



SYNTHESIS AND BIOLOGICAL EVOLUTION OF NOVEL 2-[4'-(6,7-DIHYDRO-4H-THIENO[3,2-C]PYRIDIN-5-YLMETHYL)-BIPHENYL-2-YL]-4-METHYL-THIAZOLE-5-CARBOXYLIC ACID DERIVATIVES AS ANTIPLATELET AGENTS

Vasantha Mittapelli¹, Nageswar Rao Challa², Venkateswara Rao Vallua³, Pratap Reddy Padi⁴, Mahesh Reddy Ghanta⁵

¹ Department of Chemistry and Pharmaceutical Sciences, Mahatma Gandhi University, Nalagonda, Telangana-508 254, India.

² Process Research Laboratory, Research & Development Centre, Macleods Pharmaceuticals Ltd, G-2, Mahakali Caves Road, Shantinagar, Andheri (East), Mumbai-400 093, Maharashtra, India.

³ Department of Chemistry, Pacific University, Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur-313024, Rajasthan, India.

⁴ Process Research Laboratory, Brundavan Laboratories PVT Ltd, Survey No.60-62, Yellegiri Village, Choutuppal, Nalagonda, Telangana-508 254, India.

***Corresponding Author: Vasantha Mittapelli**

Department of Chemistry and Pharmaceutical Sciences, Mahatma Gandhi University, Nalagonda, Telangana-508 254, India.

Article Received on 21/02/2016

Article Revised on 12/03/2016

Article Accepted on 03/04/2016

ABSTRACT

Series of novel derivatives of 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (**6**) are prepared from 4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-carbonitrile (**2**) via easy to operate methodology and tested for their antiplatelet activity using in vitro method. All the derivatives (**6a-k**) showed moderate to good antiplatelet activity.

KEY WORDS: Antiplatelet activity, in vitro, synthesis, saponification, thiazole.

INTRODUCTION

Thienopyridines (4,5,6,7-tetrahydrothieno[3,2-c]pyridines) and their derivatives are important heterocyclic compounds that are widely distributed in nature. Many of the compounds containing tetrahydrothienopyridine skeleton are reported as antibacterial^[1,2], potent non-peptide and non-amidinophenyl GPIIb/IIIa antagonist^[3], antiplatelet aggregators^[4,5] and antithrombotic agents.^[4] Biphenyl system is the key structural unit in most of the sartans and nonpeptide antagonists of angiotensin II receptors. Along with their well-known antihypertensive activity^[6,7], biphenyl derivatives have also been demonstrated as stimulators of growth hormone release^[8], metallo protease inhibitors,^[9,10] complement inhibition activity^[11], and antibiotics.^[12] In this context, biphenyl derivatives have received substantial attention.

Recently, we described the synthesis of 4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-carboxylic acid derivatives as promising anti-thrombotic agents.^[13] In continuation of our efforts to synthesis new thienopyridine derivatives containing a heterocyclic moiety on biphenyl system, we arrived to incorporate a thiazole ring at the 2nd position of biphenyl system as

there are reports which is responsible for the treatment of cardiovascular diseases.^[14]

We have selected the 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (**5**) as key intermediate and synthesized from compound **2** as reported in the literature. We had explored to use of various thionating agents like elemental sulfur, Lawesson's reagent, P₄S₁₀, H₂S etc, for this transformation of cyanide **2** to thioamide **3**. Because of the tedious work up procedures and purification of the isolated compounds using column chromatographic conditions resulted in the search of alternative reagents. Hence, we switched over to use of NaSH as a thionating agent for this transformation under optimized conditions. In this paper, we are reporting the synthesis, characterization and antiplatelet activity of novel 2-[4'-(6,7-dihydro-4H-thieno [3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid derivatives (**6a-k**) in pure and quantitatively.

EXPERIMENTAL

MATERIALS AND INSTRUMENTS

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- d_6 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.

SYNTHESIS AND CHARACTERIZATION

Synthesis of 4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-carbothioic acid amide (3). The compound **2** (10 g, 0.03 mole), NaSH.H₂O (7 g, 0.09 mole) and MgCl₂.6H₂O (7 g, 0.03 mole) were added into the RBF containing DMF (100 mL). The resultant solution was stirred at ambient temperature for 10-12 hr. The progress of the reaction mass was monitored using TLC. After completion of the reaction, the reaction mass was poured into the water (50 mL) under stirring, the precipitated yellow solid was filtered off and washed with water (20 mL). The obtained crude material was suspended in water (50 mL) and adjusted the pH to 7-8 with dilute HCl to afford the title compound **3** as a solid. The wet compound was dried under vacuum at room temperature for 5-6 hr. Yield: 91%; mp: 95°C; FT-IR (cm⁻¹, KBr): 1610 (C=C), 2920 (ArH), 3384 (NH₂). MS (m/z): 365.5 (M+1); ^1H NMR (400 MHz; CDCl₃): δ 1.65 (s, 2H, NH₂), 2.81-2.91 (m, 4H, 2 x CH₂), 3.6 (s, 2H, CH₂), 3.71 (s, 2H, CH₂), 6.52-7.88 (aromatic, 10H).

Synthesis of 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid ethyl ester (4). Ethyl-2-chloro acetoacetate (1 mL) was added to a solution of thioamide **3** (5 g, 0.01 mol) in ethanol (25 mL) at ambient temperature, heated to 55-60°C and maintained for 2-3 hr. Second lot of ethyl-2-chloro acetoacetate (0.5 mL) was added and maintained for 2-3 hr. Then added third lot of ethyl-2-chloro acetoacetate (0.5 mL) and maintained for 2-3 hr. The progress of the reaction mass was monitored using TLC. After completion of the reaction, the reaction mass was cooled to 25-30°C and water (15 mL) was slowly added under stirring. The separated solid was filtered and washed with a mixture of ethanol and water (1:1.5 mL). The wet material was recrystallized from ethanol and dried the compound under vacuum at 60°C for 4-5 hr to obtain compound **4**. Yield: 70%; mp: 110°C; FT-IR (cm⁻¹, KBr): 1707 (C=O), 2926 (ArH); MS (m/z): 475.0 (M+1); ^1H NMR (400 MHz; CDCl₃): δ 1.27 (t, *J* 7.2 Hz; 3H, CH₃), 2.69 (s, 3H, CH₃), 2.84 (t, *J* 5.2 Hz, 2H, allylic CH₂), 2.90 (t, *J* 5.2 Hz, 2H; CH₂, piperidine ring), 3.59 (s, 2H, CH₂), 3.76 (s, 2H, benzylic CH₂), 4.23 (q, *J* 7.2 Hz, 2H, CH₂), 6.72-8.00 (aromatic, 10H). ^{13}C NMR (100 MHz, CDCl₃): δ 14.30 (CH₃, thiazole ring), 17.41 (CH₃ ester), 25.46 (CH₂ piperidine ring), 50.51 (CH₂, allylic), 53.05 (CH₂ piperidine ring), 61.04 (benzylic CH₂), 61.86 (CH₂ ester), 122.54, 123.76, 125.31, 127.73, 129.26, 129.77, 129.84,

129.97, 130.82, 132.03, 133.41, 133.89, 138.05, 139.05, 141.36, 159.23 (thiazole ring), 162.34 (C=O), 169.16 (thiazole ring).

Synthesis of 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (5). A solution of acetonitrile (100 mL) and thiazole ester (**4**, 10 g, 0.02 mole), KOH powder (1.76 g, 0.03) was heated to reflux and maintained for 3-4 hr. The progress of the reaction mass was monitored using TLC. After completion of the reaction, the reaction mass was cooled to 25-30°C and maintained for 1 hr. The separated potassium salt of compound **5** was filtered and washed with acetonitrile (10 mL). The wet cake was dissolved in water (100 mL) and washed with dichloromethane (2x50 mL). The pH of the aqueous layer was adjusted to 3-4 with 5% acetic acid. The precipitated compound was filtered off and dried at 60°C under reduced pressure for 4-5 hr to yield compound **5**. Yield: 75%, mp: 208°C; FT-IR (cm⁻¹, KBr): 1645 (C=O), 2935 (ArH), 3405 (OH); MS (m/z): 447.1 (M+1); ^1H NMR (400 MHz; DMSO- d_6): δ 2.61 (s, 3H, CH₃), 2.78-2.91 (m, 4H, 2xCH₂, pyridine ring), 3.60 (s, 2H (CH₂, allylic), 3.72 (s, 2H; CH₂, benzylic), 6.78-8.08 (aromatic, 10H). ^{13}C NMR (100 MHz, CDCl₃): δ 14.33 (-CH₃, thiazole ring), 25.45 (CH₂ pyridine ring), 50.54 (-CH₂, allylic), 53.12 (CH₂ pyridine ring), 61.12 (benzylic CH₂), 122.51, 123.42, 125.12, 127.63, 129.32, 129.75, 129.82, 129.95, 130.91, 132.12, 133.39, 133.90, 138.15, 139.12, 141.41, 159.13 (thiazole ring), 162.13 (C=O), 169.21 (thiazole ring).

General synthetic procedure for 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid amides (6a-k)

To a solution of thiazole acid (**5**, 1 g, 0.002 mole) in dichloromethane (10 mL), hydroxy benzotriazole (0.3 g, 0.02 moles) was added at 0 to -5°C under nitrogen atmosphere. Triethyl amine (0.49 g, 0.004 moles) and thionyl chloride (0.2 g, 0.002 moles) were added subsequently, allowed to 10-15°C and maintained for 1-2 hr to form the active ester of thiazole acid **5**. The water (5 mL) was added to the reaction mass, stirred for 5-10 minutes and then separated the organic layer. Appropriate amine (0.002 mole) in dichloromethane (10 mL) was added to the organic layer over a period of 30-40 min. at 10-15°C. The temperature of the reaction mixture was raised to 25-30°C after completion of the addition and maintained for 10-12 hr. Progress of the reaction was monitored by TLC. Water (10 mL) was added to the reaction mass under stirring, separated the layers, extracted aqueous layer with dichloromethane. The pooled organic layer was washed with K₂CO₃ solution (5 mL) followed by water (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The solvent was co-distilled with diisopropyl ether and the resultant residue was treated with diisopropyl ether to isolate compounds **6a-k** in quantitatively.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxamide (6a): Pale yellow solid. Yield: 60%; mp: 176-179°C; IR (cm⁻¹, KBr): 1718 (C=O), 3400 (NH₂); MS (m/z): 446.9 (M+1); ¹H NMR (400 MHz; DMSO-*d*₆): δ 2.15 (s, 3H, CH₃), 2.78-2.83 (m, 4H, 2 x CH₂), 3.50 (s, 2H (CH₂, allylic), 3.73 (s, 2H, CH₂, benzylic), 6.79-7.69 (10H, aromatic). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.62, 25.63, 50.58 53.14, 61.44, 122.33, 122.95, 124.82, 127.28, 129.10, 129.38, 129.65, 130.00, 130.28, 132.01, 133.23, 133.19, 137.52, 138.75, 141.04, 159.24, 161.91, 168.76. Elemental analysis: (CHNS) Anal calcd for C₂₅H₂₃N₃OS₂. C, 67.39; H, 5.20; N, 9.43; S, 14.39. Found: C, 67.41; H, 5.31; N, 9.42; S, 14.51.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid phenyl amide (6b): Pale yellow solid. Yield: 75%; mp: 130-33°C; IR (cm⁻¹, KBr): 1680(C=O), 3350 (NH); MS (m/z): 522.1 (M+1); ¹H NMR (400 MHz; DMSO-*d*₆): δ 2.71 (s, 3H, CH₃), 2.83-2.91 (m, 4H, 2 x CH₂), 3.61 (s, 2H (CH₂, allylic), 3.74 (s, 2H, CH₂, benzylic), 6.61-8.11 (16H, aromatic). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 17.33, 25.42, 50.70, 53.09, 61.96, 120.31, 122.69, 124.85, 125.31, 127.40, 127.84, 129.11, 129.42, 129.79, 129.88, 130.02, 130.85, 131.75, 133.33, 133.70, 137.41, 138.28, 139.02, 141.26, 155.39, 160.04, 166.66.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (2-chlorophenyl) amide (6c): Pale yellow solid. Yield: 70%; mp: 259-261°C; IR (cm⁻¹, KBr): 1667(C=O), 3453 (NH); MS (m/z): 555.9 (M+1); ¹H NMR (400 MHz; DMSO-*d*₆): δ 2.72 (s, 3H, CH₃), 2.86-2.90 (m, 4H, 2 x CH₂), 3.59 (s, 2H (CH₂, allylic), 3.76 (s, 2H, CH₂, benzylic), 6.60-8.05 (15H, aromatic). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 17.25, 21.43, 49.18, 49.93, 58.40, 111.02, 111.17, 119.59, 122.92, 124.65, 124.99, 126.64, 126.79, 128.13, 128.58, 129.59, 129.93, 130.24, 130.34, 131.16, 131.59, 131.70, 140.05, 141.74, 155.89, 160.53, 166.20.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (3-chlorophenyl) amide (6d): Off white solid. Yield: 65%; mp: 220-223°C; IR (cm⁻¹, KBr): 1667(C=O), 3453 (NH); MS (m/z): 555.9 (M+1); ¹H NMR (400 MHz; CDCl₃): δ 2.73 (s, 3H, CH₃), 2.85-2.91 (m, 4H, 2 x CH₂), 3.58 (s, 2H (CH₂, allylic), 3.75 (s, 2H, CH₂, benzylic), 6.59-8.05 (15H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ 17.83, 25.04, 50.57, 53.70, 62.11, 121.35, 122.77, 125.32, 126.19, 127.78, 129.11, 129.25, 129.81, 129.87, 130.01, 130.58, 131.56, 133.03, 133.06, 135.99, 138.91, 139.20, 141.42, 155.58, 160.20, 166.08.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (4-chlorophenyl)-amide (6e): Pale yellow solid. Yield: 62%; mp: 140-143°C; IR (cm⁻¹,

KBr): 1638(C=O), 3423 (NH); MS (m/z): 555.9 (M+1); ¹H NMR (400 MHz; CDCl₃): δ 2.72 (s, 3H, CH₃), 2.85-2.90 (m, 4H, 2 x CH₂), 3.58 (s, 2H (CH₂, allylic), 3.75 (s, 2H, CH₂, benzylic), 6.59-8.05 (15H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ 17.38, 25.40, 50.75, 53.07, 62.11, 121.53, 122.77, 125.23, 126.91, 127.87, 129.11, 129.52, 129.18, 129.78, 130.10, 130.85, 131.65, 133.30, 133.60, 135.99, 138.19, 139.02, 141.24, 155.85, 160.02, 166.80.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (2,3-dichlorophenyl)-amide (6f): Cream colored solid. Yield: 50%; mp: 156-160°C; IR (cm⁻¹, KBr): 1643(C=O), 3304 (NH); MS (m/z): 590.00 (M+1); ¹H NMR (400 MHz; DMSO-*d*₆): δ 2.78 (s, 3H, CH₃), 2.84-2.90 (m, 4H, 2 x CH₂), 3.58 (s, 2H (CH₂, allylic), 3.77 (s, 2H, CH₂, benzylic), 6.67-8.37 (14H, aromatic). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 17.58, 25.53, 50.74, 53.12, 61.97, 119.20, 121.26, 122.58, 125.32, 125.43, 127.55, 127.87, 127.95, 129.34, 129.59, 129.93, 130.16, 130.84, 131.58, 132.81, 133.42, 133.88, 136.15, 138.68, 138.82, 141.45, 155.66, 159.75, 167.39.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (3,4-dichlorophenyl)-amide (6g): Cream colored solid. Yield: 60%; mp: 161-164°C; IR (cm⁻¹, KBr): 1643(C=O), 3304 (NH); MS (m/z): 590.00 (M+1); ¹H NMR (400 MHz; CDCl₃): δ 2.71 (s, 3H, CH₃), 2.84-2.90 (m, 4H, 2 x CH₂), 3.56 (s, 2H (CH₂, allylic), 3.74 (s, 2H, CH₂, benzylic), 6.62-8.04 (14H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ 17.43, 25.47, 50.79, 53.09, 62.03, 119.40, 121.90, 122.76, 125.20, 126.53, 127.88, 128.01, 129.49, 129.79, 129.85, 130.17, 130.55, 130.89, 131.56, 132.91, 133.31, 133.67, 136.89, 138.37, 138.92, 141.28, 156.22, 160.00, 167.01.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (2-fluorophenyl)-amide (6h): Off white colored solid. Yield: 65%; mp: 118-121°C; IR (cm⁻¹, KBr): 1656(C=O), 3451(NH); MS (m/z): 540.00 (M+1); ¹H NMR (400 MHz; CDCl₃): δ 2.78 (s, 3H, CH₃), 2.87-2.90 (m, 4H, 2 x CH₂), 3.61 (s, 2H (CH₂, allylic), 3.80 (s, 2H, CH₂, benzylic), 6.70-8.34 (15H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ 17.39, 25.47, 50.66, 53.10, 62.92, 114.75, 114.94, 121.69, 122.57, 124.70, 124.74, 125.35, 126.09, 126.19, 127.63, 127.84, 129.35, 129.68, 129.88, 130.07, 130.82, 131.69, 133.41, 133.86, 138.55, 138.87, 141.38, 151.24, 153.66, 155.25, 159.77, 167.19.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (4-fluorophenyl)-amide (6i): Off white colored solid. Yield: 70%; mp: 146-149°C; IR (cm⁻¹, KBr): 1653 (C=O), 3423 (NH); MS (m/z): 540.10 (M+1); ¹H NMR (400 MHz; CDCl₃): δ 2.72 (s, 3H, CH₃), 2.85-2.91 (m, 4H, 2 x CH₂), 3.58 (s, 2H (CH₂, allylic), 3.75 (s, 2H, CH₂, benzylic), 6.64-8.05 (15H,

aromatic). ^{13}C NMR (100 MHz, CDCl_3): δ 17.35, 25.47, 50.73, 53.12, 62.02, 115.68, 115.90, 122.22, 122.30, 122.69, 125.25, 126.95, 127.85, 129.44, 129.80, 129.86, 130.06, 130.87, 131.69, 133.34, 133.73, 138.34, 138.98, 141.25, 155.69, 158.47, 160.06, 160.90, 166.70.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (2,4-difluorophenyl)-amide (6j): Off white colored solid. Yield: 72%; mp: 163-166°C; IR (cm^{-1} , KBr): 1659 (C=O), 3449 (NH); MS (m/z): 558.00 (M+1); ^1H NMR (400 MHz; CDCl_3): δ 2.75 (s, 3H, CH_3), 3.05-3.21 (m, 4H, 2 x CH_2), 3.82 (s, 2H (CH_2 , allylic), 4.00 (s, 2H, CH_2 , benzylic), 6.73-8.07 (14H, aromatic). ^{13}C NMR (100 MHz, CDCl_3): δ 17.48, 29.69, 50.07, 53.12, 62.02, 108.84, 109.14, 110.45, 110.53, 110.70, 110.78, 115.12, 115.22, 115.34, 115.44, 125.20, 126.95, 128.08, 129.70, 130.21, 130.29, 130.66, 131.63, 140.94, 155.08, 159.69, 167.38.

2-[4'-(6,7-Dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (2,3,4-trifluorophenyl)-amide (6k): Off white colored solid. Yield: 80%; mp: 128-131°C; IR (cm^{-1} , KBr): 1654 (C=O), 3421 (NH); MS (m/z): 575.90 (M+1); ^1H NMR (400 MHz; CDCl_3): δ 2.77 (s, 3H, CH_3), 2.83-2.90 (m, 4H, 2 x CH_2), 3.56 (s, 2H (CH_2 , allylic), 3.75 (s, 2H, CH_2 , benzylic), 6.66-8.08 (13H, aromatic). ^{13}C NMR (100 MHz, CDCl_3): δ 17.55, 25.53, 50.72, 53.14, 62.01, 121.30, 122.55, 122.64, 124.84, 125.36, 127.84, 127.88, 127.93, 129.04, 129.33, 129.59, 129.91, 130.07, 130.82, 131.69, 133.43, 133.92, 134.51, 138.65, 138.85, 141.43, 155.22, 159.73, 167.24.

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

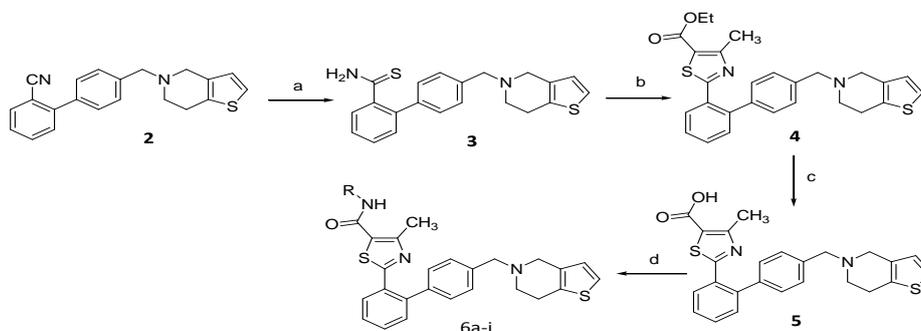
Biological activities of the new compounds were conducted at Bioworld Research Technologies, Hyderabad, Andhra Pradesh, India. Anti-platelet activity studies were evaluated by turbidimetric methods based on ADP-induced (5 μL , 2.0 $\mu\text{mol/L}$) platelet aggregation in human PRP. Platelet aggregation was studied at 37°C using Born's method in a platelet aggregation module. A final concentration of ADP 2.0 $\mu\text{mol/L}$ was used in a volume of 5 μL . The new compound at 0.05 mL different concentrations and normal saline were added to 0.45 mL PRP respectively. After 5 mins, ADP (2.0 $\mu\text{mol/L}$, 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 \pm SEM and the means were compared using Student's t-test, p value is <0.05.

RESULTS AND DISCUSSION

CHEMISTRY

The compound **2** was treated with $\text{NaSH}\cdot\text{H}_2\text{O}$ and $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ in DMF and the obtained thioamide **3** was converted into the thiazole nucleus **4** easily on reaction with 2-chloro ethyl acetoacetate in ethanol medium. The formation of thiazole nucleus **4** was confirmed by the absence of 1620 cm^{-1} and 3371 cm^{-1} peaks (thioamide) and presence of 1707 cm^{-1} (C=O) peak in IR spectrum, molecular ion peak at m/z 475.0 (M+1) in DIPMS and signals at δ 1.27 (t, 3H, CH_2CH_3), 2.69 (s, 3H, CH_3), 4.23 (q, 2H, CH_2CH_3) in the ^1H NMR spectrum. The compound **4** on saponification with KOH powder in acetonitrile medium affords 2-[4'-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid (**5**). The carboxylic acid function was activated by SOCl_2 and HOBt to form active ester of the component followed by treatment with ammonia affords the compound **6a** quantitatively. The structure of compound **6a** was confirmed by IR, NMR spectral and elemental analysis data.

This method extended to ten other derivatives and in all these cases, the corresponding thiazoles (Scheme 1) were obtained as final products.



R = 6a: H; 6b: C_6H_5 ; 6c: 2-Cl- C_6H_4 ; 6d: 3-Cl- C_6H_4 ; 6e: 4-Cl- C_6H_4 ; 6f: 2,3-Cl- C_6H_3 ; 6g: 3,4-Cl- C_6H_3 ; 6h: 2-F- C_6H_4 ; 6i: 4-F- C_6H_4 ; 6j: 2,5-F- C_6H_3 ; 6k: 2,3,4-F- C_6H_2

Scheme 1: a) NaSH , $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ b) Ethyl-2-chloro acetoacetate, ethanol, 55-60°C c) KOH, acetonitrile, aq.HCl; d) Triethyl amine, SOCl_2 , dichloromethane, HOBt, R-NH₂

ANTIPLATELET ACTIVITY

Anti-platelet activity for all the new compounds 6a-k was performed in vitro following Born's turbidimetric methods available in the literature.^[15] 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

$$\text{Inhibition \%} = (A - B) / A \times 100$$

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

Among all the compounds 6a-k, compound 6a showed significant anti-platelet activity induced by ADP. The activity of the 6k and 6b is on par with Clopidogrel and the compound 6c showed poor antiplatelet activity over the other derivatives (Table I). The results were expressed as mean ± SEM and the means were compared using Student's t-test, p value is <0.05.

Table I: In vitro potency of compounds 6a-k (n=6-8 experiments) in the inhibition of ADP induced platelet aggregation in human PRP

Compound	% of Inhibition of platelet aggregation
Vehicle	
6a	56±1
6b	38±2
6c	18±1
6d	25±2
6e	22±1
6f	28±2
6g	28±4
6h	26±2
6i	23±1
6j	33±2
6k	39±3
Clopidogrel	39±3

CONCLUSIONS

2-[4'-(6,7-Dihydro-4*H*-thieno[3,2-*c*]pyridin-5-ylmethyl)-biphenyl-2-yl]-4-methyl-thiazole-5-carboxylic acid derivatives (6) can be easily synthesized from 4'-(6,7-dihydro-4*H*-thieno[3,2-*c*]pyridin-5-ylmethyl)-biphenyl-2-carbonitrile (2). Studies of antiplatelet activity of the synthesized compounds have revealed potentiality of new compounds. Compound 6a exhibited a higher activity as compared with Clopidogrel. It was observed that the substituted aromatic amides did not show any improved activity over the free amide instead they possess moderate activity only. Hence, it can be concluded that the entire compounds showed moderate to significant antiplatelet activity compared to Clopidogrel and compound 6a can be developed as a drug.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Macleods Pharmaceuticals Ltd., Research & Development Centre, Andheri (E) for providing necessary facilities. Authors would like to thank Analytical Research & Development Department for their co-operation in carrying out this work.

REFERENCES

1. Srivastava BK, Solanki M, Mishra B, Soni R, Jayadav S, Valani D, Jain M, Patel PR, Synthesis and antibacterial activity of 4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine quinolones. *Bioorg Med Chem Lett*, 2007; 17(7): 1924–1929.
2. Maske PV, Makhija SJ, Synthesis and antibacterial activity of 5-chloro-N-cyclohexyl-6-thio substituted-nicotinamide derivatives. *Der Pharmacia Lettre*, 2011; 3(5): 110-114.
3. Katano K, Shitara E, Shimizu M, Sasaki K, Miura T, Isomura Y, Kawaguchi M, Ohuchi S, Tsuruoka T, Tetrahydrothienopyridine derivatives as novel GPIIb/IIIa antagonists. *Bioorg Med Chem Lett*, 1996; 6(21): 2601-2606.
4. Esanu A, Thienopyridine derivatives and anti-thrombotic compositions containing the same, US Patent, US 4681888, 1987.
5. Rao CN, Balaji M, Reddy GM, Reddy PP, Synthesis and pharmacological evaluation of 5-[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine derivatives as platelet aggregation inhibitors. *J Saudi Chem Soc*, 2014; 18(5): 513–519.

6. Duncia JV, Chiu AT, Carini DJ, Gregory GB, Johnson AL, Price WA, Wells GJ, Wong PC, Calabrese JC, Timmermans PBMWM, The discovery of potent nonpeptide angiotensin II receptor antagonists: a new class of potent antihypertensives. *J Med Chem*, 1990; 33(5): 1312-1329.
7. Dickstein K, Timmermans P, Segal R, Losartan: A selective angiotensin II type 1 (AT1) receptor antagonist for the treatment of heart failure, *Exp Opin Invest Drugs*, 1998; 7(11): 1897-1914.
8. Smith RG, Cheng K, Schoen WR, Pong SS, Hickey G, Jacks T, Butler B, Chan WW, Chaung LY, Judith F, A nonpeptidyl growth hormone secretagogue. *Science*, 1993; 260(5114): 1640-1643.
9. Green BG, Toney JH, Kozarich JW, Grant SK, Inhibition of bacterial peptide deformylase by biaryl acid analogs. *Arch Biochem & Biophys*, 2000; 375(2): 355-358.
10. Toney JH, Cleary KA, Hammond GG, Yuan X, May WJ, Hutchins SM, Ashton WT, Vanderwall DE, Structure-activity relationships of biphenyl tetrazoles as metallo- β -lactamase inhibitors. *Bioorg Med Chem Lett*, 1999; 9(18): 2741-2746.
11. Master HE, Khan SI, Poojari KA, Design and synthesis of non-cytotoxic tetrahydro-thieno[3,2-c]pyridine derivatives exhibiting complement inhibition activity. *Indian J Chem*, 2008; 47B(1): 97-105.
12. Toney JH, Fitzgerald PM, Sharma NG, Olson SH, May WJ, Sundelof JG, Vanderwall DE, Cleary KA, Grant SK, Wu JK, Kozarich JW, Pompliano DL, Hammond GG, Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of *Bacteroides fragilis* metallo-beta-lactamase. *Chem Biol*, 1998; 5(4): 185-96.
13. Rao CN, Reddy GM, Reddy PP, Synthesis and Antithrombotic Activity Study of Some New Thienopyridine Derivatives. *Lett in Org Chem*, 2011; 8(6): 412-415.
14. Kurup A, Garg R, Carini DJ, Hansch C, Comparative QSAR: Angiotensin II antagonists. *Chem Rev*, 2001; 101(9): 2727-2750.
15. Born GVR, Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (London)*, 1962; 194(9 June 1962): 927-929.