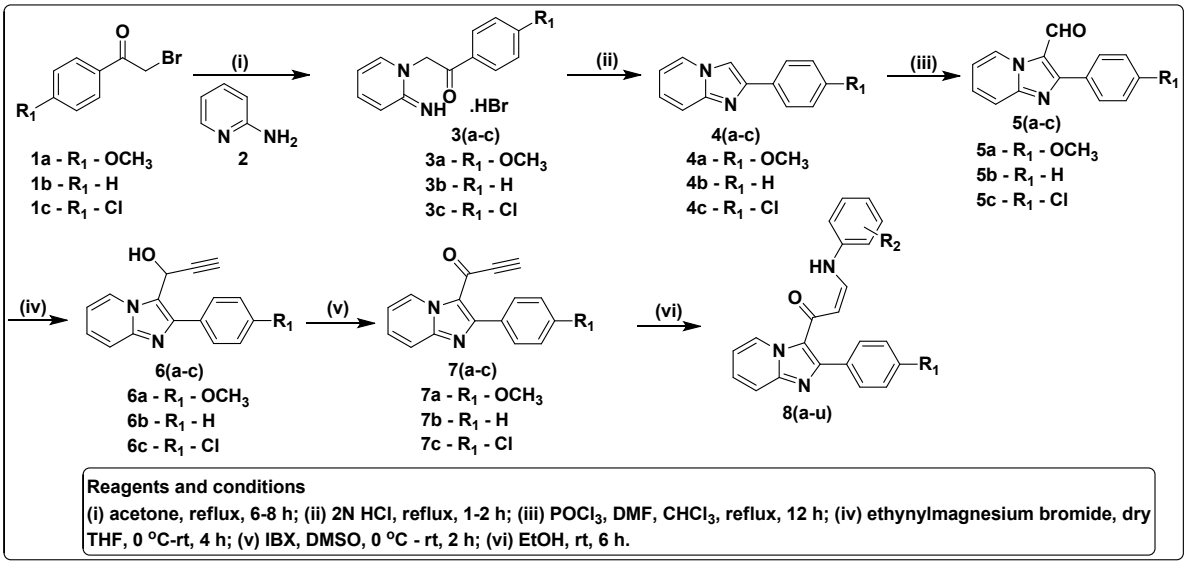
Experimental Section

Chemistry

All chemicals and reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Reactions were monitored by TLC performed on silica gel coated glass plates containing 60 GF254 and visualized by UV light or iodine staining. Column chromatography was performed with Merck 60–120 mesh silica gel. NMR spectra were recorded on Bruker UXNMR/ XWIN-NMR (300 MHz) or Inova Varian-VXR-unity (400 or 500 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from an internal TMS standard. Data are reported as follows: chemical shift (ppm) (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). ESI spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS–MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus and are uncorrected.



Scheme 1: Synthesis of imidazopyridine-propenones

Synthesis of 2-(aryl)imidazo[1,2-a]pyridine (4a-c)

2-(4-methoxyphenyl)imidazo[1,2-a]pyridine: 2-Bromo-1-(4-methoxyphenyl) ethanone (**1a**, 6.026g, 26 mmol) and 2-aminopyridine (**2**, 2.46g, 26 mmol) were dissolved in acetone and the reaction mixture was refluxed for 4–5 h. The resulting salt (**3a**) was collected by filtration, washed with acetone, dissolved in 3N HCl (200 mL) and refluxed again for 1 h. Before complete cooling, the solution was cautiously basified by drop wise addition of 15% aq. NH_4OH to pH 8.The resulting base was collected by filtration and crystallized from EtOH to afford compound 2-(4-methoxyphenyl)imidazo[1,2-a]pyridine (**4a**) as a white solid (5g, 85% yield); M.p: 130-132 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.09 (d, *J* = 6.8 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.75 (s, 1H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.14 (t, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 2H), 6.68 (t, *J* = 6.8 Hz, 1H), 3.79 (s, 3H); MS (ESI): m/z 225 $[M+H]^+$.

2-phenylimidazo[1,2-a]pyridine (**4b**): Compound **4b** was prepared according to the method described for compound **4a**, employing 2-bromo-1-(phenyl)ethanone (**1b**, 5.026g, 25 mmol) and 2-aminopyridine (**2**, 2.36g, 25 mmol) to obtain the pure product **4b** as a white solid (4.5g, 90% yield); M.p: 144–146 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.08 (d, *J* = 6.7 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.75 (s, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.29 (dt, *J* = 6.7, 1.5 Hz, 1H), 7.07 (dt, *J* = 6.7, 1.5 Hz, 1H), 6.70 (t, *J* = 6.7 Hz, 1H); MS (ESI): m/z 195 $[M+H]^+$.

2-(4-Chlorophenyl)imidazo[1,2-a]pyridine (**4c**): Compound **4c** was prepared according to the method described for compound **4a**, employing 2-bromo-1-(4-chlorophenyl)ethanone (**1c**, 6g, 25.7 mmol) and 2-aminopyridine (**2**, 2.42g, 25.7 mmol) to obtain the pure product **4c** as a white solid (5g, 87% yield); M.p: 171–173 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (d, *J* = 6.8 Hz, 1H); 7.87-7.96 (m, 2H), 7.77 (s, 1H), 7.65 (d, *J* = 9.1 Hz, 1H), 7.07-7.21 (m, 3H), 6.76 (t, *J* = 6.6 Hz, 1H); MS (ESI): m/z 229 $[M+H]^+$.

Synthesis of 2-(aryl)imidazo[1,2-a]pyridine -3-carbaldehyde (5a-c)

2-(4-methoxyphenyl)imidazo[1,2-a]pyridine-3-carbaldehyde (**5a**): Vilsmeier reagent was prepared by addition of $POCl_3$ (10 mL, 111 mmol) to a stirred solution of DMF (8.6 mL, 111 mmol) in $CHCl_3$ (10 mL) at 0–5 ^{0}C. To this reagent, 2-phenylimidazo[1,2-a]pyridine **4a** (5 g, 22 mmol) in chloroform (20 mL) was added while maintaining cold conditions. After complete addition, the reaction mixture was stirred at room temperature for 3 h and at reflux conditions for 10–12 h. After completion of the reaction, as indicated on TLC, chloroform was removed under reduced pressure and the resulting oily liquid was poured onto ice. The aldehyde **5a** was collected by filtration and crystallised from EtOH (5 mL) to obtain the pure product **5a** as a white solid (5g, 90% yield); M.p: 146-148 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.06 (s, 1H), 9.68 (d, *J* = 6.4 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 6.4 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H,), 3.88 (s, 3H); MS (ESI): m/z 253 $[M+H]^+$.

2-Phenylimidazo[1,2-a]pyridine-3-carbaldehyde (**5b**): compound **5b** was prepared according to the method described above for **5a**, employing 2-(phenyl)imidazo[1,2-a]pyridine (**4b**, 4.5g, 23mmol) to obtain the pure product **5b** as a white solid (4.3g, 83% yield); M.p: 155–158 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.06 (s, 1H), 9.66 (d, *J* = 6.7 Hz, 1H), 7.81-7.86 (m, 3H), 7.50-7.63 (m, 4H), 7.15 (t, *J* = 6.8 Hz, 1H); MS (ESI): m/z 223 $[M+H]^+$.

2-(4-Chlorophenyl)imidazo[1,2-a]pyridine-3-carbaldehyde (**5c**): Compound **5c** was prepared according to the method described for compound **5a**, employing 2-(4-chlorophenyl)imidazo[1,2-a]pyridine (**4c**, 5g, 21.9 mmol) to obtain the pure product **5c** as a white solid (5.2g, 92% yield); M.p: 151–153 ^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.06 (s, 1H), 9.65 (d, *J* = 6.9 Hz, 1H), 7.75-7.83 (m, 3H), 7.60 (t, *J* = 6.9 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.15 (t, *J* = 6.9 Hz, 1H); MS (ESI): m/z 257 $[M+H]^+$.

Synthesis of 1-(2-(aryl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (6a-c)

1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (**6a**): Compound **6a** was obtained by stirring solution of 2-(4-methoxyphenyl)imidazo[1,2-a]pyridine-3-carbaldehyde (**5a**, 5g, 19.8 mmol) with ethynyl magnesium bromide solution (60 mL, 30.0 mmol) (0.5 M) in tetrahydrofuran at 0 ^{0}C and then stirred at room temperature for 4-5 h. After completion of reaction saturated aqueous ammonium chloride solution (5-10mL)

was added, THF removed under vacuum, followed by addition of ethyl acetate. The organic layer was extracted and washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain pure compound **6a** as brown solid (4g, 72% yield); M.p: 194–196 ^{0}C; ^{1}H NMR ($CDCl_3$+DMSO, 300 MHz): δ 8.72 (d, *J* = 6.9 Hz, 1H), 7.61-7.51 (m, 3H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.08-7.00 (m, 2H), 6.95 (d, *J* = 6.3 Hz, 1H), 6.35 (s, 1H), 5.97 (s, 1H), 3.85 (s, 3H), 2.86 (s, 1H); MS (ESI): m/z 279 $[M+H]^+$.

1-(2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (**6b**): Compound **6b** was obtained using the above described method, employing 2 (phenyl)imidazo[1,2-a]pyridine -3-carbaldehyde (**5b**, 4.3g, 19.3 mmol) to the ethynyl magnesium bromide solution (58.1 mL, 29 mmol) to obtain pure product as brown solid (3g, 62.7% yield); M.p: 175–177 ^{0}C; 1H NMR (300 MHz, CDCl3+DMSO) δ 8.74 (d, *J* = 6.9 Hz, 1H), 7.71-7.69 (m, 3H), 7.60 – 7.53 (m, 1H), 7.45 – 7.39 (m, 2H), 7.30 (t, *J* = 8.5 Hz, 1H), 6.92 (t, *J* = 6.6 Hz, 1H), 6.02 (d, *J* = 2.1 Hz, 1H), 2.93 (s, 1H); MS (ESI): m/z 249 $[M+H]^+$.

1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1ol (**6c**): Compound **6c** was obtained using the above described method by adding 2-(4-chlorophenyl)imidazo[1,2-a]pyridine -3-carbaldehyde (**5c**, 5g, 19.5 mmol) to the ethynyl magnesium bromide solution (58.6 mL, 29.2 mmol) to obtain pure product as white solid (3.8g, 69% yield); M.p: 151–153 ^{0}C; ^{1}H NMR ($CDCl_3$+DMSO, 300 MHz) δ 8.75 (d, *J* = 6.7 Hz, 1H), 7.71-7.65 (m, 3H),7.58 (d, *J* = 9.1 Hz, 1H), 7.49 – 7.40 (m, 2H), 6.93 (t, *J* = 6.8 Hz, 1H), 6.01 (s, 1H), 3.05 (s, 1H); MS (ESI): m/z 283 $[M+H]^+$.

Synthesis of 1-(2-(aryl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (7a-c)

1-(2-(4methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**): A solution of 2-iodoxy benzoic acid (IBX) (6.02g, 21.5 mmol) and dimethyl sulfoxide (DMSO) was stirred for 10 min at room temperature until homogeneous solution. A solution of 1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (**6a**, 4g, 14.3 mmol) in dimethyl sulfoxide was added slowly and stirred for 2 h. After completion of reaction, ice water was added to reaction mixture and the mixture was stirred for another 10 min. To this mixture ethyl acetate was added and filtered through celite. The organic layer was separated and washed sequentially with water, saturated Na_2CO_3 solution and brine; dried over anhydrous Na_2SO_4 and evaporated in vacuum. It was recrystallized from methanol to obtain the pure compound **7a** (2.8g, 70% yield); M.p: 147–149 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.73 (d, *J* = 6.9 Hz, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 7.69-7.66 (m, 2H), 7.61-7.57 (m, 1H), 7.14 (td, *J* = 6.9 & 1.1 Hz, 1H), 7.00-6.96 (m, 2H), 3.88 (s, 3H), 2.89 (s, 1H); MS (ESI): 277 $[M+H]^+$.

1-(2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7b**): Compound **7b** was obtained using the method described for **7a** by adding 1-(2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (**6b**, 3g, 12 mmol) to the 2-iodoxy benzoic acid (5.08g, 18 mmol) in DMSO solution (20 mL) resulting compound obtain as pale yellow solid (2.3g, 77% yield); M.p: 151–153 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.73 (dt, *J* = 6.9 & 1.0 Hz, 1H), 7.80 (d, *J* = 8.9 Hz, 1H), 7.71-7.67 (m, 2H), 7.60-7.55 (m, 1H), 7.47-7.41 (m, 3H), 7.16 (td, *J* = 6.9 & 1.1 Hz, 1H), 2.80 (s, 1H); MS (ESI): 247 $[M+H]^+$.

1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**): Compound **7c** was obtained using the method described for **7a** by adding 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (**6c**, 3g, 10.6 mmol) to the 2- iodoxy benzoic acid (4.46g, 15.95 mmol) in DMSO solution (20 mL) as pale yellow solid (2.0 g, 67.3% yield); M.p.: 151–153 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.73 (d, *J* = 6.9 Hz, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.69-7.57 (m, 3H), 7.48-7.41 (m, 2H), 7.18 (td, *J* = 6.9 & 1.1 Hz, 1H), 2.90 (s, 1H); ESI-MS: m/z 281 $[M+H]^+$.

Synthesis of (Z)-3-(arylamino)-1-(2-arylimidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (8a-u)

(Z)-3-((3,5-dimethoxyphenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8a**): (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) was added to stirred solution of 3,5-dimethoxyaniline (55.43mg, 0.362 mmol) in ethanol (5 mL) and stirred at 25 ^{0}C for 4 h. The progress of the reaction was monitored by TLC (Hexane/EtOAc=6:4). After completion of reaction (TLC), the yellow color solid, that precipitated on addition of water, was filtered and washed with ethanol to give the titled compound in good yield (115 mg). M.p: 152–154 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.70 (d, *J* = 12.2 Hz, 1H), 9.69 (d, *J* = 7.0 Hz, 1H), 7.72-7.64 (m, 3H), 7.43-7.37 (m, 1H), 7.14 (dd, *J* = 12.2 & 8.1 Hz, 1H), 7.02-6.96 (m, 3H), 6.21-6.15 (m, 3H), 5.46 (d, *J* = 8.1 Hz, 1H), 3.88 (s, 3H), 3.79 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.5, 161.8, 160.3, 151.1, 146.4, 142.7, 142.2, 131.6, 128.4, 127.8, 127.4, 120.9, 117.1, 113.8, 98.4, 95.4, 94.6, 55.5, 55.1; ESI-MS: m/z 430 $[M+H]^+$; HRMS (ESI m/z) for $C_{25}H_{24}O_4N_3$ calc: 430.1761; found 430.1755 [M+H]+.

(Z)-3-((4-methoxyphenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8b**): The titled compound was synthesized by using the method described for compound **8a**, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and 4-methoxyaniline (44.56mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (109 mg). M.p: 130–132 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.80 (d, *J* = 12.3 Hz, 1H), 9.67 (d, *J* = 7.0 Hz, 1H), 7.70-7.65 (m, 3H), 7.38 (t, *J* = 11.5, 1H), 7.11 (dd, *J* = 12.4 & 7.9 Hz, 1H), 6.99 (d, *J* = 8.8 Hz, 4H), 6.95 (d, *J* = 6.3 Hz, 1H), 6.87 (d, *J* = 8.9 Hz, 2H), 5.41 (d, *J* = 7.8 Hz, 1H), 3.88 (s, 3H), 3.79 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.2, 160.2, 156.1, 146.2, 144.0, 134.0, 131.6, 128.4, 127.6, 120.9, 117.6, 117.0, 115.0, 113.7, 97.5, 55.5, 55.3; ESI-MS: m/z 400 $[M+H]^+$; HRMS (ESI m/z) for $C_{24}H_{22}O_3N_3$ calc: 400.1656; found: 400.1663 $[M+H]^+$.

(Z)-3-((4-bromophenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8c**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and 4-bromoaniline (62.26mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (115 mg). M.p 174–176 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.76 (d, *J* = 12.1 Hz, 1H), 9.69 (d, *J* = 7.0 Hz, 1H), 7.72-7.64 (m, 3H), 7.43-7.39 (m, 3H), 7.11 (dd, *J* = 12.1 & 8.1 Hz, 1H), 7.02-6.97 (m, 3H), 6.91 (d, *J* = 8.7 Hz, 2H), 5.49 (d, *J* = 8.1 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.7, 160.3, 151.4, 146.5, 142.2, 139.6, 132.6, 132.8, 131.6, 128.5, 128.0, 127.4, 120.9, 117.4, 117.2, 115.5, 113.8, 98.9, 55.4; ESI-MS: m/z 448 $[M+H]^+$; HRMS (ESI m/z) for $C_{23}H_{19}O_2N_3Br$ calc: 448.0655; found: 448.0660 $[M+H]^+$.

(Z)-3-((4-fluorophenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8d**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-

methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and 4-fluoroaniline (40.18mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (103). M.p: 118–120 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.79 (d, *J* = 12.1 Hz, 1H), 9.68 (d, *J* = 7.0 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.68-7.65 (m, 2H), 7.43-7.38 (m, 1H), 7.10 (dd, *J* = 12.2 & 8.0 Hz, 1H), 7.05-6.96 (m, 7H), 5.46 (d, *J* = 8.0 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.5, 160.3, 160.0, 158.0, 151.0, 146.4, 143.3, 136.8, 131.6, 128.4, 127.8, 127.4, 120.8, 117.6, 117.1, 116.6, 116.4, 113.8, 98.3, 55.4; ESI-MS: m/z 388 $[M+H]^+$; HRMS (ESI m/z) for $C_{23}H_{19}O_2N_3F$ calc: 388.1454; found: 388.1470 $[M+H]^+$.

(Z)-3-((1H-indol-5-yl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8e**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and 1H-indol-5-amine (47.78mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (105 mg). M.p: 218–220 ^{0}C; ^{1}H NMR ($CDCl_3$+DMSO, 300 MHz): δ 11.99 (d, *J* = 12.5 Hz, 1H), 10.31 (s, 1H), 9.69 (d, *J* = 6.9 Hz, 1H), 7.71-7.64 (m, 3H), 7.52 (s, 1H), 7.43-7.21 (m, 5H), 7.05-6.91 (m, 3H), 6.43 (s, 1H), 5.42 (d, *J* = 7.8 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 181.9, 159.6, 145.4, 145.3, 131.3, 127.01, 126.9, 126.6, 120.8, 120.6, 120.3, 120.0, 113.8, 112.4, 106.5, 101.0, 96.2, 55.2; ESI-MS: m/z 409 $[M+H]^+$; HRMS (ESI m/z) for $C_{21}H_{21}O_4N_4$ calc: 409.1659; found: 409.1665 $[M+H]^+$.

(Z)-3-((1H-indol-6-yl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8f**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and 1H-indol-6-amine (47.78mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (98mg). M.p: 198–200 ^{0}C; ^{1}H NMR ($CDCl_3$+DMSO, 300 MHz): δ 11.99 (d, *J* = 12.3 Hz, 1H), 10.22 (s, 1H), 9.69 (d, *J* = 6.9 Hz, 1H), 7.72-7.64 (m, 3H), 7.56-7.48 (m, 2H), 7.30 (dd, *J* = 12.4 & 7.9 Hz, 1H), 7.14 (d, *J* = 10.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 3H), 6.86 (d, *J* = 7.6 Hz, 1H), 6.44 (s, 1H), 5.44 (d, *J* = 7.8 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 181.4, 158.8, 144.7, 143.5, 142.9, 135.5, 133.8, 130.4, 127.2, 126.4, 124.0, 120.5, 118.9, 115.7, 113.1, 108.7, 100.3, 97.4, 96.0, 54.2; ESI-MS: m/z 409 $[M+H]^+$; HRMS (ESI m/z) for $C_{21}H_{21}O_4N_4$ calc: 409.1659; found: 409.1664 $[M+H]^+$.

(Z)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)-3-(quinolin-3-ylamino)prop-2-en-1-one (**8g**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and quinolin-3-amine (52.12mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (95 mg). M.p: 210–212 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.99 (d, *J* = 11.9 Hz, 1H), 9.76 (d, *J* = 7.0 Hz, 1H), 8.77 (d, *J* = 2.7 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 1H), 7.74-7,65 (m, 5H), 7.62-7.57 (m, 1H), 7.55-7.51 (m, 1H), 7.47-7.43 (m, 1H), 7.33-7.28 (m, 1H), 7.03 (dd, *J* = 7.1 & 5.4 Hz, 3H), 5.62 (d, *J* = 8.1 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.8, 160.4, 151.9, 146.7, 144.9, 142.9, 141.8, 134.2, 131.7, 129.3, 128.6, 128.3, 127.7, 127.4, 126.8, 120.9, 117.0, 114.0, 100.1, 55.4; ESI-MS: m/z 421 $[M+H]^+$; HRMS (ESI m/z) for $C_{26}H_{21}O_2N_4$ calc: 421.1659; found: 421.1663 $[M+ H]^+$.

(Z)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)-3-(quinolin-6-ylamino)prop-2-en-1-one (**8h**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(4-

methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7a**, 100mg, 0.362 mmol) and quinolin-6-amine (52.12mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (98 mg). M.p: 232–234 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.99 (d, *J* = 12.0 Hz, 1H), 9.74 (d, *J* = 6.9 Hz, 1H), 8.84-8.74 (m, 1H), 8.09-8.01 (m, 2H), 7.74-7.66 (m, 3H), 7.50-7.40 (m, 2H), 7.39-7.33 (m, 2H), 7.32-7.28 (m, 1H), 7.01 (t, *J* = 7.2 Hz, 3H), 5.56 (d, *J* = 8.1 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.7, 160.3, 151.5, 148.8, 146.5, 145.2, 142.2, 138.5, 135.0, 131.7, 131.2, 129.2, 128.5, 128.1, 127.4, 121.7, 120.6, 117.1, 113.9, 110.25, 99.4, 55.4; ESI-MS: m/z 421 $[M+H]^+$; HRMS (ESI m/z) for $C_{26}H_{21}O_2N_4$ calc: 421.1659; found: 421.1665 $[M+H]^+$.

(Z)-3-((3,5-dimethoxyphenyl)amino)-1-(2-phenylimidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8i**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7b**, 100mg, 0.406 mmol) and 3,5-dimethoxyaniline (62.19mg, 0.406 mmol) in ethanol (5 mL) as yellow color solid in good yield (126 mg). M.p: 151–153 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.71 (d, *J* = 12.1 Hz, 1H), 9.71 (d, *J* = 6.8 Hz, 1H), 7.71 (d, *J* = 7.1 Hz, 3H), 7.47 (d, *J* = 5.7 Hz, 3H), 7.44-7.38 (m, 1H), 7.13 (dd, *J* = 12.0 & 8.1 Hz, 1H), 6.99 (t, *J* = 6.7 Hz, 1H), 6.18 (d, *J* = 10.8 Hz, 3H), 5.41 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 183.4, 161.8, 151.2, 146.4, 142.9, 141.7, 135.2, 130.3, 128.9, 128.5, 127.9, 117.3, 113.9, 98.4, 95.4, 94.63, 55.4; ESI-MS: m/z 400 $[M+H]^+$; HRMS (ESI m/z) for $C_{24}H_{22}O_3N_3$ calc: 400.1656; found: 400.1654 $[M+H]^+$.

(Z)-3-((4-methoxyphenyl)amino)-1-(2-phenylimidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8j**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7b**, 100mg, 0.406 mmol) and 4-methoxyaniline (49.93mg, 0.406 mmol) in ethanol (5 mL) as yellow color solid in good yield (123 mg). M.p: 172–174 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.81 (d, *J* = 12.4 Hz, 1H), 9.69 (d, *J* = 7.0 Hz, 1H), 7.76-7.68 (m, 3H), 7.49-7.43 (m, 3H), 7.41-7.36 (m, 1H), 7.09 (dd, *J* = 12.4, 7.9 Hz, 1H), 7.01-6.94 (m, 3H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.36 (d, *J* = 7.8 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.1, 156.1, 150.6, 146.3, 144.1, 135.3, 133.9, 130.3, 129.1, 128.9, 127.6, 121.2, 117.6, 117.3, 115.0, 113.7, 97.5, 55.6; ESI-MS: m/z 370 $[M+H]^+$; HRMS (ESI m/z) for $C_{23}H_{20}O_2N_3$ calc: 370.1550; found: 370.1534 $[M+H]^+$.

(Z)-3-((4-bromophenyl)amino)-1-(2-phenylimidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8k**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7b**, 100mg, 0.406 mmol) and 4-bromoaniline (69.83mg, 0.406 mmol) in ethanol (5 mL) as yellow color solid in good yield (128 mg,). M.p: 160–162 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.77 (d, *J* = 11.8 Hz, 1H), 9.71 (d, *J* = 6.8 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 3H), 7.47 (d, *J* = 5.1 Hz, 3H), 7.42 (t, *J* = 7.9 Hz, 3H), 7.09 (dd, *J* = 12.0 & 8.2 Hz, 1H), 7.01 (t, *J* = 6.6 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 2H), 5.43 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.5, 151.5, 146.5, 142.4, 139.6, 135.2, 132.6, 130.3, 128.9, 128.5, 128.0, 121.1, 117.4, 115.6, 114.0, 98.9; ESI-MS: m/z 418 $[M+H]^+$; HRMS (ESI m/z) for $C_{22}H_{17}ON_3Br$ calc: 418.055; found: 418.0549 $[M+H]^+$.

(Z)-1-(2-phenylimidazo[1,2-a]pyridin-3-yl)-3-(quinolin-3-ylamino)prop-2-en-1-one (**8l**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(phenyl)imidazo[1,2-a]pyridin-3-

yl)prop-2-yn-1-one (**7b**, 100mg, 0.406 mmol) and quinolin-3-amine (58.46mg, 0.406 mmol) in ethanol (5 mL) as yellow color solid in good yield (104 mg). M.p: 188–190 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.99 (d, *J* = 11.9 Hz, 1H), 9.77 (d, *J* = 7.0 Hz, 1H), 8.76 (d, *J* = 2.6 Hz, 1H), 8.04 (d, *J* = 8.3 Hz, 1H), 7.75-7.72 (m, 3H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.64 (d, *J* = 2.4 Hz, 1H), 7.61-7.56 (m, 1H), 7.53-7.48 (m, 3H), 7.47-7.43 (m, 2H), 7.31-7.24 (m, 1H), 7.04 (t, *J* = 6.9 Hz, 1H), 5.55 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.6, 151.9, 146.6, 144.9, 142.0, 142.4, 142.0, 135.0, 134.1, 130.3, 129.3, 129.1, 128.7, 126.8, 121.1, 114.2, 100.0; ESI-MS: m/z 391 $[M+H]^+$; HRMS (ESI m/z) for $C_{25}H_{19}ON_4$ calc: 391.1553; found: 391.1557 $[M+H]^+$.

(Z)-1-(2-phenylimidazo[1,2-a]pyridin-3-yl)-3-(quinolin-6-ylamino)prop-2-en-1-one (**8m**): The titled compound was prepared using the method described for compound **8a**, by addition of (2-(phenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7b**, 100mg, 0.406 mmol) and quinolin-6-amine (58.46mg, 0.406 mmol) in ethanol (5 mL) as yellow color solid in good yield (109 mg). M.p: 204–206 ^{0}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 12.00 (d, *J* = 11.4 Hz, 1H), 9.76 (d, *J* = 6.0 Hz, 1H), 8.71 (s, 1H), 8.10-8.0 (m, 2H), 7.74 (s, 3H), 7.46 (d, 5H), 7.39-7.23 (m, 3H), 7.03 (s, 1H), 5.51 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$+DMSO, 75 MHz): δ 180.7, 146.5, 146.0, 141.2, 140.4, 137.3, 136.3, 133.0, 128.6, 128.3, 128.1, 126.3, 119.3, 118.8, 112.1, 111.9, 108.6, 101.4, 96.7; ESI-MS: m/z 391 $[M+H]^+$; HRMS (ESI m/z) for $C_{26}H_{21}O_2N_4$ calc: 391.1553; found: 391.1559 $[M+H]^+$.

(Z)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)-3-((3,4,5-trimethoxyphenyl)amino)prop-2-en-1-one (**8n**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 3,4,5-trimethoxyaniline (65.50mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (119 mg). M.p: 194–196 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.81 (d, *J* = 11.9 Hz, 1H), 9.67 (d, *J* = 6.2 Hz, 1H), 7.82-7.63 (m, 3H), 7.46 (d, *J* = 6.6 Hz, 3H), 7.23-7.12 (m, 1H), 7.03 (s, 1H), 6.28 (s, 2H), 5.36 (d, *J* = 7.4 Hz, 1H), 3.86 (s, 6H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.8, 154.20, 148.7, 146.0, 143.9, 136.4, 131.5, 130.4, 128.6, 117.1, 114.3, 97.9, 93.8, 61.0, 56.2; ESI-MS: m/z 464 $[M+H]^+$; HRMS (ESI m/z) for $C_{25}H_{23}O_4N_3Cl$ calc: 464.1372; found: 464.1343 $[M+H]^+$.

(Z)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)-3-((3,5-dimethoxyphenyl)amino)prop-2-en-1-one (**8o**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 3,5-dimethoxyaniline (54.62mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (113 mg). M.p: 120–122 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.73 (d, *J* = 12.4 Hz, 1H), 9.66 (d, *J* = 7.0 Hz, 1H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.70-7.67 (m, 2H), 7.47-7.44 (m, 3H), 7.18 (dd, *J* = 12.3 & 8.1 Hz, 1H), 7.02 (t, *J* = 6.9 & 1.1 Hz, 1H), 6.22-6.17 (m, 3H), 5.37 (d, *J* = 8.0 Hz, 1H), 3.80 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.1, 161.9, 149.4, 146.3, 143.3, 142.0, 135.1, 133.4, 131.6, 128.6, 121.1, 117.2, 114.1, 98.2, 95.7, 94.7, 55.5; ESI-MS: m/z 434 $[M+H]^+$; HRMS (ESI m/z) for $C_{24}H_{21}O_3N_3Cl$ calc: 434.1266; found: 434.1273 $[M+H]^+$.

(Z)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)-3-((4-methoxyphenyl)amino)prop-2-en-1-one (**8p**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 4-methoxyaniline (43.91 mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield(97 mg). M.p: 98–100 ^{0}C; ^{1}H NMR

($CDCl_3$, 500 MHz): δ 11.84 (d, *J* = 12.3 Hz, 1H), 9.64 (d, *J* = 6.9 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 3H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.14 (dd, *J* = 12.4 & 7.9 Hz, 1H), 7.03-6.97 (m, 3H), 6.90-6.87 (m, 2H), 5.33 (d, *J* = 7.8 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.7, 156.3, 144.7, 135.0, 133.7, 133.2, 128.7, 128.4, 128.0, 127.9, 117.8, 117.1, 115.0, 114.0, 97.4, 55.6; ESI-MS: m/z 404 $[M+H]^+$; HRMS (ESI m/z) for $C_{23}H_{19}O_2N_3Cl$ calc: 404.1160; found: 404.1171 $[M+H]^+$.

(Z)-3-((4-bromophenyl)amino)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8q**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 4-bromoaniline (61.40mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (128 mg). M.p: 208–210 ^0C; ^1H NMR ($CDCl_3$, 400 MHz): δ 11.80 (d, *J* = 11.9 Hz, 1H), 9.66 (d, *J* = 6.6 Hz, 1H), 7.81 (d, *J* = 6.6 Hz, 1H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 8.5 Hz, 5H), 7.17 (dd, *J* = 11.9, 7.9 Hz, 1H), 7.07 (d, *J* = 6.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 5.39 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.0, 148.6, 145.9, 143.2, 139.3, 135.4, 132.7, 131.7, 128.8, 128.5, 121.0, 117.6, 117.1, 116.0, 114.6, 98.6; ESI-MS: m/z 391 $[M+H]^+$; HRMS (ESI m/z) for $C_{26}H_{21}O_2N_4$ calc: 391.1553; found: 391.1559 $[M+H]^+$.

(Z)-3-((1H-indol-5-yl)amino)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8r**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 1H-indol-5-amine (47.12mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (90 mg). M.p: 251–253 ^0C; ^1H NMR ($CDCl_3$+DMSO, 300 MHz): δ 12.07 (d, *J* = 12.6 Hz, 1H), 10.32 (s, 1H), 9.68 (d, *J* = 7.0 Hz, 1H), 7.74 (dd, *J* = 15.5, 8.7 Hz, 3H), 7.54-7.40 (m, 5H), 7.37 (t, *J* = 6.3 Hz, 2H), 7.09-6.93 (m, 2H), 6.47 (s, 1H), 5.38 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR (DMSO, 75 MHz): δ 181.4, 145.9, 145.4, 145.0, 132.4, 131.6, 128.4, 126.7, 126.45, 121.3, 120.7, 114.1, 113.8, 112.4, 111.7, 106.7, 101.0, 96.2; ESI-MS: m/z 413 $[M+H]^+$; HRMS (ESI m/z) for $C_{24}H_{18}ON_4Cl$ calc: 413.1164; found: 413.1169 $[M+H]^+$.

(Z)-3-((1H-indol-6-yl)amino)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-one (**8s**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and 1H-indol-6-amine (47.12mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (95 mg). M.p: 221–223 ^0C; ^1H NMR ($CDCl_3$, 500 MHz): δ 12.00 (d, *J* = 12.5 Hz, 1H), 9.66 (d, *J* = 7.0 Hz, 1H), 8.20 (s, 1H), 7.71 (t, *J* = 8.6 Hz, 3H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.43-7.38 (m, 1H), 7.31-7.27 (m, 1H), 7.19-7.16 (m, 1H), 7.09 (s, 1H), 7.00 (dd, *J* = 9.8 & 3.9 Hz, 1H), 6.91 (dd, *J* = 8.4 & 1.9 Hz, 1H), 6.52 (s, 1H), 5.37 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$+DMSO, 75 MHz): δ 179.9, 145.8, 143.0, 134.6, 132.5, 129.7, 126.4, 125.9, 123.3, 119.10, 114.8, 112.1, 107.7, 99.4, 96.6, 94.9; ESI-MS: m/z 413 $[M+H]^+$; HRMS (ESI m/z) for $C_{24}H_{18}ON_4Cl$ calcd: 413.1164; found: 413.1173 $[M+H]^+$.

(Z)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)-3-(quinolin-3-ylamino)prop-2-en-1-one (**8t**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and quinolin-3-amine

(53.28mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (106 mg). M.p: 229–231 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.02 (d, *J* = 12.0 Hz, 1H), 9.73 (d, *J* = 7.0 Hz, 1H), 8.79 (d, *J* = 2.7 Hz, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 7.76-7.72 (m, 2H), 7.71-7.68 (m, 3H), 7.64-7.59 (m, 1H), 7.57-7.52 (m, 1H), 7.50-7.45 (m, 3H), 7.33 (dd, *J* = 12.0 & 8.1 Hz, 1H), 7.06 (td, *J* = 6.9 & 1.1 Hz, 1H), 5.52 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.4, 150.3, 146.6, 144.9, 142.8, 142.4, 135.3, 134.1, 133.5, 131.6, 129.2, 128.7, 127.9, 126.9, 117.4, 117.3, 114.4, 99.9; ESI-MS: m/z 425 $[M+H]^+$; HRMS (ESI m/z) for $C_{25}H_{18}ON_4Cl$ calc: 425.1164; found: 425.1175 $[M+ H]^+$.

(Z)-1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)-3-(quinolin-6-ylamino)prop-2-en-1-one (**8u**): The titled compound was prepared using the method described for compound **8a**, by addition of 1-(2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-one (**7c**, 100mg, 0.357 mmol) and quinolin-6-amine (53.28mg, 0.357 mmol) in ethanol (5 mL) as yellow color solid in good yield (109 mg). M.p: 214–216 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.01 (d, J=12.0 Hz, 1H), 9.71 (d, J=6.9 Hz, 1H), 8.80 (d, J=2.9 Hz, 1H), 8.07 (dd, J=15.0 & 8.6 Hz, 2H), 7.76 – 7.67 (m, 3H), 7.51 – 7.43 (m, 4H), 7.41 – 7.33 (m, 3H), 7.04 (t, J=6.7 Hz, 1H), 5.48 (d, J=8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.3, 150.1, 148.9, 146.6, 145.3, 142.7, 138.4, 135.1, 133.6, 131.6, 131.3, 128.6, 122.0, 120.7, 117.4, 114.2, 110.5, 99.2; ESI-MS: m/z 425 $[M+H]^+$; HRMS (ESI m/z) for $C_{25}H_{18}ON_4Cl$ calc: 425.1164, found: 425.1177 $[M+H]^+$.

Biology

Anti cancer activity

The Anticancer activity of the compounds was determined using MTT assay.[24] 1×10^4 cells/well were seeded in 100 µL DMEM supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 ^{0}C in a CO_2 incubator. After 24 h of incubation, all the synthesized compounds were added to the respective wells and incubated for 48 h. After 48 h of drug treatment, 10 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. The supernatant from each well was carefully removed, formazan crystals were dissolved in 100 µL of DMSO and absorbance at 570 nm wavelength was recorded.

Cell cycle analysis

Flow cytometric analysis (FACS) was performed to evaluate the distribution of the cells through the cell cycle phases. A549 cells were treated with compound **8m** and **8q** at 0.5 µM and 1 µM concentrations for 48 h. Untreated and treated cells were harvested, washed with phosphate-buffered saline (PBS), fixed in ice-cold 70% ethanol and stained with propidium iodide (Sigma–Aldrich). Cell-cycle analysis was performed by flow cytometry (Becton Dickinson FACS Caliber instrument).[28]

Tubulin polymerization assay

A fluorescence based in vitro tubulin polymerization assay was performed according to the manufacturer's protocol (BK011, Cytoskeleton, Inc.). Briefly, the reaction mixture in a total volume of 10 µL contained PEM buffer, GTP (1 µM) in the presence or absence of test compounds (final concentration of 3 µM). Tubulin polymerization was followed by a time dependent increase in fluorescence due to the incorporation of a

fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured by using a Varioscan multimode plate reader (Thermo scientific Inc.) using Nocodazole as reference compound. The IC_{50} value was defined as the drug concentration required for inhibiting 50% of tubulin assembly compared to control. The reaction mixture for these experiments include: tubulin (3 mg/mL) in PEM buffer, GTP (1 μM), in the presence or absence of test compounds at varying concentrations. Polymerization was monitored by increase in the fluorescence as mentioned above at 37 ^{0}C. To determine the IC_{50} values of the compounds against tubulin polymerization, the compounds were pre-incubated with tubulin at varying concentrations. Assay was performed under similar conditions as employed for polymerization assays as described above.

Immunohistochemistry

A549 cells were seeded on glass cover slips, incubated for 48 h in the presence or absence of test compounds (**8m** and **8q)** at 0.5 μM concentration. Following the termination of incubation, cells were fixed with 3% paraformaldehyde, 0.02% glutaraldehyde in PBS and permeabilized by dipping the cells in 100% methanol followed by overnight incubation in 4 ^{0}C. Later, cover slips were blocked with 1% BSA in phosphate buffered saline for 1 h followed by incubation with a primary anti tubulin (mouse monoclonal) antibody and FITC conjugated secondary mouse anti IgG antibody. Photographs were taken using the fluorescence microscope equipped with FITC settings and the pictures were analyzed for the integrity of microtubule network.

Hoechst staining

A549 cells were seeded at a density of 10,000 cells over 18 mm cover slips and incubated for 24 h. After incubation, cells were treated with the compounds **8m** and **8q** at 0.5 μM concentration for 48 h. Hoechst 33258 (Sigma Aldrich) was added to the cells at a concentration of 0.5 mg/mL and incubated for 30 min at 37 ^{0}C. Later, the cells were washed with phosphate buffered saline (PBS). Cells from each cover slip were captured from randomly selected fields under fluorescent microscope (Olympus microscope) to qualitatively determine the proportion of viable and apoptotic cells based on their relative fluorescence and nuclear fragmentation.[29]

Measurement of mitochondrial membrane potential (ΔΨm)

A549 (1×10^6 cells/well) cells were cultured in six-well plates. After plating, cells were treated with compounds **8m** and **8q** at 0.5 and 1 μM concentrations for 48 h. After 48 h of treatment, cells were collected by trypsinization and washed with PBS followed by resuspending in JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolocarbocyanine iodide-5μg/mL) and incubated at 37 ^{0}C for 15 min. Cells were rinsed three times with medium and suspended in pre warmed medium. The cells were then subjected to flow cytometric analysis on a flow cytometer (Becton Dickinson) in the FL1, FL2 channel to detect mitochondrial potential.[30]

Annexin V-FITC assay for apoptosis

A549 (1×10^6) cells were seeded in six-well plates and allowed to grow overnight. The medium was then replaced with complete medium containing compounds **8m** and **8q** at 0.5 and 1 μM concentrations for 48 h.

After 48 h of drug treatment, cells from the supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS at 5000 rpm. Then the cells were stained with Annexin VFITC and propidium iodide using the Annexin-V-FITC apoptosis detection kit (Sigma aldrich). The samples were analyzed by flow cytometry as described earlier.[31]

Note: The references cited in the supplementary file are given in the manuscript.