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Short communication

Assessment of antiplatelet activity of 2-aminopyrimidines

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ABSTRACT

A series of 4,6-diaryl-2-aminopyrimidines was developed as antiplatelet agents and their potency was evaluated by *in vitro* assay. Compound **14k** was found to be two times more potent than aspirin. These encouraging results could be helpful for the development of new antiplatelet compounds.

1. Introduction

Approximately 40% of morbidity and mortality in western countries is due to atherothrombotic vascular disease [1]. Platelets play a pivotal role in thrombosis and haemeostasis. The major physiological role of platelets is to maintain the integrity of the circulatory system and responds rapidly and robustly at sites of vascular injury. Platelets are activated when brought into contact with agents such as collagen, thrombin and ADP. Such activation leads to a range of responses that play a critical role in arterial thrombosis, including platelet aggregation, dense and α granule secretion and procoagulant activity [2]. Currently, aspirin **1** and thienopyridine drugs (clopidrogel **2a** and ticlopidine **2b**) are widely used antiplatelet agents (Fig. 1). Nearly 50% of the global population are resistant to these drugs resulting in increased risk of recurrent myocardial infarction and stroke, or cardiac related mortality [3,4]. Although recent advances for the treatment of coronary syndromes have been made by implementing dual antiplatelet therapy using a combination of aspirin with a thienopyridine drug during the acute phase and for secondary prevention, the atherothrombotic disease remains a leading cause of morbidity and mortality [5]. The limitations of the currently available antiplatelet agents have triggered the development of newer drugs.

Compounds derived from 2-aminopyrimidine have provided the basis for different medicinal applications (Fig. 2). Cyclohexyl-substituted aminopyrimidine **3** was found to act as a potential JNK inhibitor [6], while the cyano-substituted pyrimidine structure **4** shows remarkable inhibition for the vascular endothelial growth factor receptor 2 (VEGF-R2) [7] and a piperidine-substituted aminopyrrolopyrimidine **5** serves as a Y5 receptor antagonist [8]. Piperazine-substituted 2-aminopyrimidine structure **6** displays histamine H4 receptor properties [9], while compound **7** acts as a reverse-transcriptase inhibitor [10] and benzopyrano-2-aminopyrimidines [11] **8** and 4,5-diaryl-2-aminopyrimidine **9** have been shown to possess antiplatelet property [12].

As a part of our research program, aiming at the discovery of novel diaryl heterocycles based antiplatelet agents, we recently reported the potent antiaggregatory effect of the lead compound which contains a diaryl fragment as a key structural element and 1,4-diazepine as a heterocyclic core [13] **10** (Fig. 3). Encouraged by this finding and taking into account the well-documented antiplatelet activity associated with the 2-aminopyrimidine pharmacophoric unit [11,12], we report the synthesis and preliminary results of antiplatelet activity of the 4,6-diaryl-2-aminopyrimidines **14**.

2. Results and discussion

2.1. Chemistry

The diarylpyrimidine derivatives presented in this paper were prepared according to the synthetic route described in Scheme 1. The 1,3-diketones **12a**–**q** required for the work were obtained from the esters **11a**–**q**, by base-catalyzed Baker-Venkataraman transformation [14]. Condensation of 2-hydroxyacetophenones with various substituted benzoic acids in dry pyridine and $POCl_3$

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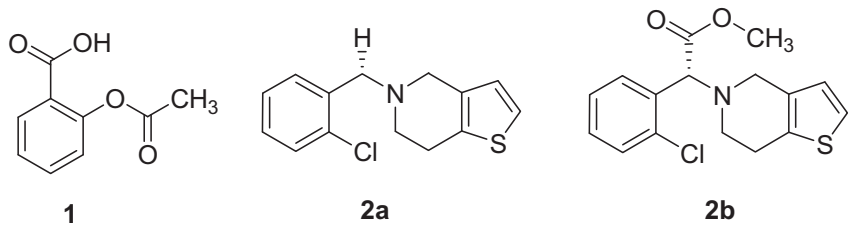

Fig. 1. Clinically used antiplatelet agents.

furnished the esters **11a**–**q**. In the IR spectra, the 1,3-diketones showed absorption bands for C=O in the range 1615–1625 cm^{-1}. Treatment of the diketone with concd H_2SO_4 leads to formation of flavone (**13a**–**q**) [15]. The formation of the flavones **13a**–**q** was confirmed by the appearance of C=O absorption bands at 1640–1660 cm^{-1}. Reaction of the flavones **13a**–**q** with a slight excess of guanidine hydrochloride in alkaline medium afforded 4,6-diaryl-2-aminopyrimidines **14a**–**q** [10].

Compounds **14a**–**q** showed a characteristic peak at 3200 cm^{-1} for O–H stretching, asymmetric and symmetric stretching N–H bands at 3500 and 3350 cm^{-1} respectively in the IR spectrum. In the ^{1}H NMR spectra, NH_2 protons δ 5.1–6.0 and the pyrimidinyl proton (C_5–H) appeared as a sharp singlet at δ 7.3–7.5. The aromatic protons appeared as a multiplet at δ 6.5–8.0. The evidence of partial ring formation due to internal hydrogen bonding of the hydroxyl protons is clearly observed at higher δ 13–14. In the ^{13}C NMR spectra the δ values of the carbons are in conformation with the structures of the compounds synthesized.

2.2. Antiplatelet activity

The newly synthesized compounds were studied for their *in vitro* platelet aggregation inhibitory activity on whole human blood [16]. The study was performed using Whole Blood Aggregometer, Chronolog Corporation, Haverton, PA, USA. The inhibitory activity of seventeen compounds was measured to compare with inhibition induced by standard drug Aspirin (ASA) (10 μg/ml). Each assay was performed three times, taking control and aspirin for comparative assay each time. Whole blood aggregometer measures inhibition of platelet aggregation induced by ADP (10 μM) in ohms which is the resistance produced by accumulation of aggregates on the electrode. The control or normal platelet aggregation was found to be 10.92 ± 1.92 Ω and for aspirin (10 μg/ml) the inhibition of aggregation 6.82 ± 1.21 Ω (37%). The standard range for inhibition of ADP (10 μM) induced aggregation for aspirin is 6–24 Ω. From the comparative study of test compounds (**14a**–**q**) at same concentration of aspirin, eleven compounds were found active as shown in Table 1.

From eleven compounds **14k** is the most potent compound being two times more potent than aspirin. Also **14 (a, d, j, n, o)** produced more than 60% inhibition and **14 (c, g, m** and **q)** found more active than aspirin (Fig. 4).

2.3. Structure–activity relationships

The effect of electron withdrawing and donating groups on the basic scaffold, 4,6-diaryl 2-aminopyrimidine was studied with respect to antiplatelet activity of the synthesized compounds (Table 1). Among all the compounds in the series, **14k** was found to be the most potent inhibitor. This suggests that in the 4′–OCH_3 series; 2″,4″-dichloro substitution favors antiplatelet activity. Dichloro substitution at these positions increases the lipophilic nature of the molecule. This could be one of the reasons for highest % inhibition of platelet aggregation for compound **14k**. Compounds with mono substituted chlorine at 4″ position and methyl at 3″ position (**14h** & **14i** respectively) showed drastic decrease in activity whereas 3″-chloro substituted compound (**14j**) showed higher % inhibition than that of **14h** & **14i**. In case of 5′–OCH_3 series, substitution of chlorine at 3″ position retained similar activity (**14d**) as that of 4′–OCH_3 series. Surprisingly fluorine at 4″ position has shown diminished activity (**14e**). Replacement of phenyl ring with a heterocyclic furan ring has shown complete loss of activity (**14f**).

3. Conclusions

To summarize, design and synthesis of a series of 4,6-diaryl-2-aminopyrimidines was carried out, in which several electron withdrawing, donating as well as bulky functions were introduced to replace the phenyl ring substitutions. Biological evaluation

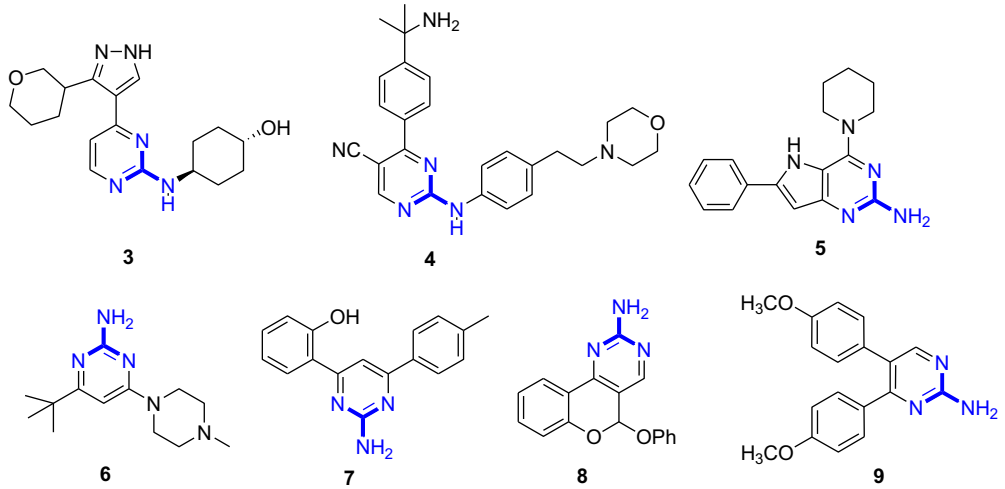

Fig. 2. Biologically interesting 2-aminopyrimidine compounds.

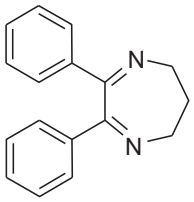

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Fig. 3. General structure of the diaryl-1,4-diazepine.

indicated that some compounds showed moderate to potent antiplatelet activities which will be further helpful for the development of new antiplatelet compounds.

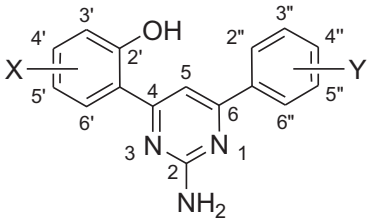
4. Experimental

Melting points were determined in capillaries using Toshniwal melting point apparatus and are uncorrected. IR (in cm^{-1}) spectra in KBr pellets on a Shimadzu 8300 instrument; and ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II spectrometer (400 MHz), using tetramethylsilane as an internal standard. Chemical shift data are reported in parts per million (δ in ppm) where s, br, t and m designate singlet, broad, triplet and multiplet, respectively. Elemental analyses were recorded on a Perkin Elmer PE 2400 CHNS analyzer. Mass spectra were recorded on APISciEX mass spectrometer equipped with an electrospray ionization (ESI) interface. Column chromatography was carried out using silica gel (100–200 mesh). Thin-layer chromatography (TLC) was performed on precoated Silica gel Merck plates. Compounds were visualized by illuminating with UV light (254 nm) or exposure to iodine vapors. All chemicals and solvents were of reagent grade and were purified and dried using standard methods. Flavones **13a**–**q** was prepared according to literature method [15].

4.1. General method for the preparation of 4,6-diaryl-2-aminopyrimidine derivatives (*14a*–*q*)

A mixture of the respective flavone **13a**–**q** (0.0019 mol), guanidine hydrochloride (0.01 mol) and potassium hydroxide (1.0 g) was refluxed in methanol (30 mL) for 4–6 h. After the completion of the reaction, the mixture was poured on to crushed ice containing acetic acid. The yellow solid obtained was filtered, washed with water and recrystallized from methanol to give **14a**–**q**.

Table 1
Structure and activity of compounds **14a**–**q**.



14a-q

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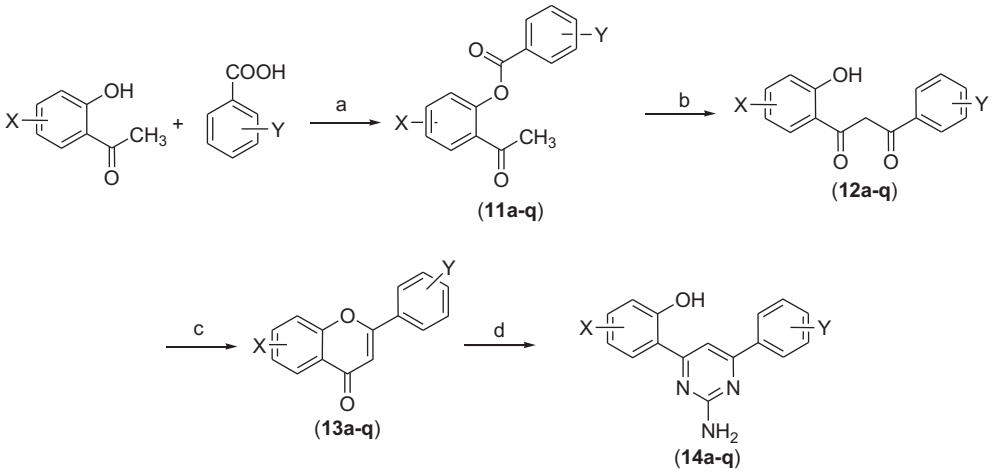
Comp. no.	X	Y	% Inhibition of platelet aggregation
14a	5′-OCH_3	4″-OCH_3	64.28
14b	5′-OCH_3	3″-OCH_3	ND[a]
14c	5′-OCH_3	2″-Cl	55.13
14d	5′-OCH_3	3″-Cl	66.85
14e	5′-OCH_3	4″-F	8.79
14f	5′-OCH_3	2-furyl	1.46
14g	4′-OCH_3	H	42.67
14h	4′-OCH_3	4″-Cl	8.42
14i	4′-OCH_3	3″-CH_3	23.08
14j	4′-OCH_3	3″-Cl	65.84
14k	4′-OCH_3	2″,4″-Cl_2	77.47
14l	4′-OCH_3	4″-OCH_3	ND[a]
14m	4′-OCH_3	3″-OCH_3	51.19
14n	4′-OCH_3	4″-F	64.01
14o	4′-OCH_3	4″-CH_3	62.82
14p	4′-OCH_3	4″-NO_2	58.52
14q	5′-OC_2H_5	H	39.92
Aspirin	–	–	37.55

ND[a]:Not determined.

4.1.1. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(4″-methoxyphenyl)-2-aminopyrimidine (*14a*)

Yield 29%, Mp. 184–185 °C (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3520, 3368, 3200, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 3.86 (s, 6H, OCH_3), 5.48 (s, 2H, NH_2), 7.24–7.26 (d, $J = 7.2$ Hz, 2H, ArH), 7.30–7.32 (d, $J = 7.6$ Hz, 2H, ArH), 7.36–7.38 (d, $J = 8.4$ Hz, 2H, ArH), 7.49 (s, 1H, ArH), 8.06 (s, 1H, pyri). MS *m/z*; 324.05 (M+1). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.0. Found: C, 67.04; H, 5.28; N, 13.10.



Scheme 1. Synthesis of 4,6-diaryl-2-aminopyrimidines. Reagents and conditions: (a) $POCl_3$, dry pyridine, rt, 2 h; (b) fused KOH, dry pyridine, rt, 2 h; (c) $_AcO_H$, catalytic H_2SO_4, 100 °C, 1 h; (d) Guanidine hydrochloride, KOH, MeOH, reflux, 4–6 h.

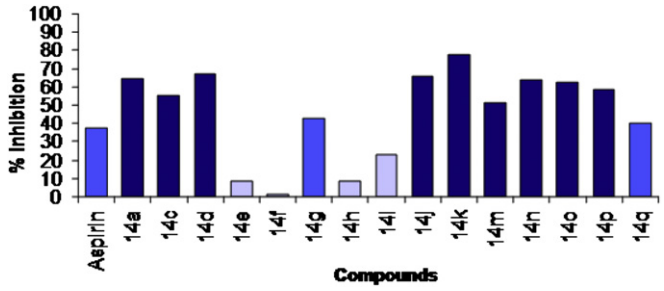


Fig. 4. Graphical illustration of percentage inhibition of platelet aggregation of the test compounds **14a**–**q** and aspirin.

*4.1.2. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(3″-methoxyphenyl)-2-aminopyrimidine (**14b**)*

Yield 54%, Mp. 158–160 °C (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3430, 3320, 3200, 1590 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 3.84 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.18 (s, 2H, NH_2), 6.95–6.97 (m, 1H, ArH), 7.0–7.02 (m, 1H, ArH), 7.05–7.07 (m, 1H, ArH), 7.34–7.35 (d, 1H, ArH), 7.40–7.44 (m, 1H, ArH), 7.48 (s, 1H, pyri), 7.59–7.61 (m, 2H, ArH), 13.19 (br, 1H, OH). MS *m/z*; 324.1 (M + 1). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.0. Found: C, 66.78; H, 5.42; N, 13.04.

*4.1.3. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(2″-chlorophenyl)-2-aminopyrimidine (**14c**)*

Yield 52%, Mp. 163–165 °C (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3480, 3310 1590 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.81 (s, 3H, OCH_3), 5.24 (s, 2H, NH_2), 6.97 (s, 1H, ArH), 7.0–7.02 (m, 1H, ArH), 7.39–7.42 (m, 3H, ArH), 7.50–7.52 (m, 1H, ArH), 7.60–7.62 (m, 1H, ArH), 7.83 (s, 1H, pyri), 13.09 (br, 1H, OH); MS *m/z*; 328.1 (M + 1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.18; 4.42; N, 12.76.

*4.1.4. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(3″-chlorophenyl)-2-aminopyrimidine (**14d**)*

Yield 70%, Mp. 178–179 °C (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3456, 3352, 1639, 1571 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.85 (s, 3H, OCH_3), 5.26 (s, 2H, NH_2), 7.04–7.26 (m, 3H, ArH), 7.32–7.37 (m, 2H, ArH), 7.53 (s, 1H, pyri), 7.98–8.12 (m, 2H, ArH), 13.12 (br, 1H, OH); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 56.13, 102.10, 111.48, 117.36, 119.37, 119.84, 125.27, 127.38, 130.08, 130.78, 135.01, 139.14, 152.24, 154.94, 160.79, 165.16, 166.00; MS *m/z*; 328.1 (M + 1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.24; 4.25; N, 12.92.

*4.1.5. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(4″-flurophenyl)-2-aminopyrimidine (**14e**)*

Yield 56%; Mp. 202–204 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3410, 3220, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.85 (s, 3H, OCH_3), 5.16 (s, 2H, NH_2), 6.95 (s, 1H, ArH), 7.0–7.03 (m, 1H, ArH), 7.17–7.19 (d, J = 6.68, 2H), 7.33–7.44 (m, 1H, ArH), 7.45 (s, 1H, pyri) 8.06–8.08 (d, J = 6.8, 2H), 13.16 (s, 1H, OH); MS *m/z*; 312.1 (M + 1); Anal. Calcd for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.78; H, 4.64; N, 13.46.

*4.1.6. 4-(2′-hydroxy-5′-methoxyphenyl)-6-(2″-furyl)-2-aminopyrimidine (**14f**)*

Yield 48%, Mp. 216–217 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3460, 3310, 3220, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.85 (s, 3H, OCH_3), 5.12 (s, 2H, NH_2), 6.58–6.60 (m, 1H, furan) 6.94–6.96 (m, 1H, furan), 7.0–7.02 (m, 1H, ArH) 7.22–7.23 (m, 1H, ArH) 7.33–7.34 (m, 1H, ArH), 7.46 (s, 1H, pyri), 7.62–7.63 (m, 1H, furan), 13.18 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 56.09, 100.00, 111.32, 112.39, 112.48, 117.34, 119.33, 119.97, 145.02, 151.89, 152.20, 154.99, 157.27, 160.59, 165.80; MS *m/z*; 284.1 (M + 1); Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.64; H, 4.52; N, 14.98.

*4.1.7. 4-(2′-hydroxy-4′-methoxyphenyl)-6-phenyl-2-aminopyrimidine (**14g**)*

Yield 24%, Mp. 186–189 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3508, 3354, 1625 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H, OCH_3), 5.17 (s, 2H, NH_2), 6.57 (m, 2H, ArH), 7.29–7.32 (m, 2H, ArH), 7.37 (s,1H, pyri.), 7.54–7.57 (m, 4H, ArH), 13.84 (s, 1H, OH); MS *m/z*; 294.75 (M + 1); Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.64; H, 5.02; N, 14.28.

*4.1.8. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(4″-chlorophenyl)-2-aminopyrimidine (**14h**)*

Yield 33%, Mp. 215–217 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3494, 3365, 2923, 1618 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.89 (s, 3H, OCH_3), 5.19 (s, 2H, NH_2), 6.59 (s, 1H, ArH), 7.29–7.30 (m, 2H, ArH), 7.32–7.34 (d, J = 7.6, 2H, ArH), 7.40 (1H, Pyri), 7.42–7.44 (d, J = 7.4, 2H, ArH); MS *m/z*; 328.76 (M + 1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.35; H, 4.22; N, 13.02.

*4.1.9. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(3″-methylphenyl)-2-aminopyrimidine (**14i**)*

Yield 26%, Mp. 230–232 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3498, 3281, 3173, 1618 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.45 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 5.94 (s, 2H, NH_2), 6.47–6.51 (m, 3H, ArH), 7.36–7.39 (m, 3H, ArH), 7.46 (1H, pyri), 7.80–7.86 (m, 1H, ArH), 14.22 (br, 1H, OH); MS *m/z*; 308.16 (M + 1); Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.36, H, 5.52; N, 13.49.

*4.1.10. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(3″-chlorophenyl)-2-aminopyrimidine (**14j**)*

Yield 35%, Mp. 189–190 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3425, 3310, 3173, 1618 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 3.86 (s, 3H, OCH_3), 5.37 (s, 2H, NH_2), 6.51–6.54 (m, 2H, ArH), 7.40 (s, 1H, pyri), 7.44–7.48 (m, 2H, ArH), 7.77–7.79 (d, J = 8.6, 1H, ArH), 7.92–7.94 (d, J = 8.92, 1H, ArH), 8.04 (s, 1H, ArH), 13.74 (br, 1H, OH); MS *m/z*; 328.76 (M + 1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.24; H, 4.44; N, 12.72.

*4.1.11. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(2″,4″-dichlorophenyl)-2-aminopyrimidine (**14k**)*

Yield 54%, Mp. 204–206 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3649, 3575, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.84 (s, 3H, OCH_3), 5.15 (s, 2H, NH_2), 6.48–6.51 (m, 2H, ArH), 7.33 (s,1H, pyri), 7.36–7.38 (dd, J = 8.36, 1H, ArH), 7.52–7.53 (m, 1H, ArH), 7.56–7.58 (d, J = 8.32, 1H, ArH), 7.67–7.69 (dd, J = 9.52, 1H, ArH), 13.19 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 55.48, 101.89, 105.84, 107.69, 110.34, 127.60, 128.66, 130.28, 131.79, 132.94, 135.56, 136.12, 160.05, 163.19, 163.90, 164.05, 165.55; MS *m/z*; 362.1 (M + 1); Anal. Calcd for $C_{17}H_{13}Cl_2N_3O_2$: C, 56.37; H, 3.62; N, 11.60. Found: C, 56.48; H, 3.46; N, 11.72.

*4.1.12. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(4″-methoxyphenyl)-2-aminopyrimidine (**14l**)*

Yield 35%, Mp. 223–225 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3410, 3300 (NH_2), 3173.9 (OH), 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.15 (s, 2H, NH_2), 6.54–6.57 (m, 2H, ArH), 6.60 (s, 1H, ArH), 7.35 (s, 1H, pyri), 7.36–7.37 (d, J = 7.8, 2H, ArH), 7.42–7.43 (d, J = 7.84, 2H, ArH); MS

m/z; 324.13 (M + 1); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.78; H, 5.21, N, 13.0.

4.1.13. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(3″-methoxyphenyl)-2-aminopyrimidine (**14m**)

Yield 22%, Mp. 186–188 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3390, 3260, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.09 (s, 2H, NH_2), 6.46–6.48 (m, 1H, ArH), 6.49–6.51 (m, 1H, ArH), 7.01–7.04 (m, 1H, ArH), 7.38–7.42 (m, 2H, ArH), 7.56 (s, 1H, pyri), 7.61–7.63 (m, 1H, ArH), 7.81 (d, 1H, ArH), 14.25 (s, 1H, OH); MS *m/z*; 324.1 (M + 1); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.90; H, 5.24, N, 13.02.

4.1.14. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(4″-fluorophenyl)-2-aminopyrimidine (**14n**)

Yield 25%, Mp. 205–207 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3500, 3380, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H, OCH_3), 5.12 (s, 2H, NH_2), 6.50–6.53 (m, 2H, ArH), 7.16–7.18 (d, J = 8.4 Hz, 2H, ArH), 7.39 (s, 1H, pyri), 7.75–7.78 (m, 1H, ArH), 8.04–8.06 (d, J = 8.84 Hz, 2H, ArH), 14.08 (br, 1H, OH); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 55.44, 100.91, 101.90, 107.47, 110.56, 115.78, 116.00, 128.35, 129.20, 129.28, 133.25, 160.22, 163.08, 163.28, 163.80, 164.47, 165.87; MS *m/z*; 312.1 (M + 1); Anal. Calcd for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.67; H, 4.56; N, 13.52.

4.1.15. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(4″-methylphenyl)-2-aminopyrimidine (**14o**)

Yield 16%, Mp. 195–196 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3400, 3390, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.44 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 5.94 (s, 2H, NH_2), 6.47–6.51 (m, 2H, ArH), 7.36–7.39 (m, 2H, ArH), 7.46 (s, 1H, pyri), 7.80–7.86 (m, 3H, ArH), 13.96 (s, 1H, OH); MS *m/z*; 308.1 (M + 1); Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.36, H, 5.50; N, 13.48.

4.1.16. 4-(2′-hydroxy-4′-methoxyphenyl)-6-(4″-nitrophenyl)-2-aminopyrimidine (**14p**)

Yield 35%, Mp. 290 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3490, 3310, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H, OCH_3), 5.5 (s, 2H, NH_2), 6.56–6.58 (m, 2H, ArH), 7.45 (s, 1H, pyri), 7.52–7.53 (m, 1H, ArH), 8.05–8.07 (d, J = 8.0 Hz, 2H, ArH), 8.21–8.23 (d, J = 8.2 Hz, 2H, ArH), 14.12 (s, 1H, OH); MS *m/z*; 339.1 (M + 1); Anal. Calcd for $C_{17}H_{14}N_4O_4$: C, 60.35; H, 4.17; N, 16.56. Found: C, 60.32; H, 4.18; N, 16.36.

4.1.17. 4-(2′-hydroxy-5′-ethoxyphenyl)-6-phenyl-2-aminopyrimidine (**14q**)

Yield 42%, Mp. 148–150 °C, (Yellow crystals from MeOH), IR (KBr) ν_{max}: 3410, 3390, 3173, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.42–1.45 (t, J = 13.96 Hz, 3H, CH_3), 4.12–4.16 (q, J = 13.96 Hz, 2H, CH_2), 5.86 (s, 2H, NH_2), 6.82 (s, 1H, pyri), 6.96–7.01 (m, 3H, ArH), 7.91–7.94 (m, 3H, ArH), 8.03–8.06 (m, 2H, ArH); MS *m/z*; 308.2 (M + 1); Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.34; H, 5.50; N, 14.00.

4.2. Antiplatelet activity

Platelet aggregation experiments were carried out using Chrono-Log model 592VS dual channel whole blood aggregometer obtained from Chrono-Log Corporation (Havertown, PA, USA). The whole blood aggregation assay was conducted using the electrical impedance method. 450 μl of pre-warmed (37 °C) whole blood sample was diluted with an equal volume of pre-warmed PBS (pH 7.4). The diluted blood sample was stirred at a constant speed of 1000 rpm and maintained at 37 °C throughout the experiment. The diluted blood sample was equilibrated for at least 2 min until a stable baseline reading was achieved and 10 μl of test sample (or control) was added. After 2 min incubation, 10 μl of ADP (10 μM of final concentration) was added to initiate the aggregation. The aggregation was monitored for 6 min and the results were analyzed using Aggro/link version 4.75 software. The impedance value (in ohms) was determined from the impedance graph, starting from the lowest point after the start of reaction to the end of the 6 min time interval. The blood samples were used within 4 h after collection. To ensure consistent platelet function during the experimental period, controls were analyzed at regular intervals between the test samples. The results of antiplatelet activity are given in Table 1. Percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(A - B)}{A} \times 100$$

where A is maximal impedance value (in ohms) obtained in the presence of control and B is maximal impedance value (in ohms) obtained in the presence of sample.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.01.035.

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