Asian Journal of Chemistry; Vol. 30, No. 9 (2018), 2063-2068

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21418

Synthesis, Characterization and Anticancer Activity of 5-Substituted 4,5,6,7-Tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide Derivatives

N. SREE LAKSHMANA RAO[1], MANDAVA V. BASAVESWARA RAO[2,*] and K.R.S. PRASAD[1]

[1]Department of Chemistry, K.L. University, Vaddeswaram, Guntur-522 502, India
[2]Department of Chemistry, Krishna University, Machilipatnam-521 001, India

*Corresponding author: E-mail: mandavabasaveswararaov@gmail.com

Received: 2 May 2018; *Accepted*: 9 June 2018; *Published online*: 31 July 2018; AJC-19021

4,5,6,7-Tetrahydrothieno pyridine (THTP) and their derivatives are an important heterocyclic compounds that exhibits various biological activities *viz.*,antimicrobial activity, antileishmanial activity, antiarrhythmic activity, antiinflammatory activity, antihyperlipidemic activity, antidepressant activity, anticancer activity, antiplatelet activity and antidiabetic *etc*. The present study describes the synthesis, characterization and *in vitro* cytotoxic potential against human lung carcinoma of some novel derivatives of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine **(7A-M)** with benzylic and amide substitution on the nitrogen atom of tetrahydro theino pyridine ring. The synthetic steps involves (i) Vilsmeyer protocol in step 1 (ii) formation of tetrahydothieno[3,2-c]pyridine ring in presence of 2-mercaptoacetate in step 2 (iii) alkaline hydrolysis followed by amide coupling with tetrahydro-2*H*-pyran-4-amine in step 3 and step 4. The newly synthesized compounds **7A-M** was sufficiently characterized by ^{1}H NMR, IR and mass techniques. Furthermore, these derivatives were screened for their *in vitro* cytotoxic potential against human lung carcinoma (HCT-116) cell line using the MTT assay. Compound **7B** (IC_{50}: 69.52 µg) and compound **7K** (IC_{50}: 66.35 µg) exhibited significant activity at micro molar concentration when compared to standard drug camptothecin.

Keywords: Anticancer activity, Camptothecin, Synthesis, Tetrahydothieno[3,2-c]pyridine, Villsmeyer-Hack.

INTRODUCTION

4,5,6,7-Tetrahydrothieno-pyridine (THTP) represents a widely used lead structure with multitude of interesting applications in numerous pharmacological fields. Thus, various biological activities such as antimicrobial activity [1-15], antileishmanial activity [16], antiarrhythmic activity [17], antiinflammatory activity [18-22], antihyperlipidemic activity [23], antidepressant activity [24], anticancer activity [25,26], antiplatelet activity [27,28], antidiabetic [29-31] and antituberculosis activity [32,33] have been reported and explored till date. Fusion of other nuclei like benzene, indole, oxadiazole, triazole rings to THTP nucleus enhances the pharmacological activities than its parent nucleus.

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 [34-36]. Hence, different substitutions at nitrogen of the biological activity of the new chemical entities (NCEs). The present study describes the synthesis, characterization and *in vitro* cytotoxic potential against human lung carcinoma of some novel derivatives of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine (**7A-M**) with benzylic and amide substitution on the nitrogen of the thieno pyridine ring.

EXPERIMENTAL

The solvents were purified according to standard procedures prior to use and all commercial chemicals were used as received. For thin-layer chromatography (TLC) analysis, Merck pre-coated plates (silica gel 60 F254) were used and eluting solvents are indicated in the procedures. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography. Melting point determinations were performed by using Meltemp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity instrument at room temperature at 400 MHz. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent and coupling constants in Hz. The mass spectra were recorded on Agilent ion trap MS. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrometer.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License, which allows others to copy and redistribute the material in any medium or format, remix, transform, and build upon the material, as long as appropriate credit is given and the new creations are licensed under the identical terms.

***tert*-Butyl 3-formyl-4-oxopiperidine-1-carboxylate (2):** To a stirred solution of *tert*-butyl 4-oxopiperidine-1-carboxylate (**1**) (40 g, 0.20 mol) in dichloromethane (500 mL) was added DMF (30.9 mL, 0.40 mol) followed by $POCl_3$ (30 mL, 0.32 mol) at 0 °C and the reaction mixture was stirred at room temperature for 4 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with aqueous sodium acetate solution (at 0 °C), diluted with water and extracted with ethyl acetate (2 × 100 mL). The organic layer was washed with water (3 × 50 mL), brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain compound **2**. The crude compound was used in the next step without any purification. Yellow oil; Yield: 30 g, 65 %; Elemental analysis, $C_{11}H_{17}NO_4$, calcd. (found) %: C 58.14 (58.11), H 7.54 (7.56), N 6.16 (6.12). IR (KBr, ν_{max}, cm^{-1}): 2926 (-CH *str*.), 1695, 1635 (-C=O *str*.), 1122 (-C-OC- *str*.); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.14 (s, 1H), 4.15 (d, *J* = 7.0 Hz, 2H), 3.72 (t, *J* = 6.0 Hz, 2H), 2.44 (t, *J* = 6.0 Hz, 2H), 2.09 (brs, 2H), 1.45 (s, 9H); MS: calcd. for $C_{15}H_{21}NO_4S$ [*m/z*] 311.40; found: 312.6 $[M+H]^+$.

5-*tert*-Butyl 2-ethyl-6,7-dihydrothieno[3,2-c]pyridine-2,5-(4*H*)-dicarboxylate (3): To a stirred solution of compound **2** (30 g, 0.132 mol) in dichloromethane (150 mL) was added triethyl amine (29.1 mL, 0.211 mol) and ethyl 2-mercaptoacetate (17.3 mL, 0.158 mol) at 0 °C. The reaction mixture was stirred at 45 °C for 16 h. After completion of reaction, the reaction mixture was diluted with ice cold water and extracted with dichloromethane (3 × 50 mL). The organic layer was washed with water, brine solution, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to afford compound **3**. The crude compound (30 g) was used in the next step without any further purification. Elemental analysis, $C_{15}H_{21}NO_4S$, calcd. (found) %: C 57.86 (57.84), H 6.80 (6.77), N 4.50 (4.53).

5-(*tert*-Butoxycarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylic acid (4): To a stirred solution of compound **3** (30g, 0.096 mol) in a mixture of tetrahydrofuran, methanol and water (250 mL, 3:1:1) was added sodium hydroxide (11.5 g, 0.288 mol) and stirred at room temperature for 16 h. After completion of reaction, the reaction mixture was evaporated under reduced pressure to obtain a yellow viscous residue. The residue was acidified with aqueous citric acid solution to about pH 4 and the resulting mixture was stirred at 0 °C for 15 min. The obtained solid was filtered, washed with water and dried under vacuum to afford compound **4**. Yellow solid; Yield: 15 g, 55 %; m.p.: 179-181 °C; Elemental analysis, $C_{13}H_{17}NO_4S$, calcd. (found) %: C 55.11 (55.15), H 6.05 (6.09), N 4.94 (4.97). IR (KBr, ν_{max}, cm^{-1}): 3598, 3492, 2982, 2441, 1658, 1560, 1465, 1416, 1256, 1156, 1012, 947, 856, 759, 601, 506. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (s, 1H), 4.50 (brs, 2H), 3.73 (d, *J* = 6.0 Hz, 2H), 2.89 (brs, 2H), 1.49 (s, 9H); MS: calcd. for $C_{13}H_{17}NO_4S$ [*m/z*] 283.34; found: 282.21 $[M-H]^+$.

***tert*-Butyl 2-(tetrahydro-2*H*-pyran-4-ylcarbamoyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-carboxylate (5):** The a stirred mixture of compound **4** (8 g, 28.26 mmol) in DMF (80 mL) was sequentially added EDC·HCl (8.2 g, 42.3 mmol), HOBt (4.5 g, 33.8 mmol), diisopropylethylamine (14.7 mL, 84.6 mmol) and tetrahydro-2*H*-pyran-4-amine (4.2 g, 31.0 mmol) at room temperature stirred for 5 h. After completion of reaction, the reaction mixture was quenched with water and extracted with ethyl acetate (4 × 25 mL). The organic layer was washed with water, brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford compound **5**. White solid; Yield: 5 g, 77 %; m.p.: 146-149 °C. Elemental analysis, $C_{18}H_{26}N_2O_4S$, calcd. (found) %: C 58.99 (58.96), H 7.15 (7.12), N 7.64 (7.62). IR (KBr, ν_{max}, cm^{-1}): 3274, 2964, 2845, 2360, 1696, 1616, 1539, 1402, 1244, 1164, 1093, 869, 777, 661; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.21 (d, *J* = 6.4 Hz, 1H), 7.53 (s, 1H), 4.40 (s, 1H), 3.94-3.85 (m, 3H), 3.63 (t, *J* = 5.6 Hz, 2H), 3.38-3.33(m, 2H), 2.78 (s, 2H), 1.74-1.70 (m, 2H), 1.58-1.54 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.3, 153.9, 137.9, 137.3, 132.9, 125.9, 79.2, 66.0 (2C), 45.6 (2C), 43.5 (2C), 32.4 (2C), 28.0 (4C); MS: calcd. for $C_{18}H_{26}N_2O_4S$ [*m/z*] 366.48; found: 367.30 $[M+H]^+$.

N-(Tetrahydro-2*H*-pyran-4-yl)-4,5,6,7-Tetrahydrothieno[3,2-c]pyridine-2-carboxamide.TFA salt (6): To a stirred solution of compound **5** (2 g, 5.46 mmol) in DCM (25 mL) was added trifluoroacetic acid (0.8 mL, 10.0 mmol) and the reaction mixture was stirred at 0 °C for 16 h. After completion of reaction, the reaction mixture was evaporated under reduced pressure to afford compound **6**. White solid; Yield: 1.2 g, 77 %; m.p.: 194-197 °C; Elemental analysis, $C_{13}H_{18}N_2O_2S$, calcd. (found) %: C 58.62 (58.60), H 6.81 (6.78), N 10.52 (10.54). IR (KBr, ν_{max}, cm^{-1}): 3295, 2991, 2839, 2360, 1687, 1623, 1687, 1623, 1538, 1204, 1145, 836, 792, 719; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.06 (brs, 1H), 8.33 (d, *J* = 7.6 Hz, 1H), 7.56 (s, 1H), 4.20 (brs, 2H), 3.93-3.84 (m, 3H), 3.0 (t, *J* = 5.6 Hz, 2H), 1.74-1.71 (m, 2H), 1.58-1.49 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.1, 138.48, 136.42, 128.84, 126.0, 66.1, 45.81, 41.97, 40.75, 32.42, 21.75. MS: calcd. for $C_{15}H_{18}F_3N_2O_3S$ [*m/z*] 363.38; found: 267.0 $[M-TFA]^+$.

General experimental procedure for the synthesis of 5-substituted 4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide derivatives (7A-7M): To a stirred solution of compound **6** (150 mg, 0.57 mmol) in DMF (5 mL) was added K_2CO_3 (0.23 g, 1.7 mmol) followed by respective aryl/hetero aryl halides (0.08 mL, 0.67 mmol). The reaction mixture was stirred at 0 °C for 15 min and later to room temperature for 6 h. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (2 × 10 mL). The organic layer was washed with water, brine solution, dried over anhydrous *s*odium sulfate, filtered and evaporated under reduced pressure to produce compound **7A-M**. Yields of the compounds vary between 74-92 %.

5-Benzyl-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7A): White solid; Yield: 135 mg, 92 %; m.p.: 181-183 °C; Elemental analysis, $C_{20}H_{24}N_2O_2S$, calcd. (found) %: C 67.38 (67.36), H 6.79 (6.77), N 7.86 (7.85). IR (KBr, ν_{max}, cm^{-1}): 3445, 3209, 3053, 2966, 2925, 2843, 2791, 1695, 1607, 1542, 1460, 1331, 1305, 1275, 1242, 1142, 1086, 1054, 1008, 885, 745, 706, 605, 570; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.37-7.20 (m, 5H), 7.12 (s, 1H), 5.65 (d, *J* = 8.0 Hz, 1H), 4.17-4.16 (m, 1H), 3.98 (t, *J* = 2.0 Hz, 2H), 3.70 (s, 2H), 3.54-3.47 (m, 4H), 2.88 (d, *J* = 4.8 Hz, 2H), 2.81 (t, *J* = 4.5 Hz, 2H), 1.99-1.95 (m, 2H); ^{13}C NMR (100 MHz,

DMSO-d_6): δ 161.1, 141.6, 137.7, 137.0, 135.9, 135.6, 128.9 (2C), 64.0 (2C), 60.0, 55.1, 52.8, 51.9, 36.6 (2C), 24.5; MS: calcd. for $C_{20}H_{24}N_2O_2S$ [*m/z*] 356.48; found: 357.41 $[M+H]^+$.

5-(2-Chlorobenzyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7B): Light brown solid; Yield: 139 mg, 86 %; m.p.: 153-155 °C; Elemental analysis, $C_{20}H_{23}N_2O_2SCl$, calcd. (found) %: C 61.45 (61.46), H 5.93 (5.91), N 7.17 (7.15). IR (KBr, ν_{max}, cm^{-1}): 3280, 3065, 2949, 2924, 2846, 1614, 1571, 1535, 1466, 1440, 1327, 1245, 1135, 1090, 1047, 874, 798, 747, 657; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.15 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 6.8 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.36 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 7.2 Hz, 1H), 3.92-3.84 (m, 2H), 3.77 (s, 2H), 3.54 (s, 2H), 3.38-3.33 (m, 3H), 2.82-2.78 (m, 4H), 1.72 (t, *J* = 10.8 Hz, 2H), 1.57-1.47 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.4, 138.1, 136.8, 135.7, 134.5, 133.2, 130.6, 129.2, 128.6, 127, 126.2, 66.1 (2C), 57.5, 52.1, 49.6, 45.6, 32.4 (2C), 25.1; MS: calcd. for $C_{20}H_{23}N_2O_2SCl$ [*m/z*] 390.93; found: 391.76 $[M+H]^+$.

5-(2-Fluorobenzyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7C): Off white solid; Yield: 140 mg, 90 %; m.p.: 139-141 °C. Elemental analysis, $C_{20}H_{23}N_2O_2SF$, calcd. (found) %: C 64.15 (64.13), H 6.19 (6.20), N 7.48 (7.46). IR (KBr, ν_{max}, cm^{-1}): 3429, 3266, 2948, 2847, 1631, 1541, 1448, 1383, 1309, 1237, 1139, 1079, 878, 770, 605; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.40 (d, *J* = 7.6 Hz, 1H), 7.68-7.61 (m, 3H), 7.42-7.34 (m, 2H), 4.90 (d, *J* = 13.2 Hz, 2H), 4.63 (d, *J* = 13.2 Hz, 2H), 4.33 (s, 2H), 3.95-3.80 (m, 6H), 3.40-3.28 (m, 1H), 1.72 (t, *J* = 10.4 Hz, 2H), 1.59-1.53 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.4, 161.1, 141.6, 137.7, 137.0, 135.9, 130.5, 128.9, 125.4, 124.1, 115.2, 64.0 (2C), 51.9, 55.1, 52.8, 49.2, 36.6 (2C), 24.5; MS: calcd. for $C_{20}H_{23}N_2O_2SF$ [*m/z*] 374.47; found: 375.37 $[M+H]^+$.

5-(3-Fluorobenzyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7D): Brown solid; Yield: 145 mg, 92 %; Elemental analysis, $C_{20}H_{23}N_2O_2SF$, calcd. (found) %: C 64.12 (64.10), H 6.16 (6.17), N 7.46 (7.44); IR (KBr, ν_{max}, cm^{-1}): 3248, 2952, 2923, 2224, 1613, 1535, 1447, 1331, 1137, 1088, 1011, 761; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 8.12 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 2H), 3.87 (t, *J* = 10.4 Hz, 4H), 3.73 (s, 2H), 3.48-3.44 (m, 2H), 2.79 (d, *J* = 5.2 Hz, 2H), 2.74 (t, *J* = 4.8 Hz, 2H), 1.71 (d, *J* = 11.2 Hz, 2H), 1.56-1.49 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.56, 161.17, 141.6, 137.7, 136.9, 135.9, 130.5, 128.9, 125.4, 124.1, 115.2, 65.5 (2C), 52.0, 55.7, 53.1, 50.1, 36.8 (2C), 24.2; MS: calcd. for $C_{20}H_{23}N_2O_2SF$ [*m/z*] 374.14; found: 374.47 $[M+H]^+$.

5-(2-Cyanobenzyl)-N-(tetrahydro-2*H*-pyran-4-yl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxamide (7E): Off white solid; Yield: 145 mg, 92 %; m.p.: 142-144 °C; Elemental analysis, $C_{21}H_{23}N_3O_2S$, calcd. (found) %: C 66.12 (66.09), H 6.08 (6.10), N 11.01 (11.04). IR (KBr, ν_{max}, cm^{-1}): 3248, 2952, 2923, 2224, 1613, 1535, 1447, 1331, 1137, 1088, 1011, 761; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 8.14 (d, *J* = 7.6 Hz, 1H), 7.83 (d, *J* = 8 Hz, 1H), 7.71-7.61 (m, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.51-7.45 (m, 2H), 3.92-3.85 (m, 5H), 3.53 (s, 2H), 3.80-3.32 (m, 2H), 2.81-2.77 (m, 4H), 1.73-1.69 (m, 2H), 1.56-1.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.62, 140.6, 138.7, 136.7, 135.5, 134.3 (2C), 132.3, 131.5, 128.3, 115.8, 112.8, 65.09 (2C), 59.12, 56.21, 55.32, 55.16, 36.44 (2C), 22.2; MS: calcd. for $C_{21}H_{23}N_3O_2S$ [*m/z*] 381.49; found: 382.53 $[M+H]^+$.

5-(2,4,6-Triethylbenzyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7F): Off white solid; Yield: 155 mg, 85 %; m.p.: 163-165 °C; Elemental analysis, $C_{26}H_{36}N_2O_2S$, calcd. (found) %: C 70.87 (70.89), H 8.23 (8.25), N 6.36 (6.38). IR (KBr, ν_{max}, cm^{-1}): 3307, 2962, 2837, 2763, 1618, 1568, 1532, 1459, 1367, 1323, 1240, 1139, 1093, 1008, 872, 618; ^{1}H NMR (400 MHz, DMSO-d_6)) δ: 8.10 (d, *J* = 8.0 Hz, 1H), 7.45 (s, 1H), 6.87 (s, 2H), 3.85 (d, *J* = 11.6 Hz, 3H), 3.64 (s, 1H), 3.38-3.28 (m, 2H), 2.72-2.68 (m, 8H), 2.56-2.50 (m, 3H), 1.69 (brs, 2H), 1.54-1.48 (m, 2H), 1.23-1.10 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.92, 146.6, 142.7, 136.7, 136.2 (2C), 135.5, 134.3 (2C), 131.5, 65.11 (2C), 57.12, 56.6, 55.7, 55.16, 36.34 (2C), 27.8, 26.2 (2C), 22.2, 14.6 (2C), 14.3; MS: calcd. for $C_{26}H_{36}N_2O_2S$ [*m/z*] 440.64; found: 441.33 $[M+H]^+$.

4,5,6,7-Tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)-5-[(1-methyl-1*H*-pyrazol-4-yl)methyl]thieno[3,2-c]pyridine-2-carboxamide (7G): White solid; Yield: 124 mg, 83 %; m.p.: 164-168 °C; Elemental analysis, $C_{18}H_{24}N_4O_2S$, calcd. (found) %: C 59.97 (59.95), H 6.71 (6.73), N 15.54 (15.52). IR (KBr, ν_{max}, cm^{-1}): 3494, 3274, 2930, 2841, 1618, 1540, 1461, 1324, 1139, 1087, 984, 871, 792, 677; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.45 (s, 1H), 8.25 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.97 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.44 (s, 1H), 3.90 (s, 2H), 3.88-3.84 (m, 5H), 3.68 (s, 2H), 3.48 (s, 2H), 2.80 (s, 2H), 2.73 (d, *J* = 5.2 Hz, 2H), 1.71 (d, *J* = 1.6 Hz, 2H), 1.53-1.49 (m, 2H);); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.1, 139.5, 137.4 (2C), 136.9, 135.7, 125.2, 116.0, 65.6 (2C), 56.9, 55.7, 55.2 (2C), 40.5, 36.7 (2C), MS: calcd. for $C_{18}H_{24}N_4O_2S$ [*m/z*] 360.47; found: 361.43 $[M+H]^+$.

4,5,6,7-Tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)-5-[(6-(1-methyl-1*H*-pyrazol-4-yl)pyridin-3-yl)methyl]thieno-[3,2-c]pyridine-2-carboxamide (7H): Light yellow solid; Yield: 140 mg, 77 %; m.p.: 171-174 °C; Elemental analysis, $C_{23}H_{27}N_5O_2S$, calcd. (found) %: C 63.13 (63.11), H 6.22 (6.20), N 16.01 (15.99). IR (KBr, ν_{max}, cm^{-1}): 3297, 2944, 2356, 1613, 1531, 1454, 1319, 1290, 1137, 1089, 968, 863; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.11 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H), 7.43 (s, 1H), 7.33 (s, 1H), 3.98-3.84 (m, 3H), 3.82 (s, 3H), 3.54 (s, 2H), 3.42-3.32 (m, 5H), 2.80 (s, 2H), 2.73 (s, 2H), 1.65-1.62 (m, 2H), 1.51-1.40 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.45, 150.67, 149.64, 138.21, 137.26, 136.96, 134.48, 130.78, 129.35, 126.28, 122.68, 118.89, 66.10 (2C), 57.86, 51.96, 49.33, 45.63, 38.70, 32.48 (2C), 25.09. MS: calcd. for $C_{23}H_{27}N_5O_2S$ [*m/z*] 437.56; found: 438.4 $[M+H]^+$.

5-(Cyclobutylmethyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7I): Off white solid; Yield: 103 mg, 74 %; m.p.: 151-153 °C; Elemental analysis, $C_{18}H_{26}N_2O_2S$, calcd. (found) %: C 64.64 (64.65), H 7.84 (7.86), N 8.38 (8.37). IR (KBr, ν_{max}, cm^{-1}): 3531, 3275, 2955, 2929, 2759, 1705, 1615, 1537, 1459, 1434, 1368, 1326, 1238, 1131, 1086, 1009, 977, 873, 793, 660, 539, 508; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.19 (d, *J* = 7.6

Hz, 1H), 7.46 (s, 1H), 3.91-3.84 (m, 3H), 3.39-3.34 (m, 4H), 3.29-3.23 (m, 2H), 2.77-2.67 (m, 4H), 2.06-1.99 (m, 2H), 1.91-1.65 (m, 6H), 1.58-1.51 (m, 2H), 1.30 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.17, 140.55, 136.44, 134.79, 126.31, 65.10 (2C), 61.34, 50.96, 49.61, 45.31, 34.47 (2C), 29.22, 24.8 (2C), 22.51, 18.74; MS: calcd. for $C_{18}H_{26}N_2O_2S$ [*m/z*] 334.48; found: 335.21 $[M+H]^+$.

5-(Cyclopropylmethyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7J): Off white solid; Yield: 101 mg, 76 %; m.p.: 154-156 °C; Elemental analysis, $C_{17}H_{24}N_2O_2S$, calcd. (found) %: C 63.72 (63.70), H 7.55 (7.53), N 8.74 (8.75). IR (KBr, ν_{max}, cm^{-1}): 3289, 2923, 2842, 1619, 1539, 1462, 1322, 1239, 1137, 1085, 873, 660; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.15 (d, *J* = 6.4 Hz, 1H), 7.48 (s, 1H), 3.91-3.68 (m, 3H), 3.51 (s, 2H), 3.38-3.33 (m, 2H), 2.80-2.76 (m, 4H), 2.37 (d, *J* = 5.2 Hz, 2H), 1.72 (d, *J* = 10.0 Hz, 2H), 1.56-1.50 (m, 2H), 0.89 (d, *J* = 4.4 Hz, 1H), 0.49 (d, 6.0 Hz, 2H), 0.12 (d, *J* = 3.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.47, 138.23, 136.74, 134.79, 126.31, 66.10 (2C), 61.84, 51.96, 49.81, 45.61, 32.47 (2C), 25.22, 8.51, 3.71 (2C); MS: calcd. for $C_{17}H_{24}N_2O_2S$ [*m/z*] 320.45; found: 321.5 $[M+H]^+$.

4,5,6,7-Tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)-5-(2-(naphthalen-2-yl)-2-oxoethyl)thieno[3,2-c]pyridine-2-carboxamide (7K): Light yellow solid; Yield: 143 mg, 80 %; m.p.: 160-163 °C; Elemental analysis, $C_{25}H_{26}N_2O_3S$, calcd. (found) %: C 69.10 (69.13), H 6.03 (6.06), N 6.45 (6.46). IR (KBr, ν_{max}, cm^{-1}): 3248, 3056, 2953, 2914, 2841, 1687, 1608, 1530, 1457, 1375, 1332, 1293, 1242, 1165, 1123, 1091, 1004, 869, 804, 752, 675, 579; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.72 (s, 1H), 8.17-8.11 (m, 2H), 8.02-7.96 (m, 2H), 7.99 (s, 1H), 7.69-7.60 (m, 2H), 7.46 (s, 1H), 4.25 (s, 2H), 3.92-3.84 (m, 3H), 3.69 (s, 2H), 3.38-3.32 (m, 2H), 2.94 (brs, 2H), 2.83 (brs, 2H), 1.72 (d, *J* = 12.4 Hz, 2H), 1.56-1.48 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.11, 160.43, 138.02, 136.84, 135.02, 134.40, 133.09, 132.07, 129.76, 129.53, 128.58, 128.14, 127.61, 126.87, 126.18, 123.68, 66.08 (2C), 62.59, 51.69, 49.76, 45.61, 32.45 (2C), 25.10; MS: calcd. for $C_{25}H_{26}N_2O_3S$ [*m/z*] 434.55; found: 435.53 $[M+H]^+$.

5-[2-(3,4-Difluorophenyl)-2-oxoethyl]-N-(tetrahydro-2*H*-pyran-4-yl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxamide (7L): Light Brown solid; Yield: 145 mg, 83 %; m.p.: 158-162 °C; Elemental analysis, $C_{21}H_{22}N_2O_3SF_2$, calcd. (found) %: C 59.99 (59.94), H 5.27 (5.28), N 6.66 (6.64). IR (KBr, ν_{max}, cm^{-1}): 3248, 2955, 2914, 2841, 1695, 1608, 1530, 1467, 1332, 1293, 1175, 1123, 1091, 1004, 869, 806, 754, 675, 670; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.16 (d, *J* = 6.0 Hz, 1H), 8.06-8.02 (m, 2H), 7.91 (s, 1H), 7.44 (s, 1H), 4.06 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.6, 160.78, 152.75, 149.1, 141.73, 137.75, 136.92, 135.83, 132.96, 126.08, 125.44, 115.34, 71.26, 66.06 (2C), 54.57, 52.88, 51.60, 32.44 (2C), 22.43; MS: calcd. for $C_{21}H_{22}N_2O_3SF_2$ [*m/z*] 420.47; found: 421.53 $[M+H]^+$.

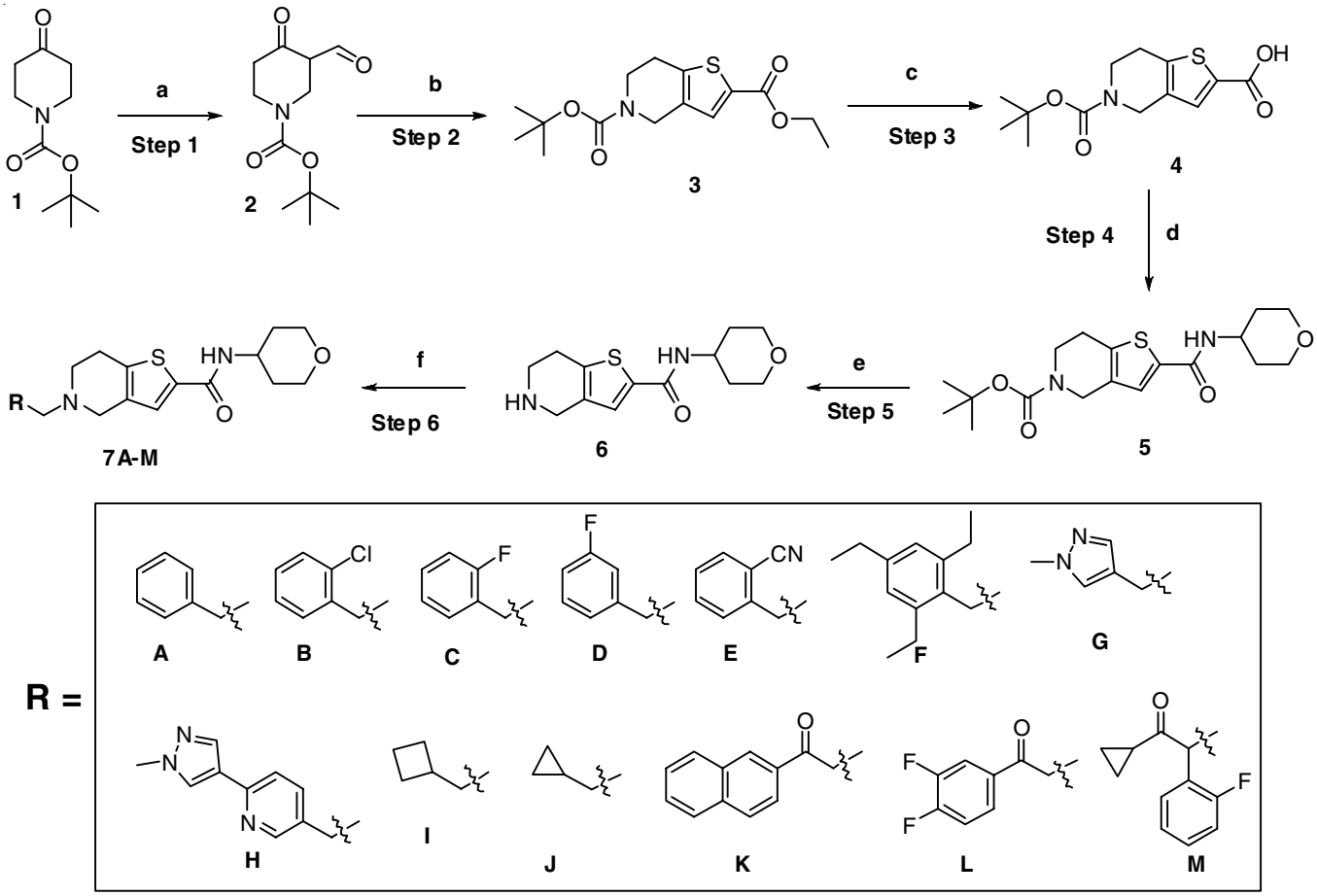
5-(2-Cyclopropyl-2-oxo-1-phenylethyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (7M): Semi solid; Yield: 155 mg, 85 %; Elemental analysis, $C_{24}H_{28}N_2O_3S$, calcd. (found) %: C 67.90 (67.87), H 6.65 (66.67), N 6.60 (6.56). IR (KBr, ν_{max}, cm^{-1}): 3322, 2925, 2849, 1702, 1626, 1572, 1532, 1485, 1456, 1379, 1238, 1088, 1009, 757, 575; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.10 (d, *J* = 6.0 Hz, 1H), 7.52 (t, *J* = 5.6 Hz, 1H), 7.43-7.41 (m, 2H), 7.28-7.25 (m, 2H), 4.81 (s, 1H), 3.90-3.84 (m, 3H), 3.54 (q, *J* = 5.2 Hz, 2H), 3.37-3.32 (m, 1H), 2.83 (brs, 3H), 2.71 (t, *J* = 7.2 Hz, 1H), 2.38 (d, *J* = 3.6 Hz, 1H), 1.71 (d, *J* = 9.6 Hz, 2H), 1.54-1.47 (m, 2H), 1.23 (s, 1H), 0.90-0.89 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 207.39, 170.31, 161.58, 160.38, 138.05, 136.98, 134.13, 130.72, 130.17, 126.27, 124.70, 115.83, 115.65, 71.26, 66.06 (2C), 49.67, 47.56, 45.60, 32.44 (2C), 25.29, 17.71, 11.58, 11.19; MS: calcd. for $C_{24}H_{28}N_2O_3S$ [*m/z*] 442.5; found: 443.6 $[M+H]^+$.

Anticancer activity: Human lung carcinoma (HCT-116) cell lines were obtained from NCCS Pune and were kept in RPMI 1640 medium (#AL199A, Himedia) supplemented with 10 % Fetal Bovine Serum (#RM10432, Himedia). The cells were made to confluency at 37 °C in a CO_2 incubator (Healforce, China) with humidified atmosphere and 5 % CO_2. Seeded 200 µL cell suspension in a 96-well plate at required cell density (20,000 cells per well) without the test agent and allowed the cells to grow for about 12 h. Test samples in the concentrations of 25 µg, 50 µg, 75 µg, 100 µg, 125 µg/mL of compounds and 50 µg/mL drugs were added for the growth of the cells separately and incubated the plate for 24 h at 37 °C in a 5 % CO_2 atmosphere. After the incubation period, the plates were taken out of the incubator and removed spent media and added MTT reagent (camptothecin) to a final concentration of 0.5 mg/mL of total volume and the plates were incubated for 3 h, removed the MTT reagent and 100 µL of solubilization solution (DMSO) was added. The absorbance was recorded on an ELISA reader at 570nm and 630nm used as the reference wavelength. The IC_{50} value was obtained by using linear regre-ssion equation *i.e.*, y = mx + c where, y = 50, m and c values were derived from the viability graph.

RESULTS AND DISCUSSION

The synthesis of 5-substituted 4,5,6,7-tetra-hydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide derivatives (**7A-M**) is illustrated in **Scheme-I**. Formylation of *tert*-butyl 4-oxopiperidine-1-carboxylate (**1**) under Villsmeyer-Hack protocol was carried out in presence of DMF and $POCl_3$ to achieve the desired compound *tert*-butyl 3-formyl-4-oxopiperidine-1-carboxylate (**2**). Treatment of aldehyde **2** with 2-mercaptoacetate in presence of triethyl amine in dichloromethane at 45 °C for 16 h produced the cyclized product, 5-*tert*-butyl-2-ethyl-6,7-dihydrothieno[3,2-c]pyridine-2,5(4*H*)-dicarboxylate (**3**). Alkaline hydrolysis of ethyl ester **3** followed by coupling with tetrahydro-2*H*-pyran-4-amine in presence of EDC·HCl, HOBt and di-isopropyl amine in DMF at room temperature for 5 h produced the desired compound N-(tetrahydro-2*H*-pyran-4-yl)-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-2-carboxamide (**6**). De-protection of the Boc group in presence of trifluoroacetic acid followed by coupling with various aryl/alkyl/acyl halides in presence of K_2CO_3 in DMF at room temperature for 16 h resulted in the formation of desired compounds **7A-M** in moderate yields.

These derivatives have been characterized by ^{1}H NMR, mass and IR spectroscopic tools. The structural determination of 5-(2-chlorobenzyl)-4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-



Reaction conditions: a) DMF, $POCl_3$, DCM, 0 °C-room temperature, 4 h; b) 2-mercaptoacetate, triethyl amine, DCM, 45 °C, 16 h; c) NaOH, THF:MeOH:H_2O, room temperature, 16 h; d) tetrahydro-2*H*-pyran-4-amine, EDC·HCl, HOBt, di-isopropyl amine, DMF, room temperature, 5 h; e) TFA, DCM, 0 °C-room temperature, 16h; f) R-X, K_2CO_3, DMF, room temperature, 16 h

Scheme-I: Synthesis of novel 5-substituted 4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide derivatives (**7A-7M**)

pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide (**7B**). In ^{1}H NMR, the proton signals resonating at 8.15 ppm (doublet) and 7.36 ppm (singlet) corresponds to –NH and thiophene ring proton, while the proton signals resonating at 7.53, 7.45, 7.33 and 7.23 ppm as doublets is assigned to 2-chloro-phenyl ring, respectively. The characteristic benzylic -CH_2- resonated at 3.77 ppm as singlet and the remaining aliphatic protons such as piperidine and morpholine ring protons resonated in the expected region. The structure of compound **7B** was further confirming by its molecular weight *m/z*, 390.76 (LC-MS purity: 93.86 %) which was found to be in agreement with the desired structure. In the IR spectra of compound **7B**, the characteristic peaks in the region 3280, 3065, 2949, 1614, 1571 cm^{-1} are assigned to –NH, Ar-C-H, aliph. -C-H, -CO-NH- and –C=C- groups respectively. The above spectroscopic techniques, thus confirms the structure of compound **7B**. Similarly, the structures of remaining compounds in the series have been confirmed.

Anticancer activity: Compounds (**7A-M**) were screened for their *in vitro* cytotoxic potential against human lung carcinoma (HCT-116) cell line using the MTT assay and the obtained IC_{50} values are tabulated in Table-1. Among all the compound **7B** (IC_{50}: 69.52 µg) and compound **7K** (IC_{50}: 66.35 µg) showed significant potent activity at micro molar concentration when compared with the standard drug camptothecin, while the compounds **7C**, **7D**, **7E**, **7G**, **7H** and **7M** exhibited moderate activity and compounds **7A**, **7F**, **7I**, **7J** and **7L** showed weak activity (Table-1). It is evident from the cytotoxic activity results that the compounds containing 2-chloro-benzyl (**7B**) and 2-acetyl naphthalene (**7K**) ring substituted to the main scaffold exhibited potent anticancer activities compared to the standard drug.

TABLE-1
in vitro CYTOTOXIC ACTIVITY OF COMPOUNDS (**7A-M**)

Compound	IC_{50} (µg/mL)	Compound	IC_{50} (µg/mL)
7A	82.36 ± 0.02	**7H**	72.15 ± 0.01
7B	69.52 ± 0.02	**7I**	80.35 ± 0.01
7C	78.48 ± 0.01	**7J**	80.21 ± 0.02
7D	77.21 ± 0.02	**7K**	66.35 ± 0.02
7E	75.35 ± 0.01	**7L**	83.25 ± 0.01
7F	80.05 ± 0.01	**7M**	71.25 ± 0.01
7G	79.45 ± 0.01	Camptothecin (SD)	47.36 ± 0.02

Conclusion

In conclusion, we have described the synthesis and characterization of some novel 5-substituted 4,5,6,7-tetrahydro-N-(tetrahydro-2*H*-pyran-4-yl)thieno[3,2-c]pyridine-2-carboxamide derivatives (**7A-M**). These derivatives were screened for their *in vitro* cytotoxic potential against human lung carci-

noma (HCT-116) cell line using the MTT assay. The anticancer activity results revealed that compound **7B** (IC_{50}: 69.52 µg) and compound **7K** (IC_{50}: 66.35 µg) showed moderate potent activity at micro molar concentration when compared with the standard drug camptothecin, while compounds **7C**, **7D**, **7E**, **7G**, **7H** and **7M** exhibited moderate activity and compounds **7A**, **7F**, **7I**, **7J** and **7L** showed weak activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J.N. Sangshetti, P.P. Dharmadhikari, R.S. Chouthe, B. Fatema, V. Lad, V. Karande, S.N. Darandale and D.B. Shinde, *Bioorg. Med. Chem. Lett.*, **23**, 2250 (2013); https://doi.org/10.1016/j.bmcl.2013.01.041.
2. R. Kalaria, M. Mittal, C. Rajyaguru and J.J. Upadhyay, *J. Chem. Pharm. Res.*, **4**, 1566 (2012).
3. R. Kalaria, R.J. Odedara, R.S. Dave, C. Rajyaguru and J.J. Upadhyay, *J. Appl. Technol. Environ. Sanit.*, **2**, 109 (2012).
4. J. Kedia, K.S. Nimavant and K.B. Vyas, *J. Chem. Pharm. Res.*, **4**, 1864 (2012).
5. Y.S. Patel, P.N. Patel and H.S. Patel, *Int. Res. J. Pure Appl. Chem.*, **4**, 315 (2014); https://doi.org/10.9734/IRJPAC/2014/5955.
6. V.P. Modi, P.N. Patel and H.S. Patel, *Der Pharm. Lett.*, **3**, 120 (2011).
7. J.N. Sangshetti, A.S. Zambare, F.A. Khan, I. Gonjari and Z. Zaheer, *Mini Rev. Med. Chem.*, **14**, 988 (2014); https://doi.org/10.2174/1389557514666141106131425.
8. M. Mittal, S.M. Sarode and G. Vidyasagar, *Int. J. Pharm. Biosci.*, **2**, 188 (2011).
9. D.P. Kawade and P.B. Khedekar, *Der. Pharm. Chem.*, **4**, 1856 (2012).
10. P.N. Patel, D.J. Patel and H.S. Patel, *Appl. Organomet. Chem.*, **25**, 454 (2011); https://doi.org/10.1002/aoc.1786.
11. H.M. Parekh, P.B. Pansuriya and M.N. Patel, *Pol. J. Chem.*, **79**, 1843 (2005).
12. B.G. Tweedy, *Phytopathology*, **55**, 910 (1964).
13. B.B. Lohray, V.B. Lohray, B.K. Srivastava, P.B. Kapadnis and P. Pandya, *Bioorg. Med. Chem.*, **12**, 4557 (2004); https://doi.org/10.1016/j.bmc.2004.07.019.
14. S.N. Darandale, N.A. Mulla, D.N. Pansare, J.N. Sangshetti and D.B. Shinde, *Eur. J. Med. Chem.*, **65**, 527 (2013); https://doi.org/10.1016/j.ejmech.2013.04.045.
15. J.N. Sangshetti, F.A. Kalam Khan, R.S. Chouthe, M.G. Damale and D.B. Shinde, *Chin. Chem. Lett.*, **25**, 1033 (2014); https://doi.org/10.1016/j.cclet.2014.04.003.
16. J.N. Sangshetti, R.I. Shaikh, F.A.K. Khan, H. Patil, S.D. Marathe, W.N. Gade and D.B. Shinde, *Bioorg. Med. Chem. Lett.*, **24**, 1605 (2014); https://doi.org/10.1016/j.bmcl.2014.01.035.
17. A.M.R. El-Sayed, A.G. Abdel-Hafez, N.N.S. Mohamed and S.F. Abdalla, *Turk. J. Chem.*, **33**, 421 (2009).
18. J.P. Maffrand and J.P. Frehel, Antiinflammatory Thieno[2,3-c]pyridine Derivatives, US Patent 4,496,568 (1985).
19. A. Amselen, Thieno-pyridine Derivatives, Process for their Preparation and their Applications, US Patent 3,983,125 (1976).
20. A. Amselem, 5-O-Cyanobenzyl-4,5,6,7-tetrahydrothieno[3,2-c]-pyridine Maleate, US Patent 4,097,482 (1978).
21. A.R.J. Castaigne, Thieno[3,2-c]pyridine Derivatives, US Patent 4,051,141 (1977).
22. J.P. Maffrand, Thieno[2,3-C]pyridine Derivatives and Therapeutic Composition Containing same, US Patent 4,075,340 (1978).
23. M. Fujita, T. Seki, H. Inada and N. Ikeda, *Bioorg. Med. Chem. Lett.*, **12**, 1607 (2002); https://doi.org/10.1016/S0960-894X(02)00228-7.
24. C.S. Schneider, K.H. Weber, H. Daniel, W.D. Bechtel and K. Boeke-Kuhn, *J. Med. Chem.*, **27**, 1150 (1984); https://doi.org/10.1021/jm00375a011.
25. L. Zheng, J. Xiang and X.A. Bai, *J. Heterocycl. Chem.*, **43**, 321 (2006); https://doi.org/10.1002/jhet.5570430211.
26. R. Romagnoli, P.G. Baraldi, M.D. Carrion, O. Cruz-Lopez, C.L. Cara, M. Tolomeo, S. Grimaudo, A.D. Cristina, M.R. Pipitone, J. Balzarini, S. Kandil, A. Brancale, T. Sarkar and E. Hamel, *Bioorg. Med. Chem. Lett.*, **18**, 5041 (2008); https://doi.org/10.1016/j.bmcl.2008.08.006.
27. R. Busacca, Compounds having Antiplatelet Aggregation Activity and Pharmaceutical Compositions Containing them, US Patent 4,737,502 (1988).
28. H. Koike, F. Asai, A. Sugidachi, T. Kimura, T. Inoue, S. Nishino and Y. Tsuzaki, Tetrahydrothienopyridine Derivatives, Furo and Pyrrolo Analogs Thereof for Inhibiting Blood Platelet Aggregation, US Patent 5,436,242 (1995).
29. P. Madsen, J.M. Lundbeck, P. Jakobsen, A.R. Varming and N. Westergaard, *Bioorg. Med. Chem.*, **8**, 2277 (2000); https://doi.org/10.1016/S0968-0896(00)00153-X.
30. P. Madsen, J.M. Lundbeck, N. Westergaard and P. Jakobsen, 4,5,6,7-Tetrahydro-thieno(2,3-C)pyridine Derivatives, US Patent 6,090,797 (2000).
31. G. Samala, P.B. Devi, R. Nallangi, J.P. Sridevi, S. Saxena, P. Yogeeswari and D. Sriram, *Bioorg. Med. Chem. Lett.*, **22**, 1938 (2014); https://doi.org/10.1016/j.bmc.2014.01.030.
32. R. Nallangi, G. Samala, G.P. Sridevi, P. Yogeeswari and D. Sriram, *Eur. J. Med. Chem.*, **76**, 110 (2014); https://doi.org/10.1016/j.ejmech.2014.02.028.
33. B.K. Srivastava, M. Solanki, B. Mishra, R. Soni, S. Jayadev, D. Valani, M. Jain and P.R. Patel, *Bioorg. Med. Chem. Lett.*, **17**, 1924 (2007); https://doi.org/10.1016/j.bmcl.2007.01.038.
34. K. Katano, E. Shitara, M. Shimizu, K. Sasaki, T. Miura, Y. Isomura, M. Kawaguchi, S. Ohuchi and T. Tsuruoka, *Bioorg. Med. Chem. Lett.*, **6**, 2601 (1996); https://doi.org/10.1016/0960-894X(96)00476-3.
35. A. Esanu, Thienopyridine Derivatives and Anti-thrombotic Compositions Containing the same US Patent 4,681,888 (1987).
36. H. Koike, F. Asai, A. Sugidachi, T. Kimura, T. Inoue, S. Nishino and Y. Tsuzaki, Tetrahydrothienopyridine Derivatives, Furo and Pyrrolo Analogs Thereof and Their Preparation and Uses for Inhibiting Blood Platelet Aggregation, US Patent 5,288,726 (1994).