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# New (3-(1H-benzo[d]imidazol-2-yl)/(3-(3H-imidazo[4,5-b]pyridin-2-yl)-(1H-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone conjugates as tubulin polymerization inhibitors

**Kishore Mullagiri,[a] V. Lakshma Nayak,[a] Satish Sunkari,[a] Geetha Sai Mani,[b] Sravanthi Devi Guggilapu,[b] Burri Nagaraju,[a] Abdulla Alarifi,[c] and Ahmed Kamal*[a]**

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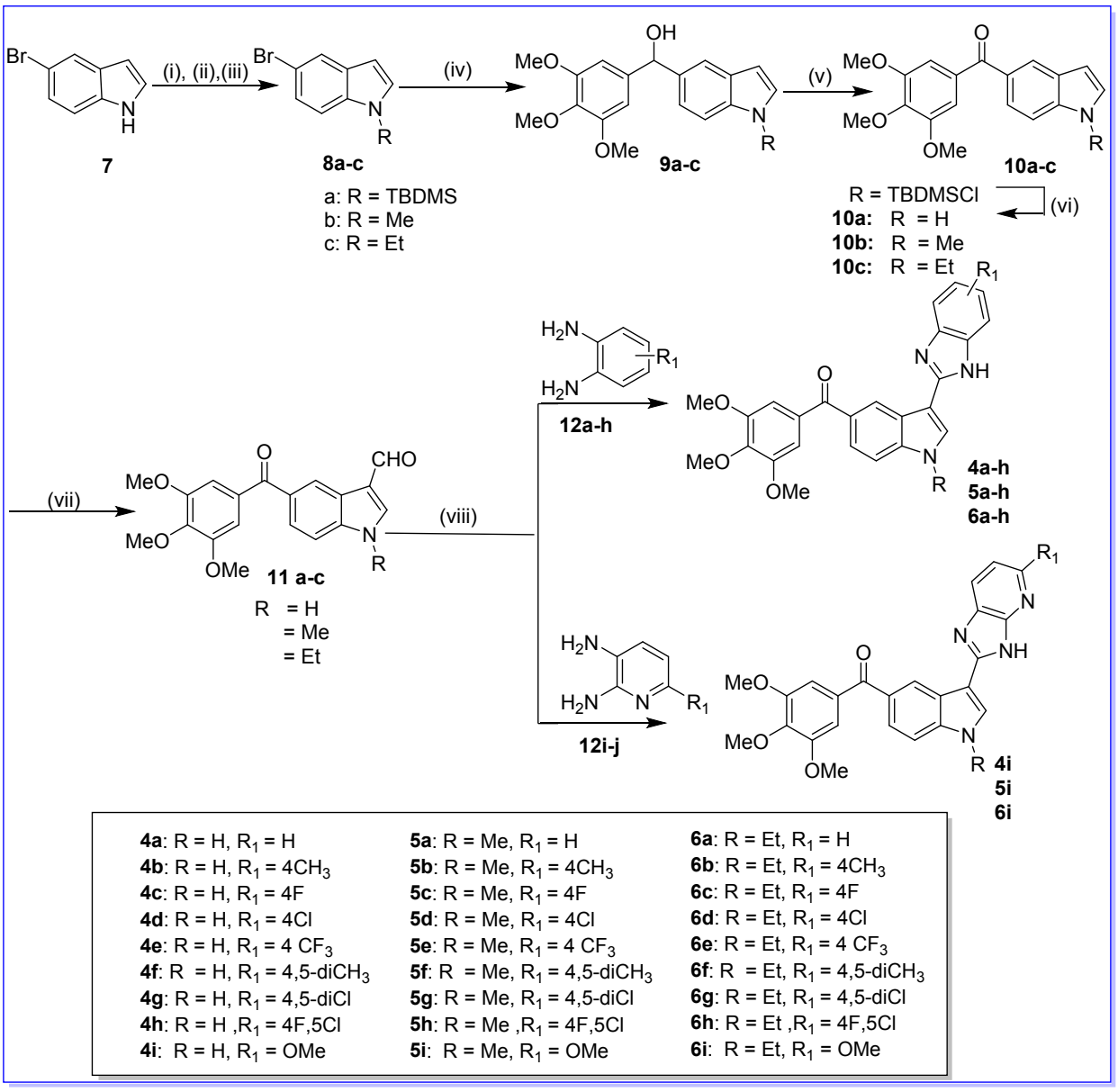
## Experimental section

### Chemistry

**Materials and methods.** $^{1}H$ NMR spectra were recorded on Avance 300, Inova 400, Avance 500, and Bruker 600 MHz spectrometers using tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethyl silane. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and or m (multiplet). Coupling constants are reported in Hertz (Hz). Melting points were determined in a capillary tube using an electrothermal apparatus (Model IA9200) and are uncorrected. The IR spectra were recorded by employing a Nicolet FTIR model MX-1spectrophotometer. Analytical thin layer chromatography (TLC) was performed on MERCK precoated silica gel 60-F254 (0.5 mm) glass plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapour or UV light or by dipping the plates into methanolic sulphuric acid-β-naphthol or to ethanolic anisaldehyde-sulphuric acid–acetic acid or to ethanolic ninhydrin solution and heating the plates to 120 °C. Column chromatography was performed using silica gel 60–120 and 100–200 mesh. Moisture sensitive reactions were carried out using standard syringe septum Techniques and under inert atmosphere of nitrogen. All solvents and reagents were purified by standard techniques. All evaporation of solvents was carried out under reduced pressure on Laborota-4000 rotary evaporator below 45 °C. The names of all the compounds given in the experimental section were taken from Chem Ultra, Version 11.0. All the cell lines were purchased from The National Center for Cell Science (NCCS), Pune, India.

**Scheme 1: Synthesis of trimethoxy aroyl indole-benzimidazole conjugates**




***Reagents and conditions*:** (i) TBDMSCl, NaH, THF, 0 °C-rt, 3 h, 93%; (ii) MeI, NaOH, DMSO, 0 °C-rt, 3 h, 95%; (iii) EtBr, NaH, THF, 0 °C-rt, 3 h, 95% (iv) 3,4,5-trimethoxy benzaldehyde, n-BuLi, THF, -78 °C, 4 h, 73-75%, (v) IBX, DMSO, 0 °C–rt, 3 h, 95%; (vi) TBAF, THF, 0 °C-rt, 4 h, 90% (vii) $POCl_3$, DMF, $CHCl_3$, Reflux, 12 h, 78-82%; (viii) $Na_2S_2O_5$, EtOH:$H_2O$, 80 °C, 2 h, 68-85%.

**General procedure for the synthesis of substituted 5-Bromo-1*H*-indole (8a–c):** To the solution of 5-bromo-1*H*-indole (1 equiv.) in dry THF, sodium hydride (NaH, 1.5 equiv. 60% dispersion in mineral oil) was added portion wise at 0 °C and the reaction mixture was stirred at room temperature. After 30 minutes methyl iodide/ethyl bromide/tert-butyldimethylsilyl chloride (1.2 equiv.) were added at 0 °C, afterwards the reaction mixture was allowed to warm to room temperature for 3 h. Then ice cold water was added carefully and the aqueous phase was extracted using $CH_2Cl_2$. The combined organic layers were washed with $H_2O$ and saturated brine solution. Then the combined organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. Then the obtained residue was purified by column chromatography over silica gel to give pure compounds **8a-c** using ethyl acetate and petroleum ether as an eluent.

**General reaction procedure for the synthesis of substituted (1*H*-Indol-5-yl)(3,4,5-trimethoxyphenyl)methanol derivatives (9a-c):**

To a stirred solution of compounds **8a-c** (1 equiv.) in dry THF was added n-BuLi (1.2 equiv., 1.6 M in hexane) at -78 $^oC$ under argon atmosphere and stirred for 1 h. Then 3,4,5-trimethoxybenzaldehyde (1 equiv.) was slowly added to the reaction mixture and allowing the reaction mixture slowly to reach the room temperature and stirred for 4 h. Then reaction mixture was poured into ice-cold water and extracted with $CH_2Cl_2$, washed with water and saturated brine solution. Then the combined organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. Then the obtained residue was purified by column chromatography over silica gel to give pure compounds **9a-c** using ethyl acetate and petroleum ether as an eluent.

**(1-(Tert-butyldimethylsilyl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanol (9a):**

Red solid: yield 75%; mp: 234 - 235 °C; $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.61 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.19 (d, *J* = 3.2 Hz, 1H ), 7.15 (dd, *J* = 1.6, 8.5 Hz, 1H), 6.68 (s, 2H), 6.60 (d, *J* = 3.0 Hz, 1H ), 5.88 (s, 1H), 3.83 (s, 9H), 0.92 (s, 9H), 0.59 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 153.0, 140.5, 140.0, 136.8, 135.3, 131.6, 131.2, 120.4, 118.4, 114.0, 104.9, 103.4, 60.7, 56.0, 26.2, 19.4, -4.0; MS (ESI): *m/z*: 427 $[M + H]^+$.

**General reaction procedure for the synthesis of substituted (1*H*-Indol-5-yl)(3,4,5-trimethoxyphenyl)methanone derivatives (10a-c):**

To a stirred solution of compounds **9a-c** (1 equiv.) in DMSO was added IBX (2 equiv.) at 0 °C and stirred for 3 h at room temperature. The reaction mixture was poured into ice-water and extracted with $CH_2Cl_2$, washed with water and saturated brine solution. Then the combined organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. Then the obtained residue was purified by column chromatography over silica gel to get pure compounds **10a-c** using ethyl acetate and petroleum ether as an eluent.

**(1*H*-Indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (10a):**

Brown solid: yield 95%; mp: 238-239 °C; $^1$H NMR (300 MHz, CDCl3) δ: 8.94 (s, 1H), 8.17 (s, 1H), 7.66 (dd, *J* = 1.1, 8.4 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 1H), 7.36 (t, *J* = 2.6 Hz, 1H), 7.09 (s, 2H), 6.63 (s, 1H ), 3.95 (s, 3H), 3.84 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 195.8, 152.8, 141.3, 139.3, 134.0, 130.0, 127.3, 124.3, 122.2, 110.8, 104.5, 103.5, 60.8, 56.1; MS (ESI): *m/z*: 311 $[M + H]^+$.

**General reaction procedure for the synthesis of substituted 5-(3,4,5-Trimethoxybenzoyl)-1*H*-indole-3-carbaldehydes (11a-c)**

The Vilsmeier reagent was prepared at 0–58 °C by dropping $POCl_3$ (2 equiv.) into a stirred solution of DMF (2.5 equiv.) in $CHCl_3$ (5 mL). Compounds **10a-c** (1 equiv.) in $CHCl_3$ (60 mL) was added drop wise to the Vilsmeier reagent while maintaining cooling and stirring. The reaction mixture was kept at 25 °C for 2 h and under reflux for 12 h. The solvent was removed under reduced pressure, and the resulting oil was poured onto ice. The crude aldehydes **11a-c** thus obtained was collected by filtration and crystallized from ethanol.

**5-(3,4,5-Trimethoxybenzoyl)-1*H*-indole-3-carbaldehyde (11a):**

Pale yellow solid: yield 74%; mp: 250 - 251 °C; $^1$H NMR (300 MHz, $CDCl_3$+ DMSO-$d_6$) δ: 11.44 (s, 1H), 9.16 (s, 1H), 7.81 (s, 1H), 7.33 (d, *J* = 3.0 Hz, 1H), 6.91 (dd, *J* = 1.3, 8.2 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.22 (s, 2H), 3.04 (s, 3H), 3.01 (s, 6H): $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 195.3, 184.8, 152.6, 141.6, 138.5, 134.0, 130.1, 128.4, 126.0, 122.4, 118.3, 110.0, 105.3, 60.8, 56.2; MS (ESI): *m/z*: 339 $[M + H]^+$.

**General reaction procedure for the synthesis of substituted (1*H*-Indol-5-yl)(3,4,5-trimethoxyphenyl)methanone derivatives (4-6(a-i)):**

To a stirred solution of aldehydes **11a-c** (1 equiv.) in ethanol was added minimum amount of water dissolved sodiummetabisulphite (1.5 equiv.) at 0 °C and stirred for 10

min. Then to this reaction mixture substituted benzene-1,2-diamine (1equiv.) were added and reaction mixture was stirred at 80 $^{o}$C for 2 h. After the completion of the reaction by monitoring TLC, reaction mixture cooled to room temperature and ice-cold water was added, the resulted precipitate was filtered and washed with water, dried under vacuum to give the crude compounds. The obtained residue was purified by column chromatography over silica gel to get pure compounds **4-6(a-i)** using ethyl acetate and petroleum ether as an eluent.

**(3-(1*H*-Benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4a):**

Yellow solid: yield 80%; mp: 249–250 °C; $^{1}$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.53 (s, 1H), 9.22 (s, 1H), 8.17 (d, *J* = 2.4 Hz, 1H), 7.79 (dd, *J* = 1.5, 8.4 Hz, 1H), 7.71 (s, 1H), 7.59 (s,1H), 7.56-7.52 (m, 2H), 7.20 (s, 2H), 7.18-7.14 (m, 2H), 3.94 (s, 3H), 3.91 (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.5, 172.0, 152.4, 148.7, 140.9, 138.7, 133.2, 129.3, 127.4, 125.6, 124.5, 123.9, 121.2, 111.7, 108.1, 107.6, 60.1, 55.1; MS (ESI): *m/z* 427 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{25}H_{22}N_3O_4$: 428.15808; found:428.15922 $[M + H].^+$

**(3-(6-Methyl-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4b):**

Yellow solid: yield 83%; mp: 215–216 °C; $^{1}$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.79 (s, 1H), 12.08 (s, 1H), 9.11 (s, 1H), 8.28 (s, 1H), 7.74 (dd, *J* = 1.5, 8.5 Hz, 1H ), 7.65 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.34 (s, 1H), 7.15 (s, 2H), 7.01 (d, *J* = 8.5 Hz, 1H), 3.80 (s, 6H) 3.82 (s, 3H), 2.43 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.0, 152.2, 147.6, 140.7, 139.4, 137.9, 132.8, 132.2, 130.8, 130.2, 129.3, 129.2, 125.5, 124.7, 123.8, 122.6, 113.9, 109.5, 107.3, 59.9, 55.7, 40.9, 21.2; MS (ESI): *m/z* 441 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{24}N_3O_4$: 442.17613; found:442.17464 $[M + H].^+$

**(3-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5 trimethoxyphenyl)methanone (4c):**

Pale yellow solid: yield 76%; mp: 244–246 °C; $^{1}$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.68 (s, 1H), 9.08 (s, 1H), 8.11 (s, 1H), 7.85-7.79 (m, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.42-7.35 (m, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.10 (s, 2H), 6.84

(dt, *J* = 1.5, 10.3 Hz, 1H), 3.82 (d, *J* = 4.3 Hz, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 195.2, 152.4, 141.1, 138.9, 133.2, 130.1, 128.8, 124.6, 124.4, 124.5, 111.8, 107.1, 60.4, 56.0, 55.8; MS (ESI): *m/z* 445 $[M + H]^+$; HRMS (ESI): *m/z* 445: calcd for $C_{25}H_{21}FN_3O_4$: 446.15106; found: 446.15070 $[M + H].^+$

**(3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4d):**

yellow solid: yield 78%; mp: 208–209 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.86 (s, 1H), 9.01 (s, 1H), 8.24 (s, 1H), 7.77 (s, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.57-7.41 (m, 3H), 7.10 (d, *J* = 7.4 Hz, 3H), 3.83 (d, *J* = 7.7 Hz, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.6, 185.4, 152.5, 141.0, 140.0, 139.3, 138.8, 133.1, 125.2, 124.5, 124.1, 123.4, 118.8, 112.5, 112.0, 107.6, 107.4, 60.3.9, 56.0; MS (ESI): *m/z* 462 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{25}H_{21}ClN_3O_4$: 462.12151; found: 462.12164 $[M + H].^+$

**(3-(6-(Trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4e):**

Pale yellow solid: yield 72%; mp: 202–203 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.00 (s, 1H), 8.98 (s, 1H), 8.37 (s, 1H), 7.81 (s, 1H), 7.71-7.62 (m, 3H), 7.55 ( d, *J* = 8.5 Hz, 1H,) 7.43 ( d, *J* = 8.5 Hz, 1H,) 7.08 (s, 2H), 3.88-3.78 (d, *J* = 10.4 Hz, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.5, 152.3, 150.1, 140.9, 138.8, 132.9, 130.2, 126.3, 124.0 (d, *J* = 6.6 Hz), 119.1, 113.9, 111.9, 111.2, 107.4, 104.9, 60.1, 55.8; MS (ESI): *m/z* 495 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{21}F_3N_3O_4$: 496.14787; found: 496.14539 $[M + H].^+$

**(3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl) methanone (4f):**

yellow solid: yield 85%; mp: 264–265 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.86 (s, 1H), 9.01 (s, 1H), 8.24 (s, 1H), 7.77 (s, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.57-7.41 (m, 3H), 7.10 (d, *J* = 7.4 Hz, 3H), 3.83 (d, *J* = 7.7 Hz, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.4, 152.4, 147.8, 140.8, 138.7, 133.2, 129.5, 129.2, 127.1, 125.6, 124.5, 123.9, 111.4, 108.3, 107.5, 60.1, 55.9, 19.9; MS (ESI): *m/z* 455 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{26}N_3O_4$: 456.19178; found: 456.19020 $[M + H].^+$

**(3-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4g):**

yellow solid: yield 76%; mp: 180–181 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 11.49 (s, 1H), 9.13 (s, 1H), 8.15 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.70-7.50 (m, 4H), 7.20 (s, 2H), 3.96 (s, 3H), 3.91 (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 195.0, 152.1, 150.9, 140.8, 138.6, 133.0, 129.6, 127.6, 125.0, 124.2, 124.0, 111.3, 107.4, 60.2, 55.7; MS (ESI): *m/z* 496 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{25}H_{20}Cl_2N_3O_4$: 496.08254; found:496.08276 $[M + H].^+$

**(3-(6-Chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4h):**

yellow solid: yield 74%; mp: 272–273 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 9.10 (s, 1H), 8.25 (s, 1H), 7.95-7.86 (m, 1H), 7.75 (dd, *J* = 1.5, 8.4 Hz, 1H), 7.62-7.56 (m, 2H), 7.38 (d, *J* = 9.6 Hz, 1H) 7.16 (s, 2H), 3.92-3.88 (m, 9H): $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.4, 154.9, 152.2, 151.7, 150.4, 140.8, 138.6, 132.9, 129.7, 128.4, 124.6, 124.1, 114.2, 113.8, 113.5, 111.5, 107.4, 106.5, 101.0, 60.0, 55.7; MS (ESI): *m/z* 480 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{25}H_{20}ClFN_3O_4$: 480.11209; found: 480.11123 $[M + H].^+$

**(3-(5-Cethoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (4i):**

Pale green : yield 85%; mp: 206–208 °C; $^1$H NMR (300 MHz, DMSO $d_6$) δ: 13.32 (s, 1H), 12.06 (s, 1H), 8.12 (s, 1H), 8.26 (s, 1H), 7.89-7.62 (m, 3H), 7.14 (s, 2H,) 6.63 ( d, *J* = 8.2 Hz, 1H,) 3.91-3.78 (m, 12H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ: 194.4, 160.0, 152.5, 140.9, 138.7, 133.2, 129.3, 127.5, 125.4, 124.3, 124.1, 112.0, 107.9, 104.3, 60.1, 56.0, 53.1; MS (ESI): *m/z* 458 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{25}H_{23}N_4O_5$: 459.16630; found: 459.16461 $[M + H].^+$

**(3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5a):**

Pale yellow solid: yield 80%; mp: 211–212 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.62 (s, 1H), 9.16 (s, 1H), 8.19 (s, 1H), 7.80 (dd, *J* = 1.5, 6.8 Hz 1H), 7.74-7.69 (m, 1H), 7.54-7.48 (m, 2H,) 7.18-7.12 (m, 4H) 3.98 (s, 3H), 3.85-3.80 (d, *J* = 6.8 Hz, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.4, 155.3, 152.5, 148.3, 140.9, 139.1, 133.1, 131.5, 129.4, 125.7, 124.8, 124.0, 121.5, 110.4, 107.6, 107.2, 106.2, 60.2, 56.0, 33.2; MS

(ESI): *m/z* 441 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{24}N_3O_4$: 442.17613; found: 442.17475 [M + H].$^+$

**(1-Methyl-3-(6-methyl-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5b):**

Yellow solid: yield 84%; mp: 140–141 °C; $^1$H NMR (300 MHz $CDCl_3$ + DMSO-$d_6$) δ: 9.96 (s, 1H), 8.30 (s, 1H), 7..72 (d, *J* = 8.5 Hz, 1H), 7.51 (s, 1H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.29 (s, 1H), 7.07 (s, 2H), 6.95 (d, *J* = 7.9 Hz, 1H) 3.88-3.78 (m, 12H), 2.36 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.5, 152.3, 146.1, 141.0, 138.9, 138.1, 137.2, 133.6, 132.7, 130.5, 124.5, 124.0, 123.6, 117.2, 113.3, 113.0, 111.3, 109.9, 107.4, 60.0, 55.7, 33.3, 21.1; MS (ESI): *m/z* 456 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{26}N_3O_4$: 456.19178; found: 456.19215 [M + H].$^+$

**(3-(6-Fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5c):**

yellow solid: yield 79%; mp: 270–272 °C; $^1$H NMR (300 MHz, $CDCl_3$ + Methanol-$d_4$) *δ*: 9.05 (s, 1H), 7.93 (s, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.50-7.34 (m, 3H), 7.16-7.07 (m, 3H), 6.88-6.79 (m, 1H), 3.90-3.79 (m, 12H), $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$ + Methanol-$d_4$) δ: 195.3, 152.5, 141.4, 139.3, 135.3, 134.7, 132.9, 132.6, 131.5, 129.1, 125.1, 124.3, 124.1, 112.9, 114.5, 113.4, 110.0, 107.4, 60.4, 56.0, 33.6; MS (ESI): *m/z* 476 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{23}ClN_3O_4$: 476.13716; found: 476.13828 [M + H].$^+$

**(3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5d):**

Pale yellow solid: yield 76%; mp: 198–200 °C; $^1$H NMR (300 MHz, $CDCl_3$ + Acetione-$d_6$) δ: $CDCl_3$) δ: 9.05 (s, 1H), 7.94 (s, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.50-7.34 (m, 3H), 7.15-7.10 (m, 3H), 6.88-6.79 (m, 1H), 3.90-3.79 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$ + Acetione-$d_6$) 194.1, 159.7, 156.6, 155.1, 152.3, 149.4,140.8, 138.8, 132.9, 131.3, 129.4, 125.5, 125.3, 124.6, 123.9, 109.8, 109.0, 108.6, 107.4, 107.0, 106.1, 59.9, 56.1, 55.7, 33.1; MS (ESI): *m/z* 459 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{23}FN_3O_4$: 460.16671; found: 460.16736 [M + H].$^+$

**(1-Methyl-3-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5e):**

yellow solid: yield 68%; mp: 178–180 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 9.09 (s, 1H) ), 7.88- 7.80 (m, 2H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.62 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.18-7.15 (m, 2H), 4.00-3.88 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.5, 155.2, 152.3, 149.9, 141.0, 139.0, 133.1, 132.8, 130.3, 124.5, (d, *J* = 6.0, Hz ), 118.8, 114.8, 111.4, 110.0, 107.4, 106.1, 105.0, 60.1, 55.8, 33.3; MS (ESI): *m/z* 509 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{23}F_3N_3O_4$: 510.16352; found:510.15997 $[M + H].^+$

**(3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5f):**

yellow solid: yield 74%; mp: 180–182 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 8.91 (s, 1H), 7.73-7.66 (m, 2H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.27 (s, 2H), 7.05 (s, 2H), 3.89-3.78 (m, 12H), 2.26 (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.3, 152.2, 145.8, 140.9, 138.8, 132.8, 131.2, 130.1, 124.9, 124.2, 124.1, 122.3, 113.6, 109.7, 107.3, 59.9, 56.0, 33.2, 19.8; MS (ESI): *m/z* 470 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{28}H_{28}N_3O_4$: 470.20743; found: 470.20593 $[M + H].^+$

**(3-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5g):**

Pale yellow solid: yield 69%; mp: 297–298 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.95 (s, 1H), 9.08 (s, 1H), 8.26 (s, 1H), 7.82-7.75 (m, 2H), 7.75-7.70 (m, 2H), 7.13 (s, 2H), 3.98 (s, 3H), 3.85-3.80 (s, 9H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 194.4, 155.2, 152.4, 143.7, 140.9, 139.0, 132.9, 132.3, 129.8, 125.0, 124.7, 124.2, 123.5, 111.7, 110.4, 107.5, 106.2, 60.1, 55.9, 33.3; MS (ESI): *m/z* 510 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{22}Cl_2N_3O_4$: 510.09819; found: 510.09990 $[M + H]^+$; HPLC: C18 tR: 7.063 min.

**(3-(6-Chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5h):**

yellow solid: yield 68%; mp: 250–251 °C; $^1$H NMR (300 MHz, CDCl3 + DMSO $d_6$) δ: 12.28 (s, 1H), 8.07 (s, 1H), 8.15 (s, 1H), 8.08 (s, 2H), 7.75 (d, *J* = 7.4 Hz, 1H) 7.58 (d, *J* = 8.8 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H) 7.11 (s, 1H) 3.96 (s, 3H), 3.86-3.80 (m, 9H); 13C NMR (75 MHz, CDCl3 + DMSO-d6) δ: 194.2, 152.4, 150.3, 139.9, 138.1, 132.6, 129.9, 129.7, 125.6, 124.8, 124.0, 110.1, 107.3, 107.0, 59.8, 55.6, 33.9; MS (ESI): *m/z* 494 [M +

H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{22}ClFN_3O_4$: 494.12774; found: 494.12940 [M + H].$^+$

**(3-(5-Methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5i):**

yellow solid: yield 73%; mp: 150–152 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 9.19(s, 1H), 8.27 (s, 1H), 7.92 (s, 1H), 7.78 (d, *J* = 9.9 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.29 (q, *J* = 3.0, 6.3 Hz, 2H), 7.14 (s 2H), 3.98 (s, 3H), 3.93-3.80 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.2, 155.2, 152.3, 140.9, 139.0, 132.9, 132.7, 129.7, 127.3, 125.4, 124.9, 124.1, 122.8, 110.1, 107.5, 106.7, 106.1, 60.0, 56.2, 55.8, 33.2; MS (ESI): *m/z* 473 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{26}H_{25}N_4O_5$: 473.18198; found:473.19213 [M + H].$^+$

**(3-(1*H*-Benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6a):**

Pale yellow solid: yield 70%; mp: 130–131 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.60 (s, 1H) ), 9.20 (s, 1H), 8.29 (d, *J* = 2.2 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.52 (s 2H), 7.19-7.12 (m, 4H), 4.40 (q, *J* = 7.1, 14.1 Hz, 2H), 3.90-3.84 (m, 9H), 1.54 (t, *J* = 7.1, 14.3 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.4, 171.0, 152.5, 148.4, 140.8, 138.2, 133.0, 129.7, 129.3, 125.9, 124.9, 123.9, 121.4, 110.4, 107.5, 60.1, 55.9, 41.0, 15.2; MS (ESI): *m/z* 456 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{26}N_3O_4$: 456.19178; found:456.19195 [M + H].$^+$

**(1-Ethyl-3-(6-methyl-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6b):**

Yellow solid: yield 72%; mp: 270–272 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 8.12 (d, *J* = 1.1, Hz 1H), 8.15 (s, 1H), 7.91 (s, 1H), 7.74 (dd, *J* = 1.7, 8.6 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.23 (s, 1H), 7.11( s, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 4.30 (q, *J* = 7.1, 14.3 Hz, 2H), 3.85-3.82 (m, 9H), 2.39 (s, 3H), 1.52 (t, *J* = 7.1, 14.3 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.0, 152.4, 147.6, 140.7, 137.9, 132.8, 130.4, 129.3, 129.2, 125.5, 124.7, 123.8, 122.6, 109.5, 107.3, 59.9, 55.1, 40.9, 21.1, 14.9; MS (ESI): *m/z* 470 [M + H]$^+$; HRMS (ESI): *m/z*: calcd for $C_{28}H_{28}N_3O_4$: 470.20743; found: 470.20814 [M + H].$^+$

**(3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6c):**

yellow solid: yield 68%; mp: 154–155 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 10.01 (s, 1H), 9.91 (d, *J* = 1.1 Hz, 1H), 7.84 (dd, *J* = 1.7, 8.6 Hz, 1H), 7.71 (s, 1H), 7.57(s, 1H), 7.54 (s, 1H), 7.50 (s, 1H), 7.19 (s, 2H), 7.12 (dd, *J* = 1.8, 8.4 Hz, 1H), 4.34 (q, *J* = 7.1, 14.5 Hz, 2H), 3.96-3.89 (m, 9H), 1.61 (t, *J* = 7.1, 14.5 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.1, 152.2, 149.5, 138.0, 133.6, 132.8, 129.8, 129.5, 125.7, 125.5, 124.8, 123.9, 122.8, 121.2, 109.8, 107.4, 107.1, 60.0, 55.7, 41.0, 15.0; MS (ESI): *m/z* 490 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{25}ClN_3O_4$: 490.15281; found:490.15251 $[M + H].^+$

**(1-Ethyl-3-(6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6d):**

Pale yellow solid: yield 68%; mp: 230–232 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ: 12.95 (s, 1H), 9.09 (s, 1H), 8.39 (s 1H), 7.83-7.73 (m, 4H), 7.15(s, 2H), 7.13 (s, 1H), 4.40 (q, *J* = 7.1, 14.3 Hz, 2H), 3.87-3.80 (m, 9H) 1.51 (t, *J* = 7.1, 14. Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ: 194.3, 152.5, 152.4, 151.0, 143.9, 140.9, 139.5, 138.9, 138.2, 130.7, 125.8, 124.2 (d, *J* = 5.4 Hz), 123.7, 123.5, 118.7, 113.8, 112.4, 111.7, 110.5, 107.5, 107.4, 106.6, 60.1, 56.0, 15.1, 15.0; MS (ESI): *m/z* 473 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{25}FN_3O_4$: 473.17007; found:474.18065 $[M + H].^+$

**(1-Ethyl-3-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6e):**

yellow solid: yield 68%; mp: 152–153 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 12.70 (s 1H), 9.13 (s, 1H), 8.28 (s, 1H), 8.12 (s, 1H), 7.79 (dd, *J* = 1.1, 8.4 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 1H ), 7.42 (d, *J* = 9.4 Hz, 1H), 7.16 (s, 2H), 4.39 (q, *J* = 7.1, 14.5, Hz, 2H), 3.89-3.86 (m 9H), 1.56 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 196.6, 152.6, 150.0, 141.8, 138.4, 132.9, 131.0, 130.9, 124.9 (d, *J* = 4.5 Hz), 124.0, 119.2, 115.8, 109.3, 107.9, 106.3, 60.9, 56.1, 41.6, 14.9; MS (ESI): *m/z* 523 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{28}H_{25}F_3N_3O_4$: 524.17917; found: 524.17605 $[M + H].^+$

**(3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6f):**

yellow solid: yield 73%; mp: 242–243 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.37 (s 1H), 9.13 (s, 1H), 8.25 (s, 1H), 7.79-7.73 (m, 2H), 7.41-7.24 (m, 2H), 7.15 (s, 2H), 4.38 (q, $J$ = 7.2, 14.4 Hz, 2H), 3.85 (s, 6H), 3.82 (s, 3H), 2.31 (s, 6H), 1.49 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 194.2, 152.4, 147.4, 147.0, 140.8, 138.1, 133.0, 129.6, 129.3, 129.2, 125.8, 124.8, 124.1, 123.8, 110.3, 107.8, 107.5, 60.1, 55.9, 40.9, 19.9, 15.2; MS (ESI): *m/z* 484 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{29}H_{30}N_3O_4$: 484.22308; found: 484.22123 $[M + H]^+$; HPLC: C18 tR: 5.248 min.

**(3-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6g):**

yellow solid: yield 68%; mp: 250–253 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.68 (s, 1H), 9.15 (s, 1H), 8.29 (s, 1H), 7.79 (dd, $J$ = 1.5, 8.6 Hz, 1H), 7.75-7.66 (m, 1H), 7.52-7.45 (m, 2H), 7.16 (s, 2H), 6.99-6.91 (dt, $J$ = 1.8, 10.5 Hz, 1H), 4.39 (q, $J$ = 7.1, 14.3 Hz, 2H), 3.88-3.82 (m, 9H), 1.54 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.0, 152.2, 149.4, 140.8, 137.8, 132.8, 129.7, 129.5, 125.4, 124.7, 123.9, 109.8, 109.0, 108.7,107.4, 107.2, 107.0, 59.9, 55.7, 40.9, 15.0; MS (ESI): *m/z* 524 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{24}Cl_2N_3O_4$: 524.11384; found: 524.11387 $[M + H].^+$

**(3-(6-Chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6h):**

yellow solid: yield 68%; mp: 250–251 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.70 (s, 1H), 9.13 (s 1H), 8.28 (s, 1H), 8.12 (s, 1H), 7.79 (dd, $J$ = 1.1, 8.4 Hz, 1H), 7.65 (d, $J$ = 8.6 Hz, 1H), 7.42 (d, $J$ = 9.4 Hz, 1H), 7.16 (s 2H), 4.39 (q, $J$ = 7.1, 14.5 Hz, 2H), 3.89-3.86 (m, 9H), 1.56 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 194.1, 152.3, 150.5, 140.8, 138.0, 132.8, 130.0, 129.6, 125.3, 124.7, 123.9, 109.9, 107.4, 106.9, 59.9, 55.7, 41.0, 15.0; MS (ESI): *m/z* 508 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{24}ClFN_3O_4$: 508.14339; found: 508.14249 $[M + H].^+$

**(1-Ethyl-3-(5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6i):**

yellow solid: yield 72%; mp: 123–125 °C; $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) δ: 12.70 (s, 1H), 9.13 (s, 1H), 8.28 (s, 1H), 8.12 (s, 1H), 7.79 (dd, $J$ = 1.1, 8.4 Hz, 1H), 7.65 (d, $J$ = 8.6 Hz 1H), 7.42 (d, $J$ = 9.4 Hz 1H), 7.16 (s 2H), 4.39 (q, $J$ = 7.1, 14.5 Hz, 2H), 3.89-3.86 (m, 12H),

1.56 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 195.9, 161.2, 152.7, 141.6, 138.4, 133.3, 130.7, 129.5, 125.7, 125.5, 124.8, 124.4, 109.5, 107.9, 107.7, 105.6, 60.9, 56.3, 53.7, 41.6, 15.1; MS (ESI): *m/z* 487 $[M + H]^+$; HRMS (ESI): *m/z*: calcd for $C_{27}H_{27}ClN_4O_5$: 487.19760; found: 487.19602 [M + H].

**Biology**

**Anti-cancer activity**

The Anticancer activity of the compounds was determined using MTT assay[1]. $1\times10^4$ cells/well were seeded in 100µl DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37°C in a $CO_2$ incubator. After 24 h of incubation all the synthesized compounds were added to the cells 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. Then the supernatant from each well was carefully removed, formazon 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. DU-145 cells were treated with compound **5g** and **6f** at 0.5 and 1µM 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).[2]

**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.). Nocodazole was used as reference compound. The IC50 value was defined as the drug concentration required 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 °C.[3-4]

**Immunohistochemistry**

DU-145 cells were seeded on glass cover slips, incubated for 48 h in the presence or absence of test compounds (**5g** and **6f)** 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 °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.[3-4].

**Hoechst staining**

DU-145 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 **5g** and **6f** 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 °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.[5]

**Measurement of mitochondrial membrane potential (ΔΨm)**

DU-145 ($1\times10^6$ cells/well) cells were cultured in six well plates. After plating, cells were treated with compounds **5g** and **6f** 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.[6]

**Annexin V-FITC assay for apoptosis**

DU-145 ($1\times10^6$) cells were seeded in six-well plates and allowed to grow overnight. The medium was then replaced with complete medium containing compounds **5g** and **6f** 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). Then the samples were analyzed by flowcytometry as described earlier.[7]

**ROS generation**

The production of ROS (reactive oxygen species) was measured by flow cytometry using DCFDA (2',7'-dichlorofluorescindiacetate) as previously described.[8] In this study, DU-145 cells were treated with compounds **5g** and **6f** at 0.5 and 1 μM concentrations for 48 h. After 48 h of treatment, cells were incubated with DCFDA (1.5 μM) at 37 $^0$C for 30 min and then measured with the flow cytometer (FACS).

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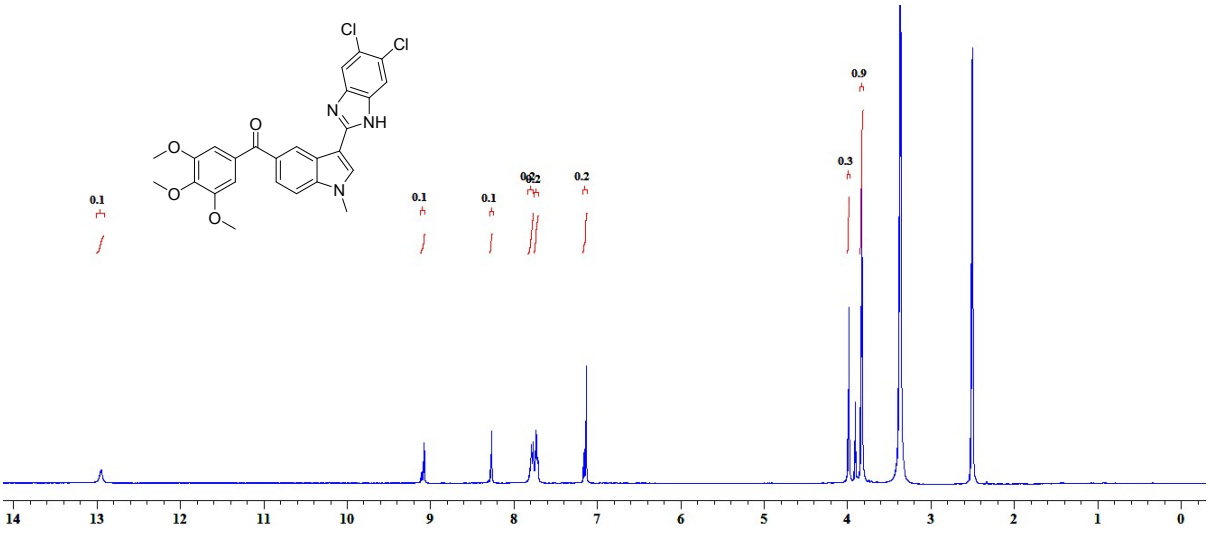
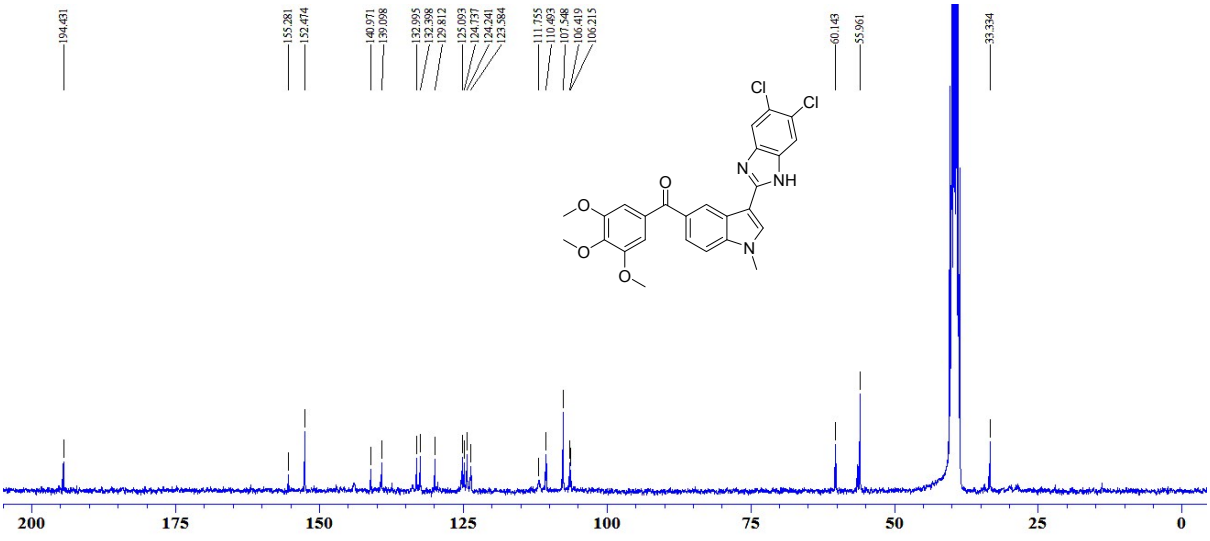
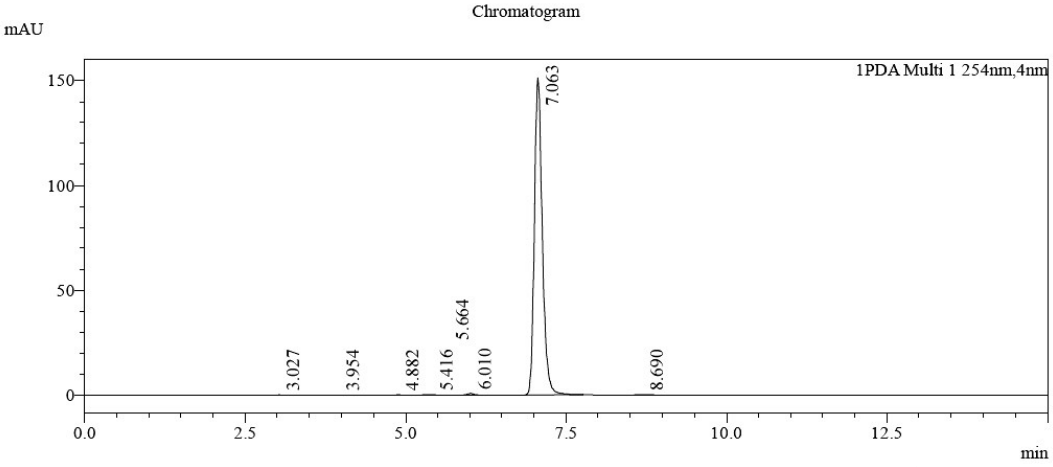
**(3-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5g):**




$^{1}$H NMR spectrum (400 MHz)



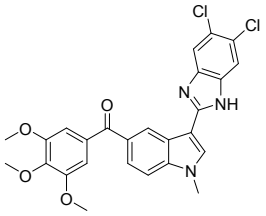

$^{13}$C NMR spectrum (400 MHz)




Peak Table

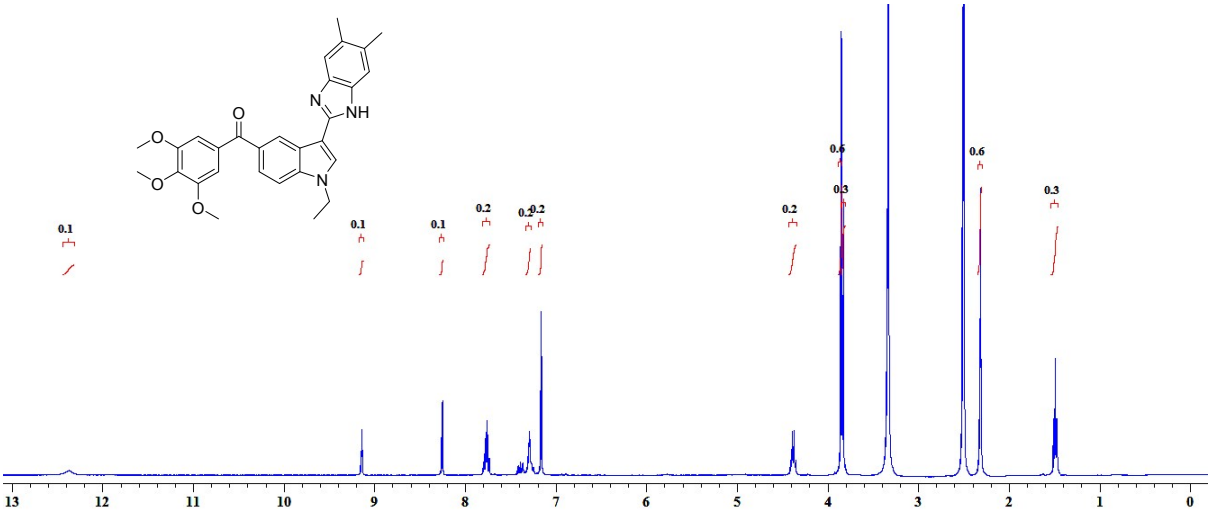
PDA Ch1 254nm

| Peak# | Ret. Time | Area | Area% | Height |
|---|---|---|---|---|
| 1 | 3.027 | 845 | 0.064 | 187 |
| 2 | 3.954 | 837 | 0.064 | 76 |
| 3 | 4.882 | 828 | 0.063 | 123 |
| 4 | 5.416 | 2181 | 0.166 | 204 |
| 5 | 5.664 | 297 | 0.023 | 40 |
| 6 | 6.010 | 5451 | 0.415 | 664 |
| 7 | 7.063 | 1300306 | 98.990 | 151418 |
| 8 | 8.690 | 2832 | 0.216 | 142 |
| Total | | 1313576 | 100.000 | 152852 |

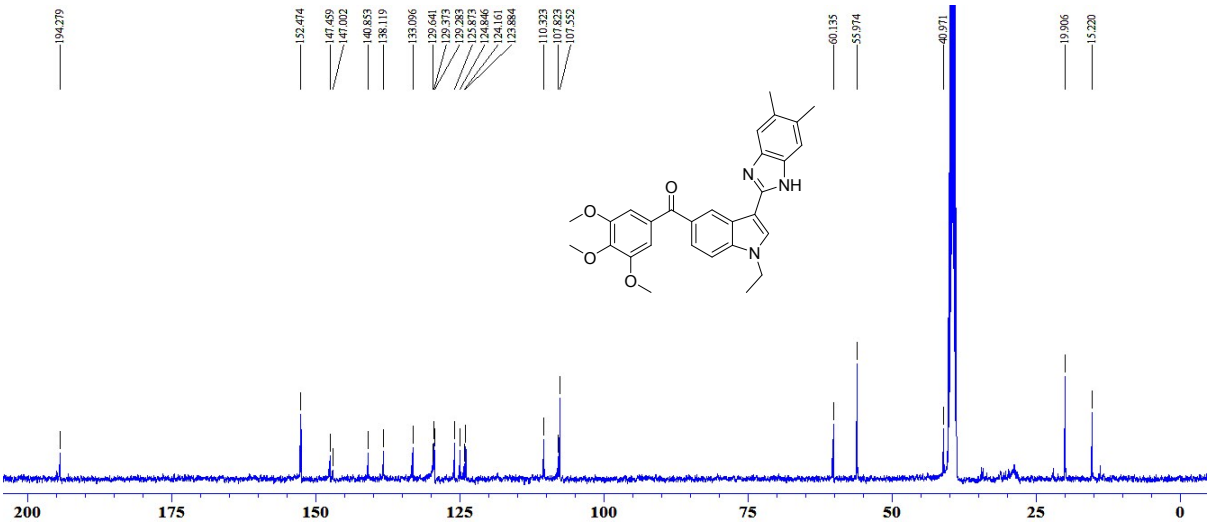



HPLC data

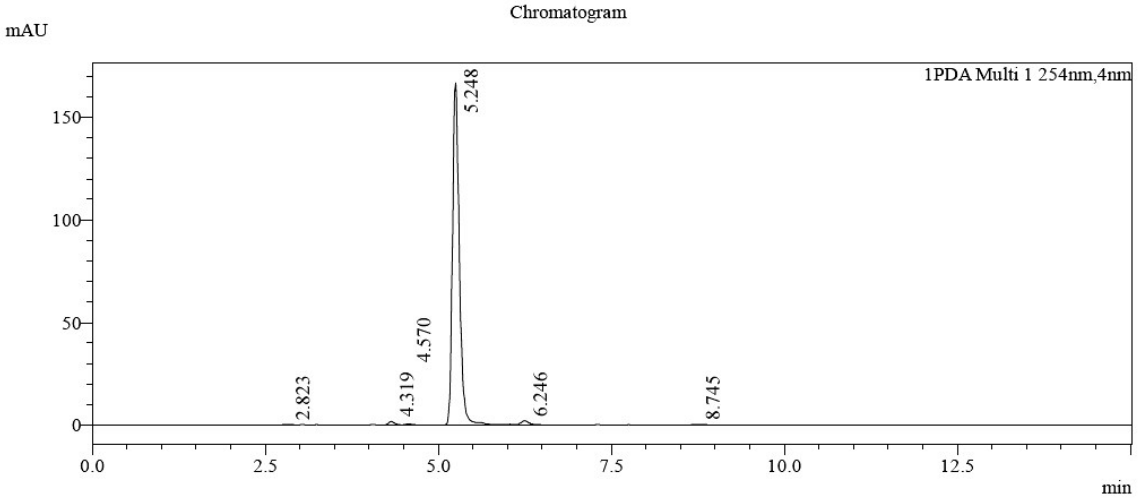
**(3-(5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-1-ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (6f):**



$^{1}$H NMR spectrum (400 MHz)




$^{13}$C NMR spectrum (400 MHz)




Peak Table

PDA Ch1 254nm

| Peak# | Ret. Time | Area | Area% | Height |
|---|---|---|---|---|
| 1 | 2.823 | 3229 | 0.273 | 289 |
| 2 | 4.319 | 10021 | 0.847 | 1740 |
| 3 | 4.570 | 3401 | 0.287 | 504 |
| 4 | 5.248 | 1145796 | 96.853 | 166779 |
| 5 | 6.246 | 16867 | 1.426 | 1992 |
| 6 | 8.745 | 3711 | 0.314 | 347 |
| Total | | 1183024 | 100.000 | 171651 |

HPLC data