Novel amide functionalized pyridine derivatives and their anticancer activity and molecular docking studies

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Abstract

A series of novel amide functionalized trifluoromethyl substituted pyridine derivatives were prepared starting from pyridine 1 on reaction with bromoethylacetate followed by reaction with different primary aliphatic amines, cyclic secondary amines or L-amino acids under different set of conditions.

All the synthesized compounds 4a-j and 5a-d were screened for anticancer activity against four 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). 4j and 5d showed good activity.

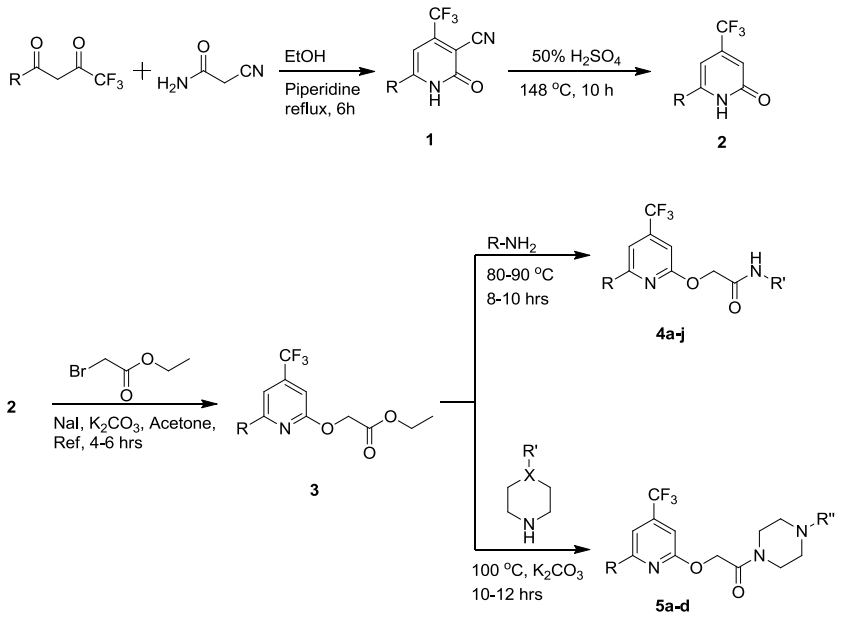
Keywords: Pyridine, Alkyl amide, Anticancer activity, Molecular docking studies.

Introduction

Modern research is driving more towards synthesis of potential organic molecules which are safe and effective molecules used as drugs, pharmaceuticals, agrochemicals. Among all organic molecules, heterocyclic molecules are more effective.[13,16,22,28] Among heterocyclic compounds, pyridine based heterocyclic compounds are paid great attention.[2,7,9,15,20] Pyridine was isolated first by heating of bones and synthesized by William Ramsay in 1876. Pyridine exists in many biologically active compounds like drugs, vitamins such as vitamin B_3 (niacin), vitamin B_6 (pyridoxin), natural alkaloids and nucleic acids.[3] 2-pyridone is main core moiety of 7,000 more drugs.[1,8]

Pyridine based compounds belong to an important class of heterocyclic compounds due to their broad range of activities like anticancer,[6,11,14] antimicrobial,[18,19,24,29] anti-viral,[10,27] anti-HIV virus,[26] antioxidant[4] and anti-inflammatory agents.[12,25] Combination of a particular functional group like amide group with pyridine moiety may lead to enhance the activity. Based on the importance of pyridine, it was decided to synthesize amide functionalized pyridine derivatives. Trifluoromethyl group at particular position on molecule alters the properties like lipophilicity and there by enhancement of biological activity of that molecule.

In our research program, A series of novel amide functionalized trifluoromethyl substituted pyridine derivatives 4a-j and 5a-d was prepared starting from pyridine 1. All the synthesized compounds 4a-j and 5a-d were screened for anticancer activity against four cancer cell lines such as HeLa-Cervical cancer (CCL-2), COLO 205-Colon cancer (CCL-222), HepG2- Liver cancer (HB-8065) and MCF7-Breast cancer (HTB-22). Compounds 4j and 5d showed good activity.



Scheme 1: Preparation of compounds 4a-j and 5a-d

Material and Methods

The pyrazole substituted pyridine scaffolds 4a-j and 5a-d were synthesized starting with 1,1,1-trifluoro-4-substituted-butane-2,4-dione. Compound 1,1,1-trifluoro-4-substituted-butane-2,4-dione on reaction with 2-cyanoacetamide gave compound 1. Compound 2 is formed by hydrolyzing and decarboxylating compound 1. Compound 2 was treated with bromoethyl acetate to yield an ester derivative 3. Compound 3 was heated in a reaction with various amines to produce amide derivatives, compounds 4a-j. Compound 3 was also reacted with piperidine to produce compounds 5a-d. Synthetic sequence is shown in scheme 1.

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 300MHz in $CDCl_3$ and 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.

Preparation of 2-oxo-6-(thiophen-2-yl)-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (1): 4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (0.02 mol) was slowly stirred into a cyanoacetamide (0.02 mol) in 95% EtOH (10 ml) solution. After producing a homogeneous mixture, piperidine (0.20 mL) was added drop by drop. The reacting mixture was allowed to cool at 35 ºC after being refluxed for 3hrs.The resulting solid was collected, cleaned in cold C_2H_5OH and dried at 85 ° C.

2-oxo-6-(thiophen-2-yl)-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (1): Yield (%):89, m. p. (ºC): 211-213, IR (KBr, cm^{-1}): 3423 (-CONH-), 1662 (-CONH-), 2223 (CN), ^{1}H NMR ($CDCl_3$, 300 MHz):δ 7.43 (dd, 1H, Ar-H), 7.53 (dd, 1H, Ar-H), 7.82 (dd, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 10.23 (br. s., 1H, -NH-), MS (ESI):m/z [(M+H)$^+$]: 271, [(M+Na)$^+$]: 293, CHN Analysis: calc. for $C_{13}H_{10}F_3N_4O$: C 48.89, H 1.86, N 10.37 %. Found: C 48.88, H 1.87, N 10.39%.

Compound 1 was charged in a sealed tube with % H_2SO_4 and then refluxed at 148 °C for 10 hours before being placed on ice and forming a white solid. Compound 2 was obtained by filtering, washing with water and drying this white solid.

Preparation of 6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin-2(1H)-one (2): Yield (%): 92 m. p. (ºC): 189-191 IR (KBr, cm^{-1}): 3425 (-CONH-), 1652 (-CONH-) ^{1}H NMR ($CDCl_3$, 300 MHz) :δ 7.41 (dd, 1H, Ar-H), 7.61 (dd, 1H, Ar-H), 7.89 (dd, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 8.41(s, 1H, Ar-H), 10.41 (br. s., 1H, -NH-); MS (ESI): m/z [(M+H)$^+$]: 246 CHN Analysis: Calc. for $C_{13}H_{10}F_3N_4O$: C 48.98, H 2.47, N5.71 %. Found: C 48.99, H 2.48, N 5.73%.

Preparation of ethyl-2-((6-(thiophen-2-yl)-4-(trifloromethyl)pyridine-2-yl)oxy)acetate (3): The compound 2 was placed in a dry round-bottom flask, to which bromoethyl acetate (1:1) was gradually added followed by acetone as a solvent. Calculated amount of NaI and K_2CO_3 was added. Reaction mixture was heated for 4-5 hours and allowed to cool, CH_3COCH_3 was taken out under vacuum, H_2O was gently and a solid was isolated by filtration.

Yield (%) 85, m. p. (ºC) 142-144, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.21 (d, 3H, -CH_3), 4.12 (q, 2H, -OCH_2), 4.95 (s, 2H, -OCH_2), 7.32 (dd, 1H, Ar-H), 7.45 (dd, 1H, Ar-H), 7.81 (dd, 1H, Ar-H), 8.11(s, 1H, Ar-H), 8.41(s, 1H, Ar-H) MS (ESI):m/z [(M+H)$^+$]: 332, CHN Analysis : calc. for $C_{14}H_{12}F_3NO_3S$: C 50.75, H 3.65, N4.23 %. Found: C 50.76, H 3.67, N 4.24 %.

N-Methyl-2-((6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin -2-yl)oxy)acetamide (4a): Yield (%): 91 m. p. (ºC): 162-164 I.R.(KBr, cm^{-1}):1664 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.76 (s, 3H, -N-CH_3), 4.95 (s, 2H, -OCH_2), 7.28 (dd, 1H, Ar-H), 7.32 (br. s., 1H, -NH-), 7.40 (dd, 1H, Ar-H), 7.79 (dd, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d6, 75 MHz):δ ppm 25.2, 68.2, 119.3,123.2, 125.8, 128.1, 129.0, 130.6, 133.6, 134.9, 137.3, 142.2, 160.9, MS (ESI):m/z [(M+H)$^+$]: 317 CHN Analysis: calc. for $C_{13}H_{11}F_3N_2O_2S$: C 49.36, H 3.51, N8.86 %. Found: C 49.37, H 3.52, N 8.85 %.

N-Ehyl-2-((6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin-2-yl)oxy)acetamide (4b): Yield (%):89, m. p. (ºC): 171-173, I.R. (KBr, cm^{-1}): 1662 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz):δ 1.02 (t, 3H, -CH_3), 3.12 (q, 2H, -OCH_2),4.95 (s, 2H, -OCH_2), 7.28 (dd, 1H, Ar-H), 7.32 (br. s., 1H, -NH-), 7.40 (dd, 1H, Ar-H), 7.79 (dd, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz) : δ ppm 25.2, 68.2, 119.3,123.2, 125.8, 128.1, 129.0, 130.6, 133.6, 134.9, 137.3, 142.2, 160.9, MS (ESI): m/z [(M+H)$^+$]: 317, CHN Analysis: calc. for $C_{13}H_{11}F_3N_2O_2S$: C 49.36, H 3.51, N8.86 %. Found: C 49.37, H 3.52, N 8.85 %.

N-Propyl-2-((6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin-2-yl)oxy)acetamide (4c): Yield (%):72, m. p. (ºC):182-184, I.R.(KBr, cm^{-1})1658 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz):δ 0.92 (t, 3H, -CH_3), 1.62 (m, 2H, -CH_2), 3.42 (t, 2H, -N-CH_3), 4.91 (s, 2H, -OCH_2), 7.27 (dd, 1H, Ar-H), 7.31 (br. s., 1H, -NH-), 7.41 (dd, 1H, Ar-H), 7.78 (dd, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz):δ ppm 10.2, 23.5, 42.7, 67.2, 120.2,122.3, 124.6, 128.7, 129.2, 130.1, 132.4, 134.4, 135.4, 142.1, 159.6, MS (ESI): m/z [(M+H)$^+$]: 345, CHN Analysis:

calc. for $C_{15}H_{15}F_3N_2O_2S$: C 52.32, H 4.39, N8.14 %. Found: C 52.33, H 4.41, N 8.15 %.

N-Cyclopentyl-2-((6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin-2-yl)oxy)acetamide (4d): Yield (%): 75, m. p. (ºC): 171-173, I.R.(KBr, cm^{-1}):1662 (-CONH-)^{1}H NMR ($CDCl_3$, 300 MHz) :δ 1.42-1.52 (m, 8H, -CH_2), 3.58 (m, 1H, -N-CH-), 4.93 (s, 2H, -OCH_2), 7.26 (dd, 1H, Ar-H), 7.32 (br. s., 1H, -NH-), 7.40 (dd, 1H, Ar-H), 7.68 (dd, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 8.30 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz) :δ ppm 23.1, 32.4, 56.7, 68.4, 121.2,122.6, 125.7, 128.6, 129.1, 130.7, 132.5, 133.8, 135.6, 142.3, 160.4; MS (ESI):m/z [(M+H)$^+$]: 371, CHN Analysis: calc. for $C_{17}H_{17}F_3N_2O_2S$: C 55.13, H 4.63, N7.56 %. Found: C 55.14, H 4.64, N 7.58 %.

N-Benzyl-2-((6-(thiophen-2-yl)-4-(trifluoromethyl) pyridin-2-yl)oxy)acetamide (4e): Yield (%): 62, m. p. (ºC): 205-207, I.R.(KBr, cm^{-1}): 1668 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.21 (s, 2H, -OCH_2), 4.93 (s, 2H, -OCH_2), 7.24-7.26 (m, 3H, -Ar-H), 7.29 (dd, 1H, Ar-H), 7.34 (br. s., 1H, -NH-), 7.36-7.38 (m, 2H, ArH), 7.41 (dd, 1H, Ar-H), 7.65 (dd, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz):δ ppm 43.8, 69.2, 118.3, 120.5, 121.3,122.4, 124.6, 126.7, 127.6, 129.1, 130.2, 132.2, 133.7, 136.4, 140.2, 142.3, 160.1, MS (ESI): m/z [(M+H)$^+$]: 393, CHN Analysis: calc. for $C_{19}H_{15}F_3N_2O_2S$: C 58.16, H 3.85, N7.14 %. Found: C 58.17, H 3.87, N 7.15 %.

N-Methyl-2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl) oxy)acetamide (4f): Yield (%): 72m. p. (ºC): 164-166, I.R.(KBr, cm^{-1}) : 1672 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz):δ 2.74 (s, 3H, -N-CH_3), 4.96 (s, 2H, -OCH_2), 7.26-7.29 (m, 3H, -Ar-H), , 7.32 (br. s., 1H, -NH-), 7.36-7.38 (m, 2H, ArH), 8.03 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 26.3, 67.3, 119.5, 120.6, 121.5,123.7, 125.3, 126.4, 127.9, 130.2, 133.7, 142.3, 160.1, MS (ESI): m/z [(M+H)$^+$]: 311, CHN Analysis : calc. for $C_{15}H_{13}F_3N_2O_2$: C 58.07, H 4.22, N9.03 %. Found: C 58.09, H 4.23, N 9.05 %.

N-Ethyl-2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl) oxy)acetamide (4g): Yield (%)**:** 76, **m. p.** (ºC)**:** 182-184, **I.R.**(KBr, cm^{-1})**:** 1665 (-CONH-), **^{1}H NMR** ($CDCl_3$, 300 MHz)**:**δ 1.04 (t, 3H, -CH_3), 3.13 (q, 2H, -OCH_2),4.96 (s, 2H, -OCH_2), 7.27-7.31 (m, 3H, -Ar-H), 7.35 (br. s., 1H, -NH-), 7.38-7.40 (m, 2H, ArH), 8.02 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), **^{13}C NMR** (DMSO-d_6, 75 MHz)**:** δ ppm 14.2, 32.1, 68.3, 119.4, 120.8, 122.6, 124.7, 125.2, 126.2, 127.5, 130.1, 134.5, 142.1, 160.4, **MS** (ESI)**:** m/z [(M+H)$^+$]: 325, **CHN Analysis:** calc.for $C_{16}H_{15}F_3N_2O_2$: C 59.26, H 4.66, N8.64 %. Found: C 59.27, H 4.67, N 8.65 %.

2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl)oxy)-N-propylacetamide (4h): Yield (%):65, m. p. (ºC):175-177, I.R. (KBr, cm^{-1}): 1661 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (t, 3H, -CH_3), 1.62 (m, 2H, -CH_2), 3.38 (t, 2H, -CH_2),4.95 (s, 2H, -OCH_2), 7.24-7.27 (m, 3H, -Ar-H), , 7.36 (br. s., 1H, -NH-), 7.38-7.40 (m, 2H, ArH), 7.92 (s, 1H, Ar-H), 8.26 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 12.2, 23.4, 42.6, 68.2, 118.6, 120.7, 122.4,124.8, 125.1, 126.7, 128.4, 130.2, 132.6, 141.4, 159.6, MS (ESI) : m/z [(M+H)$^+$]: 339, CHN Analysis: calc. for $C_{17}H_{17}F_3N_2O_2$: C 60.35, H 5.06, N8.28 %. Found: C 60.34, H 5.07, N 8.29 %.

N-Cyclopentyl-2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl)oxy)acetamide (4i): Yield (%): 65, m. p. (ºC) :175-177, I.R.(KBr, cm^{-1}): 1661 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.39-1.48 (m, 8H, -CH_2), 3.55 (m, 1H, -N-CH-), 4.91 (s, 2H, -OCH_2), 7.27-7.30 (m, 3H, -Ar-H), 7.34 (br. s., 1H, -NH-), 7.36-7.39 (m, 2H, ArH), 7.99 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 23.2, 36.5, 55.1, 68.1, 120.5, 121.4, 122.7,124.6, 125.6, 127.4, 129.5, 130.6, 132.3, 141.2, 160.1, MS (ESI): m/z [(M+H)$^+$]: 365 CHN Analysis :calc. for $C_{19}H_{19}F_3N_2O_2$: C 62.63, H 5.26, N 7.69 %. Found: C 62.61, H 5.27, N 7.70 %.

N-benzyl-2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl) oxy)acetamide (4j): Yield (%): 58, m. p. (ºC): 152-154, I.R.(KBr, cm^{-1}):1669 (-CONH-), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.23 (s, 2H, -OCH_2), 4.91 (s, 2H, -OCH_2), 7.25-7.32 (m, 6H, -Ar-H), 7.36 (br. s., 1H, -NH-), 7.39-7.41 (m, 2H, ArH), 7.44-7.46 (m, 2H, ArH), 7.91 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), ^{13}C NMR (DMSO-d_6, 75 MHz) : δ ppm 43.5, 68.6, 118.2, 119.6, 120.2, 121.6, 122.4,124.5, 125.1, 127.8, 129.4, 130.3, 132.2, 134.2, 141.2, 143.5, 160.3, MS (ESI) : m/z [(M+H)$^+$]: 387, CHN Analysis : calc. for $C_{21}H_{17}F_3N_2O_2$: C 65.28, H 4.43, N 7.25 %. Found: C 65.29, H 4.41, N 7.26 %.

1-(4-methylpiperazin-1-yl)-2-((6-(thiophen-2-yl)-4-(trifluoromethyl)pyridin-2-yl)oxy)ethanone(5a): Appearance:Pale yellow solid, Yield (%): 68, m. pt.:152–154, ^{1}H NMR ($CDCl_3$, 300 MHz) :δ ppm 2.31 (s, 3H, -CH_3), 2.83-2.87 (m, 4H, -$(CH_2)_2$-), 3.18-3.24 (m, 4H, -$(CH_2)_2$-), 7.28 (dd, 1H, Ar-H), 7.44 (dd, 1H, Ar-H), 7.68 (dd, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 13C NMR (DMSO-d6, 75 MHz):δ ppm 45.2, 49.6, 51.6, 64.8, 121.4, 123.4, 125.1, 126.2, 128.3, 129.2, 130.2, 132.4, 134.7, 142.1, 160.3, MS (ESI): m/z [(M+H)$^+$]: 386, CHN Analysis: calc. for $C_{17}H_{18}F_3N_3O_2S$: C 52.98, H 4.71, N 10.90 %. Found: C 52.99, H 4.72, N 10.91 %.

1-(4-phenylpiperazin-1-yl)-2-((6-(thiophen-2-yl)-4-(trifluoromethyl)pyridin-2-yl)oxy)ethanone (5b): Appearance: Pale white solid, Yield (%) : 56, m. pt.: 178–180, ^{1}H NMR ($CDCl_3$, 300 MHz) : δ ppm 2.85-2.89 (m, 4H, -$(CH_2)_2$-), 3.19-3.26 (m, 4H, -$(CH_2)_2$-), 7.26 (dd, 1H, Ar-H), 7.28-7.32 (m, 3H, -Ar-H) 7.41 (dd, 1H, Ar-H), 7.44-7.48 (m, 2H, Ar-H) 7.65 (dd, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 13C NMR (DMSO-d6, 75 MHz): δ ppm 49.6, 52.6, 65.1, 119.5, 121.1, 122.7, 123.4, 124.7, 125.1, 126.2, 128.3, 129.2, 130.2, 132.4, 134.7, 136.4, 142.1, 160.3 MS (ESI) : m/z [(M+H)$^+$]:448, CHN Analysis: calc. for $C_{22}H_{20}F_3N_3O_2S$: C 59.05, H 4.51, N 9.39 %. Found: C 59.06, H 4.52, N 9.41 %.

1-(4-methylpiperazin-1-yl)-2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl)oxy)ethanone (5c): Appearance : Pale white solid, Yield (%): 59, m. pt. :162–164, ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.32 (s, 3H, -CH_3), 2.82-2.87 (m, 4H, -$(CH_2)_2$-), 3.18-3.24 (m, 4H, -$(CH_2)_2$-), 7.25-7.29 (m, 3H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 8.01 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 13C NMR (DMSO-d6, 75 MHz): δ ppm 45.2, 49.5, 51.2, 64.7, 120.4, 122.5, 123.3, 125.4, 126.3, 128.9, 129.1, 130.4, 132.4, 134.6, 142.3, 160.1, MS (ESI) : m/z [$(M+H)^+$]:380, CHN Analysis : calc. for $C_{19}H_{20}F_3N_3O_2$: C 60.15, H 5.31, N 11.08 %. Found: C 60.16, H 5.32, N 11.06 %.

2-((6-phenyl-4-(trifluoromethyl)pyridin-2-yl)oxy)-1-(4-phenylpiperazin-1-yl)ethanone (5d): Appearance : Pale white solid, Yield (%): 55, m. pt.: 151–153, ^{1}H NMR ($CDCl_3$, 300 MHz) : δ ppm 2.80-2.85 (m, 4H, -$(CH_2)_2$-), 3.19-3.24 (m, 4H, -$(CH_2)_2$-), 7.27-7.33 (m, 6H, Ar-H), 7.36-7.39(m, 2H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 7.98 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 13C NMR (DMSO-d6, 75 MHz): δ ppm 49.5, 51.2, 64.7, 119.4, 120.3, 121.5, 122.6, 123.3, 125.4, 126.3, 128.8, 129.1, 130.2, 132.4, 134.6, 136.3, 142.3, 160.2, MS (ESI): m/z [$(M+H)^+$]:442, CHN Analysis : calc. for $C_{24}H_{22}F_3N_3O_2$: C 65.30, H 5.02, N 9.52 %. Found: C 65.31, H 5.03, N 9.51 %.

Results and Discussion

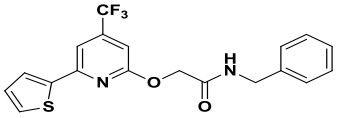
All synthesized compounds 4a–j and 5a–d were screened for *in vitro* against four human cancer cell lines such as HeLa-Cervical cancer (CCL-2), COLO 205-Colon cancer (CCL-222), HepG2- Liver cancer (HB-8065), MCF7-Breast cancer (HTB-22) using MTT assay.[21] IC_{50} values of the test compounds for 24 h on each cell line were calculated and presented in table 1.

All the compounds except 4a showed activity at micro molar concentration. Among all the compounds, 4j and 5d showed promising activity, while the remaining compounds showed moderate activity. The structure–activity relationship studies revealed that amide functional group containing benzyl and piperazine with hydroxyl group containing molecules shows more activity compared to other functional groups.

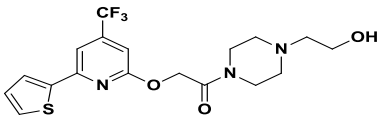
Table 1
***In vitro* cytotoxicity (anticancer activity results) of compounds 4a-j and 5a-d**

Compounds	IC_{50} values (in µM)			
	HeLa	COLO205	HepG2	MCF-7
4a	---	---	---	---
4b	55.7 ± 4.22	---	---	---
4c	37.5 ± 2.12	64.6 ± 5.21	---	75.5 ± 6.25
4d	---	76.2± 6.12	---	---
4e	41.3 ± 3.21	36.5 ± 2.34	51.2 ± 4.41	---
4f	64.2 ± 5.12	80.6 ± 7.29	78.6 ± 6.51	---
4g	32.2 ± 2.12	51.7 ± 3.28	64.1 ± 5.20	---
4h	38.5 ± 0.23	---	---	126.8 ±6.10
4i	---	48.9 ± 3.31	---	---
4j	21.5 ± 1.23	18.7 ± 0.36	41.3 ± 3.13	---
5a	73.2 ± 0.52	---	---	---
5b	65.3 ± 4.22	58.9 ± 3.31	41.8 ± 0.43	31.8 ± 3.52
5c	---	---	68.8 ± 5.23	63.7 ± 4.38
5d	20.2 ± 1.42	32.5 ± 2.36	---	27.3±0.32
5-Fluorouracil	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07

… Indicates IC_{50} value >126.8 µg/mL. Cell lines used: HeLa - Cervical cancer (CCL-2), COLO 205- Colon cancer (CCL-222), HepG2- Liver cancer (HB-8065), MCF7 - Breast cancer (HTB-22),



4j



5d

Tubulin belongs to the super family of globular proteins. Alpha and beta tubulins polymerize to form microtubules. Microtubules play an important role in cellular processes like DNA segregation, cell division, mitosis etc. Tubulin binding compounds are good targets for anticancer drugs as they inhibit microtubule dynamics. The docking studies of the compounds 4a-4j, 5a-5d and 5-fluorouracil were performed using tubulin-colchicine protein (PDB-ID: 1SA0).[21,23] PyRx software and Autodock Vina[17] were used to conduct molecular docking studies. The compounds 4a-4j, 5a-5d were drawn using Chem Draw Ultra 12.0. The universal forcefield was used to minimise the structure and the files were converted to pdbqt files.

For the purpose of determining binding affinity, the cognate ligand of the 1SA0 protein was used. The coordinates were center_x = 116.1, center_y = 89.5, center_z = 5.8, size_x= 17.9, size_y = 29.8, size_z = 19.2. The docking scores are given in table 2. The docking showed that all compounds have good hydrogen bond interactions, electrostatic and Vander Waal interactions along with π-sigma, π-alkyl interactions. The cocrystal, 5-flourouracil, has binding energy of the –6.7 kcal/mol and -4.9 kcal/mol.Among them, the compound 4j has the best IC_{50} value and bonding energy of -8.6 (Kcal/mol) with H-bonding between the NH of the acetamide group and the THR-A-179, the NH group of the pyridine moiety with ASN-B-258 and a halogen bond between fluorine and LEU-B-255.

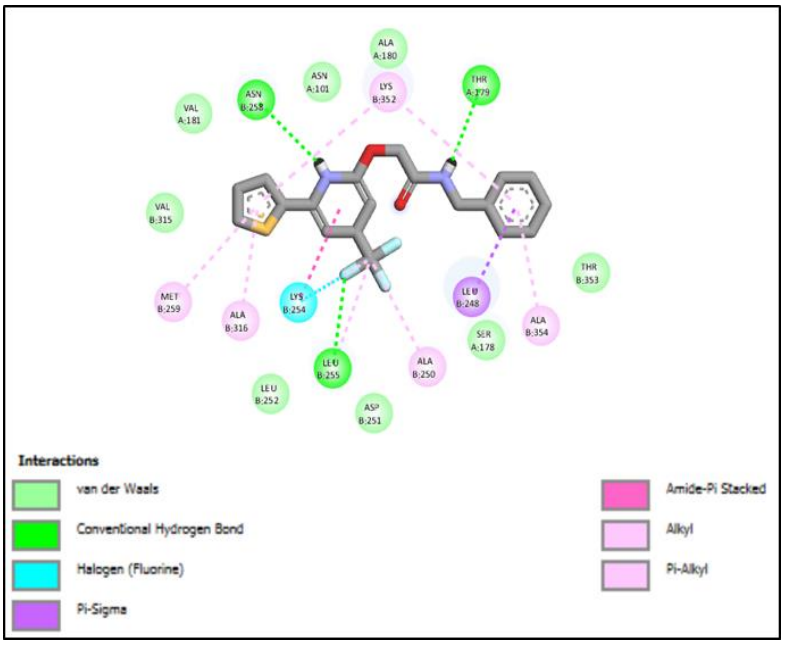


Figure 1: Interaction image of 4j with thetubulin-colchicine: stathmin-like domain complex (pdb id- 1sa0)

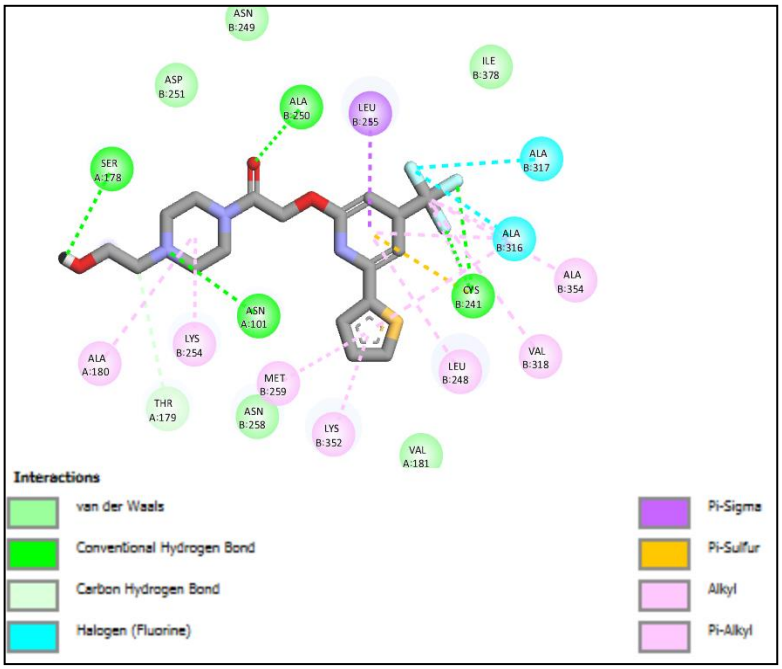


Figure 2: Interaction image of 5d with the tubulin-colchicine: stathmin-like domain complex (pdb id- 1sa0)

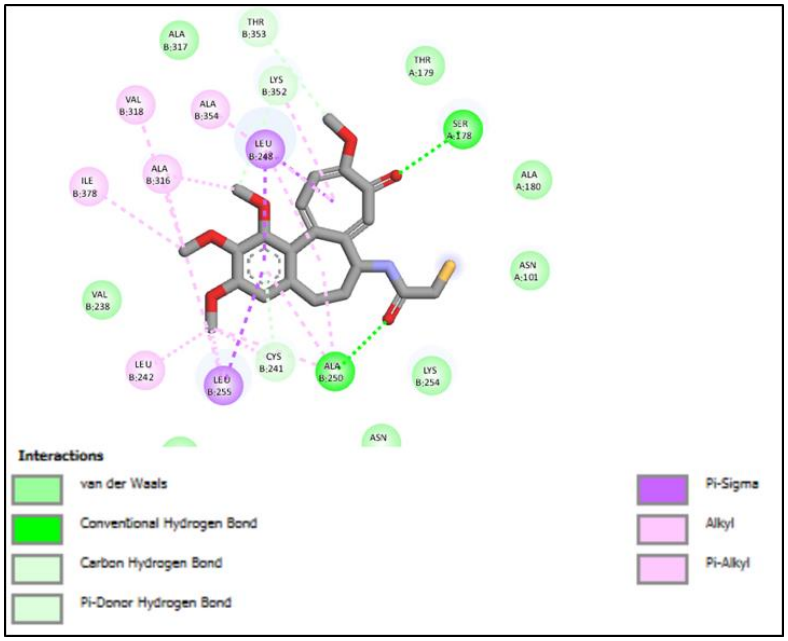


Figure 3: Interaction image of co-crystal with the tubulin-colchicine: stathmin-like domain complex (pdb id- 1sa0)

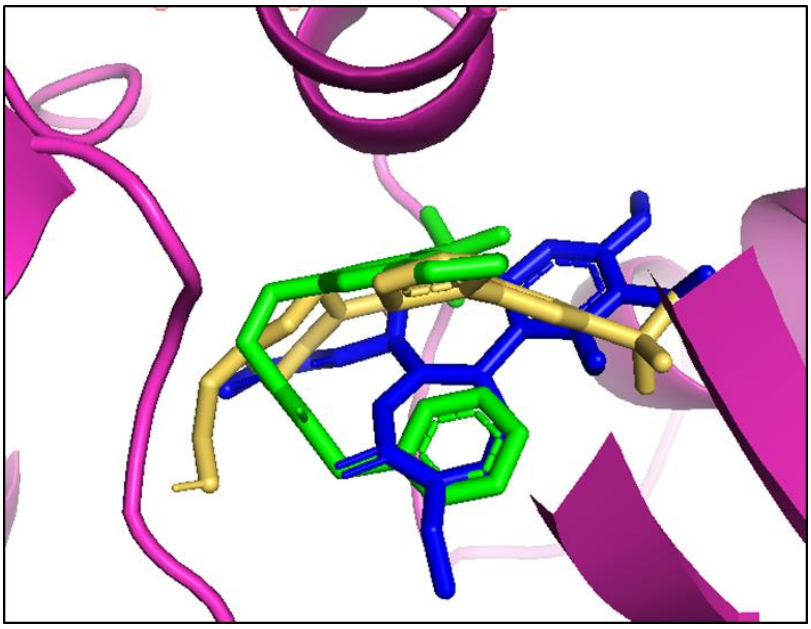


Figure 4: image of co-crystal(blue) with 4j (green), 5d (yellow) the tubulin-colchicine: stathmin-like domain complex (pdb id- 1sa0)

The benzene moiety of acetamide showed a Pi-sigma bond with LEU-B-248 whereas the second-best of the molecule 5d had a bonding energy of -8.2 (Kcal/mol), the trifluoromethyl pyridine moiety showed a Pi-sigma bond between LEU-B-255 and pyridine, a halogen bond between fluorine and ALA-B-316 and ALA-B-317 and the piperazine moiety showed a hydrogen bond between NH and ASN-A-101 and the OH of hydroxy ethyl with SER-A-178. The images figures 1 and 2 shows the interaction images. The figures 3 and 4 indicate co-crystal structure, 4j and 5d docked at the same active site.

Cytotoxicity assay: 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 using the MTT assay[5] against a panel of four different human tumor cell lines: HeLa derived from human cervical cancer cells (ATCC No. CCL-2), COLO 205 derived from human colon cancer cells (ATCC No. CCL-222), HepG2 derived from human liver cancer cells (ATCC No. HB-8065), MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22). 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.

Table 2
Molecular docking binding energy (kcal/mol)

Compound Name	Docking Score (Kcal/mol)
4a	-8.0
4b	-8.7
4c	-9.2
4d	-8.0
4e	-8.1
4f	-7.5
4g	-7.6
4h	-8.3
4i	-7.5
4j	-8.6
5a	-8.8
5b	-8.5
5c	-8.3
5d	-8.2
Co-crystal	-6.7
Standard(5-fluorouracil)	-4.9

Conclusion

In conclusion, a series of novel amide functionalized trifluoromethyl substituted pyridine derivatives 4a-j and 5a-d were prepared and evaluated for anticancer activity against four human cancer cell lines. Among all the compounds screened, the compounds 4j and 5d showed significant activity against all cell lines at micro molar concentration.

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