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Synthesis of 1-[4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-ylmethyl]-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid amide derivatives and evaluation of their platelet aggregation inhibition activity.

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Abstract : In the present study, a new kind of 1-[4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-ylmethyl]-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid amide derivatives (**6a-p**) were synthesized. All newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The synthesized compounds were screened for their Anti-Platelet Aggregation activity. All the compounds showed moderate to significant biological activity compared with Clopidogrel bisulfate.
Key words : Antiplatetelet aggregation activity, triazole, thieno[3,2-c]pyridine, in vitro, aggregometry.

Introduction

1,2,3-Triazole and their derivatives are attractive due to their unique chemical properties and structure which are having many applications in medicinal, pharmacological and agricultural fields. For example, Cefatrizine[1] and Rufinamide[2] are sold as antibacterial and anticonvulsant respectively. Likewise, members of this family have been widely used to their profound antibacterial[3,4], Antifungal [4,5], antimicrobial[6,7], cytotoxic[6], Antiprotozoal[8], cannabinoid ligands[9], HIV-1 Protease Inhibitors[10], anticancer agents[3,11], antineoplastic[12,13], AT1-non-peptide angiotensin (II) receptor antagonists[14]. Biphenyl system is the structural unit in the most of the sartans, nonpeptide of angiotensin II receptors which have been used for the treatment of hypertensive disorders.[15-21]

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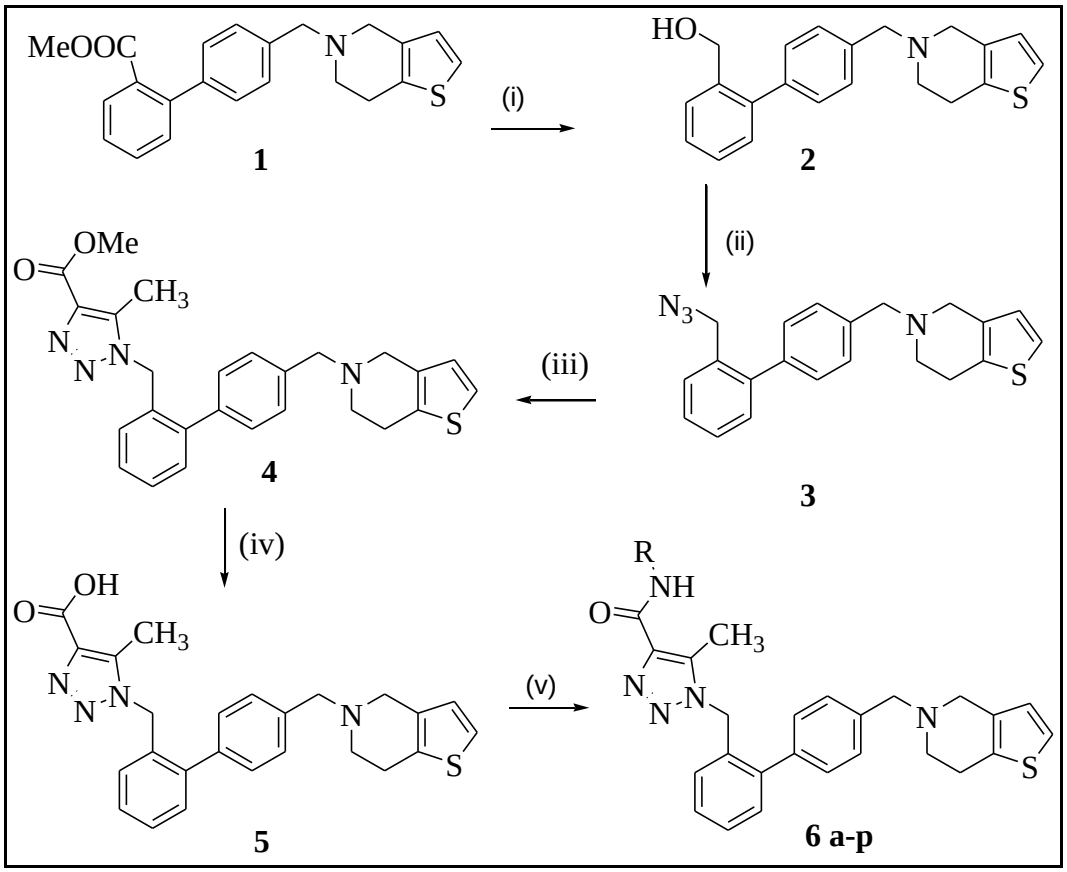
In our previous study, we have revealed that 5-[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine are potent platelet aggregation inhibitors.[22,23] In order to synthesize diverse modified compounds and investigate the effect of the triazole derivative at 2 position of the biphenyl ring, known 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-carboxylic acid methyl ester (**1**) was selected as the starting material.[24] The present study was conducted for the synthesis and anti-platelet aggregation activity of 1-[4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-ylmethyl]-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid amide derivatives (**6a-p**).

Results and Discussion

Chemistry

The synthesis amide derivatives **6a-p** is summarized in Scheme 1. Reduction of ester **1** with lithium aluminum hydride in tetrahydrofuran to 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methanol (**2**), transformation of the alcohol **2** to halogen derivative fpllowed by conversion to 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methyl azide (**3**), then azide derivarive **3** cyclization with methyl acetoacetate yielded key triazole intermediate 1-[4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-ylmethyl]-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid methyl ester (**4**). The triazole ester **4** was then treated with potassium hydroxide in acetonitrile to afford 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid (**5**) which was chlorinated with phosphorus pentachloride to give chlorinated compound followed by nucleophilic substitution with various amines to yield the corresponding 1-[4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-ylmethyl]-5-methyl-1*H*-[1,2,3]-triazole-4-carboxylic acid amide derivatives (**6a-p**). IR, ^{1}H NMR, ^{13}C NMR and Mass spectral characterization data is consistent with the proposed structures.



Scheme 1. Synthesis of series 6a-p (i) LAH, THF, reflux, 5-6 h; (ii) PBr_3, acetonitrile, rt, 5-6h, NaN_3, acetonitrile, 35°C, overnight; (iii) methyl acetoacetate, DMSO, 60-65°C, 45-50h; (iv) KOH, acetonitrile, reflux, 4-5 h (v) PCl_5, CCl_4, py, reflux, 4 h, RNH_2.

Antiplatetelet aggregation activity

Antiplatelet aggregation activity for all the new compounds **6a-p** was performed in vitro following Born's turbidimetric methods available in the literature.[25] The potency of the new compounds was estimated and compared with Clopidogrel. All the test compounds were dissolved in DMSO at 300 µg/mL concentration. Different concentrations of new compounds were prepared in normal saline and 0.05 mL of each was added to aliquots of 0.45 mL PRP. The test samples were pre-incubated with platelets for 5 min at 37°C and then ADP (5 µL, 2.0 µmol/L) was added. All the tests were performed within 3 hr after collection of blood sample. Maximal change in light transmission was assumed to represent maximal platelet aggregation. Platelet aggregation was measured and the maximal deflection was obtained after 5 mins. Corresponding solvents were used as blank controls for the corresponding tests. The anti-platelet aggregation potency is expressed as inhibition (%) which is calculated as follows:

Inhibition %= (A-B) / A x 100

Where A and B were the absorbance values of corresponding blank controls and test samples, respectively.

Table 1. Results of In vitro potency of compounds 6a-p in the inhibition of ADP induced platelet aggregation in human PRP

S. No	Compound 6	% of inhibition of platelet aggregation
1.	a	60±2
2.	b	25±3
3.	c	20±1
4.	d	21±2
5.	e	22±1
6.	f	20±2
7.	g	24±4
8.	h	21±2
9.	i	18±1
10.	j	21±1
11.	k	15±3
12.	l	50±3
13.	m	50±1
14.	n	33±3
15.	o	30±3
16.	p	22±2
17.	Clopidogrel	39±2

Among all the compounds **6a-p**, compound **6a, 6l** and **6m** showed significant anti-platelet activity induced by ADP and are potent than Clopidogrel. Remaining compounds showed moderate to good antiplatelet activity (Table I). The results were expressed as mean ± SEM and the means were compared using Student's t-test, p value is <0.05.

Experimental Section

Materials and methods

Melting point Range reported was uncorrected and taken on a Polmon Melting apparatus. The FT-IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer 1650 FT-IR spectrophotometer. Thin layer chromatography was performed on Merck precoated Silica-gel 60F254 plates using UV light as visualizing agent. 1H NMR spectra were recorded on 400 MHz Gemini Varian spectrometer using DMSO-d6 as solvent and tetramethylsilane as an internal standard. The mass spectra were recorded on an Agilent 6310 Ion Trap. Microanalyses of compounds 6a-k were performed by Elementar vario micro analyzer.

Chemistry

Synthesis of 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methanol (2): Lithium aluminum hydride (LAH) was added slowly to a mixture of 4-(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-carboxylic acid methyl ester (**1**, 23 g, 0.06 mol) and THF (230 mL) into a four necked round bottom flask under nitrogen atmosphere at 0-5^{o}C in 5 minutes of interval. The reaction mass temperature was raised to reflux and maintained for 5-6 hr. the progress of the reaction was monitored using TLC. After completion of the reaction, reaction mass was cooled to 0-5^{o}C, diluted with ethyl acetate (230 mL) slowly over a period of 40 minutes, treated with saturated NH_4Cl and separated the layers. The organic layer was concentrated and treated with diisopropyl ether to obtain 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methanol (**2**) as a cream color solid in 86% yield. mp 90-94 ^{o}C; IR (KBr, cm^{-1}): 3453; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.89-2.93 (m, 4H, 2 x CH_2), 3.63 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.73-7.57 (m, 10H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 25.43, 50.68, 53.11, 61.84, 63.12, 122.46, 122.50, 125.15, 125.19, 127.47, 127.55, 128.34, 128.78, 129.07, 130.02, 133.47, 133.96, 137.65, 138.36, 139.71, 141.35. MS (m/z): 336.1 (M+1)

Synthesis of 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methyl azide (3): Phosphorus tribromide (0.03 mol) was added to solution of alcohol **3** in acetonitrile at 0-5^{o}C over a period of 40 min. The temperature of the obtained clear solution was brought to ambient temperature and maintained for 5-6 hr. The reaction mass was concentrated to half of its total volume at 35^{o}C. The obtained mass diluted with acetonitrile (50 mL) and sodium azide (5.8 g, 0.08 mol) was added at 0-5^{o}C over a period of 40 min. The reaction mass temperature rose to 35^{o}C and maintained for overnight. The reaction mass poured into ice-cold water (100 mL) under stirring, separated solid was filtered and dried at 60^{o}C to obtain 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-2-methyl azide (**3**) as a off-white color solid in 93% yield. mp 173-180 ^{o}C; IR (KBr, cm^{-1}): 2095; ^{1}H NMR (400 MHz, DMSO d_6): δ 3.24 -3.40 (m, 4H, 2 x CH_2), 4.01(s, 2H, CH_2), 4.28 (s, 2H, CH_2), 4.29 (s, 2H, CH_2), 6.76-7.74 (m, 10H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 22.38, 49.41, 50.73, 52.76, 58.86, 122.42, 122.51, 124.83, 124.88, 128.08, 128.40, 129.69, 129.85, 130.28, 130.72, 131.75, 132.90, 137.50, 138.42, 141.33, 141.62. MS (m/z): 361.0 (M+1).

Synthesis of 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid methyl ester (4): A mixture of azide **3** (18 g, 0.04 mol) and methyl acetoacetate (9.3 g, 0.07 mol) in DMSO (180 mL) was heated to 60-65^{o}C for 45-50 h. The progress of the reaction was monitored using TLC. The reaction mixture was cooled to 0-5^{o}C and water (180 mL) was added drop wise to precipitate the compound. The pH of the reaction mass was adjusted to 7-8% with 5% HCl. The isolated solid was filtered and washed with water (50 mL). the wet cake was dissolved in dichloromethane (270 mL) and separated the layers.the organic layer was dried over anhy. Na_2SO_4 and concentrated under reduced pressure. The obtained residue was suspended in diisopropyl ether (54 mL) and adjusted the pH to 1-2 with methanolic HCl at 0-5^{o}C to isolate 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid methyl ester (**4**) as off-white color solid in 85% yield. mp 120-125 ^{o}C; IR (KBr, cm^{-1}): 1731; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.20 (s, 3H, CH_3), 3.24 -3.62 (m, 4H, 2 x CH_2), 3.88 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.76-7.84 (m, 10H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 8.87, 14.29, 21.36, 49.01, 50.26, 57.77, 60.75, 125.06, 125.48, 126.65, 128.25, 128.58, 129.77, 130.36, 131.27, 131.67, 136.81, 138.16, 140.56, 141.80, 161.62. MS (m/z): 459.1(M+1)

Synthesis of 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid (5): A mixture of ester **4** derivative (15 g, 0.03 mol) and KOH (2.5 g, 0.04 mol) in acetonitrile (180 mL) was heated to reflux for 4-5 h. After completion of the reaction, the reaction mass was cooled to ambient temperature, precipitated potassium salt of the title compound was filtered and washed with acetonitrile (15 mL). The wet cake was dissolved in water (225 mL) and washed with dichloromethane (75 mL). Dichloromethane (300 mL) was added to the aqueous layer and adjusted the pH to 3-4 with 5% AcOH. The layers were separated and aquous layer extracted with dichloromethane (100 mL). The pooled organic layers were died over Na_2SO_4, concentrated under reduced pressure upto to half of its total volume, cooled 0-5^{o}C. The separated solid was filtered and dried under reduced pressure for 4-6 h. at below 60^{o}C to afford 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid (**5**) as a cream color solid in 74% yield. mp 145-150 ^{o}C; IR (KBr, cm^{-1}): 1631, 3480; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.13 (s, 3H, CH_3), 2.79-2.82 (brs, 4H, 2 x CH_2), 3.54 (s, 3H, CH_2), 3.74 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.79-7.44 (m, 10H, ArH).^{13}C NMR (100 MHz, DMSO-d_6) : δ 8.79, 25.39, 49.46, 50.61, 52.91, 61.27, 123.21, 125.76, 128.23,

128.48, 129.21, 129.27, 130.52, 132.73, 133.25, 134.19, 136.75, 138.11, 138.55, 138.87, 141.44, 162.94. MS (m/z): 445.2 (M+1)

Synthesis of 4-(6,7-dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)-biphenyl-1,2,3-triazole-2-carboxylic acid amide (6a-p): Phosphorus pentachloride was added to a mixture of carboxylic acid **5**, carbon tetrachloride and pyridine under nitrogen atmosphere and under stirring, heated to reflux, and maintained for 4 hr. The reaction mass was concentrated under reduced pressure, cooled to 0-5^{o}C, chloroform (10 mL) was added to the obtained residue and amine was added. The progress of the reaction was monitored by TLC. The reaction mass was diluted with chloroform(10 mL) and filtered the undissolved salts. The organic layer was washed with dil. HCl (5%, 2 X 10mL), saturated $NaHCO_3$ solution (2 X 10 mL) and water (2 X 10 mL). the organic layer was dried over anhy. Na_2SO_4, concentrated under reduced pressure and isolated the solid from ether.

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid amide (6a): Off white solid; mp 155-158 ^{o}C; yield -50%; IR (KBr, cm^{-1}):1622, 3340; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.15(s, 3H, CH_3), 2.78-2.83 (m, 4H, 2 x CH_2), 3.50 (m, 2H, CH_2), 3.74 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.79-7.45 (m, 10H, ArH), 7.70 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 8.42, 25.46, 49.27, 50.67, 52.95, 61.29, 123.27, 125.90, 128.29, 128.45, 128.52, 129.25, 130.57, 132.94, 133.32, 134.46, 136.57, 138.43, 138.81, 141.43, 163.29. MS (m/z): 444.1 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid phenyl amide (6b): Off white solid; mp 105-110 ^{o}C; yield -55%; IR (KBr, cm^{-1}):1682, 3368; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.08 (brs, 4H, 2 x CH_2), 3.83 (m, 2H, CH_2), 3.98 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.73-7.67 (m, 15H, ArH), 9.02 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 8.44, 24.34, 49.73, 50.22, 52.27, 60.59, 119.90, 123.36, 124.14, 125.08, 127.88, 128.20, 128.37, 128.90, 129.15, 129.80, 130.32, 131.63, 132.84, 136.73, 137.84, 138.65, 139.61, 141.18, 159.25. MS (m/z): 520.2 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 2-chlorophenyl amide (6c): Off white solid; mp 133-138 ^{o}C; yield -60%; IR (KBr, cm^{-1}):1683, 3349; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 2.91-2.95 (m, 4H, 2 x CH_2), 3.64 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 6.74-8.15 (m, 14H, ArH), 9.64 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.39, 25.46, 49.58, 50.70, 53.14, 61.71, 121.45, 122.63, 123.40, 124.44, 125.18, 127.49, 127.73, 128.15, 128.33, 129.00, 129.16, 129.22, 130.33, 131.58, 133.42, 133.80, 134.75, 136.90, 138.44, 138.58, 138.76, 141.30, 159.32. MS (m/z): 554.2 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 4-chlorophenyl amide (6d): Off white solid; mp 152-158 ^{o}C; yield -58%; IR (KBr, cm^{-1}):1681, 3371; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.8 (t, J=5.2 Hz, 2H, CH_2), 2.94 (t, J=5.2 Hz, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.75-7.64 (m, 14H, ArH), 9.03 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.34, 49.67, 50.72, 53.10, 61.66, 121.00, 122.57, 125.14, 127.67, 128.08, 128.31, 128.93, 128.97, 129.14, 129.27, 130.36, 131.58, 131.58, 133.43, 133.87, 136.44, 136.83, 138.44, 138.63, 138.75, 141.43, 159.22. MS (m/z): 554.2 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 3-chlorophenyl amide (6e): Cream cloured solid; mp 112-116 ^{o}C; yield -61%; IR (KBr, cm^{-1}):1686, 3374; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.26 (s, 3H, CH_3), 3.03 (brs, 4H, 2 X CH_2), 3.37 (s, 2H, CH_2), 3.92 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.76-7.87 (m, 14H, ArH), 9.04 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO d_6) : δ 8.31, 22.81, 49.78, 50.32, 52.46, 60.76, 117.76, 119.98, 123.17, 124.17, 125.02, 125.07, 127.88, 128.18, 128.39, 129.09, 129.63, 129.81, 130.35, 131.54, 132.94, 134.90, 133.87, 136.94, 138.44, 138.36, 139.01, 141.29, 159.25. MS (m/z): 554.2 (M+1).

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 2,3-dichlorophenyl amide (6f): Cream cloured solid; mp 138-142 ^{o}C; yield -48%; IR (KBr, cm^{-1}):1688, 3346; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.14 (s, 3H, CH_3), 2.78-2.85 (m, 4H, 2 X CH_2), 3.54 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 5.42 (s, 2H, CH_2), 6.63-8.37 (m, 13H, ArH), 9.62 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO d_6) : δ 8.31, 25.38, 49.63, 50.68, 53.03, 61.58, 119.29, 119.46, 122.11, 122.55, 122.70, 125.03, 125.12, 127.46, 127.60, 127.87, 128.09, 130.34, 131.57, 133.00, 133.41, 133.57 (2), 133.79, 133.96, 136.55, 141.46. MS (m/z): 588.1 (M+1).

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 3,4-dichlorophenyl amide (6g): Off white solid; mp 133-135 ^{o}C; yield -56%; IR (KBr, cm^{-1}):1688, 3364; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.01 (brs, 4H, 2 X CH_2), 3.76 (s, 2H, CH_2), 3.91 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.75-7.98 (m, 13H, ArH), 9.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO d_6) : δ 8.46, 24.67, 50.03, 50.50, 52.62, 61.00, 119.01, 119.22, 121.75, 123.32, 125.19, 127.57, 128.10, 128.32, 128,59, 129.20, 129.79, 130.55, 131.66, 132.56, 133.05, 137.23, 137.57, 138.37, 139,56, 141.53, 159.41. MS (m/z): 588.1 (M+1).

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 2-fluorophenyl amide (6h): Cream colored solid; mp 122-124 ^{o}C; yield -68%; IR (KBr, cm^{-1}):1690, 3379; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 2.96-2.97 (brs, 4H, 2 x CH_2), 3.69 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.75-8.43 (m, 14H, ArH), 9.24 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.34, 25.20, 49.65, 50.52, 52.93, 61.23, 114.77, 114.96, 121.82, 122.79, 124.18, 124.27, 125.09, 126.34, 126.44, 127.80, 128.12, 128.30, 129.00, 129.01, 129.29, 130.31, 131.62, 133.26, 136.81, 138.52, 141.35, 154.39, 159.31. MS (m/z): 538.3 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 4-fluorophenyl amide (6i): Off white solid; mp 122-126 ^{o}C; yield -63%; IR (KBr, cm^{-1}):1681, 3374; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 2.93-2.97 (m, 4H, 2 x CH_2), 3.68 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.75-7.65 (m, 14H, ArH), 9.01 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.39, 25.19, 49.67, 50.60, 52.93, 61.47, 115.47, 115.70, 121.50, 121.58, 122.82, 125.14, 127.63, 128.16, 128.35, 129.01, 129.35, 130.35, 131.55, 133.28, 133.78, 136.78, 138.46, 139.92, 141.26, 158.22, 159.21, 160.64. MS (m/z): 538.2 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 2,5-difluorophenyl amide (6j): Cream colored solid; mp 157-163 ^{o}C; yield -62%; IR (KBr, cm^{-1}):1694, 3376; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.92-2.96 (m, 4H, 2 x CH_2), 3.66 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.75-8.3 (m, 13H, ArH), 9.31 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.32, 25.22, 49.57, 50.12, 53.93, 61.32, 114.47, 115.12, 121.28, 122.18, 122.92, 124.32, 127.57, 128.18, 128.44, 129.20, 129.15, 130.17, 131.14, 133.18, 133.92, 135.28, 138.12, 138.99, 141.66, 158.13, 159.22, 159.12. MS (m/z): 556.2 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 2,3,4-trifluorophenyl amide (6k): White solid; mp 155-156 ^{o}C; yield -70%; IR (KBr, cm^{-1}):1698, 3373; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.86-2.90 (m, 4H, 2 x CH_2), 3.61 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 5.49 (s, 2H, CH_2), 6.70-8.09 (m, 12H, ArH), 9.12 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.32, 25.41, 49.66, 50.69, 53.10, 61.62, 111.29, 111.46, 115.27, 122.59, 123.81, 125.07, 125.13, 127.74, 128.11, 128.35(2), 128.95, 129.16, 130.37, 131.49, 133.41, 133.80, 137.07, 138.07, 138.57, 138.76, 141.43, 159.39. MS (m/z): 574.2 (M+1).

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid benzyl amide (6l): Cream colored solid; mp 108-114 ^{o}C; yield -50%; IR (KBr, cm^{-1}):1660, 3419; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.18-3.28 (m, 4H, 2 x CH_2), 3.66 (t, *J*=6.0 Hz, 1H, NH), 4.01 (s, 2H, CH_2), 4.13 (s, 2H, CH_2), 4.61 (d, *J*=5.6 Hz, 2H, CH_2), 5.46 (s, 2H, CH_2), 6.77-7.69 (m, 15H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.42, 22.81, 42.92, 49.79, 51.22, 59.31, 68.34, 124.25, 124.96, 127.29, 127.70, 128.10, 128.30, 128.40, 128.56, 129.38, 130.25, 131.69, 132.10, 136.09, 138.40, 138.55, 140.88, 161.25. MS (m/z): 534.3 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 4-fluorobenzyl amide (6m): Off white solid; mp 120-122 ^{o}C; yield -64%; IR (KBr, cm^{-1}):1651, 3412; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.31-3.48 (m, 4H, 2 x CH_2), 3.66 (t, *J*=6.0 Hz, 1H, NH), 4.30 (s, 2H, CH_2), 4.57 (d, *J*=5.6 Hz, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.80-7.76 (m, 15H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.44, 21.79, 22.77, 42.19, 50.07, 50.40, 58.19, 115.19, 115.40, 124.89, 125.07, 128.36, 128.43, 128.50, 129.34, 129.42, 129.53, 130.25, 131.25, 131.51, 131.66, 134.40, 136.10, 138.53, 140.79, 141.54, 161.26. MS (m/z): 552.2(M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 3-methoxyphenylamide (6n): Beige colored solid; mp 98-104 ^{o}C; yield -48%; IR (KBr, cm^{-1}): 1681, 3474; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 2.99 (brs, 4H, 2 x CH_2), 3.72 (s, 2H, CH_2), 3.84 (s, 2H, CH_2),

3.87 (s, 3H, OCH_3), 5.51 (s, 2H, CH_2), 6.69-7.45 (m, 14H, ArH), 9.03 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.52, 25.95, 49.62, 51.43, 55.19, 61.42, 115.03, 121.59, 121.93, 122.73, 125.19, 127.08, 128.12, 128.25, 129.09, 129.31, 130.53, 131.31, 131.91, 133.62, 134.09, 136.19, 138.62, 138.92, 138.99, 141.82, 156.12, 160.18. MS (m/z): 550.3 (M+1)

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 4-methoxyphenylamide (6o): Beige colored solid; mp 165-169 ^oC; yield -50%; IR (KBr, cm^{-1}): 1633, 3204; ^1H NMR (400 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 2.86 (t, *J*=5.2 Hz, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 5.48 (s, 2H, CH_2), 6.60-7.40 (m, 14H, ArH), 9.01 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.42, 25.59, 49.74, 50.86, 53.26, 55.60, 61.76, 114.60, 121.73, 122.65, 122.68, 125.29, 127.84, 128.21, 128.38, 129.09, 129.27, 130.47, 131.26, 131.89, 133.59, 134.04, 136.19, 138.63, 138.74, 138.92, 138.96, 141.56, 156.83, 159.27. MS (m/z): 550.3 (M+1).

4(6,7-Dihydro-4*H*-thieno[3,2-c]pyridine-5-ylmethyl)biphenyl-1,2,3-triazole-2-carboxylic acid 4-bromophenylamide (6p): Cream colored solid; mp 157-163 °C; yield -40%; IR (KBr, cm^{-1}): 1681, 3371; ^1H NMR (400 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 2.28-2.93 (m, 4H, 2 x CH_2), 3.62 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 5.50 (s, 2H, CH_2), 6.63-7.57 (m, 14H, ArH), 9.01 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 8.40, 25.48, 49.66, 50.73, 53.13, 61.73, 116.75, 121.28, 122.25, 125.18, 127.58, 128.14, 128.36, 128.97, 129.22, 130.38, 131.51, 131.97, 133.42, 133.82, 136.88, 136.92, 138.39, 138.48, 138.70, 141.31, 159.21. MS (m/z): 600 (M+1).

Antiplatelet activity

Preparation of Platelet Rich Plasma (PRP): The blood samples were collected from the healthy human volunteer, mixed with 1.0 mL of 3.8% trisodium citrate and centrifuged at 180 g for 10 minutes. The upper two-third fraction, PRP was transferred to another centrifuge tube leaving behind lower one-third layer to avoid contamination with WBC's and RBC's. This is further centrifuged at 2500 g for 10 min to obtain Platelet Poor Plasma (PPP).

In vitro anti-platelet aggregation activity studies by Aggregometry: Biological activities of the new compounds were conducted at Bioworld Research Technologies, Hyderabad, Andhra Pradesh, India. Anti-platelet activity studies were evaluated by turbidimetric methods based on ADP-induced (5 µL, 2.0 µmol/L) platelet aggragation in human PRP. Platelet aggregation was studied at 37°C using Born's method in a platelet aggregation module. A final concentration of ADP 2.0 µmol/L was used in a volume of 5 µL. The new compound at 0.05 mL different concentrations an normal saline were added to 0.45 mL PRP respectively. After 5 mins, ADP (2.0 µmol/L, 5 µL) was given. Maximal change in light transmission was assumed to represent maximal platelet aggregation. Platelet aggregation was measured and the maximal deflection was obtained in 5 min. The results were expressed as mean ± SEM and the means were compared using Student's t-test, p value is <0.05.

Conclusions

In summary, new series of triazole derivatives **6a-p** have been synthesized and evaluated for antiplatelet activity. It was observed that compounds displayed moderate to high potent antiplatelet activity. Out of sixteen Compounds, triazole **6a, 6l** and **6m** exhibited a higher activity as compared with Clopidogrel. These results provide impetus for future application.

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