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Synthesis and Anticancer Activity of Novel Oxadiazole Functionalized Pyrazolo[3,4-*b*]pyridine Derivatives

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A series of novel oxadiazole functionalized pyrazolo[3,4-*b*]pyridine derivatives (**6a-n**) was synthesized using 6-thiophenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**1**) through reaction with 2-bromoethyl acetate, followed by hydrazine hydrate to afford hydrazide derivatives (**5**). These compounds were further treated with aromatic acids in the presence of phosphoryl chloride and obtained oxadiazole functionalized pyrazolo[3,4-*b*]pyridine derivatives (**6a-n**). All the synthesized compounds **6a-n** 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). Compounds **6i, 6m** and **6n** were found to have more prominent anticancer activity at micromolar concentration.

Keywords: Pyrazolo[3,4-*b*]pyridine, Oxadiazole, Anticancer activity.

INTRODUCTION

Heterocyclic ring systems are very important in medicinal and industrial applications. Among them, 1,3,4-oxadiazoles derivatives have wide range of biological activities like antimicrobial [1], anti-HIV [1], antitubercular [2], antimalarial [3], analgesic [4], anti-inflammatory [5], anticonvulsant [6], hypoglycemic [7-9]. Pyrazolo[3,4-*b*]pyridine ring system also present in many biologically active and pharmaceutically important compounds and these pyrazolo[3,4-*b*]pyridine derivatives acted like anticancer agents [10,11], glycogen synthase kinase-3 (GSK-3) inhibitors [12-14], A1 adenosine receptor antagonist [15], phosphodi esterase 4(PDE4) inhibitors [16], antipyretic and ACTH (adrenocorticotropichormone) releasing factor antagonist activity.

Pyrazolopyridine and their heterocyclic derivatives are bioactive molecules [17-19]. The pyrazolo[3,4-*b*]pyridine ring system belongs to interesting class in heterocyclic chemistry and some their derivatives are more prominent as anticancer agents with low toxicity [20], anti-inflammatory agents [21,22], blood platelet aggregation inhibitors [23], bone metabolism improvers [24], adenosine antagonists [21,22] and controlling herbicides. Some of the drugs pyrazolopyridine classes are shown in Fig. 1.

Further fluorine or trifluoromethyl group containing molecules showed wide range of spectrum antimicrobial and biological properties. Keeping in view of these and in continuation of our work on biologically active molecules, we hereby reported the synthesis of some novel 1,3,4-oxadiazoles functionalized pyrazolo[3,4-*b*]pyridine derivatives and screened them for anticancer activity against four human cancer cell lines.

EXPERIMENTAL

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Bruker AV 300MHz in $CDCl_3$ & 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.

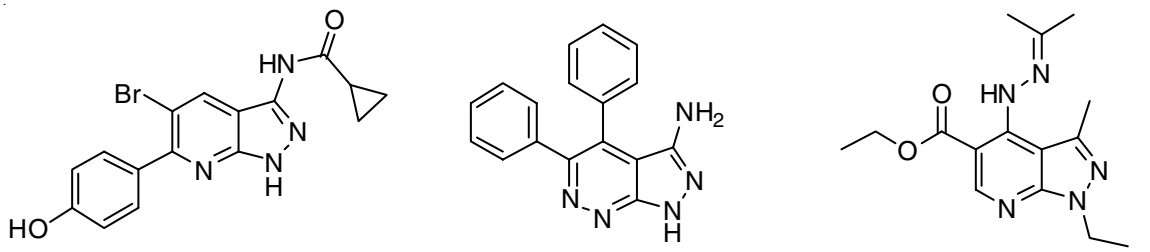


Fig. 1. Bio-active compounds based on pyrazole[3,4-*b*]pyridine derivatives

General procedure for the synthesis of oxadiazole functionalized pyrazolo[3,4-*b*]pyridine derivatives (6a-n): 2-(3-Amino-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-1-yl)acetohydrazide (**5**) (1 mmol) on reaction with diverse substituted aromatic acids in the presence of $POCl_3$ at 120 ºC for 6-8 h to afford 6-phenyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-3-amine (**6**) (**Scheme-I**).

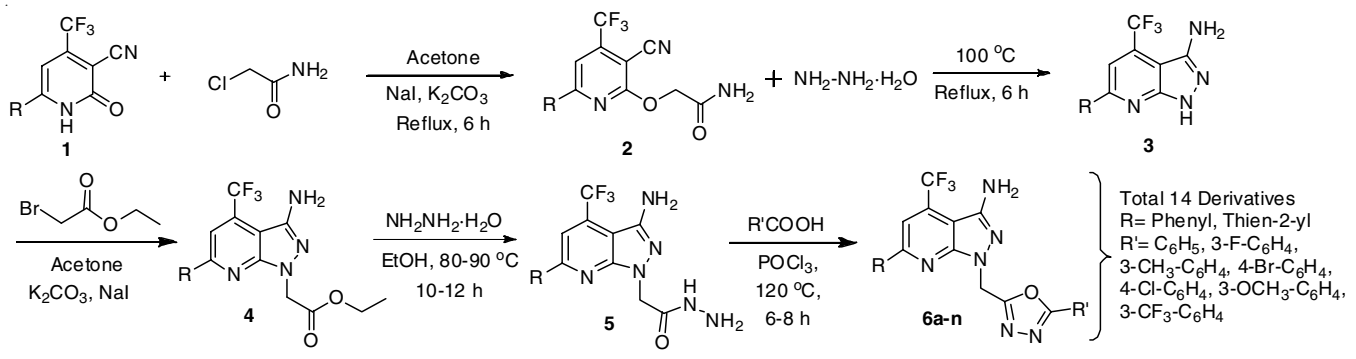
6-Phenyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6a): Yield: 85%; yellow solid; m.p. 152-154 ºC; IR (KBr, ν_{max}, cm^{-1}): 3475, 3321 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.34 (br.s, 2H, -NH_2), 5.03 (s, 2H, -CH_2-N-), 7.38-7.43 (m, 6H, Ar-H), 7.51-7.58 (m, 2H, Ar-H), 7.62-7.66 (m, 2H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 48.9, 118.9, 119.4, 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; MS (ESI): *m/z* [(M+H)$^+$]: 437. HRMS *m/z* calcd. for $C_{22}H_{15}N_6OF_3$ [(M+H)$^+$]: 437.0115 Found: 437.0118.

1-((5-(3-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6b): Yield: 80%; yellow solid; m.p. 169-171 ºC; IR (KBr, ν_{max}, cm^{-1}): 3316, 3452 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 4.32 (br.s, 2H, -NH_2), 5.10 (s, 2H, -CH_2-N-), 7.29-7.36 (m, 5H, Ar-H), 7.46 (s, 1H, Ar-H), 7.53-7.58 (m, 3H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm: 48.6, 118.3, 120.5, 121.6, 123.0, 123.1, 123.7, 125.2, 126.0, 128.0, 129.0, 129.8, 129.9, 131.3, 133.4, 133.8, 134.6, 136.8, 142.1, 143.8; MS (ESI): *m/z* [(M+H)$^+$]: 455. HRMS *m/z* calcd. for $C_{22}H_{14}N_6OF_4$ [(M+H)$^+$]: 455.0247 Found: 455.0250.

6-Phenyl-1-((5-(*m*-tolyl)-1,3,4-oxadiazol-2-yl)methyl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6c): Yield: 78%; yellow solid; m.p. 186-188 ºC; IR (KBr, ν_{max}, cm^{-1}): 3442, 3295 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.28 (s, 3H, -CH_3) 4.31 (br.s, 2H, -NH_2), 5.12 (s, 2H, -CH_2-N-), 7.26-7.31 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H), 7.49 (s, 1H, Ar-H), 7.53-7.58 (m, 2H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm: 22.3, 48.9, 119.4, 120.9, 121.5, 122.4, 123.2, 124.8, 125.5, 126.3, 126.8, 128.0, 129.1, 130.5, 131.2, 131.4, 133.4, 136.5, 137.4, 138.7, 141.5; MS (ESI): *m/z* [(M+H)$^+$]: 451. HRMS *m/z* calcd. for $C_{23}H_{17}N_6OF_3$ [(M+H)$^+$]: 451.0584 Found: 451.0587.

1-((5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6d): Yield: 69%; yellow solid; m.p. 153-154 ºC; IR (KBr, ν_{max}, cm^{-1}): 3425, 3285 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: δ 4.32 (br.s, 2H, -NH_2), 5.14 (s, 2H, -CH_2-N-), 7.28-7.33 (m, 3H, Ar-H), 7.37 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.46-7.49 (m, 2H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 48.5, 120.8, 122.9, 123.5, 124.8, 126.0, 126.6, 128.2, 128.9, 129.9, 130.2, 131.6, 133.9, 134.1, 134.8, 139.8, 141.2, 144.6; MS (ESI): *m/z* [(M+H)$^+$]: 516. HRMS *m/z* calcd. for $C_{22}H_{14}N_6OBrF_3$ [(M+H)$^+$]: 516.0154 Found: 516.0156.

1-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6e): Yield: 75%; yellow solid; m.p. 168-170 ºC; IR (KBr, ν_{max}, cm^{-1}): 3414, 3278 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.30 (br.s, 2H, -NH_2), 5.12 (s, 2H, -CH_2-N-), 7.27-7.32 (m, 3H, Ar-H), 7.38 (d, 2H, Ar-H), 7.46 (d, 2H, Ar-H), 7.49-7.53 (m, 2H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 48.6, 120.3, 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; MS (ESI): *m/z* [(M+H)$^+$]: 471. HRMS *m/z* calcd. for $C_{22}H_{14}N_6OClF_3$ [(M+H)$^+$]: 471.1052 Found: 471.1055.



Scheme-I: Synthetic rout of oxadiazole tagged pyrazolopyridine derivatives (**6a-n**)

1-((5-(3-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6f): Yield: 70%; yellow solid; m.p. 145-147 ºC; IR (KBr, ν_{max}, cm^{-1}): 3443, 3289 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.81 (s, 3H, -OCH_3), 4.29 (br.s, 2H, -NH_2), 5.13 (s, 2H, -CH_2-N-), 7.28-7.34 (m, 6H, Ar-H), 7.42-7.49 (m, 3H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 48.3, 50.2, 119.5, 120.8, 121.2, 121.9, 122.9, 123.6, 125.0, 125.4, 126.1, 126.8, 131.5, 131.6, 132.5, 133.8, 136.2, 139.0, 140.3, 142.5, 144.6; MS (ESI): *m/z* [(M+H)$^+$]: 467. HRMS *m/z* calcd. for $C_{23}H_{17}N_6O_2F_3$ [(M+H)$^+$]: 467.0105 Found: 467.0107.

6-Phenyl-4-(trifluoromethyl)-1-((5-(3-(trifluoromethyl)-phenyl)-1,3,4-oxadiazol-2-yl)methyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-3-amine (6g): Yield: 82%; yellow solid; m.p. 162-164 ºC; IR (KBr, ν_{max}, cm^{-1}): 3439, 3295 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.32 (br.s, 2H, -NH_2), 5.11 (s, 2H, -CH_2-N-), 7.26-7.29 (m, 3H, Ar-H), 7.33-7.36 (m, 3H, Ar-H), 7.45-7.48 (m, 2H, Ar-H), 7.64 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 48.7, 118.9, 119.7, 121.0, 123.2, 123.3, 125.7, 126.8, 128.3, 129.6, 131.1, 132.6, 133.1, 134.1, 137.8, 140.0, 142.0, 144.3, 146.5, 148.7, 152.0; MS (ESI): *m/z* [(M+H)$^+$]: 505. HRMS *m/z* calcd. for $C_{23}H_{14}N_6OF_6$ [(M+H)$^+$]: 505.1125 Found: 505.1127.

1-((5-Phenyl-1,3,4-oxadiazol-2-yl)methyl)-6-(thiophen-2-yl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6h): Yield: 78%; dark yellow solid; m.p. 152-154 ºC; IR (KBr, ν_{max}, cm^{-1}): 3425, 3304 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.29 (br.s, 2H, -NH_2), 5.13 (s, 2H, -CH_2-N-), 7.19 (dd, 1H, Ar-H), 7.24-7.26 (m, 3H, Ar-H), 7.29 (dd, 1H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 7.48 (dd, 1H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 50.6, 120.6, 121.0, 122.1, 124.1, 125.0, 125.7, 126.3, 127.4, 129.5, 129.6, 130.6, 131.8, 133.0, 135.8, 137.0, 139.8, 143.2, 148.9; MS (ESI): *m/z* [(M+H)$^+$]: 443. HRMS *m/z* calcd. for $C_{20}H_{13}N_6OSF_3$ [(M+H)$^+$]: 443.0056 Found: 443.0059.

1-((5-(3-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-(thiophen-2-yl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6i): Yield: 82%; dark yellow solid; m.p. 152-154 ºC; IR (KBr, ν_{max}, cm^{-1}): 3425, 3304 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.29 (br.s, 2H, -NH_2), 5.13 (s, 2H, -CH_2-N-), 7.19 (dd, 1H, Ar-H), 7.24-7.26 (m, 3H, Ar-H), 7.29 (dd, 1H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 7.48 (dd, 1H, Ar-H), 7.88 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 50.6, 120.6, 121.0, 122.1, 124.1, 125.0, 125.7, 126.3, 127.4, 129.5, 129.6, 130.6, 131.8, 133.0, 135.8, 137.0, 139.8, 143.2, 148.9; MS (ESI): *m/z* [(M+H)$^+$]: 443. HRMS *m/z* calcd. for $C_{20}H_{13}N_6OSF_3$ [(M+H)$^+$]: 443.0056 Found: 443.0059.

6-(Thiophen-2-yl)-1-((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)-methyl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6j): Yield: 73%; dark yellow solid; m.p. 163-165 ºC; IR (KBr, ν_{max}, cm^{-1}): 3415, 3318 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.29 (s, 3H, -CH_3) 4.32 (br.s, 2H, -NH_2), 5.11 (s, 2H, -CH_2-N-), 7.20 (dd, 1H, Ar-H), 7.26-7.32 (m, 3H, Ar-H), 7.41 (dd, 1H, Ar-H), 7.43-7.46 (m, 2H, Ar-H), 7.52 (dd, 1H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 23.1, 50.6, 120.6, 121.0, 122.1, 124.1, 125.0, 125.7, 126.3, 127.4, 129.5, 129.6, 130.6, 131.8, 133.0, 135.8, 139.8, 143.2, 148.9; MS (ESI): *m/z* [(M+H)$^+$]: 457. HRMS *m/z* calcd. for $C_{21}H_{15}N_6OSF_3$ [(M+H)$^+$]: 457.0105 Found: 457.0107.

1-((5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-(thiophen-2-yl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-3-amine (6k): Yield: 65%; dark yellow solid; m.p. 173-165 ºC; IR (KBr, ν_{max}, cm^{-1}): 3432, 3325 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.31 (br.s, 2H, -NH_2), 5.12 (s, 2H, -CH_2-N-), 7.21 (dd, 1H, Ar-H), 7.28 (d, 2H, Ar-H), 7.38 (dd, 1H, Ar-H), 7.49 (d, 2H, Ar-H), 7.53 (dd, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 50.3, 120.9, 121.5, 122.4, 123.2, 124.8, 125.5, 126.3, 128.0, 129.1, 130.5, 131.2, 131.4, 133.4, 136.5, 137.4, 141.5, 146.4; MS (ESI): *m/z* [(M+H)$^+$]: 522. HRMS *m/z* calcd. for $C_{20}H_{12}N_6OSBrF_3$ [(M+H)$^+$]: 522.2304 Found: 522.2306.

1-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-(thiophen-2-yl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-3-amine (6l): Yield: 68%; dark yellow solid; m.p. 148-150 ºC; IR (KBr, ν_{max}, cm^{-1}): 3318, 3417 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.28 (br.s, 2H, -NH_2), 5.14 (s, 2H, -CH_2-N-), 7.19 (dd, 1H, Ar-H), 7.26 (d, 2H, Ar-H), 7.32 (dd, 1H, Ar-H), 7.42 (d, 2H, Ar-H), 7.49 (dd, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 49.6, 121.9, 122.8, 123.2, 124.3, 125.4, 125.9, 127.0, 128.0, 128.7, 129.9, 131.4, 132.0, 135.4, 138.0, 140.8, 143.7, 144.9; MS (ESI): *m/z* [(M+H)$^+$]: 477. HRMS *m/z* calcd. for $C_{20}H_{12}N_6OSClF_3$ [(M+H)$^+$]: 477.0046 Found: 477.0048.

1-((5-(3-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-(thiophen-2-yl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]-pyridin-3-amine (6m): Yield: 76%; dellow solid; m.p. 175-177 ºC; IR (KBr, ν_{max}, cm^{-1}): 3389,3298 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.29 (br.s, 2H, -NH_2), 5.15 (s, 2H, -CH_2-N-), 7.19 (dd, 1H, Ar-H), 7.26-7.29 (m, 3H, Ar-H), 7.32 (dd, 1H, Ar-H), 7.46 (dd, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm: 48.6, 50.5, 120.3, 121.6, 122.1, 123.2, 124.0, 125.3, 126.0, 126.7, 128.7, 130.2, 130.7, 132.8, 135.0, 135.9, 138.0, 139.2, 144.1, 146.8, 153.2; MS (ESI): *m/z* [(M+H)$^+$]: 473. HRMS *m/z* calcd. for $C_{21}H_{15}N_6O_2SF_3$ [(M+H)$^+$]: 473.0105 Found: 473.0107.

6-(Thiophen-2-yl)-4-(trifluoromethyl)-1-((5-(3-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)methyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6n): Yield: 81%; dark yellow solid; m.p. 168-170 ºC; IR (KBr, ν_{max}, cm^{-1}): 3425, 3320 (-NH_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 4.32 (br.s, 2H, -NH_2), 5.11 (s, 2H, -CH_2-N-), 7.21 (dd, 1H, Ar-H), 7.25-7.29 (m, 3H, Ar-H), 7.36 (dd, 1H, Ar-H), 7.42 (dd, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 49.3, 119.0, 121.3, 122.8, 123.3, 125.4, 126.5, 127.1, 128.0, 131.6, 132.2, 133.8, 136.1, 139.0, 140.7, 142.3, 143.7, 144.8, 146.6, 148.5, 151.7; MS (ESI): *m/z* [(M+H)$^+$]: 511. HRMS *m/z* calcd. for $C_{21}H_{12}N_6OSF_6$ [(M+H)$^+$]: 511.0024 Found: 511.0026.

Cytotoxicity assay: By using 5-fluorouracil as standard, the cytotoxicity of the synthesized compounds was estimated in 96-well plates by measuring the *in vitro* growth inhibition of tumour cell lines through the cell-mediated reduction of tetrazolium salt to water-insoluble formazan crystals. Cytotoxicity was estimated against the panel of five human tumour cell lines: COLO 205 derived from human colon cancer cells (ATCC

No. CCL-222), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) and HepG2 derived from human liver cancer cells (ATCC No. HB-8065), by using the MTT assay [25]. The IC_{50} values (in mM) were calculated using the absorbance data plotted for the dose-response curves and are represented as the mean ± SD of the three independent experiments.

RESULTS AND DISCUSSION

Chemistry: 2-Oxo-6-(thiophen-2-yl)-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (**1**) on reaction with chloroacetamide in the presence of K_2CO_3, 2-((3-cyano-6-(thiophen-2-yl)-4-(trifluoromethyl)pyridin-2-yl)oxy)acetamide (**2**) (O-tagged acetamide derivative) was obtained, which further reaction with hydrazine hydrate under refluxing conditions to afford cyclized pyrazolopyridine (**3**) [23]. Cyclized pyrazolopyridine (**3**) on further treated with bromoethyl acetate in basic conditions and obtained ethyl 2-(3-amino-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl)acetate (**4**). Compound **4** on reaction with hydrazine hydrate to afford 2-(3-amino-6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl)acetohydrazide (**5**) derivatives. The hydrazide compound **5** on reaction with diverse substituted aromatic acids in the presence of $POCl_3$ to obtain 6-phenyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**6a-n**) derivatives. The synthetic route are outlined in **Scheme-I** and products are tabulated in Table-1.

TABLE-1
In vitro CYTOTOXICITY OF COMPOUNDS **6a-n**

Compd.	IC_{50} values (μM)			
	HeLa	COLO205	HepG2	MCF7
6a	53.2 ± 0.13	39.6 ± 0.36	45.2 ± 0.21	–
6b	46.1 ± 0.24	32.3 ± 0.23	–	42.1 ± 0.25
6c	56.5 ± 0.19	64.6 ± 0.31	110.4 ± 0.37	–
6d	–	–	–	–
6e	41.3 ± 0.28	54.5 ± 0.24	89.4 ± 0.21	–
6f	51.2 ± 0.23	46.6 ± 0.19	58.6 ± 0.51	–
6g	35.2 ± 0.12	–	41.1 ± 0.15	42.8 ± 0.32
6h	28.5 ± 0.24	37.7 ± 0.21	21.5 ± 0.35	26.8 ± 0.27
6i	18.2 ± 0.11	12.3 ± 0.31	11.3 ± 0.17	14.4 ± 0.21
6j	31.1 ± 0.42	29.4 ± 0.17	51.2 ± 0.27	62.6 ± 0.52
6k	63.5 ± 0.21	–	42.7 ± 0.38	–
6l	55.3 ± 0.16	–	–	51.7 ± 0.52
6m	10.3 ± 0.22	12.6 ± 0.32	19.3 ± 0.41	22.3 ± 0.28
6n	9.3 ± 0.35	14.5 ± 0.18	11.4 ± 0.23	21.2 ± 0.24
Control	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07

Control = 5-Fluorouracil (standard control)
– Indicates IC_{50} value >110.4 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)

SAR studies: Among all the synthesized oxadiazole functionalized pyrazolo[3,4-*b*]pyridine derivatives (**6a-n**) screened, many compounds showed activity against four cancer cell lines at micromolar concentration. Among all the synthesized compounds **6i**, **6m** and **6n** showed promising activity, while the rest of compounds showed medium activity. Compound **6i** found to be the more potent towards all the cancer cell lines. The structure activity relationship studies revealed that fluorine substitutent containing compound shows more activity compared to simple and all other substitutes and at 6th position thieno-2-yl group containing additional advantage in promoting cytotoxicity activity.

Conclusion

In conclusion, a series of novel oxadiazole functionalized pyranopyrazole derivatives **6a-n** were synthesized, characterized and also evaluated for anticancer activity against four human cancer cell lines. Among all the compounds screened **6i, 6m** and **6n** compounds showed significant activity against all cell lines at micromolar concentration.

CONFLICT OF INTEREST

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

REFERENCES

1. A.A. El-Emam, O.A. Al-Deeb, M. Al-Omar and J. Lehmann, *Bioorg. Med. Chem.*, **12**, 5107 (2004); https://doi.org/10.1016/j.bmc.2004.07.033
2. S.G. Kucukguzel, E.E. Oruc, S. Rollas, F. Sahin and A. Ozbek, *Eur. J. Med. Chem.*, **37**, 197 (2002); https://doi.org/10.1016/S0223-5234(01)01326-5
3. P.R. Kagthara, N.S. Shah, R.K. Doshi and H.H. Parekh, *Indian J. Chem.*, **38B**, 572 (1999).
4. M. Akhter, A. Husain, B. Azad and M. Ajmal, *Eur. J. Med. Chem.*, **44**, 2372 (2009); https://doi.org/10.1016/j.ejmech.2008.09.005
5. P.C. Unangst, G.P. Shrum, D.T. Connor, R.D. Dyer and D.J. Schrier, *J. Med. Chem.*, **35**, 3691 (1992); https://doi.org/10.1021/jm00098a015
6. M.S.Y. Khan, R.M. Khan and S. Drabu, *Indian J. Heterocycl. Chem.*, **11**, 119 (2001).
7. J.B. O'Neal, H. Rosen, P.B. Russell, A.C. Adams and A. Blumenthal, *J. Med. Chem.*, **5**, 617 (1962); https://doi.org/10.1021/jm01238a019
8. A.O. Maslat, M. Abussaud, H. Tashtoush and M. Al-Talib, *Pol. J. Pharmacol.*, **54**, 55 (2002).
9. A.A. Farghaly, A.A. Bekhit and J.Y. Park, *Arch. Pharm.*, **333**, 53 (2000); https://doi.org/10.1002/(SICI)1521-4184(200002)333:2/3<53::AID-ARDP53>3.0.CO;2-E
10. S. Ravula, R.R. Bobbala and B. Kolli, *J. Heterocycl. Chem.*, **57**, 2535 (2020); https://doi.org/10.1002/jhet.3968
11. M.S. Salem and M.A.M. Ali, *Biol. Pharm. Bull.*, **39**, 473 (2016); https://doi.org/10.1248/bpb.b15-00586
12. J. Witherington, V. Bordas, S.L. Garland, D.M.B. Hickey, R.J. Ife, J. Liddle, M. Saunders, D.G. Smith and R.W. Ward, *Bioorg. Med. Chem. Lett.*, **13**, 1577 (2003); https://doi.org/10.1016/S0960-894X(03)00134-3
13. J. Witherington, V. Bordas, A. Gaiba, N.S. Garton, A. Naylor, A.D. Rawlings, B.D. Slingsby, D.G. Smith, A.K. Takle and R.W. Ward, *Bioorg. Med. Chem. Lett.*, **13**, 3055 (2003); https://doi.org/10.1016/S0960-894X(03)00645-0
14. J. Witherington, V. Bordas, A. Gaiba, A. Naylor, A.D. Rawlings, B.P. Slingsby, D.G. Smith, A.K. Takle and R.W. Ward, *Bioorg. Med. Chem. Lett.*, **13**, 3059 (2003); https://doi.org/10.1016/S0960-894X(03)00646-2
15. F. Manetti, S. Schenone, F. Bondavalli, C. Brullo, O. Bruno, A. Ranise, L. Mosti, G. Menozzi, P. Fossa, M.L. Trincavelli, C. Martini, A. Martinelli, C. Tintori and M. Botta, *J. Med. Chem.*, **48**, 7172 (2005); https://doi.org/10.1021/jm050407k

16. J.N. Hamblin, T.D.R. Angell, S.P. Ballantine, C.M. Cook, A.W.J. Cooper, J. Dawson, C.J. Delves, P.S. Jones, M. Lindvall, F.S. Lucas, C.J. Mitchell, M.Y. Neu, L.E. Ranshaw, Y.E. Solanke, D.O. Somers and J.O. Wiseman, *Bioorg. Med. Chem. Lett.*, **18**, 4237 (2008); https://doi.org/10.1016/j.bmcl.2008.05.052
17. M. Michailidou, V. Giannouli, V. Kotsikoris, O. Papadodima, G. Kontogianni, I.K. Kostakis, N. Lougiakis, A. Chatziioannou, F.N. Kolisis, P. Marakos, N. Pouli and H. Loutrari, *Eur. J. Med. Chem*., **121**, 143 (2016); https://doi.org/10.1016/j.ejmech.2016.05.035
18. D. Matsuda, Y. Kobashi, A. Mikami, M. Kawamura, F. Shiozawa, K. Kawabe, M. Hamada, K. Oda, S. Nishimoto, K. Kimura, M. Miyoshi, N. Takayama, H. Kakinuma and N. Ohtake, *Bioorg. Med. Chem. Lett*., **26**, 3441 (2016); https://doi.org/10.1016/j.bmcl.2016.06.050
19. N.S. El-Gohary and M.I. Shaaban, *Eur. J. Med. Chem*., **152**, 126 (2018); https://doi.org/10.1016/j.ejmech.2018.04.025
20. I.H. Eissa, A.M. El-Naggar and M.A. El-Hashash, *Bioorg. Chem*., **67**, 43 (2016); https://doi.org/10.1016/j.bioorg.2016.05.006
21. H. El-Kashef, T. El-Emary, P. Verhaeghe, P. Vanelle and M. Samy, *Molecules*, **23**, 2657 (2018); https://doi.org/10.3390/molecules23102657
22. L.W. Mohamed, M.A. Shaaban, A.F. Zaher, S.M. Alhamaky and A.M. Elsahar, *Bioorg. Chem*., **83**, 47 (2019); https://doi.org/10.1016/j.bioorg.2018.10.014
23. A. Ramzan, A. Nazeer, A. Irfan, A.G. Al-Sehemi, F. Verpoort, Z.A. Khatak, A. Ahmad, M.A. Munawar, M.A. Khan and M.A.R. Basra, *Z. Phys. Chem*., **233**, 1741 (2019); https://doi.org/10.1515/zpch-2018-1316
24. K. Imaizumi and T. Sado, *Chem. Abstr*., **121**, 91797 (1994).
25. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4