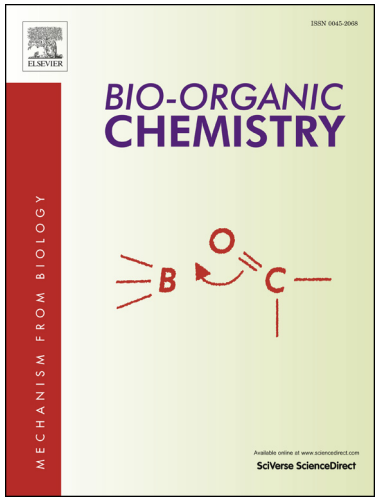


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# Synthesis and Biological Evaluation of Benzimidazole-Oxindole Conjugates as Microtubule-Targeting Agents

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**Abstract:** A series of benzimidazole-oxindole conjugates were synthesized and evaluated for their cytotoxic activity. The cytotoxicity assay results suggest that conjugates **5c** and **5p** exhibit promising cytotoxicity against human breast cancer cell line (MCF-7). The Cell cycle analysis revealed that these conjugates induced cell cycle arrest at G2/M phase in MCF-7 cells. The tubulin polymerization assay results suggested that these conjugates inhibit tubulin polymerization with $IC_{50}$ values 1.12 and 1.59 μM respectively. Immuofluorescence analysis also suggested that these conjugates effectively inhibited the microtubule assembly in MCF-7 cells. Further, molecular docking studies indicated that these conjugates **5c** and **5p** interact and binds efficiently with the tubulin protein. By and large, the results demonstrated that these benzimidazole-oxindole conjugates possess cytotoxic property by inhibiting the tubulin polymerization.

***Keywords*:** Benzimidazoles-Oxindoles; Cytotoxic activity; Cell cycle; Tubulin polymerization; Docking.

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## 1. Introduction

Microtubule is an important component of the cytoskeleton formed by α and β tubulin heterodimers, and are critical in many cellular processes such as cell shape maintenance, intracellular transport, cell division, cell growth and mitosis [1–3]. As the key structural basis of microtubule, tubulin is considered as a highly attractive target for anticancer therapy [4–12]. Several tubulin inhibitors have been approved as the first line chemotherapeutic agents for different types of human cancer [13,14]. Tubulin inhibitors are categorized as three types based on their binding site in the tubulin protein, that includes taxol-site inhibitors, (paclitaxel **1**, and epothilones) [15,16], vinblastine-site inhibitors, including (vinblastine, **2**, and vincristine) [17] and colchicine-site inhibitors (colchicines [18] and combretastatin [19,20] A-4) as shown in Fig. 1. Most of the tubulin-binding agents are derived from natural products with complex chemical structures that restrict chemical modifications, however compounds with relatively simple chemical structures could be valuable candidates for the development of newer molecules.

*<Insert Figure 1>*

Benzimidazole is a bicyclic heteroaromatic molecule which is structural isostere of naturally occurring nucleotides hence, it has been extensively utilized as a useful scaffold in the medicinal chemistry. Benzimidazole derivatives have shown different therapeutic properties including antitumor activity [21], and exert their antitumor activity by acting on various targets such as topoisomerases inhibitors [22], DNA-alkylating agents [23], tubulin polymerization inhibitors [24] and antiangiogenic agents [25]. Nocodazole (**3**, Fig. 1) having benzimidazole moiety as its basic scaffold is a well known agent that inhibits tubulin polymerization. On the other hand oxindoles are versatile molecules that display diverse biological activities, including anticancer activity [26,27]. For example, Indirubin (**4**, Fig. 1) which has an oxindole moiety as its basic structure, is reported as a potent anticancer agent that inhibits cyclin dependent kinase (CDK) [28]. Recently, it has been reported that oxindoles not only inhibited CDK but also tubulin polymerization by binding at the colchicine binding site [29]. Some of the potent hybrid molecules that have been recently developed as new anticancer agents are obtained by the combination of different pharmacophores [26,30]. The promising biological activity exhibited by these conjugates prompted us to explore newer conjugates by combining benzimidazole and oxindole

scaffolds with a view to enhance the cytotoxic activity. In this context, we have synthesized such conjugates (**5a–t** and **6a–d**) and evaluated them for their cytotoxic potential. Interestingly, some of the conjugates such as **5c, 5d, 5f, 5i, 5k, 5m, 5o, 5p** and **5s** exhibited considerable antiproliferative activity with $GI_{50}$ 0.26–28 µM against different human cancer cell lines (Table 2). Further, the results of cell cycle and mitotic arrest showed that these conjugates affect the G2/M phase along with the inhibition of tubulin assembly. The flow cytometric analysis was performed to determine the effect of active conjugates in altering the cell cycle phase distribution in MCF-7 cells.

## 2. Results and Discussion

### *2.1. Chemistry*

The synthetic strategy for the preparation of benzimidazole-oxindole conjugates is depicted in Scheme 1. Initially, oxidative cyclization of methyl ester of 3,4-diaminobenzoic acid (**8**) with various substituted benzaldehydes (**9a–e**) and thiophene-2-carbaldehyde in the presence of $Na_2S_2O_5$ afforded the corresponding methyl benzimidazole carboxylate derivatives (**10a–e** and **13**). The reduction of these esters **10a–e** and **13** with lithium aluminium hydride in dry THF produced the corresponding alcohols, which were taken to further oxidation by Dess–Martin periodinane in $CH_2Cl_2$ to the respective benzimidazole carbaldehydes (**11a–e** and **14**) in good yields. Finally, the required benzimidazole-oxindole conjugates (**5a–t** and **6a–d**) were synthesized by employing the Knoevenagel condensation between equimolar mixture of these carbaldehydes (**11a–e** and **14**) and oxindoles (**12a–d**) as shown in Scheme 1. The compound compounds were characterized by means of $^1$H NMR, $^{13}$C NMR, HRMS, and IR spectral data. All the compounds were obtained as Z isomers and confirmed by comparing the data of the previous reports [31,32].

*<Insert Scheme 1>*

### *2.2. Cytotoxicity*

To evaluate the cytotoxic potential of these conjugates (MTT) assay [33] was performed against four human cancer cell lines, A549 (lung), MCF-7 (breast), DU-145 (prostate) and HT-29 (colon) in comparison to nocodazole and the results are summarized as $IC_{50}$ values in Table 1. The results revealed that these conjugates showed promising cytotoxic activity with $IC_{50}$ values ranging from 1.84–19.9 µM against different cancer cell lines. In

this assay conjugates **5c** and **5p** showed significant anticancer activity against human breast cancer cell line (MCF-7) with $IC_{50}$ values 1.84 µM and 1.97 µM respectively.

*<Insert Table 1 >*

To further validate their potency in other cell lines, benzimidazole-oxindole conjugates were evaluated by NCI-USA with regard to anticancer activity against 60 cancer cell lines some of the conjugates (**5c, 5d, 5f, 5i, 5k, 5m, 5o, 5p** and **5s)** in this series showed noticeable cytotoxicity and were taken up for the five dose screening for further evaluation. These selected conjugates **5c, 5d, 5f, 5i, 5k, 5m, 5o, 5p** and **5s** exhibited considerable cytotoxicity with $GI_{50}$ values ranging from 0.28-28.3 µM. Interestingly, conjugates **5c** and **5p** exhibited $GI_{50}$ values ranging from 0.28–13.8 and 0.57–6.34 µM respectively and these results are showed in Table 2. Conjugate **5c**, which contains a methoxy group at C4 position of the C-ring and chloro group at C5 position of the B-ring showed significant cytotoxic activity. However change the position of chloro group from C5 to C6 in the B-ring decreased the activity as in case of in **5d**. Conjugate **5p** bearing fluoro groups at C3 and C5 positions of the C-ring and chlorine at C6 position of the B-ring showed prominent activity. On the other hand, change in the position of chloro group from C6 to C5 in the B-ring resulted in moderate activity. On the basis of the structure of nocodazole (**4**, Fig. 1), we decided to replace the C-ring with 2-thienyl scaffold, while the corresponding compounds (**6a–d**) were found to be moderate by active. The SAR revealed that among these compounds, conjugates with chloro group on the B-ring showed better activity than other compounds, while substituents on the C-ring have little effect on the activity. By and large, the conjugates **5c** and **5p** were considered as promising among these conjugates assayed and were taken up for detailed biological studies like cell cycle analysis, tubulin polymerization studies and immunohistochemistry studies. Finally, the molecular docking studies also performed to find out the mode binding on colchicines binding site on tubulin.

*<Insert Table 2 >*

### *2.3. Cell cycle analysis*

Anticancer drugs are known to interact with cells leading to cell growth arrest or cell death. To shed more light on the mechanism responsible for anticancer activity the potential

conjugates **5c** and **5p** were examined for their effect on the cell cycle analysis [34]. In this study, MCF-7 cells were treated with these conjugates for 48 h and nocodazole was used as the reference compound in this study. Interestingly, the results obtained from this assay clearly indicated that these conjugates showed G2/M cell cycle arrest as compared to the untreated control cells. These conjugates showed 52.41% and 37.62% of cell accumulation in G2/M phase at 1 μM concentration, whereas they exhibited 63.20 and 53.00 % of cell accumulation in G2/M phase at 2 μM concentration, respectively (Fig. 2 and Table 2). The reference compound nocodazole showed 54.65 % cell accumulation in G2/M phase at 2 μM concentration, whereas in control (untreated cells) 11.41 % of G2/M phase was observed.

*<Insert Figure 2 >*

*<Insert Table 3 >*

### *2.4. Tubulin polymerization study*

Conjugates that alter cell-cycle parameters with preference to G2/M blockade are known to exhibit effects on tubulin assembly. Moreover, inhibition of tubulin polymerization is strongly associated with G2/M cell cycle arrest [35]. These conjugates arrest the cell cycle at G2/M phase, hence it was considered important to investigate the tubulin polymerization aspect. As tubulin subunits heterodimerize and self-assemble to form microtubules in a time dependent manner and have investigated the progression of tubulin polymerization [36] by monitoring the increase in fluorescence emission at 420 nm (excitation wavelength is 360 nm) in 384 well plate for 1 h at 37 °C with and without the conjugates at 1 μM concentration. In this assay, conjugates **5c** and **5p** inhibited tubulin polymerization by 70.5 and 65.9 %, respectively, and in comparison, 68.2 % of inhibition was observed with nocodazole. (Fig. 3). This was followed by the evaluation of $IC_{50}$ values for these compounds and the results showed that both **5c** and **5p** displayed inhibition of tubulin assembly in comparison to nocodazole as shown in Table 3. Moreover, the effect of these conjugates on the inhibition of tubulin assembly correlated well with their significant antiproliferative activity.

*<Insert Figure 3 >*

*<Insert Table 4 >*

### *2.5. Immunohistochemistry studies on tubulin*

We also investigated alterations in the microtubule network in the MCF-7 cells induced by these conjugates (**5c** and **5p)** by carrying out immunohistochemistry studies using fluorescence microscope, as most antimitotic agents affect microtubules [37]. Therefore, MCF-7 cells were treated with **5c** and **5p** at 1 μM concentration for 48 h and the results demonstrated a well-organized microtubular network in control cells. However, cells treated with these conjugates and using nocodazole as standard showed disrupted microtubule organization as shown in Fig. 4, thus confirming the inhibition of tubulin polymerization.

*<Insert Figure 4 >*

### *2.6. Molecular modeling studies*

The conjugates that have been synthesised in the present study are designed based on nocodazole an aminobenzimidazole scaffold a well known tubulin polymerization inhibitor. It has been proved that the binding of nocodazole to the tubulin affects the colchicine binding, indicating that its binding site is closer to colchicine, that is between the α and β tubulin subunits [38]. We performed docking studies to investigate the possible binding mode of these conjugates. Coordinates of protein structure of tubulin-colchicine were obtained from the Protein Data Bank (PDB ID 3E22) [39] and the docking was accomplished into the colchicine binding site of the tubulin using AutoDock 4.2 software and analyzed by PYMOL software [40]. Fig. 5 shows that carbonyl group on the oxindole ring of **5c** establishes hydrogen binding with αGly11, βAsn249 and βLys254 and this oxindole ring is surrounded by αTyr224, αSer178 and αThr179 residues. Whereas the benzimidazole ring of **5c** shows hydrophobic contacts with βLeu254, βLeu255 and αAsn101 and the methoxyphenyl group of the benzimidazole ring is buried in the hydrophobic pocket by βLys352 βAsn258, βAsn350, βVal351, βVal181 and βMet259 residues located in β-tubulin, this is similar to that of trimethoxyphenyl group in case of colchicines. Similarly carbonyl group on oxindole moiety in conjugates **5p** showed hydrogen binding with βAsn249 and βLys254. Figure 6 shows the molecular docking pose of conjugate **5p** (red) and **5c** (yellow) overlaying with that of nocodazole (pink). Whereas Fig. 7 shows that the binding of conjugate **5c** (yellow) and **5p** (red) is very similar to the pose of the colchicine (green) with oxindole ring of both **5c** and **5p** placed towards the α-tubulin (blue) chain. Thus these docking studies suggest that these conjugates interact with both α-and β-tubulin in the colchicine-binding pocket.

*<Insert Table 5 >*

*<Insert Figure 5 >*

*<Insert Figure 6 >*

*<Insert Figure 7 >*

## 3. Conclusion

In conclusion, we have designed, synthesized some benzimidazole-oxindole conjugates that were evaluated for their cytotoxic activity and tubulin polymerization inhibitory potential. Conjugates **5c** and **5p** showed significant cytotoxic activity against human breast cancer cell line (MCF-7) with $IC_{50}$ values of 1.84 and 1.97 μM respectively. Further, the flow cytometric analysis revealed that these conjugates caused cell cycle arrest and accumulated cells in the G2/M phase. Furthermore, these conjugates effectively inhibited microtubule assembly in MCF-7 cells. Moreover, docking experiments showed that they interact and bind efficiently with the tubulin protein. The results suggest that these new benzimidazole-oxindole conjugates are potent inhibitors of tubulin polymerization, and are amenable for further structural modifications in the discovery and development of effective chemotherapeutics for the treatment of breast cancer.

## 4. Experimental

*4.1. General*

The majority of the solvents were purified by distillation under nitrogen from the indicated drying agent and used fresh: dichloromethane (calcium hydride), tetrahydrofuran (sodium benzophenone ketyl), acetone (potassium permanganate), and acetonitrile (phosphorous pentoxide). Reaction progress was monitored by thin-layer chromatography (TLC) using GF254 silica gel with fluorescent indicator on glass plates. Visualization was achieved with UV light and iodine vapor unless otherwise stated. Chromatography was performed using Acme silica gel (100–200 mesh). $^{1}H$ and $^{13}C$ NMR spectra were recorded on INOVA (400 MHz) or Gemini Varian-VXR-unity (200 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities

are described as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants are reported in Hertz (Hz). Mass spectra were recorded on a VG-7070H Micromass mass spectrometer at 200 _C, 70 eV with trap current of 200 lA, and 4 kV acceleration voltage. FABMS spectra were recorded on LSIMS-VG-AUTOSPEC-Micromass. Melting points were recorded on Electrothermal 9100 and are uncorrected. All computational studies were done with a Red Hat Enterprise Linux version 5.0 using Maestro software version 9.5 (Schrödinger, LLC, New York, NY, 2013).

### *4.2. Chemistry*

*4.2.1. Methyl 2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**10a**).*

A mixture of methyl 3,4-diaminobenzoate (**8**, 830 mg, 5 mmol) and 4-methoxy benzaldehyde (**9a,** 680 mg, 5 mmol) in ethanol (25 mL) was heated with an aqueous solution of sodium pyrosulfite (1.42 gm, 1.5 mmol, 5 mL) and then refluxed for 4 to 6 h, until TLC indicated that the reaction was complete. The ethanol was evaporated under vacuum and the residue was dissolved in $CHCl_3$ (2x15 mL) and washed with water. The combined organic phases were dried over anhydrous $Na_2SO_4$,and the solvent was removed under vacuum and purified by column chromatography MeOH-$CH_2Cl_2$ (0.5: 9.5) to obtain the pure product **10a** as a white solid (952 mg, 68%); mp: 231–234 $^oC$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.12 (d, *J* = 9.1 Hz, 2H), 7.92 (d, *J* = 9.0 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 2H), 3.94 (s, 3H), 3.85 (s, 3H); MS (ESI): *m/z* 283 $[M+H]^+$.

*4.2.2. Methyl 2-(3-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**10b**).*

Compound **10b** was prepared according to the method described for compound **10a**, employing 3-fluorobenzaldehyde (**9b**, 620 mg, 5 mmol) and methyl 3,4-diaminobenzoate (**8**, 830 mg, 5mmol) to obtain the pure product **10b** as a white solid (938 mg, 70%); mp: 238–240 $^oC$; $^1H$ NMR (500 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.28 (dd, *J* = 8.3, 5.4 Hz, 2H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.25 (t, *J* = 8.3 Hz, 2H), 3.98 (s, 3H); MS (ESI): *m/z* 271 $[M+H]^+$.

*4.2.3. Methyl 2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**10c**).*

Compound **10c** was prepared according to the method described for compound **10a**, employing 4-(trifluoromethyl)benzaldehyde (**9c**, 870 mg, 5 mmol) and methyl 3,4-diaminobenzoate (**8**, 830 mg 5 mmol mmol) to obtain the pure product **10c** as a white solid (1.14 gm, 72%); mp: 275–277 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$+DMSO–$d_6$): δ 8.39 (d, *J* = 7.8

Hz, 2H), 7.95 (t, *J* = 7.9 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.72 (s, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 3.94 (s, 3H); MS (ESI): *m/z* 331 $[M+H]^+$.

*4.2.4. Methyl 2-(3,5-difluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**10d**).*

Compound **10d** was prepared according to the method described for compound **10a**, employing 3,5-difluorobenzaldehyde (**9d**, 710 mg, 5 mmol) and methyl 3,4-diaminobenzoate (**8**, 830 mg 5 mmol) to obtain the pure product **10d** as a white solid (1.0 gm, 70%); mp: 295–297 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.44 (d, *J* = 8.3 Hz, 2H), 7.98 (t, *J* = 8.6 Hz, 1H), 7.90–7.75 (m, 3H), 3.98 (s, 3H); MS (ESI): *m/z* 289 $[M+H]^+$.

*4.2.5. Methyl 2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate.(**10e**).*

Compound **10e** was prepared according to the method described for compound **10a**, employing 3,4,5-trimethoxybenzaldehyde (**9e**, 980 mg, 5 mmol) and methyl 3,4-diamino benzoate (**8**, 830 mg, 5 mmol) to obtain the pure product **10e** as a white solid (1.1 gm, 68%); mp: 243–245 $^oC$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.1 Hz, 1H), 7.63 (s, 1H), 7.53 (s, 2H), 7.46 (s, 1H), 3.98 (s, 6H), 3.95 (s, 3H), 3.91 (s, 3H); MS (ESI): *m/z* 343 $[M+H]^+$.

*4.2.6. Methyl 2-(thiophen-2-yl)-1H-benzo[d]imidazole-5-carboxylate (**13**).*

Compound **13** was prepared according to the method described for compound **10a**, employing thiophene-2-carbaldehyde (560 mg, 5 mmol) and methyl 3,4-diaminobenzoate (**8**, 830 mg, 5 mmol) to obtain the pure product **13** as a white solid (782 mg, 62%); mp: 212–214 $^oC$; $^1H$ NMR (300 MHz, DMSO–$d_6$): 8.23 (s, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 8.07 (s, 1H), 7.84 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 3.86 (s, 3H); MS (ESI): *m/z* 259 $[M+H]^+$.

**4.3. General procedure for the preparation of compounds (11a-e & 14).**

$LiAlH_4$ (4 mmol) was added to a fine suspension of compounds **10a–e & 13** (3 mmol) in dry THF (60 mL) at 0 $^oC$ and was stirred for 4 h at RT. The reaction mixture was then quenched with saturated aq $NH_4Cl$ and diluted with EtOAc (150 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The obtained crude was used directly for the next step without any further purification. The crude obtained was taken in dry $CH_2Cl_2$ (100 mL). To that Dess–Martine periodinane (DMP) (4 mmol) was added, stirred at room temperature for about 3 h. The reaction mixture was then quenched with a 1:1 mixtureof aq sodium thiosulfate and $NaHCO_3$ (40 mL) and dilutedwith $CH_2Cl_2$ (50 mL). The organic layer

was dried over anhydrous $Na_2SO_4$ and concentrated under vacuum to obtain the crude product. The resulting crude product was purified by column chromatography to afford products **11a–e & 14** in high purity.

*4.3.1. 2-(4-Methoxyphenyl)-1H-benzo[d]imidazole-5-carbaldehyde (**11a**).*

Compound **11a** was prepared according to the general procedure. Compound **11a** obtained as a white solid: (604 mg, 80% yield); mp: 179–181 $^{o}$C; $^{1}$H NMR (500 MHz, $CDCl_3$): δ 9.98 (s, 1H), 8.28 (s, 1H), 8.02 (d, *J* = 9.0 Hz, 2H), 7.94 (dd, *J* = 9.0, 1.1 Hz, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 2H), 3.83 (s, 1H); MS (ESI): *m/z* 253 $[M+H]^+$.

*4.3.2. 2-(3-Fluorophenyl)-1H-benzo[d]imidazole-5-carbaldehyde (**11b**).*

Compound **11b** was prepared according to the general procedure. Compound **11b** obtained as a white solid: (547 mg, 76% yield); mp: 188–190 $^{o}$C; $^{1}$H NMR (300 MHz, $CDCl_3$+DMSO–$d_6$): δ 10.04 (s, 1H), 8.34–8.15 (m, 2H), 7.90 (s, 1H), 7.78 (s, 1H), 7.64 (s, 1H), 7.26 (t, *J* = 8.6 Hz, 2H); MS (ESI): *m/z* 241 $[M+H]^+$.

*4.3.3. 2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carbaldehyde (**11c**).*

Compound **11c** was prepared according to the general procedure. Compound **11c** obtained as a white solid: (617 mg, 71% yield) ; mp: 208–210 $^{o}$C; $^{1}$H NMR (500 MHz, $CDCl_3$): δ 10.05 (s, 1H), 8.38 (d, *J* = 7.5 Hz, 2H), 8.23 (s, 1H), 7.84–7.80 (m, 2H), 7.78 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 8.1 Hz, 1H); MS (ESI): *m/z* 291 $[M+H]^+$.

*4.3.4. 2-(3,5-Difluorophenyl)-1H-benzo[d]imidazole-5-carbaldehyde (**11d**).*

Compound **11d** was prepared according to the general procedure. Compound **11d** obtained as a white solid (526 mg, 68% yield) yield; mp: 217–219 $^{o}$C; $^{1}$H NMR (300 MHz, $CDCl_3$+DMSO–$d_6$): δ 10.05 (s, 1H), 8.38 (d, *J* = 8.2 Hz, 2H), 7.78 (t, *J* = 8.4 Hz, 1H), 7.84–7.71 (m, 3H); MS (ESI): *m/z* 259 $[M+H]^+$.

*4.3.5. 2-(3,4,5-Trimethoxyphenyl)-1H-benzo[d]imidazole-5-carbaldehyde (**11e**).*

Compound **11e** was prepared according to the general procedure. Compound **11e** obtained as a white solid: (651 mg, 70% yield); mp: 216–218 $^{o}$C; $^{1}$H NMR (300 MHz, $CDCl_3$+DMSO–$d_6$): δ 10.07 (s, 1H), 8.16 (s, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.42 (s, 2H), 7.28 (s, 1H), 3.91 (s, 3H), 3.79 (s, 6H); MS (ESI): *m/z* 313 $[M+H]^+$.

*4.3.6. 2-(Thiophen-2-yl)-1H-benzo[d]imidazole-5-carbaldehyde (**14**).*

Compound **14** was prepared according to the general procedure. Compound **14** obtained as a white solid: (471 mg, 69% yield); mp: 196–198 $^{o}$C; $^{1}$H NMR (300 MHz, DMSO–$d_6$): δ 10.38 (s, 1H), 8.32 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 8.13 (s, 1H), 7.86 (s, 1H), 7.78 (dd, *J* = 6.4, 3.6 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 1H); MS (ESI): *m/z* 229 $[M+H]^+$.

**4.4. General procedure for the preparation of compounds (5a-t and 6a–d).**

*4.4.1. (Z)-3-((2-(4-Methoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5a**).*

A mixture of aldehyde **11a** (126 mg, 0.5 mmol) and indolin-2-one (**12a,** 66.5 mg, 0.5 mmol) were dissolved in EtOH (10 mL) and piperidine (2–3 drops) was added. The reaction mixture was refluxed for 3–5 h. After the completion of the reaction, the reaction mixture was allowed to cool to room temperature and the precipitated product was collected by vacuum filtration and washed with EtOH (5 mL), then recrystallized from ethanol to afford pure compound **5a** as a yellow solid in 113 mg, 62% yield. mp: 172–174 $^{o}$C; IR (KBr): 3420, 3157, 2931, 2357, 1683, 1674, 1570, 1470, 1432, 1257, 1174, 1027, 964, 847 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.45 (bs, 1H), 8.20–8.07 (m, 3H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.78 (s, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.59 (t, *J* = 8.3 Hz, 1H), 7.34 (d, *J* = 2.2 Hz, 1H), 7.14 (d, *J* = 7.5 Hz, 2H), 6.89–6.74 (m, 2H), 3.86 (s, 3H); $^{13}$C NMR (75 MHz, DMSO−$d_6$): δ 168.9, 160.7, 153.1, 142.5, 137.4, 129.3, 128.1, 127.9, 127.7, 125.5, 124.0, 123.8, 122.1, 121.8, 121.2, 120.7, 114.1, 109.8, 55.1; MS (ESI): *m/z* 368 $[M+H]^+$; HRMS (ESI): calcd for $C_{23}H_{18}O_2N_3$ *m/z* 368.13869 $[M+H]^+$; found 368.13935.

*4.4.2. (Z)-5-Methoxy-3-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5b**).*

Compound **5b** was prepared according to the method described for compound **5a**, employing aldehyde **11a** (126 mg, 0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 81 mg, 0.5 mmol) to obtain the pure product **5c** as a yellow solid (114 mg, 58%); mp: 177–179 $^{o}$C; IR (KBr): 3422, 3153, 2931, 2352, 1681, 1642, 1576, 1474, 1435, 1254, 1179, 1029, 961, 864 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.41 (bs, 1H), 8.25–8.16 (m, 3H), 7.82 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 1H), 7.40 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.82–6.70 (m, 2H), 3.90 (s, 3H), 3.68 (s, 3H); $^{13}$C NMR (75 MHz, DMSO−$d_6$): δ 168.9, 160.6, 154.3, 153.7, 153.1, 137.4, 136.2, 128.0, 127.9, 127.6, 126.1, 124.5, 123.7, 122.0, 121.9, 113.9, 109.9, 108.5, 55.0; MS (ESI): *m/z* 398 $[M+H]^+$; HRMS (ESI): calcd for $C_{24}H_{20}O_3N_3$ *m/z* 398.14923 $[M+H]^+$; found 398.14992.

*4.4.3. (Z)-5-Chloro-3-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5c**).*

Compound **5c** was prepared according to the method described for compound **5a**, employing aldehyde **11a** (126 mg, 0.5 mmol) and 5-chloroindolin-2-one **(12c,** 83 mg, 0.5 mmol) to obtain the pure product **5c** as a yellow solid (128 mg, 64%); mp: 194–196 $^{o}$C; IR (KBr): 3422, 3170, 2359, 1702, 1665, 1594, 1477, 1250, 1175, 1114, 1026, 967, 740 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.76 (bs, 1H), 8.17 (d, *J* = 8.3 Hz, 2H), 8.09 (s, 1H), 7.88 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.5 Hz, 1H),7.58 (s, 1H), 7.25 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 2H), 6.94–6.75 (m, 1H), 3.85 (s, 3H); $^{13}$C NMR (75 MHz, DMSO–$d_6$): δ 168.5, 167.1, 160.9, 153.1, 141.3, 140.5, 138.6, 129.1, 128.2, 127.4, 125.2, 124.7, 122.8, 122.0, 121.2, 119.2, 114.3, 111.3, 110.4, 55.3; MS (ESI): *m/z* 402 $[M+H]^+$; HRMS (ESI): calcd for $C_{23}H_{17}O_2N_3Cl$ *m/z* 402.09674 $[M+H]^+$; found 402.09587.

*4.4.4. (Z)-6-Chloro-3-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5d**).*

Compound **5d** was prepared according to the method described for compound **5a,** aldehyde **11a** (126 mg, 0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **5d** as a yellow solid (122 mg, 61%); mp: 199–201 $^{o}$C; IR (KBr): 3426, 3172, 2360, 1669, 1647, 1587, 1469, 1257, 1170, 1118, 1028, 961, 742 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.46 (bs, 1H), 8.16 (d, *J* = 8.6 Hz, 2H), 7.86 (s, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 7.1 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.97–6.75 (m, 2H), 3.90 (s, 3H); $^{13}$C NMR (75 MHz, DMSO−$d_6$): δ 168.9, 160.7, 153.1, 142.5, 137.4, 129.3, 128.1, 127.9, 127.7, 125.5, 124.0, 123.8, 122.1, 121.8, 121.2, 120.7, 114.1, 109.8, 55.1; MS (ESI): *m/z* 402 $[M+H]^+$; HRMS (ESI): calcd for $C_{23}H_{17}O_2N_3Cl$ *m/z* 402.09674 $[M+H]^+$; found 402.09587.

*4.4.5. (Z)-3-((2-(3-Fluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5e**).*

Compound **5e** was prepared according to the method described for compound **5a**, employing aldehyde **11b** (120 mg,0.5 mmol) and indolin-2-one (**12a,** 66.5 mg, 0.5 mmol) to obtain the pure product **5e** as a yellow solid (106 mg, 60%); mp: 194–196 $^{o}$C; IR (KBr): 3420, 3223, 3160, 2356, 1703, 1610, 1466, 1347, 1222, 1164, 1113, 964, 864, 742 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.67 (bs, 1H), 8.37–8.19 (m, 2H), 7.97 (s, 1H), 7.82 (s, 1H), 7.73 (d, *J* = 7.5 Hz, 1H), 7.68 (d, *J* = 7.3 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 2H),7.17 (t, *J* = 7.3 Hz, 1H), 6.95–6.80 (m, 2H); $^{13}$C NMR (75 MHz, DMSO−$d_6$): δ 168.9, 164.3 and

161.2 (d, *J* = 248.1 Hz ) 152.2, 142.7, 140.1, 137.4, 129.7, 129.0, 128.9 (d, *J* = 8.2 Hz ), 128.2, 126.3 (d, *J* = 2.7 Hz ), 125.8, 124.3, 121.9, 121.2 ,120.9, 116.0 (d, *J* = 22.0 Hz ), 110.0, 109.1; MS (ESI): *m/z* 356 $[M+H]^+$; HRMS (ESI): calcd for $C_{22}H_{15}ON_3F$ *m/z* 356.11868 $[M+H]^+$; found 356.11937.

*4.4.6. (Z)-3-((2-(3-Fluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)-5-methoxyindolin-2-one (**5f**).*

Compound **5f** was prepared according to the method described for compound **5a**, employing aldehyde **11b** (120 mg,0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 111 mg, 0.83 mmol) to obtain the pure product **5f** as a yellow solid (109 mg, 57%); mp: 196–198 $^oC$; IR (KBr): 3424, 3167, 2359, 2340, 1697, 1608, 1452, 1414, 1231, 1207, 1020, 840, 746 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.43 (bs, 1H), 8.29–8.22 (m, 2H), 7.99 (s, 1H), 7.79 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.44 (t, *J* = 8.8 Hz, 2H), 7.32 (d, *J* = 1.5 Hz, 1H), 6.89–6.72 (m, 2H), 3.62 (s, 3H); $^{13}C$ NMR (75 MHz, DMSO–$d_6$): δ 168.8, 164.9 and 161.6 (d, *J* = 248.4 Hz ), 153.8, 152.0, 137.4, 136.4, 129.0 (d, *J* = 8.2 Hz ), 128.3, 126.5, 125.9, 124.3, 121.9, 116.0 (d, *J* = 22.0 Hz ), 14.6, 110.2, 108.6, 55.2; MS (ESI): *m/z* 386 $[M+H]^+$; HRMS (ESI): calcd for $C_{22}H_{17}O_2N_3F$ *m/z* 386.12946 $[M+H]^+$; found 386.12993.

*4.4.7. (Z)-5-Chloro-3-((2-(3-fluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5g**).*

Compound **5g** was prepared according to the method described for compound **5a**, employing aldehyde **11b** (120 mg,0.5 mmol) and 5-chloroindolin-2-one (**12c,** 83 mg, 0.5 mmol) to obtain the pure product **5g** as a yellow solid (126 mg, 65%); mp: 195–197 $^oC$; IR (KBr): 3420, 3166, 2924, 2351, 1708, 1672, 1607, 1556, 1415, 1235, 1159, 1072, 1019, 847 $cm^{-1}$; 1H NMR (300 MHz, DMSO−$d_6$): δ 10.77 (bs, 1H), 8.33–8.23 (m, 2H), 7.99 (s, 1H), 7.83 (s, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.59 (d, *J* = 8.9 Hz, 1H), 7.42 (t, *J* = 8.8Hz, 2H), 7.05–6.81 (m, 2H); $^{13}C$ NMR (75 MHz, DMSO–$d_6$): δ 168.8, 164.9 and 161.6 (d, *J* = 247.5 Hz ), 152.3, 144.0, 141.2, 138.3, 133.6, 132.2, 129.0 (d, *J* = 8.2 Hz ), 128.1 (d, *J* = 12.1 Hz ), 124.4, 123.1, 120.7, 120.1, 116.0 (d, *J* = 21.4 Hz ), 109.9, 109.1; MS (ESI): *m/z* 390 $[M+H]^+$; HRMS (ESI): calcd for $C_{22}H_{14}ON_3ClF$ *m/z* 390.07317 $[M+H]^+$; found 390.07482.

*4.4.8. (Z)-6-Chloro-3-((2-(3-fluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5h**).*

Compound **5h** was prepared according to the method described for compound **5a**, employing aldehyde **11b** (120mg, 0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **5h** as a yellow solid (122 mg, 63%); mp: 206–208 $^{o}$C; IR (KBr): 3427, 3160, 2918, 1710, 1678, 1614, 1550, 1420, 1230, 1162, 1020, 840, 749 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO–$d_6$): δ 10.78 (bs, 1H), 8.33–8.21 (m, 2H), 7.99 (s, 1H), 7.80 (s, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.71 (d, *J* = 7.3 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.42 (t, *J* = 8.6 Hz, 2H), 7.00–6.78 (m, 2H); $^{13}$C NMR (75 MHz, DMSO–$d_6$): δ 168.8, 165.0 and 161.5 (d, *J* = 248.6 Hz ), 152.1, 144.0, 138.1, 133.6, 129.0 (d, *J* = 8.2 Hz ), 128.1, 126.1 (d, *J* = 2.4 Hz ), 124.8, 123.1, 120.7, 120.1, 116.1 (d, *J* = 22.0 Hz ), 109.9, 108.9; MS (ESI): *m/z* 390 [M+H]$^{+}$; HRMS (ESI): calcd for $C_{22}H_{14}ON_3ClF$ *m/z* 390.07317 [M+H]$^{+}$; found 390.07482.

*4.4.9. (Z)-3-((2-(4-(Trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5i**).*

Compound **5i** was prepared according to the method described for compound **5a**, employing aldehyde **11c** (145mg, 0.5 mmol) and indolin-2-one (**12a,** 66.5 mg, 0.5 mmol) to obtain the pure product **5i** as a yellow solid (129 mg, 64%); mp: 313–315 $^{o}$C; ; IR (KBr): 3420, 3170, 2352, 1668, 1628, 1468, 1320, 1115, 1010, 847, 809, 740 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO–$d_6$): δ 10.69 (bs, 1H), 9.21 (s, 1H), 8.43 (d, *J* = 7.9 Hz, 2H), 8.17 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 9.1 Hz, 3H), 7.75 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.19 (t, *J* = 7.3 Hz, 1H), 6.99 (t, *J* = 7.3 Hz, 1H), 6.83 (d, *J* = 7.5 Hz, 1H); $^{13}$C NMR (75 MHz, DMSO–$d_6$): δ 167.4, 151.6, 140.2, 138.2, 133.4, 130.1, 129.7, 129.2, 128.9, 128.3, 127.2, 125.9 (q, *J* = 2.2 Hz ), 125.3, 124.7, 119.3, 109.1; MS (ESI): *m/z* 406 [M+H]$^{+}$; HRMS (ESI): calcd for $C_{23}H_{15}ON_3F_3$ *m/z* 406.10782 [M+H]$^{+}$; found 406.10587.

*4.4.10. (Z)-5-Methoxy-3-((2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6-yl)methylene) indolin-2-one (**5j**).*

Compound **5j** was prepared according to the method described for compound **5a**, employing aldehyde **11c** (145 mg,0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 81, 0.5 mmol) to obtain the pure product **5j** as a yellow solid (132 mg, 61%); mp: 293–295 $^{o}$C; IR (KBr): 3420, 3160, 2355, 1672, 1618, 1470, 1328, 1113, 1015, 915, 845, 741 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO–$d_6$): δ 10.48 (bs, 1H), 9.22 (s, 1H), 8.44 (d, *J* = 8.1 Hz, 2H), 8.17 (s, 1H), 7.99 (s, 1H), 7.96 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.45 (s, 1H), 6.87–6.68 (m, 2H), 3.79 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.8, 154.6, 153.8, 138.4, 137.3, 136.4, 134.0, 133.4, 127.2, 126.6, 125.9 , (q, *J* = 2.7 Hz ), 125.2, 125.0, 122.9, 121.9, 114.9, 110.2, 109.6,

108.6, 105.5, 55.2; MS (ESI): *m/z* 436 $[M+H]^+$; HRMS (ESI): calcd for $C_{24}H_{17}ON_3F_3$ *m/z* 436.12073 $[M+H]^+$; found 436.12437.

*4.4.11. (Z)-5-Chloro-3-((2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6-yl) methylene) indoli-2-one (**5k**).*

Compound **5k** was prepared according to the method described for compound **5a**, employing aldehyde **11c** (145 mg,0.5 mmol) and 5-chloroindolin-2-one (**12c,** 83 mg, 0.5 mmol) to obtain the pure product **5k** as a yellow solid (151 mg, 69%); mp: 317–319 $^oC$; IR (KBr): 3417, 3166, 2351, 1668, 1628, 1471, 1325, 1115, 1015, 947, 847, 741 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.63 (bs, 1H), 8.39 (d, *J* = 7.9 Hz, 2H), 7.95 (s, 1H), 7.87–7.80 (m, 3H).), 7.76 (s, 1H), 7.64 (s, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.15 (dd *J* = 8.3, 1.7 Hz, 1H), 6.89–675(m, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.7, 151.2, 141.2, 135.0, 133.4, 132.3, 130.1, 128.9, 128.2, 127.2, 125.9 (q, *J* = 2.2 Hz ), 123.6, 123.1, 122.2, 120.7, 120.6, 118.6, 115.3, 109.9, 109.0; MS (ESI): *m/z* 440 $[M+H]^+$; HRMS (ESI): calcd for $C_{23}H_{14}ON_3ClF_3$ *m/z* 440.07648 $[M+H]^+$; found 440.07691.

*4.4.12. (Z)-6-Chloro-3-((2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6-yl)methylene) indolin-2-one (**5l**).*

Compound **5l** was prepared according to the method described for compound **5a**, employing aldehyde **11c** (145 mg,0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **5l** as a yellow solid (146 mg, 67%); mp: 318–320 $^oC$; IR (KBr): 3420, 3160, 2358, 1670, 1637, 1471, 1320, 1114, 1020, 845, 742 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.83 (bs, 1H), 8.41 (d, *J* = 7.7 Hz, 2H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.99 (s, 1H), 7.94 (d, *J* = 7.3 Hz, 2H), 7.84 (s, 1H), 7.75 (d, *J* = 7.9 Hz, 2H), 7.62 (s, 1H), 7.08–6.80 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.5, 151.4, 141.2, 139.7, 138.6, 133.2, 128.8, 128.3, 127.5, 126.9, 125.5, 125.2 (q, *J* = 2.8 Hz), 124.8, 123.5, 122.6, 121.9, 121.3, 119.0, 110.9, 110.1; MS (ESI): *m/z* 440 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{14}ON_3ClF_3$ *m/z* 440.07648 $[M+H]^+$; found 440.07720.

*4.4.13. (Z)-3-((2-(3,5-Difluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5m**).*

Compound **5m** was prepared according to the method described for compound **5a**, employing aldehyde **11d** (129 mg,0.5 mmol) and indolin-2-one (**12a,** 66.5 mg, 0.5 mmol) to obtain the pure product **5m** as a yellow solid (111 mg, 60%); mp: 306–308 $^oC$; IR (KBr): 3427, 3165,

2924, 2352, 1664, 1620, 1556, 1454, 1414, 1121, 945, 866, 740 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.63 (bs, 1H), 8.02 (s, 1H), 7.90 (d, *J* = 6.1 Hz, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 2.8 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.43 (t, *J* = 9.0 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.00 (t, *J* = 7.3 Hz, 1H), 6.93–6.80 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.8, 164.4 and 161.1(d, *J* = 245.9 Hz), 164.2 and 160.9 (d, *J* = 246.4 Hz) , 150.7, 142.7, 138.1, 137.1, 132.9, 129.8, 128.9 (d, *J* = 7.7 Hz),, 128.3, 126.1, 124.6, 122.0, 121.1 (t, *J* = 7.8 Hz), 110.0, 109.7 (d, *J* = 8.8 Hz), 109.5 (d, *J* = 8.8 Hz), 109.1, 105.4; MS (ESI): *m/z* 374 $[M+H]^+$; HRMS (ESI): calcd for $C_{22}H_{14}ON_3F_2$ *m/z* 374.10965 $[M+H]^+$; found 374.10995.

*4.4.14. (Z)-3-((2-(3,5-Difluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)-5-methoxyindolin-2-one (**5n**).*

Compound **5n** was prepared according to the method described for compound **5a**, employing aldehyde **11d** (129 mg,0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 81 mg, 0.5 mmol) to obtain the pure product **5n** as a yellow solid (110 mg, 55%); mp: 296–298 $^oC$; IR (KBr): 3425, 3165, 2925, 2357, 1673, 1640, 1560, 1410, 1282, 1190, 1120, 942, 866, 745 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.69 (bs 1H), 8.04 (s, 1H), 7.91 (s, 3H), 7.75 (s, 1H), 7.62 (s, 2H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.05–6.87 (m, 2H), 3.79 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.8, 164.4 and 161.3 (d, *J* = 246.1 Hz), 163.7 and 161.7 (d, *J* = 246.1 Hz) 160.7,154.6, 149.7, 141.2, 137.5, 134.6, 128.7 (d, *J* = 4.9 Hz), 127.6, 125.4, 124.8, 122.7, 120.6, 118.6, 109.8, 109.4, 105.7, 55.2; MS (ESI): *m/z* 404 $[M+H]^+$; HRMS (ESI): calcd for $C_{23}H_{16}O_2N_3F_2$ *m/z* 404.12308 $[M+H]^+$; found 404.12758.

*4.4.15. (Z)-5-Chloro-3-((2-(3,5-difluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5o**).*

Compound **5o** was prepared according to the method described for compound **5a**, employing aldehyde **11d** (129 mg,0.5 mmol) and 5-chloroindolin-2-one (**12c,** 83 mg, 0.5 mmol) to obtain the pure product **5o** as a yellow solid (127 mg, 63%); mp: 316–318 $^oC$; IR (KBr): 3427, 3164, 2924, 2357, 2340, 1673, 1642, 1570, 1410, 1280, 1190, 1121, 956, 866 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.79 (bs 1H), 8.09 (s, 1H), 7.88 (s, 3H), 7.75 (bs, 1H), 7.62 (s, 2H), 7.44 (t, *J* = 8.1 Hz, 1H), 7.34–7.17 (m, 1H), 6.95–6.78 (m, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.6, 164.3 and 161.0 (d, *J* = 246.4Hz), 164.1 and 160.9 (d, *J* = 245.9Hz), 150.7, 140.1, 138.7, 134.5, 129.1, 128.6 (d, *J* = 3.3 Hz), 128.4 (d, *J* = 4.9 Hz), 127.5, 127.2, 125.2, 124.7, 123.6, 121.1 (d, *J* = 4.9 Hz), 122.7, 119.3, 110.4, 109.7 (d, *J* = 9.3

Hz), 109.5 (d, *J* = 9.3 Hz), 105.7; MS (ESI): *m/z* 408 $[M+H]^+$; HRMS (ESI): calcd for $C_{22}H_{13}ON_3ClF_2$ *m/z* 408.07004 $[M+H]^+$; found: 408.07097.

*4.4.16. (Z)-6-Chloro-3-((2-(3,5-difluorophenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5p**).*

Compound **5p** was prepared according to the method described for compound **5a**, employing aldehyde **11d** (129 mg,0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **5p** as a yellow solid (123 mg, 61%); mp: 315–317 $^oC$; IR (KBr): 3428, 3170, 2930, 2354, 1667, 1645, 1556, 1476, 1320, 1116, 1011, 953, 847, 752 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.09 (s, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 7.24 (s, 1H), 7.12 (s, 2H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.67 (dt, *J* = 9.1, 1.9 Hz, 1H), 6.27 (d, *J* = 8.0 Hz, 1H), 6.06 (d, *J* = 1.6 Hz, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.7, 163.7 and 161.7 (d, *J* = 246.1 Hz), 163.6 and 161.5 (d, *J* = 246.9 Hz), 150.5, 141.3, 139.1, 132.9, 129.1, 128.3 (t, *J* = 5.4 Hz), 124.3, 123.7, 120.8, 120.7 (d, *J* = 5.4 Hz), 120.6, 118.7, 109.9, 109.7 (d, *J* = 4.5 Hz), 109.5 (d, *J* = 4.5 Hz), 105.5; MS (ESI): *m/z* 408 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{13}ON_3ClF_2$ *m/z* 408.07004 $[M+H]^+$; found 408.07097.

*4.4.17. (Z)-3-((2-(3,4,5-Trimethoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**5q**).*

Compound **5q** was prepared according to the method described for compound **5a**, employing aldehyde **11e** (156 mg, 0.5 mmol) and indolin-2-one (**12a,** 66.5 mg, 0.5 mmol) to obtain the pure product **5q** as a yellow solid (132 mg, 62%); mp: 171–173 $^oC$; IR (KBr): 3419, 3169, 2935, 2357, 1694, 1642, 1590, 1477, 1421, 1284, 1127, 1035, 999, 840, 749 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO–$d_6$): δ 10.50 (bs, 1H), 7.90 (s, 1H), 7.78 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.58 (s, 1H), 7.54 (s, 2H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 3.92 (s, 6H), 3.76 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO–$d_6$): δ 168.8, 152.9, 139.7, 134.2, 133.7, 127.6, 126.5, 124.8, 121.9, 119.4, 114.7, 110.9, 108.8, 103.6, 59.5, 55.6; MS (ESI): *m/z* 428 $[M+H]^+$; HRMS (ESI): calcd for $C_{25}H_{22}O_4N_3$ *m/z* 428.15878 $[M+H]^+$; found 428.16048.

*4.4.18. (Z)-5-Methoxy-3-((2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazol-6-yl) methylene) indolin-2-one (**5r**).*

Compound **5r** was prepared according to the method described for compound **5a**, employing aldehyde **11e** (156 mg,0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 81 mg, 0.5 mmol) to obtain the pure product **5r** as a yellow solid (134 mg, 59%); mp: 168–166 $^{o}$C; IR (KBr): 3425, 3174, 2354, 1690, 1640, 1575, 1488, 1435, 1280, 1120, 1032, 992, 846, 742 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.34 (bs, 1H), 7.89 (s, 1H), 7.77 (s, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.58 (s, 1H), 7.54 (s, 2H), 7.36 (s, 1H), 6.89–6.66 (m, 2H), 3.92 (s, 6H), 3.76 (s, 3H), 3.62 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.9, 153.7, 153.0, 139.0, 137.3, 136.3, 128.2, 127.7, 126.2, 124.8, 121.9, 114.2, 113.8, 111.2, 111.1, 110.0, 108.9, 108.5, 103.8, 59.9, 55.8, 55.0; MS (ESI): *m/z* 458 $[M+H]^+$; HRMS (ESI) calcd for $C_{26}H_{24}O_5N_3$ *m/z* 458.16992 $[M+H]^+$; found 458.17105.

*4.4.19. (Z)-5-Chloro-3-((2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene) indolin-2-one (**5s**).*

Compound **5s** was prepared according to the method described for compound **5a**, employing aldehyde **11e** (156 mg, 0.5 mmol) and 5-chloroindolin-2-one (**12c,** 83 mg, 0.5 mmol) to obtain the pure product **5s** as a yellow solid (147 mg, 64%); mp: 185–187 $^{o}$C; IR (KBr): 3420, 3172, 2930, 2357, 1693, 1642, 1594, 1478, 1292, 1132, 1039, 987, 845, 753 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.70 (bs, 1H), 8.10 (s, 1H), 7.93 (s, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 1.7 Hz, 1H), 7.63 - 7.56 (m, 3H), 7.31–7.21 (m, 1H), 6.88 (dd, *J* = 30.6, 8.6 Hz, 1H), 3.94 (s, 6H), 3.77 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.8, 153.0, 142.7, 139.2, 134.6, 133.2, 127.6, 124.8, 124.2, 122.7, 120.5, 119.6, 112.4, 109.7, 103.7, 59.7, 55.6; MS (ESI): *m/z* 462 $[M+H]^+$; HRMS (ESI): calcd for $C_{25}H_{21}O_4N_3Cl$ *m/z* 462.12079 $[M+H]^+$; found 462.12151.

*4.4.20. (Z)-6-Chloro-3-((2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazol-6-yl)methylene) indolin-2-one (**5t**).*

Compound **5t** was prepared according to the method described for compound **5a**, employing aldehyde **11e** (156 mg, 0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **5t** as a yellow solid (144 mg, 63%); mp: 189–191 $^{o}$C; IR (KBr): 3427, 3170, 2357, 1692, 1642, 1590, 1470, 1425, 1284, 1127, 1035, 999, 840, 748 cm$^{-1}$; $^{1}$H NMR (300 MHz, DMSO−$d_6$): δ 10.73 (bs, 1H), 8.06–7.88 (m, 2H), 7.82 (s, 1H), 7.74 (s, 1H), 7.55 (s, 3H), 7.08–6.79 (m, 2H), 3.92 (s, 6H), 3.75 (s, 3H); $^{13}$C NMR (75 MHz,

$CDCl_3$+DMSO−$d_6$): δ 168.5, 152.7, 143.5, 138.8, 134.1, 133.2, 127.8, 126.2, 124.5, 124.3, 122.6, 120.1, 119.7, 112.2, 110.7, 109.5, 103.6, 59.6, 55.5; MS (ESI): *m/z* 462 $[M+H]^+$; HRMS (ESI): calcd for $C_{25}H_{21}O_4N_3Cl$ *m/z* 462.12079 $[M+H]^+$; found 462.12273.

*4.4.21. (Z)-3-((2-(Thiophen-2-yl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**6a**).*

Compound **6a** was prepared according to the method described for compound **5a**, employing aldehyde **14** (114 mg, 0.5 mmol) and indolin-2-one (**12a**, 66.5 mg, 0.5 mmol) to obtain the pure product **6a** as a yellow solid (102 mg, 60%); mp: 185–187 $^oC$; IR (KBr): 3417, 2927, 2355, 1689, 1603, 1476, 1435, 1308, 1203, 1089, 1035, 860 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO−$d_6$): δ 10.70 (bs, 1H), 7.96 (s, 1H), 7.91 (d, *J* = 3.2 Hz, 1H), 7.83–7.74 (m, 3H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.29–7.17 (m, 2H), 6.93–6.81 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.8, 148.9, 142.6, 140.1, 138.4, 137.4, 133.2, 129.7, 129.3, 128.4, 128.2, 127.3, 125.7, 124.3, 121.9, 121.1, 120.9, 110.0, 109.1; MS (ESI): *m/z* 344 $[M+H]^+$; HRMS (ESI): calcd for $C_{20}H_{14}ON_3S$ *m/z* 344.08466 $[M+H]^+$; found 344.08521.

*4.4.22. (Z)-5-Methoxy-3-((2-(thiophen-2-yl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**6b**).*

Compound **6b** was prepared according to the method described for compound **5a**, employing aldehyde **14** (114 mg, 0.5 mmol) and 5-methoxyindolin-2-one (**12b,** 81 mg, 0.5 mmol) to obtain the pure product **6b** as a yellow solid (104 mg, 56%); mp: 189–191 $^oC$; IR (KBr): 3420, 3152, 2920, 2330, 1689, 1640, 1603, 1476, 1430, 1308, 1209, 1092, 1037, 862 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO−$d_6$): δ 10.33 (bs, 1H), 7.94–7.83 (m, 2H), 7.75 (s, 1H), 7.68 (s, 1H), 7.67 (s, 1H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.20 (t, *J* = 4.9 Hz, 1H), 6.84–6.64 (m, 2H), 3.62 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.8, 153.7, 148.6, 137.2, 136.3, 133.0, 128.8, 127.0, 126.3, 124.0, 121.9, 114.1, 113.6, 109.9, 109.4, 108.6, 55.0; MS (ESI): *m/z* 374 $[M+H]^+$; HRMS (ESI): calcd for $C_{21}H_{16}O_2N_3S$ *m/z* 374.09561 $[M+H]^+$; found 374.09577.

*4.4.23. (Z)-5-Chloro-3-((2-(thiophen-2-yl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**6c**).*

Compound **6c** was prepared according to the method described for compound **5a**, employing aldehyde **14** (114 mg,0.5 mmol) and 5-chloroindolin-2-one (**12c,** 83 mg, 0.5 mmol) to obtain the pure product **6c** as a yellow solid (120 mg, 64%); mp: 204–206 $^oC$; IR (KBr): 3425, 3417, 2927, 2350, 1697, 1642, 1605, 1470, 1438, 1312, 1210, 1097, 1042, 872 $cm^{-1}$; $^1H$ NMR (300

MHz, DMSO−$d_6$): δ 10.76 (bs, 1H), 8.08 (s, 1H), 7.98–7.84 (m, 3H), 7.78 (d, *J* = 4.9 Hz, 1H), 7.75–7.55 (m, 2H), 7.33–7.17 (m, 2H), 6.94–6.80 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.5, 141.4, 139.3, 138.7, 133.1, 129.4, 129.1, 128.3, 127.4, 125.2, 124.9, 124.7, 123.2, 122.8, 121.2, 124.9, 124.7, 123.2, 122.8, 121.2, 119.2, 111.3, 110.4; MS (ESI): *m/z* 378[M+H]$^+$; HRMS (ESI): calcd for $C_{20}H_{13}ON_3ClS$ *m/z* 378.04627 [M+H]$^+$; found 378.04514.

*4.4.24. (Z)-6-Chloro-3-((2-(thiophen-2-yl)-1H-benzo[d]imidazol-6-yl)methylene)indolin-2-one (**6d**).*

Compound **6d** was prepared according to the method described for compound **5a**, employing aldehyde **14** (114 mg,0.5 mmol) and 6-chloroindolin-2-one (**12d,** 83 mg, 0.5 mmol) to obtain the pure product **6d** as a yellow solid (116 mg, 62%); mp: 210–212 $^o$C; IR (KBr): 3425, 3162, 2936, 2924, 2360, 1692, 1648, 1607, 1478, 1435, 1310, 1210, 1095, 1038, 845 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO−$d_6$): δ 10.74 (bs, 1H), 8.04 (s, 1H), 7.87–7.82 (m, 2H), 7.85 (d, *J* = 3.9 Hz, 1H), 7.76 (d, *J* = 4.9 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.28–7.17 (m, 2H), 6.91–6.78 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$+DMSO−$d_6$): δ 168.8, 148.7, 143.9, 141.1, 139.3, 138.0, 133.6, 133.0, 132.1, 129.0, 128.1, 127.2, 124.6, 124.2, 122.9, 120.5, 120.0, 109.8, 109.0; MS (ESI): *m/z* 378[M+H]$^+$; HRMS (ESI): calcd for $C_{20}H_{13}ON_3ClS$ *m/z* 378.04627 [M+H]$^+$; found 378.04514.

*4.5. Biology*

*4.5.1. Evaluation of in vitro anti-cancer activity*

The anticancer activity of the compounds was determined using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay [33]. $1\times10^6$ cells/well were seeded in 100 µl DMEM, supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 h at 37 $^o$C in a $CO_2$ incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 h of incubation, MTT (10 µl, 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, formazon crystals were dissolved in DMSO (100 µl) and absorbance at 570 nm wavelength was recorded.

*4.5.2 In vitro growth inhibition*

The screening of anticancer activity was evaluated by the NCI, USA, according to standard procedures (http://dtp.nci.nih.gov/ branches/btb/ivclsp.html) [41].

*4.5.3. Cell cycle analysis*

Flow cytometric analysis (FACS) was performed to evaluate the distribution of the cells through the cell-cycle phases. MCF-7 cells were incubated for 48 h with compounds **5c** and **5p** at concentrations of 1 and 2 µM. The reference compound nocodazole was treated at 2 µM concentration in this experiment. 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) [34].

*4.5.4 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 (General tubulin buffer, contains 80 mM PIPES pH 6.9, 2 mM $MgCl_2$ and 0.5 mM EGTA and used as a tubulin polymerization buffer), GTP (1 µM) in the presence or absence of test compounds **5c** and **5p** (final concentration of 1 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 Vario scan multimode plate reader (Thermo scientific Inc.). Nocodazole was used as positive control in each assay. The $IC_{50}$ value is 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 tested compounds at varying concentrations. Polymerization was monitored by increase in the fluorescence as mentioned above at $37^0$C [36,37].

*4.5.5. Immunohistochemistry*

A549 cells were seeded on glass cover slips and incubated for 48 h in the presence or absence of the test compounds **5c**, **5p**, and nocodazole (1 µM). After treatment, the cover slips were fixed with a paraformaldehyde solution (4% in 1×PBS) for 20 min at room temperature. Cell permeabilization was achieved by administration of a Triton X-100 solution (0.2% in 1×PBS) for 5 min. The cover slips were left in 100% MeOH overnight at $-4^0$C. Subsequently, the

cover slips were blocked with a 1% bovine serum albumin (BSA) solution for 60 min and then incubated with anti-α-tubulin antibody (1:1000) at room temperature for 2 h. The slides were washed three times for 5 min each with PBST. Next, the cover slips were incubated with FITC-conjugated anti-mouse secondary antibody (Sigma–Aldrich) for 1 h and then washed three times with PBST solution. Finally, the cells were observed under a fluorescence microscope (Leica, Germany), and the pictures were analyzed for the integrity of the microtubule network [37].

*4.5.6. Molecular modelling experimental procedure*

Optimizations of all compounds were performed in Gaussian 09 by using PM3 semiempirical methods. The protein cocrystal structure was downloaded from RSCB-Protein Data Bank with ligand colchicine (PDB ID 3E22) [39]. Autodock 4.2 software [42] was used to perform the docking studies. The visualization and analysis of the interactions were performed by using PyMOL (ver. 0.99) [40].

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## Figures

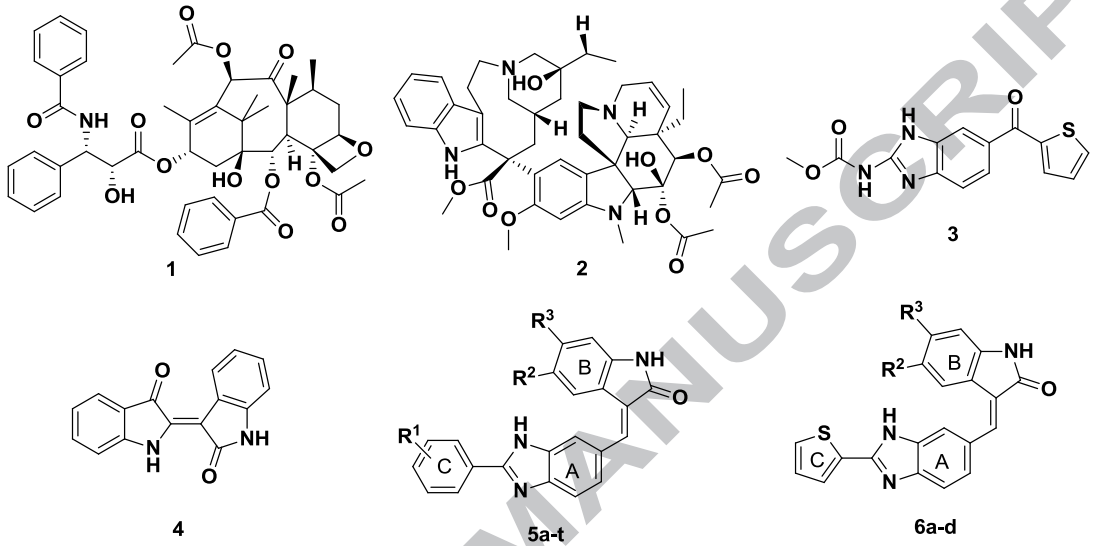



**Fig. 1.** Structures of standard anticancer molecules Paclitaxel (**1**), vinblastine (**2**), indirubin (**3**), nocodazole (**4**) and benzimidazole–oxindole analogues (**5a–t** and **6a–d**).

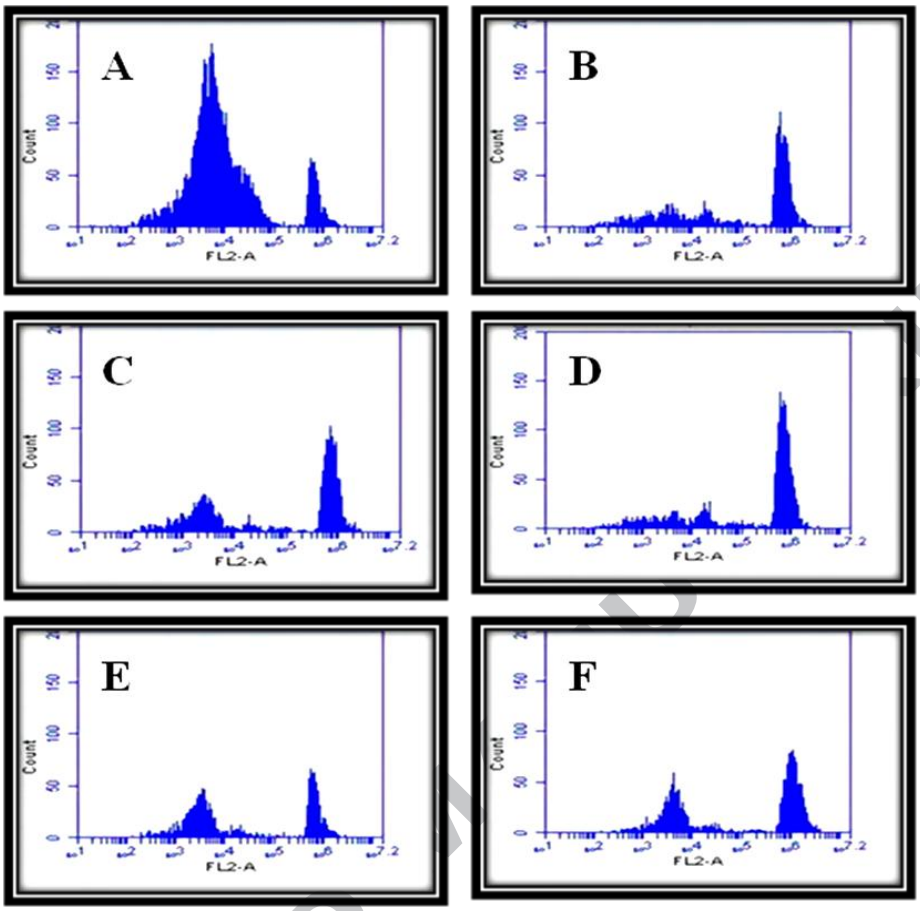



**Fig. 2.** Flow cytometric analysis in the MCF-7 breast cancer cell line after treatment with conjugates**5c** and **5p** at 1 µM and 2 µM concentrations for 48 h; A. control; B. Nocodazole (2 µM); C. **5c** (1 µM); D. **5c** (2 µM); E. **5p** (1 µM) and F. **5p** (2 µM).

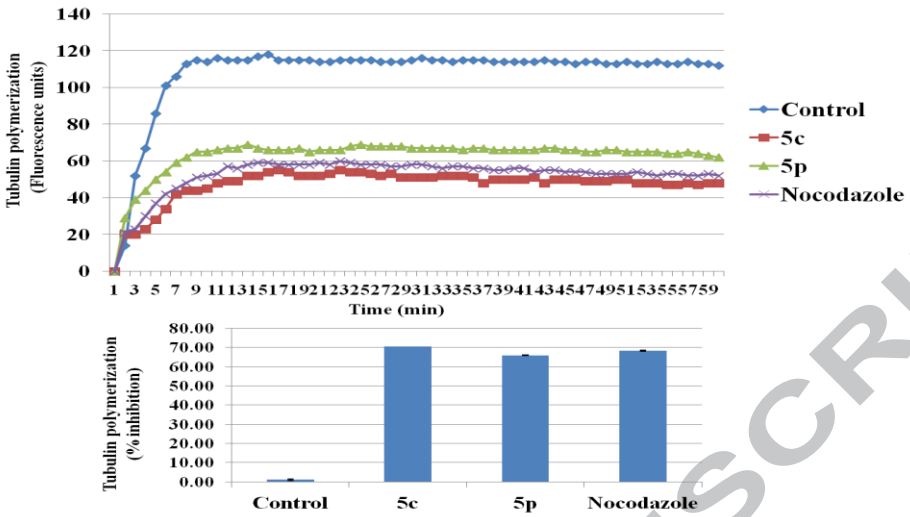



**Fig. 3.** Effect of conjugates on tubulin polymerization: tubulin polymerization was monitored by the increase in fluorescence at 360 nm (excitation) and 420 nm (emission) for 1 h at 37°C. All the conjugateswere included at a final concentration of 1 µM. Nocodazole was used as a positive control. Values indicated are the mean ± SD of two different experiments performed in triplicates.

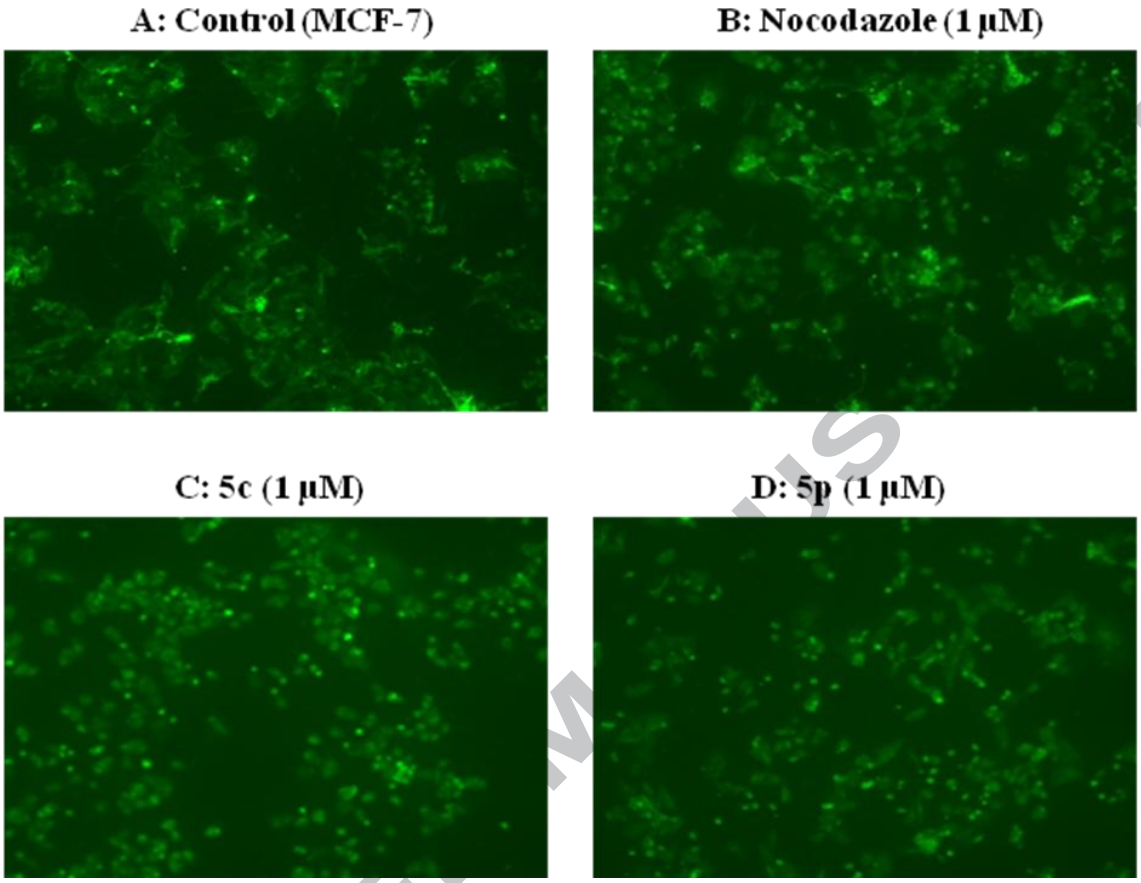
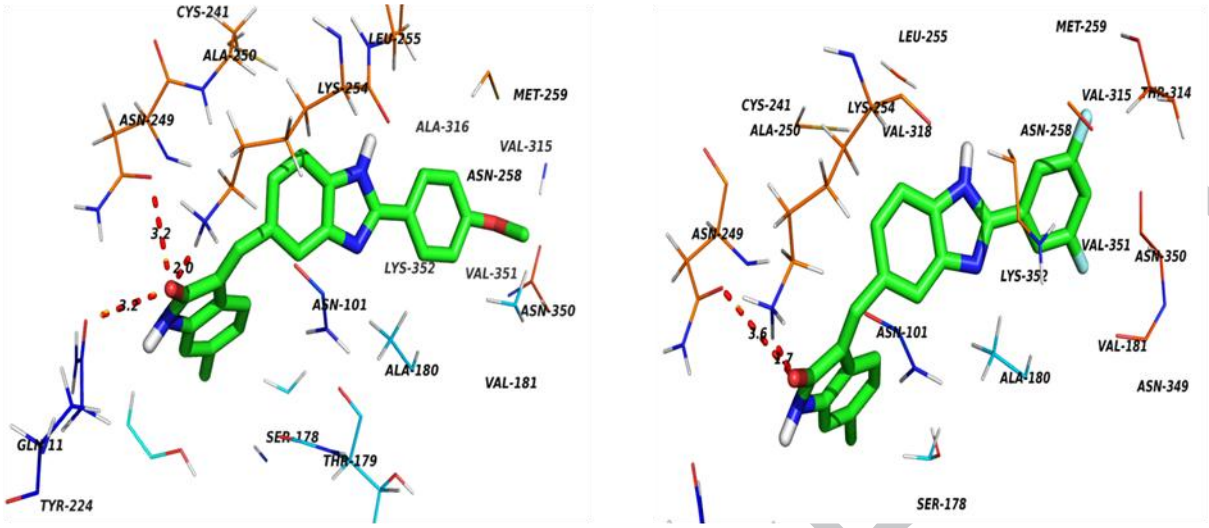



**Fig. 4.** IHC analysis of conjugates on the microtubule network: MCF-7 cells were treated with conjugates **5c**, **5p** and nocadazole at 1 µM concentration for 48 h.




**5c** **5p**

**Fig. 5.** Predicted binding model using Autodock Software: Interaction of the conjugate **5c** and **5p** with colchicines binding site of tubulin. The probable hydrogen bonds found were shown in red color. This figure has been generated using the software PYMOL from the tubulin-colchicine crystal structure.

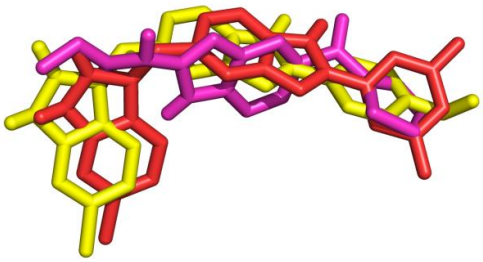


**Fig. 6.** Predicted binding model using Autodock Software: Overlay of conjugate **5c** (yellow), **5p** (red) and nocodazole (pink).

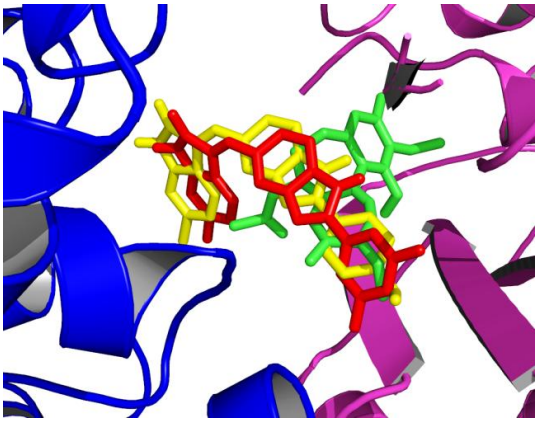


**Fig. 7.** Predicted binding model using Autodock Software: Super positioning of conjugate **5c** (yellow), **5p** (red) and colchicine (green).**5c** and **5p** is predicted to bind in colchicine-binding domain but with different orientation. β-chain shown in pink color and α-chain shown in blue color.

## Tables

**Table 1.**
Cytotoxicity ($IC_{50}$ μM) of benzimidazole-oxindole conjugates **5a**–**t** and **6a**–**d** against a panel of human cancer[a].

| Conjugate | A549[b] | MCF-7[c] | DU-145[d] | HT-29[e] |
|---|---|---|---|---|
| **5a** | 7.01 ± 0.59 | 4.91±0.37 | 8.16±0.03 | 17.6±0.01 |
| **5b** | 4.77±0.32 | 2.62±0.45 | 3.83±0.47 | 6.99±0.02 |
| **5c** | 3.57±1.42 | 1.84±0.15 | 1.91±1.19 | 2.22±0.36 |
| **5d** | 14.2±1.04 | 4.12±0.24 | 7.78±0.76 | 13.5±1.18 |
| **5e** | 16.2±0.66 | 15.7±1.61 | 17.1±0.33 | 20.0±2.04 |
| **5f** | 5.67±1.10 | 5.60±0.63 | 7.13±0.18 | 7.24±0.46 |
| **5g** | 11.3±0.20 | 10.8±0.26 | 12.5±0.46 | 13.1±0.47 |
| **5h** | 9.27±0.47 | 11.4±0.82 | 14.9±0.25 | 14.8±1.59 |
| **5i** | 8.81±056 | 6.01±2.25 | 6.81±0.77 | 9.83±0.14 |
| **5j** | 7.34±0.78 | 5.53±0.12 | 8.85±0.32 | 7.64±1.02 |
| **5k** | 4.84±1.09 | 2.41±0.28 | 4.89±0.93 | 5.66±0.05 |
| **5l** | 5.65±0.13 | 3.89±0.01 | 9.28±0.41 | 11.6±0.43 |
| **5m** | 5.99±0.66 | 3.80±0.25 | 12.5±2.35 | 12.9±1.23 |
| **5o** | 18.7±1.65 | 4.78±0.65 | 6.96±0.37 | 14.9±1.55 |
| **5p** | 3.27±0.74 | 1.97±0.59 | 4.11±0.32 | 10.3±0.17 |
| **5q** | 4.30±0.33 | 3.42±0.76 | 6.31±0.21 | 10.3±0.24 |
| **5r** | 3.41±0.18 | 2.70±0.23 | 6.77±1.44 | 5.11±0.07 |
| **5s** | 4.70±0.04 | 2.83±0.35 | 3.40±0.12 | 6.99±0.06 |
| **5t** | 3.75±1.69 | 3.21±0.35 | 6.60±0.98 | 9.40±1.3 |
| **6a** | 3.50±0.05 | 2.76±0.92 | 4.80±0.54 | 7.88±0.06 |
| **6b** | 3.66±0.31 | 2.79±0.86 | 5.02±0.52 | 7.63±0.13 |
| **6c** | 11.2±2.05 | 7.12±0.60 | 16.4±0.75 | 19.1±0.69 |
| **6d** | 5.13±1.05 | 2.64±0.31 | 6.09±0.57 | 6.65±1.49 |
| **Nocodazole** | 1.90±0.30 | 2.03±0.03 | 1.86±0.36 | 0.97±0.68 |

[a]50% Inhibitory concentration; values are the mean ±SD of three individual experiments determined after 48h of treatment..[b]lung cancer, [c]prostate cancer, [d]breast cancer, [e]colon cancer.

**Table 2.**
Cytotoxic activity of some benzimidazole–oxindole conjugates against a panel of sixty human cancer cell lines.

| Cancer panel/cell line | Growth Inhibition ($GI_{50}$)[a] in μM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **5c**[b] | **5d**[c] | **5f**[d] | **5i**[e] | **5k**[f] | **5m**[g] | **5o**[h] | **5p**[i] | **5s**[J] |
| ***Leukemia*** | | | | | | | | | |
| CCRF-CEM | 1.97 | 1.41 | 4.14 | 1.65 | 2.43 | 2.44 | 2.91 | 0.65 | 2.38 |
| HL-60(TB) | 1.51 | 1.97 | 28.3 | 2.26 | 2.61 | 12.6 | 3.39 | NT[K] | 3.17 |
| K-562 | 1.00 | 2.18 | 9.14 | 1.41 | 2.60 | 3.28 | 3.12 | 0.82 | 3.88 |
| MOLT-4 | 1.99 | 4.85 | 13.9 | 1.67 | 2.41 | 3.53 | 3.32 | 1.45 | 2.54 |
| RPMI-8226 | 1.66 | 1.73 | 3.01 | 1.34 | 2.21 | 3.23 | 3.31 | 2.38 | 1.85 |
| SR | 0.69 | 1.13 | 4.95 | 1.70 | 2.43 | 2.43 | 2.98 | 2.22 | 1.95 |
| ***Non-small lung*** | | | | | | | | | |
| A549/ATCC | 1.83 | 10.1 | 3.11 | 4.81 | 3.00 | 4.65 | NT[K] | 1.64 | 2.86 |
| EKVX | NT[K] | NT[K] | 14.0 | 4.34 | 3.16 | 2.63 | 2.77 | 5.27 | NT[K] |
| HOP-62 | 1.82 | 12.4 | 1.93 | 4.94 | 2.28 | 10.4 | 3.77 | 1.35 | 2.01 |
| HOP-92 | 0.28 | 0.55 | 2.06 | NT[K] | 1.68 | NT[K] | 2.41 | 0.74 | 0.91 |
| NCI-H226 | 13.8 | NT[K] | 2.50 | 14.5 | 3.13 | 11.4 | NA[l] | 5.78 | 13.1 |
| NCI-H23 | 1.54 | 10.2 | 3.14 | 3.40 | 3.08 | 4.13 | 5.01 | 2.47 | 2.26 |
| NCI-H322M | 2.12 | 17.0 | 3.05 | 7.06 | 4.36 | 11.0 | NA[l] | 4.81 | 5.92 |
| NCI-H460 | 1.84 | 8.01 | 3.35 | 2.13 | 1.88 | 7.04 | 2.18 | 1.64 | 2.66 |
| NCI-H522 | 1.73 | 7.07 | 2.79 | 1.97 | 1.66 | 3.64 | 2.14 | 1.19 | 505 |
| ***Colon*** | | | | | | | | | |
| COLO 205 | 1.93 | 7.19 | 5.13 | 9.89 | 2.10 | 8.45 | 4.28 | 2.24 | 2.52 |
| HCC-2998 | 1.24 | 10.2 | NA[l] | 3.77 | 2.10 | 5.65 | 7.55 | 4.86 | 4.65 |
| HCT-116 | 1.55 | 2.38 | 1.63 | 1.37 | 1.98 | 2.43 | 1.74 | 1.17 | 1.44 |
| HCT-15 | 1.26 | 3.00 | 5.83 | 4.09 | 2.64 | 3.52 | 3.96 | 3.37 | 2.76 |
| HT29 | 1.83 | 3.06 | 3.62 | 1.61 | 1.78 | 3.60 | 2.18 | 1.72 | 2.07 |
| KM12 | 1.22 | 2.49 | 4.54 | 1.09 | 2.08 | 4.36 | 3.55 | 2.20 | 3.00 |
| SW-620 | 1.41 | 3.01 | 4.34 | 1.92 | 1.97 | 3.55 | 2.46 | 1.10 | 1.90 |
| ***CNS*** | | | | | | | | | |
| SF-268 | 2.09 | 12.9 | 3.76 | 3.46 | 2.72 | 9.44 | 3.70 | 2.35 | 1.90 |
| SF-295 | 1.53 | 12.3 | 7.33 | 2.70 | 1.87 | 2.84 | 2.42 | 0.59 | 13.0 |
| SF-539 | 1.78 | 11.4 | 3.83 | 7.45 | 2.51 | 13.6 | 8.70 | 2.04 | 4.36 |
| SNB-19 | 1.84 | 15.1 | 9.47 | 5.38 | 3.12 | 9.62 | NA[l] | 6.22 | 2.69 |
| SNB-75 | 2.36 | 3.10 | 1.55 | 2.79 | 2.03 | 3.22 | 2.06 | 1.60 | 2.43 |
| U251 | 1.55 | 2.08 | 3.65 | 2.83 | 1.74 | 3.89 | NT[K] | 106 | 1.62 |
| ***Melanoma*** | | | | | | | | | |
| LOX IMVI | NT[K] | NT[K] | 2.92 | 1.82 | 1.77 | 3.94 | 3.52 | 2.22 | NT[K] |
| MALME-3M | 1.67 | 2.87 | 2.58 | 2.90 | 2.34 | 10.1 | 57.5 | 6.06 | 1.65 |
| M14 | | | | | | | | | |
| MDA-MB-435 | 1.29 | 4.17 | 2.66 | 2.05 | 1.67 | 2.70 | 2.18 | 1.52 | 1.72 |
| SK-MEL-2 | 0.31 | 0.26 | 3.20 | 1.17 | 1.90 | 1.92 | 2.08 | 0.57 | 1.79 |
| SK-MEL-28 | | | | | | | | | |
| SK-MEL-5 | 1.75 | 8.86 | 5.72 | 5.08 | 1.97 | 5.71 | 3.24 | 2.08 | 8.76 |
| UACC-257 | 1.59 | 1.55 | 4.34 | 3.11 | 2.35 | 7.24 | 5.06 | 2.78 | 5.76 |
| UACC-62 | 1.68 | 10.7 | NT[K] | 8.72 | 1.70 | 5.82 | 4.07 | 1.68 | 1.53 |
| | 1.53 | 11.0 | 3.92 | 7.40 | 2.08 | 10.6 | NT[l] | 0.92 | 1.98 |
| | 1.46 | 8.95 | 6.11 | 2.62 | 1.71 | 4.18 | 3.43 | 0.71 | 1.60 |
| ***Ovarian*** | | | | | | | | | |
| IGROV1 | 2.16 | 17.6 | 3.27 | 3.01 | 3.35 | 10.5 | 6.21 | 3.29 | 7.06 |
| OVCAR-3 | 1.53 | 2.43 | 3.25 | 0.74 | 1.56 | 5.00 | 2.26 | 1.41 | 2.29 |
| OVCAR-4 | 1.80 | 3.22 | 2.00 | 3.94 | 2.73 | 3.33 | 2.77 | 4.24 | 2.94 |
| OVCAR-5 | 1.86 | 22.0 | 7.10 | 10.1 | 3.10 | 16.8 | NA[l] | 6.34 | 14.0 |
| OVCAR-8 | 2.05 | 7.68 | 2.07 | 5.94 | 3.17 | 4.56 | NT[K] | 2.92 | 1.99 |
| NCI/ADR-RES | 2.16 | 5.00 | 3.78 | 3.98 | 2.60 | 4.18 | 2.32 | 2.03 | 4.77 |
| SK-OV-3 | 2.23 | 1.24 | 2.62 | 12.1 | 4.19 | 14.2 | 31.9 | 2.38 | 19.9 |
| ***Renal*** | | | | | | | | | |
| 786-0 | 1.94 | 7.85 | 1.69 | 1.48 | 1.75 | 10.0 | 3.66 | 3.31 | 3.87 |
| A498 | 1.33 | 5.24 | 1.46 | 1.54 | 1.32 | 1.53 | 2.39 | 1.24 | 20.5 |
| ACHN | 1.83 | 17.3 | 2.14 | 3.24 | 3.24 | 7.39 | 7.42 | 4.99 | 8.05 |
| CAKI-1 | 1.75 | 14.5 | 7.12 | 3.11 | 2.74 | 2.47 | 5.10 | 1.73 | 6.08 |
| RXF 393 | 1.58 | 3.16 | 1.95 | 2.21 | 1.88 | 8.20 | 3.37 | 2.09 | 3.27 |
| SN12C | 1.90 | 13.9 | 4.88 | 6.91 | 2.37 | 11.4 | 5.41 | 3.89 | 2.29 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TK-10 | 2.45 | 21.0 | 3.01 | 10.4 | 3.62 | 14.0 | 7.22 | 5.53 | 6.23 |
| UO-31 | 1.46 | 10.2 | 1.95 | 2.48 | 2.56 | 3.42 | 8.73 | 3.68 | 3.44 |
| ***Prostate*** | | | | | | | | | |
| PC-3 | 1.56 | 3.18 | 4.64 | 1.54 | 2.28 | 3.37 | 3.44 | 2.12 | 3.04 |
| DU-145 | 1.63 | 6.60 | 2.23 | 4.11 | 3.12 | 10.2 | 4.82 | 1.98 | 2.38 |
| ***Breast*** | | | | | | | | | |
| MCF7 | 1.62 | 2.42 | 4.53 | 2.98 | 2.25 | 2.84 | 2.39 | 1.62 | 1.40 |
| MDA-MB 231/ATCC | 1.73 | 5.23 | 2.25 | 2.04 | 1.75 | 3.63 | 3.86 | 2.78 | 1.62 |
| HS 578T | 1.73 | 10.6 | 2.73 | 3.09 | 2.40 | 5.08 | 2.90 | 1.55 | 1.69 |
| BT-549 | 1.74 | 3.65 | 1.75 | 2.04 | 1.87 | 2.13 | 2.09 | 2.10 | 3.08 |
| T-47D | 2.22 | 10.7 | 2.01 | 3.58 | 2.86 | 4.55 | 4.47 | 4.27 | 8.66 |
| MDA-MB-468 | 1.48 | 3.06 | 4.14 | 2.39 | 1.94 | 2.62 | 3.46 | 2.51 | 1.97 |

[a]Conjugates concentration required to decrease cell growth to half that of untreated cells. **5c**[b] ( NSC:763684/1), 5d[c] (NSC:763703/1), 5f[d] (NSC:763617/1), 5i[e] (NSC:763621/1), 5k [f](NSC:763643/1), 5m[g] (NSC:763618/1), 5o[h] (NSC:763633/1), 5p[i] (NSC:763642/1), 5p[j] (NSC:763692/1),NT[K] Not Tested, NA[l] Not Active.

**Table 3.**

Effect of conjugates **5c**, **5p** and nocodazole on cell cycle phase distribution in MCF-7 cells.

| Sample | Sub G1 % | G0/G1 % | S % | G2/M % |
|---|---|---|---|---|
| A: Control | 1.74 | 85.98 | 0.48 | 11.41 |
| B: Nocodazole (2 µM) | 3.01 | 40.17 | 1.56 | 54.65 |
| C: **5c** (1 µM) | 2.78 | 43.39 | 1.15 | 52.41 |
| D: **5c** (2 µM) | 2.16 | 33.12 | 1.42 | 63.20 |
| E: **5p** (1 µM) | 1.64 | 59.78 | 0.75 | 37.62 |
| F: **5p** (2 µM) | 0.83 | 45.06 | 0.50 | 53.00 |

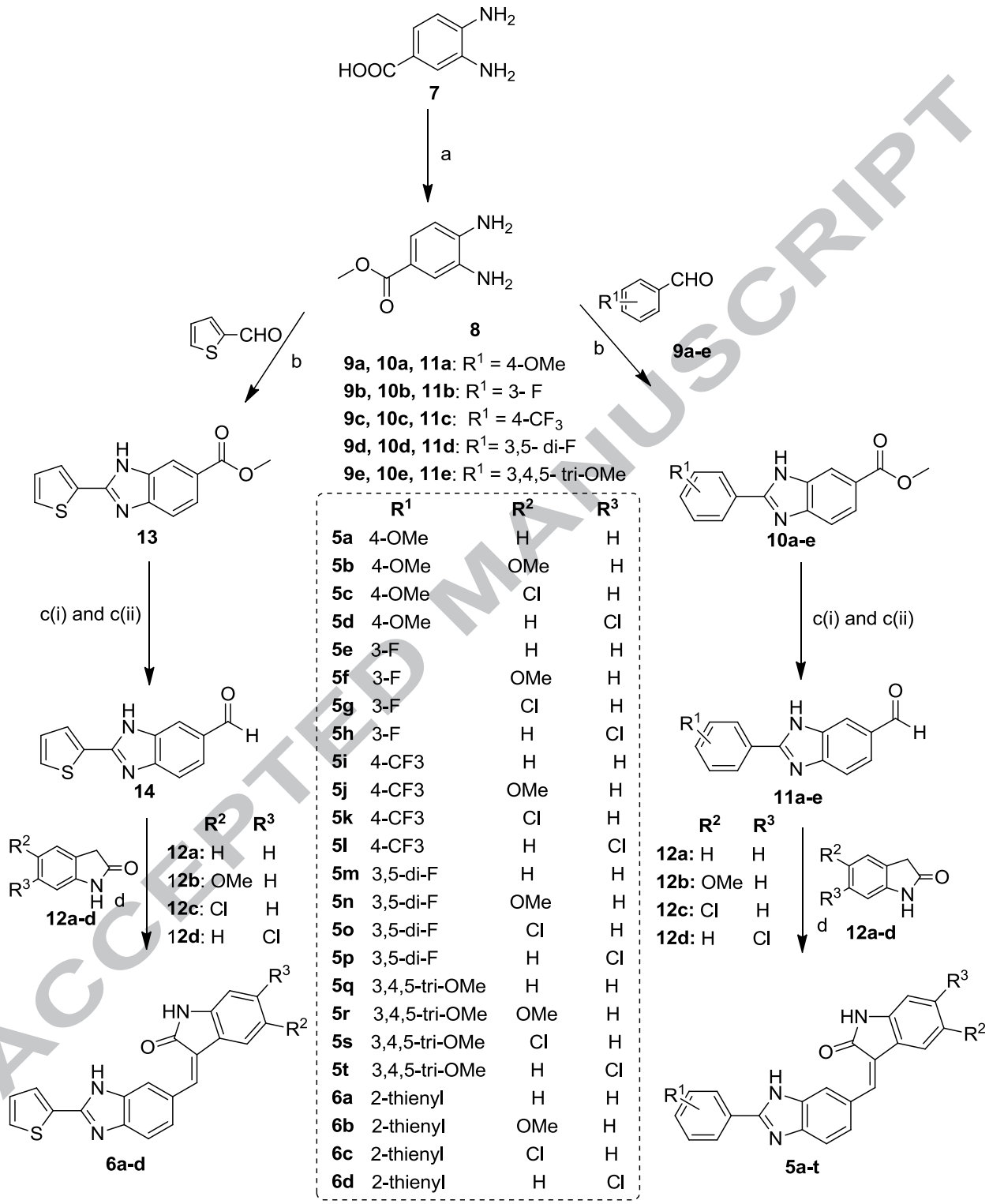
**Table 4.**
Inhibition of tubulin polymerization ($IC_{50}$) of conjugates **5c** and **5p**.

| conjugate | $IC_{50}$ ± SD (μM) |
|---|---|
| **5c** | 1.12±0.07 |
| **5p** | 1.59±0.10 |
| Nocodazole | 1.57±0.18 |

**Table 5.**
Docking results analysis of synthesized conjugates.

| conjugate | Docking score | Hydrogen bonding residues | No. of hydrogen bond |
|---|---|---|---|
| **5c** | -9.67 | αGly11, βAsn249 βLys254 | 3 |
| **5p** | -9.05 | βAsn249,βLys254 | 2 |

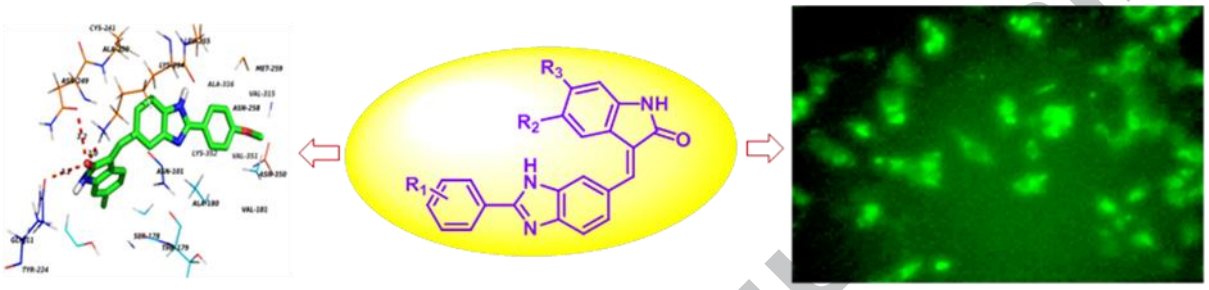
## Scheme




| | R1 | R2 | R3 |
|---|---|---|---|
| 5a | 4-OMe | H | H |
| 5b | 4-OMe | OMe | H |
| 5c | 4-OMe | Cl | H |
| 5d | 4-OMe | H | Cl |
| 5e | 3-F | H | H |
| 5f | 3-F | OMe | H |
| 5g | 3-F | Cl | H |
| 5h | 3-F | H | Cl |
| 5i | 4-CF3 | H | H |
| 5j | 4-CF3 | OMe | H |
| 5k | 4-CF3 | Cl | H |
| 5l | 4-CF3 | H | Cl |
| 5m | 3,5-di-F | H | H |
| 5n | 3,5-di-F | OMe | H |
| 5o | 3,5-di-F | Cl | H |
| 5p | 3,5-di-F | H | Cl |
| 5q | 3,4,5-tri-OMe | H | H |
| 5r | 3,4,5-tri-OMe | OMe | H |
| 5s | 3,4,5-tri-OMe | Cl | H |
| 5t | 3,4,5-tri-OMe | H | Cl |
| 6a | 2-thienyl | H | H |
| 6b | 2-thienyl | OMe | H |
| 6c | 2-thienyl | Cl | H |
| 6d | 2-thienyl | H | Cl |

**Scheme 1.** *Reagents & conditions*: a) $H_2SO_4$, MeOH, reflux, 4 h; b) substituted benzaldehydes **9a–e** and thiophene-2-carbaldehyde, $Na_2S_2O_5$, EtOH, reflux, 4h; c) i. $LiAlH_4$, THF, 0 $^oC$–RT, 4 h; ii. DMP, $CH_2Cl_2$, 0 $^oC$–RT, 3 h, 80–85%; d) piperidine, EtOH, reflux 3–5 h, 50–70%.

Graphical abstract




**5c** ($IC_{50}$ = 1.81μM)
$R_1$ = 4-Ome,
$R_2$ = Cl,
$R_3$ = H.

**5p** ($IC_{50}$ = 1.96 μM)
$R_1$ = 3,4-di F,
$R_2$ = H,
$R_3$ = Cl.

**Highlights**

- A new series of benzimidazole-oxindole conjugates designed and synthesized.
- Evaluated these compounds for cytotoxicity assay.
- Conjugates **5c** and **5p** exhibit activity on human breast cancer cell line (MCF-7).
- These conjugates induced cell cycle arrest at G2/M phase in cell cycle analysis.
- These conjugates effectively inhibit tubulin polymerization.