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Synthesis and Anticancer Activity of Novel Carbohydrazide and Carboxamide Derivatives of Pyridine Fused Heterocyclic Derivatives

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A series of novel carbohydrazide and carboxamide derivatives of hetero-fused pyridine derivatives were synthesized starting from *bis*-trifluoromethyl containing pyridine **1**. Compound **1** on Smiles rearrangement obtained compound **2**, then compound **2** on coupling with diethyl ethoxymethylenemalonate (EMME), further reaction with $POCl_3$ and hydrazine hydrate to obtain pyridopyrimidine hydrazide derivatives. This hydrazide derivative reacts with different substituted aromatic aldehydes to produce title carbohydrazide derivatives **6a-h**. Pyridopyrimidine carboxylate compound on reaction with aliphatic amine to obtain carboxamide derivatives **7a-e**. All the final products evaluated for their anticancer activity, against four human cancer cell lines and promising compounds have been identified. Compounds **7a** and **7b** exhibits remarkable activity.

Keywords: Pyridopyridine, Smiles rearrangement, Carboxamide, Carbohydrazide, Schiff's base, Anticancer activity.

INTRODUCTION

Several natural and synthetic compounds were being used to treat cancer, but using limited due to their unwanted side effects, it has been a very tough and challenging task to develop newer and safer anticancer drugs for organic and medicinal chemists. A heterocyclic compound which contains nitrogen plays a major role to promote the activity [1-5]. Small heterocyclic ring systems containing activity enhancing functionalized groups such as Schiff base and amide group derivatives are also very important in medicinal and industrial. These type of heterocycles are very important class and exists as core moiety in many biologically active compounds and drugs. Especially pyrimidine heterocycles presents in DNA and RNA, pyridopyrimidine derivatives are also exhibits diverse biological activities [6,7], antitumour [8], antibacterial [9], anticonvulsant [10], antipyretic [11], analgesic [12] and CNS depressant activities [13-16].

Fluorinated organic and heterocyclic molecules are very important by known to have broad range of biological applications. Trifluoromethyl (-CF_3) group containing drugs such as nilutamide, flutamide, hydroxyflutamide and bicalutamide are non-steroidal anti-androgens, which they are widely used for the treatment of metastatic prostate cancer [17-20].

All these reports encouraged and in this research we designed and synthesized novel carbohydrazide and carboxamide derivatives of pyridopyrimidine derivatives. All the final derivatives evaluated for their anticancer activity, 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) and promising compounds have been identified.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer FT-IR 240C 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 2-amino-4,6-*bis*(trifluoromethyl)nicotinonitrile (2): 2-Oxo-4,6-*bis*(trifluoromethyl)-1,2-dihydropyridine-

3-carbonitrile (**1**, 2.5 mmol) was taken in dry DMF (50 mL) and make it homogeneous solution, then slowly added 2-chloroacetamide (0.23 g, 2.5 mmol), potassium carbonate (0.745 g, 5.1 mmol) and a pinch of sodium iodide (0.010 g). The reaction mixture was allowed to reflux for 6-8 h at 100 ºC and cooled to room temperature. To this, added some ice cold water and solid was formed, this solid was filtered and dried. It involves Smiles rearrangement (**Scheme-I**). White solid; yield: 71%; IR (KBr, ν_{max}, cm^{-1}): 3490 (-NH_2), 3441 (-NH_2), 2231 (CN); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.71 (br s, 2H, NH_2), 7.71 (s, 1H, Ar-H); MS (ESI): *m/z* [$(M+H)^+$]: 256; Anal. calcd. (found) % for $C_8H_3N_3F_6$: C 37.66 (37.67); H, 1.19 (1.21); N, 16.47 (16.48).

Synthesis of diethyl 2-(((3-cyano-4,6-*bis*(trifluoromethyl)pyridin-2-yl)amino)methylene)malonate (3): 2-Amino-4,6-*bis*(trifluoromethyl)nicotinonitrile (**2**, 0.500 g, 0.002 mol) dissolved in ethanol was mixed with diethyl 2-(ethoxy-methylene)malonate (0.450 g, 0.002 mol) and refluxed for 10 h. After the completion of reaction by checking with TLC, ethanol was completely removed under vacuum, crude product was washed with *n*-hexane and dried to afford diethyl 2-(((3-cyano-4,6-*bis*(trifluoromethyl)pyridin-2-yl)amino)-methylene)malonate (**3**) (**Scheme-I**). White solid; yield 89%; IR (KBr, ν_{max}, cm^{-1}): 3314 (-NH-), 2231 (CN); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.37 (t, 6H, –CH_3), 4.32 (q, 4H, –CH_2–), 7.82 (s, 1H, Ar-H), 9.21 (d, *J* = 12.46 Hz, 1H, –NCH=), 11.21 (d, *J* = 12.46 Hz, 1H, –NHC=); MS (ESI): *m/z* [$(M+H)^+$]: 426; Anal. calcd. (found) % for $C_{16}H_{13}N_3O_4F_6$: C, 45.19 (45.20), H, 3.08 (3.10); N, 9.88 (9.89).

Synthesis of ethyl 9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (4): Phosphorus oxychloride (5 mL) was slowly added to diethyl 2-(((3-cyano-4,6-*bis*(trifluoromethyl)pyridin-2-yl)amino)methylene)-malonate (**3**) (0.500 g, 0.001 mol) and then the reaction mixture was refluxed for 4-5 h at 140 ºC. After the completion of reaction by checking with TLC, excess $POCl_3$ was distilled under vacuum and the crude product was poured on crushed ice. Yellow colour solid was formed, filtered and washed with excess water to get yellow solid (**Scheme-I**). White solid; yield 65%; IR (KBr, ν_{max}, cm^{-1}): 2232 (NH_2), 1726 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 1.26 (t, 3H, -CH_3), 4.21 (q, 2H, -CH_2), 7.81 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H); MS (ESI): *m/z* [$(M+H)^+$]: 380; Anal. calcd. (found) % for $C_{14}H_7N_3O_3F_6$: C, 44.34 (44.35); H, 1.86 (1.87); N, 11.08 (11.09).

Synthesis of 9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (5): Ethyl 9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]-pyrimidine-3-carboxylate dissolved in absolute ethanol (30 mL) and then added hydrazine hydrate (5 mL). The reaction mixture was refluxed for 3-4 h. After confirmation of product formation by TLC, cooled and the ethanol was removed under vacuum. The residue was stirred with water and filtered, washed with *n*-hexane and dried under vacuum to give a yellow solid which was pure enough (**Scheme-I**). White solid; yield 90%; IR (KBr, ν_{max}, cm^{-1}): 3371, 3494 (NH_2), 3211 (CONH), 1682 (CO); ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 4.16 (br. s, 2H, N-NH_2), 7.65 (s, lH, CONH), 7.85 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H); MS (ESI): *m/z* [$(M+H)^+$]: 366; Anal. calcd. (found) % for $C_{12}H_5N_5O_2F_6$: C, 39.47 (39.48); H, 1.38 (1.39); N, 19.18 (19.20).

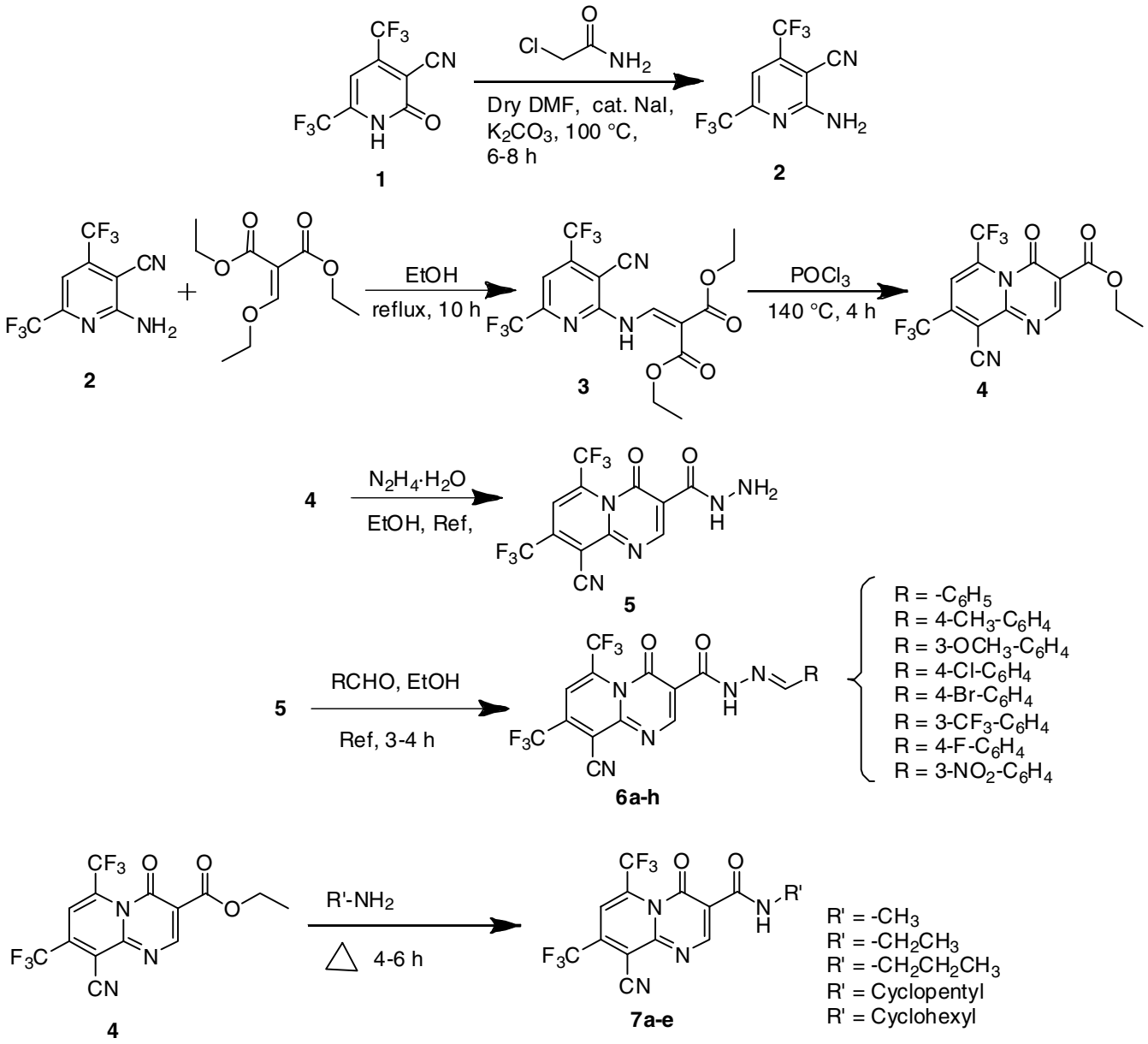
Synthesis of (*E*)-*N*′-benzylidene-9-cyano-4-oxo-6,8-*bis*-(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbo-hydrazide (6): 9-Cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (**5**) (2.5 mmol) dissolved in 10 mL absolute ethanol was added slowly to piperidine (0.1 mL) followed by benzaldehyde (3 mmol). The reaction mixture was allowed to reflux for 3 to 4 h and the reaction completion was confirmed by TLC, a reaction mixture was allowed to cool and ethanol was removed under vacuum. The residue was washed with *n*-hexane and then water was added to give yellow solid which was filtered and dried (**Scheme-I**).

(*E*)-*N*′-Benzylidene-9-cyano-4-oxo-6,8-*bis*(trifluoro-methyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6a): Yellow solid; Yield 86%; m.p.: 209-211 ºC; IR (KBr, ν_{max}, cm^{-1}): 3212, 1622 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 7.28-7.33 (m, 2H, Ar-H), 7.52-7.58 (m, 3H, Ar-H), 7.81 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.41 (s, 1H, CH=N), 11.40 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 118.2, 121.4, 122.8, 123.2, 124.7, 125.6, 126.8, 129.1, 131.8, 133.3, 136.5, 140.5, 145.3, 147.4, 148.8, 157.8, 163.3; MS (ESI): *m/z* [$(M+H)^+$]: 454; Anal. calcd. (found) % for $C_{19}H_9N_5O_2F_6$: C, 50.34 (50.35); H, 2.00 (2.01); N, 15.45 (15.46).

(*E*)-9-Cyano-*N*′-(4-methylbenzylidene)-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6b): Yellow solid; yield 72%; m.p.: 221-223 ºC; IR (KBr, ν_{max}, cm^{-1}): 3214, 1625 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 2.39 (s, 3H, -CH_3) 7.32 (d, 2H, Ar-H), 7.48 (d, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 8.46 (s, 1H, -CH=N), 11.37 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 23.6, 119.4, 121.5, 122.7, 123.5, 124.6, 125.7, 127.3, 129.2, 132.5, 134.6, 136.7, 140.4, 142.6, 145.7, 148.6, 156.8, 162.7; MS (ESI): *m/z* [$(M+H)^+$]: 468; Anal. calcd. for $C_{20}H_{11}N_5O_2F_6$: C, 51.40 (51.41); H, 2.37 (2.38); N, 14.99 (14.98).

(*E*)-9-Cyano-*N*′-(3-methoxybenzylidene)-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6c): Yellow solid; yield 86%; m.p.: 231-233 ºC; IR (KBr, ν_{max}, cm^{-1}): 3211, 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 3.78 (s, 3H, -OCH_3), 7.36-7.42 (m, 3H, Ar-H), 7.48 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 8.49 (s, 1H, -CH=N), 11.39 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 56.8, 120.1, 121.6, 122.5, 123.1, 123.8, 124.5, 125.8, 126.7, 127.5, 129.1, 132.6, 133.5, 136.8, 140.3, 142.7, 145.6, 148.3, 155.7, 162.3; MS (ESI): *m/z* [$(M+H)^+$]: 484; Anal. calcd. (found) % for $C_{20}H_{11}N_5O_3F_6$: C, 49.70 (49.71); H, 2.29 (2.31); N, 14.49 (14.51).

(*E*)-*N*′-(4-Chlorobenzylidene)-9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6d): Yellow solid; yield 81%; m.p.: 199-201 ºC; IR (KBr, ν_{max}, cm^{-1}): 3213, 1626 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.41 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.49 (s, 1H, -CH=N), 11.30 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 118.3, 120.2, 122.5, 123.6, 124.7, 125.6, 126.2, 128.4, 129.3,



Scheme-I: Synthetic route of novel carbohydrazide and carboxamide functionalized pyridopyrimidine derivatives **6a-h** and **7a-e**

131.6, 134.7, 136.6, 140.3, 142.8, 145.6, 148.3, 158.7, 162.3; MS (ESI): *m/z* [(M+H)$^+$]: 488; Anal. calcd. (found) % for $C_{19}H_8N_5O_2ClF_6$: C, 49.79 (49.80); H, 1.65 (1.66); N, 14.36 (14.37).

(*E*)-*N′*-(4-Bromobenzylidene)-9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6e): Yellow solid; yield: 76%; m.p.: 224-226 ºC; IR (KBr, ν_{max}, cm^{-1}): 3214, 1629 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): 7.42 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 8.49 (s, 1H, -CH=N), 11.32 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 119.3, 120.6, 122.2, 123.6, 124.7, 125.8, 127.4, 129.1, 132.6, 134.5, 136.8, 140.3, 142.4, 145.6, 148.7, 156.7, 162.1; MS (ESI): *m/z* [(M+H)$^+$]: 531; Anal. calcd. (found) % for $C_{19}H_8N_5O_2BrF_6$: C, 42.88 (42.89); H, 1.52 (1.53); N, 13.16 (13.17).

(*E*)-9-Cyano-4-oxo-6,8-*bis*(trifluoromethyl)-*N′*-(3-(trifluoromethyl)benzylidene)-4*H*-pyrido[1,2-*a*]-pyrimidine-3-carbohydrazide (6f): Yellow solid; yield: 78%; m.p.: 213-215 ºC; IR (KBr, ν_{max}, cm^{-1}): 3215, 1628 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.43-7.48 (m, 3H, Ar-H), 7.62 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.44 (s, 1H, -CH=N), 11.36 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 118.4, 121.3, 122.4, 123.7, 124.6, 125.3, 126.5, 128.2, 129.2, 132.4, 133.5, 134.6, 135.9, 140.2, 141.3, 142.6, 146.4, 148.5, 156.1, 162.3; MS (ESI): *m/z* [(M+H)$^+$]: 522; Anal. calcd. (found) % for $C_{20}H_8N_5O_2F_9$: C, 46.06 (46.07); H, 1.55 (1.56); N, 13.43 (13.45).

(*E*)-*N′*-(4-Fluorobenzylidene)-9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6g): Yellow solid; Yield 71%; m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 3210, 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.39 (d, 2H, Ar-H), 7.48 (d, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.45 (s, 1H,

-CH=N), 11.32 (br.s, 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 119.1, 120.3, 121.5, 122.4, 123.7, 124.6, 125.5, 128.3, 129.1, 131.7, 132.6, 134.7, 140.2, 142.5, 144.5, 158.7, 162.3; MS (ESI): *m/z* [(M+H)$^+$]: 472; Anal. calcd. (found) % for $C_{19}H_8N_5O_2F_7$: C, 48.42 (48.43); H, 1.71 (1.72); N, 14.86 (14.87).

(*E*)-9-cyano-*N*′-(3-nitrobenzylidene)-4-oxo-6,8-*bis*-(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (6h): Yellow solid; Yield 66%; m.p.: 228-230 ºC; IR (KBr, ν_{max}, cm^{-1}): 3206, 1619 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 7.39-7.43 (m, 3H, Ar-H), 7.49 (s, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.48 (s, 1H, -CH=N), 11.40 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 120.3, 121.5, 122.6, 123.2, 124.6, 125.6, 126.4, 127.6, 128.6, 129.3, 132.2, 133.6, 136.7, 140.2, 142.8, 144.5, 148.6, 156.6, 161.7; MS (ESI): *m/z* [(M+H)$^+$]: 499; Anal. calcd. (found) % for $C_{19}H_8N_6O_4F_6$: C, 45.80 (45.82); H, 1.62 (1.63); N, 16.87 (16.88).

Synthesis of 9-cyano-*N*-methyl-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (7): Ethyl 9-cyano-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]-pyrimidine-3-carboxylate (**4**, 2 mmol) was dissolved in aliphatic primary amine (4 mmol) in a sealed tube and this reaction mixture was allowed to reflux for 6 h, after the confirmation of product by TLC sealed tube kept in crushed ice. Filtered and washed with *n*-hexane, yellow solid was formed and dried to get good yield (**Scheme-I**).

9-Cyano-*N*-methyl-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (7a): Yellow solid; Yield 88%; m.p.: 195-197 ºC; IR (KBr, ν_{max}, cm^{-1}): 3356 (-NHCO-), 1635 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 3.01 (d, 3H, -CH_3), 6.38 (br. s, 1H, -CONH-), 7.51 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 25.5, 119.1, 123.2, 125.4, 127.6, 128.7, 129.2, 131.4, 133.8, 135.8, 142.2, 158.4, 160.8; MS (ESI): *m/z* [(M+H)$^+$]: 365. Anal. Calcd. (found) % for $C_{13}H_6N_4O_2F_6$: C, 42.87 (42.88); H, 1.66 (1.65); N, 15.38 (15.39).

9-Cyano-*N*-ethyl-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (7b): Yellow solid; yield: 89%; m.p.: 201-203 ºC; IR (KBr, ν_{max}, cm^{-1}): 3342 (-NHCO-), 1641 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 1.14 (t, 3H, -CH_3), 3.51 (quintet, 2H, -CH_2-), 6.33 (br. s, 1H, -CONH-), 7.52 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm):16.1, 35.2, 120.3, 124.7, 126.4, 128.2, 129.0, 131.6, 134.4, 137.0, 139.8, 142.1, 157.9, 160.5; MS (ESI): *m/z* [(M+H)$^+$]: 379. Anal. calcd. (found) % for $C_{14}H_8N_4O_2F_6$: C, 44.46 (44.47); H, 2.13 (2.14); N, 14.81 (14.83).

9-Cyano-4-oxo-*N*-propyl-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide(7c): Yellow solid; yield: 82%; m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 3359 (-NHCO-), 1640 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 1.05 (t, 3H, -CH_3), 1.59-1.64 (m, 2H, -CH_2-), 3.42-3.44 (m, 2H, -CH_2-), 6.41 (br.s, 1H, -NH-), 7.54 (s, 1H, Ar-H), 7.98 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 10.7, 23.2, 41.8, 120.2, 123.5, 125.6, 126.8, 128.3, 129.1, 130.8, 133.4, 135.1, 139.7, 142.4, 158.6, 159.8; MS (ESI): *m/z* [(M+H)$^+$]: 393. Anal. calcd. (found) % for $C_{15}H_{10}N_4O_2F_6$: C, 45.93 (45.94); H, 2.57 (2.56); N, 14.28 (14.26).

9-Cyano-*N*-cyclopentyl-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (7d): Yellow solid; yield: 79%; m.p.: 198-200 °C; IR (KBr, ν_{max}, cm^{-1}): 3341 (-NHCO-), 1638 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 1.52-1.58 (m, 2H, -CH_2-), 1.66-1.70 (m, 2H, -CH_2-), 1.73-1.76 (m, 2H, -CH_2-), 2.05-2.21 (m, 2H, -CH_2-), 4.46-4.49 (m, 1H, -CH-), 6.39 (br.d, 1H, -NHCO-), 7.55 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 20.5, 31.2, 46.1, 120.4, 123.0, 124.5, 125.9, 126.7, 130.7, 133.3, 134.0, 140.0, 142.7, 157.5, 160.2; MS (ESI): *m/z* [(M+H)$^+$]: 419. Anal calcd. (found) % for $C_{17}H_{12}N_4O_2F_6$: C, 48.81 (48.82); H, 2.81 (2.83); N, 13.39 (13.41).

9-Cyano-*N*-cyclohexyl-4-oxo-6,8-*bis*(trifluoromethyl)-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (7e): Yellow solid; yield: 79%; m.p.: 209-211 ºC; IR (KBr, ν_{max}, cm^{-1}): 3342 (-NHCO-), 1636 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 1.21-1.42 (m, 4H, -CH_2-), 1.49-1.53 (m, 4H, -CH_2-), 1.59-1.63 (m, 2H, -CH_2-), 4.46-4.49 (m, 1H, -CH-), 6.39 (br. d, 1H, -NHCO-), 7.56 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 20.4, 23.6, 31.3, 46.5, 120.7, 123.2, 124.6, 126.3, 128.8, 130.8, 133.4, 136.2, 140.4, 142.8, 158.3, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 433. Anal calcd. (found) % for $C_{18}H_{14}N_4O_2F_6$: C, 50.01 (50.02); H, 3.26 (3.27); N, 12.96 (12.98).

RESULTS AND DISCUSSION

2-Oxo-4,6-*bis*(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (**1**) on reaction with chloroacetamide and obtained 2-amino pyridine (**2**) (involving smiles rearrangement) compound 2-amino pyridne (**2**) on further treat with ethoxy methylene malonic diethyl ester (EMME) in ethanol refluxing condition to get compound **3**, which after cyclization in the presence of $POCl_3$ resulted product **4**, reaction of compound **4** with hydrazine hydrate in ethanol to obtain compound **5**. Compound **5** on reaction with diverse substituted aromatic aldehydes in ethanol for about 3-4 h of Schiff's base reaction to get carbohydrazide derivatives **6a-h**.

Another way, ester derivative **4** on reaction with different aliphatic amine at their refluxing condition for about 4-6 h and obtained carboxamide derivatives **7a-e**.

SAR studies: The pyridopyrimidine carbohydrazide derivatives and carboxamide functionalized derivatives were tested for anticancer activity among 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). 5-Fluorouracil used as standard control. Among all the synthesized compounds, some compounds showed activity up to the MIC concentration of 118.7 μM. Compounds **6a**, **6b** and **6e** doesn't show any activity. Compounds **7a** and **7b** showed the promising activity. Free hydrogen of amide functional group, participated in H-bonding (H-attached with electronegative atom like N), Most of the compounds exhibits promising activity at micromolar concentration. Among all the compounds, **7a** and **7b** showed promising activity. The activity data is shown in Table-1.

TABLE-1
In vitro CYTOTOXICITY OF COMPOUNDS **6a-h** and **7a-e**

Compound	IC_{50} values (μM)			
	HeLa	COLO205	HepG2	MCF7
6a	–	–	–	–
6b	–	–	–	–
6c	–	25.2 ± 0.23	41.3 ± 0.21	35.3 ± 0.18
6d	55.2 ± 0.22	–	–	118.7 ± 0.26
6e	–	–	–	–
6f	48.5 ± 0.32	35.5 ± 0.32	–	25.7 ± 0.38
6g	28.4 ± 0.15	33.4 ± 0.25	84.4 ± 0.65	69.7 ± 0.32
6h	–	–	–	–
7a	21.5 ± 0.16	32.2 ± 0.16	21.8 ± 0.23	19.2 ± 0.22
7b	23.1 ± 0.31	36.8 ± 0.26	29.5 ± 0.18	18.7 ± 0.38
7c	55.3 ± 0.21	68.9 ± 0.61	70.8 ± 0.71	31.5 ± 0.52
7d	–	61.5 ± 0.36	89.8 ± 0.23	59.7 ± 0.22
7e	48.6 ± 0.38	–	44.8 ± 0.22	–
5-Fluorouracil (Std. control)	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07

– indicates IC_{50} value > 118.7 μM; Cell lines used: HeLa = Cervical cancer (CCL-2); COLO 205 = Colon cancer (CCL-222); HepG2 = Liver cancer (HB-8065); MCF7 = Breast cancer (HTB-22).

Conclusion

In conclusion, a series of novel carbohydrazide and carboxamide functionalized pyridopyrimidine derivatives **6a-h** and **7a-e** were synthesized and evaluated for anticancer activity against four human cancer cell lines such as 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. Among all the compounds screened, compounds **7a** and **7b** showed significant activity against all cell lines at micromolar concentration.

CONFLICT OF INTEREST

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

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