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Synthesis of Novel Amide Functionalized Pyrido[2,3-*d*]pyrimidine Derivatives and their Anticancer Activity

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A series of novel amide functionalized pyrido[2,3-*d*]pyrimidine derivatives were synthesized from 2-amino-6-(thiophen-2-yl)-4-(trifluoromethyl)nicotinonitrile (**1**), which on reaction with trifluoroacetic acid to get pyridopyrimidine (**2**). Compound **2** when treated with bromoethyl acetate followed by hydrazine hydrate to get hydrazide derivatives **4**. Compound **4** on reaction with different substituted aromatic aldehydes to obtain Schiff base derivatives **5a-f**. In another way, compound **3** treated with different substituted amines and amino acids to obtain amide derivatives **6a-f**, **7a-d** and **8a-c**. All the compounds evaluated for anti-cancer activity against four human cancer cell lines such as A549-Lung cancer (CCL-185), MCF7-Breast cancer (HTB-22), DU145-prostate cancer (HTB-81) and HeLa-cervical cancer (CCL-2) and among the sythesized compounds, only **5d**, **7d** and **8a** were identified as the potent compounds against the anticancer activity.

Keywords: Pyrido[2,3-*d*]pyrimidines, Cyclization, Schiff's base, Anticancer activity.

INTRODUCTION

According to the World Health Organization (WHO) reports, more than 8.8 million people died due to cancer among the world in recent past year. Cancer is one of the leading causes of common death among all the worldwide death. Discovering of novel and safer drug for cancer is big challenging task to researchers and chemists. Nitrogen containing heterocycles have been attracted in drug discovery and several nitrogen containing heterocycles have been approved as a drugs [1-6].

Pyrido[2,3-*d*]pyrimidines scaffolds are very important nitrogen containing heterocycles and most of the derivatives have antimicrobial [7-9], anticancer [10-13], antiviral and antifolate [14-17], anticonvulsant [18], analgesic [19] and central nervous system depressant activities and some of compounds are more specific to dihydrofolate reductase inhibition and diuretic properties [20,21].

Meanwhile we proceed with trifluoromethyl group at the particular position on the pyrido[2,3-*d*]pyrimidines scaffolds, according to the literature [22-24], the trifluoromethyl group at a specific position of an organic and heterocyclic molecule radically alters the properties of molecule in terms of lipid solubility, oxidative thermal stability thereby enhances the transport mechanism and bio-efficacy. Although there have many reports on pyrido[2,3-*d*]pyrimidines scaffolds and their derivatives, but no article was reported on amide functionalized pyrido[2,3-*d*]pyrimidines derivatives. Based on the importance according to the literature and considering all the aspects, we decided to synthesize amide functionalized pyridopyrimidine derivatives and evaluated for anticancer activity.

There are number of reports of heterocyclic [25] and polycyclic pyrimidines [26] exhibiting antimalarial activity. Taking all these literature reports, we designed and synthesized a series of novel amide and Schiff base functionalized pyrido[1,2-*a*]-pyrimidin-4-one derivatives **5a-f, 6a-f, 7a-d** and **8a-c** and evaluated for anticancer activity.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Bruker AV 300 MHz in $CDCl_3$ & DMSO-d_6 using TMS as internal standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. All high-resolution spectra were recorded on QSTARXL hybrid MS/

MS system (Applied Biosystems, USA) under electrospray ionization. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254}; spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography.

Synthesis of 7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)-pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (2): 2-Amino-6-(thiophen-2-yl)-4-(trifluoromethyl)nicotinonitrile (**1**) (2 mmol) and trifluoroacetic acid (3 mL) and catalytic amount of conc. H_2SO_4 was heated at 120-130 ºC for about 12 h. After the completion of reaction, the reaction mixture was allowed to cool to room temperature and then poured into ice-cold water. The separated solid was collected by filtration, dried and recrystallized from ethanol. Yield 95%; IR (KBr, cm^{-1}): 3182 (-NH-),1678 (lactam CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.19 (dd, *J* = 4.81, 1H, Ar-H), 7.48 (dd, *J* = 4.81, 1H, Ar-H), 7.76 (dd, *J* = 3.72, 1H, Ar-H), 8.55 (s, 1H), 12.65 (br. S., 1H, -NH-); MS (ESI): *m/z* [(M+H)$^+$]: 366. Anal. calcd. (found) % for $C_{13}H_5N_3OSF_6$: C 42.75 (42.76); H 1.38 (1.39); N 11.50 (11.53).

Synthesis of ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*-(trifluoromethyl)pyrido[2,3-*d*] pyrimidin-3(4*H*)-yl)acetate (3): 7-(Thiophen-2-yl)-2,5-bis(trifluoromethyl)pyrido[2,3-*d*] pyrimidin-4(3*H*)-one (2.5 mL) dissolved in dry acetone (15 mL) was added in a mixture of ethyl bromoacetate (2.86 mmol), potassium carbonate (5.3 mmol) and sodium iodide (0.01 g). The reaction mixture was allowed to reflux for 6 h and cooled to room temperature. The separated solid was filtered off and washed with acetone (20 mL). The total filtrate was concentrated under vacuum and the resultant white residue was washed with *n*-hexane followed by water. Two isomers in a ratio of 3:1 were obtained and then isolated through column by using *n*-hexane and ethylacetate (3:1)

Ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-d]pyrimidin-3(4*H*)-yl)acetate (3a): Yield 65%; IR (KBr, cm^{-1}): 1712 (ester CO), 2221 (CN) ;^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31 (t, 3H, -CH_3), 4.26 (s, 2H, -CH_2-), 5.03 (q, 2H, -OCH_2-), 7.18 (dd, *J* = 4.95,1H, Ar-H), 7.59 (dd, *J* = 4.91, 1H, Ar-H), 7.72 (dd, *J* = 3.76, 1H,Ar-H), 8.86 (s, 1H, Ar-H); MS (ESI): *m/z* [(M+H)$^+$]: 452. Anal. calcd. (found) % for $C_{17}H_{11}N_3O_3SF_6$: C, 45.24 (42.25); H, 2.46 (2.26); N, 9.31 (9.33).

Synthesis of 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (4): Ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-bis(trifluoromethyl)-pyrido[2,3-*d*]pyrimidin-3(4H)-yl) acetate (3 mmol) was taken in 95% ethanol (30 mL) and then hydrazine hydrate (5 mL) was added. The mixture was refluxed for 12 h and after cooling to room temperature the ethanol was removed under vacuum. The residue was washed with *n*-hexane and then water was added to give a yellow solid, which was filtered and dried. Yellow solid; IR (KBr, cm^{-1}): 3379, 3486 (NH_2), 3211 (CONH), 1628 (CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.22 (br.s, 2H, NH_2), 4.27 (s, 2H, -CH_2-), 7.19 (dd, *J* = 4.95, 1H, Ar-H), 7.61 (dd, *J* = 4.91, 1H, Ar-H), 7.71 (dd, *J* = 3.76, 1H, Ar-H), 8.86 (s, 1H, Ar-H) MS (ESI): *m/z* [(M+H)$^+$]: 438. Anal. calcd. (found) % for $C_{15}H_9N_5O_2SF_6$: C, 41.20 (41.22); H, 2.07 (2.08); N, 16.01 (16.03).

General procedure for the synthesis of Schiff bases (5a-e): 2-(4-Oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido-[2,3-*d*]pyrimidin-3(4H)-yl) acetohydrazide (**4**) (3 mmol) was taken in 95% ethanol (10 ml) and benzaldehyde (3 mmol) was added. To this reaction mixture, piperidine (0.1 mL) was added as catalyst. The mixture was refluxed for 2 h and after completion of the reaction, allowed it to cool to room temperature. The ethanol was removed under vacuum and then the residue was washed with *n*-hexane. Finally, water was added to give yellow solid which was filtered and dried.

(*E*)-*N*′-Benzylidene-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)-acetohydrazide (5a): Yellow solid; m.p.:185-187 ºC; IR (KBr, cm^{-1}): 3231, 1628 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.26 (s, 2H, -CH_2-), 7.25 (dd, 1H, Ar-H), 7.31-7.34 (m, 3H, Ar-H), 7.38 (dd, 1H, Ar-H), 7.46-7.49 (m, 2H, Ar-H), 7.78 (dd, 1H, Ar-H), 8.38 (s, 1H, CH=N), 8.79 (s, 1H, Ar-H), 11.45 (br.s., 1H, NHCO);^{13}C NMR ($CDCl_3$, 75 MHz): δ 48.8, 120.2, 121.7, 122.3, 124.2, 125.4, 126.6, 127.3, 128.3, 129.6, 130.6, 131.3, 132.7, 133.3, 136.3, 138.4, 139.3, 141.8, 159.8, 163.2; MS (ESI): *m/z* [(M+H)$^+$]: 526; HRMS *m/z* calcd. for $C_{12}H_{13}N_5O_2SF_6$ [(M+H)$^+$]: 526.0112, found 526.0114.

(*E*)-*N*′-(4-Methylbenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (5b): Yellow solid; m.p.: 192-194 ºC; IR (KBr, cm^{-1}): 3228, 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.41 (s, 3H, -CH_3), 4.25 (s, 2H, -CH_2-), 7.21 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.55 (d, 2H, *J* = 8.21 Hz, Ar-H), 7.58 (d, *J* = 8.21 Hz, 2H, Ar-H), 7.82 (dd, *J* = 4.88 Hz, 1H, Ar-H), 7.88 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.26 (s, 1H, CH=N), 11.42 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 23.3, 49.1, 119.5, 121.3, 122.3, 124.7, 125.3, 126.6, 127.2, 128.8, 129.4, 130.3, 132.5, 133.6, 136.5, 137.2, 138.6, 142.4, 145.6, 158.9, 162.1; MS (ESI): *m/z* [(M+H)$^+$]: 540; HRMS *m/z* calcd. for $C_{23}H_{15}N_5O_2SF_6$ [(M+H)$^+$]: 540.0105, found 540.0107.

(*E*)-*N*′-(3-Methoxybenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3-(4*H*)-yl)acetohydrazide (5c): Yellow solid; m.p.: 209-211 ºC; IR (KBr, cm^{-1}): 3218, 1629 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.81 (s, 3H, -OCH_3), 4.26 (s, 2H, -CH_2-), 7.25 (dd, *J* = 3.83 Hz, 1H, Ar-H), 7.55-7.58 (m, 3H, Ar-H), 7.62 (dd, *J* = 3.83 Hz, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.87 (dd, *J* = 4.81 Hz, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 8.28 (s, 1H, CH=N), 11.41 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 49.8, 54.4, 119.4, 120.6, 121.5, 123.2, 124.8, 125.4, 126.8, 127.3, 128.9, 129.5, 130.2, 132.5, 133.6, 136.5, 137.4, 138.5, 142.2, 143.4, 145.7, 159.8, 162.5; MS (ESI): *m/z* [(M+H)$^+$]: 556; HRMS *m/z* calcd. for $C_{23}H_{15}N_5O_3SF_6$ [(M+H)$^+$]: 556.0248, found 556.0251.

(*E*)-*N*′-(4-Chlorobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (5d): Yellow solid; m.p.: 201-203 ºC; IR (KBr, cm^{-1}): 3215, 1626 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.24 (s, 2H, -CH_2-), 7.27 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.36 (d, 2H, Ar-H), 7.59 (d, *J* = 8.21 Hz, 2H, Ar-H), 7.81 (dd, *J* = 4.88 Hz, 1H, Ar-H), 7.89 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 8.26 (s, 1H, CH=N), 11.38 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 49.5, 119.2, 120.6, 122.1, 123.8, 125.4,

126.7, 127.4, 128.4, 131.2, 132.6, 134.5, 136.7, 137.4, 138.6, 141.8, 142.3, 145.6, 158.6, 162.1; MS (ESI): *m/z* [(M+H)$^+$]: 560; HRMS *m/z* calcd. for $C_{22}H_{12}N_5O_2SClF_6$ [(M+H)$^+$]: 560.0112, found 560.0115.

(*E*)-*N*′-(4-Bromobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (5e): Yellow solid; m.p.: 225-227 ºC; IR (KBr, cm^{-1}): 3221, 1628 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.24 (s, 2H, -CH_2-), 7.26 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.32 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H), 7.67 (dd, *J* = 4.88 Hz, 1H, Ar-H), 7.89 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 8.24 (s, 1H, CH=N), 11.32 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 49.2, 119.1, 120.7, 122.5, 123.8, 125.6, 126.3, 127.4, 128.6, 131.4, 132.4, 134.6, 136.7, 137.2, 138.6, 141.2, 142.7, 145.6, 158.2, 162.3; MS (ESI): *m/z* [(M+H)$^+$]: 604; HRMS *m/z* calcd. (found) % for $C_{22}H_{12}N_5O_2SBrF_6$ [(M+H)$^+$]: 604.0482, found 604.0485.

General Procedure for the synthesis of 6a-f: Ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]-pyrimidin-3(4*H*)-yl)acetate (**3**, 3 mmol) reaction with different substituted aromatic/aliphatic amines (6 mmol) in neat condition under refluxing conditions for 6-10 h. After completion of the reaction, reaction mixture was allowed to cool, concentrate and by adding water, solid was formed, which was filtered, dried and recrystallized from ethanol.

***N*-Methyl-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamide (6a):** Yellow solid; m.p.: 205-207 ºC; IR (KBr, cm^{-1}): 1652 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.81 (s, 3H, -N-CH_3), 4.73 (s, 2H, -CH_2-), 7.29 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.41 (br.s, 1H, -CONH-), 7.66 (dd, *J* = 4.82 Hz, 1H, Ar-H), 7.87 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.07 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 28.2, 49.5, 119.6, 120.2, 121.4, 123.3, 123.8, 124.5, 125.6, 126.2, 128.7, 132.4, 136.3, 142.3, 158.6, 162.2; MS (ESI): *m/z* [(M+H)$^+$]: 437; HRMS *m/z* calcd. for $C_{16}H_{10}N_4O_2SF_6$ [(M+H)$^+$]: 437.0112, found 437.0115.

***N*-Ethyl-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamide (6b):** Yellow solid; m.p.: 211-213 ºC; IR (KBr, cm^{-1}): 1658 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.05 (t, 3H, -CH_3), 3.15 (q, 3H, -N-CH_2-), 4.73 (s, 2H, -CH_2-), 7.27 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.43 (br.s, 1H, -CONH-), 7.64 (dd, *J* = 4.82 Hz, 1H, Ar-H), 7.83 (dd, *J* = 3.81 Hz, 1H, Ar-H), 8.08 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 15.1, 34.3, 48.8, 119.1, 120.5, 122.4, 123.1, 123.7, 124.3, 125.3, 127.3, 128.6, 132.4, 136.2, 142.6, 145.3, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 451; HRMS *m/z* calcd. for $C_{17}H_{12}N_4O_2SF_6$ [(M+H)$^+$]: 451.0237, found 451.0234.

2-(4-Oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)-pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)-*N*-propylacetamide (6c): Yellow solid; m.p.: 221-223 ºC; IR (KBr, cm^{-1}): 1656 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.92 (t, 3H, -CH_3), 1.41-1.50 (m, 2H, -N-CH_2-), 3.28 (q, 2H, -CH_2-N), 4.69 (s, 2H, -CH_2-), 7.25 (dd, *J* = 3.83 Hz, 1H, Ar-H), 7.41 (br.s, 1H, -CONH-), 7.61 (dd, *J* = 4.82 Hz, 1H, Ar-H), 7.81 (dd, *J* = 3.83 Hz, 1H, Ar-H), 8.09 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 12.1, 23.4, 46.5, 120.2, 120.7, 121.5, 122.6, 123.2, 124.4, 125.6, 127.2, 128.7, 131.4, 133.5, 141.6, 142.8, 158.4, 161.4; MS (ESI): *m/z* [(M+H)$^+$]: 465; HRMS *m/z* calcd. for $C_{18}H_{14}N_4O_2SF_6$ [(M+H)$^+$]: 465.0113, found 465.0115.

***N*-Cyclopentyl-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamide (6d):** Yellow solid; m.p.: 202-204 ºC; IR (KBr, cm^{-1}): 1652 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.81-1.89 (m, 4H, -CH_2-), 1.94-1.99 (m, 2H, -CH_2-), 2.31-2.38 (m, 2H, -CH_2-), 4.73 (s, 2H, -CH_2-), 5.26-5.38 (m, 1H, =CH-), 7.26 (dd, *J* = 3.84 Hz, 1H, Ar-H), 7.33 (br., s, 1H, -CONH-), 7.62 (dd, *J* = 4.81 Hz, 1H, Ar-H), 7.80 (dd, *J* = 3.84 Hz, 1H, Ar-H), 8.08 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 24.2, 32.4, 46.4, 49.5, 120.3, 122.4, 124.7, 125.3, 126.4, 127.1, 131.2, 133.4, 135.4, 140.3, 141.2, 143.2, 158.5, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 491; HRMS *m/z* calcd. for $C_{20}H_{16}N_4O_2SF_6$ [(M+H)$^+$]: 491.0105, found 491.0108.

***N*-(Furan-2-ylmethyl)-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)-acetamide (6e):** Yellow solid; m.p.: 231-233 ºC; IR (KBr, cm^{-1}): 1655 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.70 (s, 2H, -CH_2-), 5.19 (d, 2H,-CH_2-), 7.25 (dd, *J* = 3.83 Hz, 1H, Ar-H), 7.31 (br.s, 1H, -CONH-), 7.61 (dd, *J* = 4.80 Hz, 1H, Ar-H), 7.81 (dd, *J* = 3.83 Hz, 1H, Ar-H), 8.06 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.5, 49.4, 119.2, 120.3, 121.5, 122.3, 124.7, 125.1, 126.8, 128.3, 130.7, 132.6, 133.2, 135.6, 140.4, 141.7, 142.8, 143.6, 157.2, 160.5; MS (ESI): *m/z* [(M+H)$^+$]: 503; HRMS *m/z* calcd. for $C_{20}H_{12}N_4O_3SF_6$ [(M+H)$^+$]: 503.0214, found 503.0217.

***N*-Benzyl-2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamide (6f):** Yellow solid; m.p.: 236-238 ºC; IR (KBr, cm^{-1}): 1659 (CONH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.71 (s, 2H, -CH_2-), 5.15 (d, 2H,-CH_2-), 7.26 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.28-7.30 (m, 3H, Ar-H), 7.34 (br.s, 1H, -CONH-), 7.39-7.41(m, 2H, Ar-H), 7.65 (dd, *J* = 4.80 Hz, 1H, Ar-H), 7.86 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 42.2, 48.6, 119.5, 120.2, 121.5, 122.4, 124.7, 125.6, 126.2, 128.4, 129.4, 130.5, 132.2, 134.3, 135.7, 140.1, 141.7, 142.8, 157.7, 160.5; MS (ESI): *m/z* [(M+H)$^+$]: 513; HRMS *m/z* calcd. for $C_{22}H_{14}N_4O_2SF_6$ [(M+H)$^+$]: 513.0713, found 513.0715.

General Procedure for the synthesis of 7a-d: Ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]-pyrimidin-3(4*H*)-yl)acetate (**3**, 1 mmol) reacted with excess of secondary amines (1.5 mmol) in the presence of potassium carbonate (8.1 mmol) and the mixture was heated at 100 ºC for 12 h. The reaction mixture was cooled to room temperature, treated with crushed ice and neutralized with 1N HCl. The solid precipitate was filtered and washed with excess water and the crude product was recrystallized from ethanol.

3-(2-Oxo-2-(piperidin-1-yl)ethyl)-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3H)one (7a): Yellow solid; m.p.: 195-197 ºC; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59-1.65 (m, 4H, -CH_2-), 1.78-1.85 (m, 2H, -CH_2-), 3.38-3.43 (m, 4H, -CH_2-), 4.75 (s, 2H, -CH_2-), 7.26 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.48 (dd, *J* = 4.80 Hz, 1H, Ar-H), 7.82 (dd, *J* = 3.81 Hz, 1H, Ar-H), 8.06 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 23.5, 26.8, 43.7, 49.5, 120.2, 121.6, 123.3, 124.5, 125.4, 126.1, 128.2, 129.4, 130.8, 132.2, 140.2, 142.1, 158.6, 160.1; MS (ESI):

m/z [(M+H)$^+$]: 491; HRMS *m/z* calcd. for $C_{20}H_{16}N_4O_2SF_6$ [(M+H)$^+$]: 491.0121, found 491.0124.

3-(2-(4-Methylpiperazin-1-yl)-2-oxoethyl)-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4-(3*H*)one (7b): Yellow solid; m.p.: 209-211 ºC; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.42 (s, 3H, N-CH_3), 2.89-2.95 (m, 4H, -CH_2-), 3.34-3.38 (m, 4H, -CH_2-), 4.71 (s, 2H, -CH_2-), 7.28 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.44 (dd, *J* = 4.80 Hz, 1H, Ar-H), 7.69 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.96 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.5, 48.1, 49.5, 51.3, 119.8, 120.5, 122.4, 123.4, 124.5, 125.2, 126.6, 128.3, 129.6, 131.6, 132.9, 140.6, 144.7, 157.2, 161.4; MS (ESI): *m/z* [(M+H)$^+$]: 506; HRMS *m/z* calcd. for $C_{20}H_{17}N_5O_2SF_6$ [(M+H)$^+$]: 506.0025, found 506.0028.

3-(2-Oxo-2-(4-phenylpiperazin-1-yl)ethyl)-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4-(3*H*)-one (7c): Yellow solid; m.p.: 219-221 ºC; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.92-2.95 (m, 4H, -CH_2-), 3.21-3.25 (m, 4H, -CH_2-), 4.72 (s, 2H, -CH_2-), 7.29 (dd, *J* = 3.85 Hz, 1H, Ar-H), 7.32-7.36 (m, 3H, Ar-H), 7.44 (dd, *J* = 4.81 Hz, 1H, Ar-H), 7.49-7.52 (m, 2H, Ar-H), 7.72 (dd, *J* = 3.85 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.1, 49.8, 52.5, 118.7, 120.1, 121.4, 122.3, 123.5, 124.8, 125.6, 126.3, 127.3, 128.5, 129.6, 131.6, 132.9, 141.6, 143.7, 148.7, 157.2, 161.4; MS (ESI): *m/z* [(M+H)$^+$]: 568; HRMS *m/z* calcd. for $C_{25}H_{19}N_5O_2SF_6$ [(M+H)$^+$]: 568.0105, found 568.0108.

3-(2-(4-(2-Hydroxyethyl)piperazin-1-yl)-2-oxoethyl)-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]-pyrimidin-4(3H)-one (7d): Yellow solid; m.p.: 202-204 ºC; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.53 (t, 2H, *J* = 5.26 Hz, -CH_2-), 2.63-2.68 (m, 4H, -$(CH_2)_2$-), 2.75-2.81 (m, 4H, -$(CH_2)_2$-), 3.63 (t, 2H, *J* = 5.26Hz, -CH_2-OH), 4.72 (s, 2H, -CH_2-), 7.25 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.43 (dd, *J* = 4.81 Hz, 1H, Ar-H), 7.71 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.07 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 49.8, 52.5, 56.2, 59.1, 60.3, 119.3, 120.3, 121.5, 123.6, 124.8, 126.4, 127.3, 128.5, 131.6, 134.8, 140.5, 143.8, 158.5, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 536; HRMS *m/z* calcd. for $C_{25}H_{19}N_5O_3SF_6$ [(M+H)$^+$]: 536.0152, found 536.0155.

General Procedure for the synthesis of 8a-c: Ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]-pyrimidin-3(4*H*)-yl)acetate (**3**, 1 mmol), L-amino acid (2 mmol), potassium carbonate (5 mmol) and DMSO (10 mL) was mixed and refluxed for 12 h. The reaction mixture was cooled, diluted with ice water and then the aqueous solution was neutralized with 1 N HCl. The precipitated solid was filtered, dried and recrystallized from ethanol.

2-(2-(4-Oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamido)acetic acid (8a): Yellow solid; m.p.: 178-180 ºC; IR (KBr, cm^{-1}): 1661(amide), 1742 (-COOH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.21 (d, 2H, *J* = 5.61 Hz, -CH_2-), 4.81 (s, 2H, -CH_2-), 7.28 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.41 (dd, *J* = 4.81 Hz, 1H, Ar-H), 7.72 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.04 (s, 1H, Ar-H) 8.41 (t, 1H, *J* = 5.61 Hz, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 44.5, 48.4, 119.3, 120.5, 121.7, , 123.2, 124.5, 126.8, 128.1, 129.5, 132.4, 134.6, 140.6, 142.8, 158.6, 161.2, 168.9; MS (ESI): *m/z* [(M+H)$^+$]: 481; HRMS *m/z* calcd. for $C_{17}H_{10}N_4O_4SF_6$ [(M+H)$^+$]: 481.0210, found 481.0212.

2-(2-(4-Oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)-pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetamido)propanoic acid (8b): Yellow solid; m.p.: 183-185 ºC; IR (KBr, cm^{-1}): 1665 (amide), 1748 (-COOH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.45 (d, 3H, -CH_3), 4.23 (m, 1H, -CH-), 4.85 (s, 2H, -CH_2-), 7.27 (dd, *J* = 3.80 Hz, 1H, Ar-H), 7.43 (dd, *J* = 4.87 Hz, 1H, Ar-H), 7.71 (dd, *J* = 3.80 Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H) 8.45 (t, 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.2, 43.7, 49.1, 120.8, 121.4, 122.6, , 123.3, 124.7, 127.4, 128.3, 129.6, 132.5, 136.7, 140.3, 142.6, 157.5, 160.5, 168.7; MS (ESI): *m/z* [(M+H)$^+$]: 495; HRMS *m/z* calcd. for $C_{18}H_{12}N_4O_4SF_6$ [(M+H)$^+$]: 495.0258, found 495.0261.

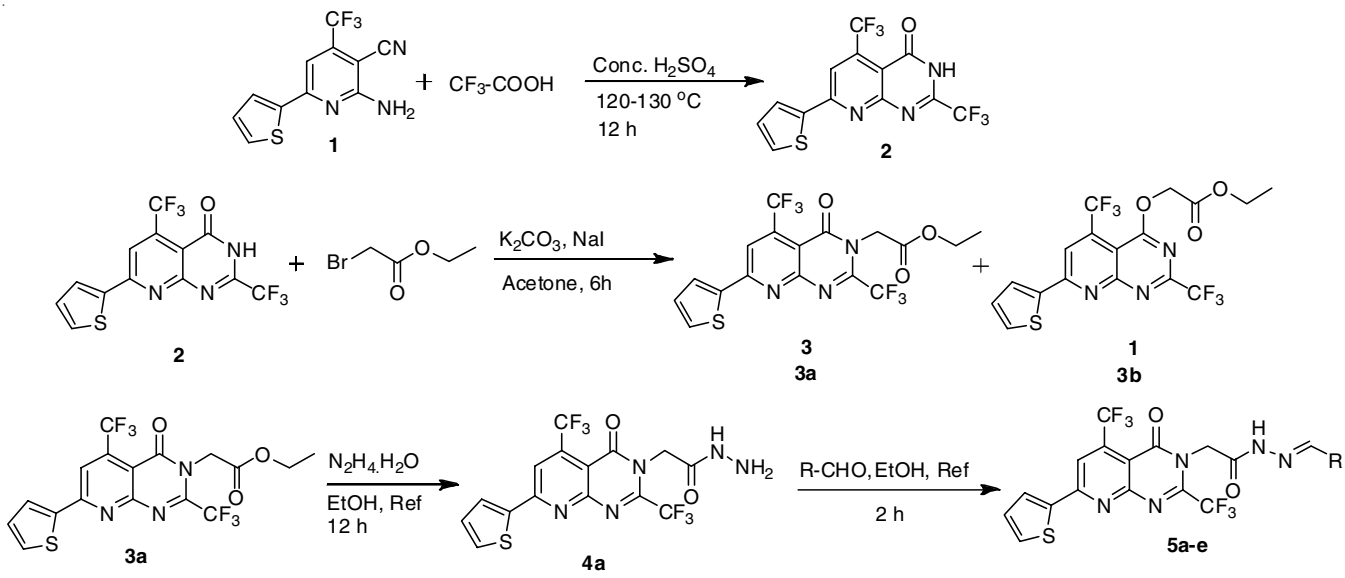
2-(2-(4-Oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)-pyrido[2,3-*d*]pyrimidin-3(4H)-yl)acetamido)-3-phenyl-propanoic acid (8c): Yellow solid; m.p.: 174-176 ºC; IR (KBr, cm^{-1}): 1662 (amide), 1742 (-COOH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.25 (d, 2H, -CH-), 4.21 (m, 1H, -CH_2-), 4.82 (s, 2H, -CH_2-), 7.26 (dd, *J*=3.81 Hz, 1H, Ar-H), 7.31-7.35 (m, 2H, Ar-H), 7.41 (dd, *J* = 4.87 Hz, 1H, Ar-H), 7.46-7.49 (m, 3H, Ar-H), 7.71 (dd, *J* = 3.81 Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H) 8.44 (d, 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 35.2, 43.2, 49.8, 120.8, 121.3, 122.1, 122.7, 123.4, 124.8, 126.4, 127.4, 128.3, 128.7, 129.6, 130.3, 132.6, 135.3, 141.2, 143.4, 158.6, 161.6, 168.3; MS (ESI): *m/z* [(M+H)$^+$]: 571; HRMS *m/z* calcd. for $C_{24}H_{16}N_4O_4SF_6$ [(M+H)$^+$]: 571.0521, found 571.0524.

RESULTS AND DISCUSSION

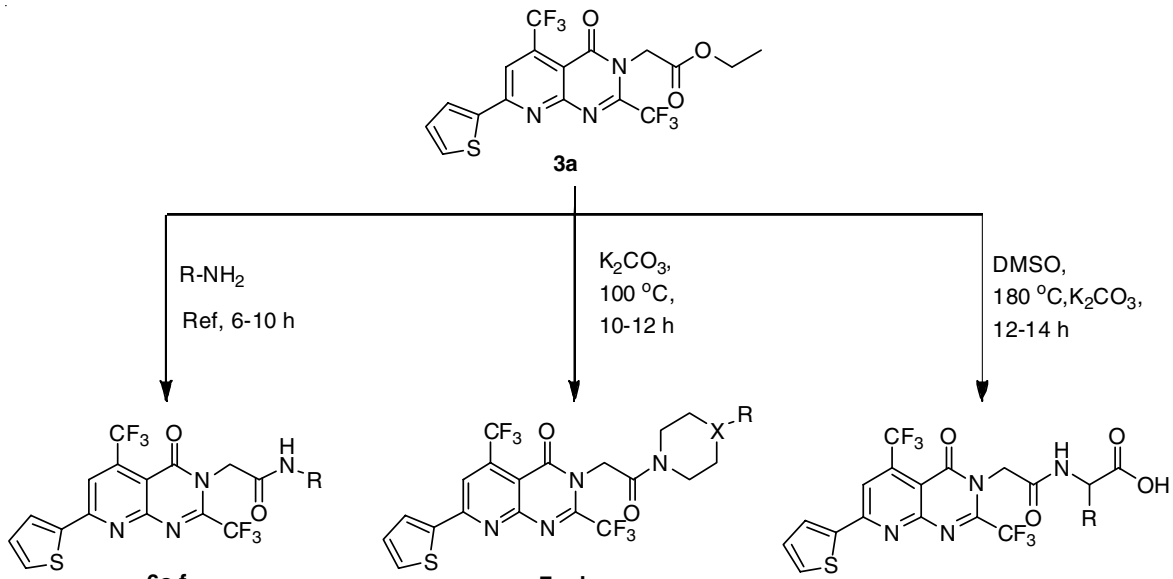
Synthesis: 2-Amino-6-(thiophen-2-yl)-4-(trifluoromethyl)-nicotinonitrile (**1**) on reaction with trifluoroacetic acid in the presence of conc. H_2SO_4 at high temperature to yield 2-methyl-7-(thiophen-2-yl)-5-(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2**). Compound 2 on further reaction with bromoethyl acetate in the presence of base media to obtain compounds **3a** and **3b** at the ratio of 3:1. Compound **3a** further reaction with hydrazine hydrate in refluxed conditions with ethanol to afford compound **4a**. Finally, compound **4a** on reaction with different substituted aromatic aldehydes in the presence of ethanol under the refluxing conditions to obtain Schiff base derivatives **5a-g** (**Scheme-I**).

In another route, when ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*] pyrimidin-3(4H)-yl)acetate (**3a**) compound on reaction with different substituted aromatic and aliphatic amines in different conditions like neat and basic media to get amide derivatives (**6a-f**, **7a-d** and **8a-c**) (**Scheme-II**).

The IR spectrum of ethyl 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetate (**3**) shows characteristic band at 1712 cm^{-1} and 2-(4-oxo-7-(thiophen-2-yl)-2,5-*bis*(trifluoromethyl) pyrido[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (**4**) shows IR bands at 3379, 3486 cm^{-1}, which indicated the presence of -NH_2 and at 3211, 1628 cm^{-1}, these bands indicate the presence of amide group, thus, the conversion of ester (**3**) to amide (**4**) and amide to Schiff's base (**5**) was confirmed. The ^{1}H NMR spectrum of compound **3** showed triplet at 1.31 ppm and quartret at 5.03 ppm which represent ester protons, Thiophenyl ring three aromatic protons appeared as AMX patern at 7.18 ppm with *J* = 4.95 as doublet



Scheme-I: Synthetic route of Schiff base (**5a-e**)



6a) R = -CH_3
6b) R = -CH_2-CH_3
6c) R = -CH_2-CH_2-CH_3
6d) R = Cyclopentyl
6e) R = Furfuryl
6f) R = Benzyl

7a) R = H, X = CH
7b) R = CH_3, X = N
7c) R = Phenyl, X = N
7d) R = -CH_2-CH_2-OH, X = N

8a) R= H
8b) R= CH_3
8c) R= Benzyl

Scheme-II: Synthetic route of amide functionalized pyrido[2,3-*d*]pyrimidine derivatives

of doublet, 7.59 ppm with J = 4.91 Hz as doublet of doublet, 7.72 ppm with J = 3.76 Hz as doublet of doublet, remaining pyridine proton appeared at 8.86 ppm as singlet. Compound **4** shows only broad singlet at 4.22 ppm indicating -NH_2 and disappearance of ester protons, thus confirmed the formation of ester to hydrazide derivative. The ^{1}H NMR spectrum of compound **5** shows imine (-HC=N) proton at 8.38 ppm as singlet and enol (-NHCO-) proton at 11.45 ppm as broad singlet, indicating the formation of Schiff's base derivatives.

The IR spectrum of compound **6** shows IR bands at 3215, 1652 cm^{-1} which indiates the presence of amide, while compound **8** exhibits the bands at 3220, 1661 cm^{-1} (amide), 3092, 1742 cm^{-1} (-COOH). The ^{1}H NMR spectrum of compound **6** shows aliphatic protons at 2.81 ppm and -N, amide attached 2 protons appeared at 4.73 ppm as singlet. The ^{1}H NMR spectrum of compounds **7** and **8** show the aliphatic protons at 1.59-3.43 ppm and enol proton appeared at 8.44 ppm.

Anticancer activity: All compounds were tested for *in vitro* against four human cancer cell lines such as HeLa-cervical cancer (CCL-2); COLO 205-colon cancer (CCL-222); HepG2-liver cancer (HB-8065); MCF7-breast cancer (HTB-22) using MTT assay [21]. The IC_{50} values of the synthesized compounds

for 24 h on each cell line were calculated and its values are shown in Table-1.

TABLE-1
in vitro CYTOTOXICITY OF SCHIFF'S BASE AND AMIDE FUNCTIONALIZED PYRIDO[2,3-*d*]PYRIMIDINE DERIVATIVES AGAINST FOUR HUMAN CANCER CELL LINES

Compd.	IC_{50} values (μM)			
	A549	MCF7	DU145	HeLa
5a	33.1	49.4	–	–
5b	56.2	42.2	–	–
5c	21.4	26.7	19.5	14.1
5d	11.3	14.6	13.4	9.1
5e	31.2	28.4	39.2	29.4
5f	61.5	46.7	68.5	–
5g	–	–	–	73.8
6a	18.2	27.3	31.4	26.3
6b	28.5	–	41.3	–
6c	41.2	39.3	61.4	46.3
6d	73.5	123.4	–	–
6e	24.8	27.3	34.2	41.9
6f	54.7	–	–	51.8
7a	71.3	44.3	31.6	–
7b	44.6	52.5	66.2	48.5
7c	19.2	25.7	12.8	14.1
7d	8.5	7.7	11.5	16.8
8a	14. 1	10.6	11.2	21.5
8b	12.7	21.5	29.8	34.7
8c	–	72.1	59.4	–
5-Fluorouracil (Std. control)	1.1	1.2	1.3	1.1

– indicates IC_{50} value > 123.4 μg/mL; Cell lines used: A549 = Lung cancer (CCL-185), MCF7 = Breast cancer (HTB-22), DU145 = Prostate cancer (HTB-81) and HeLa = Cervical cancer(CCL-2).

SAR studies: Structure activity relation studies revealed that all the synthesized Schiff's base derivatives (**5a-f**) and amide functionalized derivatives (**6a-f**, **7a-d** and **8a-c**) were screened for anticancer activity. Almost all the compounds showed activity against four cancer cell lines at micromolar concentration. Among all the compounds, only three compounds **5d**, **7d** and **8a** showed promising activity, while the remaining compounds showed medium activity. Compound **5d** was showed activity as the more potent towards all the cancer cell lines. The SAR studies revealed that Schiffs base derivatives showed less activity than amide functionalized derivatives, due to the availability of N-attached hydrogen in amide link and it is participated in H-bonding (H-attached with electronegative atom like N), the trifluoromethyl group containing molecule shows more activity compared to simple all other substitutes, containing thien-2-yl group at 6th position leads to additional advantage in promoting cytotoxicity activity.

CONFLICT OF INTEREST

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

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