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European Journal of Medicinal Chemistry 42 (2007) 1144–1150
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Short communication

Novel synthetic approach to *N*-aryl-4-(3-pyridyl)thiazol-2-amine and analogues using HMCM-41 as catalyst, and their biological evaluation as human platelet aggregation inhibitors

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Received 14 June 2006; received in revised form 3 January 2007; accepted 15 January 2007
Available online 27 January 2007

Abstract

A novel synthetic approach to *N*-aryl-4-(3-pyridyl)thiazol-2-amine and analogues using HMCM-41, a mesoporous aluminosilicate catalyst and their *in vitro* ADP-induced platelet aggregation inhibitory activity on human blood platelets is described. Among the test compounds *N*-(2′-flourophenyl)-4-(3-pyridyl)thiazol-2-amine (**9e**) was found to be the most potent, $IC_{50} = 4.84 \times 10^{-7}$ M.

Keywords: *N*-Aryl-4-(3-pyridyl)thiazol-2-amine and analogues; HMCM-41; Antiplatelet aggregation activity

1. Introduction

Platelets play a major role in development of thromboembolic disease [1], myocardial infarction, stroke, unstable angina [2], cardiovascular diseases [3], and ischemic disease [4]. Till date, several compounds possessing platelet aggregation inhibitory activity have been discovered [5]. To the best of our knowledge, aspirine **1** [6], E-5510 **2** [7], iloprost **3** [8], CV-4151 **4** [9], 2-(2′-fluorophenyl)-5-methyl-4-(3-pyridyl)imidazole **5** [10], **6a–l** series [11] and (amino benzamidino) succinyl (ABAS) series of orally active fibrinogen receptor antagonists [12], abciximab, a human–mouse monoclonal antibody for intravenous infusion, binding to glycoprotein IIb/IIIa receptor on human platelets [13] are a few (Fig. 1). However, the mechanism of drugs on inhibition varies based on the inhibitory capability of a particular enzyme [14]. For example, aspirin **1** inhibits cyclooxygenase [15], ozagrel inhibits thromboxane synthetase [16], ticlopidine activates adenylate cyclase [17], dipyridamoles and cilostazole inhibit phosphodiesterase [18] and the above-mentioned compounds have been investigated for their application to ischemic disease. In addition, there are a few ADP-induced platelet aggregation antagonists such as clopidogrel [19], 4-(4-amidinophenoxy) butanoyl aspartyl valine monohydrate [20], isoflurane [21] and propofol [22], 2-[4-[*N*-(5,6-diphenyl pyrazin-2-yl)-*N*-methylamino]-butoxy] acetic acid [23], *N*-(6-ethoxybenzo thiazol-2-yl)-2-(8-ethoxy-4-hydroxy-2,2-dioxo[1,2,4]thiadiazino[3,4-*b*]benzothiazol-3-yl)-2-oxoethane-1-sulfonamide [24], *cis*-3-(3,5-diflourophenyl)-2-(4-flourophenyl-4-hydroxy-5-methyl-4-phenyl-2-cyclopenten-1-one isomer A [25], P1-[N^6-[*N*-(4-flourophenyl)carbamoyl]-2′-*O*,3′-*O*-(2-phenylethylidene) cytidin-5′-yl]-P4-[2′-*O*,3′-(2-phenylethylidene) uridin-5′-yl]-tetra phosphate [26], 2(*S*)-(methoxalylamino)-3-[1-[3-(4-piperidinyl)propionyl]-piperidin-3(*R*)-yl-carboxamido] propionic acid [27], 1-[4-(6-ethylthieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl]-2-phenyl ethanone triflouro acetate [28]. Among them, aspirin has been studied most extensively. However, due to irreversible inhibition of cyclooxygenase, aspirin inhibits not only synthesis of thromboxane A_2 (TXA) in platelets, but also prostaglandin I_2 in vascular endothelial cells and as a result, aspirin induces stomach ulcers [29]. Keeping pharmaceutical potential of 2-(2′-flourophenyl)-5-methyl-4-(3-pyridyl)-imidazole **5** and **6a–l** series in view as most potent antiplatelet

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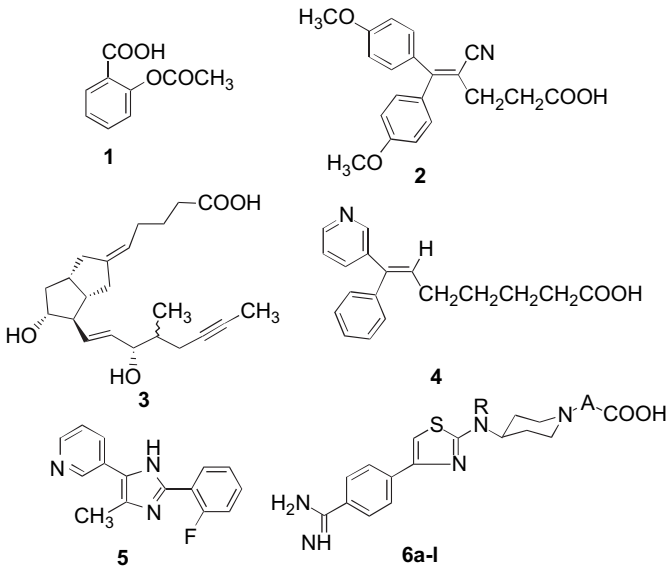
0223-5234/$ - see front matter © 2007 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2007.01.016



6a R = H; A = CH_2	**6g** R = H; A = $CH(2\text{-}ClC_6H_4)$
6b R = H; A = $CHCH_3$	**6h** R = CH_3; A = CH_2
6c R = H; A = $C(CH_3)_2$	**6i** R = $CH_2C_6H_5$; A = CH_2
6d R = H; A = $CHCH_2COOH$	**6j** R = CH_2COOH; A = CH_2
6e R = H; A = CHC_6H_5	**6k** R = $(CH_2)_2COOH$; A = CH_2
6f R = H; A = CH_2CH_2	**6l** R = $(CH_2)_3COOH$; A = CH_2

Fig. 1.

aggregation agents, it was planned to design new chemical entities with similar skeletal backbone i.e., pyridyl ring at 4-position of the thiazole system and various aryl, naphthyl and benzyl substituents on 2-amino group of the thiazole ring in order to unravel their ADP-induced antiplatelet aggregation activity on human blood platelets.

2. Chemistry

In the present approach, *N*-aryl-4-(3-pyridyl)thiazol-2-amine and analogues **9a–j** were obtained by HMCM-41 catalyzed cyclocondensation of 3-(α-bromo)-acetylpyridine hydrobromide (**7**) and *N*-arylthioureas **8a–j** based on well known Hantzsch-thiazole synthesis [30]. 3-(α-Bromo)-acetylpyridine hydrobromide (**7**) was obtained by bromination of 3-acetylpyridine using bromine and hydrobromic acid in acetic acid [31]. *N*-Arylthioureas **9a–g**, *N*-naphthylthiourea **9i** and *N*-benzylthiourea **9h** were prepared by treatment of an equimolar proportions of commercially available primary arylamines, 1-naphthylamine and benzylamine, respectively, with ammonium thiocyanate in 15% of the aqueous hydrochloric acid at 100 °C [32]. The condensation catalyst, HMCM-41, an acidic mesoporous crystalline aluminosilicate has unique physical properties which made these materials highly efficient for catalytic applications [33]. The high specific surface area is conductive to high catalytic activity. The large pore size of the cavities allows the fixation of large reactants, thus enables reaction involving bulky groups to take place, i.e., shape selectivity. Being acidic in nature, HMCM-41 protonates the carbonyl group of 3-(α-bromo)-acetylpyridine, resulting in an ionic intermediate which on nucleophilic attack by amino group of arylthiourea followed by loss of HMCM-41, dehydrobromination and dehydration in cyclocondensation furnish 2-amino-4-(3-pyridyl)-thiazole analogues **9a–j**. Depicted mechanism is given below [33f].

Mechanism

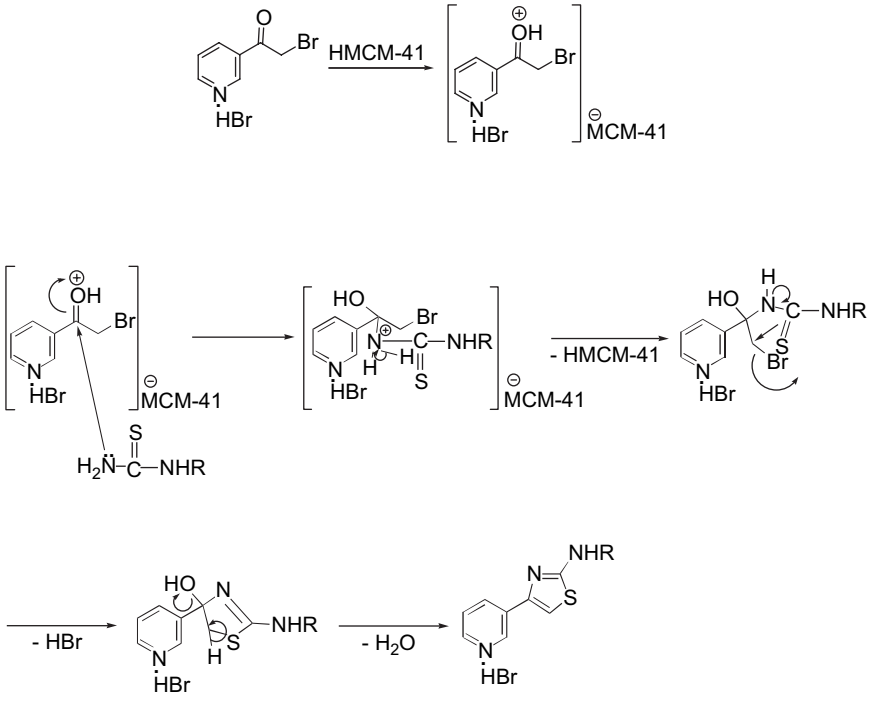


Table 1
Difference between the cyclocondensation of 3-(α-bromo)-acetylpyridine hydrobromide (**7**) with thioureas **8a–j** in presence of HMCM-41 and MeOH reflux alone

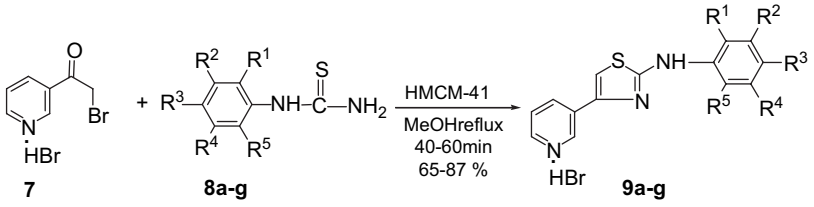
Compound	Yield (%) (reaction time) in presence of HMCM-41	Yield (%) (reaction time: 4 h) in MeOH alone
9a	87 (45 min)	60
9b	80 (45 min)	71
9c	85 (55 min)	72
9d	71 (60 min)	67
9e	85 (45 min)	68
9f	65 (55 min)	53
9g	72 (50 min)	69
9h	70 (45 min)	58
9i	63 (45 min)	50
9j	92 (55 min)	73

Thus, HMCM-41 can be recycled without any loss of shape selectivity. The use of HMCM-41 improved the yields of condensation products by 3–27%, with 45–60 min reaction time where as the reaction time for condensation in methanol by a standard procedure [11] was 4 h with 50–73% yields (Table 1). The formation of **9a** was identified by its ^{1}H NMR and mass spectroscopy. In ^{1}H NMR, the singlet for CH_2 protons of 3-(α-bromo)-acetylpyridine hydrobromide was disappeared at δ 5.10. Further, **9a** showed a singlet at δ 7.70 corresponding to CHS proton and phenyl protons appeared as multiplet between δ 7.00 and 7.34. Further, EIMS showed a stable molecular ion peak at *m*/*z* 253, confirming the structure of **9a**. Similarly, **9b–i** were identified. In the oxidation of **9j**, formation of **10** was confirmed by its IR spectrum which showed characteristic absorption band corresponding to S=O at ~1100 cm^{-1} (S=O str) (Schemes 1 and 2).

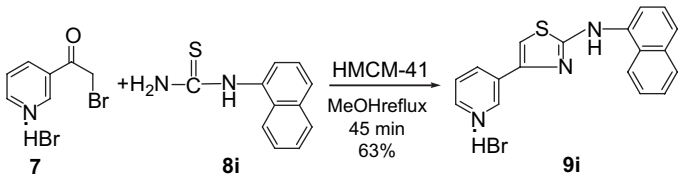
3. Results and discussion

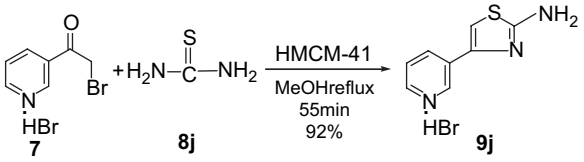
The *N*-aryl-4-(3-pyridyl)thiazol-2-amine analogues **9a–j** and **10** were tested for their *in vitro* ADP-induced platelet aggregation inhibitory activity on human blood platelets. All the test compounds showed inhibitory effect on platelet aggregation in a concentration dependant manner. Among the test compounds, *N*-(2′-flourophenyl)-4-(3-pyridyl)thiazol-2-amine (**9e**) was found to be the most potent, IC_{50} 4.84×10^{-7} M. The results showed that lipophilicity attributed to the decreased platelet aggregation inhibitory activity. However, the mechanism of inhibition is not known.

Since the sulfoxide **10** showed IC_{50} 1.41×10^{-6} M, the synthesis of sulfoxides of the other analogues [34] has been proposed to prepare and investigate for their ADP-induced platelet aggregation inhibitory activity (Fig. 2; Table 2).

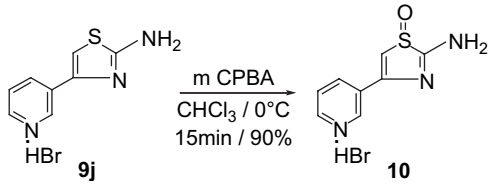


9a : $R^1 = R^2 = R^3 = R^4 = R^5 = H$;
9b : $R^1 = R^2 = R^4 = R^5 = H$; $R^3 = CH_3$
9c : $R^1 = R^2 = R^4 = R^5 = H$; $R^3 = NO_2$
9d : $R^2 = R^3 = R^4 = R^5 = H$; $R^1 = C_2H_5$
9e : $R^2 = R^3 = R^4 = R^5 = H$; $R^1 = F$
9f : $R^1 = R^2 = R^4 = R^5 = H$; R^3 = n-butyl
9g : $R^2 = R^3 = R^4 = H$; $R^1 = R^5 = C_2H_5$





Scheme 1.



Scheme 2.

3.1. Structure–activity relationships

The SAR studies of the *N*-aryl-4-(3-pyridyl)thiazol-2-amine derivatives were established through the structural analysis of the compounds that differ in the functionalisation.

Among the series, *N*-(2′-flourophenyl)-4-(3-pyridyl)thiazol-2-amine (**9e**) was found to be most active. This compound is flanked by flourine at R^1. The high electronegativity of the flourine, relatively of small size, and its ability to form hydrogen bond facilitating hydrophilicity might be contributing for the enhanced activity. There are structural similarities of **9e** with most potent compound **5** which has imidazole instead of thiazole system flanked by 2-flourophenyl ring. Compound **9a** without any substitution on phenyl ring showed moderate activity. But **9d** showed better inhibition than **9a** indicating lipophilicity and steric factors on introducing one ethyl group at R^1. However, substitution of two ethyl groups at R^1 and R^5 in **9g** did not substantially improve potency. On the other hand amine directly attached to naphthyl system **9i**, drastically decreased the activity.

Compound **10** showed good inhibitory activity but less than **9d**. This suggests that NH_2 flanked by aryl substituents are shown to exhibit competitive activity. Introduction of methyl group at R^3 of **9b** or NH_2 directly linked to benzyl system **9h** resulted in almost equivalent or less potent derivatives.

Examination of the biological activities of these compounds indicates that the variation of the substituents on aromatic system in terms of position, bulkiness and electronegativity resulted in the variation of inhibitory activity on platelet aggregation. Introduction of substituents at R^1 or R^5 showed good activity compared to the substituents at R^3 position. Therefore, it is theoretically assumed that **9c** with NO_2 group at R^3 might not be encouraging due to electron withdrawing nature of the nitro group. Similarly *n*-butyl group at R^3 theoretically increases lipophilicity and steric factors compared to methyl substituent in **9b**. Results showed that substitution at R^3 did not exhibit encouraging activity profile. Substitution of amine group with aryl or cycloalkyl ring systems exhibited moderate to good activity like in compounds **5** and **6a–l**. This is also proven by experimental efforts in case of compound **10**. Therefore, compound **9j** might not be an encouraging target. On the whole, the small size of flourine at favorable position and high electronegativity in compound **9e** proved to be the most potent among the analogues.

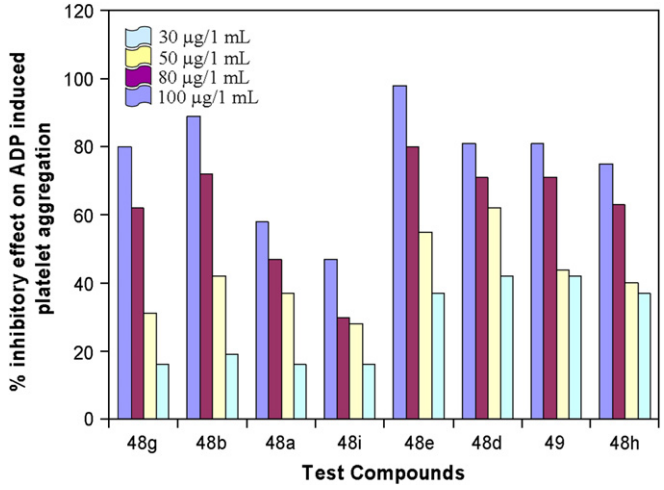


Fig. 2. Percent inhibitory effect of the test compounds at four different concentrations on ADP-induced human platelet aggregation.

Table 2
The ADP-induced antiplatelet aggregation activity of the test compounds **9a–j** and **6a–l** which were carried out under identical conditions [11]

Entry	Inhibition of platelet aggregation[a] IC_{50} (M)
9a	1.45×10^{-6}
9b	2×10^{-6}
9d	1.35×10^{-6}
9e	4.84×10^{-7}
9g	2.19×10^{-6}
9h	2.1×10^{-6}
9i	2.89×10^{-6}
10	1.41×10^{-6}
6a	0.042×10^{-6}
6b	0.25×10^{-6}
6c	$>10 \times 10^{-6}$
6d	0.085×10^{-6}
6e	0.065×10^{-6}
6f	1.8×10^{-6}
6g	6×10^{-6}
6h	0.16×10^{-6}
6i	0.23×10^{-6}
6j	0.17×10^{-6}
6k	0.046×10^{-6}
6l	0.096×10^{-6}

[a] *In vitro* inhibition of ADP-induced (2.5 μM) human platelet aggregation.

4. Experimental section

4.1. Chemistry

All chemicals were reagent grade and used as purchased. All the solvents were purified using standard literature procedures. Column chromatography was performed using ACME silica gel (100–200 mesh). ^{1}H NMR (200 MHz, DMSO-d_6) spectra were recorded on a Varian Gemini 200 spectrometer and the chemical shifts were expressed in δ (ppm) using TMS (tetra methyl silane) as an internal standard. Mass spectra were recorded on VG 70-70H spectrometer (SHRADER Laboratories Inc). Progress of the reaction was monitored by TLC. ^{1}H NMR refers to hydrobromide salts while elemental analysis refers to the free bases of the analogues.

*4.1.1. Typical procedure for the preparation of hydrobromide salt of N-phenyl-4-(3-pyridyl)thiazol-2-amine (**9a**)*

A mixture of 3-(α-bromo)-acetylpyridine hydrobromide (**7**) (2.81 g, 10 mmol), phenyl thiourea **9a** (1.52 g, 10 mmol) and a catalytic amount of HMCM-41 catalyst in methanol (15 mL) was allowed to reflux for 45–60 min. After the reaction was complete (TLC), solvent was filtered giving methanol washings (50 mL) to remove HMCM-41 catalyst. Combined filtrate was evaporated to afford a residue which was purified by column chromatography using ethyl acetate:pet ether (1:9) to furnish hydrobromide salt of 2-phenylamino-4-(3-pyridyl)thiazole (**9a**) as a solid. Similarly, **9b–j** were prepared.

*4.1.2. Hydrobromide salt of N-phenyl-4-(3-pyridyl)thiazol-2-amine (**9a**)*

Yield 87%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.00 (m, 1H), 7.15–7.32 (m, 4H), 7.53 (m, 1H), 7.70 (s, 1H), 7.65 (m, 1H), 8.70 (m, 1H), 9.01 (s, 1H), 10.19 (br s, 1H); MS (EI): *m/z* 253 (M^+, 100%), 252 ($M^+ - 1$, 70%); mp 168 °C; Anal. Calcd for $C_{14}H_{11}N_3S$: C, 66.38; H, 4.38; N, 16.59. Found: C, 66.57; H, 4.50; N, 16.78.

*4.1.3. Hydrobromide salt of N-(p-toluyl)-4-(3-pyridyl)-thiazol-2-amine (**9b**)*

Yield 80%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.25 (s, 3H), 7.09 (d, $J = 8.4$ Hz, 2H), 7.50 (d, $J = 8.7$ Hz, 2H), 7.65 (m, 1H), 8.02 (m, 1H), 8.80 (m, 1H), 8.95 (m, 1H), 9.42 (s, 1H), 10.1 (s, 1H); MS (EI): *m/z* 267(M^+, 100%), 266 ($M^+ - 1$, 50%); mp 155 °C; Anal. Calcd for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72. Found: C, 67.58; H, 4.93; N, 15.80.

*4.1.4. Hydrobromide salt of N-(p-nitrophenyl)-4-(3-pyridyl)thiazol-2-amine (**9c**)*

Yield 85%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.20 (s, 1H), 7.40 (m, 1H), 7.49 (s, 1H), 7.79 (d, 2H, $J = 7.2$ Hz), 8.2 (d, $J = 7.4$ Hz, 2H), 8.58 (m, 1H), 9.20 (s, 1H), 10.60 (s, 1H); MS (EI): *m/z* 298 (M^+, 100%), 252 ($M^+ - NO_2$, 80%); mp 187 °C; Anal. Calcd for $C_{14}H_{10}N_4SO_2$: C, 56.37; H, 3.38; N, 18.78. Found: C, 56.40; H, 3.43; N, 18.82.

*4.1.5. Hydrobromide salt of N-(2′-ethyl phenyl)-4-(3-pyridyl)thiazol-2-amine (**9d**)*

Yield 71%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.30 (t, $J = 3$ Hz, 3H), 2.72 (m, 2H), 6.83 (s, 1H), 7.19 (m, 1H), 7.30 (m, 3H), 7.39 (s, 1H), 7.63 (m, 1H), 8.10 (m, 1H), 8.51 (m, 1H), 9.40 (s, 1H); MS (EI): *m/z* 281 (M^+, 100%), 248 (M^+ – ethyl, 50%); mp 155 °C; Anal. Calcd for $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93. Found: C, 68.48; H, 5.40; N, 14.98.

*4.1.6. Hydrobromide salt of N-(2′-flourophenyl)-4-(3-pyridyl)thiazol-2-amine (**9e**)*

Yield 85%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.00 (m, 4H), 7.38 (m, 1H), 8.15 (m, 1H), 8.44 (m, 1H), 8.60 (m, 1H), 9.20 (m, 1H), 9.65 (s, 1H); MS (EI): *m/z* 271 (M^+, 100%), 252 ($M^+ - F$, 50%); mp 150 °C; Anal. Calcd for $C_{14}H_{10}N_3SF$: C, 61.98; H, 3.72; N, 15.79. Found: C, 62.04; H, 3.75; N, 15.56.

*4.1.7. Hydrobromide salt of N-(4′-n-butyl phenyl)-4-(3-pyridyl)thiazol-2-amine (**9f**)*

Yield 65%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.90 (t, 3 Hz, 3H), 1.40 (m, 2H), 1.60 (m, 2H), 2.58 (t, $J = 3$ Hz, 2H), 6.90 (s, 1H), 7.10 (d, $J = 8.4$ Hz, 2H), 7.30 (m, 1H), 7.50 (d, $J = 8.5$ Hz, 2H), 8.15 (m, 1H), 8.50 (m, 1H), 9.12 (s, 1H), 9.55 (s, 1H); MS (EI): *m/z* 309 (M^+, 87%), 266 ($M^+ - 43$, 100%); mp 158 °C; Anal. Calcd for $C_{18}H_{19}N_3S$: C, 69.87; H, 6.19; N, 13.58. Found: C, 69.97; H, 6.20; N, 13.65.

*4.1.8. Hydrobromide salt of N-(2,6-diethyl phenyl)-4-(3-pyridyl)thiazol-2-amine (**9g**)*

Yield 72%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.20 (t, $J = 3$ Hz, 6H, 2 × CH_3), 2.70 (q, $J = 5.7$ Hz, 4H, 2 × CH_2), 6.61 (s, 1H), 7.05 (m, 1H), 7.80 (m, 1H), 7.20 (s, 1H), 7.30 (m, 1H), 7.85(m, 1H), 8.40 (m, 1H), 8.81 (s, 1H), 9.20 (s, 1H); MS (EI): *m/z* 253 (M^+, 100%); mp 153 °C; Anal. Calcd for $C_{18}H_{19}N_3S$: C, 69.87; H, 6.19; N, 13.58. Found: C, 69.96; H, 6.22; N, 13.63.

*4.1.9. Hydrobromide salt of N-benzyl-4-(3-pyridyl)thiazol-2-amine (**9h**)*

Yield 70%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.57 (s, 2H), 6.80 (s, 1H), 7.30 (m, 5H), 7.80 (m, 1H), 8.10 (m, 1H), 8.42 (m, 1H), 9.00 (s, 1H); MS (EI): *m/z* 267 (M^+, 100%), 91(benzyl, 100%); mp 192 °C; Anal. Calcd for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72. Found: C, 67.56; H, 4.91; N, 15.87.

*4.1.10. Hydrobromide salt of N-(1-naphthyl)-4-(3-pyridyl)-thiazol-2-amine (**9i**)*

Yield 63%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.00 (s, 1H), 7.34 (m, 1H), 7.48 (m, 3H), 7.62 (m, 1H), 8.16 (m, 2H), 8.26 (m, 1H), 8.48 (br s, 1H), 9.10 (br s, 1H), 9.82 (br s, 1H); MS (EI): *m/z* 303 (M^+, 100%), 304 ($M^+ + 1$, 98%); mp 187 °C; Anal. Calcd for $C_{18}H_{13}N_3S$: C, 71.26; H, 4.32; N, 13.85. Found: C, 71.38; H, 4.44; N, 13.97.

*4.1.11. Hydrobromide salt of 4-(3-pyridyl)thiazol-2-amine (**9j**)*

Yield 92%, ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.60 (s, 1H), 8.20 (m, 1H), 8.80 (m, 1H), 8.83 (m, 1H), 9.14 (s, 14); MS (EI): *m/z* 177 (M^+, 100%), 178 ($M^+ + 1$, 10%); mp 204 °C; Anal. Calcd for $C_8H_7N_3S$: C, 54.22; H, 3.98; N, 23.71. Found: C, 54.39; H, 3.98; N, 23.81.

*4.1.12. Preparation of S-oxo-4-(3-pyridyl)thiazol-2-amine (**10**)*

A solution of **9j** (100 mg, 0.32 mmol) and *m*-chloroperbenzoic acid (55 mg, 0.32 mmol) in chloroform (10 mL) was stirred at 0 °C for 15 min under N_2 atmosphere. Reaction was monitored by TLC. After reaction was complete (TLC), reaction mixture was quenched with aqueous sodium bisulfate

solution to remove the traces of *m*-CPBA and extracted with chloroform (5 × 5 mL). Combined organic layer was washed with aqueous $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a solid which was purified by column chromatography using silica gel (100–200 mesh), ethyl acetate and pet ether (2:8) as eluent to afford sulfoxide **10** as a white solid (mp 232 °C, 90%); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.73 (s, 2H), 6.83 (s, 1H), 7.30 (m, 1H), 8.14 (m, 1H), 8.40 (m, 1H), 8.99 (s, 1H); MS (EI): *m/z* 177 ($M^+ - O$); mp 232 °C; Anal. Calcd for $C_8H_7N_3SO$: C, 49.73; H, 3.65; N, 21.76. Found: C, 49.88; H, 3.78; N, 21.79.

4.2. Biology

4.2.1. In vitro antiplatelet aggregation activity

Blood was collected from cubital vein of healthy male volunteers who did not take any medication for 45 days and who were fasting over night prior to start of the study. Platelet rich plasma (PRP) was obtained by centrifugation of citrated blood (0.38% final concentration) at 800 rpm for 10 min. The supernatant fraction was called platelet rich plasma (PRP). The residual blood was centrifuged at a speed of 2500 rpm for 10 min. This supernatant fraction was called platelet poor plasma (PPP). Aliquots of 250 μL of PRP were distributed in the test cuvettes and inserted in incubation chamber of Aggregometer (Whole Blood Lumi-Aggregometer (Ca^{2+}); CHRONO-LOG corporation, Havertown, PA, USA) at 37 °C. Platelet aggregation was measured using PRP on Chrono Log after activation by 2.5 μM ADP according to Born [35]. The test compounds were dissolved in DMSO (at 0.01% final concentration) and added to the PRP, 2 min before activation with ADP. The extent of aggregation was quantified by determining the maximum height of the curve. The platelet aggregation inhibitory activity was expressed as percent inhibition by comparison with that measured in presence of vehicle (DMSO) alone. The platelet aggregation inhibitory activity of test compounds was expressed as IC_{50} values.

Procedure for determining the IC_{50} value: The percent inhibition values of platelet aggregation were plotted against concentration and linear regression equation was obtained. IC_{50} values were obtained from the linear regression equation. By definition, IC_{50} is the concentration of the test compounds required which produces 50% inhibition of ADP-induced platelet aggregation:

$$y = 38.448x + 5.2586,$$

$$r^2 = 0.8771 \ (r = \text{correlation coefficient}).$$

Acknowledgements

U.B. thanks Dr. Gafoorunnisa (NIN) for providing with bioassay facilities and Late Dr. A.K. Singh. Financial support from CSIR, New Delhi is acknowledged.

References

[1] A. Badorc, M.F. Bordes, P. Cointet, P. Savi, A. Bernat, A. Lale, M. Petitou, J.P. Maffrand, J.M. Herbert, J. Med. Chem. 40 (1997) 3393–3401.
[2] (a) J. Moran, The Market, Clinical Background and Future Therapies, SCRIP-PJB Publications, New York, 1990;
(b) M.G. Bousser, E. Eschwege, M. Haguenau, J.M. Lefaucconier, D. Thibult, P.J. Touboul, Stroke 14 (1983) 5–14;
(c) Anti platelet Trialist's Collaboration, Br. Med. J. 296 (1988) 320–331;
(d) P.S. Sorensene, H. Pedersen, J. Marquardsen, H. Petersson, A. Heltberg, N. Simonsen, O. Munck, L.A. Andersen, Stroke 14 (1983) 15;
(e) H. Lewis, J. Davis, D. Archibald, W. Steinke, T. Smitherman, J.E. Doeherty, H.W. Schnaper, M.M. LeWinter, E. Linares, J.M. Pouget, S.C. Sabherwel, E. Chesler, H. Demost, New. Engl. J. Med. 309 (1983) 396–403;
(f) J.A. Cairns, M. Gent, J. Singer, K.J. Finnie, G.M. Froggalt, D.A. Holder, G. Jablonsky, W.J. Kostik, L.J. Meledenz, M.G. Myers, L.D. Sackett, B.J. Sealey, P.H. Tauser, New. Engl. J. Med. 313 (1985) 1369–1375.
[3] (a) B.S. Coller, New. Engl. J. Med. 322 (1990) 33–42;
(b) M.D. Trip, V.M. Cats, F.J.L. Van Capelle, J. Vreekan, New. Engl. J. Med. 322 (1990) 1549–1554;
(c) W.G. Henderson, S. Goldman, J.G. Copeland, T.E. Moritz, L.A. Harker, Ann. Int. Med. 111 (1989) 743–750;
(d) B. Stein, J. Badimon, V. Fuster, D.M. Israel, M. Cohen, L. Badimon, V. Chesebro, J. Am. Coll. Cardiol. 14 (1989) 813–836.
[4] N. Seko, K. Yoshino, K. Yokota, G. Tsukamoto, Chem. Pharm. Bull. 39 (1991) 651–657.
[5] (a) A.J. Marcus, M.J. Broekman, J. Lab. Clin. Med. 132 (1998) 446–447;
(b) S.R. Kim, J.Y. Lee, M.Y. Lee, S.M. Chung, O.N. Bae, J.H. Chung, Toxicol. Sci. 62 (2001) 176–182;
(c) C.M. Shah, I.C. Locke, H.S. Chowdrey, M.P. Gordge, Biochem. Soc. Trans. 31 (2003) 1450–1452;
(d) M. Paciaroni, V. Galli, Cereb. Vasc. Dis. 10 (2000) 36–39;
(e) R.A. Kulkarni, J. Postgrad. Med. 46 (2000) 312–313;
(f) Y. Wang, Z. Tang, M. Dong, X. Liu, S. Peng, Acta Pharmocol. Sci. 25 (2004) 469–473.
[6] (a) R. De Caterina, D. Gianessi, A. Boem, W. Bernini, D. Battaglia, C. Michelassi, F. Dell'Amico, A. L'Abbate, P. Patrignani, C. Patrono, Thromb. Haemat. 54 (1985) 528–532;
(b) W.S. Fields, R.F. Lemak, R.F. Frankowski, R.J. Hardy, Stroke 8 (1977) 301;
(c) The Canadian Cooperative Study Group, New. Engl. J. Med. 299 (1978) 53–59.
[7] J. Fujimori, K. Harada, T. Saeki, M. Koguchi, K. Akasaka, Y. Yamagishi, I. Yamatsu, Arzneim. Forsch. Drug. Res. 10 (1987) 1143–1148.
[8] E. Tremoli, S. Colli, R. Paoletti, Thromb. Res. Suppl. 11 (1990) 33.
[9] I.S. Watts, B.P. White, K.A. Wharton, P. Lumley, Br. J. Pharm. 98 (1989) 842.
[10] A. Tanaka, K. Ito, S. Nishino, Y. Motoyama, H. Takasugi, Chem. Pharm. Bull. 40 (1992) 3206–3213.
[11] A. Badorc, M.F. Bordes, P. de Cointet, P. Savi, A. Bernat, A. Lale, M. Petitou, J.P. Maffrand, J.M. Herbert, J. Med. Chem. 40 (1997) 3393–3401.
[12] J.A. Zablocki, J.G. Rico, R.B. Garland, T.E. Rogers, K. Williams, L.A. Schretzman, A.R. Sashidhar, P.R. Bovy, F.S. Tjoeng, R.J. Lindmark, M.V. Toth, M.E. Zupec, D.E. McMackins, S.P. Adams, M. Miyano, C.S. Markos, M.N. Milton, S. Paulson, M. Herin, P. Jacqmin, N.S. Nicholson, S.G. Panzer-Knodle, N.F. Haas, J.D. Page, J.A. Szalony, B.B. Taite, K.S. Anita, L.W. King, J.G. Campion, L.P. Feigen, J. Med. Chem. 38 (1995) 2378–2394.
[13] Abciximab, Drugs Future 20 (1995) 457–463.
[14] See Ref. [4].
[15] H.J. Weiss, L.M. Aledort, S. Kochwa, J. Clin. Invest. 47 (1968) 2169–2180.

[16] K. Iizuka, K. Akahane, D. Momose, M. Nakazawa, J. Med. Chem. 24 (1981) 1139–1148.
[17] S. Ashida, Thromb. Haemostas. 40 (1979) 542–544.
[18] (a) M.P. Cucuianu, E.E. Nishizawa, J.F. Mustard, J. Lab. Clin. Med. 77 (1971) 958–974;
(b) K. Watanabe, T. Kanbe, T. Igawa, H. Hidaka, Abstracts of papers, Eighth International Congress of Pharmacology, 1981, pp. 781.
[19] J.V. Mitsios, A.I. Papathanasiou, F.D. Rodis, J.A. Goudevenos, A.D. Tselepsis, Circulation 109 (2004) 1335–1338.
[20] T. Aoki, K. Harada, J. Seki, A. Tanaka, H. Taksug, Y. Motoyama, Cardiovasc. Drug Res. 17 (1999) 147–159.
[21] G. Blaise, M. Parent, S. Laurin, A. Omri, T. Reader, J. Motquin, J. Cardiothorac. Vasc. Anesth. 8 (1994) 175–181.
[22] H. Aoki, T. Mizobe, S. Nozuchi, N. Hiramatsu, Anesthesiology 88 (1998) 362–370.
[23] T. Asaki, WO 0288084.
[24] Drug Data Rep. 23 (2001) 985.
[25] Drug Data Rep. 25 (2003) 895.
[26] (a) J.L. Boyer, WO 0216381;
(b) J.L. Boyer, US 2002052337;
(c) J.L. Boyer, Pharmocologist 44 (2002) 146.
[27] M. Ohkubo, WO 0160813.
[28] D.E. Levy, WO 0322214.
[29] C. Patrono, Trends Pharm. Sci. 10 (1989) 453–458.
[30] J.V. Metzger, in: K.T. Potts (Ed.), first ed., Comphrehensive Heterocyclic Chemistry, vol. 6 Pergamon Press, New York, 1984, pp. 235–331.
[31] US Patent WO 2000048997.
[32] A.E. Dixon, J. Chem. Soc. 59 (1891) 551–569.
[33] (a) C.R. Venkat, J.C. Vartuli, Proceedings of the IIChE Golden Jubilee Congress, 2002, pp. 642–657;
(b) J.S. Beck, J.C. Vartuli, M.E. Roth, M.E. Leonowicz, C.T. Kresge, K.D. Schmitt, C.T.-W. Chu, D.H. Olson, E.W. Sheppard, S.B. Mc Cullen, J.B. Higgins, J.L. Schlenker, J. Am. Chem. Soc. 114 (1992) 10843–10854;
(c) J.Y. Ying, C.P. Menhert, M.S. Wong, Angew. Chem. Int. Ed. 38 (1999) 57–77;
(d) E. Armengol, M.L. Cono, A. Corma, H. Garcia, M. Navarro, J. Chem. Soc. Chem. Commun. 8 (1995) 519–520;
(e) K. Richard Kloestra, H. van Bekkum, J. Chem. Soc. Chem. Commun. 9 (1995) 1005–1006;
(f) N. Narender, P. Srinivasu, S.J. Kulkarni, K.V. Raghavan, J. Catal. 202 (2001) 430–433.
[34] B.B. Lohray, V.B. Lohray, G. Prasuna, K. Narayan Reddy, N. Premkumar, R. Rajagopalan, WO 98/54172.
[35] G.V.R. Born, Nature 194 (1962) 927–929.