Novel carboxamide and carbohydrazide functionalized pyridopyrimidine derivatives and their anticancer activity

Dasari Raghu, Gali Srinivas and Vaddiraju Namrata*
Department of Chemistry, Satavahana University, Karimnagar, Telangana, 505001, INDIA
*namrathav69@gmail.com

Abstract

A series of novel carboxamide and carbohydrazide functionalized pyridopyrimidine derivatives was prepared starting from 6-methyl/ethyl-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile 1. Compound 1 on reaction with sulphuric acid gave compound 2. Compound 2 on reaction with chloroacetamide followed by reaction with EMME coupling and further cyclization gave compound 5. Compound 5 on reaction with hydrazine hydrate produced hydrazide derivatives 6.

Compound 6 on reaction with diverse substituted aromatic aldehyde gave Schiff's base derivatives 7a-j. Ester derivatives 5 on reaction with different aliphatic amine gave carboxamide derivatives 8a-f. All the final 7a-j and 8a-f compounds were evaluated for anti cancer 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 7e, 7h and 7j have been identified.

Keywords: Pyridopyrimidine, Carboxamide, Carbohydrazide, Schiff's base, Anticancer activity.

Introduction

Heterocyclic chemistry occupied an important place in synthetic organic chemistry. Many of the drugs in the market are heterocycles. Nitrogen-heterocycles are acting as core moiety of many biological active compounds and drugs.[3,5,8,10,15,16,19,20,22,23,26,27] Six-member nitrogen ring heterocycles exhibits more biological activities such as antitumor, antibacterial, anti-inflammatory, anti hepatitis B virus and analgesic activities.[4,7,9,11,13,14,17,24,29] Pyridopyrimidine structure has a long distinguished history as they had wide range of biological activities and applications such as antitumor,[21] antibacterial,[28] antifungal,[6,2] antiviral,[1] anti diabetic[18] and calcium channel blocking activity.[12] pyridopyridine scaffold exhists in many bioactive compounds like in tranquilizer pirenperone, the antiallergic agent ramastine, an antiulcerative agent and an antiasthmatic agent (Fig. 1). Amide and Schiff's base functionalized derivatives have broad spectrum of biological activities and promote the activity when they associate with aromatic heterocyclic atoms.

In our research work, we designed and synthesized novel carboxamide and carbohydrazide functionalized pyridopyrimidine derivatives and tested for 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).

Chemistry: 6-methyl/ethyl-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile 1 on reaction with 50% H_2SO_4 followe by decarbonylation and deamination gave 6-methyl/ethyl-4-(trifluoromethyl)pyridin-2(1H)-one 2. Compound 2 on reaction with chloroacetamide gave 2-amino pyridne 3 by Smiles rearrangement, compound 3 on reaction with ethoxy methylene malonic diethyl ester (EMME) in ethanol refluxing condition produced compound 4 which after in cyclization under reflux in the presence of $POCl_3$ resulted product 5.

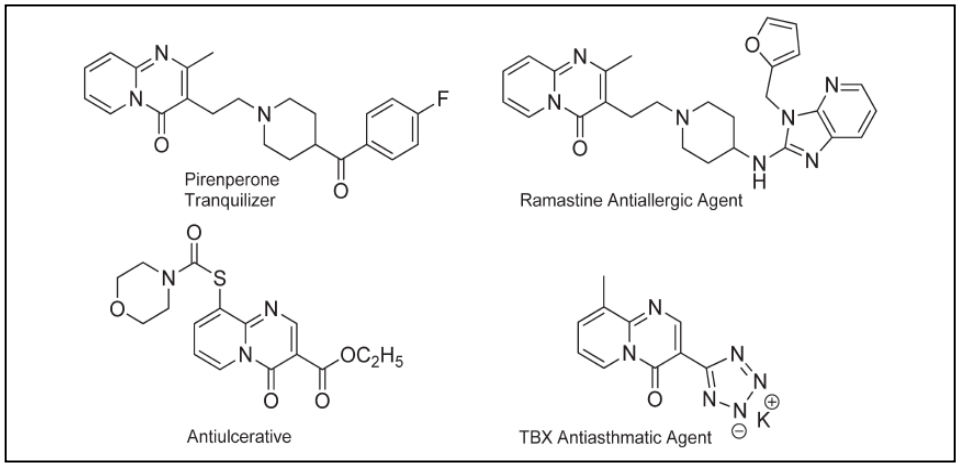
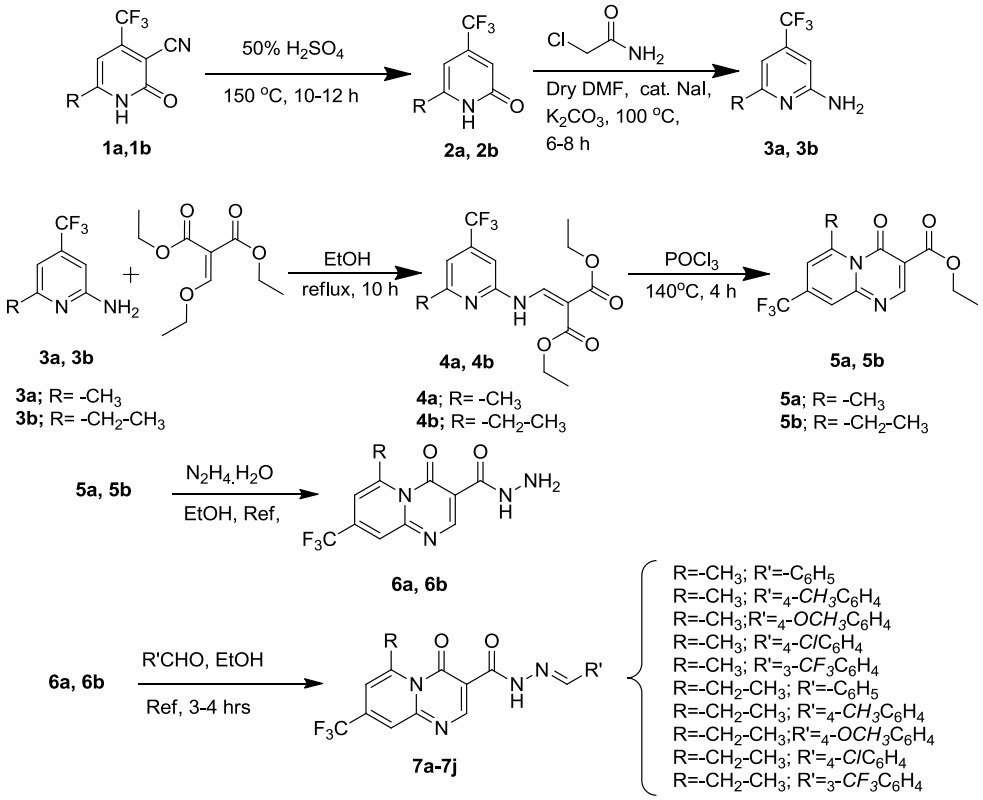


Figure 1: Pyridopyridine based drugs

Reaction of compound 5 with hydrazine hydrate in refluxed EtOH gave compound 6, compound 6 on reaction with different substituted aromatic aldehydes in piperidine and EtOH refluxing condition for about 3-4 hours of Schiff's base reaction produced imine derivatives 7a-j. Reaction condition and details are outlined in scheme 1.

Ester derivative 5 on reaction with different aliphatic amines at their refluxing condition for about 4-6 hrs gave amide derivatives 8a-f. Reaction condition and details are outlined in scheme 2.

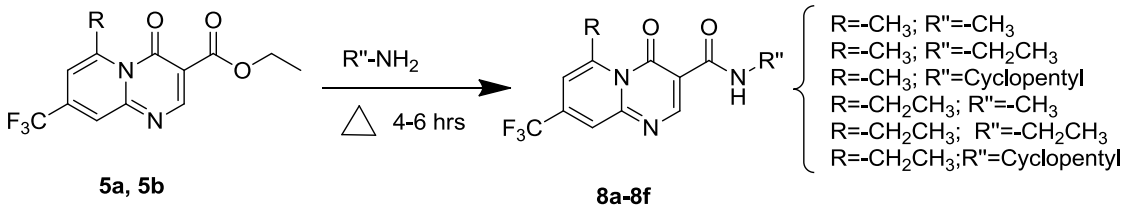


Scheme 1: Synthesis of carbohydrazide derivatives 7a-7j

Table 1
***In vitro* cytotoxicity (anticancer activity results) of compounds 7a-j and 8a-f**

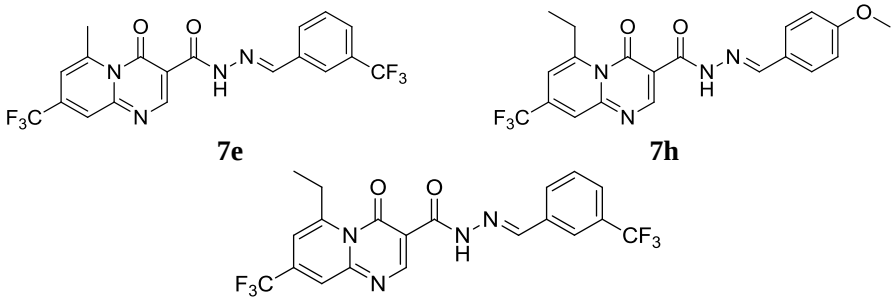
Compounds	IC_{50} values (in µM)			
	HeLa	**COLO205**	**HepG2**	**MCF-7**
7a	---	---	---	---
7b	63.7 ± 4.22	64.6 ± 5.37	75.5 ± 6.25	---
7c	27.5 ± 2.12	24.6 ± 5.21	---	35.5 ± 6.25
7d	56.2± 6.12	66.2± 5.12	---	---
7e	21.3 ± 3.21	26.5 ± 2.34	31.2 ± 4.41	28.3 ± 3.51
7f	---	---	128.2 ± 6.10	---
7g	---	91.7 ± 8.28	---	---
7h	48.5 ± 0.23	28.7 ± 2.81	32.5 ± 3.41	20.4 ± 2.35
7i	66.5 ± 5.30	68.7 ± 2.41	85.5 ± 3.41	---
7j	21.4 ± 2.43	28.7 ± 0.36	31.3 ± 3.13	38.8 ± 3.15
8a	73.2 ± 0.52	---	48.8 ± 4.23	---
8b	---	---	---	---
8c	---	---	58.8 ± 5.23	---
8d	---	---	---	---
8e	---	---	---	---
8f	---	118.5 ± 3.31	---	---
5-Fluorouracil	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07

--- Indicates IC_{50} value >128.2 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),



5a, 5b **8a-8f**

Scheme 2: Synthesis of carboxamide derivatives 8a-8f



7e **7h** **7j**

Scheme 3: Promising compounds 7e, 7h and 7j which showed good activity have been identified

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 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 6-methyl-4-(trifluoromethyl)pyridin-2(1H)-one (1): 2-Oxo-6-methyl-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (10 g, 0.03 mol) was slowly added to 150 mL of 50% sulfuric acid. The reaction mixture was heated with stirring for 10 h at 148°C. The reaction mixture was allowed to cool and poured on to crushed ice. White solid was formed and filtered.

Preparation of 6-methyl-4-(trifluoromethyl)pyridin-2-amine (2): The 3-cyano-4-trifluoromethyl-6-substituted-2(1H)-pyridone 1 (2.6 mmol) was dissolved in dry acetone (50 mL). To the homogeneous solution, 2-chloroacetamide (0.243 g, 2.6 mmol), potassium carbonate (0.730 g, 5.3 mmol) and a pinch of sodium iodide (0.010 g) were added. The reaction mixture was refluxed for 6–8 h at 60°C and cooled to room temperature. The separated salt was filtered off and washed with acetone (30 mL). The total filtrate was concentrated under vacuum and the residue was treated with water. The separated white solid was filtered, dried and recrystallized from ethyl alcohol.

6-Methyl-4-(trifluoromethyl)pyridin-2(1H)-one (2a): White solid; Yield 96%; IR (KBr, cm^{-1}): 3326, (CONH), 1655 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.36 (s, 3H, -CH_3), 6.39 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H); MS (ESI): m/z [(M+H)$^+$]: 178; Anal. calc. for $C_7H_6F_3NO$: C 47.47, H 3.41, N 7.91 %. Found: C 47.48, H 3.42, N 7.93%.

6-Ethyl-4-(trifluoromethyl)pyridin-2(1H)-one (2b): White solid; Yield 96%; IR (KBr, cm^{-1}): 3325, (CONH), 1656 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.19 (t, 3H, -CH_3), 2.48 (q, 2H, -CH_2), 6.33 (s, 1H, Ar-H), 6.68 (s, 1H, Ar-H); MS (ESI): m/z [(M+H)$^+$]: 192; Anal. calc. for $C_8H_8F_3NO$: C 50.27, H 4.22, N 7.33 %. Found: C 50.28, H 4.21, N 7.35%

Preparation of diethyl 2-(((6-methyl-4-(trifluoromethyl)pyridin-2-yl) amino) methylene)malonate (3): The 6-methyl-4-(trifluoromethyl) pyridin-2-amine (0.500 g, 0.002 mol) and diethyl 2-(ethoxymethylene) malonate (0.450 g, 0.002 mol) were taken in absolute ethanol and refluxed for 10 h. After completion of the reaction, ethanol was completely removed under vacuum, washed with n-hexane and dried to afford diethyl 2-(((6-methyl-4-(trifluoromethyl)- pyridin-2-yl) amino) methylene) malonate 4.

6-Methyl-4-(trifluoromethyl)pyridin-2-amine (3a): White solid; Yield 82%; IR (KBr, cm^{-1}): 3374, 3492 (NH_2), 1631 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.68 (br, 2H), 2.36 (s, 3H, -CH_3), 6.38 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H); MS (ESI): m/z [(M+H)$^+$]: 177; Anal. calc. for $C_7H_7F_3N_2$: C 47.73, H 4.01, N 15.90 %. Found: C 47.74, H 4.03, N 15.92%.

6-Ethyl-4-(trifluoromethyl)pyridin-2-amine (3b): White solid; Yield 80%; IR (KBr, cm^{-1}): 3371, 3489 (NH_2), 1632 (CO); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.70 (br, 2H), 1.18 (t, 3H, $-CH_3$), 2.46 (q, 2H, $-CH_2$), 6.32 (s, 1H, Ar-H), 6.69 (s, 1H, Ar-H); MS (ESI): m/z [$(M+H)^+$]: 191; Anal. calc. for $C_8H_9F_3N_2$: C 50.53, H 4.77, N 14.73 %. Found: C 50.54, H 4.78, N 14.72%.

Prepartion of ethyl 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (4): The diethyl 2-(((6-methyl-4-(trifluoromethyl) pyridin-2-yl)-amino) methylene) malonate 4 (0.500 g, 0.001 mol) was taken in 5 mL of phosphorus oxychloride ($POCl_3$) and the reaction mixture was refluxed for 4–5 h at 140°C. After completion of the reaction, the excess $POCl_3$ was distilled under vacuum and the residue was treated with crushed ice. Yellow color solid was formed, filtered and washed with excess water.

Diethyl 2-(((6-methyl-4-(trifluoromethyl)pyridin-2-yl)amino)methylene)malonate (4a): White solid; Yield 86%; IR (KBr, cm^{-1}): 1731 (CO); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.38 (t, 6H, $–CH_3$), 2.36 (s, 3H, $-CH_3$), 4.31 (q, 4H, $–CH_2–$), 6.39 (s, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 9.28 (d, *J*=12.46 Hz, 1H, –NCH=), 11.30 (d, *J*=12.46 Hz, 1H, –NHC=); MS (ESI): m/z [$(M+H)^+$]: 347; Anal. calc. for $C_{15}H_{17}F_3N_2O_4$: C 52.02, H 4.95, N 8.09 %. Found: C 52.04, H 4.96, N 8.10%.

Diethyl 2-(((6-ethyl-4-(trifluoromethyl)pyridin-2-yl)amino)methylene)malonate (4b): White solid; Yield 79%; IR (KBr, cm^{-1}): 1732 (COOEt); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.17 (t, 3H, $-CH_3$), 1.37 (t, 6H, $–CH_3$), 2.41 (q, 2H, $-CH_3$), 4.32 (q, 4H, $–CH_2–$), 6.38 (s, 1H, Ar-H), 6.82 (s, 1H, Ar-H) 9.29 (d, *J*=12.46 Hz, 1H, –NCH=), 11.30 (d, *J*=12.46 Hz, 1H, –NHC=); MS (ESI): m/z [$(M+H)^+$]: 361; Anal. calc. for $C_{16}H_{19}F_3N_2O_4$: C 53.33, H 5.31, N 7.77 %. Found: C 53.34, H 5.32, N 7.79 %.

Preparation of 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (5): Ethyl 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate was taken in 95% ethanol (30 ml) and hydrazine hydrate (5 ml) was added. The mixture was refluxed for 3-4 hrs. After cooling to room temperature, 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.

Ethyl 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido [1,2-a]pyrimidine-3-carboxylate (5a): Yellow solid; Yield 75%; IR (KBr, cm^{-1}): 1725 (COOEt); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, 3H, $-CH_3$), 2.39 (s, 3H, $-CH_3$), 4.21(q, 2H, $-CH_2$), 6.81 (s, 1H, Ar-H), 7.71(s, 1H, Ar-H), 8.10(s, 1H, Ar-H); MS (ESI): m/z [$(M+H)^+$]: 301; Anal. calc. for $C_{13}H_{11}F_3N_2O_3$: C 52.01, H 3.69, N 9.33 %. Found: C 52.02, H 3.71, N 9.36 %.

Ethyl 6-ethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (5b): Yellow solid; Yield 70%; IR (KBr, cm^{-1}): 1728 (COOEt); ^1H NMR ($CDCl_3$, 300 MHz): δ 1.21 (t, 3H, $-CH_3$), 1.24 (t, 3H, $-CH_3$), 2.40 (q, 2H, $-CH_2$), 4.29 (q, 2H, $-CH_2$), 6.73 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 8.096 (s, 1H, Ar-H); MS (ESI): m/z [$(M+H)^+$]: 315; Anal. calc. for $C_{14}H_{13}F_3N_2O_3$: C 53.01, H 3.69, N 9.33 %. Found: C 53.02, H 3.71, N 9.36 %.

Preparation of (E)-N'-benzylidene-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a] pyrimidine-3-carbohydrazide (6): The 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide 4 (3 mmol) was taken in 95% ethanol (10 ml) and benzaldehyde (3 mmol) was added. Piperidine (0.1 ml) was added as catalyst. The mixture was refluxed for 2 h and after cooling to room temperature, the ethanol was removed under vacuum. The residue was washed with *n*-hexane and then water was added to give yellow solid which was filtered with water and dried.

6-Methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a] pyrimidine-3-carbohydrazide (6a): White solid; Yield 71%; IR (KBr, cm^{-1}): 3372, 3493 (NH_2), 3210 (CONH), 1682 (CO); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.51 (s, 3H, $-CH_3$), 4.15 (br. s, 2H, N-NH_2), 6.91(s, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.72 (s, lH, CONH), 8.98 (s, 1H, Ar-H); MS (ESI): m/z [$(M+H)^+$]: 287; Anal. calc. for $C_{11}H_9F_3N_4O_2$: C 46.16, H 3.17, N 19.58 %. Found: C 46.17, H 3.18, N 19.59 %.

6-Ethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a] pyrimidine-3-carbohydrazide (6b): White solid; Yield 66%; IR (KBr, cm^{-1}): 3378, 3491 (NH_2), 3212 (CONH), 1690 (CO); ^1H NMR (DMSO-d_6, 300 MHz): δ 1.25 (t, 3H, $-CH_3$), 2.81(q, 2H, $-CH_2$), 4.18 (br, 2H, N-NH_2), 6.95(s, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.70 (s, lH, CONH), 8.91 (s, 1H, Ar-H); MS (ESI): m/z [$(M+H)^+$]: 301; Anal. calc. for $C_{12}H_{11}F_3N_4O_2$: C 48.00, H 3.69, N 18.66 %. Found: C 48.02, H 3.71, N 18.65 %.

Preparation of N,6-dimethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (7): The ethyl 6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (0.4 g, 0.001 mol) and alkyl amine (0.6 g, 0.002 mol) were taken in sealed tube and refluxed for 6 h. After completion of the reaction, the reaction mixture was allowed to cool, filtered, washed with n-hexane followed by water to give a yellow solid and dried.

(E)-N'-benzylidene-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7a): Yellow solid; m. p. 198-200 °C; I.R. (KBr, cm^{-1}): 3212, 1619 (CONH); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.51 (s, 3H, $-CH_3$), 6.98 (s, 1H, Ar-H), 7.28-7.32 (m, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.48-7.53 (m, 3H, Ar-H), 8.41 (s, 1H, CH=N), 8.91 (s, 1H, Ar-H), 11.42 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.8, 121.4, 122.9, 123.2, 125.6, 126.4, 129.5, 132.8,

134.2, 138.4, 142.1, 144.7, 147.1, 148.9, 157.2, 162.1; MS (ESI): m/z [(M+H)$^+$]: 375; Anal. calc. for $C_{18}H_{13}F_3N_4O_2$: C 57.76, H 3.50, N 14.97%. Found: C 57.75, H 3.51, N 14.98%.

(E)-6-methyl-N'-(4-methylbenzylidene)-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7b): Yellow solid; m. p. 202-204 °C; I.R. (KBr, cm^{-1}): 3210, 1618 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.33 (s, 3H, -CH_3), 2.51 (s, 3H, -CH_3), 6.99 (s, 1H, Ar-H), 7.33 (d, 2H, Ar-H), 7.42 (s, 1H, Ar-H), 7.53 (d, 2H, Ar-H), 8.45 (s, 1H, CH=N), 8.98 (s, 1H, Ar-H), 11.41 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.6, 24.6, 121.5, 122.9, 124.4, 125.7, 128.1, 129.6, 130.5, 132.3, 134.3, 138.7, 142.2, 144.8, 148.9, 158.3, 161.2; MS (ESI): m/z [(M+H)$^+$]: 389; Anal. calc. for $C_{19}H_{15}F_3N_4O_2$: C 58.76, H 3.89, N 14.43%. Found: C 58.75, H 3.90, N 14.45%.

(E)-N'-(4-methoxybenzylidene)-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7c): Yellow solid; m. p. 219-221 °C; I.R. (KBr, cm^{-1}): 3209, 1619 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.68 (s, 3H, -CH_3), 3.83 (s, 3H, -OCH_3), 6.89 (s, 1H, Ar-H), 7.29 (d, 2H, Ar-H), 7.45 (s, 1H, Ar-H), 7.55 (d, 2H, Ar-H), 8.47 (s, 1H, CH=N), 8.99 (s, 1H, Ar-H), 11.40 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.5, 55.8, 119.5, 122.8, 124.5, 126.2, 128.2, 129.8, 130.6, 132.4, 135.7, 139.8, 142.3, 144.5, 148.8, 157.8, 160.3; MS (ESI): m/z [(M+H)$^+$]: 405; Anal. calc. for $C_{19}H_{15}F_3N_4O_2$: C 56.44, H 3.74, N 13.86%. Found: C 56.45, H 3.75, N 13.88%.

(E)-N'-(4-chlorobenzylidene)-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7d): Yellow solid; m. p. 205-207 °C; I.R. (KBr, cm^{-1}): 3212, 1618 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.58 (s, 3H, -CH_3), 6.91 (s, 1H, Ar-H), 7.28 (d, 2H, Ar-H), 7.41 (s, 1H, Ar-H), 7.56 (d, 2H, Ar-H), 8.48 (s, 1H, CH=N), 8.89 (s, 1H, Ar-H), 11.41 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.5, 119.5, 122.5, 124.4, 126.6, 128.6, 129.4, 130.2, 132.3, 135.7, 139.5, 142.1, 144.3, 148.5, 159.3, 161.2; MS (ESI): m/z [(M+H)$^+$]: 409; Anal. calc. for $C_{18}H_{12}ClF_3N_4O_2$: C 52.89, H 2.96, N 13.71%. Found: C 52.90, H 2.97, N 13.72%.

(E)-6-methyl-4-oxo-8-(trifluoromethyl)-N'-(3-(trifluoromethyl)benzylidene)-4H-pyrido[1,2-a] pyrimidine -3-carbohydrazide (7e): Yellow solid; m. p. 226-228 °C; I.R. (KBr, cm^{-1}): 3219, 1625 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.57 (s, 3H, -CH_3), 6.92 (s, 1H, Ar-H), 7.28-7.35 (m, 3H, Ar-H), 7.46 (s, 1H, Ar-H), 7.58 (s, 1H, Ar-H), 8.45 (s, 1H, CH=N), 8.82 (s, 1H, Ar-H), 11.39 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.6, 120.4, 122.5, 124.3, 125.4, 126.7, 127.3, 128.7, 129.3, 130.1, 132.4, 135.8, 138.6, 140.2, 142.6, 144.2, 148.6, 159.4, 160.7; MS (ESI): m/z [(M+H)$^+$]: 443; Anal. calc. for $C_{19}H_{12}F_6N_4O_2$: C 51.59, H 2.73, N 12.67%. Found: C 51.60, H 2.73, N 12.68%.

(E)-N'-benzylidene-6-ethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7f): Yellow solid; m. p. 185-187 °C; I.R. (KBr, cm^{-1}): 3216, 1618 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.23 (t, 3H, -CH_3), 2.81 (q, 2H, -CH_2), 6.95 (s, 1H, Ar-H), 7.29-7.34 (m, 2H, Ar-H), 7.41 (s, 1H, Ar-H), 7.50-7.54 (m, 3H, Ar-H), 8.43 (s, 1H, CH=N), 8.93 (s, 1H, Ar-H), 11.43 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 13.1, 29.7, 120.7, 122.8, 123.3, 125.7, 128.1, 129.6, 132.6, 134.7, 138.5, 142.2, 144.4, 147.3, 148.6, 158.3, 162.2; MS (ESI): m/z [(M+H)$^+$]: 389; Anal. calc. for $C_{19}H_{15}F_3N_4O_2$: C 58.76, H 3.89, N 14.43%. Found: C 58.75, H 3.90, N 14.45%.

(E)-6-ethyl-N'-(4-methylbenzylidene)-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7g): Yellow solid; m. p. 210-212 °C; I.R. (KBr, cm^{-1}): 3216, 1616 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 3H, -CH_3), 2.34 (s, 3H, -CH_3), 2.86 (q, 2H, -CH_2), 6.93 (s, 1H, Ar-H), 7.34 (d, 2H, Ar-H), 7.41 (s, 1H, Ar-H), 7.52 (d, 2H, Ar-H), 8.46 (s, 1H, CH=N), 8.98 (s, 1H, Ar-H), 11.45 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.9, 21.5, 29.5, 121.6, 122.9, 124.6, 125.8, 127.2, 129.4, 130.6, 132.7, 134.4, 138.8, 142.3, 144.7, 148.3, 158.8, 161.3; MS (ESI): m/z [(M+H)$^+$]: 403; Anal. calc. for $C_{20}H_{17}F_3N_4O_2$: C 59.70, H 4.26, N 13.92%. Found: C 59.71, H 4.27, N 13.93%.

(E)-6-ethyl-N'-(4-methoxybenzylidene)-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7h): Yellow solid; m. p. 225-227 °C; I.R. (KBr, cm^{-1}): 3219, 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 3H, -CH_3), 2.85 (q, 2H, -CH_2), 3.84 (s, 3H, -OCH_3), 6.90 (s, 1H, Ar-H), 7.28 (d, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.52 (d, 2H, Ar-H), 8.48 (s, 1H, CH=N), 8.98 (s, 1H, Ar-H), 11.40 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.8, 21.6, 55.7, 118.6, 121.7, 124.4, 126.3, 128.1, 129.7, 130.6, 132.3, 135.6, 139.7, 142.2, 144.6, 148.7, 157.9, 160.2; MS (ESI): m/z [(M+H)$^+$]: 405; Anal. calc. for $C_{20}H_{17}F_3N_4O_3$: C 57.42, H 4.10, N 13.39%. Found: C 57.43, H 4.11, N 13.41%.

(E)-N'-(4-chlorobenzylidene)-6-ethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (7i): Yellow solid; m. p. 210-212 °C; I.R. (KBr, cm^{-1}): 3211, 1617 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 3H, -CH_3) 2.87 (s, 3H, -CH_3), 6.88 (s, 1H, Ar-H), 7.31 (d, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.53 (d, 2H, Ar-H), 8.49 (s, 1H, CH=N), 8.88 (s, 1H, Ar-H), 11.42 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.9, 21.7, 120.2, 121.6, 124.5, 126.4, 128.7, 129.5, 130.4, 132.6, 135.8, 139.4, 142.2, 144.2, 148.6, 158.7, 161.3; MS (ESI): m/z [(M+H)$^+$]: 423; Anal. calc. for $C_{19}H_{14}ClF_3N_4O_2$: C 53.98, H 3.34, N 13.25%. Found: C 53.99, H 3.35, N 13.26%.

(E)-6-ethyl-4-oxo-8-(trifluoromethyl)-N'-(3-(trifluoromethyl)benzylidene)-4H-pyrido[1,2-a] pyrimidine-3-carbohydrazide (7j): Yellow solid; m. p. 231-233 °C; I.R. (KBr, cm^{-1}): 3221, 1620 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.22 (t, 3H, -CH_3) 2.89 (s, 3H, -CH_3), 6.89 (s, 1H, Ar-H), 7.29-7.35 (m, 3H, Ar-H), 7.45 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 8.46 (s, 1H, CH=N), 8.81 (s, 1H, Ar-H), 11.38 (br.s., 1H, NHCO); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.9, 21.9,

120.5, 122.8, 124.4, 125.6, 126.8, 127.4, 128.6, 129.2, 130.2, 132.5, 135.4, 138.5, 140.3, 142.7, 143.8, 148.7, 159.5, 160.3; MS (ESI): m/z [(M+H)$^+$]: 457; Anal. calc. for $C_{20}H_{14}F_6N_4O_2$: C 52.64, H 3.09, N 12.28%. Found: C 52.65, H 3.10, N 12.30%.

Preparation of N,6-dimethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (8): In a sealed tube, compound ethyl 6-substituted-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxyla te 5 (3 mmol) and aliphatic primary amines (6 mmol) were taken and refluxed at primary amine boiling point for 4-6h and after cooling to room temperature, were kept in crushed ice. The reaction mixture was filtered, washed with *n*-hexane followed by water to give a white solid and dried.

N,6-dimethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (8a): White solid; m. p. 182-184 ^{o}C; I.R. (KBr, cm^{-1}): 3211, 1611 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.52 (s, 3H, -CH_3), 3.03 (s, 3H, -CH_3), 6.36 (br. s, 1H, -CONH-), 6.92 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 8.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.5, 25.3, 122.5, 123.6, 125.4, 127.2, 132.1, 134.4, 138.7, 142.3, 154.7, 158.3; MS (ESI): m/z [(M+H)$^+$]: 286; Anal. calc. for $C_{12}H_{10}F_3N_3O_2$: C 50.53, H 3.53, N 14.73%. Found: C 50.54, H 3.52, N 14.74%.

N-ethyl-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido [1,2-a]pyrimidine-3-carboxamide (8b): White solid; m. p. 191-193 ^{o}C; I.R. (KBr, cm^{-1}): 3209, 1611 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (s, 3H, -CH_3), 2.91 (s, 3H, -CH_3), 3.21 (q, 2H, -CH_2), 6.37 (br. s, 1H, -CONH-), 6.91 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 13.8, 25.1, 42.6, 122.6, 123.8, 125.3, 127.1, 132.2, 134.6, 138.8, 142.4, 154.6, 158.8; MS (ESI): m/z [(M+H)$^+$]: 300; Anal. calc. for $C_{13}H_{12}F_3N_3O_2$: C 52.18, H 4.04, N 14.04%. Found: C 52.19, H 4.05, N 14.06%.

N-cyclopentyl-6-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (8c): White solid; m. p. 168-170 ^{o}C; I.R. (KBr, cm^{-1}): 3218, 1608 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.49-1.55 (m, 2H, -CH_2-), 1.64-1.68 (m, 2H, -CH_2-), 1.73-1.78 (m, 2H, -CH_2-), 2.04-2.11 (m, 2H, -CH_2-), 2.46 (s, 3H, -CH_3), 4.41-4.46 (m, 1H, -CH-), 6.39 (br. s, 1H, -CONH-), 6.92 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 23.6, 24.3, 32.1, 55.3, 119.3, 121.8, 123.7, 125.3, 127.1, 132.2, 134.5, 142.4, 157.6, 158.4; MS (ESI): m/z [(M+H)$^+$]: 340; Anal. calc. for $C_{16}H_{16}F_3N_3O_2$: C 56.64, H 4.75, N 12.38%. Found: C 56.65, H 4.74, N 12.39%.

6-Ethyl-N-methyl-4-oxo-8-(trifluoromethyl)-4H-pyrido [1,2-a]pyrimidine-3-carboxamide (8d): White solid; m. p. 156-158 ^{o}C; I.R. (KBr, cm^{-1}): 3213, 1612 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.22 (t, 3H, -CH_3) 2.89 (s, 3H, -CH_3), 2.98 (s, 3H, -CH_3), 6.38 (br. s, 1H, -CONH-), 6.90 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 8.87 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.8, 25.2, 27.7, 120.4, 122.8, 125.1, 127.3, 132.2, 134.5, 138.8, 142.2, 154.5, 158.2; MS (ESI): m/z [(M+H)$^+$]: 300; Anal. calc. for $C_{13}H_{12}F_3N_3O_2$: C 52.18, H 4.04, N 14.04%. Found: C 52.19, H 4.05, N 14.06%.

N,6-diethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a] pyrimidine-3-carboxamide (8e): White solid; m. p. 175-177 ^{o}C; I.R. (KBr, cm^{-1}): 3228, 1619 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, -CH_3), 1.21 (t, 3H, -CH_3), 2.91 (q, 3H, -CH_3), 3.21 (q, 2H, -CH_2), 6.34 (br. s, 1H, -CONH-), 6.92 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 8.96 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.8, 13.4, 27.8, 42.1, 120.4, 123.2, 125.2, 127.4, 132.1, 134.7, 138.5, 142.5, 155.8, 158.8; MS (ESI): m/z [(M+H)$^+$]: 314; Anal. calc. for $C_{14}H_{14}F_3N_3O_2$: C 53.67, H 4.50, N 13.41%. Found: C 53.68, H 4.49, N 13.42%.

N-cyclopentyl-6-ethyl-4-oxo-8-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (8f): White solid; m. p. 178-180 ^{o}C; I.R. (KBr, cm^{-1}): 3219, 1619 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, -CH_3), 1.48-1.54 (m, 2H, -CH_2-), 1.64-1.68 (m, 2H, -CH_2-), 1.73-1.78 (m, 2H, -CH_2-), 2.04-2.11 (m, 2H, -CH_2-), 2.87 (q, 3H, -CH_3), 4.41-4.46 (m, 1H, -CH-), 6.38 (br. s, 1H, -CONH-), 6.90 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 8.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 12.7, 23.6, 27.8, 32.1, 55.2, 118.2, 121.1, 123.8, 125.2, 127.2, 132.4, 134.6, 142.3, 157.7, 158.3; MS (ESI): m/z [(M+H)$^+$]: 354; Anal. calc. for $C_{17}H_{18}F_3N_3O_2$: C 57.79, H 5.13, N 11.89%. Found: C 57.80, H 5.12, N 11.90%.

Results and Discussion

All synthesized compounds 7a–j and 8a–f 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.[20] 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 7a, 8b, 8d and 8e showed activity at micro molar concentration. Among all the compounds, 7e, 7h and 7j showed promising activity, while the remaining compounds showed moderate activity. The structure–activity relationship studies revealed that amide functional group containing trifluoromethyl group containing Schiff's base derivatives showed good activity. Benzyl and piperazine with hydroxyl group containing molecules show more activity compared to other functional groups.

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[18] 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) are indicated as means ± SD of three independent experiments.

Conclusion

In conclusion, a series of novel carboxamide and carbohydrazide functionalized pyridopyrimidine derivatives 7a-j and 8a-f were prepared and evaluated for anticancer activity against four human cancer cell lines. Among all the compounds screened, the compounds 7e, 7h and 7j showed significant activity against all cell lines at micro molar concentration.

Acknowledgements

Authors are thankful to the Department of Chemistry, Satavahana University, Karimnagar for providing lab facility.

References

1. Agarwal H., Swathi, Yadav A.K. and Prakash L., Synthesis of ribonucleosides of 2-thioxopyrido [2,3-d] pyrimidines by phase transfer catalysis and their antimicrobial activity, *Phosphorous, Sulphur, Silicon and Related Elements*, **141**, 159 **(1998)**

2. Agrafoglio L., Suhas E., Farese A., Condom R., Challand S.R., Earl R.A. and Guedj R., Synthesis of carbocyclic nucleosides, *Tetrahedron*, **50**, 10611 **(1994)**

3. Amr A.G.E. and Abdulla M.M., Anti-inflammatory profile ofsome synthesized heterocyclic pyridone and pyridine derivatives fused with steroidal structure, *Bioorg. Med. Chem*., **14**, 4341–4352 **(2006)**

4. Balakrishna Pai S., Liu S.H., Zhu Y.L., Chu C.K. and Cheng Y.C., Inhibition of hepatitis B virus by a novel L-nucleoside, 2′-fluoro-5-methyl-β-L-arabinofuranosyl uracil, *Antimicrob. Agents Chemother*., **40**, 380 **(1996)**

5. Carter P.J., Cheng C.C. and Thorp H.H., Oxidation of DNA and RNA by oxoruthenium (IV) metallointercalators: visualizing the recognition properties of dipyridophenazine by high-resolution electrophoresis, *J. Am. Chem. Soc*., **120**, 632 **(1998)**

6. Cocco M.T., Congiu C., Onnis V., Morelli M. and Cauli O., Synthesis of ibuprofen heterocyclic amides and investigation of their analgesic and toxicological properties, *Eur. J. Med. Chem*., **38**, 513–518 (**2003)**

7. Focher F., Ubiali D. and Pregnolato M., Zhic., Bambio., Nucleosides and nucleotides. 152. 1-(3-C-Ethynyl-β-D-ribo-pentofuranosyl)uracil as a broad spectrum antitumor nucleoside, *Bioorg. Med. Chem. Lett*., **6**, 1887 **(1996)**

8. Forrestall K.L., Burley D.E., Cash M.K., Pottie I.R. and Darvesh S., 2-Pyridone natural products as inhibitors of SARSCoV- 2 main protease, *Chem. Biol. Interact.*, **335**, 109348 **(2021)**

9. Forterre P., Gribaldo S., Gadelle D. and Serre M.C., Origin and evolution of DNA topoisomerases, *Biochimie*, **89**, 427 **(2007)**

10. Heller M. and Schubert U.S., 2-Thienyl-4-furyl-6-aryl pyridine derivatives: Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity and structure–activity relationship study, *Macromol. Rapid Commun*., **23**, 411 **(2002)**

11. Holden J.A., DNA Topoisomerases as anticancer drug targets from the laboratory to the clinic, *Curr. Med. Chem. Anticancer Agent*, **1**, 1 **(2001)**

12. Hhigh D. and Rami H.K., Int PCT. Int. Appl. WO 9604261, *Chem. Abstr*., **125**, 336234 **(1996)**

13. Kellner U., Rudolph P. and Parwaresch R., Human DNA-topoisomerases–diagnostic and therapeutic implications for cancer, *Onkologie*, **23**, 424 **(2000)**

14. Kerru N., Gummidi L., Maddila S., Gangu K.K. and Jonnalagadda S.B., A review on recent advances in nitrogencontaining molecules and their biological applications, *Molecules*, **25**, 1909 **(2020)**

15. Kim D.S.H.L., Ashendel C.L., Zhou Q., Lee E.S., Chang C.T. and Chang C.J., Novel protein kinase C inhibitors: α-terthiophene derivatives, *Bioorg. Med. Chem. Lett.*, **8**, 2695 **(1998)**

16. Magedov I.V., Manpadi M., Evdokimov N.M., Elias E.M., Rozhkova E., Ogasawara M.A., Bettale J.D., Przheval'skii N.M., Rogelj S. and Kornienko A., Antiproliferative and apoptosis inducing properties of pyrano [3, 2-c] pyridines accessible by a one-step multi component synthesis, *Bioorg. Med. Chem. Lett.*, **17**, 3872-3876 **(2007)**

17. Mitscher L.A., Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents, *Chem. Rev.*, **105**, 559–592 (**2005)**

18. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, **65**, 55-63 **(1983)**

19. Mukkala V.M., Helenius M., Hemmila I., Knakare J. and Takalo H., 2-Thienyl-4-furyl-6-aryl pyridine derivatives: Synthesis, topoisomerase I and II inhibitory activity, cytotoxicity and structure–activity relationship study, *Helv. Chim. Acta*, **76**, 1361 **(1993)**

20. Patel K.K., Plummer E.A., Darwish M., Rodger A. and Hannon M.J., Aryl substituted ruthenium bis-terpyridine complexes: intercalation and groove binding with DNA, *J. Inorg. Biochem*., **91**, 220 **(2002)**

21. Singh G., Singh G., Yadav A.K. and Mishra A.K., Synthesis of Some Novel 4-Imino-3,5,7-Trisubstituted Pyrido[2,3-d]Pyrimidine-2(1H)-Thiones and Their Nucleosides as Potential Therapeutic Agents, *Phosphorous, Sulphur, Silicon and Related Elements*, **165**, 107 **(2000)**

22. Sos´nicki J.G. and Idzik T.J., Pyridones–powerful precursors for the synthesis of Alkaloids, their derivatives and alkaloid-inspired compounds, *Synthesis*, **51**, 3369–3396 **(2019)**

23. Teena Suresh and Suma Sarojini, In vivo, in vitro and in silico screening of a potent Angiotensin Converting Enzyme (ACE) inhibitor from Trigonella foenum-graecum extract using Zebrafish as a model organism to reduce hypertension, *Res. J. Biotech.*, **15(2)**, 9-16 **(2020)**

24. Topcu Z.J., DNA topoisomerases as targets for anticancer drugs, *Clin. Pharm. Ther*., **26**, 405 **(2001)**

25. Wang J.C., DNA topoisomerases, *Annu. Rev. Biochem*., **65**, 635 **(1996)**

26. Wang J.C., Interaction between DNA and an Escherichia coli protein ω, *J. Mol. Biol*., **55**, 523 **(1971)**

27. Wei F., Kang D., Cherukupalli S., Zalloum W.A., Zhang T., Liu X. and Zhan P., Discovery and optimizing polycyclic pyridine compounds as anti-HBV agents, *Expert Opin. Ther. Pat.*, **30**, 715–721 **(2020)**

28. Xu W.C., Zhou Q., Ashendel C.L., Chang C.T. and Chang C.J., Novel protein kinase C inhibitors: synthesis and PKC inhibition of β-substituted polythiophene derivatives, *Bioorg. Med. Chem. Lett.*, **9**, 2279 **(1999)**

29. Ztu¨ rk G.O., Erol D.D., Uzbay T. and Aytemir M.D., Synthesis of 4 (1H)-pyridinone derivatives and investigation of analgesic and anti-inflammatory activities, *Il Farmaco*, **56**, 251–256 **(2001)**.

(Received 26th April 2022, accepted 29th June 2022)