CH_2C_6H_5$	1.5	**4e**	82

Subsequent nucleophilic displacement of the chlorine atom of **4** by the various amines conveniently afforded the target compounds **5**, as is shown in Table 3. The reaction is compatible with different primary amines in the presence of triethylamine. However, the results indicate that the effect of steric hindrance has a negative influence on the nucleophilic substitution. When a sterically hindered structure of amine, such as 1-phenylethylamine (Table 3, entry 2) was used as the nucleophilic reagent, the reaction proceeds more slowly to keep the yield lower even for the reaction time extended to 38 h (the yield: 73%). As for aniline, unfortunately, no reaction occurred due to the low nucleophilicity of aniline, even though the reaction time was prolonged.

Table 3
Nucleophilic substitution of 2,4-dialkyl(aryl)thio-6-chloropyrimidines (**4a**–**e**)



Entry	Compound **4**	R	R^1	R^2	Time (h)	Product **5**	Yield (%)
1	**4a**	Me	*n*-Pr	$C_6H_5CH_2CH_2$	12	**5a**	84
2	**4a**	Me	*n*-Pr	$C_6H_5CH(CH_3)$	38	**5b**	73
3	**4b**	*n*-Pr	*n*-Pr	$C_6H_5CH_2$	13	**5c**	85
4	**4b**	*n*-Pr	*n*-Pr	*p*-$MeC_6H_4CH_2$	14	**5d**	84
5	**4b**	*n*-Pr	*n*-Pr	*m*-$MeOC_6H_4CH_2CH_2$	16	**5e**	84
6	**4c**	*n*-Pr	*i*-Pr	*p*-$MeOC_6H_4CH_2$	15	**5f**	86
7	**4d**	*n*-Pr	C_6H_5	cyclohexyl	10	**5g**	83
8	**4d**	*n*-Pr	C_6H_5	$C_6H_5CH_2CH_2$	16	**5h**	88
9	**4e**	*n*-Pr	$CH_2C_6H_5$	*p*-$MeOC_6H_4CH_2$	15	**5i**	83
10	**4c**	*n*-Pr	*i*-Pr	C_6H_5	40	No reaction	

2.2. Antiplatelet activity evaluation

The antiplatelet activity of all the synthetic compounds was assayed on human platelet-rich plasma by using the Born's reported turbidimetric method,[25] where ADP (adenosine 5′-diphosphate) was employed as the agonist, and Cangrelor (AR-C69931MX) was used as the positive control (PIC_{50} was 9.4,[26] 30 μM ADP, human washed platelets), in the experiment, the percentage of platelet aggregation was 0% at the final concentrations of 100 nM. The results indicate that compounds **5a** and **5h** show some inhibitive effect on antiplatelet activities both at their final concentrations of 10 μM and 100 μM (Table 4). In contrast, compounds **5c**–**g** and **5i** show partial inhibition effect (≤10%) on platelet aggregation at the high concentration of 100 μM. The anitplatelet activities are comparable to those of 4-alkoxyl-2-alkylthio-6-aminopyrimidines reported by Cattaneo et al.[11] in the case that the same concentration of ADP (10 μM) was used. For all the observed partial antiplatelet activity, however, the structural difference of the alkyl(aryl)thio groups and *N*-monosubstituents resulted in no remarkable variation on the antiplatelet-activity.

Table 4
The antiplatelet activity of compounds **5**

Compounds **5**	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**
10 μM (%)[a]	107	101	98	101	103	102	97	102	99
100 μM (%)[a]	104	99	91	94	97	96	90	100	94

[a] The antiplatelet activity was expressed as percentage of the platelet aggregation measured in the presence of each compound to that in presence of the vehicle (DMSO) alone as blank. ADP was used as 10 μM.

3. Conclusions

We have developed a new and efficient synthesis of 6-alkylamino-2,4-dialkyl(aryl)thiopyrimidines, where two same or different kinds of alkylthio groups were introduced with two different methods into the pyrimidine ring skeleton. Commercially available starting material, 4-amino-6-hydroxyl-2-mercaptopyrimidine **1** was converted to 2-alkylthio-4-amino-6-chloropyrimidines (**3a**,**b**) via S-alkylation and chlorination. Then, the second alkylthio group was introduced by diazotization of **3** with *i*-AmONO and then reacted with dialkyl(aryl) disulfide to afford 2,4-dialkyl(aryl)thio-6-chloropyrimidines (**4a**–**e**). The conversion of aminopyrimidine derivatives via the diazotization–alkylthionation has been achieved in this study for the first time. Subsequently, nucleophilic substitution with various amines successfully gives the designed compounds (**5a**–**i**). All the synthesized compounds were evaluated for their inhibition activities on the human platelet aggregation caused by ADP as agonist (ADP concentration: 10 μM). Some compounds (**5c**–**g** and **5i**) show partial effect (≤10%) against platelet aggregation at their final concentration of 100 μM. Although the examined series of *N*-monoalkylated and dialkyl(aryl)thio-substituted pyrimidines currently have no remarkable activity, the observed partial effect seems to suggest the importance of further screening for other substituents for the purpose of increasing the activity. In this view, our developed synthesis is effective tool for the access of various 6-alkylamino-2,4-dialkyl(aryl) thiopyrimidines not to be investigated yet.

4. Experimental section

4.1. General

The chemicals were obtained from commercial sources, and the solvents used in reactions were dried by standard procedures prior to use. Melting points were measured on a Yanaco MP-500 melting point apparatus without being corrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 200 spectrometer, Plus 300 spectrometer or Bruker 400 plus. Chemical shifts are reported relative to TMS (^{1}H) or DMSO-d_6 (^{13}C). IR spectra were recorded on a Perkin–Elmer Fourier transform infrared spectrometer Spectrum 100. High Resolution Mass spectra (HRMS-ESI) were obtained on an Agilent LC/TOF mass spectrometer. P.E. denotes petroleum ether (bp 60–90 °C). Cangrelor (AR-C69931MX) was a gift from AstraZeneca (Loughborough, UK). ADP was purchased from Chrono-Log (Havertown, PA, USA); sodium citrate was purchased from Sigma (St Louis, MO).

4.2. General procedure for the synthesis of 2-alkylthio-4-amino-6-hydroxylpyrimidines (2a,b)

Haloalkane (12.4 mmol) was added dropwise into a solution of 4-amino-6-hydroxyl-2-mercaptopyrimidine (**1**) (1.78 g, 12.4 mmol) in 1 mol/L NaOH (12.4 mL). The reaction mixture was kept under stirring at room temperature for 1–3 days, until TLC (EtOAc/MeOH, 15:1, v/v) monitoring indicated no unreacted materials existed. The mixture was neutralized with acetic acid. The resulting solid was collected by filtration, washed with P.E. (10 mL) and water (10 mL), and dried.

*4.2.1. 4-Amino-6-hydroxyl-2-methylthiopyrimidine (**2a**).* Colorless crystals, yield: 1.85 g (95%), mp 264–266 °C (lit.[15] 268–269 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ=11.84 (br s, 1H, OH), 6.44 (br s, 2H, NH_2), 4.89 (s, 1H, CH), 2.41 (s, 3H, SCH_3). ^{13}C NMR (50 MHz, DMSO-d_6) δ=164.6, 163.6, 163.0, 81.3, 12.7.

*4.2.2. 4-Amino-6-hydroxyl-2-propylthiopyrimidine (**2b**).* Colorless crystals, yield: 2.09 g (91%), mp 206–208 °C (lit.[15] 209–210 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ=11.50 (br s, 1H, OH), 6.46 (br s, 2H, NH_2), 4.87 (s, 1H, CH), 3.03 (t, J=7.2 Hz, 2H, SCH_2), 1.62 (sextet, J=7.2 Hz, 2H, SCH_2CH_2), 0.95 (t, J=7.2 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6) δ=164.4, 163.5, 162.2, 81.3, 31.2, 22.4, 13.2.

4.3. General procedure for the synthesis of 2-alkylthio-4-amino-6-chloropyrimidines (3a,b)

2-Alkylthio-4-amino-6-hydroxylpyrimidine (**2a**,**b**) (27 mmol), $POCl_3$ (15 mL, 162 mmol), and $PhNMe_2$ (6.8 mL, 54 m mol) were refluxed together for 1 h. The excess $POCl_3$ was removed in vacuo, and then the residue was poured into 60 mL (1:1, v/v) of cooled concentrated ammonium hydroxide and chloroform. The solution was kept stirring at room temperature. After $POCl_3$ was dissolved completely, the aqueous phase was extracted with $CHCl_3$ (3×20 mL), the combined organic phases were dried with $MgSO_4$, concentrated, and purified by flash column chromatography (Et_3N-neutralized silica gel, gradient elution separation with EtOAc/P.E., 1:50–1:2, v/v) and then by recrystallization from cyclohexane to afford the product as colorless crystals.

*4.3.1. 4-Amino-6-chloro-2-methylthiopyrimidine (**3a**).* Colorless crystals, yield: 3.94 g (83%), mp 122–124 °C (lit.[18] 126–127 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.16 (s, 1H, CH), 5.18 (br s, 2H, NH_2), 2.50 (s, 3H, SCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=172.4, 163.2, 159.3, 99.1, 13.9.

*4.3.2. 4-Amino-6-chloro-2-propylthiopyrimidine (**3b**).* Colorless crystals, yield: 3.08 g (56%), mp 98–100 °C (lit.[16a] 101–102 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.11 (s, 1H, CH), 5.03 (br s, 2H, NH_2), 3.04 (t, J=7.3 Hz, 2H, SCH_2), 1.70 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.00 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=172.2, 163.2, 159.4, 99.0, 32.7, 22.4, 13.4.

4.4. General procedure for the synthesis of 2,4-dialkyl(aryl) thio-6-chloropyrimidines (4a–e)

Compound **3a** or **3b** (5.85 mmol) was added into the dry acetonitrile (35 mL) containing appropriate dialkyl(aryl) disulfide (40.95 mmol) and CuCl (0.29 m mol). The mixture was purged thoroughly with N_2 and stirred at room temperature for 30 min. Isoamyl nitrite (4.25 g, 36.3 mmol) was added to the solution immediately, the reaction was stirred for 30 min at room temperature, and then the mixture was heated at 60 °C. The reaction time was listed in Table 2. The disappearance of the starting material was monitored by TLC (EtOAc/P.E., 1:10, v/v). After removing CuCl, the solvent and other volatiles were removed under reduced pressure. The residue was purified by column chromatography (Et_3N-neutralized silica gel, gradient elution separation with EtOAc/P.E., 1:40–1:20, v/v) to afford the product as colorless or yellow oil.

*4.4.1. 4-Chloro-2-methylthio-6-propylthiopyrimidine (**4a**).* Colorless oil, yield: 1.11 g (81%), IR (film): 2961, 2875, 1732, 1358, 810 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ=6.80 (s, 1H, CH), 3.12 (t, J=7.3 Hz, 2H, SCH_2), 2.52 (s, 3H, SCH_3), 1.72 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.01 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ=172.6, 171.9, 158.7, 113.0, 31.7, 22.6, 14.3, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_8H_{12}ClN_2S_2$: 235.0125; found: 235.0138.

*4.4.2. 4-Chloro-2,6-dipropylthiopyrimidine (**4b**).* Yellow oil, yield: 1.15 g (75%), IR (film): 2965, 2872, 1665, 1361, 811 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.72 (s, 1H, CH), 3.04 (t, J=7.3 Hz, 2H, SCH_2), 3.01 (t, J=7.3 Hz, 2H, SCH_2), 1.67 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.65 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 0.94 (t, J=7.3 Hz, 3H, CH_3), 0.95 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=172.3, 171.7, 158.5, 112.9, 33.0, 31.5, 22.5 (2 carbons), 13.4, 13.3. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{10}H_{16}ClN_2S_2$: 263.0438; found: 263.0447.

*4.4.3. 4-Chloro-6-isopropylthio-2-propylthiopyrimidine (**4c**).* Yellow oil, yield: 1.11 g (72%), IR (film): 2962, 2872, 1700, 1361, 808 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.78 (s, 1H, CH), 4.05 (heptet, J=6.8 Hz, 1H, SCH), 3.10 (t, J=7.3 Hz, 2H, SCH_2), 1.77 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.42 (d, J=6.8 Hz, 6H, SCH(CH_3)$_2$), 1.05 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=172.4, 171.8, 158.6, 112.9, 35.1, 32.9, 22.8 (2 carbons), 22.6, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{10}H_{16}ClN_2S_2$: 263.0438; found: 263.0447.

*4.4.4. 4-Chloro-6-phenylthio-2-propylthiopyrimidine (**4d**).* Yellow oil, yield: 0.90 g (52%), IR (film): 2962, 2872, 1732, 1359, 809, 748, 690 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.61–7.47 (m, 5H, ArH), 6.43 (s, 1H, CH), 2.94 (t, J=7.3 Hz, 2H, SCH_2), 1.62 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 0.95 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=173.7, 172.6, 159.8, 135.8, 130.3, 129.8, 126.9, 111.2, 32.9, 22.3, 13.3. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{13}H_{14}ClN_2S_2$: 297.0281; found: 297.0263.

*4.4.5. 4-Benzylthio-6-chloro-2-propylthiopyrimidine (**4e**).* Yellow oil, yield: 1.49 g (82%), IR: 2962, 2867, 1718, 1361, 808, 774, 698 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.40–7.23 (m, 5H, ArH), 6.83 (s, 1H, CH), 4.44 (s, 2H, PhCH_2), 3.11 (t, J=7.3 Hz, 2H, SCH_2), 1.76 (sextet, J=7.3 Hz,

2H, SCH_2CH_2), 1.03 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=172.6, 170.9, 158.8, 136.3, 128.8, 128.7, 127.5, 112.6, 33.8, 33.0, 22.4, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{14}H_{16}ClN_2S_2$: 311.0438; found: 311.0409.

4.5. General procedure for the synthesis of 6-alkylamino-2,4-dialky(aryl)thiopyrimidines (5a–i)

A solution of 2,4-dialkyl(aryl)thio-6-chloropyrimidine (**4a**–**e**) (0.7 mmol), the appropriate amine (4.2 mmol) and triethylamine (1.4 mmol) in anhydrous ethanol (20 mL) was refluxed for the given time as reported in Table 3. Then the reaction mixture was evaporated in vacuo, and the residue was purified by flash column chromatography (Et_3N-neutralized silica gel, gradient elution separation with EtOAc/P.E., 1:40–1:20, v/v) and then by recrystallization from cyclohexane to afford the product as colorless crystals.

*4.5.1. 2-Methylthio-4-phenethylamino-6-propylthiopyrimidine (**5a**).* Colorless crystals, yield: 187.9 mg (84%), mp 96–98 °C. IR (neat): 3260, 2959, 698, 796, 833 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ=7.34–7.29 (m, 3H, ArH), 7.24–7.18 (m, 2H, ArH), 5.87 (s, 1H, CH), 4.76 (br s, 1H, *NH*), 3.52 (m, 2H, $NHCH_2$), 3.09 (t, J=7.3 Hz, 2H, SCH_2), 2.86 (t, J=6.9 Hz, 2H, $NHCH_2CH_2$), 2.50 (s, 3H, SCH_3), 1.72 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.01 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.4, 167.6, 160.8, 138.5, 128.7, 128.6, 126.5, 94.6, 42.5, 35.4, 31.1, 23.0, 13.9, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{16}H_{22}N_3S_2$: 320.1250; found: 320.1256.

*4.5.2. 2-Methylthio-4-(1-phenylethyl)amino-6-propylthiopyrimidine (**5b**).* Colorless crystals, yield: 163.3 mg (73%), mp 80–82 °C. IR (neat): 3352, 2967, 814, 758, 695 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ=7.39–7.20 (m, 5H, ArH), 5.70 (s, 1H, CH), 5.16 (m, 1H, NH*CH*), 4.67 (br s, 1H, *NH*), 2.98 (t, J=7.3 Hz, 2H, SCH_2), 2.45 (s, 3H, SCH_3), 1.62 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.50 (d, J=6.8 Hz, 3H, NHCH(CH_3)), 0.95 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.2, 168.0, 160.2, 143.3, 128.7, 127.3, 125.6, 94.8, 51.3, 31.1, 23.7, 22.9, 13.8, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{16}H_{22}N_3S_2$: 320.1250; found: 320.1256.

*4.5.3. 4-Benzylamino-2,6-dipropylthiopyrimidine (**5c**).* Colorless crystals, yield: 181.8 mg (85%), mp 66–67 °C. IR (neat): 3250, 2965, 849, 799, 695 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ=7.35–7.27 (m, 5H, ArH), 5.86 (s, 1H, CH), 5.47 (br s, 1H, *NH*), 4.45 (d, J=5.6 Hz, 2H, $NHCH_2$), 3.05 (t, J=7.3 Hz, 2H, SCH_2), 3.04 (t, J=7.3 Hz, 2H, SCH_2), 1.72 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.68 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.00 (t, J=7.3 Hz, 3H, CH_3), 0.99 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=170.1, 167.7, 161.0, 137.9, 128.6, 127.4, 127.2, 94.8, 45.3, 32.6, 31.1, 23.0 (2 carbons), 13.5, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{17}H_{24}N_3S_2$: 334.1406; found: 334.1412.

*4.5.4. 4-(4-Methylbenzyl)amino-2,6-dipropylthiopyrimidine (**5d**).* Colorless crystals, yield: 204.4 mg (84%), mp 92–94 °C. IR (neat): 3249, 2966, 828, 799, 685 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ=7.19 (d, J=8.1 Hz, 2H, ArH), 7.14 (d, J=8.1 Hz, 2H, ArH), 5.88 (s, 1H, CH), 5.32 (br s, 1H, *NH*), 4.42 (d, J=5.2 Hz, 2H, $NHCH_2$), 3.06 (t, J=7.3 Hz, 2H, SCH_2), 3.05 (t, J=7.3 Hz, 2H, SCH_2), 2.35 (s, 3H, $C_6H_4CH_3$), 1.75 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.70 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.02 (t, J=7.3 Hz, 3H, CH_3), 1.03 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=170.2, 167.7, 161.0, 137.1, 134.8, 129.3, 127.2, 94.8, 45.1, 32.6, 31.1, 23.1 (2 carbons), 21.0, 13.5, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{18}H_{26}N_3S_2$: 348.1563; found: 348.1569.

*4.5.5. 4-(3-Methoxyphenethyl)amino-2,6-dipropylthiopyrimidine (**5e**).* Colorless crystals, yield: 222.0 mg (84%), mp 46–48 °C. IR (neat): 3253, 2964, 829, 772, 693 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ=7.26–7.18 (m, 1H, ArH), 6.80–6.74 (m, 3H, ArH), 5.85 (s, 1H, CH), 4.89 (br s, 1H, *NH*), 3.79 (s, 3H, OCH_3), 3.51 (dt, J=6.5 Hz, 2H, $NHCH_2$), 3.08 (t, J=7.3 Hz, 2H, SCH_2), 3.07 (t, J=7.3 Hz, 2H, SCH_2), 2.84 (t, J=6.9 Hz, 2H, $NHCH_2CH_2$), 1.75 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.71 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.03 (t, J=7.3 Hz, 3H, CH_3), 1.02 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ=170.2, 167.5, 160.8, 159.8, 140.1, 129.6, 121.0, 114.5, 111.8, 94.6, 55.1, 42.3, 35.4, 32.6, 31.1, 23.1 (2 carbons), 13.5, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{19}H_{28}N_3OS_2$: 378.1668; found: 378.1675.

*4.5.6. 4-(4-Methoxybenzyl)amino-6-isopropylthio-2-propylthiopyrimidine (**5f**).* Colorless crystals, yield: 244.3 mg (86%), mp 112–113 °C. IR (neat): 3248, 2964, 1169, 803, 743, 679 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ=7.20 (d, J=8.7 Hz, 2H, ArH), 6.85 (d, J=8.7 Hz, 2H, ArH), 5.85 (s, 1H, CH), 5.35 (br s, 1H, NH), 4.37 (d, J=5.7 Hz, 2H, $NHCH_2$), 3.98 (heptet, J=6.8 Hz, 1H, SCH), 3.78 (s, 3H, OCH_3), 3.04 (t, J=7.3 Hz, 2H, SCH_2), 1.73 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 1.36 (d, J=6.8 Hz, 6H, $SCH(CH_3)_2$), 1.00 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.2, 167.6, 161.0, 158.9, 129.8, 128.6, 114.0, 95.1, 55.2, 44.8, 34.3, 32.6, 23.2, 23.1, 13.5. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{18}H_{26}N_3OS_2$: 364.1512; found: 364.1518.

*4.5.7. 4-Cyclohexylamino-6-phenylthio-2-propylthiopyrimidine (**5g**).* Colorless crystals, yield: 208.9 mg (83%), mp 80–81 °C. IR (neat): 3252, 2927, 827, 749, 690 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ=7.61–7.40 (m, 5H, ArH), 5.40 (s, 1H, CH), 4.68 (br s, 1H, NH), 2.92 (t, J=7.3 Hz, 2H, SCH_2), 1.90–1.06 (m, 13H, SCH_2CH_2+cyclohexyl), 0.95 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.6, 169.5, 160.5, 135.8, 129.4, 129.3, 129.1, 94.2, 49.7, 32.7, 32.5, 25.4, 24.6, 22.9, 13.4. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{19}H_{26}N_3S_2$: 360.1563; found: 360.1570.

*4.5.8. 4-Phenethylamino-6-phenylthio-2-propylthiopyrimidine (**5h**).* Colorless crystals, yield: 235.0 mg (88%), mp 98–100 °C. IR (neat): 3251, 2964, 802, 751, 700 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ=7.63–7.09 (m, 10H, ArH), 5.49 (s, 1H, CH), 5.19 (br s, 1H, NH), 3.42 (m, 2H, $NHCH_2$), 2.98 (t, J=7.3 Hz, 2H, SCH_2), 2.80 (t, J=7.2 Hz, 2H, $NHCH_2CH_2$), 1.67 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 0.98 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.2, 161.1, 138.4, 135.9, 129.6, 129.5, 128.9, 128.7, 128.6, 126.6, 94.0, 42.5, 35.4, 32.7, 22.9, 13.5. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{21}H_{24}N_3S_2$: 382.1406; found: 382.1399.

*4.5.9. 4-Benzylthio-6-(4-methoxybenzyl)amino-2-propylthiopyrimidine (**5i**).* Colorless crystals, yield: 239.1 mg (83%), mp 110–112 °C. IR (neat): 3253, 2965, 1175, 833, 712, 696 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ=7.23–7.19 (m, 5H, ArH), 7.20 (d, J=8.7 Hz, 2H, ArH), 6.86 (d, J=8.7 Hz, 2H, $NHCH_2$), 5.88 (s, 1H, CH), 5.01 (br s, 1H, NH), 4.39 (s, 2H, SCH_2), 4.36 (m, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 3.06 (t, J=7.3 Hz, 2H, SCH_2), 1.73 (sextet, J=7.3 Hz, 2H, SCH_2CH_2), 0.99 (t, J=7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.4, 167.0, 161.0, 159.0, 137.7, 129.8, 128.8, 128.6, 128.5, 127.1, 114.1, 94.8, 55.2, 44.9, 33.3, 32.6, 23.0, 13.5. ESI-HRMS: m/z $[M+H]^+$ calcd for $C_{22}H_{26}N_3OS_2$: 412.1512; found: 412.1526.

4.6. In vitro examination for inhibition of platelet aggregation

Blood was sampled from the cubital vein of healthy volunteers without taking aspirin or other nonsteroidal anti-inflammatory drugs for at least 14 days on the basis of informed consent before blood collection. The blood sample was collected in 50 mL sample tubes containing 3.8% sodium citrate (1:9, v/v), and centrifuged at 300 rpm for 20 min to generate platelet-rich plasma (PRP). The residual blood was centrifuged at 900 rpm for 10 min. The

supernatant fraction was called platelet-poor plasma (PPP). Aliquots of 500 μL of PRP were distributed in test cuvettes and inserted into the incubation chamber of an aggregometer (Model 400VS, Chrono-Log, Haverston, PA) at 37 °C. Platelet aggregation was measured on the aggregometer using the PRP fractions after activation by ADP (final concentration 10 μM) according to Born,[25] DMSO was used as a vehicle control and Cangrelor was used as the positive control. The test compounds were dissolved in DMSO (below at 0.5% final concentration) and added to the PRP for 1 min before activation with agonist. The extent of aggregation was quantified by determining the maximum height of the analysis curve. Each sample was allowed to aggregate for at least 3 min. The chart recorder (Model 707, Chrono-Log, Haverston, PA, USA) was set for 1 cm/min. The baseline was set using the PPP fraction as blank. The anti-platelet aggregation activity was expressed as percent inhibition by comparison with that measured in presence of vehicle (DMSO) alone.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.056.

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