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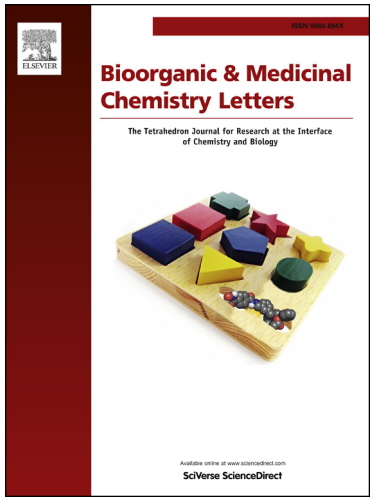

Design, synthesis and biological studies of novel tubulin inhibitors

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Design, synthesis and biological studies of novel tubulin inhibitors

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ABSTRACT: A series of compounds originally derived from the vascular endothelial growth factor receptor tyrosine kinase inhibitor, SU5416, were synthesized and evaluated. The most potent compound in this series, compound **3**, structurally resemble the potent anti-microtubule agent Combretastatin A-4, and inhibited tubulin polymerization and showed potent growth inhibitory activities on both prostate and breast cancer lines with IC_{50} values in the low nanomolar range.

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Anti-microtubule agents such as Taxol and Vinca alkaloids have been used for the treatment of cancer for many years. However, among the 3 classes of tubulin binding agents (i. microtubule stabilizing agents, ii. vinca site binding agents and iii. colchicine site binding agents), colchicine site binding agents are the only class of agents that do not have a representative drug in clinical use for cancer. This class includes many natural products as well as synthetic small molecules.[1,2] Among all the colchicine site agents, Combretastatin A-4 (CA-4, Fig. 1) has received special attention in the last decade or so.[3] In addition to its potent cytotoxicity and inhibitory activity on tubulin polymerization, CA-4 is one of the few anti-microtubule agents reported to have selective vascular targeting activity, and it is an agent that selectively destroyed tumor blood vessels and resulted in tumor cell death.[4,5] The anti-tumor and anti-vascular activities of CA-4 and its water-soluble prodrug, Combretastatin A-4 phosphate (CA-4P), have been demonstrated in both animal and human studies.[6-9]

Many analogs of CA-4 have been designed to study the structure-activity-relationship of the molecule in order to enhance both its cytotoxic and selective vascular targeting activity.[3,10] We have been using SU5416,[11,12] a potent ATP competitive VEGF receptor tyrosine kinase inhibitor, as a template for the design of inhibitors for other growth factor receptors. Previously, we discovered and reported a 2-indolinone containing compound (compound **1**, Fig. 1) with potent growth inhibitory activities on hormone-independent prostate (PC-3) and breast (MDA-MB-231) cancer cell lines with IC_{50} values in low to subnanomolar range.[13] The anti-proliferative profile of compound **1** was also evaluated at the National Cancer Institute (NCI) against 53 human cancer cell lines. Compound **1** was extremely potent with a GI_{50} below 10 nM towards 46 out of 53 human cancer cell lines tested. The compound is active in colon, CNS, prostate and renal cancer cell lines.[14] Structure-activity-relationship studies showed that compound **1** isomerized to compound **2** which has no tubulin polymerization inhibitory activity. In our continuous search for potent inhibitors of tubulin polymerization and vascular disrupting agents, we here report the synthesis and biological studies of seven analogs of compound **1** (Fig. 2). One of our analogs **3**, exhibits potent cytotoxicity against prostate (PC-3) and breast cancer cells (MDA-MB-231) in *vitro*, as well as inhibition of tubulin polymerization.

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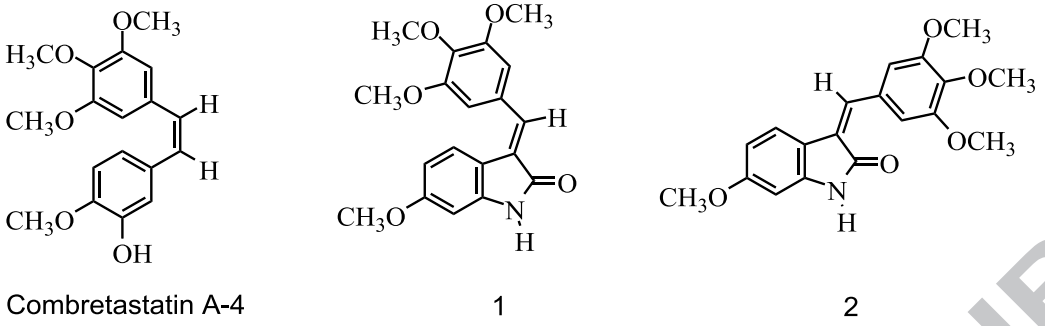

Figure 1. Structures of Combretastatin A-4 (CA-4), compounds **1** and **2**.

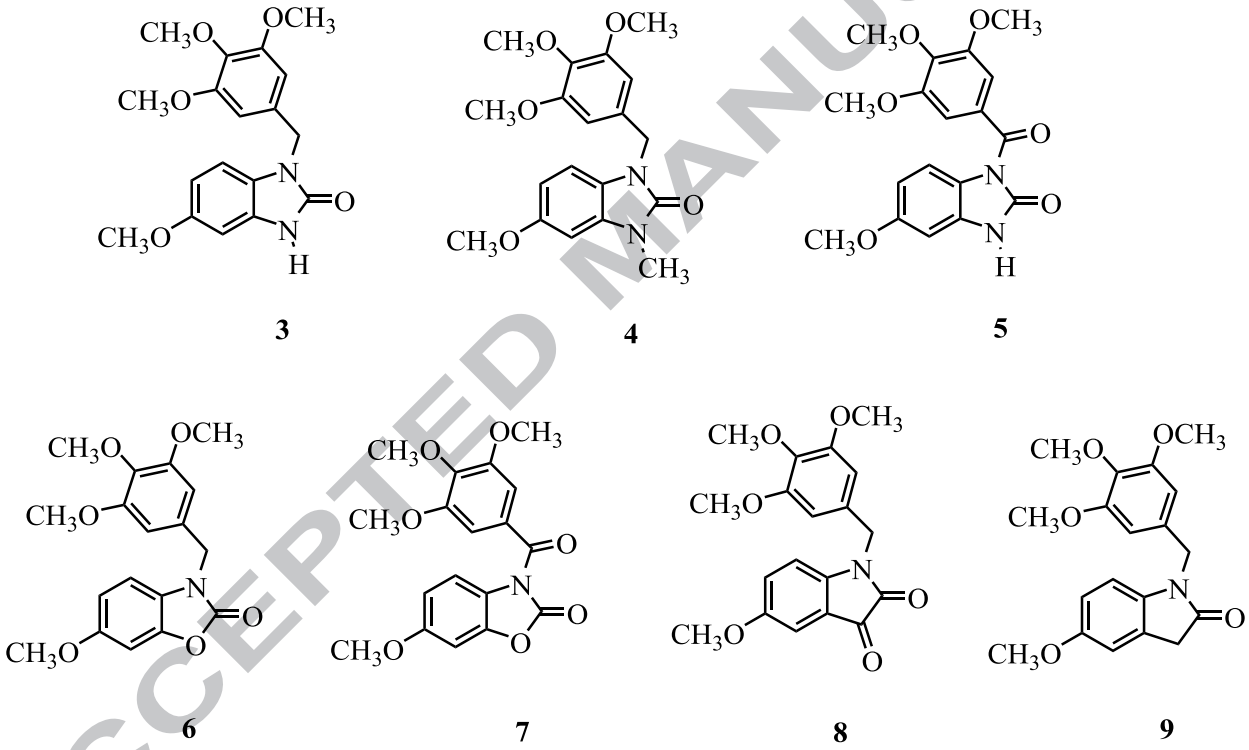

Figure 2. Proposed compound **1** analogs.

Compound 1 has potent anti-proliferative and tubulin polymerization inhibitory activity, but tends to isomerize to compound 2 in solution which has no tubulin polymerization inhibitory activity. This phenomenon is also observed in the KDR inhibitor SU5416.[15] In our continuous effort to design potent anti-microtubule agents; we have designed, synthesized and biologically evaluated analogs of compound **1** that do not have the ability to be deactivated through

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isomerization. To design these types of analogs, the indolin-2-one in compound **1** has been replaced with a benzimidazol-2-one (**3**, **4** and **5**), benzoxazol-2-one (**6** and **7**), or 1*H*-indole-2,3-dione (**8**). For compound **1**, the 6-methoxy group in the indolin-one and the trimethoxy group on the benzylidene ring are essential for potent cytotoxicity and inhibition of tubulin polymerization. Compounds **3** – **9** were designed with a methoxy in the position corresponding to the 6-methoxy group of compound **1**. In addition, the compounds also have 3,4,5-trimethoxy phenyl ring that mimic the same group in compound **1**.

The synthetic route used for the synthesis of **3** and **4** are shown in Fig. 3. In brief, benzylation of 4-methoxy-2-nitroaniline yielded **10**. The nitro group of **10** was reduced using using Zn/HOAc to afford **11**. Compound **3** was prepared by cyclization of **11** with N,N-carbonyldiimidazole (CDI). Methylation of **3** with methyl iodide provided **4**.

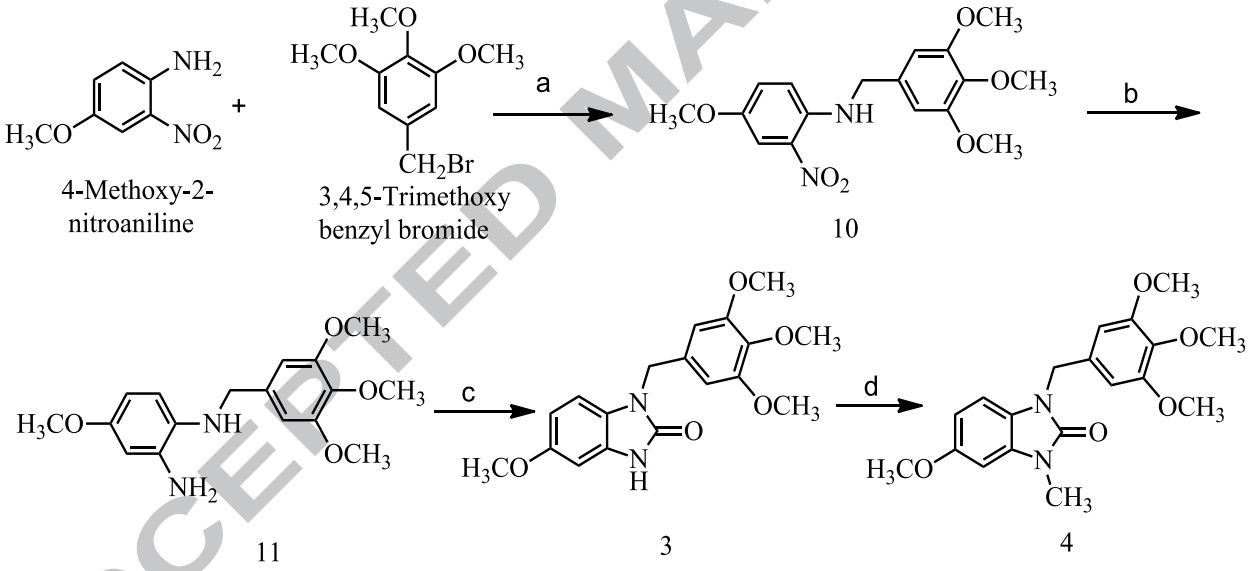

Figure 3. Synthesis of **3** and **4**: a. K_2CO_3, KI, DMF, rt, 4h; b. Zn, HOAc, 110°C, 2h; c. CDI, THF, rt, overnight; d. MeI, K_2CO_3, KI, DMF, rt, 24h.

The conversion of **10** to **11** was carried out using two different reaction conditions; $SnCl_2$/HCl (reaction condition a or c in Fig 4) and Zn/HOAc (condition b in Fig. 4). Compound **11** appeared to be formed under both reaction conditions, when monitored by TLC. The reactions were worked up and the product was extracted with ethyl acetate. Surprisingly only compound

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12 was isolated (Fig 4). The structure of **12** was confirmed by 1D and 2D NMR spectra as well as high resolution mass spectrometry. Interestingly, compound **11** was recovered instead of **12,** when dichloromethane was used for extraction instead of ethyl acetate (Fig. 4).

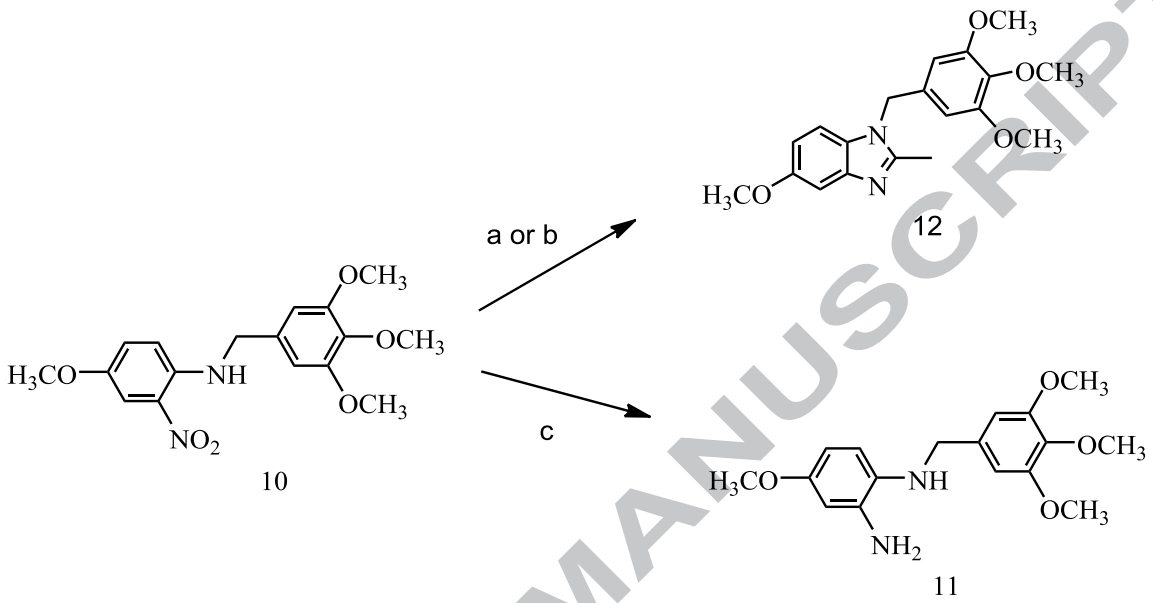

Figure 4. Formation of compound **12**: a. $SnCl_2 \cdot 2H_2O$, HCl, EtOH, 80°C-90°C, 30 min (workup with EtOAc); b. Zn, HOAc, 110°C, 2h (workup with EtOAc); c. $SnCl_2 \cdot 2H_2O$, HCl, EtOH, 80°C-90°C, 30 min (workup with CH_2Cl_2).

We postulated that compound **12** was formed from the reaction between **11** and ethyl acetate (Fig. 5). The reaction is proposed to start with the acetylation of **11** to form the intermediate, N-acetamide, which went through intramolecular cyclization to form the intermediate, benzoimidazol-2-ol. Compound **12** was then formed by dehydration of the benzoimidazol-2-ol intermediate.

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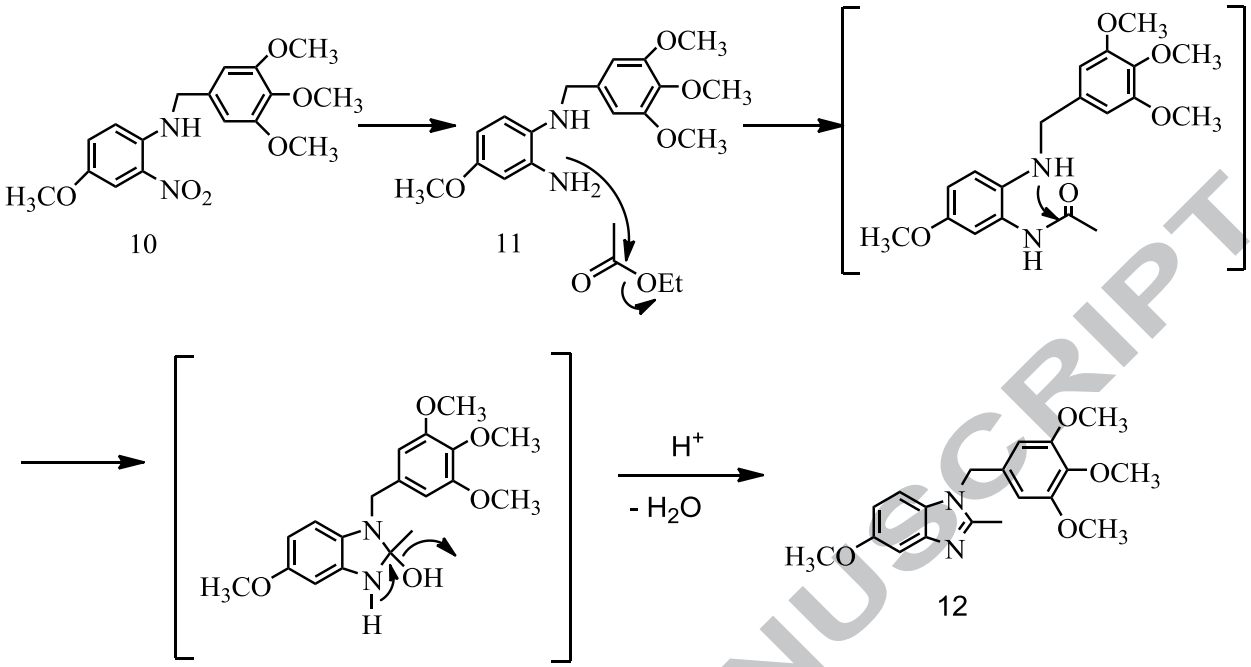

Figure 5. Proposed mechanism for the formation of **12** from **11**.

Preparation of compound **5** was achieved by using a procedure similar to the one described in Fig. 3, where the corresponding commercially available 3,4,5-trimethoxy benzoyl chloride was used instead of 3,4,5-trimethoxy benzyl bromide (Fig. 6).

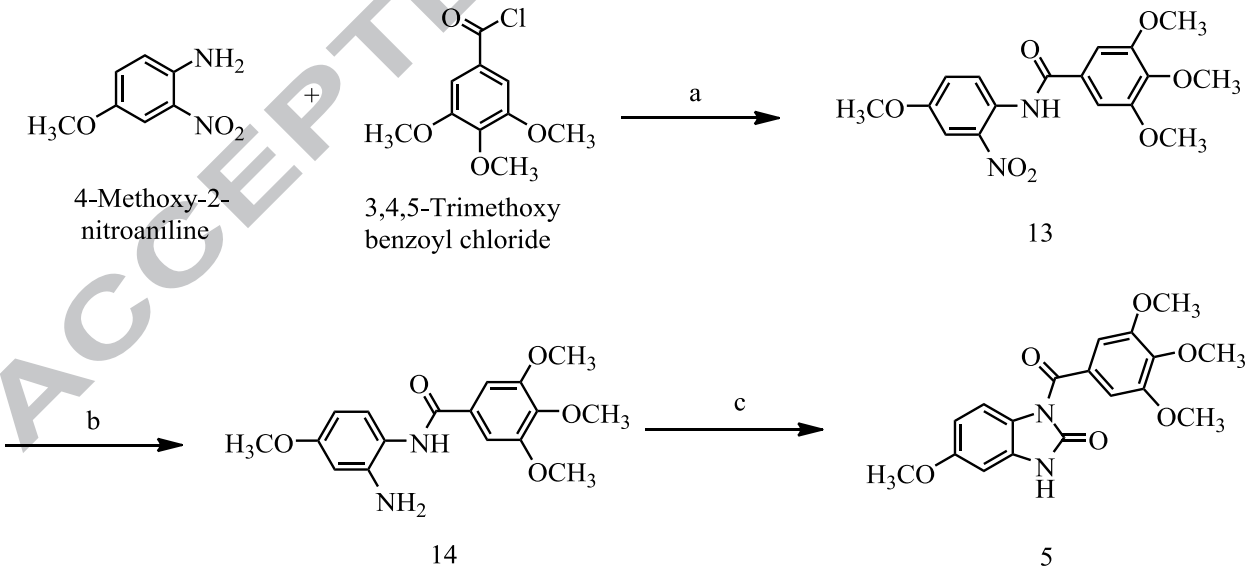

Figure 6. Synthesis of compound **5**: a. Et_3N, CH_2Cl_2, rt, 2h ; b. $SnCl_2{\cdot}2H_2O$, HCl, EtOH, 80°C-90°C, 30 min; c. CDI, THF, rt, overnight.

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Both compounds **6** and **7** were synthesized via a one-step reaction (Fig 7). For the synthesis of **6**, 6-methoxy-3*H*-benzooxazol-2-one was stirred with 3,4,5-trimethoxybenzyl bromide in DMF at 65°C for 3 hours. A milder reaction condition was used for the preparation of **7**; 6-methoxy-3*H*-benzooxazol-2-one was stirred with triethylamine and methylene chloride overnight at room temperature.

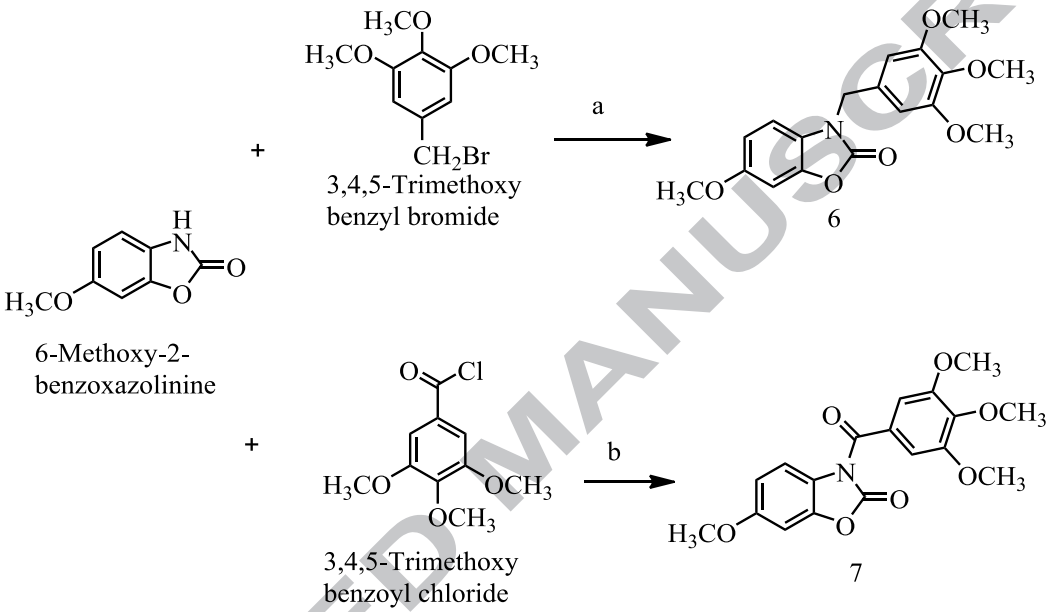

Figure 7. Synthesis of compound **6** and **7**: a. K_2CO_3, DMF, 65°C, 3h; b. Et_3N, CH_2Cl_2, rt, overnight.

For the synthesis of **9**, we first adopted a procedure similar to the synthesis of **6**, the reaction failed to produce the desired product (Fig. 8). Instead, compound **9** was obtained through Wolff Kisner reduction of indole-2,3-dione (**8**), which was readily synthesized by stirring 5-methoxy-1*H*-2,3-dione with 3,4,5-trimethyl benzyl bromide.

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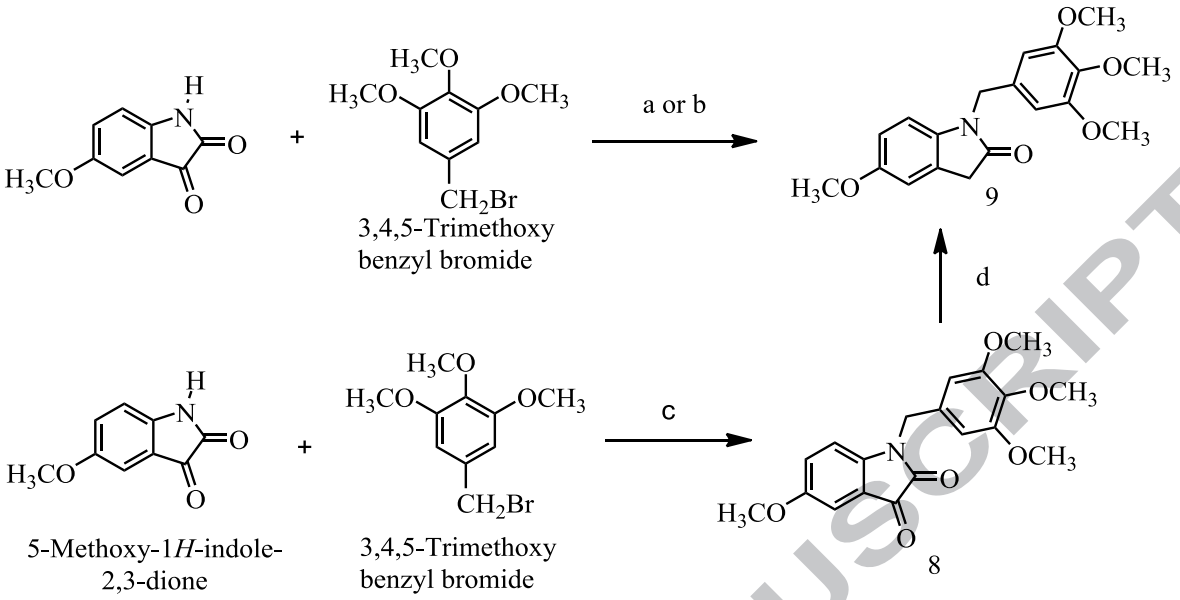

Figure 8. Synthesis of compounds 8 and 9: a. K_2CO_3, DMF, 65°C, 3h; b. Et_3N, CH_2Cl_2, rt, overnight. c. K_2CO_3, KI, DMF, rt, 4h; d. H_2NNH_2, reflux, overnight.

The anti-proliferative activities of compounds **3** – **9** were first examined in PC-3, and MDA-MB-231 cancer cells via the standard MTS assay (Table 1). Compounds **3** – **5** all contain the benimidazol-2-one moiety. Compound **3** exhibited potent anti-proliferative activities on both PC-3 and MDA-MB-231 cells with IC_{50} of 44.25 and 52.75 nM respectively. Replacing the trimethoxybenzyl group of **3** with a trimethoxybenzoyl group (**5**) caused a slight decrease in the anti-proliferative activities in both cancer cell lines. In addition, replacing the NH group of **3** with a CH_2 (**9**) also caused a slight decrease in anti-proliferative activities in both cancer cell lines to the same extent as **5**. However, the N-methylated version of compound **3,** which is **4,** resulted in more than a 10 fold decrease in the anti-proliferative activities in PC-3 cells and more than a 50 fold decrease in MDA-MB-231 cells when compared to **3**. Lastly, replacement of the indol-2-one moiety of **3** with a benzoxazol-2-one (compounds **6** and **7**) proved to be detrimental to the anti-proliferative activities. Both compounds **6** and **7** are at least 100 fold less active than compound **3**.

In addition to anti-proliferative activities, all of the compounds were also tested on their abilities to inhibit tubulin polymerization in purified porcine brain tubulin. We observed a direct

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correlation between anti-proliferative activities and inhibition of tubulin polymerization. Compound **3** is the most potent inhibitor of tubulin polymerization whereas compounds **6** and **7** are inactive.

Compound #	PC-3 (IC_{50} nM)	MDA-MB-231 (IC_{50} nM)	Tubulin polymerization inhibition (IC_{50} μM)
1	8.2 ± 1.1	0.88 ± 0.02	4.5
3	44.25 ± 2.3	52.75 ± 4.20	16.4
4	555.1 ± 10.1	3027.5	>40
5	125.5 ± 5.5	108.5 ± 4.7	19.14
6	4486	4520	>40
7	>20000	>20000	n.d
8*	318.5 ± 9.8	934.6 ± 22.9	n.d
9	165.2 ± 6.6	193.3 ± 8.8	31.2

* Solubility was poor in DMSO, IC_{50} values not accurate

Table 1. Inhibition of cancer cell proliferation and tubulin polymerization by compounds **3 - 9**. Cells (2,500 cells/well) were treated with varying concentration of compounds and cell associated protein was determined using MTS assay. The IC_{50} values represent the means of 3 independent experiments that were run in triplicate. Tubulin polymerization inhibition was determined as described in reference **13**.

In conclusion, we have designed and synthesized several tubulin inhibitors derived from our lead molecule - compound **1**. The most potent compound in this series, compound **3**, exhibited potent anti-proliferative activities on both PC-3 and MDA-MB-231 cells as well as potent inhibitory activities on tubulin polymerization.

Acknowledgment

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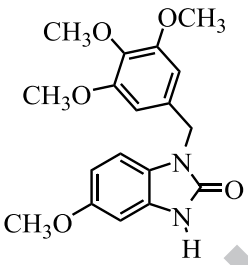

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