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Synthesis of Novel Triazolothione, Thiadiazole, Triazole and Oxadiazole Functionalized Tri-fluoromethylnaphthyridine Derivatives and their Anticancer Activity & Antimicrobial Activity

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Novel triazolothione, thiadiazole, triazole and oxadiazole-tagged trifluoromethyl group containing naphthyridine derivatives (**6a-l** and **7a-d**) were synthesized from 2-amino-6-(thiophen-2-yl)-4-(trifluoromethyl)nicotinonitrile (**1**) on treatment with acetophenone and obtained 2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (**2**), compound **2** on reaction with bromoethylacetate and after that reaction with hydrazine hydrate and obtained carbohydrazide derivatives (**4**), compound **4** on reaction with different substituted phenyl isothiocyanates to obtain phenyl hydrazine carbothiamide derivatives (**5**). Compound **5** is independently reaction with NaOH, H_2SO_4 and $N_2H_4 \cdot H_2O$ to obtain triazolothione, thiadiazole, triazole-substituted naphthyridine derivatives (**6a-l**), respectively. The carbohydrazide compounds **4** on reaction with diverse substituted aromatic acids and obtained oxadiazole derivatives (**7a-d**). All the synthesized compounds (**6a-l** and **7a-d**) were tested 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) and one normal cell line (HEK 293)". Compounds **6b, 6d** and **6l** are known to have good anticancer activity at micro molar concentration and found to be non-toxic on normal cell line. And all the products **6a-o** and **7a-d** were tested against Gram-positive, Gram-negative bacteria and fungal strains. All the compounds, compounds **6e-h** showed more activity against *Bacillus subtilis* (MTCC-121) at < 6.8 micromolar concentration. Compounds (which showed more activity) further screened for minimum bactericidal concentration against *B. subtilis* MTCC 121 using ciprofloxacin as standard and known to show optimistic activity. These compounds further tested for biofilm inhibition activity against *B. subtilis* MTCC 121 using erythromycin as standard which confirmed the high activity.

Keywords: Triazolothione, Thiadiazole, Triazole, Oxadiazole, Anticancer activity, Antimicrobial activity.

INTRODUCTION

Heterocyclic compounds containing nitrogen, oxygen and sulfur are acting like the key building blocks towards biological active compounds. Many of nitrogen containing heterocyclic building blocks have applications in pharmaceuticals, agrochemical research and drug discovery [1,2]. Among different types of naphthyridines, 1,8-naphthyridine derivatives have been received great attention due to their broad spectrum of biological activity. The 1,8-naphthyridine skeleton is present in many biologically active compounds and wide spectrum of biological activities such as antibacterial [3-5], antimycobacterial [6], antitumor [7], anti-inflammatory [8-10], antiplatelet [11,12], gastric antisecretary [13], antiallergic [14], local anaesthetic [15], anti-HIV [16], anticancer [17] and benzodiazepine receptor activity [18]. Azoles, especially 1,2,4-triazolothione, 1,2,4-triazoles and 1,3,4-thiadiazoles and oxadiazoles are known to interact with particular receptors on enzyme receptor sites [19-21]. Recently, it was found that these azoles consists interesting biological activity such as antimicrobial [22], antituberculosis [23,24], anti-inflammatory [25-28], anticonvulsant [29], antihypertensive [30], local anesthetic [31], anticancer activities [32,33]. Further, it was noticed that fluorine [34] or trifluoromethyl [35,36] group at appropriate position of an organic molecule will definitely alters the properties of molecule in terms of lipid solubility, oxidative thermal stability thereby enhances the transport mechanism and bioefficacy.

Based on the importance, it is assumed and planned to design and synthesized a series of triazolothione, thiadiazole, triazole-functionalized trifluoromethylnaphthyridine derivatives and submitted for anticancer activity against four cancer cell lines such as HeLa-cervical cancer, COLO 205-colon

cancer, HepG2-liver cancer, MCF7-breast cancer and one (healthy) normal cell line (HEK 293). Compounds **6b**, **6d** and **6l** are found to have promising anticancer activity at micro molar concentration and found to be non-toxic to normal cell line.

All the products **6a-l** and **7a-d** were tested against Gram-positive, Gram-negative bacteria and fungal strains. Among these compounds **6e-h** showed good activity against *Bacillus subtilis* microbial-type culture collection (MTCC) 121 at < 7.0 micromolar concentration. Compounds which showed good activity, further screened for minimum bactericidal concentration against *B. subtilis* MTCC 121 using ciprofloxacin as standard and found to show very good activity. These compounds also tested for biofilm inhibition activity against *B. subtilis* MTCC 121 using erythromycin as standard and confirmed the high activity. Compounds **6b**, **6d** and **6l** are known to have promising anticancer activity at micromolar concentration and found to be non-toxic on normal cell line.

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 $CDCl_3$ 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 electro spray ionization. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254}; spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography.

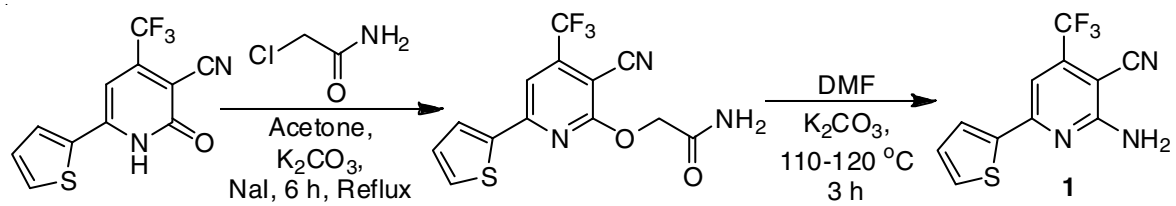
Synthesis of 2-((3-cyano-6-(thiophen-2-yl)-4-(trifluoromethyl)pyridin-2-yl)oxy)acetamide: 2-Oxo-6-(thiophen-2-yl)-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (0.7 g, 0.0026 mol) was dissolved in dry acetone (50 mL). To the homogeneous solution, 2-chloroacetamide (0.243 g, 0.0026 mol), K_2CO_3 (0.73 g, 0.0053 mol) and a pinch of sodium iodide (0.01 g) were added. The reaction mixture was refluxed for 6 h at 60 ºC and cooled to room temperature.The separated salt was filtered off and washed withacetone (30 mL). The total filtrate was concentrated under vacuum and the residue treated with water. The separated white solid was filtered, dried and recrystallizedfrom ethyl alcohol.

Synthesis of 2-amino-6-(thiophen-2-yl)-4-(trifluoromethyl)nicotinonitrile (1): 2-O-Acetamido-3-cyano-4-trifluoromethyl-6-substituted pyridines (0.001 mol) were suspended in DMF (6 mL) and treated with K_2CO_3 (0.276 g, 0.002 mol) at 110-120 ºC for 2 h. The dark reaction mixture was cooled and poured into crushed ice. The separated solid was filtered, washed with water and dried. The isolated compound was purified by passing through alumina in chloroform (**Scheme-I**). IR (KBr, cm^{-1}): 3452, 3281 (NH_2), 2220 (C≡N); ^{1}H NMR ($CDCl_3$): δ 5.51 (s, 2H, NH_2), 7.18 (dd, *J* = 4.91, 1H, Ar-H), 7.58 (dd, *J* = 4.91, 1H, Ar-H), 7.74 (dd, *J* = 3.76, 1H, Ar-H), 8.91 (s, 1H, Ar-H); MS (ESI): *m/z* [(M+H)$^+$]: 270. Elemental anal. calcd. (found) % for $C_{11}H_6N_3SF_3$; C, 49.07 (49.10); H, 2.25 (2.26); N, 15.61 (15.62).

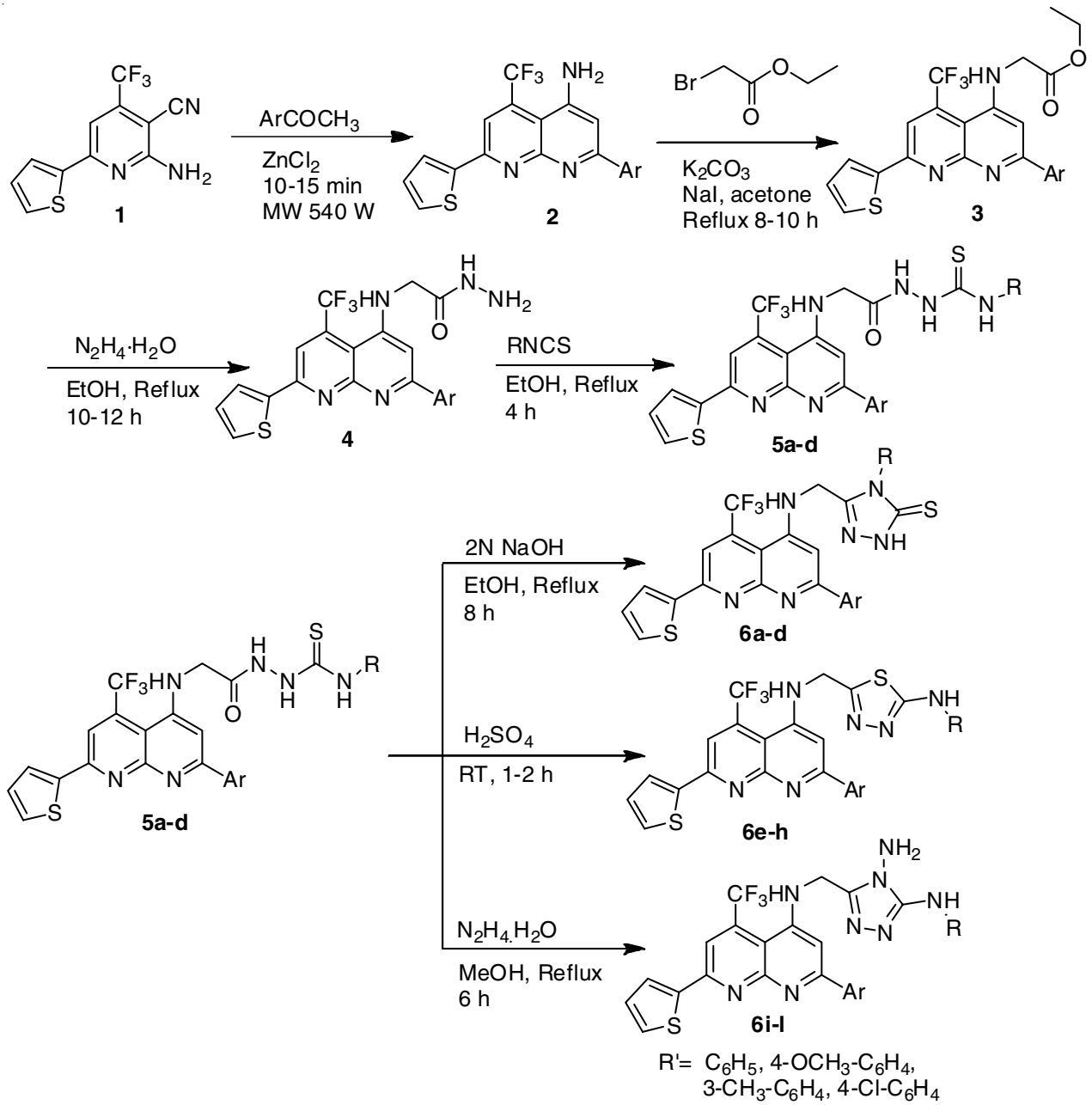
Synthesis of 2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (2): 2-Amino-6-(thiophen-2-yl)-4-(trifluoromethyl) nicotinonitrile (**1**) (2 mmol) was reacted with acetophenone (5 mmol) in presence of $ZnCl_2$ (0.8 mmol) under microwave irradiation conditions with 540 W power during 15 min and product was obtained in high yield. The product was purified by passing through a column packed with silica gel and *n*-hexane:ethylacetate (3:2) as eluents (**Scheme-II**). IR (KBr, cm^{-1}): 3464, 3291 (NH_2); ^{1}H NMR ($CDCl_3$): δ 5.55 (s, 2H, NH_2), 7.20 (dd, *J* = 4.93,1H, Ar-H), 7.28-7.32 (m, 2H, Ar-H), 7.42 (dd, J = 4.93, 1H, Ar-H), 7.51-7.56 (m, 3H, Ar-H), 7.71 (dd, *J* = 3.76, 1H,Ar-H) 7.88 (s, 1H, Ar-H); 8.13 (s, 1H, Ar-H); MS (ESI): *m/z* [(M+H)$^+$]: 372. Elemental anal. calcd. (found) % for $C_{19}H_{12}N_3SF_3$; C, 61.45 (61.46), H, 3.26 (3.28); N, 11.31 (11.32).

Synthesis of ethyl 2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetate (5): Compound **2** (2.6 mmol) was dissolved in dry acetone (30 mL). To the solution was added ethyl bromoacetate (2.86 mmol), K_2CO_3 (5.3 mmol) and sodium iodide (0.01 g). The reaction mixture was refluxed for 10 h and cooled to room temperature. The separated salt was filtered off and washed with acetone (20 mL). The total filtrate was concentrated under vacuum and the resultant white residue was washed with *n*-hexane followed by water. The white solid separated was filtered and dried to obtain good yield of product in pure form (**Scheme-II**). IR (KBr, cm^{-1}): 3421, 1719; ^{1}H NMR ($CDCl_3$): δ 1.28 (t, 3H, -CH_3), 3.82 (d, 2H, -CH_2-), 4.28 (q, 2H, -CH_2-), 5.32 (d, 1H, -NH-), 7.21 (dd, *J* = 4.91, 1H, Ar-H), 7.32-7.36 (m, 2H, Ar-H), 7.45 (dd, *J* = 4.91, 1H, Ar-H), 7.55-7.59 (m, 3H, Ar-H), 7.81 (dd, *J* = 3.71, 1H, Ar-H), 7.93 (s, 1H, Ar-H); 8.11 (s, 1H, Ar-H); MS (ESI): *m/z* [(M+H)$^+$]: 458. Elemental anal. calcd. (found) % for $C_{23}H_{18}N_3O_2SF_3$; C, 60.39; H, 3.97 (3.98); N, 9.19 (9.21).

Syntehsis of 2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetohydrazide (6):



Scheme-I

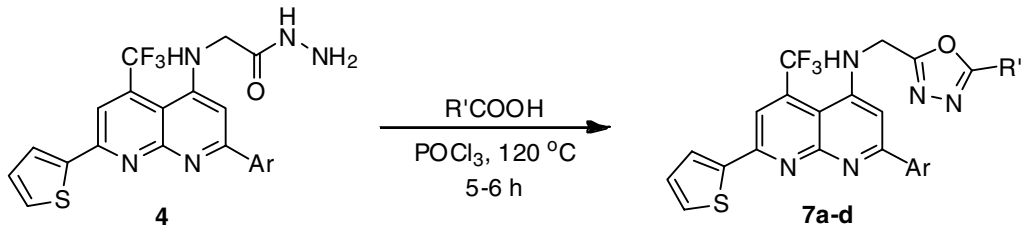


Scheme-II

Ethyl 2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetate (0.5 g) (3 mmol) in 95% ethanol (30 mL) was mixed hydrazine hydrate (5 mL). The mixture was refluxed for 6 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 a yellow solid which was filtered and dried. IR (KBr, cm^{-1}): 3390, 3471 (NH_2), 3214 (CONH), 1622 (CO); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.81(d, 2H, -CH_2-), 4.21 (br. s, 2H, NH_2), 5.35 (d, 1H, -NH-), 7.18 (dd, *J* = 4.74, 1H, Ar-H),7.39 (dd, *J* = 4.74, 1H, Ar-H), 7.50-7.54 (m, 2H, Ar-H), 7.66-7.69 (m, 3H, Ar-H), 7.81 (dd, *J* = 3.72, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 9.13 (s, 1H, -CONH-); MS (ESI): *m/z* [$(M+H)^+$]: 444. Elemental anal. calcd. (found) % for $C_{23}H_{18}N_3O_2SF_3$; C, 56.88 (56.89); H, 3.64 (3.66); N, 15.79 (15.81).

Synthesis of *N*-substituted-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)-acetyl)hydrazinecarbothioamide derivatives (7a-d): 2-((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetohydrazide (**4**) (1.3 g) (3 mmol) was taken in 95% ethanol (20 mL) and phenylisothiocyanate (3 mmol) was added, the reaction mixture was refluxed for 3-4 h and after completion of reaction, reaction mixture was cooled to room temperature the ethanol was removed under vacuum. The residue was washed with *n*-hexane and it gives light yellow solidand dried (**Scheme-III**).

***N*-Phenyl-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)hydrazine-carbothioamide (7a):** IR (KBr, cm^{-1}): 3390, 3471 (NH_2), 3214 (CONH), 1622 (CO); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.83



Scheme-III

(d, 2H, -CH_2-), 5.36 (d, 1H, -NH-), 7.18 (dd, *J* = 4.90, 1H, Ar-H), 7.41-7.46 (m, 4H, Ar-H), 7.51-7.56 (m, 6H, Ar-H) 7.61 (dd, *J* = 4.90, 1H, Ar-H), 7.78 (dd, *J* = 3.76, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 9.15 (br, s, 1H, -NH-), 9.90 (br. s., 1H, -NHCO-); MS (ESI): *m/z* [(M+H)$^+$]: 579. Elemental anal. calcd. (found) % for $C_{28}H_{21}N_6OS_2F_3$: C, 58.12 (58.14); H, 3.66 (3.65); N, 14.52 (14.53).

***N*-(4-Methoxyphenyl)-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)-hydrazinecarbothioamide (7b):** IR (KBr, cm^{-1}): 3332, 3410 (NH_2), 3219 (CONH), 1615 (CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.62 (s, 3H, -OCH_3), 3.86 (d, 2H, -CH_2-), 5.32 (d, 1H, -NH-), 7.17 (dd, *J* = 4.82, 1H, Ar-H), 7.21 (d, *J* = 8.49, 2H, Ar-H), 7.26-7.29 (m, 2H, Ar-H), 7.32 (d, *J* = 8.49, 2H, Ar-H), 7.52-7.56 (m, 3H, Ar-H) 7.63 (dd, *J* = 4.82, 1H, Ar-H), 7.81 (dd, *J* = 3.72, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 9.28 (br, s, 1H, -NH-), 9.93 (br. s., 1H, -NHCO-); MS (ESI): *m/z* [(M+H)$^+$]: 609. Elemental anal. calcd. (found) % for $C_{29}H_{23}N_6O_2S_2F_3$: C, 57.23 (57.24); H, 3.81 (3.83); N, 13.81 (13.79).

2-(2-((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)-*N*-(*m*-tolyl)hydrazine-carbothioamide (7c): IR (KBr, cm^{-1}): 3345, 3428 (NH_2), 3223 (CONH), 1621 (CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.41 (s, 3H, -CH_3), 3.85 (d, 2H, -CH_2-), 5.38 (d, 1H, -NH-), 7.21 (dd, *J* = 4.89, 1H, Ar-H), 7.26-7.31 (m, 5H, Ar-H), 7.41 (s, 1H, Ar-H), 7.48-7.52 (m, 3H, Ar-H) 7.66 (dd, *J* = 4.89, 1H, Ar-H), 7.76 (dd, *J* = 3.71, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 9.24 (br, s, 1H, -NH-), 9.91 (br. s., 1H, -NHCO-); MS (ESI): *m/z* [(M+H)$^+$]: 593. Elemental anal. calcd. (found) % for $C_{29}H_{23}N_6OS_2F_3$: C, 58.77 (58.78); H, 3.91 (3.93); N, 14.18 (14.17).

***N*-(4-Chlorophenyl)-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)-hydrazinecarbothioamide (7d):** IR (KBr, cm^{-1}): 3328, 3405 (NH_2), 3204 (CONH), 1622 (CO); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.82 (d, 2H, -CH_2-), 5.36 (d, 1H, -NH-), 7.19 (dd, *J* = 4.74, 1H, Ar-H), 7.25 (d, *J* = 8.52, 2H, Ar-H), 7.28-7.30 (m, 2H, Ar-H), 7.34 (d, *J* = 8.52, 2H, Ar-H), 7.44-7.49 (m, 3H, Ar-H) 7.65 (dd, *J* = 4.74, 1H, Ar-H), 7.79 (dd, *J* = 3.85, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 9.21 (br, s, 1H, -NH-), 9.96 (br. s., 1H, -NHCO-); MS (ESI): *m/z* [(M+H)$^+$]: 614. Elemental anal. calcd. (found) % for $C_{28}H_{20}N_6OS_2ClF_3$: C, 54.85 (54.86); H, 3.29 (3.30); N, 13.71 (13.70).

Synthesis of 4-phenyl-3-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl) amino)methyl) -1*H*-1,2,4-triazole-5-(4*H*)-thione derivatives (8a-d): *N*-Phenyl-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)hydrazine carbothioamide (**7a**) (0.5 g) (0.1 mmol) in 2 N NaOH was allowed to reflux for 6-8 h. The resulting reaction mixture was cooled to room temperature and acidified to pH 3-4 with 37% HCl. The precipitate formed was filtered, washed with distilled water and dried to afford compound **8a**.

4-Phenyl-3-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8a): Yield 82% (0.45 g); m.p.: 191-193 °C; IR (KBr, cm^{-1}): 3281 (-NH-), 1221 (-NHCS-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.02 (d, 2H, -CH_2-), 5.41 (d, 1H, -NH-), 7.18 (dd, *J* = 4.90, 1H, Ar-H), 7.32-7.38 (m, 4H, Ar-H), 7.43 (dd, *J* = 4.90, 1H, Ar-H), 7.46-7.51 (m, 6H, Ar-H), 7.72 (dd, *J* = 3.76, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 13.28 (br. s., 1H, -NHCS-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 58.2, 120.6 (q, -CF_3), 122.1, 122.7, 123.8, 124.3, 125.2, 125.8, 126.9, 128.4, 129.0, 129.3, 132.0, 133.0, 134.7, 136.2, 136.9, 138.4, 140.5, 143.0, 147.6 (q, C-CF_3), 152.2, 159.4, 171.5; MS (ESI): *m/z* [(M+H)$^+$]: 561. HRMS *m/z* calcd. for $C_{28}H_{19}N_6S_2F_3$ [(M+H)$^+$]: 561.0575. Found: 561.0578.

4-(4-Methoxyphenyl)-3-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8b): Yield 85% (0.50 g); (m.p.: 182-183 °C; IR (KBr, cm^{-1}): 3282 (-NH-), 1223 (-NHCS-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.62 (s, 3H, -OCH_3), 4.03 (d, 2H, -CH_2-), 5.43 (d, 1H, -NH-), 7.20 (dd, *J* = 4.94, 1H, Ar-H), 7.24 (d, *J* = 8.41, 2H, Ar-H), 7.27-7.30 (m, 2H, Ar-H), 7.33 (d, *J* = 8.41, 2H, Ar-H), 7.52-7.56 (m, 3H, Ar-H) 7.63 (dd, *J* = 4.94, 1H, Ar-H), 7.81 (dd, *J* = 3.72, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 13.22 (br. s., 1H, -NHCS-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.1, 57.6, 118.8 (q, -CF_3), 120.3, 121.8, 122.5, 123.7, 124.0, 125.0, 125.9, 126.8, 127.7, 128.0, 128.8, 131.3, 132.5, 134.7, 135.2, 135.8, 136.4, 137.8, 143.0, 147.7 (q, C-CF_3), 151.2, 170.9; MS (ESI): *m/z* [(M+H)$^+$]: 591. HRMS *m/z* calcd. for $C_{29}H_{21}N_6OS_2F_3$ [(M+H)$^+$]: 591.0116. Found: 591.0118.

3-(((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-4-(m-tolyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8c): Yield 80% (0.45 g); m.p.: 202–204 °C; IR (KBr, cm^{-1}): 3273 (-NH-), 1225 (-NHCS-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 2.43 (s, 3H, -CH_3), 4.02 (d, 2H, -CH_2-), 5.41 (d, 1H, -NH-), 7.21 (dd, *J* = 4.97, 1H, Ar-H), 7.27-7.32 (m, 5H, Ar-H), 7.47 (dd, *J* = 4.97, 1H, Ar-H), 7.52-7.56 (m, 4H, Ar-H) 7.81 (dd, *J* = 3.71, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 13.25 (br. s., 1H, -NHCS-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 21.7, 57.1, 119.8 (q, -CF_3), 120.4, 121.0, 122.7, 123.4, 124.6, 125.0, 125.9, 126.7, 127.8, 128.6, 129.0, 129.4, 131.3, 132.4, 134.7, 135.3, 135.8, 136.4, 137.2, 143.0, 147.3 (q, C-CF_3), 151.1, 159.9, 171.3; MS (ESI): *m/z* [(M+H)$^+$]: 575. HRMS *m/z* calcd. for $C_{29}H_{21}N_6S_2F_3$ [(M+H)$^+$]: 575.0314. Found: 575.0316.

4-(4-Chlorophenyl)-3-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8d): Yield 88% (0.52 g); m.p.: 185–187 °C; IR (KBr, cm^{-1}): 3279 (-NH-), 1221 (-NHCS-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.05 (d, 2H, -CH_2-), 5.39 (d, 1H, -NH-), 7.21 (dd, *J* = 4.92, 1H, Ar-H), 7.26 (d, *J* = 8.64, 2H, Ar-H), 7.29-7.32 (m, 2H, Ar-H), 7.35 (d, *J* = 8.64, 2H, Ar-H), 7.45-7.49 (m, 3H, Ar-H) 7.67 (dd, *J* = 4.92, 1H, Ar-H), 7.81 (dd, *J* = 3.78, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 13.26 (br. s., 1H, -NHCS-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 57.3, 119.3 (q, -CF_3), 120.6, 122.1, 122.8, 124.0, 124.4, 125.6, 125.9, 126.5, 127.2, 128.1, 129.1, 131.5, 132.4, 134.7, 135.4, 135.9, 136.3, 137.5, 142.0, 146.7 (q, C-CF_3), 158.8, 170.6; MS (ESI): *m/z* [(M+H)$^+$]: 596. HRMS *m/z* calcd. for $C_{28}H_{18}N_6S_2ClF_3$ [(M+H)$^+$]: 596.1021. Found: 596.1023.

General procedure for the synthesis of *N*-phenyl-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1,3,4-thiadiazol-2-amine derivatives (8e-h): A mixture of 0.001 mol of *N*-phenyl-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)hydrazine carbothioamide (**7a**) (0.5 g) (0.1 mmol) and concentrated H_2SO_4 (1 mL) was stirred at room temperature for 1-2 h. Then the reaction mixture was poured over crushed ice. The precipitated solid was washed with sodium carbonate solution followed by water to afford compound (**8e**).

***N*-Phenyl-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1,3,4-thiadiazol-2-amine (8e):** Yield 81% (0.45 g); m.p.: 175-177 °C; IR (KBr, cm^{-1}): 3281 (-NH-), 3211 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.02 (d, 2H, -CH_2-), 5.41 (d, 1H, -NH-), 7.21 (dd, *J* = 4.92, 1H, Ar-H), 7.41-7.46 (m, 4H, Ar-H), 7.51-7.56 (m, 6H, Ar-H) 7.61 (dd, *J* = 4.92, 1H, Ar-H), 7.76 (dd, *J* = 3.76, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 10.14 (br. s, 1H, -NH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.4, 119.4 (q, -CF_3), 120.2, 121.4, 123.7, 124.9, 125.1, 126.4, 128.0, 129.0, 129.9, 131.3, 132.7, 134.8, 135.2, 135.8, 136.6, 137.3, 138.8, 142.2, 146.8 (q, C-CF_3), 150.4, 161.5, 171.3; MS (ESI): *m/z* [(M+H)$^+$]: 561. HRMS *m/z* calcd. for $C_{28}H_{19}N_6S_2F_3$ [(M+H)$^+$]: 561.1012. Found: 561.1015.

N-(4-Methoxyphenyl)-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl) amino)methyl)-1,3,4-thiadiazol-2-amine (8f): Yield 83% (0.48 g); m.p.: 206-208 °C; IR (KBr, cm^{-1}): 3305 (-NH-), 3224 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.61 (s, 3H, -OCH_3),4.06 (d, 2H, -CH_2-), 5.46 (d, 1H, -NH-), 7.19 (dd, *J* = 4.94, 1H, Ar-H), 7.27 (d, *J* = 8.72, 2H, Ar-H), 7.29-7.32 (m, 2H, Ar-H), 7.36 (d, *J* = 8.72, 2H, Ar-H), 7.48-7.52 (m, 3H, Ar-H) 7.63 (dd, *J* = 4.94, 1H, Ar-H), 7.83 (dd, *J* = 3.76, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 10.12 (br, s, 1H, -NH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.5, 56.3, 119.6 (q, -CF_3), 120.3, 121.4, 122.6, 123.5, 125.0, 126.8, 127.1, 129.4, 130.8, 132.5, 134.9, 136.5, 137.1, 137.9, 138.3, 138.9, 142.3, 146.4 (q, C-CF_3), 151.3, 153.6, 160.2, 171.3; MS (ESI): *m/z* [(M+H)$^+$]: 591. HRMS *m/z* calcd. for $C_{29}H_{21}N_6OS_2F_3$ [(M+H)$^+$]: 591.0347. Found: 591.0349.

5-(((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-*N*-(*m*-tolyl)-1,3,4-thiadiazol-2-amine (8g): Yield 79% (0.45 g); m.p.: 178-180 °C; IR (KBr, cm^{-1}): 3311 (-NH-), 3235 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 2.41 (s, 3H, -CH_3), 4.02 (d, 2H, -CH_2-), 5.46 (d, 1H, -NH-), 7.22 (dd, *J* = 4.74, 1H, Ar-H), 7.25-7.30 (m, 5H, Ar-H), 7.44 (dd, *J* = 4.74, 1H, Ar-H), 7.51-7.55 (m, 4H, Ar-H) 7.82 (dd, *J* = 3.74, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 10.12 (br, s, 1H, -NH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 21.5, 52.6, 118.5, 119.2 (q, -CF_3), 120.6, 121.4, 122.6, 123.5, 125.0, 126.8, 127.1, 129.4, 130.8, 132.4, 134.9, 136.5, 137.2, 137.8, 138.6, 139.7, 140.6, 142.3, 146.4, 148.5 (q, C-CF_3), 152.5, 160.2, 170.5; MS (ESI): *m/z* [(M+H)$^+$]: 575. HRMS *m/z* calcd. for $C_{29}H_{21}N_6S_2F_3$ [(M+H)$^+$]: 575.0118. Found: 575.0119.

N-(4-Chlorophenyl)-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-1,3,4-thiadiazol-2-amine (8h): Yield 89% (0.52 g); m.p.: 211-213 °C; IR (KBr, cm^{-1}): 3305 (-NH-), 3228 (-NH-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.06 (d, 2H, -CH_2-), 5.38 (d, 1H, -NH-), 7.24 (dd, *J* = 4.91, 1H, Ar-H), 7.28 (d, *J* = 8.74, 2H, Ar-H), 7.32-7.35 (m, 2H, Ar-H), 7.38 (d, *J* = 8.74, 2H, Ar-H), 7.42-7.46 (m, 3H, Ar-H) 7.62 (dd, *J* = 4.91, 1H, Ar-H), 7.88 (dd, *J* = 3.72, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 10.18 (br. s, 1H, -NH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.1, 118.2, 119.5 (q, -CF_3), 120.5, 121.2, 121.8, 123.1, 123.7, 124.6, 125.2, 126.0, 132.4, 134.6, 135.1, 135.9, 136.7, 138.6, 142.1, 146.6, 148.8 (q, C-CF_3), 150.6, 152.3, 162.8, 170.1; MS (ESI): *m/z* [(M+H)$^+$]: 596. HRMS *m/z* calcd. for $C_{29}H_{21}N_6S_2F_3$ [(M+H)$^+$]: 596.0228. Found: 596.0230.

Synthesis of *N*3-phenyl-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)-methyl)-4*H*-1,2,4-triazole-3,4-diamine derivatives (8i-l): *N*-Phenyl-2-(2-((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)acetyl)hydrazine carbothioamide (**7a**) (0.5 g) (0.1 mmol) was mixed with $N_2H_4{\cdot}H_2O$ (0.01 mol) and MeOH (1 mL). The solution was refluxed for 5-6 h. After cooling to room temperature, ice water (10 mL) was added to the reaction mixture, which was then neutralized with 3 N HCl to form a precipitate. The precipitate was isolated by filtration to afford the triazole derivatives **8i-l**.

***N*3-Phenyl-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-4*H*-1,2,4-triazole-3,4-diamine (8i):** Yield 90% (0.50 g); m.p.: 182-184 °C; IR (KBr, cm^{-1}): 3482, 3325 (-NH_2-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.06 (d, 2H, -CH_2-), 5.38 (d, 1H, -NH-), 6.23 (br. s, 2H, -NH_2), 7.01 (br. s, 1H, -NH-), 7.24 (dd, *J* = 4.92, 1H, Ar-H), 7.28-7.32 (m, 4H, Ar-H), 7.36 (dd, *J* = 4.92, 1H, Ar-H), 7.42-7.47 (m, 6H, Ar-H) 7.88 (dd, *J* = 3.72, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.2, 118.5, 119.3 (q, -CF_3), 125.2, 125.5, 127.1, 128.1, 129.0, 130.6, 133.6, 134.9, 137.3, 139.9, 142.2, 142.8, 143.7, 144.5, 146.3, 146.9, 148.6 (q, C-CF_3), 150.8, 152.4, 156.3, 156.8; MS (ESI): *m/z* [(M+H)$^+$]: 559. HRMS *m/z* calcd. for $C_{28}H_{21}N_8SF_3$ [(M+H)$^+$]: 559.0362. Found: 559.0365.

***N*3-(4-Methoxyphenyl)-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)-methyl)-4*H*-1,2,4-triazole-3,4-diamine (8j):** Yield 86% (0.50 g); m.p.: 154-156 °C; IR (KBr, cm^{-1}): 3475, 3321 (-NH_2-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 3.62 (s, 3H, -OCH_3), 4.02

(d, 2H, -CH_2-), 5.41 (d, 1H, -NH-), 6.21 (br. s, 2H, -NH_2), 7.03 (br. s, 1H, -NH-), 7.21 (dd, *J* = 4.91, 1H, Ar-H), 7.26 (d, *J* = 8.42, 2H, Ar-H), 7.28-7.31 (m, 2H, Ar-H), 7.36 (d, *J* = 8.42, 2H, Ar-H), 7.48-7.52 (m, 3H, Ar-H), 7.61 (dd, *J* = 4.91, 1H, Ar-H), 7.81 (dd, *J* = 3.72, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.5, 57.6, 118.4, 119.6 (q, -CF_3), 120.1, 124.7, 126.4, 126.7, 128.2, 129.0, 131.6, 133.3, 134.4, 137.0, 139.8, 141.9, 142.2, 142.8, 144.3, 146.5, 147.5, 148.3 (q, C-CF_3), 150.7, 152.3, 155.6; MS (ESI): *m/z* [(M+H)$^+$]: 589. HRMS *m/z* calcd. for $C_{29}H_{23}N_8OSF_3$ [(M+H)$^+$]: 589.0857. Found: 589.0859.

5-(((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-*N*3-(*m*-tolyl)-4*H*-1,2,4-triazole-3,4-diamine (8k): Yield 85% (0.48 g); m.p.: 168-170 °C; IR (KBr, cm^{-1}): 3472, 3315 (-NH_2-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 2.43 (s, 3H, -CH_3), 4.04 (d, 2H, -CH_2-), 5.43 (d, 1H, -NH-), 6.25 (br. s, 2H, -NH_2), 7.01 (br. s, 1H, -NH-), 7.21 (dd, *J* = 4.95, 1H, Ar-H), 7.26-7.31 (m, 5H, Ar-H), 7.47 (dd, *J* = 4.95, 1H, Ar-H), 7.52-7.56 (m, 4H, Ar-H) 7.78 (dd, *J* = 3.72, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 23.1, 52.7, 118.1, 118.7, 119.4, 119.8 (q, -CF_3), 120.1, 123.6, 125.5, 126.7, 128.1, 129.0, 130.7, 133.3, 134.3, 135.1, 139.7, 141.9, 142.6, 143.4, 144.5, 146.3, 146.8, 148.7 (q, C-CF_3), 150.5, 153.4, 159.8; MS (ESI): *m/z* [(M+H)$^+$]: 573. HRMS *m/z* calcd. for $C_{29}H_{23}N_8SF_3$ [(M+H)$^+$]: 573.0104. Found: 573.0106.

***N*3-(4-Chlorophenyl)-5-(((2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl)amino)methyl)-4*H*-1,2,4-triazole-3,4-diamine (8l):** Yield 91% (0.53 g); m.p.: 185-187 °C; IR (KBr, cm^{-1}): 3456, 3319 (-NH_2-); ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm 4.05 (d, 2H, -CH_2-), 5.41 (d, 1H, -NH-), 6.23 (br. s, 2H, -NH_2), 7.05 (br. s, 1H, -NH-), 7.19 (dd, *J* = 4.94, 1H, Ar-H), 7.25 (d, *J* = 8.73, 2H, Ar-H), 7.28-7.32 (m, 2H, Ar-H), 7.36 (d, *J* = 8.73, 2H, Ar-H), 7.46-7.49 (m, 3H, Ar-H) 7.69 (dd, *J* = 4.94, 1H, Ar-H), 7.88 (dd, *J* = 3.78, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm 52.3, 119.5 (q, -CF_3), 120.4, 123.0, 124.5, 125.9, 126.7, 127.3, 130.7, 133.3, 134.0, 140.0, 142.7, 144.5, 144.8, 145.6, 147.3, 147.9, 148.3 (q, C-CF_3), 152.4, 152.9, 154.2, 155.3, 156.4; MS (ESI): *m/z* [(M+H)$^+$]: 594. HRMS *m/z* calcd. for $C_{28}H_{20}N_8SClF_3$ [(M+H)$^+$]: 594.0142. Found: 594.0145.

Synthesis of 2-phenyl-*N*-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine derivatives (9a-d): 2-((2-Phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-yl) amino) acetohydrazide (**6**) (1.5 g) (4 mmol) was taken in $POCl_3$ (5 mL) and aromatic acid (4 mmol) was added. The mixture was refluxed for 5-6 h and after cooling to room temperature, poured into crushed ice and neutralized with saturated $NaHCO_3$. Extracted with ethyl acetate, the organic layer was separated and dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by passing through a silica gel column using ethyl acetate-*n*-hexane as eluents.

2-Phenyl-*N*-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (9a): Yield 90% (1.90 g); m.p.: 162-164 °C; IR (KBr, cm^{-1}): 3215 (-NH-); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 4.06 (d, 2H, -CH_2-), 5.39 (d, 1H, -NH-), 7.19 (dd, *J* = 4.78, 1H, Ar-H), 7.26-7.31 (m, 6H, Ar-H), 7.36 (dd, *J* = 3.72, 1H, Ar-H), 7.43-7.47 (m, 4H, Ar-H), 7.54 (dd, *J* = 4.78, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 52.1, 118.9, 119.4 (q, -CF_3), 121.0, 123.0, 124.5, 125.8, 126.8, 127.7, 128.4, 129.5, 130.6, 131.9, 132.5, 133.5, 137.2, 139.8, 141.8, 142.5, 145.3, 146.9 (q, C-CF_3), 151.7, 158.4, 160.6; MS (ESI): *m/z* [(M+H)$^+$]: 530. HRMS *m/z* calcd. for $C_{28}H_{18}N_5OSF_3$ [(M+H)$^+$]: 530.0025. Found: 530.0027.

***N*-((5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (9b):** Yield 84% (1.87 g); m.p.: 187-189 °C; IR (KBr, cm^{-1}): 3221 (-NH-); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 3.62 (s, 3H, -OCH_3), 4.05 (d, 2H, -CH_2-), 5.36 (d, 1H, -NH-), 7.20 (dd, *J* = 4.82, 1H, Ar-H), 7.25 (d, *J* = 8.42, 2H, Ar-H), 7.29-7.32 (m, 3H, Ar-H) 7.36 (dd, *J* = 3.72, 1H, Ar-H), 7.39 (d, *J* = 8.42, 2H, Ar-H), 7.41-7.43 (m, 2H, Ar-H), 7.54 (dd, *J* = 4.82, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 52.1, 57.8, 120.0, (q, -CF_3), 121.2, 122.8, 123.2, 123.9, 124.8, 125.9, 126.7, 128.6, 129.8, 131.0, 131.5, 133.4, 135.8, 138.8, 140.1, 142.4, 143.5, 146.7 (q, C-CF_3), 149.8, 150.6, 152.7, 159.6; MS (ESI): *m/z* [(M+H)$^+$]: 560. HRMS *m/z* calcd. for $C_{29}H_{20}N_5O_2SF_3$ [(M+H)$^+$]: 560.1054. Found: 560.1056.

2-Phenyl-7-(thiophen-2-yl)-*N*-((5-(*m*-tolyl)-1,3,4-oxadiazol-2-yl)methyl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (9c): Yield 89% (1.91 g); m.p.: 165-167 °C; IR (KBr, cm^{-1}): 3228 (-NH-); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 2.41 (s, 3H, -CH_3), 4.02 (d, 2H, -CH_2-), 5.33 (d, 1H, -NH-), 7.20 (dd, *J* = 4.74, 1H, Ar-H), 7.26-7.31 (m, 6H, Ar-H), 7.33-7.36 (m, 3H, Ar-H) 7.39 (dd, *J* = 3.71, 1H, Ar-H), 7.48 (dd, *J* = 4.74, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 23.1, 52.1, 120.3 (q, -CF_3), 121.8, 122.8, 123.5, 124.9, 125.6, 126.2, 128.2, 129.9, 130.1, 132.0, 133.2, 135.4, 137.4, 138.8, 141.9, 142.8, 143.5, 145.4, 147.8 (q, C-CF_3), 148.8, 150.5, 152.3, 154.7, 161.1; MS (ESI): *m/z* [(M+H)$^+$]: 545. HRMS *m/z* calcd. for $C_{29}H_{20}N_5OSF_3$ [(M+H)$^+$]: 545.0108. Found: 545.0111.

***N*-((5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-phenyl-7-(thiophen-2-yl)-5-(trifluoromethyl)-1,8-naphthyridin-4-amine (9d):** Yield 90% (1.96 g); m.p.: 154-156 °C; IR (KBr, cm^{-1}): 3221 (-NH-); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 4.06 (d, 2H, -CH_2-), 5.36 (d, 1H, -NH-), 7.21 (dd, *J* = 4.76, 1H, Ar-H), 7.26 (d, *J* = 8.71, 2H, Ar-H), 7.30-7.33 (m, 2H, Ar-H), 7.36 (d, *J* = 8.71, 2H, Ar-H), 7.39 (dd, *J* = 3.76, 1H, Ar-H), 7.42-7.45 (m, 3H, Ar-H), 7.49 (dd, *J* = 4.76, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 52.2, 118.9, 119.7 (q, -CF_3), 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, 145.4, 147.6 (q, C-CF_3), 149.7, 152.2, 159.0, 161.7; MS (ESI): *m/z* [(M+H)$^+$]: 548. HRMS *m/z* calcd. for $C_{28}H_{17}F_4N_5OS$ [(M+H)$^+$]: 548.0549. Found: 548.0547.

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 [37] 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) 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.

Antibacterial assay: The antimicrobial activity of the synthesized compounds was determined using a well diffusion method [38] against different pathogenic bacterial and *Candida* strains procured from the MTCC and Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on surface of the media Petri plates, containing Mueller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu/mL (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds dissolved in 10% DMSO at a dose range of 125-0.97 μg were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of neomycin (bacterial strains) and miconazole (*Candida* strains) at a dose range of 125-0.97 μg/well, served as positive controls, while the well containing DMSO, served as negative control. The plates were incubated for 24 h at 30 ºC, and the well containing least concentrationshowing the inhibition zone is considered as the minimum inhibitory concentration. All experiments were performed in duplicate and mean values are represented.

Minimum bactericidal concentration assay: Bactericidal assay [39] was performed in sterile 2.0 mL microfuge tubes against a panel of pathogenic bacterial strains, including *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940, *B. subtilis* MTCC 121, *P. aeruginosa* MTCC 2453, *E. coli* MTCC 739 and *K. planticola* MTCC 530, cultured overnight in Mueller-Hinton broth. Serial dilutions of test compounds were prepared in Mueller-Hinton broth with different concentrations ranging from 0 to 150 μg/mL. To the test compounds, 100 μL of overnight cultured bacterial suspensions was added to reach a final concentration of 1.5×10^8 cfu/mL (equal to 0.5 McFarland) and incubated at 37 ºC for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 μL of suspension from the tubes onto Mueller-Hinton agar plates and were incubated for 24 h at 37 ºC to observe the growth of test organisms. All the experiments were performed in duplicate.

Biofilm inhibition assay: Test compounds were screened in sterile 96-well polystyrene microtiter plates using themodified biofilm inhibition assay [40], against a panel of pathogenic bacterial strains, including *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940, *B. subtilis* MTCC121, *P. aeruginosa* MTCC 2453 and *K. planticola* MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 μg/mL were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 cfu/mL. Aliquots of 100 μL were distributed in each well and then incubated at 37 ºC for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the nonadherentbacteria. Each well of the microtiter plate was stained with 100 μL of 0.1% crystal violet solution followed by 30 min incubation at room temper-ature. Later, the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for 3 to 4 times, and air-dried at room temperature. The crystal violet stained biofilm was solubilized in 95% ethanol (100 μL) and the absorbance was recorded at 540 nm using TRIAD multi-modereader (Dynex Technologies, Inc. USA). Blank wells were used as background check. The inhibition data were interpreted from the dose-response curves, where IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experi-ments were performed in triplicate and the values are indicated as mean ± SD.

RESULTS AND DISCUSSION

2-Oxo-6-(thiophen-2-yl)-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile was reacted with 2-chloroacetamide in the presence of potassium carbonate and obtained 2-O-acetamido-3-cyano-4-trilluoromethyl-6-substituted pyridine, this compound was suspended in *N,N*-dimethylformamide and treated with potassium carbonate at 110-120 °C for 2 h and obtained 2-amino-6-(thiophen-2-yl)-4-(trifluoromethyl)nicotinonitrile (**1**). 2-Amino-6-(thiophen-2-yl)-4-(trifluoromethyl)-nicotinonitrile (**1**) was further reacted with acetophenone in presence of $ZnCl_2$ under microwave irradiation conditions with 540 W power during 15 min and product **2** was obtained in high yield. This compound **2** reacted with ethyl bromoacetate, potassium carbonate and sodium iodide to afford the compound **3**. Compound **3** treated with hydrazine hydrate after that reaction with different substituted phenylisocyanate in the presence of ethanol solvent resulted phenylhydrazine carbothioamide derivatives (**4**). Compound **4** was treated with phenylisothiocyanate to obtain thiourea containing products **5** these compounds on further reaction under different set of conditions with different reagents obtained different azole-substituted naphthyridine derivatives **6a-l**. The products are tabulated in Table-1.

Reaction of compound **4** with diverse substituted aromatic acids in the presence of $POCl_3$ resulted in the formation of 1,3,4-oxadiazole functionalized naphthyridine derivatives (**7a-d**). All the synthesized compounds **6a-l** and **7a-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)" and one (healthy) normal cell line (HEK 293). Compounds **6b**, **6d** and **6l** are found to have promising anticancer activity at micro molar concentration. All the products **6a-o** and **7a-d** were tested against Gram-positive, Gram-negative bacteria and fungal strains. Compounds **6e-h** showed high activity.

TABLE-1
PREPARATION OF NOVEL TRIAZOLOTHIONE, THIADIAZOLE, TRIAZOLE AND OXADIAZOLE-FUNCTIONALIZED TRI FLUOROMETHYLNAPHTHYRIDINE DERIVATIVES (**6a-l** AND **7a-c**)

Entry	Compd.	R	R'	Yield (%)
1	**6a**	-C_6H_5	–	82
2	**6b**	4-*OCH_3*-C_6H_4	–	85
3	**6c**	3-*CH_3*-C_6H_4	–	80
4	**6d**	4-*Cl*-C_6H_4	–	88
5	**6e**	-C_6H_5	–	81
6	**6f**	4-*OCH_3*-C_6H_4	–	83
7	**6g**	3-*CH_3*-C_6H_4	–	79
8	**6h**	4-*Cl*-C_6H_4	–	89
9	**6i**	-C_6H_5	–	90
10	**6j**	4-*OCH_3*-C_6H_4	–	86
11	**6k**	3-*CH_3*-C_6H_4	–	85
12	**6l**	4-*Cl*-C_6H_4	–	91
13	**7a**	–	-C_6H_5	90
14	**7b**	–	4-*CH_3*-C_6H_4	84
15	**7c**	–	4-*F*-C_6H_4	89
16	**7d**	–	3-*CF_3*-C_6H_4	90

Compounds were screened for *in vitro* 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); HEK-293-human embryonic kidney cells (CRL-1573) using MTT assay [37]. IC_{50} values of the test compounds for 24 h on each cell line was calculated and are presented in Table-2.

Anticancer activity: All the compounds (except **6e-k**) showed good activity against four cancer cell lines at micro molar concentration. Among all the compounds **6b**, **6d** and **6l** showed good activity and remaining compounds showed moderate to good activity. Among all the derivatives, compound **6d** exhibited good activity on COLO205 cells at IC_{50} < 8.5 μg/mL and other derivatives **6b** and **6l** also showed promising activity on all cell lines at IC_{50} < 19.8 μg/mL. Compound **6d** was considered as the more potent towards all the cancer cell lines. The structure-activity relationship studies explained that chlorine containing phenyl at 4th position shows more activity compared to other group containing to phenyl and also (-CF_3) trifluoromethyl groups at a appropriate position. The presence of CF_3 group, it increase the properties of lipid solubility and thereby enhances the transport mechanism and bioefficacy. Thieno fused pyridine is additional advantage in promoting cytotoxicity activity compared to furo fused pyridine ring.

Antimicrobial activity and structure activity relationship: Compounds **6a-l** and **7a-d** were tested for antimicrobial activity against seven bacterial organisms such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* MTCC 530 and one fungal strain *Candida albicans* MTCC 3017. Out of 12 compounds screened, six compounds **6e**, **6f**, **6g** and **6h** showed good activity against Gram-positive *Bacillus subtilis* MTCC 121. Structure *versus* activity relationship revealed that specific variation in structure showed enhanced activity. If we consider compound **6e-h** series of 1,3,4-oxadiazole derivatives, compounds **6e**, **6f** and **6g** showed MIC 6.8 μg/mL and compound **6h** showed good activity MIC 2.9 μg/mL. It may, due to the presence of trifluoromethyl group at 4th position of phenyl. Compounds **6e-h** (1,3,4-thiadiazole derivatives) plays a promising role to promote antibacterial activity against Gram-positive *Bacillus subtilis* MTCC 121. Among all the derivatives, compound **6h** showed high activity *i.e.* MIC is 2.9 μg/mL. However, compounds **6a-l** could not show activity upto the concentration of 130 μg/mL against all organisms. The details of activity data are outlined in Tables 3-5.

TABLE-2
in vitro CYTOTOXICITY OF COMPOUNDS **5a-p**

Compd.	IC_{50} values (μM)				
	HeLa	COLO205	HepG2	MCF7	HEK293
6a	21.4 ± 0.32	26.2 ± 0.23	29.4 ± 0.21	39.3 ± 0.25	58 ± 0.28
6b	11.5 ± 0.12	19.4 ± 0.22	18.4 ± 0.26	12.5 ± 0.36	98 ± 0.34
6c	27.6 ± 0.13	34.2 ± 0.21	45.6 ± 0.16	29.3 ± 0.32	68 ± 0.26
6d	16.2 ± 0.23	08.5 ± 0.15	17.5 ± 0.23	19.8 ± 0.26	45 ± 0.66
6e	51.3 ± 0.21	–	48.2 ± 0.48	–	69 ± 0.29
6f	64.8 ± 0.58	–	72.6 ± 0.61	–	–
6g	–	–	64.7 ± 0.30	52.8 ± 0.28	83 ± 0.72
6h	–	–	51.5 ± 0.58	66.2 ± 0.52	87 ± 0.69
6i	25.6 ± 0.11	–	29.8± 0.38	–	58 ± 0.45
6j	35.2 ± 0.21	25.8 ± 0.27	–	62.6 ± 0.27	96 ± 0.47
6k	29.4 ± 0.36	34.4 ± 0.29	–	–	48 ± 0.28
6l	15.2 ± 0.28	18.2 ± 0.32	13.8 ± 0.29	11.5 ± 0.34	104 ±0.62
7a	52.8 ± 0.26	33.5 ± 0.46	41.9 ± 0.63	62.7 ± 0.18	–
7b	42.2 ± 0.28	51.3 ± 0.44	38.6 ± 0.49	32.8 ± 0.32	66 ± 0.36
7c	26.4 ± 0.22	32.5 ± 0.24	38.4 ± 0.42	28.4 ± 0.32	–
7d	2.2 ± 0.180	16.1 ± 0.11	18.3 ± 0.28	12.8 ± 0.32	–
5-Fluorouracil (Std control)	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07	19.6 ± 0.19

– indicates IC_{50} value > 112.5 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); HEK-293 – Human embryonic kidney cells (CRL-1573).

TABLE-3
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY DATA OF SYNTHESIZED COMPOUNDS

Entry	Compd.	Minimum inhibitory concentration (MIC) (µg/mL)							
		Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Bacillus subtilis* MTCC 121	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Klebsiella planticola* MTCC 530	*Candida albicans* MTCC 3017
1	**6a**	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0
2	**6b**	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0
3	**6c**	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0
4	**6d**	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0	>55.0
5	**6e**	>115.0	>115.0	>115.0	6.8	>115.0	>115.0	>115.0	>115.0
6	**6f**	>115.0	>115.0	>115.0	6.8	>115.0	>115.0	>115.0	>115.0
7	**6g**	>115.0	>125.0	>115.0	6.8	>115.0	>115.0	>115.0	>115.0
8	**6h**	>115.0	>115.0	>115.0	2.9	>115.0	>115.0	>115.0	>115.0
9	**6i**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
10	**6j**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
11	**6k**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
12	**6l**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
13	**7a**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
14	**7b**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
15	**7c**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
16	**7c**	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0	>130.0
Ciprofloxacin		0.9	0.9	0.9	0.9	0.9	0.9	0.9	–
Miconazole (Std.)		–	–	–	–	–	–	–	7.8

TABLE-4
BACTERICIDAL ACTIVITY RESULTS

Test compound	Minimum bactericidal concentration (µg/mL)
	Bacillus subtilis MTCC 121
6e	6.8
6f	6.8
6g	6.8
6h	2.9
Ciprofloxacin (Std.)	1.17

TABLE-5
BIO-FILM INHIBITION ASSAY RESULTS

Test compound	IC_{50} values (µg/mL)
	Bacillus subtilis MTCC 121
6e	4.2 ± 0.16
6f	3.8 ± 0.32
6g	3.9 ± 0.26
6h	2.5 ± 0.32
Erythromycin (Std.)	0.2 ± 0.09

Conclusion

In conclusion, we synthesized novel triazolothione, thiadiazole, triazole and oxadiazole functionalized trifluoromethyl naphthyridine derivatives and All the synthesized compounds **6a-l** and **7a-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) and one normal cell line (HEK 293)". Compounds **6b**, **6d** and **6l** are found to have promising anticancer activity at micromolar concentration and found to be non-toxic on normal cell line. And all the products **6a-o** and **7a-d** were tested against Gram-positive, Gram-negative bacteria and fungal strains. Compounds **6e-h** showed good activity against *Bacillus subtilis* microbial-type culture collection (MTCC) 121 at < 8.0 micromolar concentration. After that promising compounds screened for minimum bactericidal concentration against *B. subtilis* MTCC 121 using ciprofloxacin as standard and found to show very good activity. These compounds also screened for biofilm inhibition activity against *B. subtilis* MTCC 121 using erythromycin as standard and confirmed the high activity.

CONFLICT OF INTEREST

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

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