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Conformationally restricted analogs of Combretastatin A-4 derived from SU5416

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Abstract—A series of compounds originally derived from the vascular endothelial growth factor receptor tyrosine kinase inhibitor, SU5416, was synthesized and evaluated. The most potent compound in this series, compound **7**, structurally resembles the potent anti-microtubule agent Combretastatin A-4, inhibited tubulin polymerization, and showed potent growth inhibitory activities on both prostate and breast cancer lines with IC_{50} values in low to subnanomolar range.

Microtubules are involved in cellular functions such as cell transport, movement, and separation of chromosomes during mitosis. Microtubules are polymers of α- and β-tubulin heterodimers. During mitosis, microtubule polymerization and depolymerization are tightly controlled and disruption of this process can cause cell cycle arrest and subsequently cell death.[1] Mechanistically, anti-microtubule agents can be classified into three classes: (i) microtubule stabilizing agents, (ii) Vinca site binding agents, and (iii) colchicine site binding agents. Taxanes (paclitaxel and docetaxel) are agents that induce tubulin polymerization and stabilize microtubules.[2,3] Vinca alkaloids (vincristine, vinblastine, and vinorelbine) bind to β-tubulin and block the formation of microtubules. Like Vinca alkaloids, colchicine site binding agents also inhibit tubulin polymerization by binding to the colchicine site on β-tubulin.[4]

Currently, Taxanes and Vinca alkaloids are used clinically for the treatment of cancers. Colchicine site agents are the only class that do not have a representative drug in clinical use for cancer. However, these agents are structurally the most diverse group among the three classes of tubulin binders and include natural products such as colchicine, Combretastatin A-4, and podophyllotoxin. In addition, many small synthetic molecules are reported to bind to the colchicine site. Some of the representative agents are indanocine, oxazoline (A204197), and arylsulfonamide (T138067) (Fig. 1).[5,6]

In our search for growth factor receptor kinase inhibitors using SU5416, a potent ATP competitive vascular endothelial growth factor receptor tyrosine kinase inhibitor,[7] as the lead structure, we recently discovered a group of 2-indolinone containing compounds (Fig. 2) with potent anti-proliferative activities in both prostate and breast cancer cell lines.

The syntheses of compounds **1** and **2** were carried out by refluxing 6-methoxy-2-indolinone with 3- and 4-hydroxybenzaldehydes, respectively (Fig. 3). 6-Methoxy-2-indolinone was synthesized according to the reported procedure.[8] Demethylation of compounds **1** and **2** with boron tribromide at 0 °C yielded the respective compounds **5** and **6** (Fig. 3). The procedure for the synthesis of compounds **3** and **4** was the same as for compounds **1** and **2**, except that the commercially available 2-indolinone was used as the reagent instead of 6-methoxy-2-indolinone. Compounds **1**–**6** were purified as *E* isomers. The *E* configurations of the compounds were assigned based upon the chemical shifts of the protons at the C-2′ and C′-6′ positions in the phenyl ring at the C-3 position of compounds **1**–**6**. It has been demonstrated through NOE experiment that the chemical

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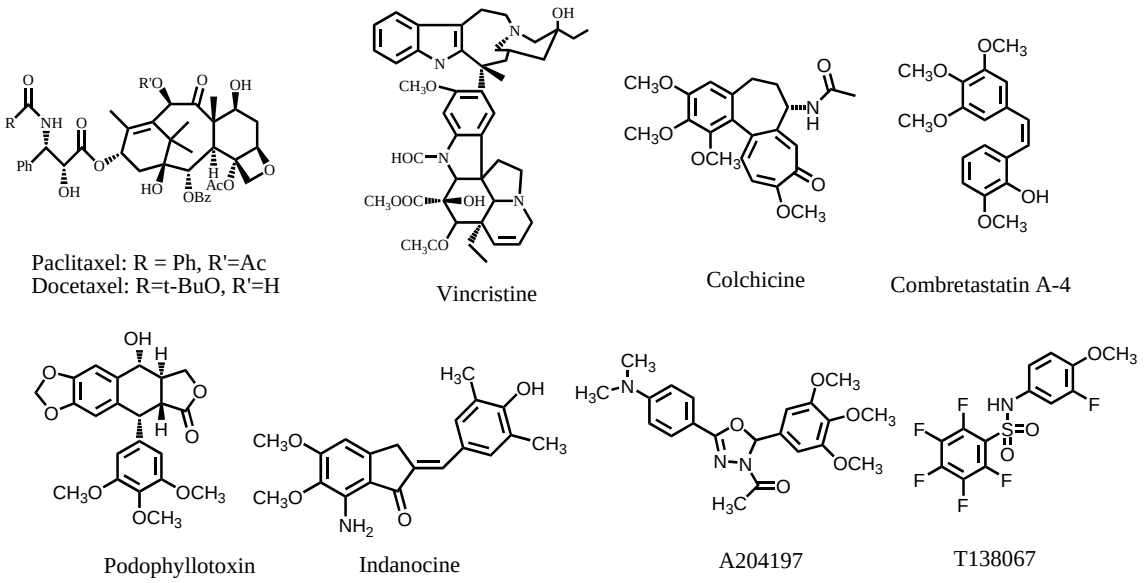


Figure 1. Structures of Taxanes, Vinca alkaloid, and colchicine site binders.

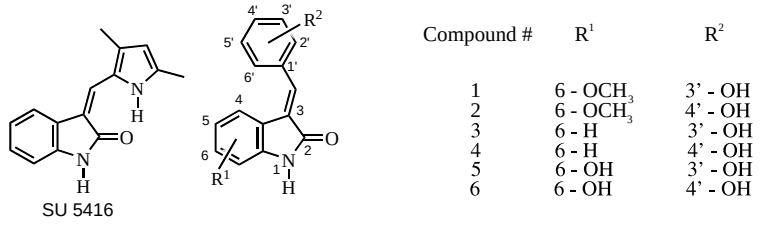


Figure 2. Structures of SU5416 and 3-(hydroxy-benzylidene)-2-indolinones (compounds **1**–**6**).

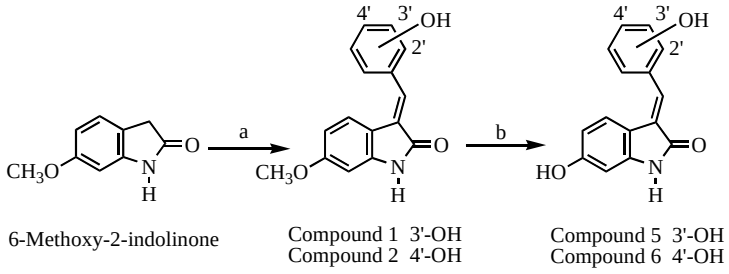


Figure 3. Synthesis of compounds **1**, **2**, **5**, and **6**. Reagents and conditions: (a) 3 or 4-hydroxybenzaldehyde/piperidine/EtOH/reflux/2–3 h; (b) BBr_3/CH_2Cl_2/0 °C, rt/overnight.

Table 1. Inhibition of cancer cell proliferation and tubulin polymerization by compounds **1**–**6**

Compound	R^1	R^2	PC-3 (IC_{50} μM)	MCF-7 (IC_{50} μM)	MDA-MB-231 (IC_{50} μM)	Inhibition of tubulin polymerization (μM)
1	6-OCH_3	3′-OH	0.54 ± 0.2	0.39 ± 0.1	1.4 ± 1.0	9.1
2	6-OCH_3	4′-OH	0.64 ± 0.2	0.23 ± 0.1	2.6 ± 1.7	18
3	H	3′-OH	>50	31.4 ± 7.5	16.9 ± 7.3	>40
4	H	4′-OH	>50	25.7 ± 5.2	17.5 ± 3.5	>40
5	6-OH	3′-OH	19.5 ± 3.7	8.6 ± 1.4	4.3 ± 0.7	>40
6	6-OH	4′-OH	44.7 ± 6.8	19.7 ± 3.8	9.4 ± 1.3	>40
SU5416	—	—	>50	>50	>50	>40
Podophyllotoxin	—	—	0.013	Not tested	0.008	1.56

Cells (1000 cells/well) were treated with varying concentrations of compounds and cell-associated protein was determined using MTS assay. The IC_{50} values represent means of three experiments in triplicate. Tubulin polymerization studies were determined as described in Ref. 9.

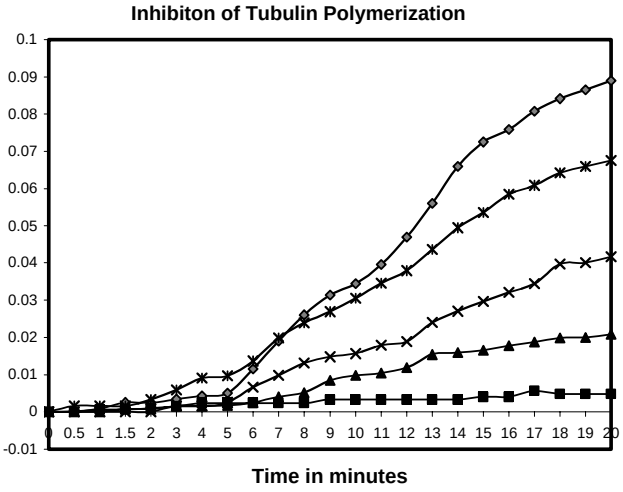
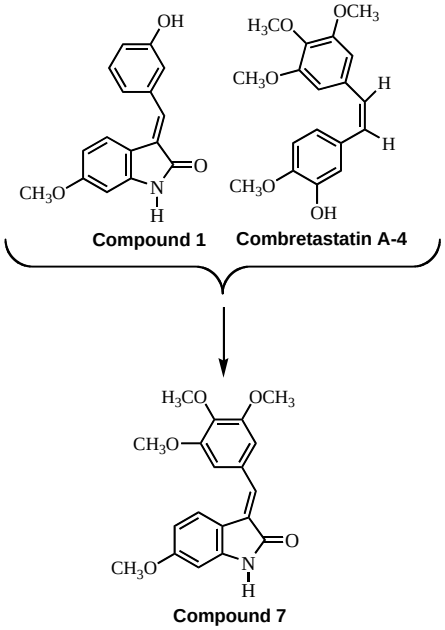


Figure 4. Effect of compound **1** on the assembly of purified porcine brain tubulin. The assembly of 1.5 mg/ml (15 μM) of purified bovine tubulin was measured by a change in the absorbance at 351 nm at 37 °C in the absence-control (♦) or presence of 5 μM (∗), 10 μM (×), and 20 μM (▲) of compound **1**. Podophyllotoxin at 5 μM (■) was used as a positive control.

shifts of C-2′ and C-6′ protons of 3-(substituted benzyldenyl)indolin-2-ones were approximately 7.45–7.84 for the E isomers and 7.85–8.53 for the *Z* isomers.[7]

Compounds **1**–**6** were examined for their anti-proliferative activities against three cancer cell lines which included: androgen-independent prostate cancer cell lines (PC-3), and estrogen-dependent (MCF-7), and estrogen-independent (MDA-MB-231) breast cancer cell lines. Cells were treated with test compounds for 72 h and cell viability was determined by the MTS assay. As shown in Table 1, compounds **1** and **2** are the most active molecules against all three cell lines tested, suggesting that the 6-OMe group is important for cytotoxicity. Removal of the 6-OMe and replacement with a 6-H (**3** and **4**) decreased cytotoxicity significantly by 100-fold against both PC-3 and MCF-7 cells, and by 6- to 10-fold against MDA-MB-231 cells. Conversion of the 6-OMe to a hydrophilic 6-OH group (compounds **5** and **6)** caused a 22- to 86-fold decrease in inhibition of the growth of PC-3 and MCF-7 cells, and there was only a slight reduction in inhibitory activity against MDA-MB-231 cell line (3- to 4-fold, Table 1). SU5416, the potent VEGF receptor tyrosine kinase inhibitor with a 2-indolinone moiety, did not show anti-proliferative activity on any of the three cell lines.

To investigate the mechanism of the anti-proliferative activities of the compounds, we examined the effects of compounds on tubulin polymerization in vitro. Using GTP-induced assembly of purified porcine brain tubulin (without microtubule-associated protein) as our assay,[9] we demonstrated that compound **1** inhibited tubulin assembly in a dose-dependent manner (Fig. 4). Quantitatively, we measured the extent of tubulin assembly after 20 min incubation and determined that the IC_{50} of tubulin inhibition for compounds **1** and **2** were 9.1 and 18 μM, respectively (Table 1). Compounds with substantially lower anti-proliferative effects on cancer cells (compounds **3**–**6**) have much lower inhibitory activities on tubulin assembly ($IC_{50} > 40$ μM). The IC_{50} of



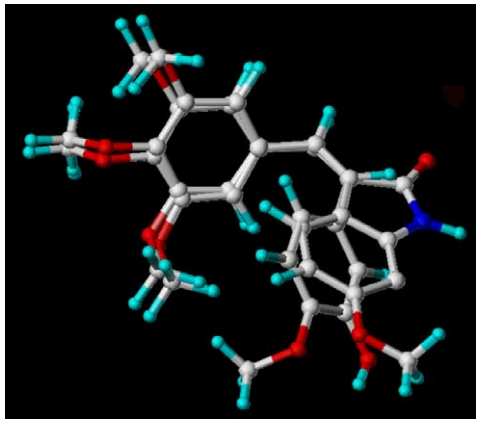


Figure 5. Illustration of the structural similarity between compound **1** and Combretastatin A-4, and the design of compound **7**. The modeling work was carried out on a SGI O_2 workstation using SYBYL 6.9 modeling software (Tripos In., St. Louis, MO). The molecules **7** and Combretastain-A-4 were minimized using Tripos force field and Gasteiger–Huckel charges, using Powell method and an energy gradient of 0.005 kcal mol^{-1} $Å^{-1}$. Conformational search of Combretastain A-4 molecule with a 30° rotation increment of the C=C–C bonds produced 6 conformers in the energy range of 44.34–34.59 kcal mol^{-1}. Among the conformers, the lowest energy conformer of Combretastain A-4 was aligned with the compound **7** using SYBYL 6.9.

Table 2. Inhibition of cancer cell proliferation and tubulin polymerization by compounds **1** and **7**

Compound	PC-3 (IC_{50} μM)	MCF-7(IC_{50} μM)	MDA-MB-231 (IC_{50} μM)	Inhibition of tubulin polymerization (μM)
1	0.54 ± 0.2	0.39 ± 0.1	1.4 ± 1.0	9.1
7	0.0082	0.0007	0.0009	4.5
SU5416	>50	>50	>50	>40
Podophyllotoxin	0.013	Not tested	0.008	1.56

Cells (1000 cells/well) were treated with varying concentration of compounds and cell-associated protein was determined using MTS assay. The IC_{50} values represent means of three experiments in triplicate. Tubulin polymerization studies were determined as described in Ref. 9.

podophyllotoxin, a potent colchicine site agent, was 1.5 μM under the same condition.

By comparing the structures of compound **1** with those of the colchicine site binders (Fig. 1), we observed a structural similarity between compound **1** and Combretastatin A-4, a potent inhibitor of tubulin polymerization derived from the South African tree *Combretum caffrum* (Fig. 5).[10] Thus, compound **7** was designed as a rigid analog of Combretastatin A-4 (Fig. 5). In addition, overlapping of the energy minimized structures of compound **7** and Combretastatin A-4 further illustrates the structural similarity of the two molecules (Fig. 5).

Compound **7** was synthesized in a similar manner as compound **1** by refluxing for 3 h of 6-methoxy-2-indolinone, 3,4,5-trimethoxybenzaldehyde, and piperidine (catalytic amount) in EtOH.[11] The anti-proliferative activities of compound **7** were examined on PC-3, MCF-7, and MDA-MB-231 cancer cells via MTS assay. Compound **7** exhibited extremely potent anti-proliferative activities against all three cell lines with IC_{50} values of 8.2, 0.7, and 0.9 nM, respectively. In addition, it also showed potent inhibitory activity against tubulin polymerization in purified porcine brain tubulin with an IC_{50} of 4.5 μM (Table 2).

In conclusion, we have discovered a series of compounds originally derived from the vascular endothelial growth factor receptor tyrosine kinase inhibitor SU5416 with potent anti-proliferative activities in both breast and prostate cancer cell lines. In addition, the compounds also exhibited potent inhibitory activities on tubulin polymerization. The most potent compound in this series, compound **7**, structurally resembled the potent anti-microtubule agent Combretastatin A-4.

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11. All the compounds showed satisfactory spectroscopic data. Selected analytical data: Compound **7**: ^{1}H NMR (300 MHz, DMSO-d_6)™ 3.74 (3H, s, –OCH_3), 3.78 (3H, s, OCH_3), 3.81 (6H, s, OCH_3), 6.43 (1H, d, J = 2.4 Hz, H-7), 6.50 (1H, dd, J = 8.4, 2.4 Hz, H-5), 7.03 (2H, s, H-2′,6′), 7.40 (1H, s, vinyl-H), 7.67 (1H, d, J = 8.4 Hz, H-4), 10.54 (1H, s, NH). HRMS: calculated for $[C_{19}H_{19}NO_5Na]^+$: 364.11554. Found: 364.11574.