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Synthesis and antiproliferative activity of 3-(substituted)-4,5,6,7-tetrahydro-6-(substituted)-1*H*-pyrazolo[3,4-c]pyridine derivatives

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ABSTRACT

A series of new molecules having 3-(substituted)-4,5,6,7-tetrahydro-6-(substituted)-1*H*-pyrazolo[3,4-*c*]pyridine and 3-(substituted)-5,6-dihydro-6-(substituted)-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one derivatives were designed and synthesized in large scale (grams range). The structures of the synthesized compounds were elucidated and confirmed by ^{1}H NMR, ^{13}C NMR, Mass spectra; and purity was also checked through LC/MS and HPLC analysis. The antiproliferative activity of the compounds was checked for lung cancer, cervical cancer, breast cancer and prostate cancer on panel of four cell lines. A few compounds (13c, 13g, 15g and 15h) showed promising antiproliferative activity in the range of 5.12-17.12 µM which were further tested for their inhibitory activity against panel of 8 human kinases at 10 µM concentrations. The compounds 13c, 13g, 15g and 15h shows prominent inhibitory activity against Aurora-A, Aurora-B, CDK_5/P_{25} and mTOR kinases.

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1. Introduction

Cancer treatment is difficult due to plethora of unwanted side effects [1]. In present study, we have chosen 4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine and 5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one as core structure as 4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine is showing diversified biological activities as anticancer activity [2], 5-HT_6 inhibitors for pain treatment [3], inflammatory disorders [4], GnRH receptor antagonists [5], $kinase_1$ inhibitors [6], cannabinoid receptors [7], inhibitor of blood coagulation factor Xa [8], PDE_4 inhibitor [9], COX-2 inhibitors [10], antimicrobial [11] and P13K inhibitors [12]. The pyrazole is known for adenine mimetic pharmacophore and is useful in inhibitors of several classes of kinases like Aurora, CDK-2 and MAP kinases as these plays key role in drug discovery [2]. The tetrahydro-1*H*-pyrazole and their derivatives show diversifying activity. By considering their biological importance herein we report the synthesis of 3-(substituted)-4,5,6,7-tetrahydro-6-(substituted)-1*H*-pyrazolo[3,4-*c*]pyridine and 3-(substituted)-5,6-dihydro-6-(substituted)-1*H*-pyrazolo[3, 4-*c*]pyridin-7(4*H*)-one and anticancer activity in cell line along with kinase inhibition study. We have optimized routes for their synthesis. The synthetic methods adopted for the preparation of the title compounds **13a-h** and **15a-h** are depicted in Schemes 1 and 2 presented below.

2. Experimental

2.1. Reagent and instrumentation

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avra Labs. The development of reactions was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F_{254} aluminum sheets, visualized by UV light. All reactions were carried out under argon inert atmosphere. Melting points were recorded on SRS OptiMelt. The purity of intermediates was pursued by TLC, NMR, and LC-MS. All final compounds and intermediates are characterized by NMR, LC-MS and purity of final compounds pursued by HPLC and all structures are consistent with proposed structures characterization. The ^{1}H NMR spectra were recorded on Varian NMR (400 MHz) spectrometer. The ^{13}C NMR spectra were recorded on Varian NMR (100 MHz) spectrometer.

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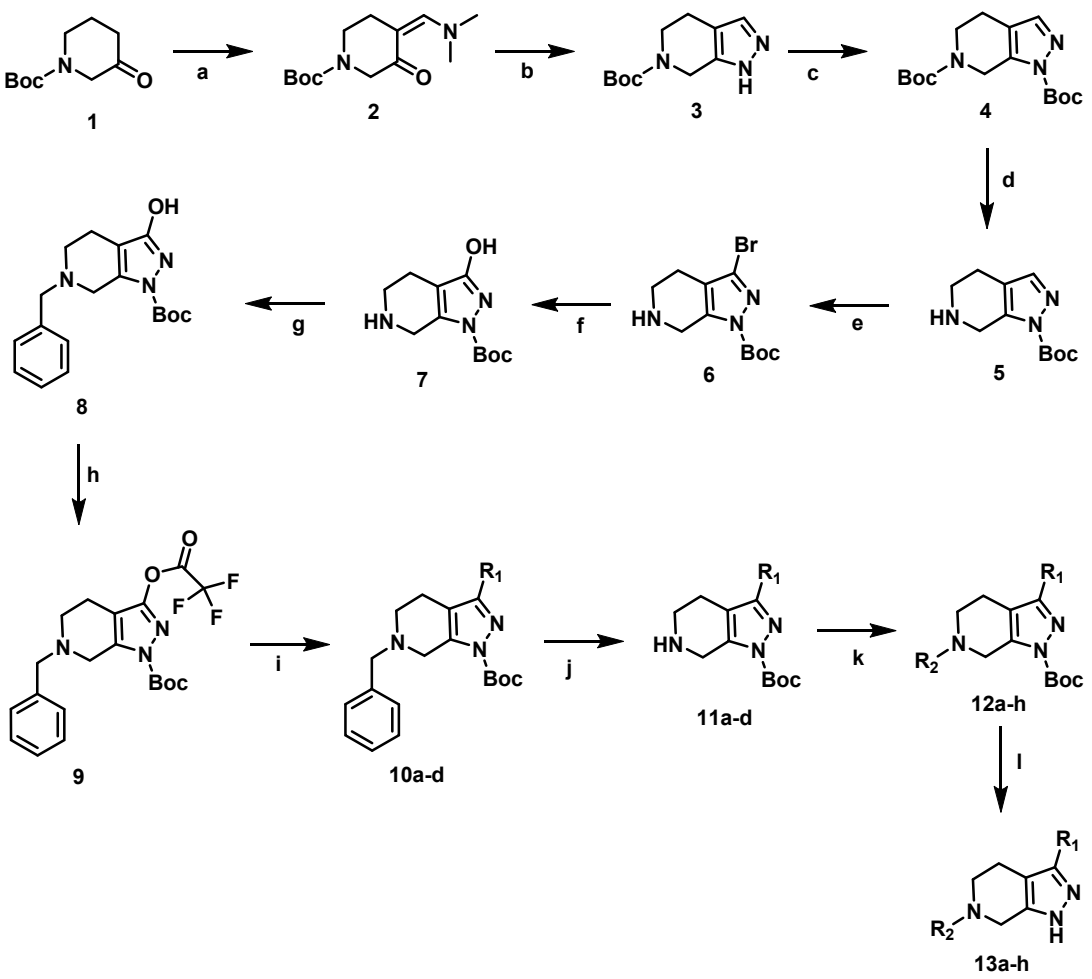


Reagents and conditions: (a) DMF-DMA at 100 °C, 1 h; (b) $N_2H_4.H_2O$, EtOH, 80 °C, 8 h; (c) $(BOC)_2O$, TEA, DCM, room temperature, 3 h; (d) 2 N aq. HCl, room temperature, 2 h; (e) Pyridine Br_2, THF, room temperature, 3 h; (f) 2 N NaOH, 100 °C, 3 h (g) benzyl bromide, 2,6-lutidine, DMAP, THF, room temperature, 8 h; (h) Triflic anhydride, TEA, DCM, room temperature, 6 h; (i) Aromatic boronic acid, $Pd_2(dba)_3$, Ruphos, Cs_2CO_3, toluene, 100 °C, 6 h, (general procedure); (j) Pd/C, H_2, MeOH, 50 psi, room temperature, 3 h (general procedure); (k) Alkyl bromide, 2,6-lutidine, DMAP, THF, room temperature, 8 h (general procedure); (l) 6 N aq. HCl, room temperature, 6 h (general procedure).

Scheme 1

The chemical shifts are reported as NMR spectra δ_{ppm} units. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken using Varian VG 7070 spectrometer at nominal 5000 resolution. The purity of final compounds was determined by HPLC on an Alltech Alltima C18 column (3.2 × 150 mm, 5 µM) eluting with 5-80% acetonitrile / 45 nM sodium bicarbonate.

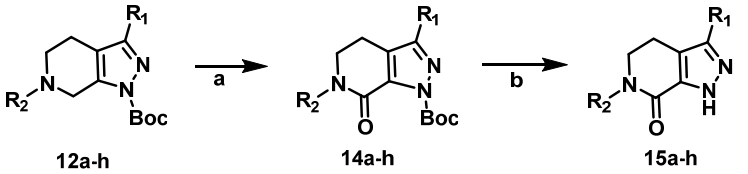
2.2. Synthesis

2.2.1. Synthesis of tert-butyl-4-((dimethylamino)methyl ene)-3-oxopiperidine-1-carboxylate (2), Step (a)

To a stirred solution of *N-tert*-butoxycarbonyl-3-piperidone (**1**) (10.0 g, 50.2 mmol) in *N*,*N*-dimethylformamide dimethylacetal (50 mL). The reaction mixture was heated at 100 °C for 1 h. Progress of reaction was monitored by LC/MS for the consumption of starting material. After completion the reaction, the reaction mixture cooled to room temperature and evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was diluted it with H_2O (100 mL) and extracted it with EtOAc (2 × 50 mL). The organic layer was washed with H_2O (50 mL) and brine (50 mL), dried it over anhydrous Na_2SO_4 to obtain yellow solid. The crude material was washed with 10% ethyl acetate:hexane (*v*:*v*, 10:90, 100 mL), hexane (100 mL) and diethyl ether (100 mL) to obtain compound **2**. Color: Yellow. Yield: 78 % (10.0 g). M.p.: 48-49 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (sS, 9H, *t*-Bu), 2.22 (d, 2H, J = 6.4 Hz, CH_2), 2.44 (s, 6H, N-$(CH_3)_2$), 2.81 (m, 2H, CH_2), 3.81 (s, 2H, NH-CH_2), 6.89 (s, 1H, N-CH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 28.55, 30.32, 43.47, 47.46, 58.89, 81.2, 101.21, 146.12, 154.42, 192.22. LC-MS (EI, *m/z*): 255 (M+H). Anal. calcd. for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.01. Found: C, 61.36; H, 8.73; N, 11.03%.

2.2.2. Synthesis of 4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c] pyridine (3), Step (b)

To a stirred solution of compound **2** (10.0 g, 39.4 mmol) was dissolved in ethanol (50 mL) and hydrazine hydrate (3.94 g, 78.7 mmol). The reaction mixture was heated at 80 °C for 8 h and the progress of reaction was monitored by LC-MS for the consumption of starting material.



Reagents and conditions: (a) $KMnO_4$, 18-Crown-6, DCM, room temperature, 6 h, (general procedure); (b) 6 N HCl, room temperature, 6 h (general procedure).

Scheme 2

The reaction mixture was cooled to room temperature and evaporated under reduced pressure to obtain yellow gummy material. The obtained crude material was purified by silica gel (100-200 mesh) column chromatography by using 10-40% ethyl acetate:hexane (*v*:*v*, 10-40:90-60). The obtained compound was washed with diethyl ether (100 mL) to obtain compound **3** [13]. Color: Yellow. Yield: 68.3%, 6 g. M.p.: 74-75 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.46 (s, 9H, t-Bu), 2.22 (d, 2H, *J* = 6.4 Hz, CH_2), 2.80 (m, 2H, CH_2), 3.84 (s, 2H, NH-CH_2), 7.31 (s, 1H, Ar-H), 12.6 (br, 1H, Ar-NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.21, 28.88, 38.41, 47.11, 80.2, 114.12, 133.57, 142.26, 152.88. LC-MS (EI, *m/z*): 225 (M+H). Anal. calcd. for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 59.14; H, 7.68; N, 18.80%.

2.2.3. Synthesis of di-tert-butyl 4,5-dihydro-7H-pyrazolo [3,4-c]pyridine-1,6-dicarboxylate (4), Step (c)

To a stirred solution of compound **3** (5.00 g, 22.4 mmol) in DCM (50 mL), triethylamine (6.00 ml, 44.8 mmol) was added BOC anhydride (7.33 g, 33.6 mmol) and stirred reaction mixture to room temperature for 3 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*, 10:90) (25 mL), hexane (50 mL) and diethyl ether (50 mL) to obtain compound **4**. Color: Yellow. Yield: 91 %, 6.6 g. M.p.: 55-56 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.56 (s, 18H, N-(t-Bu)$_2$), 2.21 (d, 2H, *J* = 6.2 Hz, CH_2), 2.79 (m, 2H, CH_2), 3.83 (s, 2H, N-CH_2), 7.31 (s, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.92, 28.41, 31.32, 44.38, 77.2, 116.6, 135.23, 143.84, 148.66. LC-MS (EI, *m/z*): 325 (M+H). Anal. calcd. for $C_{16}H_{25}N_3O_4$: C, 59.42; H, 7.79; N, 12.99. Found: C, 59.40; H, 7.80; N, 12.97%.

2.2.4. Synthesis tert-butyl 4,5,6,7-tetrahydropyrazolo[3,4-c] pyridine-1-carboxylate (5), Step (d)

To a stirred solution compound **4** (6.50 g, 20.1 mmol) was dissolved in 2 N HCl (65 mL) and stirred reaction mixture to room temperature for 2 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*,10:90, 25 mL), hexane (50 mL) and diethyl ether (50 mL) to obtain compound **5**. Color: Yellow. Yield: 89.3 %, 4 g. M.p.: 61-62 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.56 (s, 9H, N-t-Bu), 2.21 (d, 2H, *J* = 6.2 Hz, CH_2), 2.78 (m, 2H, CH_2), 3.83 (s, 2H, N-CH_2), 7.31 (s, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.26, 28.85, 31.52, 44.28, 78.84, 98.62, 134.36, 144.18, 148.77. LC-MS (EI, *m/z*): 225 (M+H). Anal. calcd. for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 59.19; H, 7.64; N, 18.80%.

2.2.5. Synthesis tert-butyl 3-bromo-4,5,6,7-tetrahydro pyrazolo[3,4-c]pyridine-1-carboxylate (6), Step (e)

To a stirred solution compound **5** (4.00 g, 17.9 mmol) in THF (40 mL) was added pyridine hydrobromide (5.74 g, 35.8 mmol) drop wise at 0 °C. The reaction mixture was stirred at room temperature for 3 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*, 10: 90, 25 mL), hexane (50 mL), and diethyl ether (50 mL), to obtain compound **6**. Color: Yellow. Yield: 92 %, 5 g. M.p.: 145-146 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, N-t-Bu), 2.49 (d, 2H, *J* = 6.2 Hz, CH_2), 2.88 (m, 2H, CH_2), 3.79 (s, 2H, N-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.33, 28.41, 30.76, 4312, 78.81, 115.61, 123.10, 139.18, 148.88. LC-MS (EI, *m/z*): 303 (M+H). Anal. calcd. for $C_{11}H_{16}BrN_3O_2$: C, 47.72; H, 5.34; N, 13.91. Found: C, 47.73; H, 5.32; N, 13.90%.

2.2.6. Synthesis tert-butyl 4,5,6,7-tetrahydro-3-hydroxy pyrazolo[3,4-c]pyridine-1-carboxylate (7), Step (f)

To a stirred solution compound **6** (5.00 g, 16.5 mmol) in 2 N NaOH (50 mL) and heat reaction mixture to 100 °C for 3 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*, 10:90, 50 mL), hexane (50 mL) and diethyl ether (50 mL) to obtain compound **7**. Color: Yellow. Yield: 88.4 %, 3.5 g. M.p.: 87-88 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.54 (s, 9H, N-t-Bu), 2.20 (d, 2H, *J* = 6.2 Hz, CH_2), 2.78 (m, 2H, CH_2), 3.79 (s, 2H, N-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.23, 28.41, 31.76, 44.36, 78.88, 99.10, 134.17, 144.16, 147.46. LC-MS (EI, *m/z*): 240 (M+H). Anal. calcd. for $C_{11}H_{17}N_3O_3$: C, 55.22; H, 7.16; N, 17.56. Found: C, 55.20; H, 7.18; N, 17.58%.

2.2.7. Synthesis of tert-butyl 6-benzyl-4,5,6,7-tetrahydro-3-hydroxypyrazolo[3,4-c]pyridine-1-carboxylate (8), Step (g)

To a stirred solution of compound **7** (3.50 g, 14.6 mmol) in THF (35 mL) was added 2,6-lutidine (3.14 g, 29.3 mmol), DMAP (0.36 g, 2.93 mmol) and benzyl bromide (2.98 g, 16.1 mmol). The reaction mixture was stirred at room temperature for 6 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*, 10:90, 50 mL), hexane (50 mL), and diethyl ether (50 mL), to obtain compound **8**. Color: Yellow. Yield: 83 %, 4.5 g. M.p.: 133-134 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.42 (s, 9H, t-Bu), 2.34 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.70 (s, 2H, N-CH_2), 7.38-7.28 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 9.88, 28.41, 43.44, 52.12, 60.18,

78.81, 98.67, 127.62, 128.21, 128.34, 128.46, 128.58, 135.11, 135.21, 144.66, 148.78. LC-MS (EI, *m/z*): 330 (M+H). Anal. calcd. for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.65; H, 7.01; N, 12.78%.

2.2.8. Synthesis of tert-butyl 3-(2,2,2-trifluoroacetoyloxy)-6-benzyl-4,5,6,7-tetrahydropyrazolo[3,4-c]pyridine-1-carboxylate (9), Step (h)

To a stirred solution of synthesis of compound **8** (3.50 g, 10.6 mmol) in DCM (35 mL), triethylamine (2.12 ml, 15.9 mmol) was added triflouromethanesulfonicanhydride (3.60 g, 12.8 mmol) drop wise at 0 °C. The reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate:hexane (*v*:*v*, 10:90, 50 mL), hexane (50 mL), and diethyl ether (50 mL), to obtain compound **9**. Color: Yellow. Yield: 88.5 %, 4 g. M.p.: 173-174 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.40 (s, 9H, t-Bu), 2.34 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.70 (s, 2H, N-CH_2), 7.38-7.28 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 9.86, 28.41, 43.44, 52.12, 60.18, 78.80, 98.68, 112.13, 127.72, 128.22, 128.32, 128.46, 128.58, 135.13, 135.22, 144.66, 148.78, 168.34. LC-MS (EI, *m/z*): 426 (M+H). Anal. calcd. for $C_{20}H_{22}F_3N_3O_4$: C, 56.47; H, 5.21; N, 9.88. Found: C, 56.44; H, 5.20; N, 9.90%.

2.2.9. General procedure for synthesis of compounds 10a-d, Step (i)

To a stirred solution of compound **9** (1 mmol) in toluene (5 mL) was substituted aromatic boronic acid (2 mmol), 2-dicyclohexylphosphino-2,6-diisopropoxybiphenyl (0.2 mmol), cesium carbonate (3 mmol) and *tris*(dibenzylideneacetone) dipalladium(0) (0.2 mmol). The reaction mixture was purged with argon for 10 min and heat reaction mixture to 100 °C for 6 h. Progress of reaction was monitored by LC-MS. After completion the reaction, the reaction mixture was filtered through a pad of celite, washed with EtOAc (10 mL) and saturated cold sodium chloride solution (2×5 mL) and organic layer was evaporated under reduced pressure to obtain crude gummy material (**10a**-**d**). The obtained crude was purified by silica gel (230-400 mesh) by using ethyl acetate:heptane (15:85, *v*:*v*) to obtain compound **10a**-**d**.

Tert-butyl 3-(benzofuran-2-yl)-6-benzyl-4, 5, 6, 7-tetrahydro pyrazolo[3,4-c]pyridine-1-carboxylate (**10a**): Color: Brown. Yield: 83%. M.p.: 131-132 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9 H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.67 (m, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.71 (s, 2H, N-CH_2), 6.65 (s, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.38-7.28 (m, 7H, Ar-H). LC-MS (EI, *m/z*): 430 (M+H). Anal. calcd. for $C_{26}H_{27}N_3O_3$: C, 72.71; H, 6.34; N, 9.78. Found: C, 72.70; H, 6.36; N, 9.75%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-6-benzyl-4, 5, 6,7-tetra hydropyrazolo[3,4-c]pyridine-1-carboxylate (**10b**): Color: Brown. Yield: 81%. M.p.: 145-146 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.7 (s, 2H, N-CH_2), 7.25 (s, 1H, Ar-H), 7.38-7.28 (m, 7H, Ar-H), 7.68-7.81 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 446 (M+H). Anal. calcd. for $C_{26}H_{27}N_3O_2S$: C, 70.09; H, 6.11; N, 9.43. Found: C, 70.06; H, 6.13; N, 9.41%.

Tert-butyl 6-benzyl-4,5,6,7-tetrahydro-3-(quinolin-3-yl)pyra zolo[3,4-c]pyridine-1-carboxylate (**10c**): Color: Brown. Yield: 63%. M.p.: 154-155 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.35 (d, 2H, *J* = 6.4 Hz, CH_2), 2.67 (m, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.70 (s, 2H, N-CH_2), 7.38-7.28 (m, 5H, Ar-H), 7.66-7.45 (m, 3H, Ar-H), 8.19-8.02 (m, 2H, Ar-H), 8.78 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 441 (M+H). Anal. calcd. for $C_{27}H_{28}N_4O_2$: C, 73.61; H, 6.41; N, 12.72. Found: C, 73.64; H, 6.39; N, 12.74%.

Tert-butyl 6-benzyl-4,5,6,7-tetrahydro-3-(quinolin-5-yl)pyra zolo[3,4-c]pyridine-1-carboxylate (**10d**): Color: Brown. Yield: 66%. M.p.: 161-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.70 (s, 2H, N-CH_2), 7.38-7.21 (m, 5H, Ar-H), 7.66-7.45 (m, 3H, Ar-H), 8.19-8.02 (m, 2H, Ar-H), 8.78 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 441 (M+H). Anal. calcd. for $C_{27}H_{28}N_4O_2$: C, 73.61; H, 6.41; N, 12.72. Found: C, 73.63; H, 6.42; N, 12.73%.

2.2.10. General procedure for synthesis of compounds (11a-d), Step (j)

To a stirred solution of compounds **10a**-**d** (1 mmol) in methanol (10 mL) was added palladium on carbon (10 mol%) and keep the reaction in Parr Shaker apparatus by applying hydrogen gas pressure of 50 psi for 3 h at room temperature. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixtures filtered through a pad of celite and obtain filtrate evaporated under reduced pressure to obtain crude semisolid material for compound **11a**-**h**. The obtained crude was washed with cold pentane and cold diethyl ether to obtain solid compounds **11a**-**h**.

Tert-butyl 3-(benzofuran-2-yl)-4, 5, 6, 7-tetrahydropyrazolo [3,4-c]pyridine-1-carboxylate (**11a**): Color: Brown. Yield: 87%. M.p.: 115-116 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.67 (m, 2H, CH_2), 3.60 (s, 2H, N-CH_2), 6.65 (s, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.18 (m, 1H, Ar-H), 7.38-7.28 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 340 (M+H). Anal. calcd. for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.22; H, 6.21; N, 12.39%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-4, 5, 6, 7-tetrahydro pyrazolo[3,4-c]pyridine-1-carboxylate (**11b**): Color: Yellow. Yield: 90%. M.p.: 121-122 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.61 (s, 2H, N-CH_2), 7.25 (s, 1H, Ar-H), 7.38-7.24 (m, 2H, Ar-H), 7.68-7.81 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 356 (M+H). Anal. calcd. for $C_{19}H_{21}N_3O_2S$: C, 64.20; H, 5.95; N, 11.82. Found: C, 64.18; H, 5.96; N, 11.84%.

Tert-butyl 4,5,6,7-tetrahydro-3-(quinolin-3-yl)pyrazolo[3, 4-c]pyridine-1-carboxylate (**11c**): Color: Yellow. Yield: 86%. M.p.: 138-139 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.35 (2H, d, *J* = 6.4 Hz, CH_2), 2.66 (m, 2H, CH_2), 3.61 (s, 2H, N-CH_2), 7.66-7.45 (m, 3H, Ar-H), 8.19-8.02 (m, 2H, Ar-H), 8.78 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 351 (M+H). Anal. calcd. for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.56; H, 6.34; N, 15.97%.

Tert-butyl 4,5,6,7-tetrahydro-3-(quinolin-5-yl)pyrazolo[3, 4-c]pyridine-1-carboxylate (**11d**): Color: Yellow. Yield: 84%. M.p.: 140-141 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (s, 9H, t-Bu), 2.33 (d, 2H, *J* = 6.4 Hz, CH_2), 2.68 (m, 2H, CH_2), 3.61 (s, 2H, N-CH_2), 7.66-7.45 (m, 3H, Ar-H), 8.19-8.02 (m, 2H, Ar-H), 8.78 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 351 (M+H). Anal. calcd. for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.57; H, 6.32; N, 15.98%.

2.2.11. General procedure for synthesis of compounds (12a-h), Step (k)

To a stirred solution of compounds **11a**-**d** (1 mmol) was dissolved in THF (10 mL). Then, added 2,6-lutidine (2 equiv.), DMAP (0.2 equiv.) and benzyl bromide (1.2 equiv.) and stirred reaction mixture to room temperature for 8 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 20% ethyl acetate:hexane (*v*:*v*, 20:80), hexane and diethyl ether to

obtain yellow semisolid compound. Crystallization of crude was done by using pentane and diethyl ether to obtain solid compounds **12a-h**.

Tert-butyl 3-(benzofuran-2-yl)-4,5, 6, 7-tetrahydro-6-(3-met hoxyphenyl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12a**): Color: Yellow. Yield: 85 %. M.p.: 134-135 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.84 (d, 2H, *J* = 8Hz, CH_2), 3.62 (s, 2H, CH_2), 3.78 (s, 3H, O-CH_3), 4.40 (d, 2H, *J* =7.8 Hz, N-CH_2), 6.97 (d, 1H, *J* =7.8 Hz, Ar-H), 7.18-7.08 (m, 2H, Ar-H), 7.41-7.21 (m, 3H, Ar-H), 7.46 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.74-7.61 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 446 (M+H). Anal. calcd. for $C_{26}H_{27}N_3O_4$: C, 70.09; H, 6.11; N, 9.43. Found: C, 70.07; H, 6.12; N, 9.44%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-4, 5, 6, 7-tetrahydro-6-(3-methoxyphenyl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12b**): Color: Yellow. Yield: 88 %. M.p.: 144-145 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.97-2.81 (m, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.76 (s, 3H, O-CH_3), 4.36 (d, 2H, *J* = 8.4 Hz, N-CH_2), 6.41 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.54 (s, 1H, Ar-H), 6.62 (m, 1H, Ar-H), 7.18-7.04 (m, 2H, Ar-H), 7.32-7.18 (m, 2H, Ar-H), 7.73-7.68 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 462 (M+H). Anal. calcd. for $C_{26}H_{27}N_3O_3S$: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.63; H, 5.92; N, 9.12%.

Tert-butyl 4,5,6,7-tetrahydro-6-(3-methoxyphenyl)-3-(quino lin-3-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12c**): Color: White. Yield: 91%. M.p.: 147-148 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.92 (d, 2H, *J* = 8.2 Hz, CH_2), 3.61 (t, 2H, *J* = 8.2 Hz, CH_2), 3.72 (s, 3H, O-CH_3), 4.42 (s, 2H, N-CH_2), 6.44 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 6.64 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.18 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.81-7.55 (dd, 2H, *J* = 8.4 Hz, 16.8 Hz, Ar-H), 8.08 (t, 2H, *J* = 8.2 Hz, Ar-H), 8.58 (s, 1H, Ar-H), 9.38 (s, 1H, Ar-H), 9.20 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 457 (M+H). Anal. calcd. for $C_{27}H_{28}N_4O_3$: C, 71.03; H, 6.18; N, 12.27. Found: C, 71.01; H, 6.20; N, 12.26%.

Tert-butyl 4,5,6,7-tetrahydro-6-(3-methoxyphenyl)-3-(quino lin-5-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12d**): Color: White. Yield: 89%. M.p.: 148-149 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 3.01 (s, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.76 (s, 3H, O-CH_3), 4.38 (d, 2H, *J* = 8 Hz, N-CH_2), 6.38 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.60 (s, 1H, Ar-H), 6.66 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.16 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.78-7.58 (m, 2H, Ar-H), 8.18-8.01 (m, 2H, Ar-H), 8.42 (s, 1H, Ar-H), 9.38 (s, 1H, Ar-H), 9.20 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 457 (M+H). Anal. calcd. for $C_{27}H_{28}N_4O_3$: C, 71.03; H, 6.18; N, 12.27. Found: C, 71.02; H, 6.19; N, 12.26%.

Tert-butyl 3-(benzofuran-2-yl)-4,5,6,7-tetrahydro-6-(pyrimi din-2-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12e**): Color: White. Yield: 86%. M.p.: 155-156 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.86 (d, 2H, *J* = 8Hz, CH_2), 4.08 (s, 2H, CH_2), 4.96 (d, 2H, *J* = 7.8 Hz, N-CH_2), 6.60 (s, 1H, Ar-H), 7.18-7.02 (d, 1H, Ar-H), 7.49-7.20 (m, 2H, Ar-H), 7.76-7.10 (m, 2H, Ar-H), 8.42 (s, 2H, Ar-H). LC-MS (EI, *m/z*): 418 (M+H). Anal. calcd. for $C_{23}H_{23}N_5O_3$: C, 66.17; H, 5.55; N, 16.78. Found: C, 66.15; H, 5.53; N, 16.79%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-4,5,6,7-tetrahydro-6-(pyrimidin-2-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12f**): Color: White. Yield: 84%. M.p.: 154-155 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 3.06 (d, 2H, *J* = 8.2 Hz, CH_2), 4.18 (s, 2H, CH_2), 4.96 (d, 2H, *J* = 7.6 Hz, N-CH_2), 6.56 (s, 1H, Ar-H), 7.16-7.02 (d, 1H, Ar-H), 7.49-7.14 (m, 2H, Ar-H), 7.86-7.54 (m, 2H, Ar-H), 8.54 (s, 2H, Ar-H). LC-MS (EI, *m/z*): 434 (M+H). Anal. calcd. for $C_{23}H_{23}N_5O_2S$: C, 63.72; H, 5.35; N, 16.15. Found: C, 63.71; H, 5.37; N, 16.16%.

Tert-butyl 4,5,6,7-tetrahydro-6-(pyrimidin-2-yl)-3-(quinolin-3-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12g**): Color: White. Yield: 84%. M.p.: 165-166 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.92 (d, 2H, *J* = 8.4 Hz, CH_2), 4.12 (q, 2H, *J* = 8.4 Hz, CH_2), 4.94 (s, 2H, N-CH_2), 6.72 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.74 (t, 1H, *J* = 8 Hz, Ar-H), 7.84 (t, 1H, *J* = 8 Hz, Ar-H), 8.12 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.22 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.42 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.56 (s, 1H, Ar-H), 9.38-9.22 (br, 2H, Ar-H). LC-MS (EI, *m/z*): 429 (M+H). Anal. calcd. for $C_{24}H_{24}N_4O_2$: C, 67.27; H, 5.65; N, 19.61. Found: C, 67.25; H, 5.64; N, 19.63%.

Tert-butyl 4,5,6,7-tetrahydro-6-(pyrimidin-2-yl)-3-(quinolin-5-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**12h**): Color: Yellow. Yield: 93%. M.p.: 161-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (s, 9H, t-Bu), 2.62 (t, 2H, *J* = 8.2 Hz, CH_2), 4.04 (q, 2H, *J* = 8.2 Hz, CH_2), 4.98 (s, 2H, N-CH_2), 6.66 (s, 1H, Ar-H), 7.44 (br, 1H, Ar-H), 7.58 (d, 1H, *J* = 8 Hz, Ar-H), 7.83-7.68 (m, 2H, Ar-H), 8.23-8.01 (m, 2H, Ar-H), 8.28 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 429 (M+H). Anal. calcd. for $C_{24}H_{24}N_4O_2$: C, 67.27; H, 5.65; N, 19.61. Found: C, 67.24; H, 5.66; N, 19.64%.

2.2.12. General procedure for synthesis of compound 13a-h, Step (l)

To a stirred solution of compounds **12a-h** (1 mmol) was dissolved in 2 N dioxane in HCl (10 mL) and stirred reaction mixture to room temperature for 6 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The crude obtained was washed with 10% ethyl acetate:hexane, hexane and diethyl ether to obtain crude **13a-h** as yellow solid material. The crude was purified by column chromatography (silica gel, 230-400 mesh) by using 25-75% ethyl acetate and hexane to obtain desired compounds **13a-h** as solid materials.

3-(Benzofuran-2-yl)-4, 5, 6, 7-tetrahydro-6-(3-methoxyphen yl)-1H-pyrazolo[3,4-c]pyridine (**13a**): Color: White. Yield: 91%. M.p.: 155-156 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.84 (d, 2H, *J* = 8 Hz, CH_2), 3.62 (s, 2H, CH_2), 3.78 (s, 3H, O-CH_3), 4.39 (d, 2H, *J* = 7.8 Hz, N-CH_2), 6.96 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.2-7.12 (m, 2H, Ar-H), 7.36-7.21 (m, 3H, Ar-H), 7.46 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.74-7.63 (m, 2H, Ar-H), 13.38 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.34, 48.55, 51.72, 55.85, 97.56, 103.11, 103.21, 106.81, 111.12, 121.18, 122.34, 123.76, 124.44, 130.84, 135.67, 150.42, 150.67, 155.67, 161.82. LC-MS (EI, *m/z*): 345 (M+H). Anal. calcd. for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 73.05; H, 5.53; N, 12.15%. HPLC: r.t. = 5.68 min, purity = 98.3%.

3-(Benzo[b]thiophen-2-yl)-4,5, 6, 7-tetrahydro-6-(3-methoxy phenyl)-1H-pyrazolo[3,4-c]pyridine (**13b**): Color: Yellow. Yield: 89%. M.p.: 161-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.97-2.81 (m, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.76 (s, 3H, O-CH_3), 4.36 (d, 2H, *J* = 8.4 Hz, N-CH_2), 6.38 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.55 (s, 1H, Ar-H), 6.63 (m, 1H, Ar-H), 7.18-7.04 (m, 2H, Ar-H), 7.32-7.2 (m, 2H, Ar-H), 7.73-7.68 (m, 2H, Ar-H), 13.4 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.24, 48.45, 51.62, 55.85, 97.66, 102.11, 102.21, 106.81, 111.2, 120.18, 122.34, 123.66, 124.44, 130.84, 135.66, 150.32, 150.67, 154.67, 161.72. LC-MS (EI, *m/z*): 361 (M+H). Anal. calcd. for $C_{21}H_{19}N_3OS$: C, 69.78; H, 5.30; N, 11.63. Found: C, 69.77; H, 5.32; N, 11.61%. HPLC: r.t. = 9.21 min, purity = 99.5%.

3-(4, 5, 6,7-Tetrahydro-6-(3-methoxyphenyl)-1H-pyrazolo[3, 4-c]pyridin-3-yl)quinolone (**13c**): Color: Yellow. Yield: 80%. M.p.: 179-180 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.92 (d, 2H, *J* = 8.2 Hz, CH_2), 3.61 (t, 2H, *J* = 8.2 Hz, CH_2), 3.72 (s, 3H, O-CH_3), 4.42 (s, 2H, N-CH_2), 6.34 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 6.64 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.16 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.81-7.59 (dd, 2H, *J* = 8.4 Hz, 16.8 Hz, Ar-H), 8.08 (t, 2H, *J* = 8.2 Hz, Ar-H), 8.56 (s, 1H, Ar-H), 9.38 (s, 1H, Ar-H), 13.26 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.72, 49.24, 52.46, 55.46, 97.8, 103.21, 106.67, 127.34, 127.84, 128.22, 128.42, 128.88, 129.42, 129.41, 130.71, 130.85, 145.21, 148.73, 150.65, 162.10. LC-MS (EI, *m/z*): 357 (M+H). Anal. calcd. for $C_{22}H_{20}N_4O$: C, 74.14; H, 5.66; N, 15.72. Found: C, 74.17; H, 5.65; N, 15.71%. HPLC: r.t. = 4.89 min, purity = 96.6%.

5-(4,5,6,7-Tetrahydro-6-(3-methoxyphenyl)-1H-pyrazolo [3, 4-c]pyridin-3-yl)quinolone (**13d**): Color: Off white. Yield: 86%. M.p.: 184-185 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.01 (s, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.76 (s, 3H, O-CH_3), 4.39 (d, 2H, *J*

= 8 Hz, N-CH_2), 6.38 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.58 (s, 1H, Ar-H), 6.65 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.16 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.8-7.58 (m, 2H, Ar-H), 8.18-8.01 (m, 2H, Ar-H), 8.43 (s, 1H, Ar-H), 9.38 & 9.20 (s, 1H, Ar-H), 13.2 (br, 1H, NH, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.62, 49.34, 52.56, 54.46, 97.86, 102.21, 106.77, 127.44, 127.84, 128.32, 128.42, 128.88, 129.42, 129.40, 130.60, 130.85, 145.31, 148.63, 151.14, 162.60. LC-MS (EI, *m/z*): 357 (M+H). Anal. calcd. for $C_{22}H_{20}N_4O$: C, 74.14; H, 5.66; N, 15.72. Found: C, 74.11; H, 5.69; N, 15.71%. HPLC: r.t. = 5.09 min, purity = 98.0%.

3-(Benzofuran-2-yl)-4, 5, 6, 7-tetrahydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3,4-c]pyridine (**13e**): Color: Yellow. Yield: 88%. M.p.: 167-168 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.86 (d, 2H, *J* = 8Hz, CH_2), 4.08 (s, 2H, CH_2), 4.96 (d, 2H, *J* = 7.8 Hz, N-CH_2), 6.65 (s, 1H, Ar-H), 7.16-7.02 (d, 1H, Ar-H), 7.49-7.21 (m, 2H, Ar-H), 7.76-7.6 (m, 2H, Ar-H), 8.42 (s, 2H, Ar-H), 13.4 & 12.9 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.41, 48.67, 51.72, 102.82, 103.03, 110.62, 111.67, 121.12, 123.46, 123.86, 135.40, 144.20, 150.66, 162.84, 175.88. LC-MS (EI, *m/z*): 318 (M+H). Anal. calcd. for $C_{18}H_{15}N_5O$: C, 68.13; H, 4.46; N, 22.07. Found: C, 68.11; H, 4.48; N, 22.09%. HPLC: r.t. = 7.58 min, purity = 93.7%.

3-(Benzo[b]thiophen-2-yl)-4, 5, 6,7-tetrahydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3,4-c]pyridine (**13f**): Color: Yellow. Yield: 91%. M.p.: 197-198 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.06 (d, 2H, *J* = 8.2 Hz, CH_2), 4.18 (s, 2H, CH_2), 4.96 (d, 2H, *J* = 7.6 Hz, N-CH_2), 6.66 (s, 1H, Ar-H), 7.16-7.04 (d, 1H, Ar-H), 7.49-7.17 (m, 2H, Ar-H), 7.86-7.59 (m, 2H, Ar-H), 8.52 (s, 2H, Ar-H), 13.4 & 12.0 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.40, 48.66, 51.72, 102.72, 103.30, 110.62, 111.67, 121.22, 123.46, 123.72, 135.48, 144.20, 148.66, 162.84, 174.78. LC-MS (EI, *m/z*): 334 (M+H). Anal. calcd. for $C_{18}H_{15}N_5S$: C, 64.84; H, 4.53; N, 21.01. Found: C, 64.86; H, 4.51; N, 21.03%. HPLC: r.t. = 5.84 min, purity = 97.5%.

3-(4,5,6,7-Tetrahydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3, 4-c]pyridin-3-yl)quinolone (**13g**): Color: White. Yield: 76 %. M.p.: 185-186 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.92 (d, 2H, *J* = 8.4 Hz, CH_2), 4.12 (q, 2H, *J* = 8.4 Hz, CH_2), 4.94 (s, 2H, N-CH_2), 6.7 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.74 (t, 1H, *J* = 8 Hz, Ar-H), 7.84 (t, 1H, *J* = 8 Hz, Ar-H), 8.14 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.22 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.42 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.58 (s, 1H, Ar-H), 9.38-9.22 (br, 2H, Ar-H),13.2 & 12.8 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.76, 49.12, 52.46, 103.24, 110.36, 128.62, 128.88, 129.42, 129.51, 129.62, 130.41, 134.83, 144.42, 145.12, 148.80, 157.90, 162.78. LC-MS (EI, *m/z*): 329 (M+H). Anal. calcd. for $C_{19}H_{16}N_6$: C, 69.50; H, 4.91; N, 25.59. Found: C, 69.52; H, 4.93; N, 25.60%. HPLC: r.t. = 6.59 min, purity = 95.8%.

5-(4,5,6,7-Tetrahydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3, 4-c]pyridin-3-yl)quinolone (**13h**): Color: White. Yield: 88 %. M.p.: 188-189 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.62 (t, 2H, *J* = 8.2 Hz, CH_2), 4.04 (q, 2H, *J* = 8.2 Hz, CH_2), 4.98 (s, 2H, N-CH_2), 6.63 (s, 1H, Ar-H), 7.44 (br, 1H, Ar-H), 7.68 (d, 1H, *J* = 8 Hz, Ar-H), 7.83-7.68 (m, 2H, Ar-H), 8.23-8.01 (m, 2H, Ar-H), 8.40 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H), 13.2 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.55, 49.33, 53.66, 110.21, 116.68, 119.7, 121.22, 125.58, 127.79, 129.10, 130.00, 130.42, 136.46, 137.12, 147.45, 150.51, 159.12, 162.84. LC-MS (EI, *m/z*): 329 (M+H). Anal. calcd. for $C_{19}H_{16}N_6$: C, 69.50; H, 4.91; N, 25.59. Found: C, 69.51; H, 4.90; N, 25.59%. HPLC: r.t. = 4,78 min, purity = 99.2%.

2.2.13. General procedure for synthesis of compounds 14a-h, Step (a)

To a stirred solution of compounds **12a**-**h** (1 mmol) was dissolved in DCM (10 mL). Then added $KMnO_4$ (2 equiv.) and 18-crown-6 (0.5 equiv.) and stirred reaction mixture to room temperature for 6 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture was diluted with DCM (10 mL) and washed it with water (3×5 mL). Separated and collected the organic layer, washed it with 5 mL of brine and dried organic layer over anhydrous Na_2SO_4 and evaporated it under reduced pressure to obtain crude compounds **14a**-**h** as semisolid compound. The crude obtained was washed with 5% ethyl acetate: hexane, hexane and diethyl ether to obtain yellow semisolid compound. The obtained compound was crystallized by using cold pentane and cold diethyl ether to obtain solid compounds **14a**-**h**.

Tert-butyl 3-(benzofuran-2-yl)-4,5,6,7-tetrahydro-6-(3-methoxyphenyl)-7-oxopyrazolo[3,4-c]pyridine-1-carboxylate (**14a**): Color: Brown. Yield: 75 %. M.p.: 165-166 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.21 (t, 2H, *J* = 7.6 Hz, 2H, CH_2), 3.31 (s, 3H, O-CH_3), 4.18 (d, 2H, *J* = 7.6 Hz, N-CH_2), 6.8 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.87-6.79 (m, 2H, Ar-H), 7.44-7.21 (m 4H, Ar-H), 7.76-7.6 (m, 2H, Ar-H). LC-MS (EI, *m/z*): 460 (M+H). Anal. calcd. for $C_{26}H_{25}N_3O_5$: C, 67.96; H, 5.48; N, 9.14. Found: C, 67.98; H, 5.47; N, 9.15%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-4, 5, 6, 7-tetrahydro-6-(3-methoxyphenyl)-7-oxopyrazolo[3,4-c]pyridine-1-carboxylate (**14b**): Color: Brown. Yield: 77%. M.p.: 171-172 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.40 (t, 2H, *J* = 8.4 Hz, CH_2), 3.74 (s, 3H, O-CH_3), 4.24 (d, 2H, *J* = 6.2 Hz, N-CH_2), 6.38 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.54 (s, 1H, Ar-H), 6.63-6.57 (m, 2H, Ar-H), 7.2-7.11 (m, 3H, Ar-H), 7.56-7.44 (dd, 2H, *J* = 8.2 Hz, 4.1 Hz, Ar-H). LC-MS (EI, *m/z*): 476 (M+H). Anal. calcd. for $C_{26}H_{25}N_3O_4S$: C, 65.67; H, 5.30; N, 8.84. Found: C, 65.65; H, 5.31; N, 8.83%.

Tert-butyl 4, 5, 6, 7-tetrahydro-6-(3-methoxyphenyl)-7-oxo-3-(quinolin-3-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**14c**): Color: Brown. Yield: 78%. M.p.: 181-182 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.21 (br s, 2H, CH_2), 3.83 (s, 3H, O-CH_3), 4.16 (s, 2H, N-CH_2), 6.93 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.12 (d, 1H, *J* = 8 Hz, Ar-H), 7.18 (s, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.58 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.72 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.84 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.14 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.56 (s, 1H, Ar-H), 9.41 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 471 (M+H). Anal. calcd. for $C_{27}H_{26}N_4O_4$: C, 68.92; H, 5.57; N, 11.91. Found: C, 68.91; H, 5.55; N, 11.92%.

Tert-butyl 4, 5, 6, 7-tetrahydro-6-(3-methoxyphenyl)-7-oxo-3-(quinolin-5-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**14d**): Color: Brown. Yield: 76%. M.p.: 188-189 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.75 (s, 3H, O-CH_3), 3.80 (d, 2H, *J* = 7.6 Hz, CH_2), 4.38 (d, 2H, *J* = 7.8 Hz, N-CH_2), 6.34 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.68 (s, 1H, Ar-H), 6.75 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.14 (m, 1H, Ar-H), 7.88 (m, 1H, Ar-H), 8.14-7.98 (m, 3H, Ar-H), 8.37-8.33 (d, 1H, *J* = 7.8 Hz, Ar-H), 9.16 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 471 (M+H). Anal. calcd. for $C_{27}H_{26}N_4O_4$: C, 68.92; H, 5.57; N, 11.91. Found: C, 68.93; H, 5.55; N, 11.92%.

Tert-butyl 3-(benzofuran-2-yl)-4, 5, 6, 7-tetrahydro-7-oxo-6-(pyrimidin-2-yl)pyrazolo[3,4-c]pyridine-1-carboxylate (**14e**): Color: White. Yield: 77%. M.p.: 163-164 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.22 (s, 2H, CH_2), 4.24 (d, 2H, *J* = 8.2 Hz, N-CH_2), 7.41-7.16 (m 4H, Ar-H), 7.68-7.54 (m, 2H, Ar-H), 8.84 (d, 2H, *J* = 7.8 Hz, Ar-H). LC-MS (EI, *m/z*): 431 (M+H). Anal. calcd. for $C_{23}H_{21}N_5O_4$: C, 64.03; H, 4.91; N, 16.23. Found: C, 64.02; H, 4.94; N, 16.25%.

Tert-butyl 3-(benzo[b]thiophen-2-yl)-4, 5, 6, 7-tetrahydro-7-oxo-6-(pyrimidin-2-yl)pyrazolo[3, 4-c]pyridine-1-carboxylate (**14f**): Color: Brown. Yield: 71 %. M.p.: 155-156 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.48 (s, 2H, CH_2), 4.44 (d, 2H, *J* = 8.2 Hz, N-CH_2), 7.48-7.16 (m 4H, Ar-H), 7.78-7.58 (m, 2H, Ar-H), 8.84 (d, 2H, *J* = 7.6 Hz, Ar-H). LC-MS (EI, *m/z*): 448 (M+H). Anal. calcd. for $C_{23}H_{21}N_5O_3S$: C, 61.73; H, 4.73; N, 15.65. Found: C, 61.72; H, 4.75; N, 15.63%.

Tert-butyl 4, 5, 6, 7-tetrahydro-7-oxo-6-(pyrimidin-2-yl)-3-(quinolin-3-yl)pyrazolo[3, 4-c]pyridine-1-carboxylate (**14g**): Color: Brown. Yield: 76 %. M.p.: 161-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 3.26 (q, 2H, *J* = 7.6 Hz, CH_2), 4.26 (t, 2H, *J* = 7.6 Hz, N-CH_2), 7.28 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.56 (q, 1H, *J* = 8.2 Hz, Ar-H), 7.82 (t, 1H, *J* = 8.2 Hz, Ar-

H), 8.18-8.02 (q, 2H, Ar-H), 8.60 (s, 1H, Ar-H), 8.83 (d, 2H, Ar-H), 9.38 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 443 (M+H). Anal. calcd. for $C_{24}H_{22}N_6O_3$: C, 65.15; H, 5.01; N, 18.99. Found: C, 65.14; H, 5.03; N, 18.98%.

Tert-butyl 4, 5, 6, 7-tetrahydro-7-oxo-6-(pyrimidin-2-yl)-3-(quinolin-5-yl)pyrazolo[3, 4-c]pyridine-1-carboxylate (**14h**): Color: Brown. Yield: 73%. M.p.: 164-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.41 (s, 9H, t-Bu), 2.8 (q, 2H, CH_2), 4.2 (q, 2H, N-CH_2), 7.26 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.42 (d, 1H, *J* = 7.4 Hz, Ar-H), 7.64 (d, 1H, *J* = 8 Hz, Ar-H), 7.82 (t, 1H, *J* = 7.6 Hz, Ar-H), 8.12 (d, 2H, *J* = 7.4 Hz, Ar-H), 8.8 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.96 (s, 1H, Ar-H). LC-MS (EI, *m/z*): 443 (M+H). Anal. calcd. for $C_{24}H_{22}N_6O_3$: C, 65.15; H, 5.01; N, 18.99. Found: C, 65.13; H, 5.02; N, 18.97%.

2.2.14. General procedure for synthesis of compounds 15a-h, Step (b)

To a stirred solution of compounds **14a**-**h** (1 mmol) was dissolved in 2 N dioxane in HCl (10 mL) and stirred reaction mixture to room temperature for 6 h. Progress of reaction was monitored by LC-MS for the consumption of starting material. After completion the reaction, the reaction mixture evaporated under reduced pressure to obtain yellow gummy material. The obtained crude was washed with 10% ethyl acetate: hexane, hexane and diethyl ether to obtain crude **15a**-**h** as yellow solid material. The obtained compound was purified by column chromatography by using silica gel (230-400 mesh) by using 25-75% ethyl acetate and hexane to obtain desired compound **15a**-**h** as solid materials.

3-(Benzofuran-2-yl)-5,6-dihydro-6-(3-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15a**): Color: White. Yield: 87%. M.p.: 191-192 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.21 (t, 2H, *J* = 7.6 Hz, CH_2), 3.31 (s, 3H, O-CH_3), 4.18 (d, 2H, *J* = 7.6 Hz, N-CH_2), 6.82 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.89-6.79 (m, 2H, Ar-H), 7.40-7.21 (m 4H, Ar-H), 7.76-7.60 (m, 2H, Ar-H), 14.4 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.64, 51.82, 55.85, 97.46, 103.31, 103.61, 106.61, 111.02, 121.18, 122.34, 123.76, 124.44, 128.84, 134.67, 150.42, 150.67, 156.61, 156.88, 161.72. LC-MS (EI, *m/z*): 360 (M+H). Anal. calcd. for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.16; H, 4.74; N, 11.70%. HPLC: r.t. = 6.18 min, purity = 99.3%.

3-(Benzo[b]thiophen-2-yl)-5, 6-dihydro-6-(3-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15b**): Color: Yellow. Yield: 88%. M.p.: 188-189 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.40 (t, 2H, *J* = 8.4 Hz, CH_2), 3.74 (s, 3H, O-CH_3), 4.24 (d, 2H, *J* = 6.2 Hz, N-CH_2), 6.38 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.50 (s, 1H, Ar-H), 6.63-6.57 (m, 2H, Ar-H), 7.21-7.11 (m, 3H, Ar-H), 7.56-7.44 (dd, 2H, *J* = 8.2 Hz, 4.1Hz, Ar-H) 14.4 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 13.54, 52.82, 55.65, 98.46, 104.31, 104.61, 108.66, 111.12, 121.18, 122.34, 123.76, 124.44, 128.84, 134.67, 150.42, 150.67, 156.8, 157.00, 161.73. LC-MS (EI, *m/z*): 375 (M+H). Anal. calcd. for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19. Found: C, 67.16; H, 4.58; N, 11.18%. HPLC: r.t. = 7.63 min, purity = 99.7%.

5,6-Dihydro-6-(3-methoxyphenyl)-3-(quinolin-3-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15c**): Color: Off white. Yield: 91%. M.p.: 193-194 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.21 (br s, 2H, CH_2), 3.83 (s, 3H, O-CH_3), 4.16 (s, 2H, N-CH_2), 6.93 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.12 (d, 1H, *J* = 8 Hz, Ar-H), 7.18 (s, 1H, Ar-H), 7.40 (m, 1H, Ar-H), 7.58 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.73 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.84 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.15 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.56 (s, 1H, Ar-H), 9.43 (s, 1H, Ar-H), 14.8 (br, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz): 12.22, 49.44, 52.46, 55.46, 97.80, 102.21, 106.60, 127.34, 127.84, 128.22, 128.42, 128.88, 129.62, 129.41, 130.71, 130.85, 145.21, 148.63, 150.60, 168.16. LC-MS (EI, *m/z*): 371 (M+H). Anal. calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.32; H, 4.91; N, 15.14%. HPLC: r.t. = 7.20 min, purity = 99.3%.

5, 6-Dihydro-6-(3-methoxyphenyl)-3-(quinolin-5-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15d**): Color: Off white. Yield: 92%. M.p.: 206-207 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.75 (s, 3H, O-CH_3), 3.80 (d, 2H, *J* = 7.6 Hz, CH_2), 4.38 (d, 2H, *J* = 7.8 Hz, N-CH_2), 6.38 (t, 1H, *J* = 7.6 Hz, Ar-H), 6.58 (s, 1H, Ar-H), 6.65 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.14 (m, 1H, Ar-H), 7.88 (m, 1H, Ar-H), 8.14-7.98 (m, 3H, Ar-H), 8.37-8.33 (d, 1H, *J* = 7.8 Hz, Ar-H), 9.16 (s, 1H, Ar-H), 12.9 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.62, 49.44, 52.56, 54.46, 97.76, 102.31, 106.76, 127.44, 127.84, 128.32, 128.42, 128.88, 129.42, 129.40, 130.60, 130.85, 145.31, 148.63, 152.14, 168.60. LC-MS (EI, *m/z*): 371 (M+H). Anal. calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.31; H, 4.92; N, 15.11%. HPLC: r.t. = 7.81 min, purity = 96.1%.

3-(Benzofuran-2-yl)-5,6-dihydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15e**): Color: Yellow. Yield: 79%. M.p.: 176-177 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.22 (q, 2H, CH_2), 4.24 (d, 2H, *J* = 8.2 Hz, N-CH_2), 7.41-7.16 (m 4H, Ar-H), 7.78-7.61 (m, 2H, Ar-H), 8.84 (d, 2H, *J* = 7.8 Hz, Ar-H), 14.4 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.24, 43.92, 102.84, 110.36, 111.62, 116.21, 121.26, 123.36, 124.82, 133.11, 133.26, 150.45, 156.46, 158.10, 169.32. LC-MS (EI, *m/z*): 332 (M+H). Anal. calcd. for $C_{18}H_{13}N_5O_2$: C, 65.25; H, 3.95; N, 21.14. Found: C, 65.23; H, 3.97; N, 21.15%. HPLC: r.t. = 5.17 min, purity = 99.6%.

3-(Benzo[b]thiophen-2-yl)-5, 6-dihydro-6-(pyrimidin-2-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15f**): Color: Yellow. Yield: 84%. M.p.: 202-203 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.48 (q, 2H, CH_2), 4.44 (d, 2H, *J* = 8.2 Hz, N-CH_2), 7.48-7.16 (m 4H, Ar-H), 7.78-7.38 (m, 2H, Ar-H), 8.84 (d, 2H, *J* = 7.6 Hz, Ar-H), 14.3 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 10.24, 43.92, 102.84, 110.36, 111.62, 116.21, 121.26, 123.36, 124.82, 133.11, 133.26, 150.45, 156.46, 158.10, 169.32. LC-MS (EI, *m/z*): 347 (M+H). Anal. calcd. for $C_{18}H_{13}N_5OS$: C, 62.23; H, 3.77; N, 20.16. Found: C, 62.21; H, 3.76; N, 20.18%. HPLC: r.t. = 8.12 min, purity = 98.1%.

5, 6-Dihydro-6-(pyrimidin-2-yl)-3-(quinolin-3-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15g**): Color: White. Yield: 85%. M.p.: 211-212 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.26 (q, 2H, *J*= 7.6 Hz, CH_2), 4.26 (t, 2H, *J* = 7.6 Hz, N-CH_2), 7.38 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.6 (q, 1H, *J* = 8.2 Hz, Ar-H), 7.80 (t, 1H, *J* = 8.2 Hz, Ar-H), 8.18-8.02 (q, 2H, Ar-H), 8.6(s, 1H, Ar-H), 8.83 (d, 2H, *J* = Hz, Ar-H), 9.38 (s, 1H, Ar-H), 14.1 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.53, 44.57, 69.36, 110.31, 116.12, 127, 127.11, 128.45, 128.85, 129.11, 129.34, 130.17, 132.23, 134.68, 157.88, 158.12. LC-MS (EI, *m/z*): 343 (M+H). Anal. calcd. for $C_{19}H_{14}N_6O$: C, 66.66; H, 4.12; N, 24.55. Found: C, 66.67; H, 4.10; N, 24.57%. HPLC: r.t. = 8.42 min, purity = 98.0%.

5, 6-Dihydro-6-(pyrimidin-2-yl)-3-(quinolin-5-yl)-1H-pyrazolo[3,4-c]pyridin-7(4H)-one (**15h**): Color: White. Yield: 80%. M.p.: 209-210 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.80 (q, 2H, CH_2), 4.20 (q, 2H, N-CH_2), 7.36 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.40 (d, 1H, *J* = 7.4 Hz, Ar-H), 7.64 (d, 2H, *J* = 8 Hz, Ar-H), 7.81 (t, 1H, *J* = 7.6 Hz, Ar-H), 8.12 (d, 1H, *J* = 7.4 Hz, Ar-H), 8.80 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.96 (s, 1H, Ar-H), 13.98-14.2 (br. s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.35, 45.33, 110.20, 116.78, 119.47, 121.20, 125.38, 127.29, 129.31, 130.00, 130.12, 136.46, 137.12, 147.45, 150.51, 156.60, 159.12, 162.84. LC-MS (EI, *m/z*): 343 (M+H). Anal. calcd. for $C_{19}H_{14}N_6O$: C, 66.66; H, 4.12; N, 24.55. Found: C, 66.67; H, 4.11; N, 24.54%. HPLC: r.t. = 6.37 min, purity = 99.3%.

2.3. Biological evaluation

All the synthesized compounds were tested for their *in vitro* anticancer activity against various cancer cell lines.

Table 1. *In vitro* anticancer screening of the synthesized compounds against five cell lines.

Compound	A-549 [a]	Si [f]	HeLa [b]	Si [f]	MCF-7 [c]	Si [f]	DU-145 [d]	Si [f]	HUVEC [e]
13a	22.72±0.11	4.04	23.87±0.08	3.84	24.12±0.06	3.80	28.86±0.22	3.18	91.8±0.28
13b	15.81±0.11	5.13	14.32±0.04	6.74	26.32±0.06	3.67	33.73±0.12	2.86	96.6±0.14
13c	6.81±0.11	12.73	11.32±0.04	7.65	17.32±0.06	5.00	10.73±0.12	8.07	86.6±0.28
13d	10.65±0.11	8.41	18.79±0.22	4.76	16.86±0.12	5.31	20.82±0.11	4.30	89.6±0.28
13e	13.86±0.08	6.39	24.38±0.06	3.63	13.63±0.12	6.50	11.52±0.22	7.69	88.7±0.12
13f	15.72±0.11	6.08	26.87±0.08	3.55	24.12±0.06	3.96	38.86±0.22	2.46	95.6±0.28
13g	5.12±0.11	17.42	9.12±0.22	9.78	9.36±0.12	9.52	13.52±0.11	6.59	89.2±0.28
13h	13.25±0.14	6.53	17.78±0.08	4.87	13.82±0.08	6.26	11.72±0.06	7.38	86.6±0.19
15a	10.82±0.11	8.69	13.39±0.22	7.02	11.36±0.12	8.28	9.52±0.11	9.88	94.1±0.26
15b	14.13±0.12	6.18	15.16±0.08	5.76	16.12±0.12	5.42	11.62±0.11	7.52	87.4±0.22
15c	23.86±0.08	3.24	14.38±0.06	6.50	20.63±0.12	4.53	11.52±0.22	8.12	93.6±0.12
15d	11.72±0.11	7.81	8.87±0.08	10.32	13.12±0.06	6.98	18.86±0.22	4.85	91.6±0.28
15e	23.82±0.11	3.55	20.99±0.22	4.03	19.36±0.12	4.36	12.52±0.11	6.75	84.6±0.28
15f	10.78±0.14	8.12	18.78±0.08	4.66	14.82±0.08	5.91	18.72±0.06	4.67	87.6±0.19
15g	10.82±0.11	8.78	8.59±0.22	11.07	8.36±0.12	11.37	17.52±0.11	5.42	95.1±0.26
15h	9.13±0.12	9.81	14.16±0.08	6.32	6.12±0.12	14.17	11.62±0.11	7.71	89.6±0.22
Doxil	1.71±0.11	51.57	1.82±0.13	48.46	1.91±0.08	46.17	1.62±0.08	54.44	88.2±0.18

[a] A-549: Human lung cancer cell line.
[b] HeLa: Human cervical cancer cell line (ATCC CCL-2).
[c] MCF-7: Human breast cancer cell line.
[d] DU-145: Human prostate cancer cell line.
[e] HUVEC: Human umbilical vein endothelial cell line (ATCC CRL-1730).
[f] Selectivity Index (SI) = IC_{50} of pure compound in normal cell line/IC_{50} of same compound in cancer cell line. IC_{50} - The concentration required to inhibit 50% of cell population.

The anticancer activity test is performed according to the proce-dure developed by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye Sulforhodamine B (SRB) to assess cell growth [14,15]. Briefly, cells are grown in 96-well plates in suspension and then exposed for 48 hours to four serial concentrations of 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4} and 1×10^{-3} M of each compound. Cells were fixed and stained with protein binding SRB stain. Excess stain is washed and bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader. Concentration of the compounds that inhibited 50% of the net cell growth, growth inhibition of 50% (GI_{50}), was calculated from the dose response curve obtained for each test compound and cell line. GI_{50} values were presented in micro molar (μM) concentration. Doxorubicin was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate on three independent experiments and their mean values are taken as a final reading. The result of this study indicates that compound **13c**, **13g**, **15g** and **15h** shows prominent anticancer activity in all cell lines, having growth inhibition of 50 (GI_{50}) values of 5.12 to 17.52 μM (Table 1). All experiments were performed in duplicate and repeated three times.

3. Results and discussion

3.1. Chemistry

In Scheme 1, Step (a) is enamine formation which is done by reacting compound **1** with DMF-DMA heating at 100 °C for obtaining compound **2** with 78% yield. The compound **2** is reacted with $N_2H_4.H_2O$ in EtOH at 80 °C for 8 h to obtain compound **3** with having 68.3% yield. The structure of 4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine is confirmed by singlet at δ 7.31 ppm in ^{1}H NMR [16]. Purification of the compound **3** required purification by using column chromatography. The overall yield obtained by this method is greater than earlier reports [13]. 4,5,6,7-Tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine (**3**) is reacted with di-*tert*-butyl dicarbonate (Boc anhydride) using triethylamine as base to obtain di-*tert*-butyl-4,5-dihydro-7*H*-pyrazolo[3,4-*c*]pyridine-1,6-dicarboxylate (**4**) with yield 91%. The compound **4** is having BOC protection on both nitrogen's confirmed by ^{1}H NMR showing singlet for 18 H at δ 1.56 ppm. The Step (d) is deportation of aliphatic N-BOC which is achieved by treating compound **4** with 2 N HCl for 2 h to obtain compound **5**, confirmed by ^{1}H NMR showing singlet for 9 H at δ 1.56 ppm. The compound **5** was treated with pyridine Br_2 at room temperature for 3 h to obtain compound **6** with 92% yield. The structure of *tert*-butyl-3-bromo-4,5,6,7-tetrahydropyrazolo[3,4-*c*]pyridine-1-carboxylate (**6**) was confirmed by disappearance of singlet at δ 7.31 ppm in ^{1}H NMR. The compound **6** reacted with aqueous NaOH in heating for 3 h. There is formation of compound **7** in 88.4% yield which is confirmed by desired mass in LC-MS. The compound **7** is protected by using benzylbromide in THF by using mixture of bases as 2,6-leutidine and DMAP at room temperature for compound **6h** to obtain compound **8** with 83% yield, with ^{1}H NMR signals at δ 7.38-7.28 ppm (m, 5H). The compound **8** having free hydroxyl group which is protected by using triflic anhydride at room temperature for 12 h to obtain compound **9** with 88.5% yield. *t*-Butyl 3-(2,2,2-trifluoroacetoyloxy)-6-benzyl-4,5,6,7-tetrahydropyrazolo[3, 4-*c*]pyridine-1-carboxylate (**9**) is key intermediate for the synthesis of final compounds **13a-h** and **15a-h**. The compound **9** was treated with different aromatic boranic acids at 100 °C for 6 h to obtain compounds **10a-d** with yields in the range from 63 to 83% yields after purifications by silica gel (100-200 mesh) column chromatography. Debenzylation of compounds **10a-d** was done by using Pd/C in EtOH for 50 psi of hydrogen for 3 h at room temperature to obtain compounds **11a-d** with 84 to 90% yields. Alkylation of compounds **11a-d** was done by using substituted aromatic bromides in THF by using mixed bases 2,6-leutidine and DMAP for 3 h at room temperature to obtain compounds **12a-h** with yields 84 to 93%. The cleavage of protecting group of compounds **12a-h** was done by using aqueous 6 N HCl at room temperature for 6 h to obtain compounds **13a-h** with yields 76 to 91%. The mixed bases used in Steps (g) and (k) to enhance the reactivity of secondary amine used for reaction.

In Scheme 2, compounds **12a-h** are treated with $KMnO_4$ in DCM and 18-crown-6 used as phase transfer catalyst at room temperature for 6 h to give compounds **14a-h** with yields 71 to 78%, which is confirmed by vanishing singlet at 3.78 in ^{1}H NMR. The compound **14a-h** are converted to compounds **15a-h** by using aqueous 6 N HCl at room temperature for 6 h with 79 to 92% yields. The final compounds **13a-h** and **15a-h** are obtained with reaction yields 76 to 92%. Purity of all final compounds and key intermediates is >95% which are further used for biological activity studies.

Table 2. Inhibitory activity of compound **13c**, **13g**, **15g** and **15h** against panel of eight human kinases.

Kinase	% Inhibition			
	Compound 13c	Compound 13g	Compound 15g	Compound 15h
Aurora-A	73	64	51	57
Aurora-B	41	70	77	73
CDK_2/cyclinA	28	37	23	33
CDK2/cyclinE	17	22	21	23
CDK5/P25	66	59	70	56
EGFR	14	21	15	18
mTOR	44	68	46	48
PDK1	23	28	19	33

3.2. Biological studies

All the newly synthesized compounds **13a-h** and **15a-h** were evaluated for their antiproliferative activities against a panel of four different human cancer cell lines. The IC_{50} for each synthesized compounds are calculated with respect to one human normal cell line Human umbilical vein endothelial cell line (ATCC CRL-1730) and results are summarized in Table 1. These values represent the concentration required to inhibit 50% cell population compared with the control cells treated with DMSO and positive control Doxorubicin under similar conditions.

From substituted *tetra*-hydro-6-(substituted)-1*H*-pyrazolo[3,4-*c*]pyridine derivatives (**13a-h** and **14a-h**), the IC_{50} value ranges from 5.12 to 38.86 μM all four cell lines. For cell line A-549, compound **13g** is most active with IC_{50} value of 5.12 μM; and compound **13c** is also active with IC_{50} value of 6.81 μM along with compounds **13b**, **13e**, **13f**, **13h**, **15b** and **15d** are moderately active with IC_{50} value of 15.81, 13.86, 15.72, 13.25, 14.13 and 11.72 μM, respectively. The compounds **13a**, **15c** and **15e** are most inactive compounds in the series. For cell line HeLa, it's have IC_{50} values are in between 8.59 to 26.87 μM. The compounds **15g** is most active with IC_{50} value of 8.59 μM along with compound **15d** and **13g** with IC_{50} values of 8.87 and 9.12 μM, respectively. The compounds **13b**, **13c**, **15a**, **15c** and **15h** are moderately active with IC_{50} values ranging in between 11.32 to 14.38 μM. Remaining compounds are less active with IC_{50} value in between 15.16 to 26.87 μM. For cell line MCF-7, the IC_{50} values are in the range of 6.12 and 26.32 μM. The compounds **15h**, **15g** and **13g** are most active IC_{50} values of 6.12, 8.63 and 9.36 μM, respectively, total five compounds are moderately active with IC_{50} values in the range of 11.36 to 14.82 μM and eight compounds are less active with IC_{50} values are in the range of 16.12 to 26.32 μM. For cell line DU-145, the IC_{50} value ranges from 9.52 to 38.56 μM. The compounds **15a** is most active with IC_{50} value of 9.52 μM along with compound **13c** having IC_{50} value of 10.37 μM are most active. The compounds **13e**, **13h**, **15b**, **15c**, **15d**, **15h** and **15e** are also active compounds in DU-145 cell line with IC_{50} values in the range of 10-12 μM total seven compounds are less active with IC_{50} values in the range of 13.52 to 38.56 μM.

Compound **13a** having 3-methoxy phenyl and benzofuran-2-yl groups is inactive compared with standard with IC_{50} values in the range of 22.72 to 28.87 μM in all cell lines. Compound **13b** is moderately active with IC_{50} value of 14.33 μM of HeLa cell line and in remaining all cell lines it is inactive. The compound **13c** is active in A-549 cell line and DU-145 and is moderately active in HeLa and it is most inactive in MCF-7 due to presence of 3-methoxy phenyl and 3-yl quinolone groups. The compound **13d** is moderately active in A-549 and in remaining cell lines, it is inactive. Compound **13e** is inactive in cell lines HeLa and in remaining cell lines, it is moderately active with IC_{50} values 11.82 to 13.86 μM, its having pyrimidine and benzo-furan group. Compound **13f** is mostly inactive in all cell lines because the presence of pyrimidine and benzo-thiophene group. The compound **13g** is active compound in all cell lines with IC_{50} values in the range of 5.12 to 9.36 μM and for DU-145, it is moderately active with IC_{50} values of 13.52 μM due to the presence of pyrimidine and 3-yl quinolone group. Compound **13h**, for HeLa cell line, is inactive with IC_{50} value of 17.78 μM and, for remaining cell lines, it is moderately active with IC_{50} values in the range of 11.72 to 13.82 μM due to presence of pyrimidine and 5-yl-quinolone group. The compound **15a** having 3-methoxy phenyl and benzofuran-2-yl groups are mostly active in all cell lines with IC_{50} values in the range of 10.82 to 13.39 μM and is active in DU-145 with IC_{50} value of 9.52 μM. The compound **15b** is moderately active in A-549 and DU-145 cell lines and it is inactive in HeLa and MCF-7 cell line. The compound **15c** is moderately active in HeLa and DU-145 (IC_{50} value of 11.52 μM) cell lines and it is inactive in A-549 and MCF-7 cell line 23.86 and 20.63 μM, respectively. The compound **15d** is active for HeLa cell line with IC_{50} value of 8.87 μM and it is also moderately active for A-549 with IC_{50} value of 11.72 μM, for MCF-7 cell line with IC_{50} value of 13.12 μM with moderate active interestingly it is in active for DU-145 cell line with IC_{50} value of 18.86 μM. The compound **15e** having pyrimidine and benzo-furan group is moderately active for DU-145 cell line with IC_{50} value of 12.52 μM and for remaining cell lines, it is inactive. The compound **15f** having IC_{50} values of 10.78 μM is active for A-549 cell line and it is inactive for remaining all cell lines with IC_{50} values of 14.82 to 18.78 μM. The compound **15g** is active for A-549, HeLa and MCF-7 cell lines with IC_{50} values of 10.82, 8.59 and 8.36 μM, respectively. It is inactive with DU-145 cell line with IC_{50} value of 17.52 μM with pyrimidine and 3-yl-quinolone groups. The compound **15h** having pyrimidine and 5-yl-quinolone groups is most active in MCF-7 cell line with IC_{50} value of 6.12 μM and A-549 with IC_{50} value of 9.13 μM also it is moderately active in HeLa and DU-145 cell lines with IC_{50} values of 14.16 and 11.62 μM, respectively. From cell line data compounds **13c**, **13g**, **15g** and **15h** are most active which are having pyrimidine-2-yl group and quinoline3/5-yl groups, compared with compounds having 3-methoxy phenyl, benzofuran and benzothiophene groups. The compound **13c** is more active than compound **13d** as both of these compounds are separated by position of nitrogen in the qunioline ring, the 3-methoxy compounds with benzofuran and benzothiophene are less active than compounds having pyrimidine-2-yl substitutions. Interestingly compounds having substituted 4,5,6,7-tetrahydro group and substituted 5,6-dihydro groups are moderate to active on all four cell lines and that substituted 5,6-dihydro groups are more active than that of substituted 4,5,6,7-tetrahydro group. These are results from both series of compounds. There is not much difference in their inhibitions in all four cancer cell lines. Further we have studied the most active compounds **13c**, **13g**, **15g** and **15h** on human kinases.

The compounds **13c**, **13g**, **15g** and **15h** are most active in cell line studies, so further we have tested for its activity against a panel of eight human kinase at 10 μM concentrations. For Aurora-A kinase compounds, they shows 73, 64, 51 and 57% inhibitions, respectively. The results are summarized in Table 2. For Aurora-B kinase, compound **13c** shows 41% inhibitions and for remaining compounds **13g** (70%), **15g** (77%) and **15h** (73%) inhibitions. For CDK/cyclinA, CDK/cyclinE, EGFR and PDK1, the inhibition is in the range of 17 to 37%. CDK_5/P_{25} kinase and mTOR kinase the inhibitions are in the range of 44 to 70%. For Aurora-A, Aurora-B, CDK_5/P_{25} and

mTOR kinase, all the compounds shows promising inhibitions to great extent. The inhibition results shows compound **13c** is active for aurora-A kinase and CDK*5*/P*25* kinase and it shows less inhibition for remaining kinases. Compounds **13g**, **15g** and **15h** shows >50% inhibitions. For EGFR, PDK*1*, CDK*2*/ cyclinE and CDK_2/cyclinA kinases, most of compounds shows <40% inhibitions.

4. Conclusion

We have synthesized 3-(substituted)-4,5,6,7-tetrahydro-6-(substituted)-1*H*-pyrazolo[3, 4-*c*]pyridine (**13a**-**h**) and 3-(substituted)-5, 6-dihydro-6-(substituted)-1*H*-pyrazolo[3, 4-*c*] pyridin-7(4*H*)-one (**15a**-**h**). The synthesis mainly required protection, deportation, N-alkylation and Suzuki coupling reactions. We have optimized all the steps for clean reaction profile and easy isolation of all intermediates and final compounds. The compounds **13a**-**h** and **15a**-**h** are tested for anti-proliferative activity on panel of four cell lines. Compounds with pyrimidine-2-yl substitutions and quinolone 3/5-yl groups are most active compared with 3-methoxy and benzofurane/benzothiophene. Compounds **13c**, **13g**, **15g** and **15h** are tested for panel of eight kinase inhibitors and most of derivatives are mostly active on Aurora-A, Aurora-B, CDK5/P25 and mTOR human kinase inhibitors.

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References

[1]. Alafeefy, A. M.; Ashour, A. E.; Prasad, O.; Sinha, L.; Pathak, S.; Alasmari, F. A.; Rishi, A. K.; Abdel-Aziz, H. A. *Eur. J. Med. Chem.* **2015**, *92*, 191-201.
[2]. Devegowda, V. N.; Seo, S. H.; Pae, A. N.; Nam, G.; Choi, K. I. *Bull. Korean Chem. Soc.* **2012**, *33*, 647-650.
[3]. Devegowda, V. N.; Hong, J. R.; Cho, S.; Lim, E. J.; Choo, H.; Keum, G.; Rhim, H.; Nam, G. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4696-4700.
[4]. Guo, Z.; Orth, P.; Zhu, Z.; Mazzola, R. D.; Chan, T. Y.; Vaccaro, H. A.; Brian, K.; Kozlowski, J. A.; Lavey, B. J.; Zhou, G.; Paliwal, S.; Wong, S. C.; Shih, N. Y.; Ting, P. C.; Rosner, K. E.; Shipps, G. W.; Siddiqui, M. A. Belanger, D. B.; Dai, C.; Li, D.; Girijavallabhan, V. M.; Popovici-Muller, J.; Yu, W.; Zhao, L. PCT Int. Appl., 2005121130, 22 Dec. 2005.
[5]. Chen, M.; Guo, Z.; Lanier, M. C.; Zhao, L.; Betz, S. F.; Huang, C. Q.; Loweth, C. J.; Ashweek, N. J.; Liu, X. J.; Struthers, R. S.; Bradbury, M. J.; Behan, J. W.; Wen, J.; Brien, Z. O.; Saunders, J.; Zhu, Y. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3845-3850.
[6]. Caruso, M.; Valsasina, B.; Ballinari, D.; Bertrand, J.; Brasca, M. G.; Caldarelli, M.; Cappella, P.; Fiorentini, F.; Gianellini, L. M.; Scolaro, A.; Beria, I. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 96-101.
[7]. Wei, Z.; Yang, H.; Liu, Z.; Tremblay, M.; Jonstone, S.; Beha, S.; Yue, S. Y.; Srivastava, S.; Tomaszewaski, M. J.; Brown, W.; Walpole, C.; St-Onge, S.; Lessard, E.; Archambault, A. J.; Groblewski, T.; Page, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3884-3889.
[8]. Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K, A.; Alexander, R. S.; Smallwood, A.; Wong, P. C.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; He, K.; Xin, B.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2007**, *50*, 5339-5356.
[9]. Duplantier, A. J.; Andersen, C. J.; Cheng, J. B.; Cohan, V. L.; Decker, C.; Dicapua, F. M.; Kraus, K. G.; Johnson, K. L.; Turner, C. R.; Umland, J. P.; Watson, J. W.; Wester, R. T.; Williams, A. S.; Williams, J. A. *J. Med. Chem.* **1998**, *41*, 2268-2277.
[10]. Khatri, C. J.; Indalkar, K. S.; Patil, C. R.; Goyal, S. N.; Chaturbhuj, G. U. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1721-1726.
[11]. Hafez, H. N.; El-Gazzar, A. R.; Al-Hussain, S. A. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2428-2433.
[12]. Bhide, R. S.; Neels, J.; Qin, L. Y.; Ruan, Z.; Stachura, S.; Weight, C.; Sack, J. S.; Stefanski, K.; Gu, X.; Xie, J. H.; Goldstine, C. B.; Skala, S.; Pedicord, D. L.; Ruepp, S.; MuraliDhar, T. G.; Carter, P. H.; Salter-Cid, L. M.; Poss, M. A.; Davies, P. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4256-4260.
[13]. Fukui, H.; Inoguchi, K.; Nakano, J. *Heterocycles* **2002**, *56*, 257-264.
[14]. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J ; Vistica, D.; Jonathan, T. W.; Heidi, B.; Susan, K.; Michael, R. B. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.
[15]. Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, *1*, 1112-1116.