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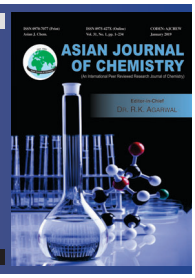

Synthesis and Anticancer Activity of Novel Hetero Ring Fused Pyridine Amide Derivatives

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A series of novel hetero ring fused pyridine amide derivatives were prepared starting from ethyl furo[2,3-*b*]pyridine-2-carboxylate (**3**) on reaction with ammonia to afford furo[2,3-*b*]pyridine-2-carboxamide (**4**), compound **4** on reaction with trifluoroacetic acid to give compound **5**, which on reaction with bromoethyl acetate followed by hydrazine hydrate to give compound **7**. Compound **7** when reacted with different substituted aromatic aldehydes to give Schiff base compounds (**8a-l**). Similarly, compound **6a** when reacted with diverse substituted aliphatic amines to give amide derivatives (**9a-h**). All the synthesized compounds **8a-l** and **9a-h** were screened for anticancer activity against four cancer cell lines such as A549-lung cancer (CCL-185); DU145-prostate cancer (HTB-81); SiHa-squamous cell carcinoma (HTB-35); MCF-7-breast cancer (HTB-22); HEK-29-human embryonic kidney cells (CRL-1573). Compounds **9e** and **9f** are found to have promising anticancer activity at micro molar concentration and found to be non-toxic on normal cell line.

Keywords: Schiff base, Amide derivatives, Trifluoromethyl group, Anticancer activity.

INTRODUCTION

Nitrogen, oxygen and sulfur containing heterocyclic compounds play an important role to promoting the biological activity. Heterocycles are considered as an extremely important class of compounds which play a key role in health care and pharmaceutical drug design [1]. Currently, a number of heterocyclic compounds are available commercially as anticancer drugs. The research interest on heterocyclic compounds is continuously going on and especially on pyrido-pyrimidine molecules, the reason is pyrido-pyrimidine compounds have wide range of biological applications like antitumor [2-5], antibacterial [6-8], antifungal [9-12], anti-inflammatory [13], antiallergic [14], antidiabetic [15], antiviral [16-18], antiherpes [19] and calcium channel blocking activity [20,21]. The trifluoromethyl group on heterocyclic molecule alters the properties like lipid solubility, oxidative thermal stability and oral bioavailability [22,23] and plays an important role in promoting activity.

Based on the literature survey, we planned and synthesized novel hetero ring fused pyridine amide derivatives and submitted for anticancer activity against four cancer cell lines such as A549-lung cancer (CCL-185); DU145-prostate cancer (HTB-81); SiHa-squamous cell carcinoma (HTB-35); MCF-7 breast cancer (HTB-22); HEK-29 human embryonic kidney cells (CRL-1573). Compounds **9e** and **9f** are found to have promising anticancer activity at micro molar concentration and found to be non-toxic to normal cell line.

In continuation to our efforts, we report the novel hetero ring fused pyridine amide derivatives. The 2(1*H*)-pyridine (**1**) was treated with 2-bromoethyl acetate in basic conditions (K_2CO_3) and got selectively 2-O-ethylacetoxy-3-cyano-4-trifluoromethyl-6-substituted pyridine derivatives (**2**). Compound **2** further reacted with K_2CO_3 in DMF and obtained furo[2,3-*b*]pyridine derivatives **3**. This cyclization involves selective O-alkylation then proton abstraction of an active methylene by base followed cyclization onto nitrile carbon. This cyclization is also known as Thorpe-Ziegler cyclization. Compound **3** on reaction with ammonia to afford compound **4**, this amide compound **4** on reaction with trifluoroacetic acid in the presence of HCl and water to give compound **5**.

EXPERIMENTAL

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. 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 pre-coated silica gel 60 F_{254}; spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography.

General procedure of the synthesis: To a stirred solution of ethyl 3-amino-6-phenyl/thiophen-2-yl-4-(trifluoromethyl)-furo[2,3-*b*] pyridine-2-carboxylate (**3**) (100 mg, 0.7 mmol) in ethanolic ammonia (2.5 mL) at 0 ºC in a 2-5 mL microwave vial in a single portion. The vial was sealed immediately and heated at 80 ºC for 4-5 h. The reaction was allowed to cool to room temperature and diluted with chloroform (25 mL) and water (25 mL). The aqueous layer was neutralized with 3 N HCl and the organic layer separated. The aqueous layer was further extracted with chloroform (2 × 25 mL) and the organic layers combined and concentrated in vacuum to afford solid compound **4**. No further purification was required.

3-Amino-6-phenyl-4-(trifluoromethyl)furo[2,3-*b*]-pyridine-2-carboxamide (4a): Yellow colour solid; yield: 62 %, IR (KBr, ν_{max}, cm^{-1}): 3121, 3172 (-NH_2), 1662 (amide CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 5.28 (br. s., 2H, -NH2), 6.89 (br. s., 2H, -NH_2), 7.48-7.54 (m, 3H, Ar-H), 7.71-7.76 (m, 2H, Ar-H), 8.12 (s, 1H, py-HC); MS (ESI): *m/z* [(M+H)$^+$]: 322. Elemental analysis calcd. (found) % for $C_{16}H_8N_3O_2F_3$: C 56.08 (56.11), H 3.14 (3.16), N 13.08 (13.10).

3-Amino-6-(thiophen-2-yl)-4-(trifluoromethyl)furo-[2,3-*b*]pyridine-2-carboxamide (4b): Yellow colour solid; yield: 55 %; IR (KBr, ν_{max}, cm^{-1}): 3118, 3169 (-NH_2), 1665 (amide CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 5.26 (br. s., 2H, -NH_2), 6.91 (br. s., 2H, -NH_2), 7.20 (dd, *J* = 3.83 Hz, 1H, Ar-H), 7.42 (dd, *J* = 4.13 Hz, 1H, Ar-H), 7.71 (dd, *J* = 3.82 Hz, 1H, Ar-H), 8.13 (s, 1H, py-HC); MS (ESI): *m/z* [(M+H)$^+$]: 328; Elemental analysis calcd. (found) % for $C_{13}H_8N_3O_2SF_3$: C 47.71 (47.72), H 2.46 (2.47), N 12.84 (12.86).

3-Amino-N-methyl-6-substituted-4-(trifluoromethyl)furo [2,3-*b*]pyridine-2-carboxamide (**4**, 4 mmol) and trifluoroacetic acid (4 mmol) were taken. To this were added 5 mL HCl and 5 mL H_2O. The mixture was refluxed at 100 ºC for 2 h and after completion of the reaction, reaction mixture was poured on crushed ice, solid was formed and washed with excess water and dried.

(*E*)-N′-Benzylidene-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (8a): m.p.: 211-213 ºC; IR (KBr, ν_{max}, cm^{-1}): 3218 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.21 (s, 2H, -CH_2-), 7.19 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.49 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.55-7.59 (m, 3H, Ar-H), 7.72 (m, 2H, Ar-H), 7.80 (dd, *J* = 4.23 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 8.51 (s, 1H, CH=N) 11.21 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 42.1, 118.9, 119.7, 121.0, 123.2, 123.3, 125.7, 126.8, 127.5, 128.3, 129.6, 131.1, 133.1, 134.1, 137.8, 140.0, 142.0, 147.6, 149.7, 152.2, 159.0, 161.7; MS (ESI): *m/z* [(M+H)$^+$]: 566. HRMS *m/z* calcd. (found) for $C_{24}H_{13}N_5O_3SF_6$ [(M+H)$^+$]: 566.0479, found: 566.0480. Anal. calcd. (found) %: C 50.98 (50.96); H 2.32 (2.33); N 12.39 (12.41).

(*E*)-N′-(4-methoxybenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8b): m.p.: 201-203 ºC; IR (KBr, ν_{max}, cm^{-1}): 3222 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.76 (s, 3H, -OCH_3), 4.22 (s, 2H, -CH_2-), 7.21 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.34 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.46 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.78 (dd, *J* = 4.23 Hz, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 8.48 (s, 1H, CH=N), 11.23 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 48.3, 56.2, 120.4, 121.7, 123.0, 123.6, 124.8, 125.6, 126.5, 127.3, 128.1, 129.4, 130.9, 132.2, 133.3, 135.8, 137.1, 139.9, 143.3, 146.7, 158.7, 161.0; MS (ESI): *m/z* [(M+H)$^+$]: 596. HRMS *m/z* calcd. (found) for $C_{25}H_{15}N_5O_4SF_6$ [(M+H)$^+$]: 596.1056, found: 596.1058. Anal. calcd. (found) %: C 50.43 (50.42); H 2.54 (2.55); N 11.76 (11.75).

(*E*)-N′-(3-methylbenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8c): m.p.: 215-217 ºC; IR (KBr, ν_{max}, cm^{-1}): 3219 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 2.36 (s, 3H, -CH_3), 4.19 (s, 2H, -CH_2-), 7.19 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.32 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.42-7.47 (m, 3H, Ar-H), 7.59 (s, 1H, Ar-H), 7.71 (dd, *J* = 4.21 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 8.49 (s, 1H, CH=N), 11.21 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 23.2, 49.5, 120.3, 121.8, 122.8, 123.5, 124.9, 125.6, 126.2, 127.1, 127.8, 128.5, 129.9, 130.1, 132.0, 133.2, 135.4, 137.4, 138.8, 141.9, 142.8, 145.2, 147.3, 158.9, 161.1; MS (ESI): *m/z* [(M+Na)$^+$]: 602. HRMS *m/z* calcd. (found) for $C_{25}H_{15}N_5O_3SF_6$ [(M+H)$^+$]: 580.0448, found: 580.0450. Anal. calcd. (found) %: C 51.82 (51.82); H 2.61 (2.62); N 12.09 (12.11).

(*E*)-N′-(4-Chlorobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8d): m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 3222 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.76 (s, 3H, -OCH_3), 4.22 (s, 2H, -CH_2-), 7.21 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.34 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.46 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.78 (dd, *J* = 4.23 Hz, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 8.48 (s, 1H, CH=N), 11.23 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 48.3, 56.2, 120.4, 121.7, 123.0, 123.6, 124.8, 125.6, 126.5, 127.3, 128.1, 129.4, 130.9, 132.2, 133.3, 135.8, 137.1, 139.9, 143.3, 146.7, 158.7, 161.0; MS (ESI): *m/z* [(M+H)$^+$]: 596. HRMS *m/z* calcd. (found) for $C_{24}H_{12}N_5O_3SClF_6$ [(M+H)$^+$]: 596.1056, found: 596.1058. Anal. calcd. (found) %: C 48.05 (48.07); H 2.02 (2.03); N 11.67 (11.68).

(*E*)-N′-(3-Fluorobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8e): m.p.: 202-204 ºC; IR (KBr, ν_{max}, cm^{-1}): 3215 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.19 (s, 2H, -CH_2-), 7.18 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.28 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.38-7.42 (m, 3H, Ar-H), 7.48 (s, 1H, Ar-H), 7.65 (dd, *J* = 4.19 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 8.42 (s, 1H, CH=N), 11.18 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 49.5, 120.3, 121.8, 122.8,

123.5, 124.9, 125.6, 126.2, 127.1, 127.8, 128.5, 129.9, 130.1, 132.0, 133.2, 135.4, 137.4, 138.8, 141.9, 142.8, 145.2, 147.3, 158.9, 161.1; MS (ESI): *m/z* [(M+H)$^+$]: 584. HRMS *m/z* calcd. (found) for $C_{24}H_{12}N_5O_3SF_7$ [(M+H)$^+$]: 584.0106. Found: 584.0109. Anal. calcd. (found) %: C 49.41 (49.42); H 2.07 (2.08); N 12.00 (12.02).

(*E*)-N′-benzylidene-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*-(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)acetohydrazide (8f): m.p.: 229-231 ºC; IR (KBr, ν_{max}, cm^{-1}): 3214 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.20 (s, 2H, -CH_2-), 7.18 (dd, *J* = 3.84 Hz, 1H, Ar-H), 7.42 (dd, *J* = 3.84 Hz, 1H, Ar-H), 7.51-7.56 (m, 3H, Ar-H), 7.71 (m, 2H, Ar-H), 7.79 (dd, J=4.19 Hz, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.55 (s, 1H, CH=N) 11.23 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 42.8, 118.9, 119.7, 121.0, 123.2, 123.3, 125.7, 126.8, 127.5, 128.3, 129.6, 131.1, 132.6, 133.1, 134.1, 137.8, 140.0, 142.0, 145.3, 147.5, 159.0, 161.7; MS (ESI): *m/z* [(M+H)$^+$]: 582. HRMS *m/z* calcd. (found) for $C_{24}H_{13}N_5O_2S_2F_6$ [(M+H)$^+$]: 582.0324, found: 582.0326. Anal. calcd. (found) %: C 49.57 (49.58); H 2.25 (2.27); N 12.04 (12.02).

(*E*)-N′-(4-Methoxybenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8g): m.p.: 225-227 ºC; IR (KBr, ν_{max}, cm^{-1}): 3226 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.72 (s, 3H, -OCH_3), 4.19 (s, 2H, -CH_2-), 7.20 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.35 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.42 (d, 2H, Ar-H), 7.59 (d, 2H, Ar-H), 7.76 (dd, *J* = 4.23 Hz, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 8.49 (s, 1H, CH=N), 11.21 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 48.9, 56.4, 120.7, 121.9, 123.3, 123.9, 124.5, 125.7, 126.5, 127.6, 128.8, 129.9, 131.5, 132.3, 133.6, 136.4, 137.4, 139.8, 142.4, 145.4, 158.5, 161.5; MS (ESI): *m/z* [(M+H)$^+$]: 612. HRMS *m/z* calcd. (found) for $C_{25}H_{15}N_5O_3S_2F_6$ [(M+H)$^+$]: 612.1185, found: 612.1188. Anal. calcd. (found) %: C 49.10 (49.11); H 2.47 (2.49); N 11.45 (11.47).

(*E*)-N′-(3-Methylbenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8h): m.p.: 232-234 ºC; IR (KBr, ν_{max}, cm^{-1}): 3223 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 2.35 (s, 3H, -CH_3), 4.18 (s, 2H, -CH_2-), 7.21 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.29 (dd, *J* = 3.81 Hz, 1H, Ar-H), 7.38-7.43 (m, 3H, Ar-H), 7.52 (s, 1H, Ar-H), 7.69 (dd, *J* = 4.21 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 8.41 (s, 1H, CH=N), 11.21 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 23.5, 49.8, 118.7, 120.4, 121.7, 122.4, 123.5, 124.9, 126.1, 127.6, 128.1, 128.7, 129.8, 130.1, 132.2, 133.4, 135.7, 136.3, 138.4, 140.6, 142.5, 144.3, 146.3, 158.5, 161.4; MS (ESI): *m/z* [(M+H)$^+$]: 596. HRMS *m/z* calcd. (found) for $C_{25}H_{15}N_5O_2S_2F_6$ [(M+H)$^+$]: 596.0258, found: 596.0261. Anal. calcd. (found) %: C 50.42 (50.43); H 2.54 (2.55); N 11.76 (11.77).

(*E*)-N′-(4-Chlorobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidin-3(4H)-yl)acetohydrazide (8i): m.p.: 203-205 ºC; IR (KBr, ν_{max}, cm^{-1}): 3215 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.18 (s, 2H, -CH_2-), 7.18 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.28 (dd, *J* = 3.79 Hz, 1H, Ar-H), 7.35 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.67 (dd, *J* = 4.20 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 8.39 (s, 1H, CH=N), 11.19 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 48.5, 121.8, 122.3, 123.4, 124.7, 125.1, 125.8, 126.7, 127.4, 128.8, 129.2, 130.4, 131.7, 132.2, 133.7, 135.9, 137.2, 139.4, 142.4, 145.6, 156.4, 162.3; MS (ESI): *m/z* [(M+H)$^+$]: 616. HRMS *m/z* calcd. (found) for $C_{24}H_{12}N_5O_2S_2ClF_6$ [(M+H)$^+$]: 616.0542, found: 616.0545. Anal. calcd. (found) %: C 46.80 (46.79); H 1.96 (1.97); N 11.37 (11.35).

(*E*)-N′-(3-fluorobenzylidene)-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidin-3(4*H*)-yl)acetohydrazide (8j): m.p.: 220-222 ºC; IR (KBr, ν_{max}, cm^{-1}): 3216 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.20 (s, 2H, -CH_2-), 7.21 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.32 (dd, *J* = 3.82 Hz, 1H, Ar-H), 7.36-7.40 (m, 3H, Ar-H), 7.47 (s, 1H, Ar-H), 7.66 (dd, *J* = 4.18 Hz, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 8.37 (s, 1H, CH=N), 11.19 (br. s., 1H, -CONH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 49.4, 120.7, 121.3, 121.8, 122.9, 123.4, 124.6, 125.7, 126.4, 127.3, 128.0, 128.6, 129.4, 130.2, 132.3, 133.4, 135.5, 136.8, 138.7, 141.7, 143.4, 145.5, 147.4, 157.6, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 600. HRMS *m/z* calcd. (found) for $C_{24}H_{12}N_5O_2S_2F_7$ [(M+H)$^+$]: 600.0557, found: 600.0559. Anal. calcd. (found) %: C 48.08 (48.09); H 2.02 (2.03); N 11.68 (11.65).

N-Methyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]fuoro[3,2-d]pyrimidin-3(4*H*)-yl)-acetamide (9a): m.p.: 186-188 ºC; IR (KBr, ν_{max}, cm^{-1}): 3208 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.03 (s, 3H, -CH_3), 4.20 (s, 2H, -CH_2-), 6.38 (br. d, 1H, -CONH-), 7.18 (dd, *J* = 4.82, 1H, Ar-H), 7.49 (dd, *J* = 4.82, 1H, Ar-H), 7.78 (dd, *J* = 3.78, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 26.5, 48.3, 121.5, 122.4, 124.7, 125.8, 127.0, 128.2, 130.4, 132.3, 134.7, 137.3, 139.9, 142.1, 143.6, 146.7, 158.6, 161.5; MS (ESI): *m/z* [(M+H)$^+$]: 477. HRMS *m/z* calcd. (found) for $C_{18}H_{10}N_4O_3SF_6$ [(M+H)$^+$]: 477.0125, found: 477.0127. Anal. calcd. (found) %: C 45.39 (45.40); H 2.12 (2.13); N 11.76 (11.78).

N-Ethyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]fuoro[3,2-*d*]pyrimidin-3(4*H*)-yl)acetamide (9b): m.p.: 195-197 °C; IR (KBr, ν_{max}, cm^{-1}): 3212 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.26 (t, 3H, -CH_3), 3.53 (quintet, 2H, -CH_2-), 4.21 (s, 2H, -CH_2-), 6.35 (br. s, 1H, -NH), 7.19 (dd, *J* = 4.87, 1H, Ar-H), 7.52 (dd, *J* = 4.87, 1H, Ar-H), 7.65 (dd, *J* = 3.79, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 14.1, 34.8, 48.5, 119.4, 120.4, 121.5, 123.1, 124.3, 125.4, 126.4, 127.9, 128.8, 130.3, 132.8, 137.9, 140.3, 143.7, 157.8, 160.5; MS (ESI): *m/z* [(M+H)$^+$]: 491. HRMS *m/z* calcd. (found) for $C_{19}H_{12}N_4O_3SF_6$ [(M+H)$^+$]: 491.0247, found: 491.0249. Anal. calcd. (found) %: C 46.54 (46.53); H 2.47 (2.46); N 11.43 (11.45).

2-(4-Oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)-pyrido[3′,2′:4,5]fuoro[3,2-*d*]pyrimidin-3(4*H*)-yl)-N-propyl-acetamide (9c): m.p.: 205-207 ºC; IR (KBr, ν_{max}, cm^{-1}): 3212 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.24 (t, 3H, -CH_3), 3.51 (quintet, 2H, -CH_2-), 3.69-3.76 (m, 2H, -CH_2-), 4.22 (s, 2H, -CH_2-), 6.36 (br. s, 1H, -NH), 7.19 (dd, *J* = 4.78,1H, Ar-H), 7.48 (dd, *J* = 4.78, 1H, Ar-H), 7.57 (dd, *J* = 3.79, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): 11.8, 24.0, 43.0, 48.2, 119.9, 121.7, 124.5, 125.2, 126.3, 129.0, 130.7, 131.5, 133.4, 134.0, 136.0, 140.0, 142.6, 145.6, 158.7, 162.5; MS (ESI): *m/z* [(M+H)$^+$]: 505. HRMS *m/z* calcd. (found) for

$C_{20}H_{14}N_4O_3SF_6$ [(M+H)$^+$]: 505.1058, found: 505.1061. Anal. calcd. (found) %: C 47.62 (47.64); H 2.80 (2.78); N 11.11 (11.13).

N-Cyclopentyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]fuoro[3,2-*d*]pyrimidin-3(4*H*)-yl)acetamide (9d): m.p.: 185-187 °C; IR (KBr, ν_{max}, cm^{-1}): 3219 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.48-1.56 (m, 2H, -CH_2-), 1.68-1.82 (m, 4H, -CH_2-CH_2-), 2.06-2.12 (m, 2H, -CH_2-), 4.21 (s, 2H, -CH_2-), (4.39-4.47 (m, 1H, -CH-), 6.36 (br. d, 1H, -NHCO-), 7.18 (dd, *J* = 4.82, 1H, Ar-H), 7.42 (dd, *J* = 4.82, 1H, Ar-H), 7.71 (dd, *J* = 3.72, 1H, Ar-H), 7.82 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): 24.0, 32.2, 46.4, 48.9, 118.6, 120.9, 122.4, 123.0, 124.5, 125.8, 126.7, 127.3, 131.5, 133.6, 135.9, 140.2, 143.4, 144.5, 158.7, 160.6; MS (ESI): *m/z* [(M+H)$^+$]: 531. HRMS *m/z* calcd. (found): for $C_{22}H_{16}N_4O_3SF_6$ [(M+H)$^+$]: 531.0157, found: 531.0159. Anal. calcd. (found) %: C 49.81 (49.82); H 3.04 (3.06); N 10.56 (10.58).

N-Methyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)acetamide (9e): m.p.: 176-178 ºC; IR (KBr, ν_{max}, cm^{-1}): 3223 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.05 (s, 3H, -CH_3), 4.18 (s, 2H, -CH_2-), 6.36 (br. d, 1H, -CONH-), 7.20 (dd, *J* = 4.81, 1H, Ar-H), 7.49 (dd, *J* = 4.81, 1H, Ar-H), 7.58 (dd, *J* = 3.78, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 25.4, 48.5, 119.3, 121.4, 122.5, 123.8, 125.5, 127.1, 128.1, 129.0, 130.6, 133.6, 134.9, 137.3, 139.9, 142.2, 158.4, 160.9; MS (ESI): *m/z* [(M+H)$^+$]: 493 HRMS *m/z* calcd. (found) for $C_{18}H_{10}N_4O_2S_2F_6$ [(M+H)$^+$]: 493.0342, found: 493.0345. Anal. calcd. (found) %: C 43.90 (43.93); H 2.05 (2.06); N 11.38 (11.40).

N-Ethyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)acetamide (9f): m.p.: 185-187 °C; IR (KBr, ν_{max}, cm^{-1}): 3218 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.28 (t, 3H, -CH_3), 3.52 (quintet, 2H, -CH_2-), 4.22 (s, 2H, -CH_2-), 6.34 (br. s, 1H, -NH), 7.26 (dd, *J* = 4.85,1H, Ar-H), 7.53 (dd, *J* = 4.85, 1H, Ar-H), 7.66 (dd, *J* = 3.78, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): 16.13, 36.3, 48.6, 120.1, 122.2, 124.7, 126.2, 126.7, 128.2, 129.0, 131.6, 133.3, 134.4, 137.0, 139.8, 141.9, 143.7, 157.9, 161.5; MS (ESI): *m/z* [(M+H)$^+$]: 507. HRMS *m/z* calcd. (found) for $C_{19}H_{12}N_4O_2S_2F_6$ [(M+H)$^+$]: 507.0768, found: 507.0771. Anal. calcd. (found) %: C 43.90 (43.92); H 2.05 (2.06); N 11.38 (11.40).

2-(4-Oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)-pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)-N-propylacetamide (9g): m.p.: 193-195 ºC; IR (KBr, ν_{max}, cm^{-1}): 3212 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.25 (t, 3H, -CH_3), 3.52 (quintet, 2H, -CH_2-), 3.66-3.74 (m, 2H, -CH_2-), 4.21 (s, 2H, -CH_2-), 6.35 (br. s, 1H, -NH), 7.25 (dd, *J* = 4.78,1H, Ar-H), 7.38 (dd, *J* = 4.78, 1H, Ar-H), 7.52 (dd, *J* = 3.77, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): 10.6, 23.1, 41.9, 48.5, 120.1, 122.4, 123.6, 125.5, 126.7, 128.1, 129.0, 130.7, 133.3, 134.3, 135.1, 139.7, 141.9, 143.6, 159.8, 162.5; MS (ESI): *m/z* [(M+H)$^+$]: 521. HRMS *m/z* calcd. (found) for $C_{20}H_{14}N_4O_2S_2F_6$ [(M+H)$^+$]: 521.0092, found: 521.0095. Anal. calcd. (found) %: C 46.15 (46.16); H 2.71 (2.73); N 11.76 (11.78).

N-Cyclopentyl-2-(4-oxo-7-(thiophen-2-yl)-2,9-*bis*(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)acetamide (9h): m.p.: 208-210 ºC; IR (KBr, ν_{max}, cm^{-1}): 3215 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 1.46-1.53 (m, 2H, -CH_2-), 1.66-1.81 (m, 4H, -CH_2-CH_2-), 2.04-2.10 (m, 2H, -CH_2-), 4.22 (s, 2H, -CH_2-), 4.41-4.45 (m, 1H, -CH-), 6.34 (br. d, 1H, -NHCO-), 7.26 (dd, *J* = 4.82, 1H, Ar-H), 7.43 (dd, *J* = 4.82, 1H, Ar-H), 7.74 (dd, *J* = 3.71, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): 20.6, 31.1, 46.0, 48.8, 120.4, 121.4, 122.8, 123.0, 124.5, 125.9, 126.7, 127.3, 130.7, 133.3, 134.0, 140.0, 142.7, 144.5, 158.4, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 547. HRMS *m/z* calcd. (found) for $C_{22}H_{16}N_4O_2S_2F_6$ [(M+H)$^+$]: 547.0105, found: 547.0107. Anal. calcd. (found) %: C 48.35 (48.36); H 2.95 (2.97); N 10.25 (10.27).

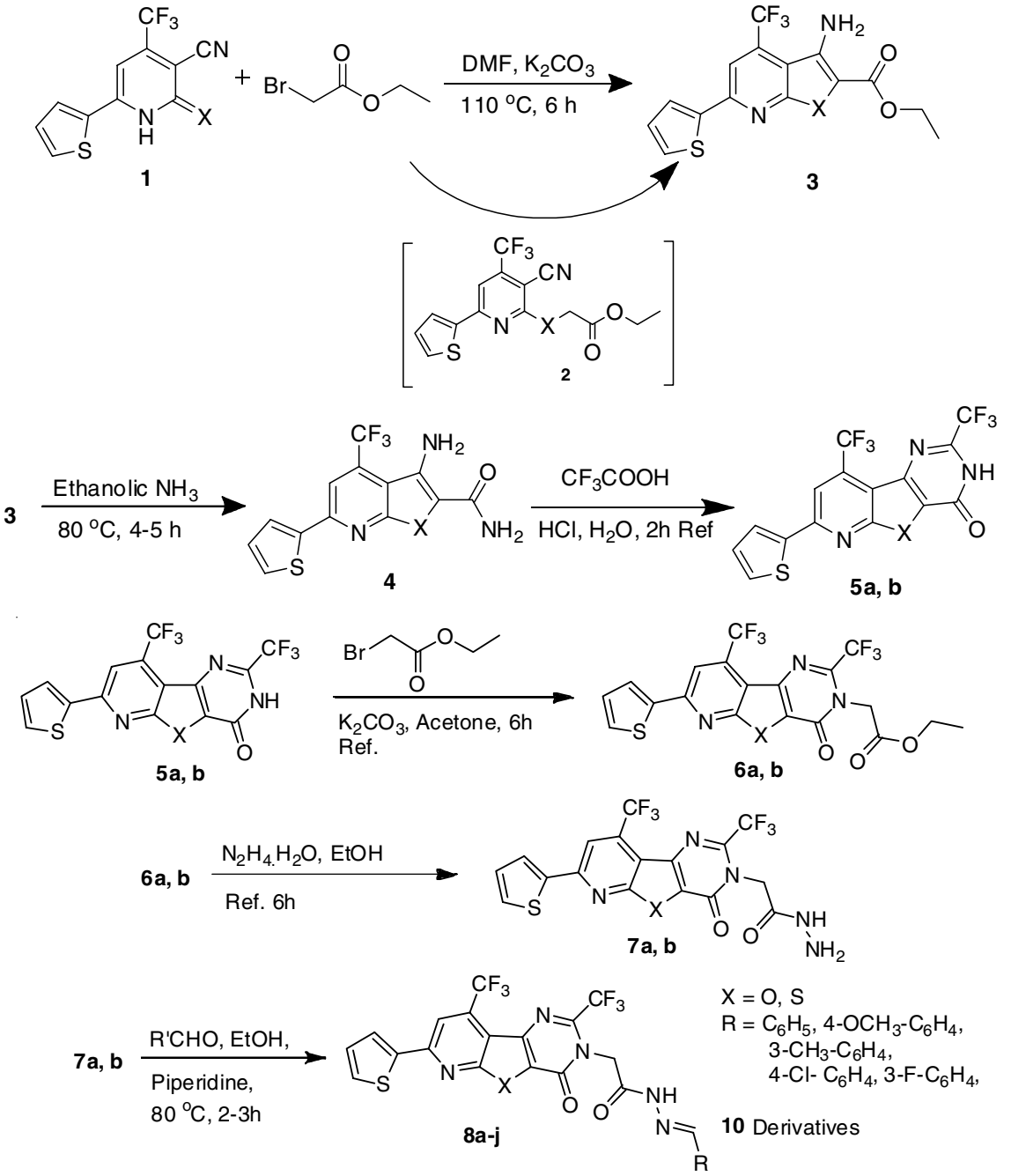
Cytotoxicity assay: The cytotoxicity of the compounds was determined on the basis of measurement of *in vitro* growth inhibition of tumor cell lines in 96 well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using 5-fluorouracil as a standard. The cytotoxicity was assessed by using the MTT assay [24] against a panel of five different human tumor cell lines: HeLa derived from human cervical cancer cells A549- Lung cancer (CCL-185); DU145 -prostate cancer (HTB-81); SiHa-Squamous cell carcinoma (HTB-35); MCF-7-breast cancer (HTB-22); and HEK-293 derived from human embryonic kidney cells (CRL-1573). The IC_{50} (50 % inhibitory concentration) values were calculated from the plotted absorbance data for the dose-response curves. IC_{50} values (in μM) are indicated as means ± SD of three independent experiments.

RESULTS AND DISCUSSION

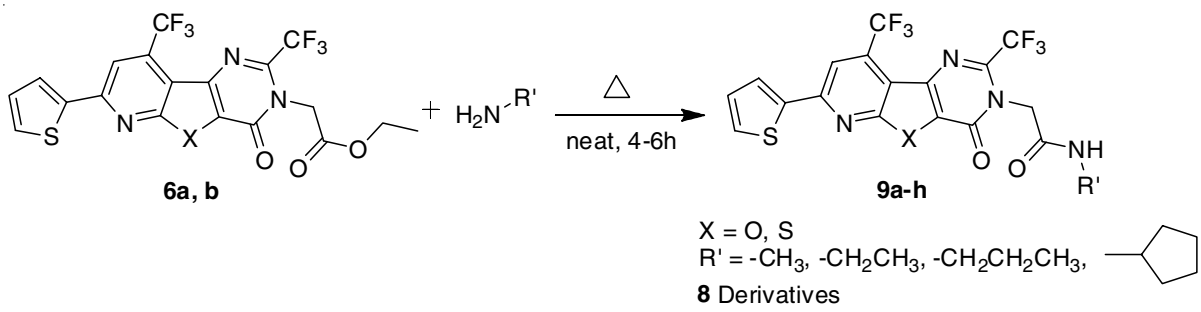
In **Scheme-I**, compound **5** on reaction with bromoethyl-acetate in basic conditions to afford compound **6** and followed by reaction with hydrazine hydrate to give compound **7**. Compound **7** on reaction with diverse substituted aryl aldehyde in the presence of piperidine to afford Schiff's base compounds **8a-l**. In **Scheme-II**, compound **6** on reaction with aliphatic primary amines at their refluxing conditions to obtain amide derivatives **9a-h** and products are tabulated in Table-1.

TABLE-1
SYNTHESIS OF NOVEL HETERO RING FUSED PYRIDINE AMIDE DERIVATIVES **8a-j** AND **9a-h**

Entry	Comd.	X	R	R'	Yield (%)
1	**8a**	O	-C_6H_5	–	79
2	**8b**	O	4-*OCH*$_3$-C_6H_4	–	82
3	**8c**	O	3-*CH*$_3$-C_6H_4	–	75
4	**8d**	O	4-*Cl*-C_6H_4	–	83
5	**8e**	O	3-*F*-C_6H_5	–	85
6	**8f**	S	-C_6H_5	–	71
7	**8g**	S	4-*OCH*$_3$-C_6H_4	–	79
8	**8h**	S	3-*CH*$_3$-C_6H_4	–	81
9	**8i**	S	4-*Cl*-C_6H_4	–	80
10	**8j**	S	3-*F*-C_6H_5	–	86
11	**9a**	O	–	-CH_3	85
12	**9b**	O	–	-CH_2CH_3	91
13	**9c**	O	–	-$CH_2CH_2CH_3$	79
14	**9d**	O	–	-Cyclopentyl	84
15	**9e**	S	–	-CH_3	89
16	**9f**	S	–	-CH_2CH_3	90
17	**9g**	S	–	-$CH_2CH_2CH_3$	85
18	**9h**	S	–	-Cyclopentyl	82



Scheme-I



Scheme-II

All the synthesized compounds **8a-j** and **9a-h** were screened for anticancer activity against four cancer cell lines such as A549 lung cancer (CCL-185); DU145 prostate cancer (HTB-81); SiHa-Squamous cell carcinoma (HTB-35); MCF-7 breast cancer (HTB-22); HEK-29 human embryonic kidney cells (CRL-1573). Compounds **9e** and **9f** are found to have promising anti-

TABLE-2
in vitro CYTOTOXICITY OF COMPOUNDS **8a-j** AND **9a-h**

Compound	IC_{50} values (μM)				
	DU145	A549	SiHa	MCF7	HEK293
8a	–[a]	52.3 ± 0.32	–	–	48.0 ± 0.18
8b	24.5 ± 0.15	39.4 ± 0.12	22.4 ± 0.46	19.5 ± 0.26	88.0 ± 0.45
8c	–	74.3 ± 0.27	–	39.3 ± 0.22	78.0 ± 0.36
8d	36.2 ± 0.33	48.5 ± 0.25	27.5 ± 0.34	39.8 ± 0.18	53.0 ± 0.46
8e	21.3 ± 0.21	–	28.2 ± 0.38	–	69.0 ± 0.24
8f	34.6 ± 0.18	58.2 ± 0.21	–	–	73.0 ± 0.22
8g	18.3 ± 0.25	15.7 ± 0.18	24.2 ± 0.20	22.6 ± 0.18	82.0 ± 0.12
8h	24.5 ± 0.14	18.6 ± 0.15	12.4 ± 0.48	26.5 ± 0.32	75.0 ± 0.39
8i	25.6 ± 0.21	–	39.4± 0.18	41.5± 0.29	52.0 ± 0.45
8j	33.4 ± 0.18	25.2 ± 0.35	–	–	96.0 ± 0.47
9a	29.4 ± 0.36	34.4 ± 0.29	–	–	48.0 ± 0.28
9b	35.3 ± 0.19	28.5 ± 0.15	18.2 ± 0.41	11.5 ± 0.34	89.0 ± 0.62
9c	42.8 ± 0.26	38.2 ± 0.46	51.9 ± 0.33	–	62.0 ± 0.18
9d	31.2 ± 0.28	41.4 ± 0.52	28.5 ± 0.39	31.4 ± 0.15	86.0 ± 0.36
9e	15.3 ± 0.31	21.6 ± 0.27	12.2 ± 0.12	19.3 ± 0.42	81.0 ± 0.28
9f	14.2 ± 0.28	19.1 ± 0.31	11.3 ± 0.18	12.8 ± 0.31	99.0 ± 0.57
9g	–	21.6 ± 0.18	19.6 ± 0.35	16.2 ± 0.24	102.0 ± 0.38
9h	37.6 ± 0.26	–	21.4 ± 0.25	18.4 ± 0.26	79.0 ± 0.45
Doxorubicine (Std control)	0.8 ± 0.11	0.8 ± 0.09	0.7 ± 0.18	0.9 ± 0.21	29.4 ± 0.19

–[a]Indicates IC_{50} value > 102 μg/mL; Cell lines used: A549 = Lung cancer (CCL-185); DU145 = Prostate cancer (HTB-81); SiHa = Squamous cell carcinoma (HTB-35); MCF-7 = Breast cancer (HTB-22); HEK-293 = Human Embryonic Kidney cells (CRL-1573).

cancer activity at micro molar concentration. By using MTT assay [24]. IC_{50} values of the test compounds for 24 h on each cell line are presented in Table-2.

Anticancer activity: All the compounds except **8a-j** and **9a-h** showed activity against four cancer cell lines at micro molar concentration such as A549- lung cancer (CCL-185); DU145 - prostate cancer (HTB-81); SiHa-squamous cell carcinoma (HTB-35); MCF-7 breast cancer (HTB-22); and one normal cell line HEK-29 human embryonic kidney cells (CRL-1573). Among all the compounds **9e** and **9f** showed promising activity, while remaining compounds showed moderate activity. All the compounds screened upto the concentration of < 102 μM. Among all the derivatives, compound **9f** exhibited significant activity on all cell lines such as < 19.1 μM. So that compound **9f** was considered as the more potent towards all the cancer cell lines. The structure-activity relationship studies revealed that the aliphatic amine (-ethyl group) and thiophenyl group at 6th position shows more activity and also (-CF_3) trifluoromethyl groups at a strategic position. The presence of CF_3 group, it increases the properties of lipid solubility and thereby enhances the transport mechanism and bioefficacy. Thieno-fused pyridine is additional advantage in promoting cytotoxicity compared to furo fused pyridine ring. All the compounds found to be non-toxic on normal cell line.

Conclusion

In conclusion, we synthesized novel hetero ring fused pyridine amide and all the synthesized compounds **8a-j** and **9a-h** were screened for anticancer activity against four cancer cell lines such as A549 lung cancer (CCL-185); DU145 prostate cancer (HTB-81); SiHa-squamous cell carcinoma (HTB-35); MCF-7 breast cancer (HTB-22); HEK-29 human embryonic kidney cells (CRL-1573); Compounds **9e** and **9f** are found to have promising anticancer activity at micro molar concentration and all compounds found to be non-toxic on normal cell line.

CONFLICT OF INTEREST

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

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