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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 5-((2-CHLOROPHENYL) (1 PHENYL-1H-TETRAZOL-5-YL) METHYL)-4, 5,6,7-TETRAHYDROTHIENO [3,2-C] PYRIDINE DERIVATIVES

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ABSTRACT

We demonstrate some novel one pot synthesis, characterization and evaluation of Antithrombotic activity of new 5-(2-chlorophenyl)(1-Phenyl-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3a–k) using amides (2a–k) were reported. The prepared 5-(2-chlorophenyl)(1phenyl-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3a) is a bioisostere 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetic acid (1), having good in vivo antithrombotic activity compared with Clopidogrel. The new tetrazole derivatives (3a–k) were screened for their in vitro activity as platelet aggregation inhibitors.

KEYWORDS: Antithrombotic activity; Bioisostere, In vitro; In vivo; Tetrazole Platelet aggregation

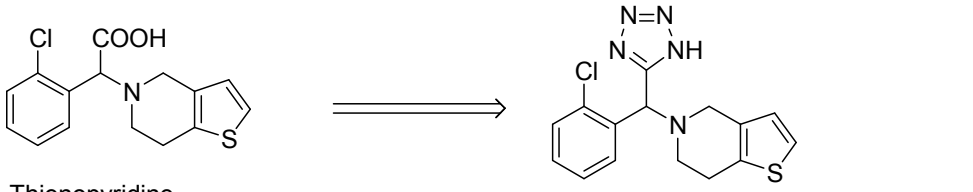
INTRODUCTION

Thienopyridines (4,5,6,7-tetrahydro thieno[3,2-c]pyridines) and their derivatives are important heterocyclic compounds that are widely distributed in nature. Many of the compounds containing tetrahydro thienopyridine skeleton are reported as antibacterial[I] non-peptide GPIIb/IIIa antagonists[II] platelet aggregators and antithrombotic agents[III]. The incorporation of benzylic or substituted benzylic groups on the nitrogen of the thienopyridine ring can bring an extensive modification in the biological activities of parent compound. Among the substitutions occurred at nitrogen of the thienopyridine moiety[IV], the increased effect in the biological activity of the parent moiety affects the good antithrombotic activity in Ticlopidine and with more increased activity in Clopidogrel. Later on, the studies proved that the Prasugrel to be more efficient drug candidate than the existing Clopidogrel by making the structural modifications to the parent thienopyridine moiety. Hence, different substitutions at nitrogen of the biological activity of the new chemical entities (NCEs).

Tetrazole system is the key structural unit in most of the sartans, non-peptide antagonists of angiotensin II recep-tors[V] Along with their well known antihypertensive activity[VI], phenyl tetrazoles have also been demonstrated as stimulators of growth hormone release and chloride

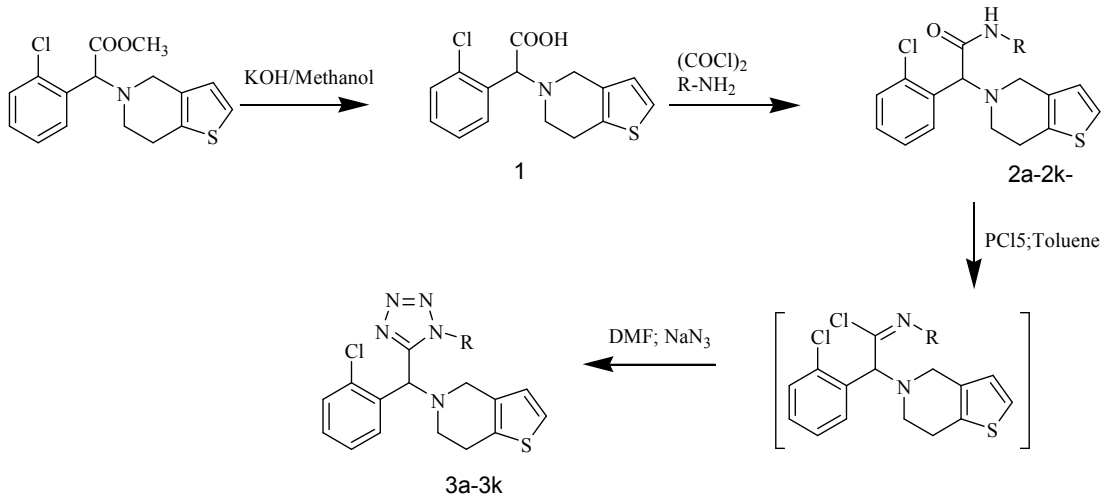
channel blockers[VII]In this context, phenyl tetrazoles and its derivatives have received substantial attention thienopyridine moiety may bring extensive improvement.

Recently, reported the synthesis of 4-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)biphenyl-2-carboxylic acid derivatives as promising antithrombotic agents[VIII] As part of our continuous efforts in the synthesis of new thienopyridine derivatives containing phenyl system, we arrived at the synthesis of 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetic acid (1) (Fig. 1) derivatives. We focused on the phenyl system to incorporate a hetero cyclic ring system on second position, i.e. on carboxylic acid position. Among the heterocyclic nucleus, we opted to introduce tetrazole ring system over the carboxylic acid function on the phenyl system as tetrazole is a bioisostere of carboxylic acid function. Tetrazoles are the nonclassical isosteres of carboxylic acids[IX,] The term ‘‘nonclassical isosterism derives from the concept that the functional groups having the similar physicochemical proper-ties can be interchangeable, while the biological activity of the initial and the new compounds will be similar. Like carboxylic acids the tetrazoles are ionized in the range of physiological pH (7.4) and have a planar structure. And at the same time the ionized tetrazoles are 10 times more lipophilic than the corresponding carboxylic acids which will enable to penetrate the membranes with greater ease. It was proved that the tetrazoles were more potent candidates over the carboxylic acids[X] For instance, the introduction of tetrazole ring into the molecule of 2-(2-chlorophenyl)-2-(6, 7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetic acid 1 and also some of the derivatives of this substrate might afford the promising metabolically stable analogs



Thienopyridine Tetrazole containing thienopyridine

Thus we have targeted the synthesis of 5-(2-chlorophenyl)(1-Phenyl1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine Fig. 2) derivatives (3a–k). For this strategy, we have chosen compound 1 as the starting material. The compound 1 is converted into tetrazole via amide transformation. Synthesis of tetrazole could be easily done using (3 + 2) cycloaddition of the classical nitrile function with sodium azide. But it suffers from the draw backs of longer reaction time cycle and substitution on the free tetrazole ring is not possible to the single regioisomer as it offers both the positions for the substitution on 1,2-positions on tetrazole ring. Tetrazole can also be syntheized from alkyl nitrile using TMS azide and trialkyl tin azides but it suffers from tedious work up procedures along with purification of the materials using chromatographic techniques like column chromatography. To overcome all the problems encountered during the preparation of tetrazole derivatives, we had developed an improved and facile synthesis of tetrazole using amide derivatives. Here, we report the synthesis of new 5-((2-chlorophenyl)(1-phenyl- 1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine



Where R=H, phenyl, 2-chloro phenyl,3-chloro phenyl, 4-chloro phenyl,2,3-dichloro phenyl, 3,4-dichloro phenyl, 2-fluoro phenyl, 4-fluoro phenyl, 2,5-difluoro phenyl, 2,3,4-trifluoro phenyl,

Reagents and Conditions

(a).KOH/Methanol, RT (b), Oxalyl chloride /MDC,reflux, R-NH2 (c) PCl_5, toluene; (d) NaN_3, DMF, water

MATERIALS AND METHODS

All solvents used were of commercial grade purchased from a qualified vendor. 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 silicagel $60F_{254}$ plates using UV light as visualizing agent. ^{1}H NMR and ^{13} C NMR spectra were recorded on 400 and 100 MHz Gemini Varian spectrometer using DMSO-d_6 as solvent and tetramethylsilane as an internal standard. The mass spectra were recorded on an Agilent 6310 Ion Trap.

(2a). **Synthesis of 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide**

A mixture of 1 (10.0 g, 0.33 mol) and oxalyl chloride 13.6g, 0.66 mol) charged into the round bottom flask (RBF) containing methylenedichloride (100 mL), added amount of DMF 0.5ml maintained the reaction mass at reflux for 2–3 h under inert atmosphere. Solvent was removed completely under reduced pressure. Then cooled the reaction mass to 0–5^0 C and charged methylene dichloride (50 mL) into the reaction mass. Stirred the reaction mass for 10–15 min to get clear solution. Then passed the ammonia gas/ amine into the reaction mass until the pH of the reaction mass becomes basic (pH should be 9–10) at 0–5^{o}C under nitrogen atmosphere to precipitate out the compound 2a as thick solid.. Maintained the stirring for 2 h, filtered the resulted solid material and was washed with water till the pH of the mother liquor becomes neutral. The crude compound was suspended in ether (100 mL) to afford the title compound quantitatively. Yield: 90%; mp: 152; FT-IR (cm^{-1}, KBr): 1643 (– C=O), 2918, (ArH), 3186, 3384 (–NH_2). MS (m/e): 307.71(M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.75 (t,2H), 2.8 (t, 2H), 3.57 (s, 2H) 4.85 (s, 1H), 6.35 - 7.3(aromatic 6H), 8.5 (s, 2H, NH2)); ^{13}C NMR (100 MHz, DMSO-d6:125.16, 127.45, 128.75, 129.04, 129.17, 130.34, 130.36, 133.43,

133.87, 134.54, 138.49, 139.19, 139.79 and 173.01.

Employing the similar procedure as mentioned for 2**a** the remaining amides (**2b-k**) were prepared in quantitatively

Characterization of **2b:**(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl) phenylacetamide Yield: 76%; mp: 162; FT-IR (cm^{-1}, KBr): 1728 (–C=O), 2920 (ArH), 3414 (amide –NH); MS (m/e) :384 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 3.07 (t, 2H), 2.99 (t, 2H), 3.75 (s, 2H)) 4.9 (s,1H), 6.8–7.68 (aromatic, 11H), 7.5 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 23.18, 49.21, 50.11, 58.11, 121.50, 125.55, 125.62, 127.60, 128.09, 128.39, 128.99, 129.12, 130.36, 130.50, 131.60, 132.04, 137.31, 138.43, 139.11, 141.48 and 169.21.

Characterization of **2c :** N,2-bis(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl) Yield: 76%; mp: 172; FT-IR (cm^{-1}, KBr): 1728 (–C=O), 2920 (ArH), 3414 (amide –NH); MS (m/e): 417(M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 3.07 (t, 2H), 3.0 (t, 2H), 3.75 (s, 2H) 5.1 (s,1H), 6.6–7.68 (aromatic, 10H), 7.5 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 22.18, 49.21, 50.11, 58.11, 121.50, 125.55, 125.62, 127.60, 128.09, 128.39, 128.99, 129.12, 130.36, 130.50, 131.60, 132.04, 137.31, 138.43, 139.11, 141.48 and 169.3.

Characterization of **2d:** (2-chlorophenyl)-N-(3–chloro phenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide Yield: 72%; mp : 175,; FT-IR (cm^{-1}, KBr): 1658 (–C=O), 2924 (ArH), 3450 (amide –NH); MS (m/e): 417. (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 3.07 (t,; 2H), 3..0 (t,; 2H), 3.75 (s, 2H)) 4.9 (s ,1H) 6.9–7.68 (aromatic, 10H), 7.5 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 22.3, 50.21, 52.11, 58.11, 121.50, 125.55, 125.62, 127.60, 128.09, 128.39, 128.99, 129.12, 130.36, 130.50, 131.60, 132.04, 137.31, 138.43, 139.11, 141.48 and 169.21.

Characterization of **2e:** 2-(2-chlorophenyl)-N-(4–chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide. Yield: 80%; mp: 190; FT-IR (cm^{-1}, KBr): 1680 (–C=O), 2922 (ArH), 3390 (amide –NH); MS (m/e): 417 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 3.07 (t,; 2H), 3.0 (t, 2H), 3.75 (s, 2H)) 5.0 (s, 1H) 6.6–7.7 (aromatic, 10H), 7.5 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 22.18, 49.21, 50.11, 58.11, 121.50, 125.55, 125.62, 127.60, 128.09, 128.39, 128.99, 129.12, 130.36, 130.50, 131.60, 132.04, 137.31, 138.43, 139.11, 141.48 ,168.21.

Characterization of **2f**: 2-(2-chlorophenyl)-N-(2,3–dichloro phenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide Yield: 73%; mp: 180; FT-IR (cm^{-1}, KBr): 1660 (–C=O), 2921 (ArH), 3433 (amide –NH); MS (m/e): 451.8 (M + 1)^{1}H NMR (400 MHz; DMSO-d6): 2.80 (t, 2H), 2.88 (t, 2H), 3.75 (s, 2H)) 5.1 (s, 1H), 6.88–7.76 (aromatic, 9H), 7.8 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 25.46, 50.65, 53.07, 61.64, 118.81, 121.26, 122.79, 125.21, 127.48, 127.99, 128.77, 129.65, 130.34, 130.37, 131.08, 132.63, 133.37, 133.70, 134.62, 137.11, 169.

Characterization of **2g :**2-(2-chlorophenyl)-N-(3,4 dichlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide,Yield: 75%; mp: 185 FT-IR (cm^{-1}, KBr): 1659 (–C=O), 2978 (ArH), 3418 (amide –NH); MS (m/e): 451.8 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.80 (t,; 2H), 2.88 (t, 2H), 3.75 (s, 2H)) 5.1 (s,1H), 6.88–7.86 (aromatic, 9H), 7. 7 (s, 1H, NH); ^{13}CNMR (100 MHz, DMSO-d6): 25.46, 50.65, 53.07, 61.64, 118.81, 121.26, 122.79, 125.21, 127.48, 127.99, 128.77, 129.65, 130.34, 130.37, 131.08, 132.63, 133.37, 133.70, 134.62, , 138

Characterization of **2h:** 2-(2-chlorophenyl)-N-(2-fluorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide Yield: 65%; mp: 162; FT-IR (cm^{-1}, KBr): 1652 (–C=O), 2926 (ArH), 3424(amide –NH); MS (m/e): 401 ((M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.74 (t,

2H), 2.83 (t, 2H), 3.54 (s, 2H) 5.1 (s, 1H) 6.78–8.36 (aromatic, 10H), 7.5 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 25.41, 50.24, 52.85, 61.67, 114.36, 121.30, 122.65, 124.14, 124.53, 125.26, 126.36, 127.82, 128.72, 129.55, 129.70, 130.49, 130.89, 133.34, 133.78, 135.14, 168

Characterization of **2i:** :2-(2-chlorophenyl)-N-(4-fluorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide Yield: 60%; mp: 168; FT-IR (cm^{-1}, KBr): 1655 (–C,O), 2924 (ArH), 3420 (amide –NH); MS (m/e): 401 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.72 (t, 2H), 2.82 (t,; 2H), 3.55 (s, 2H), 5.1 (s,1H) 6.75–8.12 (aromatic, 10H), 7.6 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6): 25.44, 50.14, 52.75, 61.77, 113.42, 120.30, 121.65, 123.14, 124.55, 125.12, 125.56, 127.80, 128.12, 129.15, 129.60, 130.52, 130.99, 133.44, 133.88, 135.44.168

Characterization of **2j:** 2-(2-chlorophenyl)-N-(2,5-difluorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide,Yield: 62%; mp: 174; FT-IR (cm^{-1}, KBr): 1664 (–C=,O), 2924 (ArH), 3402 (amide -NH); MS (m/e): 420 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.91 (t, 2H), 2.8(t,; 2H), 3.6 (s, 2H) 5.1 (1H), 6.80–7.78(aromatic 9H) ,NH-7.9 (s,1H)^{13}C NMR (100 MHz, DMSO-d6): 21.38, 48.55, 49.36, 57.62, 108.48, 110.18, 110.43, 114.70, 114.80, 114.92, 115.01, 124.75, 125.61, 126.50, 128.26, 128.44, 129.29, 129.60, 130.48, , 131.32, 169.

Characterizationof**2k:**2-(2-chlorophenyl)-N-(2,3,4-trifluorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetamide Yield: 65%; mp: 182; FT-IR (cm^{-1}, KBr): 1676 (–C=O), 2920 (ArH), 3380 (amide –NH); MS (m/e): 437 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.91 (t, 2H), 3.50(t,; 2H), 3.7 (s, 2H), 5.1 (s,1H), 6.80–7.78(aromatic 8H) ,NH-8 (s,1H) ^{13}C NMR (100 MHz, DMSO-d6): 25.44, 50.14, 52.75, 61.77, 113.42, 120.30, 121.65, 123.14, 124.55, 125.12, 125.56, 127.80, 128.12, 129.15, 129.60, 130.52, 130.99, 133.44, 133.88, 135.44, 138.11, 169.

General method for (3a) Synthesis of 5-((2-chlorophenyl)(1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno [3,2-c]pyridine.

Amide 2a derivative (10 g, 0.033 mol) and toluene (100 mL) refluxed for 30–40 minutes using dean stark apparatus to remove the moisture content in the reaction mass (removed two volumes (25mL) of toluene during the reflux. Cooled the reaction mixture to 0–5^{O}C and added PCl_5 (7.0 g; 0.03 mol) into the reaction mass under nitrogen atmosphere. Raised the temperature to 50–60^{0}C and maintained for 2–3 h, then solvent was removed under reduced pressure. Cooled the reaction mixture to 0–5^{0} C and added DMF (100 mL) into the mixture. Stirred the reaction mass for 10–15 min to get clear solution of iminyl chloride. Then charged DMF (80 mL) and finely grinded NaN_3 was added into another RBF. Then added the above iminyl chloride solution into the reaction mass containing sodium azide slowly over a period of 30–40 min under nitrogen atmosphere. After completion of the addition, temperature of the reaction mass raised to 25– 30^{0} C and maintained for 4-5 h. Then cooled the reaction mass to 0–5^{0}C and added water (80 mL) into the reaction mass slowly over a period of 30–40 min to precipitate out the compound 56a as a crude solid material during the addition. The crude material was dissolved in isopropyl acetate (30 mL) and washed the organic layer with water (20 mL). Organic layer was dried over sodium sulfate and the solvent was largely distilled off to isolated the solid in diisopropyl ether (10 mL) as an off white crystalline solid. Yield: 70%; mp: 135^{0}C,FT-IR (cm^{-1}, KBr): 1593 (– C,C), 2935 (ArH); MS (m/e): 334.84.(M + 1); ^{1}H NMR (400 MHz; DMSO-d6) 2.76 (t,2H (–CH_2, pyridine ring)), 2.86 (t, 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 7.0,(S,1H ,NH),)6.5–7.4 (aromatic, 6 H). ^{13}C

NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9, 141.2,152,152.5

Employing the similar procedure as mentioned for 3**a** the remaining amides (**3b-k**) were prepared in quantitatively

Characterization of **3b:** (5-((2-chlorophenyl)(1-phenyl-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine Yield: 65%; mp: 158; FT-IR (cm^{-1}, KBr): 1591 (–C,C), 2930 (ArH); MS (m/e): 410.11. (M + 1); ^{1}H NMR (400 MHz; DMSO-d6) 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t, 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.5–7.4 (aromatic, 11 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9, 136.8,141.2,152,152,152.5
Characterization of **3c:** (5-((2-chlorophenyl)(1-(2-chloro phenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;Yield: 60%; mp: 154; FT-IR (cm^{-1}, KBr): 1594 (–C,C), 2924 (ArH), 783 (C–Cl); MS (m/e): 445.5 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t,; 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.5–7.8 (aromatic, 10 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9, ,141.2,152,152.5
Characterization of **3d:** (5-(2-chlorophenyl)(1-(3-chlorophenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2- c]pyridine .Yield: 60%, mp: 150; FT-IR (cm^{-1}, KBr): 1593 (–C,C), 2926 (ArH), 783 (C–Cl); MS (m/e): 445..5 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t, 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.5–7.8 (aromatic, 10 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9, 141.2,152,152.5
Characterization of **3e:** 5- (2-chlorophenyl)(1-(4 -chloro phenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine. Yield: 50%; mp: 132; FT-IR (cm^{-1}, KBr): 1497 (–C,C), 2922 (ArH), 825 (C–Cl); MS (m/e): 445..5 (M + 1);); ^{1}H NMR (400 MHz; DMSO-d6): 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t, 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.5–7.8 (aromatic, 10 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, , 131.8, 134.5,134.2,135.6,136.9, ,141.2,152,152.5
Characterization of **3f:** (5-(2-chlorophenyl)(1-(2,3-dichlorophenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine Yield: 50%; mp: 128; FT-IR (cm^{-1}, KBr): 1600 (–C,C), 2921 (ArH), 789 (C–Cl); MS (m/e): 478.02 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t, 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.46–7.7 (aromatic, 9 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9,141.2,152,152.5
Characterization of **3g:** (5-(2-chlorophenyl)(1-(3,4, -dichloro phenyl)-1H-tetrazol-5-

yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine)Yield: 60%; mp: 155; FT-IR (cm^{-1}, KBr): 1612 (–C,C), 2946 (ArH), 789 (C–Cl); MS (m/e): 478.02 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.76 (t, 2H (–CH_2, pyridine ring)), 2.86 (t,; 2H (–CH_2, pyridine ring), 3.52 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.46–7.8 aromatic, 9 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.63 (–CH_2, allylic pyridine ring), 53.05 (–CH_2 pyridine ring), 49 (–CH_1,), 112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, , 131.8, 134.5,134.2,135.6,136.9, ,141.2,152,152.5

Characterization of **3h :(**5-(2-chlorophenyl)(1-(2-fluorophenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydrothieno [3,2-c]pyridineYield: 62%; mp: 170; FT-IR (cm^{-1}, KBr): 1508 (–C,C), 2922 (ArH), 767 (C–F); MS (m/e): 428.09 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.80 (t, 2H (–CH_2, pyridine ring)), 2.90(t, 2H (–CH_2, pyridine ring), 3.56 (s, 2H; –CH_2, allylic), 5.1 (s, 1H) ,6.47–7.8 (aromatic, 10H). ^{13}C NMR (100 MHz, DMSO-d6): 25.52 (–CH_2, pyridine ring), 50.51 (–CH_2, allylic pyridine ring), 54.12 (–CH_2 pyridine ring), 49 (–CH,benzylic) ,112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9,141.2,152,152.5

Characterization of **3i:** (5- (2-chlorophenyl)(1-(4-fluorophenyl)- 1H-tetrazol-5-yl) methyl)-4,5,6,7-tetrahydrothieno [3,2-c]pyridineYield: 65%; mp: 154; FT-IR (cm^{-1}, KBr): 1511 (–C,C), 2922 (ArH), 837 (C–F); MS (m/e): 428.09 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.79 (t, 2H (–CH_2, pyridine ring)), 2.91 (t, (–CH_2, pyridine ring), 3.7 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.59–7.7 (aromatic 10 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.52 (–CH_2, pyridine ring), 50.51 (–CH_2, allylic pyridine ring), 54.12 (–CH_2 pyridine ring), 49 (–CH,benzylic) ,114.7,118.2,124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.9, 141.2,152,152.5

Characterization of **3j:** (5-(2-chlorophenyl)(1-(2,5-trifluorophenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetra hydro thieno [3,2-c]pyridine Yield: 60%; mp: 167; FT-IR (cm^{-1}, KBr): 1512 (–C,C), 2903 (ArH), 873 (C–F); MS (m/e): 444.08.4 (M + 1); ^{1}H NMR (400 MHz; DMSO-d6): 2.80 (t, 2H (–CH_2, pyridine ring)), 2.88 (t,2H (–CH_2, pyridine ring), 3.56 (s, 2H; –CH_2, allylic), 5.1 (s, 1H), 6.5–7.8 (aromatic, 10 H). ^{13}C NMR (100 MHz, DMSO-d6): 25.40 (–CH_2, pyridine ring), 50.62 (–CH_2, allylic pyridine ring), 53.12 (–CH_2 pyridine ring), 49 (–CH_2, benzylic), 114.7,117.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5,134.2,135.6,136.92,141.2,158.2,158.5

Characterization of **3k:** (5-(2-chlorophenyl)(1-(2,3,4-trifluorophenyl)-1H-tetrazol-5-yl)methyl)-4,5,6,7-tetrahydro thieno[3,2-c]pyridine)Yield: 60%; mp: 104 LC; FT-IR (cm^{-1}, KBr): 1515 (–C,C), 2900 (ArH), 894 (C–F); MS (m/e): 462.07 (M + 1); ^{1}H NMR400 MHz; DMSO-d6): 2.68(t, 2H (–CH_2, pyridine ring)), 2.93 (t ,2H (–CH_2, pyridine ring), 3.7 (s, 2H; –CH_2, allylic), 5.19 (s, 1H; –CH), 6.5–7.7 (aromatic, 8H). ^{13}C NMR (100 MHz, DMSO-d6): 25.50 (–CH_2, pyridine ring), 50.76 (–CH_2, allylic pyridine ring), 53.10 (–CH_2 pyridine ring 48 (–CH_2, benzylic),112.7,114.2. 124.2, 124.8, 128.6, 128.7, 128.8, 128.8, 131.5, 131.8, 134.5, 135.3,135.6,136.92,141.2,152,152.5

RESULTS AND DISCUSSION

The conversion of an acid function of compound 1 into amide 2a–k is effected by PCl_5 to afford acid chloride which is in situ converted into amide 2a by one pot synthesis using appropriate amines (Scheme 1). The obtained amide is collected as pure material without using any chromatographic purification. The one pot synthesis of a tetrazole nucleus from an amide (2a–k) could be achieved by using PCl_5 to afford iminoyl chloride followed by treatment with sodium azide affords tetrazole (3a–k) ring very easily without any

chromatographic purifications. The process conditions applied for this conversion are appropriate to build 1,5-disubstituted phenyl tetrazoles from the corresponding substituted amides. The obtained tetrazole derivatives showed moderate to good in vitro biological activities as platelet aggregation inhibitors.

Tetrahydro thienopyridine is the core structural fragment present in all the tetrazole analogs (3a–k) and is responsible for the biological activity like Ticlopidine, Clopidogrel and Prasugrel. In general, thienopyridine skeleton is being metabolized in the body after oral administration to form its active metabolites which irreversibly binds to the $P2Y_{12}$ class of ADP receptors on the platelets, thereby inhibiting the platelet activation to avoid the aggregation. Hence, thienopyridine serves as pivotal analogs .. The results (Table 1) prompted us to derive the structure activity relation-ship between the new molecules (3a–k) which boosted us to screen the biological activity of the tetrazole analogs (3a–k). Herewith, we report the synthesis and antiplatelet activity of tetrazole containing derivatives of 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetic acid using two one pot procedures by avoiding the intermediate stages and chromatographic purifications.

ANTIPLATELET ACTIVITY

Antiplatelet activity for all the new compounds 3a–k was performed in vitro following Born's turbid metric methods available in the literature (Born, 1962). The potency of the new compounds was estimated and compared with Clopidogrel and the aggregation was induced by adenosine diphosphate (ADP; 0.3 mM). The test samples were preincubated with platelets for 5 min at 37 LC. All the test compounds were dissolved in DMSO at 300 lg/mL concentration. All the tests were performed within 3 h after collection of blood. Corresponding solvents were used as blank controls for the corresponding tests. The antiplatelet aggregation potency is expressed as inhibition (%) which is calculated as follows:

Inhibition % =_ *(A- B) /A X100*

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

Among all the compounds 3a–k, compound 3e and 3k showed significant platelet aggregation induced by ADP. It was observed that substituted tetrazoles showed good anti-platelet activity over the free tetrazole (3a). Among all the substitutions occurred on the aryl ring, it was observed that substitution at the 4th position of the aromatic ring is more prominent than the substitution at other positions. We attempted to screen the antiplatelet activity of chloro and fluoro substituted series from the examples. It was showed that 4-chloro derivative 3e to be tprominent antiplatelet aggregate-tor among the other chloro substituted derivatives. It was also noticed that 2,3,4-trisubstituted derivative **(3k)** showed good antiplatelet activity over the other derivatives (Table 1). Substitution at the 3rd position of the phenyl ring showed poor antiplatelet activity (**3d**) over the other positions. Hence, all the compounds showed moderate to significant antiplatelet activity compared with Clopidogrel (Table 1).

In vitro antiplatelet aggregation activity studies

Preparation of platelet rich plasma

The citrated blood was used for the preparation of PRP collected from the healthy human volunteer and mixed with 1.0 mL of 3.8% trisodium citrate and centrifuged at 180g for 10

min. The upper two-third fraction of plasma (PRP) was transferred to another centrifuge tube leaving behind lower one-third layer to avoid contamination with WBCs and RBCs. Platelet poor plasma (PPP) was obtained by the centrifugation of the remaining sample at 2500g for 10 min.

Aggregometry

The antiplatelet aggregation studies were evaluated by a turbidimetric method based on ADP-induced (2.0 lM, 5 lL) platelet aggregation in human PRP. Platelet aggregation was studied at 37 LC using Born's method in a platelet aggregation module. A final concentration of ADP 2.0 lmol/L was used in a volume of 5 lL. The new compound 0.05 mL at different concentrations and normal saline were added to 0.45 mL PRP, respectively. After 5 min, ADP (2.0 lmol/L, 5 lL) was given. Maximal change in light transmission was assumed to represent maximal platelet aggregation. Platelet aggregation was measured and the maximal deflection was obtained after 5 min.

The results were expressed as mean ± SEM and the means were compared using Student's t-test, p value is <0.05.

Table 1 In vitro potency of compounds 3a–k (n = 6–8 experiments) in the inhibition of ADP induced platelet aggregation in human PRP.

Compound	**% Of inhibition of platelet aggregation**
3a	29 ± 2
3b	32 ± 3
3c	48 ± 1
3d	23 ± 2
3e	50 ± 1
3f	40 ± 2
3g	35 ± 4
3h	41 ± 2
3I	37 ± 1
3J	40± 1
3k	50 ± 3
Clopidogrel	37 ± 2

Noticed that 2,3,4-trisubstituted derivative (3k) showed good antiplatelet activity over the other derivatives (Table 1). Substitution at the 3rd position of the phenyl ring showed poor antiplatelet activity (3d) over the other positions. Hence, all the compounds showed moderate to significant antiplatelet activity compared with Clopidogrel (Table 2).

APPLICATIONS

All synthesized compounds were screened for antiplatelet activity noticed that 2,3,4-trisubstituted derivative (3k) showed good antiplatelet activity over the other derivatives (Table 1). Substitution at the 3rd position of the phenyl ring showed poor antiplatelet activity (3d) over the other positions. Hence, all the compounds showed moderate to significant

antiplatelet activity compared with Clopidogrel (Table 1)

CONCLUSION

Herewith, we report the synthesis and antiplatelet activity of new thieno pyridines derivatives known as (3a–k). From the results and discussions made above it may be concluded that:The substituted derivatives should be used in future drug designing. Introduction of other heterocyclic moieties may also bring improved inhibitory activity. All the compounds showed moderate to significant platelet aggregation inhibitor activity.

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