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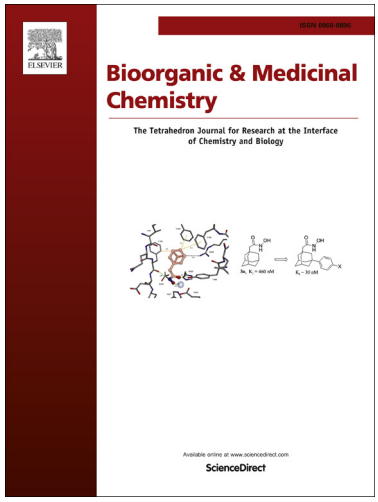

Synthesis and Evaluation of *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides as Potential Anticancer Agents that Inhibit Tubulin Polymerization

Ahmed Kamal, N.V. SubbaReddy, V. LakshmaNayak, Narasimha Rao Bolla, A.V. Subba Rao, B. Prasad

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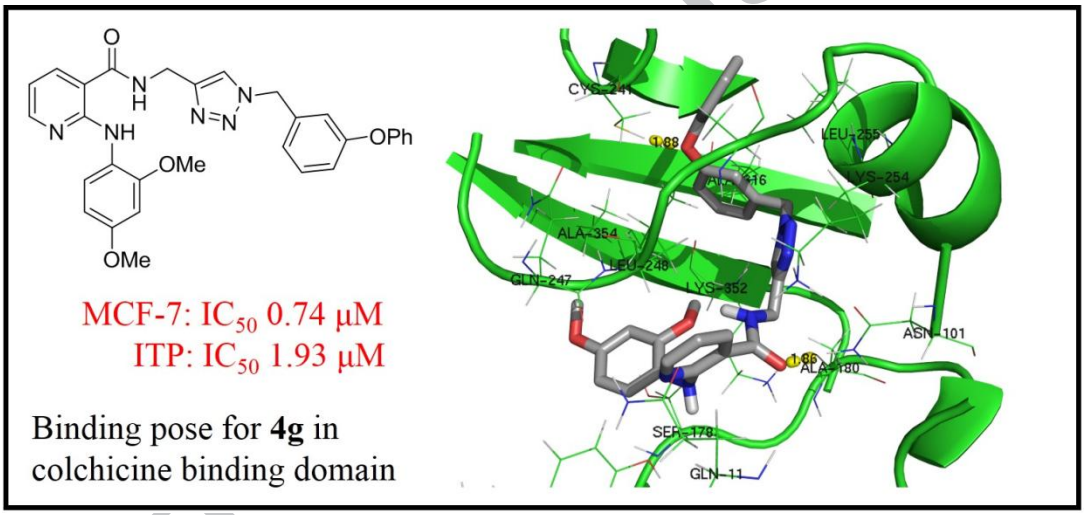
Graphical Abstract

Synthesis and Evaluation of *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides as Potential Anticancer Agents that Inhibit Tubulin Polymerization

Ahmed Kamal,*[a,b] N. V. Subba Reddy,[a] V. Lakshma Nayak,[a] Narasimha Rao Bolla,[b] A.V. Subba Rao[a]

A series of nicotinamides was synthesized and evaluated for their anticancer potential against a panel of sixty human cancer cell lines by NCI. Some of the representative compounds like **4g** and **4i** displayed good antiproliferative activity against MCF-7 cell line by inhibiting tubulin polymerization and these compounds induced apoptosis.



Synthesis and Evaluation of *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides as Potential Anticancer Agents that Inhibit Tubulin Polymerization

Ahmed Kamal,*[a,b] N. V. SubbaReddy,[a] V. LakshmaNayak,[a] Narasimha Rao Bolla,[b] A.V. Subba Rao,[a] B. Prasad[a]

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Abstract: A series of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides (**4**) was synthesized and tested for their anticancer activity against a panel of sixty human cancer cell lines. Some of the representative compounds such as **4a**, **4b**, **4f**, **4g**, **4i** and **4t** were selected for the five dose study and amongst them **4g** and **4i** displayed significant anticancer activity with GI_{50} values ranging from 0.25 to 8.34 and 1.42 to 5.86 µM respectively. Cell cycle analysis revealed that these compounds induced cell cycle arrest at G2/M phase in MCF-7 cells. The most active compound in this series **4g** also inhibited tubulin polymerization with IC_{50} value 1.93 µM superior to that of E7010. Moreover, assay to investigate the effect on Caspase-9, Hoechst staining and DNA fragmentation analysis suggested that these compounds induced cell death by apoptosis. Docking experiments showed that they interact and bind efficiently with tubulin protein. Overall, the results demonstrate that *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamide scaffold possess anticancer property by inhibiting the tubulin polymerization.

***Keywords*:** E7010, cell cycle, cytotoxicity, triazoles, tubulin polymerization.

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1. Introduction

Small molecules which affect tubulin polymerization have drawn considerable interest. Microtubules that consist of α- and β-tubulin heterodimers play a vital role in many cellular processes, such as mitosis, formation and maintenance of cellular shape and cell motility and intracellular transport.[1] Their importance to cellular functions, especially mitosis makes them prominent target for the development of anticancer agents.[2] In general, antitumor agents that inhibit the function of microtubules are known as antimitotic agents which exhibit their anticancer properties by interfering with the dynamic process of microtubule assembly and disassembly, inducing cell cycle arrest in the metaphase/anaphase transition and subsequent apoptosis.[3] Three distinct classes of antimitotic agents are identified by their mechanism of action and their different binding sites on tubulin. The vinca alkaloids (vincristine, vinblastine, vindesine, and vinorelbine) and colchicines are known as microtubule-destabilizing agents or microtubule polymerization inhibitors but with a different binding site and depolymerization mechanism. Vinca alkaloids are used in the treatment of leukemias, lymphomas, small cell lung cancer and other cancers.[4-6] The taxanes are microtubule-stabilizing agents or polymerizing agents, which are used in the treatment of lung, breast, ovarian, head and neck, and bladder carcinomas among others. However, the problems of neurotoxicity, difficult syntheses, p-glycoprotein-mediated drug resistance and isolation procedure[5] with existing amtimitotic agents encouraged medicinal chemists to develop novel antimitotic agents with novel modes of action.[6,7]

<Insert Figure 1>

E7010 (**1**), a sulphonamide that exhibits antitumor activity by inhibiting tubulin polymerisation,[8] causes cell cycle arrest and apoptosis in M phase.[9,10] *N*-Phenyl nicotinamides (**2**) are known as apoptosis inducers which blocks the cell cycle in G2/M phase and structure–activity relationship (SAR) studies indicate that the 3-pyridyl group is crucial for their activity.[11] Whereas, 1,2,3-triazoles have displayed broad range of biological properties such as antifungal, anticancer,[12-15] anti-allergic, antibacterial, anti-HIV, anticonvulsant, anti-inflammatory and antitubercular activities. Recently *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides have been evaluated as antimicrotubule agents against selective cancer cell lines.[16] In continuation to our search for identifying potent and selective anticancer agents, we incorporated triazole moiety to the 2-anilino pyridyl structural motif of

E7010 that resulted in triazolo linked nicotinamides (**4a-t**). These were evaluated for their cytotoxic potential, effect on tubulin polymerization and cell-cycle effects.

2. Results and Discussions

2.1. Chemistry.

1,4-Disubstituted triazoles can be prepared in high regioselectively by using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.[17,18] Following this strategy, the synthesis of compounds **4a-t** was carried out as shown in Scheme 1. Coupling of propargylamine with 2-chloronicotinylchloride which was prepared from corresponding acid, provided the precursor alkyne **6**.This alkyne and substituted anilines **7a-k** were refluxed in ethylene glycol to afford the intermediates 2-anilino nicotinamides **8a-k**. The azides **10a-d** that are required as another precursors were obtained from the reaction of benzyl bromides with NaN_3 in DMSO.[19] The synthesis of 1,2,3-triazoles was carried out by exposing alkynes to benzyl azides in the presence of catalytic Cu(I) and Na-Ascorbate in H_2O/*t*-BuOH mixture.

<Insert Scheme 1>

3. Biology

3.1.Cytotoxicity:

MTT assay [20] was performed to evaluate the cytotoxic potential of the newly synthesized compounds against five human cancer cell lines, A549 (lung), DU-145 (prostate), MCF-7 (breast), ACHN (renal) and HT-29 (colon) in comparison to E7010 and the results are summarized as IC_{50} values in Table 1. The results revealed that these compounds showed promising cytotoxic activity with IC_{50} values ranging from 0.74-18.6 µM against different cancer cell lines. The most active compounds **4g** and **4i** exhibited promising antiproliferative activity with IC_{50} values ranging from 0.74-1.57 and 0.93-1.93 µM and significant activity in MCF-7 cell line with IC_{50} values 0.74 and 0.93 µM respectively.

<Insert Table 1>

Further all the newly synthesized*N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides (**4a-t)** were evaluated for their cytotoxicity in preliminary screening by the National Cancer Institute (NCI), against a panel of 60 human tumor cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. These compounds can be considered as active, if they reduce the growth

of any of the cancer cell lines up to 60% or more in at least eight cancer cell lines screened. From the preliminary screen, six compounds like **4a**, **4b**, **4f**, **4g**, **4i**, and **4t** were selected for five dose screening to determine GI_{50} (the molar concentration required to cause 50% growth inhibition). These results are expressed as GI_{50} values[21] and listed in Table 2. These selected compounds (**4a**, **4f**, **4g**, **4i**, and **4t)** exhibited GI_{50} values ranging from 2.1-20.4, 2.3-5.9, 0.25-12.7, 2.40-10.5, and 2.1-9.03 µM respectively against majority of cell lines. Particularly, **4g** exhibited good cytotoxicity with GI_{50} values 0.25, 0.56, 0.62, 0.65, 0.72 and 0.75 µM against MDA-MB-435, SF-295, PC-3, MCF7, SK-MEL-5 and K-562 cell lines respectively.

<Insert Table 2>

3.2. Cell cycle analysis

To better understand the cytotoxic effects of these nicotinamides, the cell cycle analysis was performed on MCF-7 cell lines that were treated with **4g** and **4i** at 0.5 and 1µM after 48 h by flow cytometry[20]. The results demonstrate that both **4g** and **4i** induced more accumulation of cells in G2/M phase; 68.8% and 62.1% at 0.5 µM concentration and 69.9% and 67.8% at 1 µM concentration respectively relative to control (Figure. 2, Table. 3). However, in E7010, the percentage of cells (cell accumulation) at G_2/M phase increased from 20.6% to 25.7% by increasing the concentration from 0.5 to 1µM. The effect of these nicotinamides on cell cycle progression correlated well with their cytotoxic activity.

<Insert Figure 2>

<Insert Table 3>

3.3. Effect on tubulin polymerization

As these (**4g** and **4i**) compounds showed G_2/M cell cycle arrest which is a hallmark of tubulin polymerization,[22] we investigated the antiporliferative activities of these compounds on their ability to inhibit tubulin polymerization. E7010 was used for comparision. In the assembly assay,[23,24] both **4g** and **4i** inhibited 65.2% and 67.9% respectively, whereas E7010 inhibited 58.0% tubulin polymerization compared to control (Figure. 3). Next these compounds were evaluated for IC_{50} values for their ability to inhibit tubulin polymerization in vitro and the results are summarized in Table 4. Compound **4g**, which displayed maximum tubulin inhibition assembly, was found to be more potent ($IC_{50,}$ 1.93 µM) than E7010 (2.88

μM), whereas **4i** with IC_{50} value of 2.78 μM is equipotent to E7010. The effect of these compounds on the inhibition tubulin assembly also correlated well with their significant cytotoxicity.

<Insert Figure 3>

<Insert Table 4>

3.4. Effect on microtubules

The effects of **4g** and **4i** were further examined on the cellular microtubules in MCF-7 cancer cells by immunohistochemistry.[25] It is clear from the cytotoxicity studies that these compounds inhibited the growth of MCF-7 cell line. Therefore, MCF-7 cells were treated with these nicotinamides along with the standard E7010 at 1 μM concentration for 48 h. As shown in Figure 4, microtubules network displayed normal arrangement and organization in untreated human breast cancer cells. However, cells treated with **4g** and **4i** showed disrupted microtubule organization with spherical morphology thus demonstrating the inhibition of tubulin polymerization.

<Insert Figure 4>

3.5. Hoechst staining for morphological analysis of apoptosis

Apoptosis is a continuous process in which the mitochondrial membrane potential is depolarized and chromatin condensation takes place.[25] Hoechst staining was performed to investigate the apoptotic inducing effect of **4g** and **4i** in MCF-7 cell line at 1 μM concentration for 24 h. These compounds showed significant effect on the nuclear condensation in comparison to the untreated control cells as shown in Figure. 5. These results demonstrated that these compounds are effective in inducing cellular apoptosis.

<Insert Figure 5>

3.6. Effect on caspase-9

The activation of caspases play an important role in the process of cell death.[26] To ascertain the cytotoxic effect of **4g** and **4i** were due to apoptotic cell death, we examined the activitation of caspase 9 in MCF-7 cell line which lacks endogenous caspase-9.[27] MCF-7 cells were treated with these compounds along with standard E7010 at 1 and 2 μM concentrations.

The results demonstrate that there was 4 to 5 fold induction in caspase-9 activity by these nicotinamides at 2 µM. The reference compound E7010 induced 3-fold induction in caspase 9 activity as compared to untreated cells as shown in Figure 6. Therefore activation of caspase 9 by **4g** and **4i** suggest that they have ability to induce apoptosis in MCF-7 cells.

<Insert Figure 6>

3.7. DNA fragmentation assay

DNA fragmentation is a typical biochemical hallmark of apoptotic cell death. During apoptosis DNA is cleaved into small fragments by endonucleases and these fragments can be observed by gel electrophoresis as ladders.[28] It is clear from the cytotoxic studies that these compounds (**4g** and **4i**) significantly inhibited the growth of MCF-7cell lines that were treated with these compounds at 1 µM concentration for 48 h. DNA fragmentation revealed that these compounds induced significant DNA fragmentation, however, no fragmentation was observed in untreated cells as shown in Figure.7.

<Insert Figure 7>

4.0. Molecular modelling studies

To elucidate the mode of binding interactions with tubulin, we performed molecular docking studies with most active molecules **4g** and **4i**. Interestingly, binding pose of compound **4g** shows that ether oxygen attached rings are in the hydrophobic pocket of the colchicine binding domain in the β chain forming hydrophobic interaction with the Leu248, Ala316, Leu255, Cys241, Val238 and Ala250. In addition to hydrophobic contacts, hydrogen bonding is observed between the βCys241 and ether oxygen. Moreover, 2,4-dimethoxyanilino group of **4g** interact with the amino residues located deep in the colchicine binding site where the A ring of colchicine (trimethoxybenzyl ring) interacts. The structure of **4g** resembles to that of E7010, hence a comparison the docking pose of **4g** with the binding pose of E7010 was performed. Superimposed structure for cocrystallized pose of E7010 (PDB ID 3HKC) and docking pose of **4g** shows that the 2-anilinopyridinyl group of **4g** is in opposite to that of the E7010. This may be due to the longer substitution on 2-anilinopyridyl ring. In E7010, the 2-anilinopyridinyl group is in hydrophobic pocket of colchicine binding site however, it is at α,β interface for **4g**. Carbonyl oxygen forms a hydrogen bonding

interaction with the αAsn101 (Fig. 8). Some of the amino acids in hydrophobic contacts with **4g** are αSer178, αTyr224, βGln247, βLeu248, βAla354, αGln11, and βLys254 at α, β interface. Overall molecular docking studies provide information that these molecules fit well at α,β interface of the tubulin and making favourable interactions. Figure 9 gives the significant information about the interaction of the different amino acids with each part of the molecule. These interactions could be useful in rationalizing the potency observed for compound **4g** in its inhibition of tubulin polymerization.

<Insert Figure 8>

<Insert Figure 9>

5. Conclusion

In summary, a series of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamides (**4a-t**) was synthesized and evaluated for cytotoxic activity. Among them, six compounds were selected for the 60 cell line anticancer screening programme by NCI, USA. We identified two compounds (**4g** and **4i)** as the most effective antiproliferative agents. They showed significant activity in the breast cancer cell line MCF-7 and the FACS analysis showed that cell cycle arrest at G2/M phase. These compounds **4g** and **4i** also inhibited tubulin polymerization with IC_{50} 1.93 and 2.78 µM. Detailed biological studies like Hoechst staining, caspase 9 activity assay and DNA fragmentation analysis demonstrate that these compounds induced apoptotic cell death. Overall, the current study demonstrated that **4g** and **4i** are promising tubulin polymerization inhibitors and worthy of further evaluation as potential chemotherapeutic agents.

6. Experimental Section

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Reactions were performer by TLC performed on silica gel glass plate containing 60 GF-254, and visualization was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ by using Varian and Avance instruments. Chemical shifts are expressed in parts per million (δ in ppm) downfield from internal TMS and coupling constants are expressed in Hz. ^{1}H NMR spectroscopic data are reported in the following order: multiplicity (s, singlet; brs,

broad singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet), coupling constants in Hz, number of protons. ESI mass spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and an ESI mode positive ion trap detector. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

6.1. Synthesis of 2-chloro-*N*-(prop-2-ynyl)nicotinamide (6)

To a solution of 2-chloronicotinic acid (5g, 31.84 mmol) in dry DCM under nitrogen, oxalyl chloride (3.3 mL, 38.21 mmol) and catalytic amount of *N*,*N*-dimethylformamide were added carefully with stirring. The reaction was stirred for 3 h. After completion of the reaction, the reaction mixture was concentrated under vaccum to yield 2-chloronicotinyl chloride as solid which was used for next step without purification. 2-Chloronicotinyl chloride (4.6 g, 26.1 mmol) was dissolved in dry DCM, cooled to 0 °C, propargylamine hydrochloride (26.1 mmol) and triethylamine (9.04 mL, 65.25 mmol) were added. The reaction was warmed to room temperature. After stirring overnight, the reaction mixture was diluted with water and extracted with DCM. The combined organic extracts were washed with aq. $NaHCO_3$ and dried with Na_2SO_4 and concentrated *in vacuo*.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (dd, J = 3.0, 2.2 Hz, 1H), 8.10 (dd, J = 6.2, 3.0 Hz, 1H), 7.36 (d, J = 3.0 Hz, 1H), 6.94 (brs, 1H), 4.25 (m, 2H), 2.32 (t, J = 2.2 Hz, 1H).

6.2. 2-(Phenylamino)-*N*-(prop-2-ynyl)nicotinamide (8a): The compound 2-chloro-*N*-(prop-2-ynyl)nicotinamide **6** (195 mg, 1 mmol) and aniline **7a** (93 mg, 1 mmol) were taken in ethylene glycol and heated at 120 °C for 6 h. After completion of the reaction as confirmed by TLC, the reaction mixture was cooled, diluted with water and extracted with ethyl acetate and concentrated *in vacuo*. The crude product was further purified by column chromatography to afford compound **8a** as a pale yellow solid (175 mg, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.30 (dd, J = 3.0, 1.5 Hz, 1H), 7.69-7.64 (m, 3H), 7.34-7.29 (m, 2H), 7.05-7.00 (m, 1H), 6.70-6.65 (m, 1H), 6.36 (brs, 1H), 4.23-4.21 (m, 2H), 2.31 (s, 1H); MS (ESI *m/z*): 252 $[M+H]^+$.

6.3. 2-(4-Fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (8b):The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 4-fluoroaniline (**7b**, 111 mg, 1 mmol) to obtain the pure product **8b** as solid (193 mg, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 8.37 (dd, J = 3.0, 2.2 Hz, 1H), 7.82-7.76 (m, 1H), 7.72 (dd, J = 6.0, 1.5 Hz, 1H), 7.28-7.19 (m, 2H), 6.77-6.67 (m, 2H), 6.34 (brs, 1H), 4.25-4.23 (m, 2H), 2.32 (s, 1H); MS (ESI *m/z*): 270 $[M+H]^+$.

6.4. 2-(4-Chlorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (8c):The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 4-chloroaniline (**7c**, 127 mg, 1 mmol) to obtain the pure product **8c** as solid (200 mg, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.38 (s, 1H), 8.33 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.71 (dd, *J* = 6.0, 1.5 Hz, 1H), 7.63 (m, 2H), 7.27-7.24 (m, 2H), 6.73-6.69 (m, 1H), 6.35 (brs, 1H), 4.24-4.21 (m, 2H), 2.31 (s, 1H); MS (ESI *m/z*): 286 $[M+H]^+$.

6.5. 2-(4-Bromophenylamino)-*N*-(prop-2-ynyl)nicotinamide (8d):The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 4-bromoaniline (**7d**, 172 mg, 1 mmol) to obtain the pure product **8d** as solid (236 mg, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.32 (dd, *J* = 3.0, 1.6 Hz, 1H), 7.69 (dd, *J* = 6.0, 1.6 Hz, 1H), 7.63-7.60 (m, 2H), 7.24-7.20 (m, 2H), 6.72-6.69 (m, 1H), 6.34 (brs, 1H), 4.24-4.21 (m, 2H), 2.31 (s, 1H); MS (ESI *m/z*): 330 $[M+H]^+$.

6.6. 2-(4-Methoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (8e): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 4-methoxyaniline (**7e**, 123 mg, 1 mmol) to obtain the pure product **8e** as solid (202 mg, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.20 (s, 1H), 8.27 (dd, *J* = 3.4, 1.3 Hz, 1H), 7.66 (dd, *J* = 6.2, 1.5 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 6.65-6.60 (m, 1H), 6.36 (brs, 1H), 4.24-4.21 (m, 2H), 3.80 (s, 3H), 2.32 (s, 1H); MS (ESI *m/z*): 282 $[M+H]^+$.

6.7. 2-(3-Fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (8f): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 3-fluoroaniline (**7f**, 111 mg, 1 mmol) to obtain the pure product **8f** as solid (182 mg, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, 1H), 8.37 (dd, *J* = 3.2, 1.5 Hz, 1H), 7.79 (d, *J* = 11.7 Hz, 1H), 7.72 (dd, *J* = 1.5, 6.2 Hz, 1H), 7.25-7.19 (m, 3H), 6.78-6.67 (m, 2H), 6.32 (brs, 1H), 4.24-4.21 (m, 2H), 2.32 (s, 1H); MS (ESI *m/z*): 270 $[M+H]^+$.

6.8. 2-(2,4-Dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide(8g): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 2,4-dimethoxyaniline (**7g**, 153 mg, 1 mmol) to obtain the pure product **8g** as solid (217 mg, 70%); ^{1}H NMR (300 MHz,

$CDCl_3$) δ 10.21 (s, 1H), 8.30 (dd, J = 4.5, 1.5 Hz, 2H), 8.21 (d, J = 8.3 Hz, 1H), 7.69 (dd, J = 6.0, 1.5 Hz, 1H), 6.65-6.63 (m, 2H), 6.36 (brs, 1H), 4.26-4.24 (m, 2H), 3.91 (s, 3H), 3.81 (s, 3H), 2.36 (s, 1H); MS (ESI *m/z*): 312 $[M+H]^+$.

6.9. 2-(2,5-Dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (8h): The title compound was prepared according to the method described for compound **8a,** employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 2,5-dimethoxyaniline (**7h**, 153 mg, 1 mmol) to obtain the pure product **8h** as solid (211 mg, 68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.60 (s, 1H), 8.36 (s, 2H), 7.71 (d, J = 7.3 Hz, 1H), 6.81 (d, J = 8.6 Hz, 1H), 6.69-6.66 (m, 1H), 6.48 (dd, J = 8.6, 2.8 Hz, 1H), 6.36 (brs, 1H), 4.26-4.24 (m, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 2.31 (s, 1H); MS (ESI *m/z*): 312 $[M+H]^+$.

6.10. 2-(3,5-Dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (8i): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 3,5-dimethoxyaniline (**7i**, 153 mg, 1 mmol) to obtain the pure product **8i** as solid (217 mg, 70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.35 (s, 1H), 8.33 (dd, J = 3.4, 1.2 Hz, 1H), 7.72 (dd, J = 6.6, 1.2 Hz, 1H), 6.98 (s, 2H), 6.72-6.68 (m, 1H), 6.36 (brs, 1H), 6.14 (t, J = 2.0 Hz, 1H), 4.24-4.21 (m, 2H), 3.79 (s, 6H), 2.31 (s, 1H); MS (ESI *m/z*): 312 $[M+H]^+$.

6.11. *N*-(prop-2-ynyl)-2-(3,4,5-trimethoxyphenylamino)nicotinamide (8j): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and 3,4,5-trimethoxyaniline (**7j**, 183 mg, 1 mmol) to obtain the pure product **8j** as solid (235 mg, 65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, 1H), 8.34 (dd, J = 3.2, 1.5 Hz, 1H), 7.69 (dd, J = 6.2, 1.3 Hz, 1H), 6.95 (d, J = 2.0 Hz, 2H), 6.72-6.68 (m, 1H), 6.36 (brs, 1H), 4.25-4.23 (m, 2H), 3.87 (s, 6H), 3.82 (s, 3H), 2.31 (s, 1H); MS (ESI *m/z*): 342 $[M+H]^+$.

6.12. 2-(4-Chlorobenzylamino)-*N*-(prop-2-ynyl)nicotinamide (8k): The title compound was prepared according to the method described for compound **8a**, employing 2-chloro-*N*-(prop-2-ynyl)nicotinamide (**6,** 195 mg, 1 mmol) and (4-chlorophenyl)methanamine (**7k**, 141 mg, 1 mmol) to obtain the pure product **8k** as solid (212 mg, 71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.48 (brs, 1H), 8.20 (dd, J = 2.9, 1.8 Hz, 1H), 7.60 (dd, J = 6.0, 1.83 Hz, 1H), 7.32-7.27 (m, 3H), 6.56-6.50 (m, 1H), 6.22 (brs, 1H), 4.66 (d, J = 5.8 Hz, 2H) 4.19-4.16 (m, 2H), 2.28 (s, 1H); MS (ESI *m/z*): 300 $[M+H]^+$.

6.13. *N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(phenylamino) nicotinamide (4a):To a solution of 2-(phenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8a**, 150 mg, 0.59 mmol)and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 147 mg, 0.65 mmol) in 2:1 mixture of water and *tert*-butyl alcohol, sodium ascorbate (12 mg, 0.06 mmol) and copper (II) sulphate (7.5 mg, 0.03 mmol) were added sequentially. The reaction was stirred at room temperature for overnight, TLC analysis indicated completion of reaction. The solvent was concentrated under vacuum and extracted with EtOAc to give crude product. The crude was purified by column chromatography to afford pure product **4a** as brown solid (210 mg, 74%); mp: 124-126 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.51 (s, 1H), 8.25 (dd, *J* = 2.2, 1.5 Hz, 1H), 7. 84 (dd, *J* = 2.2,1.5 Hz, 1H), 7.80 (brs, 1H), 7.66-7.60 (m, 3H), 7.33-7.25 (m, 4H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.00-6.90 (m, 7H), 6.61-6.59 (m, 1H), 5.45 (s, 2H), 4.62 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 158.1, 156.3, 155.3, 151.4, 140.0, 136.2, 136.1, 130.5, 129.9, 128.7, 123.9, 122.7, 122.5, 122.3, 120.5, 119.2, 118.6, 118.1, 113.0, 110.60, 53.9, 35.1; IR (□ cm^{-1}): 3237, 2951, 2360, 1641, 1596, 1488, 1304, 1251, 1054, 826; MS (ESI *m/z*): 477 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{28}H_{25}N_6O_2$: 477.2033 $[M+H]^+$; found: 477.2002.

6.14. 2-(4-Fluorophenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol4-yl)methyl) nicotinamide (4b): Starting from 2-(4-fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8b**, 150mg, 0.55 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 138 mg, 0.61 mmol), **4b** was prepared according to the procedure described for **4a** as white solid (220 mg, 80%); mp: 160-162 ºC; ^{1} H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.25 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.76 (dd, *J* = 3.0, 1.5 Hz, 2H), 7.61-7.59 (m, 3H), 7.35-7.28 (m, 3H), 7.22 (brs, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.03-6.94 (m, 7H), 6.67-6.63 (m, 1H), 5.45 (s, 2H), 4.64 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO) δ 166.5, 157.7, 155.9, 154.9, 154.5, 153.5, 149.1, 143.8, 136.0, 135.5, 135.0, 131.2, 128.6, 122.1, 121.5, 121.2, 119.9, 119.1, 117.0, 113.5, 111.6, 109.2, 51.6, 33.6; IR (□ cm^{-1}): 3282, 2918, 1642, 1612, 1589, 1458, 1225, 1053, 825; MS (ESI *m/z*): 495 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{28}H_{24}O_2N_6F$: 495.1939, $[M+H]^+$; found: 495.1939.

6.15. 2-(4-Chlorophenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) nicotinamide (4c): Starting from 2-(4-chlorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8c**, 150 mg, 0.52 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 130 mg, 0.57 mmol), **4c** was prepared according to the procedure described for **4a** as yellow solid (204 mg, 76%); mp: 127-129 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.51 (s, 1H), 8.25 (dd, *J* = 2.2, 1.5 Hz, 1H), 7.

84 (dd, *J* = 2.2, 1.5 Hz, 2H), 7.61-7.58 (m, 3H), 7.35-7.23 (m, 5H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.98-6.91 (m, 5H), 6.67-6.63 (m, 1H), 5.45 (s, 2H), 4.62(d, *J* = 5.2 Hz, 2H); IR (□ cm^{-1}): 3283, 3146, 2360, 1641, 1613, 1589, 1488, 1309, 1256, 1052, 823; MS (ESI *m/z*): 511 $[M+H]^+$

6.16. 2-(4-Bromophenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) nicotinamide (4d): Starting from 2-(4-bromophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8d**, 150 mg, 0.45 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 112 mg, 0.5 mmol), **4d** was prepared according to the procedure described for **4a** as white solid (181 mg, 72%); mp: 149-151 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.51 (s, 1H), 8.29 (dd, *J* = 2.2, 1.5 Hz, 1H), 7.78 (dd, *J* = 2.2,1.5 Hz, 1H), 7.60-7.55 (m, 3H), 7.40-7.28 (m, 6H), 7.14-7.08 (m, 1H), 6.99-6.91 (m, 5H), 6.70-6.65 (m, 1H), 5.48 (s, 2H), 4.66 (d, *J* = 5.2Hz, 2H); IR (□ cm^{-1}): 3295, 2951, 2360, 1645, 1614, 1583, 1486, 1310, 1258, 1046, 825; MS (ESI *m/z*): 555 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{28}H_{24}O_2N_6Br$: 555.1138, $[M+H]^+$; found: 555.1124.

6.17. 2-(4-Methoxyphenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) nicotinamide (4f): Starting from 2-(4-methoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8f**, 150 mg, 0.53 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 132 mg, 0.58 mmol), **4f** was prepared according to the procedure described for **4a** as yellow solid (202 mg, 75%); mp: 95-98 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, 1H), 8.18 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.80 (dd, *J* = 3.0, 1.5 Hz, 2H), 7.59(s, 1H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.33-7.27 (m, 3H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.96-6.90 (m, 5H), 6.85-6.79 (m, 2H), 6.54-6.50 (m, 1H), 5.45 (s, 2H), 4.60 (d, *J* = 5.2 Hz, 2H) 3.78(s, 3H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 168.2, 158.0, 155.7, 155.3, 151.5, 144.8, 136.1, 135.9, 133.0, 132.7, 130.4, 129.8, 123.8, 122.8, 122.4, 120.7, 119.1, 118.5, 118.0, 113.9, 112.34, 109.9,55.4, 53.8, 35.0; IR (□ cm^{-1}): 3235, 3049, 2360, 1640, 1607, 1585, 1457, 1306, 1250, 1053, 827; MS (ESI *m/z*): 507 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{29}H_{27}O_3N_6$: 507.2145 $[M+H]^+$; found: 507.2103.

6.18. 2-(3-Fluorophenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) nicotinamide (4e): Starting from 2-(3-fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8e**, 150 mg, 0.55 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 86 mg, 0.61 mmol), **4e** was prepared according to the procedure described for **4a** as white solid (220 mg, 80%); mp: 130-132 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, 1H), 8.31 (dd, *J* = 3.0, 1.7 Hz, 1H), 7. 83 (dd, *J* = 3.0, 1.7 Hz, 1H), 7.78-7.73 (m, 1H), 7.59-7.53 (m, 2H), 7.36-7.18 (m, 5H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.99-6.91 (m, 5H), 6.71-6.66 (m, 2H), 5.48 (s, 2H), 4.66 (d, *J* = 5.6Hz, 2H); IR (□ cm^{-1}): 3345, 3142, 2360, 1642, 1608, 1588, 1488, 1259, 1053, 827; MS (ESI

m/z): 495 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{28}H_{24}O_2N_6F$: 495.1939 $[M+H]^+$; found: 495.1912.

6.19. 2-(2,4-Dimethoxyphenylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamide (4g): Starting from 2-(2,4-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8g**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 119 mg, 0.53 mmol), **4g** was prepared according to the procedure described for **4a** as yellow solid (201 mg, 78%); mp: 127-129 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.25-8.21 (m, 2H), 7.73 (d, *J* = 7.5 Hz 1H), 7.56 (s, 1H), 7.35-7.28 (m, 4H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.99-6.90 (m, 5H), 6.59-6.47 (m, 3H), 5.45 (s, 2H), 4.67 (d, *J* = 5.2 Hz, 2H), 3.87 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 158.3, 157.9, 155.3, 151.3, 150.8, 144.8, 136.1, 135.7, 133.9, 130.4, 129.8, 127.1, 123.7, 123.0, 122.3, 121.4, 119.1, 118.5, 117.9, 112.1, 110.8, 103.4, 98.8, 55.8, 55.4, 53.8, 35.0; IR (□ cm^{-1}): 3366, 2927, 2359, 1632, 1602, 1560, 1489, 1254, 1055, 827; MS (ESI *m/z*): 537 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{30}H_{29}O_4N_6$: 537.2229 $[M+H]^+$; found: 537.2244.

6.20. 2-(2,5-Dimethoxyphenylamino)-*N*-((1-(3-phenoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)nicotinamide (4h): Starting from 2-(2,5-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8h**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 119 mg, 0.53 mmol), **4h** was prepared according to the procedure described for **4a** as yellow solid (201 mg, 78%); mp: 154-156 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.71 (s, 1H), 8.34 (d, *J* = 2.2Hz, 2H), 8.32 (dd, *J* = 3.0, 1.5 Hz, 1H) ,7.76 (dd, *J* = 2.2, 1.5 Hz, 1H), 7.54 (s, 1H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.28 (d, *J* = 2.2 Hz, 1H), 7.18-7.06 (m, 2H), 6.97-6.84 (m, 4H), 6.76 (d, *J* = 8.3 Hz 1H), 6.66-6.62 (m, 1H), 6.42-6.38 (m, 1H), 5.45 (s, 2H), 4.66 (d, *J* = 5.2 Hz, 2H), 3.90 (s, 3H), 3.79(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 158.4, 156.7, 155.1, 154.2, 151.5, 145.2, 143.9, 136.7, 136.2, 131.3, 130.8, 130.2, 124.2, 122.8, 121.2, 119.6, 118.9, 118.4, 113.4, 112.1, 111.4, 107.1, 105.5, 57.2, 56.0, 54.2, 35.5; IR (□ cm^{-1}): 3335, 2942, 2360, 1635, 1602, 1578, 1488, 1291, 1054, 827; MS (ESI *m/z*): 537 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{30}H_{29}O_4N_6$: 537.2244 $[M+H]^+$; found: 537.2229.

6.21. 2-(3,5-Dimethoxyphenylamino)-*N*-((1-(3-phenoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)nicotinamide (4i):. Starting from 2-(3,5-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8i**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 119 mg, 0.53 mmol), **4i** was prepared according to the procedure described for **4a** as white solid (201 mg, 78%); mp: 123-125 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.55 (s, 1H), 8.27 (dd,

J = 2.2, 1.5 Hz, 1H), 7.82 (dd, *J* = 2.2, 1.5 Hz, 1H), 7.72 (t, *J* = 6.0 Hz, 1H), 7.58 (s, 1H), 7.33-7.28 (m, 3H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.97-6.90 (m, 7H), 6.62-6.58 (m, 1H), 6.08 (t, *J* = 2.2 Hz 1H), 5.46 (s, 2H), 4.61 (d, *J* = 5.2 Hz, 2H), 3.80 (s, 6H); IR (□ cm^{-1}): 3245, 3038, 2360, 1624, 1602, 1526, 1457, 1319, 1259, 1058, 835; MS (ESI *m/z*): 537 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{30}H_{29}O_4N_6$: 537.2244 $[M+H]^+$; found: 537.2229.

6.22. *N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3,4,5-trimethoxyphenylamino)nicotinamide(4j): Starting from *N*-(prop-2-ynyl)-2-(3,4,5-trimethoxyphenylamino)nicotinamide (**8j**, 150 mg, 0.41 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 102 mg, 0.45 mmol), **4j** was prepared according to the procedure described for **4a** as brown solid (186 mg, 75%); mp: 142-144 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 10.42 (s, 1H), 8.27 (d, *J* = 7.5, Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.75 (brs, 1H), 7.60 (s, 1H), 7.39 -7.27 (m, 3H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.99-6.83 (m, 7H), 6.65 (m, 1H), 5.47 (s, 2H), 4.66 (d, *J* = 5.2 Hz, 2H), 3.85 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 168.1, 158.3, 156.3, 153.3, 153.0, 151.4, 144.7, 136.1, 133.4, 132.7, 130.4, 129.8, 123.8, 122.8, 122.3, 120.7, 119.1, 118.5, 118.0, 112.8, 110.4, 98.3, 60.8, 56.0, 53.8, 35.0; MS (ESI *m/z*): 567 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{31}H_{31}N_6O_5$: 567.2350 $[M+H]^+$; found: 567.2336.

6.23. *N*-((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(4-fluorophenylamino) nicotinamide (4k): Starting from 2-(4-fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8b**, 150 mg, 0.55 mmol) and 1-(azidomethyl)-4-fluorobenzene (**10b**, 91 mg, 0.61 mmol), **4k** was prepared according to the procedure described for **4a** as yellow solid (187 mg, 80%); mp: 197-199 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 10.61 (s, 1H), 8.63 (brs, 1H), 8.24 (dd, *J* = 4.4, 1.4 Hz, 1H), 7. 98 (dd, *J* =, 8.0, 1.8 Hz, 1H), 7.64 (s, 1H), 7.63-7.58 (m, 2H), 7.41 (s, 1H), 7.23 (s, 1H), 6.98 (t, *J* = 8.4 Hz, 2H), 6.88 (t, *J* = 8.4 Hz, 2H), 6.69-6.65 (m, 1H), 5.44 (s, 2H), 4.63 (d, *J* = 5.5 Hz, 2H); IR (□ cm^{-1}): 3245, 2936, 2359, 1646, 1609, 1586, 1458, 1301, 1259, 1057, 827; MS (ESI *m/z*): 421 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{22}H_{19}ON_6F_2$: 421.1558$[M+H]^+$; found: 421.1556.

6.24. 2-(4-Fluorophenylamino)-*N*-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)nicotinamide (4l): Starting from 2-(4-fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8b**, 150 mg, 0.55 mmol) and 1-(azidomethyl)-4-methoxybenzene (**10c**, 99 mg, 0.61 mmol), **4l** was prepared according to the procedure described for **4a** as brown solid (192 mg, 80%); mp: 201-204 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 10.57 (s, 1H), 8.45 (brs, 1H), 8.25 (dd, *J* = 4.7, 1.4 Hz, 1H), 7. 94 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.62-7.57 (m, 3H), 7.41 (s,

1H), 7.23 (s, 1H), 6.98 (t, *J* = 8.4 Hz, 2H), 6.88 (t *J* = 8.4 Hz, 2H), 6.69-6.65 (m, 1H), 5.44 (s, 2H), 4.63 (d, *J* = 5.5 Hz, 2H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 158.6, 154.13, 149.7, 135.9, 135.5, 128.6, 126.0, 121.4, 120.6, 120.5, 113.8, 113.2, 112.0, 114.1, 109.7, 54.2, 52.3, 34.2 ; IR (□ cm^{-1}): 3235, 2978, 2359, 1635, 1584, 1456, 1305, 1248, 1056, 826; MS (ESI m/z): 433 $[M+H]^+$; HRMS (ESI m/z) calcd for $C_{23}H_{22}FN_6O_2$: 433.1782$[M+H]^+$; found: 433.17633.

6.25. *N*-((1-(3,5-dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(4-fluorophenylamino)nicotinamide (4m): Starting from 2-(4-fluorophenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8b**, 150 mg, 0.55 mmol) and 1-(azidomethyl)-3,5-dimethoxybenzene (**10d**, 116 mg, 0.61 mmol), **4m** was prepared according to the procedure described for **4a** as solid (182 mg, 71%); mp: 209-211 ºC;^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, 1H), 8.19 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.81 (dd, *J* = 3.0, 1.5 Hz, 2H), 7.52-7.47 (m, 3H), 7.20 (d, *J* = 9.0 Hz, 2H), 6.83 (t, *J* = 7.5 Hz, 3H), 6.55-6.51 (m, 1H), 5.41 (s, 2H), 4.58 (d, *J* = 4.5Hz, 2H), 3.78 (s, 3H) , 3.77 (s, 3H);^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 160.0, 157.4, 154.2, 153.2, 148.9, 143.5, 135.3, 134.8, 130.1, 128.5, 121.3, 119.6, 113.6, 111.4, 108.9, 54.2, 50.8, 33.3; IR (□ cm^{-1}): 3289, 2930, 2359, 1618, 1590, 1476, 1321, 1299, 1055, 825; MS (ESI *m/z*): 463 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{24}H_{24}O_3N_6F$: 463.1888 $[M+H]^+$; found: 463.1856.

6.26. *N*-((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(4-methoxyphenylamino) nicotinamide (4n): Starting from 2-(4-methoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8e**, 150 mg, 0.53 mmol) and 1-(azidomethyl)-4-fluorobenzene (**10b**, 88 mg, 0.58 mmol), **4n** was prepared according to the procedure described for **4a** as yellow solid (173 mg, 75%); mp: 169-171 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.26 (s, 1H), 8.23 (dd, *J* = 4.0, 1.5Hz, 1H), 7.74 (dd, *J* = 8.0,1.5 Hz, 1H), 7.57 (brs, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.29-7.24 (m, 3H), 7.06 (t, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 6.59-6.50 (m, 1H), 5.47 (s, 2H), 4.64 (d, *J* = 5.2 Hz, 2H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 164.4, 161.1, 155.7, 155.3, 151.5, 135.9, 132.9, 130.0, 129.93, 122.7, 116.2, 115.9, 114.0, 112.3, 109.9, 55.4, 53.4, 35.0; IR (□ cm^{-1}): 3282, 3147, 2360, 1646, 1602, 1585, 1458, 1303, 1246, 1057, 826; MS (ESI *m/z*):433 $[M+H]^+$; HRMS (ESI *m/z*) calcd for$C_{23}H_{22}O_2N_6F$:433.1763$[M+H]^+$found:433.1763.

6.27. *N*-((1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(4-methoxyphenylamino) nicotinamide (4o): Starting from 2-(4-methoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8e**, 150 mg, 0.53 mmol) and 1-(azidomethyl)-4-methoxybenzene (**10c**, 95 mg, 0.58 mmol), **4o** was prepared according to the procedure described for **4a** as white solid (189 mg, 80%);^{1}H

NMR (300 MHz, $CDCl_3$) δ 10.31 (s, 1H), 8.19 (dd, *J* = 4.9, 1.7 Hz, 1H), 7.81 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.52-7.48 (m, 3H), 7.25-7.19 (m, 2H), 6.85-6.81 (m, 4H), 6.55-6.51 (m, 1H), 5.42 (s, 2H), 4.55 (d, *J* = 5.4 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H); IR (□ cm^{-1}): 3254, 3049, 2359, 1637, 1608, 1506, 1457, 1246, 1035, 827;

6.28. *N*-((1-(3,5-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(4-methoxyphenylamino)nicotinamide (4p): Starting from 2-(4-methoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8e**, 150 mg, 0.53 mmol) and 1-(azidomethyl)-3,5-dimethoxybenzene (**10d**, 102 mg, 0.58 mmol), **4p** was prepared according to the procedure described for **4a** as white solid (197 mg, 78%); mp: 147-149 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.24 (dd, *J* = 4.9, 1.7 Hz, 2H), 7.70 (m, 1H), 7.53 (s, 1H), 7.32-7.26 (m,1H), 7.10-7.05 (m,1H), 6.97-6.87 (m, 3H), 6.58-6.55 (m, 1H), 6.47-6.44(m, 2H), 5.42 (s, 2H), 4.66 (d, *J* = 5.4 Hz, 2H), 3.79 (s, 3H), 3.75(s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 159.3, 153.7, 153.1, 149.1, 143.7, 136.1, 135.3, 131.8, 121.4, 120.1, 112.2, 110.8, 108.6, 104.4, 98.2, 53.7, 53.6, 51.7, 33.5; IR (□ cm^{-1}): 3264, 2948, 1621, 1599, 1584, 1459, 1310, 1295, 1049, 824; MS (ESI *m/z*): 475 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{25}H_{27}N_6O_4$: 475.2088$[M+H]^+$; found: 475.2057.

6.29. 2-(2,4-Dimethoxyphenylamino)-*N*-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamide(4q): Starting from 2-(2,4-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8g**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-4-methoxybenzene (**10c**, 86 mg, 0.53 mmol), **4q** was prepared according to the procedure described for **4a** as white solid (180 mg, 79%); mp: 178-180 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.23 (m, 2H), 7.74 (dd, *J* = 6.0,2.0 Hz, 1H), 7.51 (s, 1H), 7.34 (brs, 1H), 7.20 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.56-6.48 (m, 3H), 5.45 (s, 2H), 4.64 (d, *J* = 6.0 Hz, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 3.79 (s, 3H); MS (ESI *m/z*): 475 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{25}H_{27}O_4N_6$: 475.2088 $[M+H]^+$; found: 475.2057

6.30. 2-(2,4-Dimethoxyphenylamino)-N-((1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)nicotinamide (4r): Starting from 2-(2,4-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8g**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-4-fluorobenzene (**10b**, 79 mg, 0.53 mmol), **4r** was prepared according to the procedure described for **4a** as brown solid (167 mg, 70%); mp: 146-148 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.33 (s, 1H), 8.25 (dd, *J* = 7.5, 1.5 Hz, 2H), 7.72 (dd, *J* = 7.5,1.5 Hz, 1H), 7.53 (s, 1H), 7.28-7.23 (m, 3H), 7.18-7.13 (m, 1H), 7.03 (t, *J* = 8.3 Hz, 2H), 6.58-6.54 (m, 1H), 6.46 (s, 1H), 5.45 (s, 2H), 4.64 (d, *J* =

5.2 Hz, 2H), 3.90 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 164.3, 161.0, 155.2, 151.3, 150.8, 144.9, 135.8, 129.8, 122.9, 122.3, 121.4, 116.1, 115.8, 112.1, 110.7, 103.4, 98.7, 55.8, 55.4, 53.3, 35.0; IR (□ cm^{-1}): 3336, 2939, 2360, 1634, 1599, 1577, 1459, 1236, 1048, 827; MS (ESI *m/z*): 463 $[M+H]^+$; HRMS (ESI *m/z*) calcd for$C_{24}H_{24}O_3N_6F$: 463.1888 $[M+H]^+$; found: 463.1856.

6.31. *N*-((1-(3,5-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(2,4-dimethoxyphenylamino)nicotinamide (4s): Starting from 2-(2,4-dimethoxyphenylamino)-*N*-(prop-2-ynyl)nicotinamide (**8g**, 150 mg, 0.48 mmol) and 1-(azidomethyl)-3,5-dimethoxybenzene (**10d**, 102 mg, 0.53 mmol), **4s** was prepared according to the procedure described for **4a** as yellow solid (175 mg, 72%); mp: 147-149 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.25 (dd, *J* = 5.0,3.0 Hz, 2H), 7.73 (dd, *J* = 7.0 Hz, 1H), 7.53 (s, 1H), 7.15 (brs, 1H), 6.58-6.54 (m, 1H), 6.47-6.43 (m, 2H), 6.36 (s, 3H), 5.41 (s, 2H), 4.66 (d, *J* = 5.2 Hz, 2H), 3.91 (s, 3H), 3.80 (s, 3H),3.74 (s, 6H); IR (□ cm^{-1}): 3358, 2924, 2360, 1631, 1599, 1585, 142552, 1306, 1258, 1053, 827; MS (ESI *m/z*): 505 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{26}H_{29}O_5N_6$: 505.2193 $[M+H]^+$ found: 505.2169.

6.32. 2-(4-Chlorobenzylamino)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) nicotinamide (4t): Starting from 2-(4-chlorobenzylamino)-N-(prop-2-ynyl)nicotinamide (**8k**, 150 mg, 0.50 mmol) and 1-(azidomethyl)-3-phenoxybenzene (**10a**, 124 mg, 0.55 mmol), **4t** was prepared according to the procedure described for **4a** as white solid (184 mg, 60%); mp: 209-211 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (t, *J* = 5.2 Hz, 1H), 8.14-8.11 (m, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.50 (brs, 1H), 7.45 (s, 1H), 7.27-7.13 (m, 7H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.90-6.80 (m, 5H), 6.36-6.32 (m, 1H), 5.33 (s, 2H), 4.60 (d, *J* = 5.2 Hz, 2H), 4.49 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 158.0, 157.8, 156.3, 151.9, 145.1,139.7, 136.2, 135.8, 130.5, 129.9, 128.5, 127.5, 126.9, 123.8, 122.6, 122.4, 119.2, 118.6, 118.1, 1108, 109.4, 53.8, 44.8, 34.9; IR (□ cm^{-1}): 3288, 3054, 2359, 1629, 1579, 1458, 1307, 1252, 1055, 822; MS (ESI *m/z*): 525 $[M+H]^+$; HRMS (ESI *m/z*) calcd for $C_{29}H_{26}O_2N_6Cl$: 525.1800 $[M+H]^+$; found: 525.1778.

7. Biology

7.1. 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.[20] 1×10^6 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 °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 540 nm wavelength was recorded.

7.2. 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).[21]

7.3. 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 **4g** and **4i** at concentrations of 0.5 and 1µM. and also with E7010 in the same concentrations. 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).[21]

7.4. 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 10µL contained PEM buffer, GTP (1µM) in the presence or absence of test compounds **4g** and **4i** (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 Vario scan multimode plate reader (Thermo scientific Inc.). E7010 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 0.5 and 1 µM concentrations. Polymerization was monitored by increase in the fluorescence as mentioned above at 37°C.

7.5. Immunohistochemistry

MCF-7 cells were seeded on glass cover slips, incubated for 48 h inthe presence or absence of test compounds **4g**, **4i** and E7010 (1 µM). 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 (20 °C). The cover slips were left in

100% MeOH overnight at 4 °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.

7.6. Morphological analysis for apoptosis with Hoechst staining

MCF-7 Cells were seeded at a density of 10,000 cells over 18-mm cover slips and incubated for 24 h. The medium was replaced, and cells were treated with compounds, **4g** and **4i** at 0.5 and 1 µM concentrations for 48 h. Cells treated with vehicle (0.001% DMSO) were included as controls for all experiments. After overnight treatment, Hoechst 33258 (Sigma Aldrich) was added to medium at a concentration of 0.5 mg/mL containing 4% Para formaldehyde. After incubation for 30 min, cells from each dish were captured from randomly selected fields under fluorescent microscope (Leica, Germany) to qualitatively determine the proportion of viable and apoptotic cells based on their relative fluorescence and nuclear fragmentation.

7.7. Caspase 9 activity

To determine the caspase-9 activity of **4g, 4i** and E7010 for detection of apoptosis in breast cancer cell line (MCF-7), the commercially available apoptosis detection kit (Sigma-Caspase 9 Assay kit, Florometric) was used. MCF-7 cells were treated with compounds **4g**and **4i** at 1µM concentration for 48 h. Here the substrate used is Ac-LEHD-AFC to the cell lysate and incubation was carried out at 37 °C for 1 h. Readings were taken at excitation wavelength 400 nm and emission wavelength 505 nm.

7.8. Determination of the Apoptotic DNA Ladder.

MCF-7 Cells were seeded (1×10^6) in six-well plates. After incubation for 24 h, cells were treated with compound **4g, 4i** and E7010 at 1 µM concentration. After 48 h of treatment, cells were collected and centrifuged at 2500 rpm for 5 min at 4 °C. Pellet was collected and washed with Phosphate buffered saline (PBS). Lysis buffer was added, the pellet was collected centrifuged at 3000 rpm for 5 min at 4 °C and the supernant was collected. Sodium dodecylsulfate (SDS, 10%, 10 mL) and 50 mg/mL RNase A (10 mL) were then added, and the mixture was incubated for 2 h at 568C. After incubation, proteinase K (25 mg/mL) was added and further incubated at 37 °C for 2 h. Ammonium acetate (10M, 65 µL) and ice-cold ethanol (500 µL) were then added, and mixed well. These samples were incubated at -80 °C

for 1 h. After incubation, the samples were centrifuged at 12000 rpm for 20 min at 4 ℃. After centrifugation, the pellet was washed with 80% ethanol and air dried for 10 min at room temperature. The pellet was dissolved in 50 mL TE buffer, and DNA laddering was determined by using 2%agarose gel electrophoresis in TE buffer.

7.9. Molecular modelling procedure:

All the geometries are optimized in Gaussian 09 using PM3 semi-empirical method.[29] Protein structure was downloaded from Protein Data Bank (PDB ID 3E22).[30] Docking studies were performed using AutoDock 4.2 software.[31] The Analysis of intermolecular interactions has been performed using Pymol, v. 0.99.[32]

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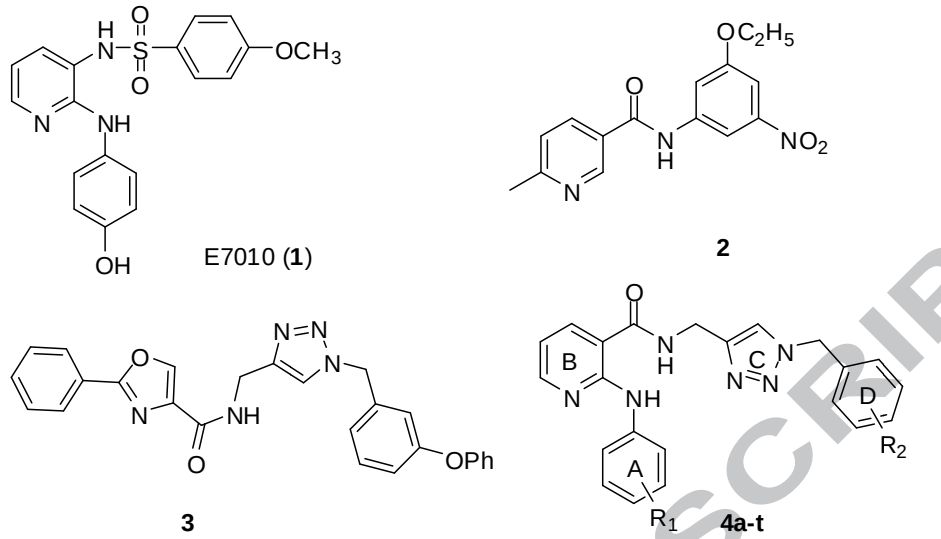


Figure 1: Chemical structures of E7010 (**1**), *N*-phenyl nicotinamides (**2**), and compounds **3-4.**

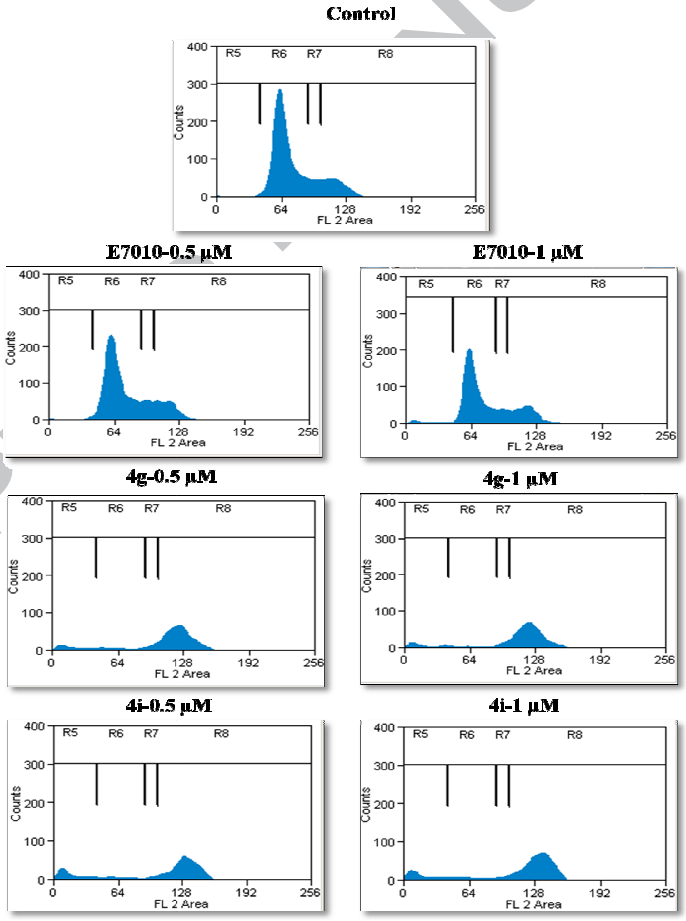


Figure 2: Flow cytometric analysis in the MCF-7 cancer cell line aftertreatment with compounds **4g,4i**and E7010 at 0.5 and 1µM for 48 h.

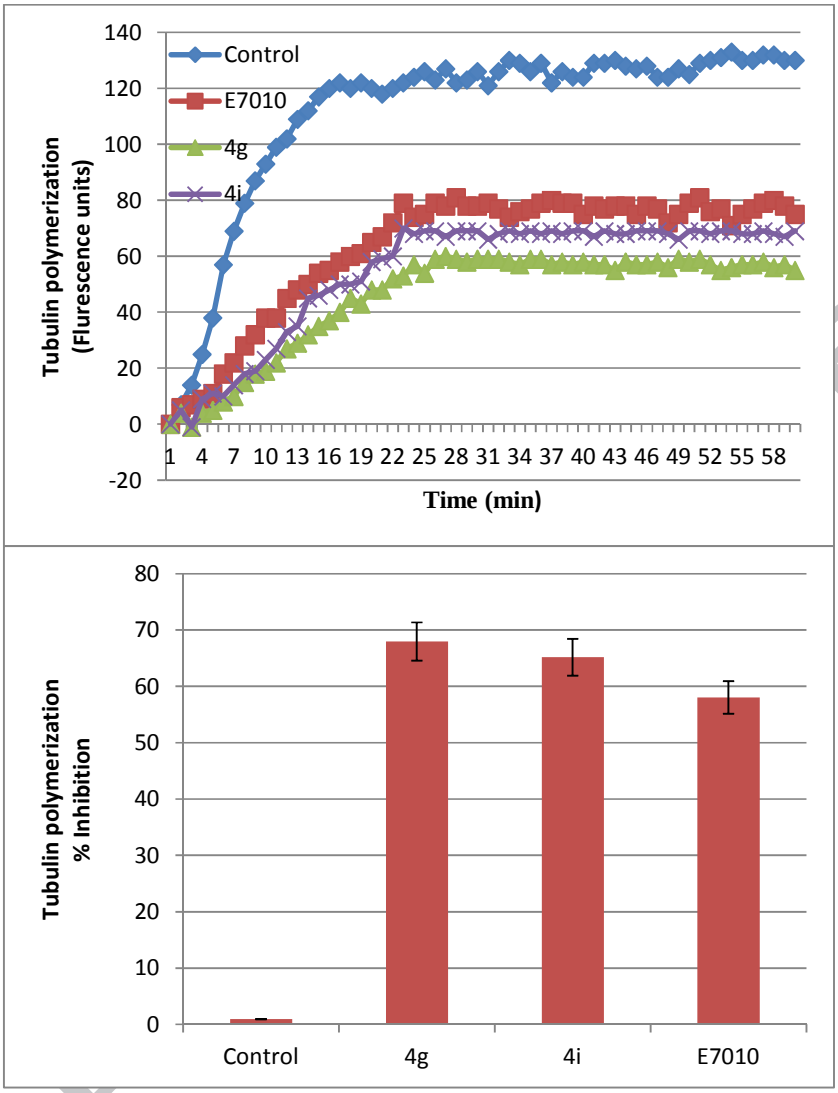


Figure 3: Effect of compounds on the tubulin polymerization: tubulin polymerization was monitored by the increase in fluorescence at 360 nm (excitation) and 420 nm (emission) for 1 h at 37 °C. All the compounds were included at a final concentration of 3 µM. Values indicated are the mean ± SD of two different experiments performed in triplicates.

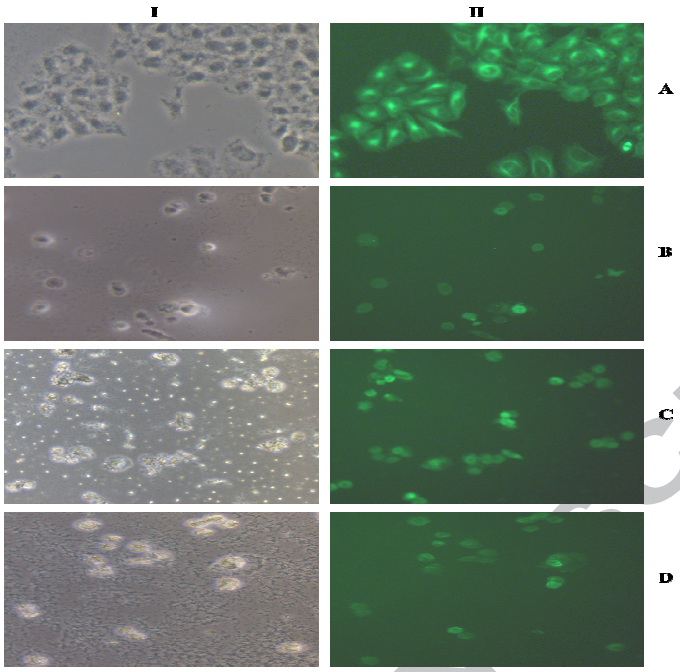


Figure 4: IHC analysis of compounds on the microtubule network: Panel, I: Phase-contrast microscopic view; Panel II: fluorescence microscopic view: A: MCF-7 control cells, B: E7010 (1µM), C:**4g** (1µM), D:**4i** (1µM).

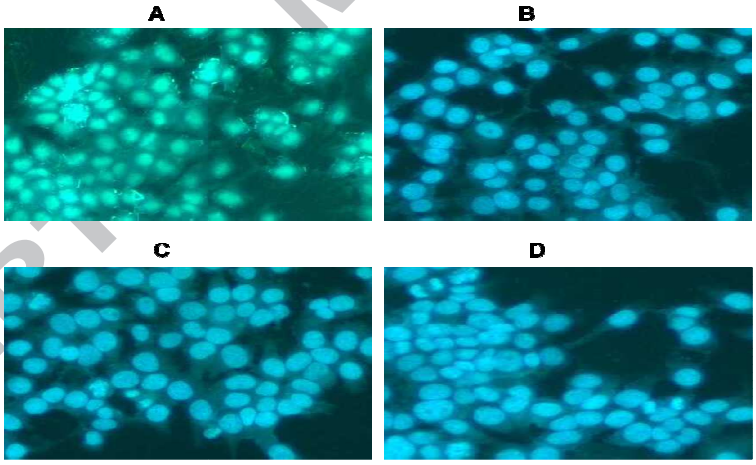


Figure 5: Hoechst staining in MCF-7 Breast cancer cell line. A: MCF-7 control cells, B: E7010 (1µM), C: **4i** (1µM), D: **4g** (1µM).

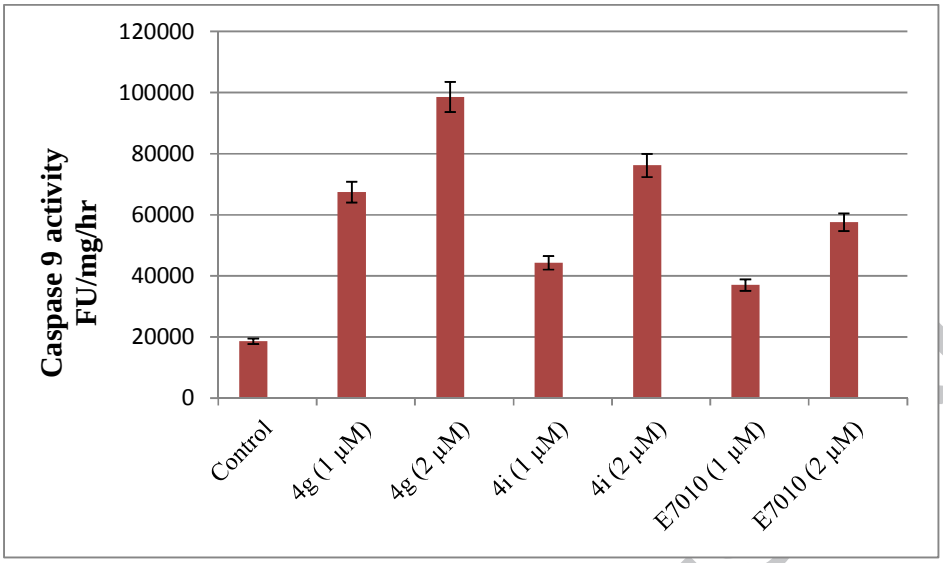


Figure 6: Effect of compounds **4g** and **4i** on caspase-9 activity: MCF-7 cells were treated with compounds at 1 and 2 µM concentration for 48 h. Values indicated are the mean ± SD of two different experiments performed in triplicates.

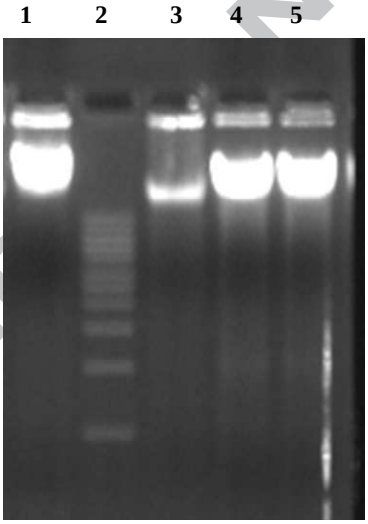


Figure 7: DNA fragmentation assay. Lane-1: Control (untreated), Lane-2: Marker (100 bp), Lane-3: E7010 (1µM), Lane-4: **4g** (1 µM), Lane-5: **4i** (1 µM).

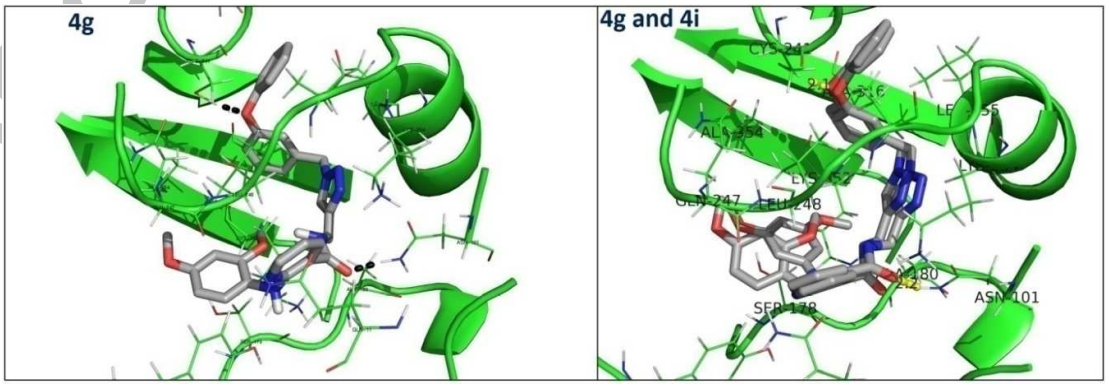


Figure 8: Binding pose for **4g** in colchicine binding site of tubulin and superimposition of **4g** and **4i** in colchicine binding site of tubulin.

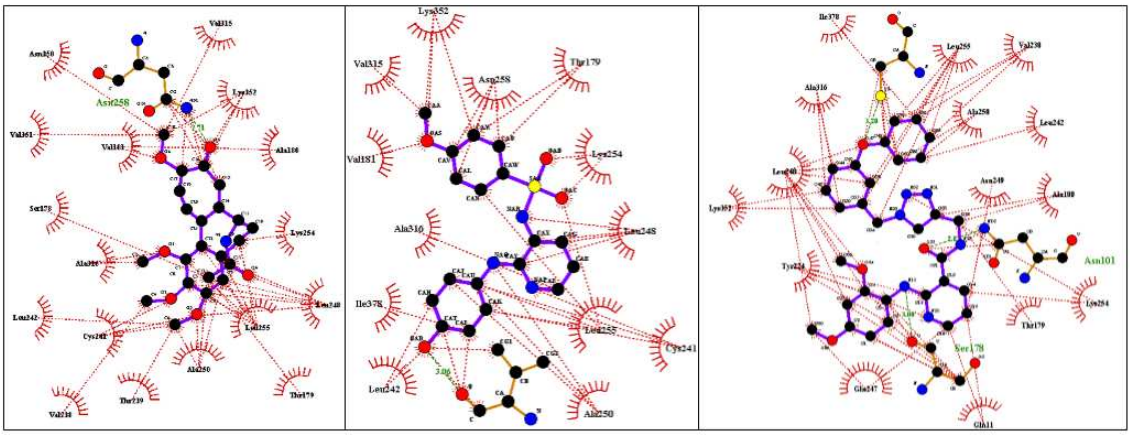


Figure 9: 2D interaction diagram of colchicine, E7010 and **4g**.

Tables

Table 1: Cytotoxicity of compounds **4a-t** and E7010 against a panel of cancercelllines**.**

Compound	IC_{50}[a] (µM)				
	A549[b]	DU-145[c]	ACHN[d]	MCF-7[e]	HT-29[f]
4a	1.54	1.13	7.37	1.65	3.09
4b	1.69	1.65	3.09	1.65	6.76
4c	3.98	1.81	6.30	2.51	4.36
4d	1.90	1.89	1.25	0.97	1.39
4e	6.60	7.07	6.91	7.24	7.41
4f	1.90	1.75	1.14	1.78	1.27
4g	1.57	1.54	0.86	0.74	0.89
4h	6.45	3.46	5.37	3.71	9.54
4i	1.93	1.61	0.98	0.93	1.34
4j	8.70	7.24	7.41	7.53	11.74
4k	7.41	5.49	11.2	6.60	11.01
4l	9.31	7.94	11.61	8.31	9.37
4m	1.25	1.34	8.31	1.0	1.41
4n	7.58	4.69	8.70	5.49	8.70
4o	18.62	10.47	13.98	11.74	11.22
4p	8.51	7.24	7.07	8.21	9.77
4q	14.45	9.55	10.96	9.41	10.23
4r	4.57	4.57	5.88	7.58	7.76
4s	1.31	1.02	3.98	1.17	3.38
4t	1.79	1.57	3.0	2.51	0.96
E7010	1.31	1.81	2.13	1.25	1.62

[a]50% Inhibitory concentration and the values are average of three individual experiments.[b]lung cancer, [c]prostatecancer, [d]renal cell carcinoma, [e]breast cancer, [f] colon cancer.

Table 2: Anticancer activity of *N*-((1-benzyl-1*H*-1, 2, 3-triazol-4-yl) methyl) nicotinamides against panel of 60 human cancer cell lines.

Cancer panel/Cell lines	GI_{50} (μM)[a]					
	[b]**4a**	[c]**4b**	[d]**4f**	[e]**4g**	[f]**4i**	[g]**4t**
Leukemia						
CCRF-CEM	3.34	NA	3.80	1.20	4.19	3.05
HL-60(TB)	3.74	NA	2.66	2.21	3.97	3.39
K-562	3.69	NA	3.48	0.75	5.05	3.24
MOLT-4	3.74	NA	2.91	3.65	4.85	3.28
RPMI-8226	3.61	NA	4.84	3.03	5.25	3.12
SR	2.56	NA	NT	NT	NT	3.31
Non-small-cell- lung						
A549/ATCC	4.81	6.11	3.44	4.15	4.02	3.21
EKVX	5.18	25.3	4.16	5.09	6.33	3.65
HOP-62	5.58	60.2	5.07	12.7	6.31	5.69
HOP-92	NT	10.7	5.67	NT	4.67	4.23
NCI-H226	3.24	38.6	3.92	2.24	9.82	3.66
NCI-H23	3.73	29.9	3.45	2.24	6.22	NT
NCI-H322M	NT	5.29	5.24	NT	6.15	NT
NCI-H460	2.85	5.25	3.52	3.05	3.98	2.85
NCI-H522	2.11	10.8	2.13	1.82	3.31	2.18
Colon						
COLO 205	2.79	6.22	2.86	2.14	4.12	2.18
HCC-2998	3.70	NA	2.53	3.33	9.60	4.17
HCT-116	3.40	5.02	4.17	3.08	4.34	3.18
HCT-15	3.81	NT	3.93	2.89	3.89	3.39
HT29	3.65	5.60	3.66	3.26	3.88	2.96
KM12	3.63	5.61	3.43	1.65	3.84	3.62
SW-620	3.97	6. 58	3.86	2.12	3.88	4.04
CNS						
SF-268	3.22	2.1	4.59	3.88	5.43	4.82
SF-295	3.69	5.66	2.94	0.56	4.06	2.80
SF-539	3.10	15.1	2.79	2.65	4.39	2.24
SNB-19	3.27	20.7	3.55	3.33	9.64	3.91
SNB-75	1.94	3.81	3.09	3.41	3.98	2.02
U251	3.61	7.12	3.91	3.60	4.58	2.66
Melanoma						
LOX IMVI	4.54	61.3	3.60	3.01	5.10	3.90
MALME-3M	1.61	15.1	3.80	4.07	5.81	5.69
M14	2.86	13.7	3.15	2.13	4.80	2.49
MDA-MB-435	2.65	2.79	1.71	0.25	2.23	2.33
SK-MEL-2	6.05	34.2	3.66	7.67	6.69	4.44
SK-MEL-28	4.93	7.98	3.88	2.60	4.14	2.95
SK-MEL-5	3.89	4.48	2.90	0.72	3.11	2.53
UACC-257	1.42	6.87	4.82	11.9	4.86	3.83
UACC-62	3.02	6.51	3.13	1.43	3.86	2.08
Ovarian						
IGROV1	5.86	30.7	5.89	5.96	NA	9.03
OVCAR-3	2.28	17.7	2.98	1.54	3.69	3.48
OVCAR-4	5.41	23.0	5.19	5.11	5.42	3.37
OVCAR-5	5.72	NA	4.23	8.34	10.0	5.62
OVCAR-8	4.39	7.44	3.72	4.10	5.96	3.63
NCI/ADR-RES	2.47	14.0	2.12	2.12	3.21	2.86
SK-OV-3	3.79	5.94	3.11	2.59	4.33	3.32

Renal						
786-0	NT	23.2	5.59	4.06	6.73	NT
A498	1.88	13.7	2.35	1.01	4.18	2.12
ACHN	5.67	11.0	4.79	4.83	6.90	4.03
CAKI-1	5.45	64.2	3.41	1.71	6.30	4.36
SN12C	4.30	52.6	4.75	1.71	7.41	3.44
TK-10	4.30	24.2	4.59	3.14	5.16	3.70
UO-31	2.49	86..3	3.00	8.06	5.25	3.00
RXF 393	2.37	5.69	2.59	7.83	3.77	2.19
Prostate						
PC-3	20.4	20.4	3.86	0.62	4.74	2.96
DU-145	8.58	8.58	3.76	3.37	10.5	4.00
Breast						
MCF7	3.76	NT	NT	0.65	NT	2.99
MDA-MB-231/ATCC	2.25	14.5	3.09	5.54	5.35	2.29
HS 578T	3.68	30.9	5.90	2.94	NA	2.72
BT-549	2.80	45.5	4.58	3.51	7.16	2.72
T-47D	5.40	21.4	4.03	4.28	4.68	3.20
MDA-MB-468	2.88	51.7	2.32	1.46	2.42	3.44

[a]Compound concentration required to decrease cell growth to half that of untreated cells.

[b] (**4a**, NSC 761812)

[c] (**4b**, NSC 758359)

[d](**4f**, NSC 758359)

[e](**4g**, NSC 758359)

[f](**4i**, NSC 758359)

[g](**4t**, NSC 758359)

NT: Not tested

NA: Not active (GI_{50} 100 µM)

Table 3. Cell cycle distribution of MCF-7 cell line treated with **4g, 4i** and E7010

Compound	Sub G1%	G0/G1%	S%	G2/M%
Control	1.44	71.43	8.25	18.88
4g (0.5 µM)	14.20	10.92	6.08	68.80
4g (1 µM)	14.50	9.43	6.17	69.90
4i (0.5 µM)	21.46	12.31	4.11	62.12
4i (1 µM)	17.43	10.90	3.84	67.83
E7010(0.5 µM)	1.92	67.87	9.58	20.63
E7010 (1 µM)	3.98	62.67	7.56	25.79

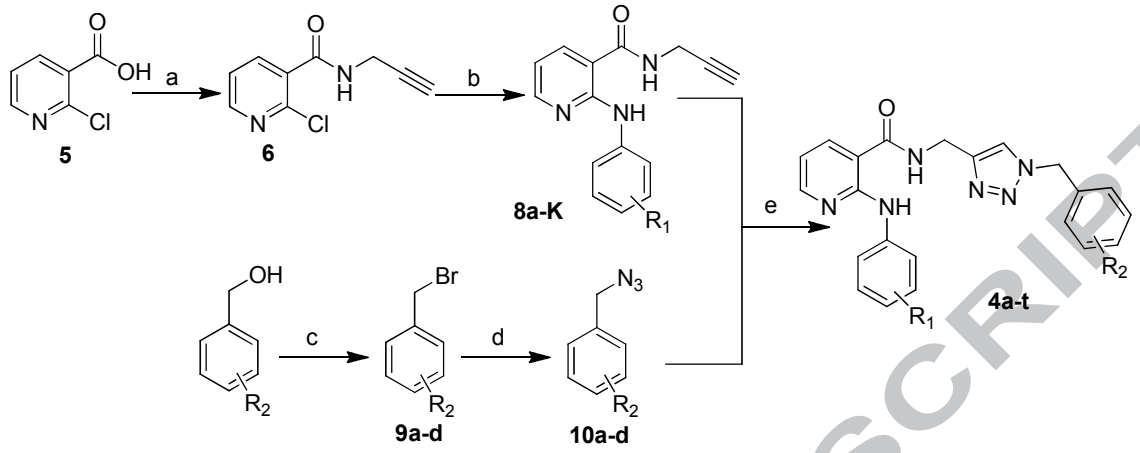
Table 4.

Inhibition of tubulin polymerization (IC_{50}) of compounds **4g**, **4i** and E7010

Compound	IC_{50}[a] ± SD (in μM)
4g	1.93±0.18
4i	2.78±0.64
E7010	2.88±0.29

[a]Concentration of drug to inhibit 50% of tubulin assembly.

Scheme 1.



R_2 = 3-OPh (**a**), 4-F (**b**), 4-OMe (**c**), 3,5-diOMe (**d**)

8a: R_1 = H
8b: R_1 = 4-F
8c: R_1 = 4-Cl
8d: R_1 = 4-Br
8e: R_1 = 4-OMe
8f: R_1 = 3-F
8g: R_1 = 2,4-diOMe
8h: R_1 = 2,5-diOMe
8i: R_1 = 3,5-diOMe
8j: R_1 = 3,4,5-triOMe
8k: R_1 = 4-ClBn

4a:R_1 = H, R_2 = 3-OPh
4b:R_1 = 4-F, R_2 = 3-OPh
4c:R_1 = 4-Cl, R_2 = 3-OPh
4d:R_1= 4-Br, R_2 = 3-OPh
4e:R_1= 4-OMe, R_2= 3-OPh
4f:R_1 = 3-F, R_2 = 3-OPh
4g:R_1 = 2,4-diOMe, R_2 = 3-OPh
4h:R_1= 2,5-diOMe, R_2 = 3-OPh
4i:R_1 = 3,5-diOMe, R_2 = 3-OPh
4j:R_1 = 3,4,5-triOMe, R_2 = 3-OPh

4k:R_1 = 4-F, R_2 = 4-F
4l:R_1 = 4-F, R_2 = 4-OMe
4m:R_1 = 4-F, R_2 = 3,5-diOMe
4n:R_1 = 4-OCH_3, R_2 = 4-F
4o:R_1 = 4-OCH_3, R_2 = 4-OCH_3
4p:R_1= 4-OCH_3, R_2 = 3,5-diOMe
4q:R_1 = 2,4-diOMe, R_2 = 4-OCH_3
4r:R_1 = 2,4-diOMe, R_2 = 4-F
4s:R_1 = 2,4-diOMe, R_2 = 3,5-diOMe
4t:R_1 = 4-ClBn, R_2 = 3-OPh

Scheme1.*Reagents and conditions*: (a) (i) oxalyl chloride, CH_2Cl_2, DMF; (ii) propagylamine hydrochloride, CH_2Cl_2, Et_3N; (b) substituted anilines**7a-k**, ethylene glycol,120 ^oC; (c) PBr_3, ether,0 ^oC; (d) NaN_3, DMSO; (e) Na Ascorbate (10 mol%), $CuSO_4.5H_2O$ (5mol%), H_2O/*t*-buOH, (2:1).