Novel trifluoromethyl-thieno[2,3-*b*]pyridine-2-carboxamide and Schiff's base derivatives and their anticancer activity

Vadla Balakishan, Puram Naveen and Betala Sailu*
Department of Chemistry, Telangana University, Dichpally, Nizamabad, 503322, TS, INDIA
*bethalasailu@gmail.com

Abstract

A series of novel trifluoromethyl-thieno[2,3-b] pyridine-2-carboxamide 3a-h and Schiff's base derivatives 5a-g was prepared starting from 6-methyl-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carb onitrile 1. Compound 1 on reaction with bromoethyl acetate produced ester derivative 2 on reaction with different amines produced amide derivatives 3. Ester derivative 2 which on reaction with hydrazine hydrate gave hydrazide 4 derivative. Further this compound on reaction with different substituted aromatic aldehydes formed Schiff's base derivatives 5.

All the products 3a-h and 5a-f were screened against four human 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). Promising compounds 3a and 3c have been identified with reference to standard control of 5-Fluorouracil.

Keywords: Furo[2,3-b]pyridine-2-carboxamide, Cyclization, Schiff's base derivatives, Anticancer activity.

Introduction

Cancer is worldwide killer. WHO also says that cancer is the second most common cause of death after heart disease.[3] Developing safer and efficient drug is very important. Heterocyclic compounds especially pyridine based compounds are found very important and play a key role in promoting biological activity. Bis-heterocyclic compounds (BHCs) are scaffolds comprising two linked, bound, spaced, or fused heterocycles. Fused heterocycles also exhibit various biological activities including antibacterial, fungicidal, tuberculostatic, antiamoebic, anthelmintic and plant growth regulative properties.[8-10,15,16,19,24-26,30]

Many of pyridine based compounds exist in herbicides, bactericides, fungicides, insecticides and pharmaceuticals[21,23]. Particularly thienopyridine derivatives are known to exhibit antibacterial[11], antifungal[22], antiviral[5], anti-inflammatory[19], antihypertensive[1], antiparasitic[6], antibacterial[2], vasodilator activities. Thieno[2,3-*b*]pyridine derivatives are useful as gonadotropin-releasing hormone antagonists.[2,7,12-14,18,28]

Suárez et al[27] reported 7-dihydrothieno[2,3-*b*]pyridines from the o-chloroformyl substituted 1,4-dihydropyridines. Balkrishen et al[4] reported one-step synthesis of 2-methoxycarbonylthieno[2,3-b]quinolines from 2-chloroquinoline-3-carbaldehydes using potassium carbonate in tetrahydrofuran with good yields. Tri fluoromethyl at particular position may alter the properties like lipophilicity and oxidative thermal stability, permeability and oral bioavailability.

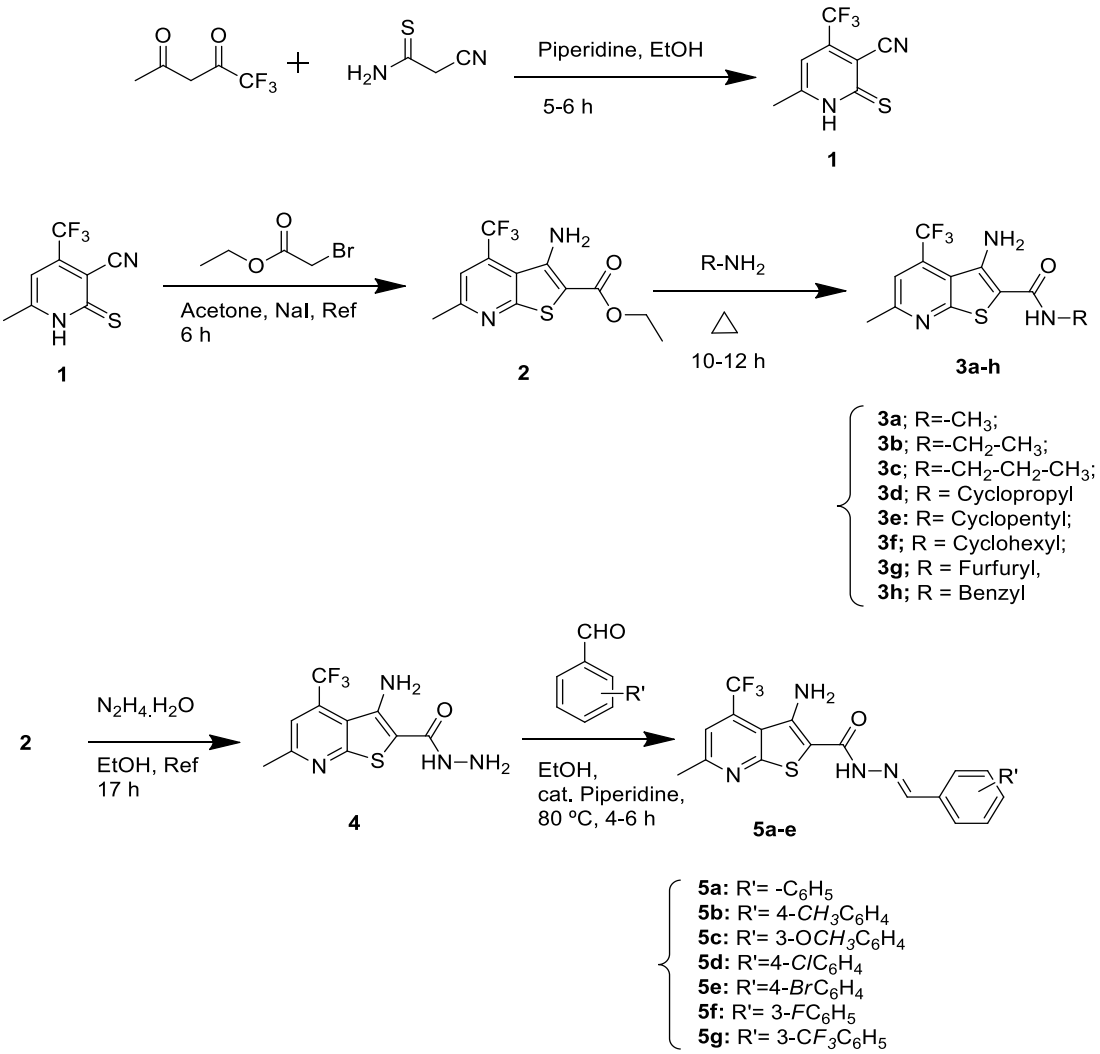
We have developed and synthesized trifluoromethyl-thieno[2,3-*b*]pyridine amide derivatives and evaluated then for anticancer activity on four human cancer cell lines. Promising compounds 3a and 3c showing good activity were identified.

A series of novel trifluoromethyl-thieno[2,3-*b*]pyridine-2-carboxamide 3a-h and Schiff's base derivatives 5a-g was prepared starting from 6-methyl-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile 1. Compound 1 on reaction with bromoethyl acetate in the presence of K_2CO_3 and catalytic amount of NaI in acetone solvent gave ester derivative 2 which on reaction with aliphatic amine gave amide 3a-h derivatives. Compound 2 on reaction with hydrazine hydrate in ethanol gave compound 4 of hydrazide derivatives. This hydrazide derivative on reaction with aromatic aldehydes gave Schiff's base derivatives 5a-g. Reaction details are outlined in scheme 1.

Material and Methods

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 $CDCl_3$ 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.

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.



Scheme 1: Synthesis of novel trifluoromethyl-thieno[2,3-b]pyridine-2-carboxamide and Schiff's base derivatives

Table 1
***In vitro* cytotoxicity of compounds 3a-h and 5a-g**

Compd.	IC_{50} values (in µM)			
	HeLa	**COLO205**	**HepG2**	**MCF7**
3a	22.2 ± 0.35	28.5 ± 0.18	---	47.2 ± 0.51
3b	55.2 ± 0.22	61.3 ± 0.43	---	---
3c	27.2 ± 0.12	18.6 ± 0.21	29.4 ± 0.32	31.5 ± 0.24
3d	63.7 ± 0.54	---	69.2 ± 0.38	---
3e	41.1 ± 0.31	---	78.2 ± 0.61	---
3f	24.2 ± 0.12	40.6 ± 0.39	32.6 ± 0.51	43.3 ± 0.32
3g	54.2 ± 0.42	90.7 ± 0.88	---	---
3h	28.5 ± 0.23	32.7 ± 0.24	41.5 ± 0.31	21.8 ± 0.15
5a	---	---	38.5 ± 0.32	---
5b	48.2± 0.32	---	24.7 ± 0.24	---
5c	65.7 ± 0.58	---	---	---
5d	45.3 ± 0.22	118.5 ± 0.62	---	41.7 ± 0.52
5e	42.2 ± 0.39	59.5 ± 0.46	49.8 ± 0.23	---
5f	---	---	79.8 ± 0.65	82.7 ± 0.78
5g	55.7 ± 0.58	---	39.8 ± 0.26	42.2 ± 0.36
5-Fluorouracil	(Std control)			
	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07

---indicates IC_{50} value > 118.5 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)

The cytotoxicity was assessed using the MTT assay[20] against a panel of five 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) were indicated as means ± SD of three independent experiments.

6-Methyl-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridine -3-carbonitrile (1)[17]: Yield 72%, white powdery crystals, mp 234".[29] mp. 232-234'. 'H NMR: 6 2.37 (s, 3H, (2%-C(6)), 6.62 **(s,** IH, H-C(5)), IR (Nujol): 3320,2225, 1655 cm-I. MS: m/z 202.

Ethyl 3-amino-6-methyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxylate (2): 6-Methyl-2-oxo-4-(trifluoro methyl)-1,2-dihydropyridine-3-carbonitrile compound 1 on reaction with bromoethyl acetate (1:2) in the presence of catalytic amount of NaI in acetone was refluxed for about 6 hours and after completion of the reaction, confirm by TLC. Reaction mixture was cooled and acetone was completely removed under vacuum and desired product was purified by using solvents EtOAc and hexane as 1:3 ratio.

Yield 81%, mp 161-163 °C, IR (KBr, cm^{-1}): 1690 (-COOEt -), 3245, 3324 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 1.28 (t, 3H, -CH_3), 2.48 (s, 3H, -CH_3), 4.28 (q, 2H, -CH_2-), 6.51 (br., s, 2H, -NH_2), 7.78 (s, 1H, Py-H), MS (ESI): m/z [(M+H)$^+$]: 289. HRMS m/z Calcd. for $C_{12}H_{11}F_3N_2O_3$[(M+H)$^+$]: 289.0112. Found: 289.0114.

3-Amino-N, 6-dimethyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxamide (3a): Ethyl 3-amino-6-methyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxylate compound 2 and aliphatic amine (1:2) were taken in sealed tube and refluxed. After confirmation of product by TLC, reaction mixture was cooled and poured on crushed ice and filtered solid was collected and dried to afford amide derivatives 3a-h as product.

Yield 85%, mp 171-173 °C, IR (KBr, cm^{-1}): 1690(-COOEt -), 3245, 3324 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.43 (s, 3H, -CH_3), 2.87(s, 3H, -CH_3), 6.53 (br., s, 2H, -NH_2), 7.61 (br. s., 1H, -NH-) 7.79 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 21.4, 25.8, 120.6, 122.4, 124.7, 126.1, 128.3, 131.2, 136.5, 142.7, 160.2, MS (ESI): m/z [(M+H)$^+$]: 290. HRMS m/z Calcd. for $C_{11}H_{10}F_3N_3OS$[(M+H)$^+$]: 290.0254. Found: 290.0257.

3-Amino-N-ethyl-6-methyl-4-(trifluoromethyl)thieno[2, 3-*b*]pyridine-2-carboxamide (3b): Yield 90%, mp 189-191 °C, IR (KBr, cm^{-1}): 1682(-COOEt-), 3265, 3318 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 1.08 (s, 3H, -CH_3), 2.48 (s, 3H, -CH_3), 3.21 (m, 2H, -CH_2-), 6.55 (br., s, 2H, -NH_2), 7.42 (br. s., 1H, -NH-) 7.77 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 16.4, 24.5, 25.9, 121.2, 122.6, 124.6, 127.3, 128.9, 132.3, 136.4, 142.2, 160.1, MS (ESI): m/z [(M+H)$^+$]: 304. HRMS m/z Calcd.for $C_{12}H_{12}F_3N_3OS$ [(M+H)$^+$]: 304.0108. Found: 304.0110.

3-Amino-6-methyl-N-propyl-4-(trifluoromethyl)thieno [2,3-*b*]pyridine-2-carboxamide (3c): Yield 82%, mp 195-197 °C, IR (KBr, cm^{-1}): 1679(-COOEt-), 3285, 3320 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 1.01 (t, 3H, -CH_3), 1.51(m, 2H, -CH_2-), 2.43 (s, 3H, -CH_3), 3.18 (m, 2H, -CH_2-), 6.54 (br., s, 2H, -NH_2), 7.45 (br. s., 1H, -NH-) 7.85 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 16.4, 24.5, 25.9, 121.2, 122.6, 124.6, 127.3, 128.9, 132.3, 136.4, 142.2, 160.1, MS (ESI): m/z [(M+H)$^+$]: 318. HRMS m/z Calcd. for $C_{13}H_{14}F_3N_3OS$ [(M+H)$^+$]: 318.0110. Found: 318.0113.

3-Amino-N-cyclopropyl-6-methyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxamide (3d): Yield 76%, mp 167-169 °C, IR (KBr, cm^{-1}): 1674 (-COOEt-), 3281, 3318 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 0.75-0.90 (m, 4H, -CH_2-CH_2), 2.60-2.69 (m, 1H, -CH-), 2.43 (s, 3H, -CH_3), 3.18 (m, 2H, -CH_2-), 6.51 (br., s, 2H, -NH_2), 7.41 (br. s., 1H, -NH-) 7.81 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 21.2, 23.5, 25.8, 120.1, 122.5, 124.8, 126.3, 127.7, 132.1, 135.3, 142.1, 159.3, MS (ESI): m/z [(M+H)$^+$]: 316. HRMS m/z Calcd.for $C_{13}H_{12}F_3N_3OS$ [(M+H)$^+$]: 316.0427. Found: 316.0429.

3-Amino-N-cyclopentyl-6-methyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxamide (3e): Yield 79%, mp 188-189 °C, IR (KBr, cm^{-1}): 1678 (-COOEt-), 3283, 3311 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 1.38-1.71 (m, 4H, -CH_2-), 1.94-2.05 (m, 4H, -CH_2-), 2.43 (s, 3H, -CH_3), 3.62 (m, 1H, -CH-), 6.54 (br., s, 2H, -NH_2), 7.45 (br. s., 1H, -NH-) 7.97 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 22.4, 31.2, 25.8, 55.2, 119.4, 121.6, 123.6, 125.2, 127.6, 132.2, 135.4, 142.2, 160.2, MS (ESI): m/z [(M+H)$^+$]: 344. HRMS m/z Calcd.for $C_{15}H_{16}F_3N_3OS$ [(M+H)$^+$]: 344.0554. Found: 344.0557.

3-Amino-N-cyclohexyl-6-methyl-4-(trifluoromethyl)thie no[2,3-*b*]pyridine-2-carboxamide (3f): Yield 68%, mp 152-154 °C, IR (KBr, cm^{-1}): 1671 (-COOEt-), 3275, 3318 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 1.41-2.08 (m, 10H, -CH_2-), 2.44 (s, 3H, -CH_3), 3.78 (m, 1H, -CH-), 6.50 (br., s, 2H, -NH_2), 7.42 (br. s., 1H, -NH-) 7.82 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.1, 25.0, 32.4, 52.4, 120.3, 121.7, 122.5, 124.8, 125.1, 127.8, 132.6, 136.5, 142.3, 158.3, MS (ESI): m/z [(M+H)$^+$]: 358. HRMS m/z Calcd.for $C_{16}H_{18}F_3N_3OS$ [(M+H)$^+$]: 358.0120. Found: 358.0123.

3-Amino-N-(furan-2-ylmethyl)-6-methyl-4-(trifluoromet hyl)thieno[2,3-b]pyridine-2-carboxamide (3g): Yield 58%, mp 196-198 °C, IR (KBr, cm^{-1}): 1668 (-COOEt-), 3268, 3321 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.45 (s, 3H, -CH_3), 4.39 (s, 2H, -CH_2), 6.51 (br., s, 2H, -NH_2),

7.44 (br. s., 1H, -NH-), 7.49 (dd, 1H, furyl-H), 7.71 (dd, 1H, furyl-H), 8.02 (s, 1H, Py-H), 8.15 (dd, 1H, furyl-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.5, 38.4, 118.4, 119.8, 120.7, 121.5, 123.4, 124.7, 125.4, 126.2, 128.5, 132.7, 136.2, 142.4, 159.8, MS (ESI): m/z [$(M+H)^+$]: 356. HRMS m/z Calcd.for $C_{15}H_{12}F_3N_3O_2S$ [$(M+H)^+$]: 356.0512. Found: 356.0515.

3-Amino-N-benzyl-6-methyl-4-(trifluoromethyl)thieno [2,3-b]pyridine-2-carboxamide (3h): Yield 60%, mp 201-203 °C, IR (KBr, cm^{-1}): 1671 (-COOEt-), 3272, 3328 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.46 (s, 3H, -CH_3), 4.41 (s, 2H, -CH_2), 6.52 (br., s, 2H, -NH_2), 7.33 (br., s, 1H, -CONH-), 7.54-7.61 (m, 2H, Ar-H), 7.76-7.82 (m, 3H, Ar-H), 8.08 (s, 1H, Py-H), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.6, 38.3, 119.6, 120.2, 121.6, 123.7, 124.8, 125.3, 126.6, 128.4, 129.9, 132.6, 136.7, 142.3, 160.4, MS (ESI): m/z [$(M+H)^+$]: 366. HRMS m/z Calcd.for $C_{17}H_{14}F_3N_3OS$ [$(M+H)^+$]: 366.0405. Found: 366.0408.

3-Amino-6-methyl-4-(trifluoromethyl)thieno[2,3-*b*]pyri dine-2-carbohydrazide (4): Ethyl 3-amino-6-methyl-4-(trifluoromethyl) thieno[2,3-*b*]pyridine-2-carboxylate compound 2 was dissolved in excess hydrazine hydrate in ethanol and refluxed for about 17 hours after completion of the reaction; reaction mixture was poured on crushed ice and solid was separated and dried.

Yield 71%, mp 207-209 °C, IR (KBr, cm^{-1}): 1694 (CONH), 3232, 3332 (-NH_2), 3195 (NH), 3214, 3358(-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.47 (s, 3H, -CH_3), 6.52 (br., s, 2H, -NH_2), 7.79 (s, 1H, Py-H), MS (ESI): m/z [$(M+H)^+$]: 291. HRMS m/z Calcd. for $C_{10}H_9F_3N_4OS$[$(M+H)^+$]: 291.0052. Found: 291.0055.

3-Amino-N'-benzylidene-6-methyl-4-(trifluoromethyl) thieno[2,3-b]pyridine-2 carbohydrazide (5a): 3-Amino-6-methyl-4-(trifluoromethyl)thieno[2,3-*b*]pyridine-2-carbohydrazide 4 and different substituted aromatic aldehydes (1:1 ratio) were taken in ethanol and add catalytic amount of piperidine. Reaction mixture was allowed to reflux for 4-6 hours. After completion of the reaction by TLC, reaction mixture was poured on crushed ice and product was filtered and dried to afford Schiff's base derivatives 5a-g.

Yield 91%, mp 211-213 °C, IR (KBr, cm^{-1}): 3281, 3332 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.48 (s, 3H, -CH_3), 6.51 (br., s, 2H, -NH_2), 7.36-7.42 (m, 2H, Ar-H), 7.71-7.78 (m, 3H, Ar-H), 8.09 (s, 1H, Py-H), 8.42(s, 1H, CH=N), 11.31(br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.9, 119.7, 120.3, 121.5, 122.8, 123.6, 124.4, 125.1, 125.8, 127.8, 128.3, 132.4, 134.5, 142.3, 160.4, MS (ESI): m/z [$(M+H)^+$]: 379. HRMS m/z Calcd.for $C_{17}H_{13}F_3N_4OS$ [$(M+H)^+$]: 379.0115. Found: 379.0118.

3-Amino-6-methyl-N'-(4-methylbenzylidene)-4-(trifluor omethyl)thieno[2,3-b]pyridine-2-carbohydrazide (5b): Yield 88%, mp 218-220 °C, IR (KBr, cm^{-1}): 3224, 3339 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.49 (s, 3H, -CH_3), 2.38 (s, 3H, -CH_3), 6.53 (br., s, 2H, -NH_2), 7.35 (d, *J*= 8.12 Hz, 2H, Ar-H), 7.68 (d, *J*= 8.12 Hz, 2H, Ar-H), 8.10 (s, 1H, Py-H), 8.41 (s, 1H, CH=N), 11.32(br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 21.8, 23.9, 120.1, 121.6, 122.5, 123.7, 124.5, 125.8, 127.9, 128.2, 132.2, 134.6, 142.2, 160.1, MS (ESI): m/z [$(M+H)^+$]: 379. HRMS m/z Calcd.for $C_{18}H_{15}F_3N_4OS$ [$(M+H)^+$]: 393.0258. Found: 393.0260.

3-Amino-N'-(3-methoxybenzylidene)-6-methyl-4-(trifluo romethyl)thieno[2,3-b]pyridine-2-carbohydrazide (5c): Yield 92%, mp 202-204 °C, IR (KBr, cm^{-1}): 3218, 3395 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.50 (s, 3H, -CH_3), 3.81 (s, 3H, -OCH_3), 6.51 (br., s, 2H, -NH_2), 7.32-7.38 (m, 3H, Ar-H), 7.69 (s, 1H, Ar-H), 8.11 (s, 1H, Py-H), 8.43 (s, 1H, CH=N), 11.34 (br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.8, 56.1, 118.8, 120.6, 121.4, 122.4, 123.7, 124.6, 125.5, 126.4, 127.8, 128.1, 129.3, 132.3, 134.7, 142.3, 146.3, 160.2, MS (ESI): m/z [$(M+H)^+$]: 409. HRMS m/z Calcd.for $C_{18}H_{15}F_3N_4O_2S$ [$(M+H)^+$]: 409.0045. Found: 409.0048.

3-amino-N'-(4-chlorobenzylidene)-6-methyl-4-(trifluoro methyl)thieno[2,3-b]pyridine-2-carbohydrazide (5d): Yield 89%, mp 198-200 °C, IR (KBr, cm^{-1}): 3225, 3383 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.49 (s, 3H, -CH_3), 6.53 (br., s, 2H, -NH_2), 7.36 (d, *J*= 8.14 Hz, 2H, Ar-H), 7.68 (d, *J*= 8.14 Hz, 2H, Ar-H), 8.12 (s, 1H, Py-H), 8.42 (s, 1H, CH=N), 11.30 (br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.5, 119.8, 120.2, 121.6, 122.5, 124.6, 125.5, 126.1, 127.6, 128.2, 132.6, 135.4, 142.4, 146.7, 159.3, MS (ESI): m/z [$(M+H)^+$]: 413. HRMS m/z Calcd.for $C_{17}H_{12}ClF_3N_4OS$ [$(M+H)^+$]: 413.0147. Found: 413.0149.

3-Amino-N'-(4-bromobenzylidene)-6-methyl-4-(trifluo romethyl)thieno[2,3-b]pyridine-2-carbohydrazide (5e): Yield 85%, mp 212-214 °C, IR (KBr, cm^{-1}): 3221, 3379 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.50 (s, 3H, -CH_3), 6.52 (br., s, 2H, -NH_2), 7.31 (d, *J*= 8.12 Hz, 2H, Ar-H), 7.62 (d, *J*= 8.12 Hz, 2H, Ar-H), 8.11 (s, 1H, Py-H), 8.39 (s, 1H, CH=N), 11.29 (br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.2, 118.6, 120.6, 121.7, 122.3, 124.5, 125.6, 126.8, 127.5, 128.8, 132.7, 135.6, 142.2, 146.8, 160.2, MS (ESI): m/z [$(M+H)^+$]: 458. HRMS m/z Calcd.for $C_{17}H_{12}BrF_3N_4OS$ [$(M+H)^+$]: 458.0018. Found: 458.0020.

3-Amino-N'-(3-fluorobenzylidene)-6-methyl-4-(trifluoro methyl)thieno[2,3-b]pyridine-2-carbohydrazide (5f): Yield 85%, mp 205-207 °C, IR (KBr, cm^{-1}): 3218, 3364 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.51 (s, 3H, -CH_3), 6.51 (br., s, 2H, -NH_2), 7.33-7.39 (m, 3H, Ar-H), 7.71 (s, 2H, Ar-H), 8.12 (s, 1H, Py-H), 8.40 (s, 1H, CH=N), 11.28 (br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.4, 119.1, 120.3, 121.6, 122.4, 124.3, 125.4, 126.1, 127.6, 128.4, 130.5, 132.1, 135.3, 140.8, 142.1, 146.6, 160.2, MS

(ESI): m/z [(M+H)$^+$]: 397. HRMS m/z Calcd.for $C_{17}H_{12}F_4N_4OS$ [(M+H)$^+$]: 397.0687. Found: 397.0690.

3-Amino-6-methyl-4-(trifluoromethyl)-N'-(3-(trifluoro methyl)benzylidene)thieno[2,3-b]pyridine-2-carbohydrazide (5g): Yield 79%, mp 221-223 °C, IR (KBr, cm^{-1}): 3215, 3362 (-NH_2), ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.52 (s, 3H, -CH_3), 6.50 (br., s, 2H, -NH_2), 7.32-7.39 (m, 3H, Ar-H), 7.69 (s, 2H, Ar-H), 8.11 (s, 1H, Py-H), 8.45 (s, 1H, CH=N), 11.29 (br., s, 1H, -CONH-), ^{13}C NMR ($CDCl_3$, 300 MHz): δ ppm 23.4, 119.5, 120.5, 121.7, 123.3, 124.5, 125.6, 126.2, 127.8, 128.9, 130.6, 132.2, 136.2, 140.7, 142.1, 143.6, 146.5, 159.6, MS (ESI): m/z [(M+H)$^+$]: 447. HRMS m/z Calcd.for $C_{18}H_{12}F_6N_4OS$ [(M+H)$^+$]: 447.0108. Found: 447.0110.

Results and Discussion

A series of novel trifluoromethyl-thieno[2,3-*b*]pyridine-2-carboxamide 3a-h and Schiff's base derivatives 5a-g were synthesized and evaluated for anticancer activity on four human 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) with reference to 5-Fluorouracil as standard control. Promising compounds which showed good activity like 3a and 3c, have been identified. All compounds responded for IC_{50} values as 18.6 to 118.5 µg/mL. Compound 3c showed very good activity 18.6 µg/mL on COLO205 cancer cell line. Structure activity relation studies explains that 3a-h are carboxamide derivatives and 5a-g are Schiff's base derivatives. Hydrogen bonding to intercat with enzymes (N-H) is present in 3a-h, but in 5a-g, all π elocrons participate in delocalisation. Activity results are outlined in table 1.

Conclusion

In conclusion, a series of novel trifluoromethyl-thieno[2,3-*b*]pyridine-2-carboxamide 3a-h and Schiff's base derivatives 5a-g was synthesized and characterized by spectra ^{1}H NMR, ^{13}C NMR and ESI Mass and evaluated for anticancer activity on four human 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) with reference to 5-Fluorouracil as standard control. Promising compounds 3a and 3c showing good activity were identified.

Acknowledgement

Authors are thankful to the Department of Chemistry, Telangana University, for providing Lab facilities.

References

1. Adachi I., Hiramatsu Y., Japan Patent 03 52 890, *Chem Abstr*, **115**, 71573 **(1991)**

2. Adachi I., Yamamori T., Hiramatsu Y., Sakai K., Mihara S.I., Kawakami M., Masui M., Uno O. and Ueda M., Studies on dihydropyridines. III. Synthesis of 4,7-dihydrothieno [2,3-b]-pyridines with vasodilator and antihypertensive activities, *Chem. Pharm. Bull.*, **36(11)**, 4389 **(1988)**

3. Anand P., Kunnumakara A.B., Sundaram C., Harikumar K.B., Tharakan S.T., Lai O.S., Sung B. and Aggarwal B.B., Cancer Is a Preventable Disease That Requires Major Lifestyle Changes, *Pharmaceutical Research*, **25**, 2097-2116 **(2008)**

4. Balkrishen B. and Bhaduri A.P., Effect of Metal Ions in Organic Synthesis; Part XXIII. Easy and High-Yield Direct Synthesis of 3-Aminocarbonyl-1-ureidopyrroles by the Copper(II) Chloride-Catalyzed Reaction of Aminocarbonylazoalkenes with 3-Oxoalkanamides, *Synthesis*, 671-673, DOI: 10.1055/s-1984-30928 **(1984)**

5. Bernardino A.M.R., Pinheiro L.C.S., Ferreira V.F., Azevedo A.R., Carneiro J.W. de M., Souza T.M.L. and Frugulhetti I.C.P.P., synthesis and antiviral activity of new 4- (phenylamino)thieno[2,3-b]pyridine derivatives, *Heterocycl. Commun*., **10**, 407 **(2004)**

6. Bernardino A.M.R., Pinheiro L.C.S., Rodrigues C.R., Loureiro N.I.V., Castro H.C., Lanfredi-Rangel A., Sabatini-Lopes J., Borges J.C., Carvalho J.M., Romeiro G.A., Ferreira V.F., Frugulhetti I.C.P.P. and Vannier-Santos M.A., *Bioorg. Med. Chem.*, **14**, 5765 **(2006)**

7. Cho N., Harada M., Imaeda T., Imada T., Matsumoto H., Hayase Y., Sasaki S., Furuya S., Suzuki N., Okubo S., Ogi K., Endo S., Onda H. and Fujino M., Discovery of a novel, potent and orally active nonpeptide antagonist of the human luteinizing hormone-releasing hormone (LHRH) receptor, *J. Med. Chem*., **41**, 4190 **(1998)**

8. Csuk R., Barthel A., Raschke C., Kluge R., Ströhl D., Trieschmann L. and Böhm G., Synthesis of monomeric and dimeric acridine compounds as potential therapeutics in Alzheimer and prion diseases, *Arch. Pharm.* (Weinheim), **342**, 699–709 **(2009)**

9. Dolle R.E., Comprehensive survey of combinatorial library synthesis: 2003, *J. Comb. Chem*., **6(5)**, 623–679 **(2004)**

10. Dolle R.E., Comprehensive Survey of Chemical Libraries for Drug Discovery and Chemical Biology: 2008, *J. Comb. Chem*., **11(5)**, 739–798 **(2009)**

11. El-Abadelah M.M., Nazer M.Z., Okasha S.F., Calas M., Bompart J. and Mion P., Thienopyridinone antibacterials: Synthesis and antibacterial activity of some 7-aryl-2-chloro-4,7-dihydro-4-oxothieno[2,3-b]pyridine-5-carboxylic acids, *Eur. J. Med. Chem*., **33**, 33-42 **(1998)**

12. Furuya S., Choh N., Kato K. and Hinuma S., PCT Int. Appl. WO, 95, 28, 405, *Chem. Abstr*., **124**, 202226t **(1996)**

13. Furuya S., Choh N., Suzuki N. and Imada T., PCT Int. Appl. WO, 00, 00, 493, *Chem. Abstr*., **132**, 64179s **(2000)**

14. Furuya S., Matsumoto H., Hayase Y., Suzuki N. and Imada T., PCT Int. Appl. WO, 97, 41, 126, *Chem. Abstr*., **128**, 13211f **(1998)**

15. Hassaneen H.M., Shawali A.S. and Saleh F.M., A convenient synthesis of novel 1,3- phenylene bridged bis-heterocyclic compounds, *J. Sulfur Chem*., **37**, 241–250 **(2016)**

16. Helal C.J., Sanner M.A., Cooper C.B., Gant T., Adam M., Lucas J.C., Kang Z., Kupchinsky S., Ahlijanian M.K., Tate B.,

Menniti F.S., Kelly K. and Peterson M., Discovery and SAR of 2-aminothiazole inhibitors of cyclin-dependent kinase 5/p25 as a potential treatment for Alzheimer's disease, *Bioorg. Med. Chem. Lett*., **14**, 5521–5525 **(2004)**

17. Lang R.W. and Wenk P.F., Synthesis of selectively trifluoromethylated pyridine derivaties as potential antihypertensives, *Helv. Chim. Acta*, **71**, 596 **(1988)**

18. Miki S., Fukuoka K., Akita M., Kawa Kami J., Furuya S. and Ishimaru Y., PCT Int. Appl. WO, 99, 09, 033, *Chem. Abstr*., **130**, 196644h **(1999)**

19. Moloney G.P., Methyl 3-Hydroxythieno[2,3-b]pyridine-2-carboxylate, *Molecules*, **6**, M203 **(2001)**

20. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, **65**, 55-63 **(1938)**

21. Muthusaravanan S., Perumal S., Yogeeswari P. and Sriram D., Facile three-component domino reactions in the regioselective synthesis and antimycobacterial evaluation of novel indolizines and pyrrolo[2,1-a]isoquinolines, *Tetrahedron Lett*., **51**, 6439 **(2010)**

22. Ooe T., Sano M., Kobayashi H., Chiba K. and Azuma H., Jpn Kokai Tokky Koho Japan Patent 0776, 586,1995, *Chem Abstr*., **123**, 55855w **(1995)**

23. Roth H.J. and Kleemann A., Pharmaceutical Chemistry, Drug Synthesis, Prentice Hall, London, **1**, 407 **(1988)**

24. Shaker R.M., Synthesis of 1,4-phenylene-bridged bis-heterocyclic compounds, *ARKIVOC*, https://doi.org/10.3998/ark.5550190.0013.101 **(2012)**

25. Shawali A.S., Sherif S.M., El-Merzabani M.M. and Darwish M.A., Synthesis and antitumor activity of novel pyrazolylenaminone and bis(pyrazolyl)ketones via hydrazonoyl halides, *J. Heterocycl. Chem.*, **46**, 548–551 **(2009)**

26. Soural M., Bouillon I. and Krchňák V., Combinatorial libraries of bis-heterocyclic compounds with skeletal diversity, *J. Comb. Chem*., **10**, 923–933 **(2008)**

27. Suarez M., Ochoa E., Pita B., Espinosa R., Gonzalez L., Martin N., Quinteiro M., Seoane C. and Soto J.L., Synthesis and structural characterization of substituted thieno[2,3-b]pyridines from o-chloroformyl-1,4-dihydropyridines, *J. Heterocycl. Chem.*, **34**, 931 **(1997)**

28. Suzuki N., Matsumoto H. and Furuya S., Eur. Pat. Appl., 781, 774, *Chem. Abstr*., **127**, 135807e **(1997)**

29. Viegas J.C., Danuello A., da Silva B.V., Barreiro E.J. and Fraga C.A.M., Molecular hybridization: a useful tool in the design of new drug prototypes, *Curr. Med. Chem.*, **14**, 1829–52 **(2007)**

30. Warszycki D., Mordalski S., Kristiansen K., Kafel R., Sylte I., Chilmonczyk Z. and Bojarski A.J., A linear combination of pharmacophore hypotheses as a new tool in search of new active compounds - An application for 5-HT1A receptor ligands, *PLoS One*, **8**, e84510 **(2013)**.

(Received 09th November 2022, accepted 13th January 2023)