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Structure Guided Design, Synthesis, and Biological Evaluation of Oxetane-Containing Indole Analogues

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Abstract

The oxetane functional group offers a variety of potential advantages when incorporated within appropriate therapeutic agents as a ketone surrogate. OXi8006, a 2-aryl-3-aroyl-indole analogue, functions as a small-molecule inhibitor of tubulin polymerization that has a dual mechanism of action as both an antiproliferative agent and a tumor-selective vascular disrupting agent. Replacement of the bridging ketone moiety in OXi8006 with an oxetane functional group has expanded structure activity relationship (SAR) knowledge and provided insights regarding oxetane incorporation within this class of molecules. A new synthetic method using an oxetane-containing tertiary alcohol subjected to Lewis acid catalyzed conditions led to successful Friedel–Crafts alkylation and yielded fourteen new oxetane-containing indole-based molecules. This synthetic approach represents the first method to successfully install an oxetane ring at the 3-position of a 2-aryl-indole system. Several analogues showed potent cytotoxicity (micromolar GI_{50} values) against human breast cancer cell lines (MCF-7 and MDA-MB-231) and a pancreatic cancer cell line (PANC-1), although they proved to be ineffective as inhibitors of tubulin polymerization. Molecular docking studies comparing colchicine with the OXi8006-oxetane analogue **5m** provided a rationale for the differential interaction of these molecules with the colchicine site on the tubulin heterodimer.

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Supporting Information

^{1}H NMR,^{13}C NMR, HRMS, HPLC for final compounds and intermediates (^{1}H NMR, ^{13}C NMR, HRMS) associated with this paper and Molecular Docking details (SI-1); Selected traces for cytotoxicity and wounding healing assay (SI-2); Wound healing assay videos for **5c** and **5h** are uploaded as separated video files.

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Keywords

Inhibition of Tubulin Polymerization; Oxetane; Friedel-Crafts Alkylation

1. Background

Decades of fundamental science coupled with translational advancement have resulted in a wide variety of FDA approved therapeutic modalities for the treatment of cancer and led to the identification of many promising biological targets associated with cancer initiation, progression, and metastasis. Despite these important discoveries, there remains a continuing quest focused on the discovery of new therapeutic agents and methods to more effectively and selectively treat cancer and to further our understanding of the associated biological mechanisms of action.

Inspired by the natural products colchicine,[1] combretastatin A-4[2] and combretastatin A-1[3] (Figure 1), our efforts to extend the known structure-activity relationship correlations associated with small-molecule inhibitors of tubulin polymerization led to the discovery of promising new molecules including dihydronaphthalenes,[4,5] benzosuberenes,[4,6–11] benzo[*b*]thiophenes,[12] benzo[*b*]furans,[13] and indoles[14–18] (Figure 1). These molecules function as potent inhibitors of tubulin polymerization and show strong antiproliferative activity against human cancer cell lines. In addition, these molecules function as tumor-selective vascular disrupting agents (VDAs)[17,19–23] that cause effective vascular damage to tumor-associated microvessels.

The promising biological activity associated with the indole-based analogue OXi8006[15–17] has inspired continued synthetic efforts towards new analogues to enhance pharmacophore kinetics and bioactivity. These efforts included the identification of alternative functional groups to serve as surrogates for the carbonyl moiety. One promising candidate was the oxetane functional group, which is a four-membered ring containing three carbon atoms and one oxygen atom. The oxetane moiety emerged as a “hot topic” in medicinal chemistry, due to the physicochemical and biological properties associated with this functional group.[24,25] The oxetane functionality offers several advantages as a good replacement group for *gem*-dimethyl[26,27] and carbonyl groups.[28] In particular, the use of oxetanes as replacements for carbonyl groups has drawn attention due to similar dipoles and H-bonding properties.[28] Given the intriguing features of oxetane as a surrogate of ketone, this functionality was incorporated into our indole-based analogue OXi8006[15,17] (Figure 2) along with related analogues. Common strategies to prepare oxetanes include intramolecular cyclization,[29–31] Paternò−Büchi [2+2] photocycloaddition,[32,33] and derivatization from oxetane containing building blocks.[26,27,34] Previously, the Bull group reported a method to a 3,3-diaryloxetane ring by Friedel–Crafts alkylation on oxetanols with a lithium catalyst.[35] Installation of the oxetane moiety into the highly sterically congested quaternary center still poses a synthetic challenge and was further investigated.

2. Results and Discussion

2.1. Synthesis

The oxetane containing tertiary alcohol **3**[35] was synthesized by performing lithium-halogen exchange on aryl bromide **1** and subsequent addition to ketone **2**, which delivered the oxetane containing building block **3** in 77% yield (Scheme 1). Our methodology drew inspiration from work accomplished by the Bull Group,[35] using lithium-catalyzed Friedel–Crafts to access 3,3-diaryloxetanes and acylation of indoles under conditions developed by the Aquino Group.[36] Through utilization of this method, $SnCl_4$ was used as a Lewis acid and CH_2Cl_2:CH_3NO_2 was employed as a co-solvent under room temperature (overall mild conditions) to install the 3,4,5-trimethoxylphenyloxetane moiety within structurally diverse 2-arylated indole analogues **4**. Various conditions utilizing different Lewis acids or Brønsted acids were also investigated in order to enhance the yield during Friedel-Crafts acylation, but $SnCl_4$ as a Lewis acid showed the highest efficiency. This approach represents the first method that facilitates successful installation of the oxetane moiety within 2-arylated indoles.

Through this Friedel–Crafts alkylation strategy, the key compound (**5m)**, oxetane-incorporated OXi8006 (Scheme 2), along with a variety of indole-based analogues (Table 1), were synthesized with functional group variation on the indole core as well as in the pendant aryl ring. Analogues selected for synthesis drew structure and functional group inspiration from OXi8006[15–18] (Pinney Group) along with related molecules from the Silvestri Group.[37]

2.2. Biological Evaluation:

The new oxetane-containing analogues were evaluated for their bioactivity against selected human cancer cell lines and their ability to inhibit tubulin polymerization. While none of the synthesized oxetane-containing analogues demonstrated significant activity as inhibitors of tubulin polymerization (IC_{50} > 20 μM), ten of the molecules displayed μM cytotoxicity as low as 0.47 ± 0.02 μM against MCF-7 cells (Table 2). The most potent analogues (**5c**, **5h**, **5k**) were further evaluated against additional cancer cell lines (MDA-MB-231 and PANC-1), and potent activity against these cell lines was also observed (Table 3 and SI Figs. S1–S6). It is instructive to note that OXi8006,[15] the ketone counterpart of oxetane **5m**, and the ketone analogues[37] of compounds **5c**, **5h**, **5k**, and **5l** function as inhibitors of tubulin polymerization while all five corresponding oxetane analogues (**5m**, **5c**, **5h**, **5k**, and **5l**) were at most weakly active as inhibitors of tubulin polymerization. Some limited preliminary structure activity relationship (SAR) patterns can be drawn from these initial antiproliferative cell-based studies. The three most active compounds (**5c**, **5h**, **5k**) each incorporate a functional group at either the R_2 position (Br and Cl, respectively for **5c** and **5k**) or the R_3 position (OCH_3 for **5h**) and include an unfunctionalized pendant phenyl ring at the 2-position. The lack of any functionalization (**5a**) and functionalization at just R_4 (**5i** and **5j**) led to significantly decreased antiproliferative activity. Interestingly, antiproliferative activity was restored (albeit at a lower level) with substitution at R_4 on the pendant ring when accompanied by substitution on the indole ring at R_2 or R_3 (**5b**, **5e**, **5f**), and in one case with additional functionalization at R_5 (**5m**), but not at R_1 (**5d**). Since these molecules

are not highly active as inhibitors of tubulin polymerization, their cytotoxicity could result from an alternative biological target, which will be the subject of future investigation.

2.2.1. Wound Healing Assay—In the multistep process that leads to cancer metastasis, cell migration is an essential element. Both compounds **5c** and **5h** inhibited PANC-1 cell migration in wound healing assays. At a higher concentration than the respective IC_{50} values, 0.5 μM, cells treated with compound **5c** (Fig. 3B) or **5h** (Fig. 4B) slowly moved into the gap introduced by the scratch as compared to controls (Figs. 3A, 4A). Upon treatment with 1 μM **5c** (Fig. 3C) or **5h** (Fig. 4C), the gaps were largely unclosed at 40 h while the control scratches were confluent (SI-2 Figs. S7, S8). Inhibition of cell migration is often associated with disruption of the microtubule cytoskeleton, which is required for cell body translocation. However, cell migration is a complex process that involves many proteins in addition to the actin and microtubule cytoskeleton. For example, anticancer agents homoharringtonine, an inhibitor of protein translation, and doxorubicin, an inhibitor of topoisomerase II, both inhibit human osteosarcoma U2OS cell migration by other mechanisms.[38]

2.3. Docking Study

Molecular docking was implemented to predict binding affinity and docking poses of the oxetane indole analogues utilizing Discovery Studio Client 4.5 (Accelrys). Compound **5m** was used as a model compound for the oxetane-containing analogues for the docking study. A previously characterized X-ray structure of *N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine (DAMA-colchicine) co-crystallized with tubulin (1SA0) served as the model structure.[39] The protein was prepared, and DAMA-colchicine was removed and re-docked (CDocker) to validate docking parameters. Agreement between X-ray structure and docked ligand bound to tubulin demonstrated a suitable protein structure and parameters to dock compound **5m**.

Based on in silico results, the energetically favored orientation of compound **5m** is dramatically different than that of known colchicine site agents. Displacement of the trimethoxy ring of **5m** in comparison to DAMA-colchicine affects a multitude of hydrophobic interactions as well as hydrogen bonding (Figure 5). Relocation of the trimethoxy ring facing the alpha-tubulin subunit indicates hydrogen bonding with αASN101, but this produces an unfavorable acceptor-acceptor clash involving the 4'-methoxy group and αTHR179. Additionally, hydrophobic interactions within the binding pocket associated with β-tubulin are lacking, including those with LEU248 and LEU242 (Figure 6).[40,41] These results suggest the importance of the bridging ketone between the aroyl and indole scaffold for maintaining potent activity as an inhibitor of tubulin polymerization.

3. Conclusions

Incorporation of the oxetane functional group (as a surrogate for the corresponding ketone moiety) was evaluated as a design paradigm in a small but focused series of indole-based analogues. Several synthetic strategies were evaluated to circumvent difficulties associated with oxetane incorporation, which led to a general synthetic route capable of accessing several new analogues. Biological studies evaluated inhibition of tubulin polymerization

and cytotoxicity against human cancer cell lines for these oxetane-bearing analogues. In comparison to their corresponding ketone counterparts, the oxetane-bearing analogues **5m**, **5c**, **5h**, **5k**, and **5l** showed no significant tubulin assembly inhibition activity, however several oxetane analogues in this series demonstrated micromolar antiproliferative activity, expanding knowledge regarding SAR for tubulin binding at the colchicine site.

4. Experimental Section

4.1. Chemistry

4.1.1. General Materials and Methods—Tetrahydrofuran (THF) was used as anhydrous. Thin-layer chromatography (TLC) plates (pre-coated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a Biotage Isolera flash purification system using silica gel (200−400 mesh, 60 Å) or RP18 prepacked columns. Intermediates and products synthesized were characterized based on their ^{1}H NMR (600 MHz) and ^{13}C NMR (151 MHz) spectroscopic data using a Bruker DPX 600 MHz instrument. Spectra were recorded in $CDCl_3$. All chemicals' shifts are expressed in ppm (δ), and peak patterns are reported as singlet (s), doublet (d), triplet (t), double doublet (dd), double double doublet (ddd), and multiplet (m). The purity of the final compounds was further analyzed at 25 °C using an Agilent 1200 HPLC system with a diode-array detector (λ = 190−400 nm), a Zorbax XDB-C18 HPLC column (150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; solvent A H_2O, solvent B CH_3CN; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 230, 240, 254, 280, 300 and 320 nm. Method A: gradient, 90% A/10% B for 5 min, 90% A/10% B to 0% A/100% B over 20 min, 0% A/100% B for 5 min; post-time 10 min; Method B: gradient, 70% A/30% B to 10% A/90% B over 25 min, 10% A/90% B for 5 min; post-time 10 min; Method C: gradient, 50% A/50% B to 10% A/90% B over 25 min; 10% A/90% B for 5 min; post-time 10 min. Mass spectrometry was carried out under either positive or negative ESI (electrospray ionization) or positive or negative APCI/APPI (atmospheric pressure chemical ionization/ atmospheric pressure photoionization) using a Thermo Scientific LTQ Orbitrap Discovery instrument. **4a** was purchased from Ambeed Inc. Known commercially-available indoles **4b-4l, 4n** were prepared following the reported procedure.[42] Compound **4m** was prepared using the procedure published previously by our group.[16]

4.1.2. Synthesis of Oxetane Analogues

4.1.2.1. 3-(3,4,5-Trimethoxyphenyl)oxetan-3-ol[35] (3): To an oven-dried flask, THF (30 mL) and 3,4,5-trimethoxyphenyl bromide (3.0 g, 12 mmol) were added, and the solution was cooled to −78 °C. n-BuLi (5.3 mL, 13 mmol) was added dropwise to the reaction mixture, which was then stirred at −78 °C for 1 h. 3-Oxetanone (0.92 mL, 13 mmol) was then added slowly to the flask, and the reaction mixture was stirred while gradually warming from −78 °C to room temperature over 12 h. The reaction was quenched with water and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate and the solvent removed by evaporation under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 100% ethyl acetate in hexanes) to afford alcohol **3** (2.2 g, 9.3 mmol, 77%) as a white solid. ^{1}H NMR

(600 MHz, $CDCl_3$) δ 6.80 (s, 2H), 4.87 (d, *J* = 1.3 Hz, 4H), 3.85 (s, 6H), 3.84 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.21, 138.44, 137.20, 101.70, 85.85, 75.46, 60.81, 56.13. HRMS (ESI+) calculated for $C_{12}H_{16}O_5$ [M+Na] 263.0890, actual 263.0889.

4.1.2.2. 2-Phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5a): To a well-stirred solution of commercially-available indole **4a** (0.10 g, 0.50 mmol) in CH_2Cl_2 (5.0 mL) under N_2 at 0 °C, $SnCl_4$ (0.12 mL, 0.80 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.15 g, 0.60 mmol) was added in small portions to the suspension, followed by nitromethane (5.0 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 50 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5a** (50 mg, 0.12 mmol, 23%) as a colorless solid: ^1H NMR (600 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.48 (d, *J* = 8.1 Hz, 1H), 7.41 – 7.36 (m, 3H), 7.30 (dd, *J* = 8.1, 2.0 Hz, 2H), 7.26 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.11 – 7.02 (m, 2H), 6.96 (s, 2H), 5.17 (d, *J* = 5.7 Hz, 2H), 4.93 (d, *J* = 5.7 Hz, 2H), 3.92 (s, 3H), 3.75 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.30, 142.62, 136.92, 136.00, 134.51, 132.96, 128.99, 128.35, 127.98, 127.34, 122.63, 120.11, 119.82, 115.17, 111.16, 103.69, 85.35, 60.88, 56.22, 47.16; HRMS (ESI+) calculated for $C_{26}H_{25}NO_4$ [M+Na] 438.1676, actual 438.1678. HPLC (Method B): retention time 15.3 min.

4.1.2.3. 6-Chloro-2-(*p*-tolyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5b): To a well-stirred solution of indole **4b** (0.30 g, 1.2 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.23 mL, 1.9 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.33 g, 1.4 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5b** (0.18 g, 0.38 mmol, 31%) as a colorless solid: ^1H NMR (600 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.43 (d, *J* = 1.8 Hz, 1H), 7.21 – 7.12 (m, 4H), 7.03 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.96 – 6.87 (m, 3H), 5.13 (d, *J* = 5.8 Hz, 2H), 4.92 (d, *J* = 5.9 Hz, 2H), 3.92 (s, 3H), 3.75 (s, 6H), 2.38 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.36, 142.49, 138.53, 136.90, 136.38, 135.60, 129.69, 129.63, 128.09, 127.17, 126.53, 120.60, 120.34, 114.42, 111.23, 103.65, 85.24, 60.89, 56.21, 47.03, 21.28; HRMS (ESI+) calculated for $C_{27}H_{26}ClNO_4$ [M+Na] 486.1443, actual 486.1443. HPLC (Method A): retention time 22.8 min.

4.1.2.4. 6-Bromo-2-phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5c): To a well-stirred solution of indole **4c** (0.4 g, 1.5 mmol) in CH_2Cl_2 (10 mL) under

N_2 at 0 °C, $SnCl_4$ (0.32 mL, 2.2 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.39 g, 1.6 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5c** (98 mg, 0.21 mmol, 14%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.62 (d, *J* = 1.7 Hz, 1H), 7.42 – 7.32 (m, 3H), 7.32 – 7.23 (m, 2H), 7.18 (dd, *J* = 8.5, 1.7 Hz, 1H), 6.97 – 6.87 (m, 3H), 5.12 (d, *J* = 5.8 Hz, 2H), 4.91 (d, *J* = 5.9 Hz, 2H), 3.92 (s, 3H), 3.75 (s, 6H); ^{13}C NMR (151 MHz, CDCl3) δ 153.37, 142.30, 137.00, 136.78, 135.26, 132.44, 129.05, 128.63, 127.31, 126.83, 123.40, 120.91, 116.05, 115.14, 114.21, 103.61, 85.20, 60.88, 56.24, 46.96.; HRMS (ESI+) calculated for $C_{26}H_{24}BrNO_4$ [M+Na] 516.0781, actual 516.0794. HPLC (Method A): retention time 22.3 min.

4.1.2.5. 5-Methoxy-2-(*p*-tolyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5d): To a well-stirred solution of indole **4d** (0.50 g, 2.1 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.42 mL, 3.2 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.56 g, 2.3 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 60% ethyl acetate in hexanes) to afford oxetane analogue **5d** (49 mg, 0.11 mmol, 5%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.39 – 7.32 (m, 1H), 7.21 – 7.13 (m, 4H), 6.97 (s, 2H), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.43 (d, *J* = 2.5 Hz, 1H), 5.17 (d, *J* = 5.7 Hz, 2H), 4.91 (d, *J* = 5.6 Hz, 2H), 3.92 (s, 3H), 3.78 (s, 3H), 3.76 (s, 6H), 2.38 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.12, 153.31, 142.63, 138.18, 136.85, 135.64, 131.23, 130.16, 129.62, 128.49, 127.12, 114.25, 111.96, 111.93, 103.70, 101.80, 85.29, 60.88, 56.21, 56.00, 47.13, 21.25; HRMS (ESI+) calculated for $C_{28}H_{29}NO_5$ [M+Na] 482.1938, actual 482.1935. HPLC (Method C): retention time 6.6 min.

4.1.2.6. 6-Chloro-2-(4-methoxyphenyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5e): To a well-stirred solution of indole **4e** (0.50 g, 2.0 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.41 mL, 3.0 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.51 g, 2.1 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product

was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5e** (0.16 g, 0.34 mmol, 17%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.39 (d, *J* = 1.8 Hz, 1H), 7.21 – 7.06 (m, 2H), 7.00 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.94 – 6.81 (m, 5H), 5.11 (d, *J* = 5.8 Hz, 2H), 4.88 (d, *J* = 5.8 Hz, 2H), 3.89 (s, 3H), 3.80 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.80, 153.33, 142.49, 136.88, 136.28, 135.41, 128.58, 127.92, 126.54, 124.90, 120.55, 120.21, 114.41, 113.94, 111.13, 103.61, 85.17, 60.85, 56.20, 55.31, 46.97; HRMS (ESI+) calculated for $C_{27}H_{26}ClNO_5$ [M+Na] 502.1392, actual 502.1395. HPLC (Method A): retention time 21.9 min.

4.1.2.7. 6-Bromo-2-(*p*-tolyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5f): To a well-stirred solution of indole **4f** (0.25 g, 0.90 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.22 mL, 1.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.23 g, 1.0 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5f** (0.15 g, 0.30 mmol, 34%) as a yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.60 (d, *J* = 1.7 Hz, 1H), 7.23 – 7.10 (m, 5H), 7.03 – 6.81 (m, 3H), 5.12 (d, *J* = 5.8 Hz, 2H), 4.91 (d, *J* = 5.9 Hz, 2H), 3.91 (s, 3H), 3.75 (s, 6H), 2.38 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.35, 142.35, 138.70, 136.97, 136.67, 135.40, 129.77, 129.50, 127.16, 126.90, 123.33, 120.81, 115.86, 114.72, 114.09, 103.59, 85.19, 60.88, 56.23, 46.98, 21.29; HRMS (ESI+) calculated for $C_{27}H_{26}BrNO_4$ [M+Na] 530.0937, actual 530.0941. HPLC (Method A): retention time 23.1 min.

4.1.2.8. 2-(3-Methoxyphenyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5g): To a well-stirred solution of indole **4g** (0.50 g, 2.2 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.42 mL, 3.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.59 g, 2.4 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 60% ethyl acetate in hexanes) to afford oxetane analogue **5g** (0.31 g, 0.67 mmol, 30%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.33 – 7.20 (m, 2H), 7.14 – 7.01 (m, 2H), 6.97 (s, 2H), 6.92 – 6.87 (m, 2H), 6.85 – 6.81 (m, 1H), 5.20 (d, *J* = 5.8 Hz, 2H), 4.95 (d, *J* = 5.9 Hz, 2H), 3.90 (s, 3H), 3.75 (s, 6H), 3.65 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.82, 153.30, 142.65, 136.93, 135.88, 134.39, 134.18, 130.07, 127.95, 122.66, 120.15, 119.78, 119.57, 115.33, 114.35, 112.44, 111.18,

103.68, 85.34, 60.86, 56.24, 55.01, 47.17; HRMS (ESI+) calculated for $C_{27}H_{27}NO_5$ [M+Na] 468.1781, actual 468.1788. HPLC (Method A): retention time 20.6 min.

4.1.2.9. 7-Methoxy-2-phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5h): To a well-stirred solution of indole **4h** (0.50 g, 2.2 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.40 mL, 3.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.59 g, 2.4 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 60% ethyl acetate in hexanes) to afford oxetane analogue **5h** (0.12g, 0.30 mmol, 13%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 – 8.42 (m, 1H), 7.47 – 7.18 (m, 5H), 7.08 – 6.90 (m, 3H), 6.71 (d, *J* = 7.7 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 5.16 (d, *J* = 5.9, 2H), 4.92 (d, *J* = 6.0, 2H), 4.04 (s, 3H), 3.91 (s, 3H), 3.76 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.28, 146.12, 142.68, 136.87, 134.13, 132.99, 129.22, 128.93, 128.24, 127.34, 126.47, 120.52, 115.55, 112.48, 103.68, 102.40, 85.37, 60.87, 56.23, 55.37, 47.21; HRMS (ESI+) calculated for $C_{27}H_{27}NO_5$ [M+Na] 468.1781, actual 468.1781. HPLC (Method A): retention time 20.7 min.

4.1.2.10. 2-(4-Methoxyphenyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5i): To a well stirred solution of indole **4i** (0.50 g, 2.2 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.41 mL, 3.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.58 g, 2.4 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 60 % ethyl acetate in hexanes) to afford oxetane analogue **5i** (96 mg, 0.22 mmol, 10%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 1H), 7.26 – 7.19 (m, 3H), 7.10 – 7.00 (m, 2H), 6.95 (s, 2H), 6.93 – 6.89 (m, 2H), 5.18 (d, *J* = 5.8 Hz, 2H), 4.92 (d, *J* = 5.9 Hz, 2H), 3.91 (s, 3H), 3.84 (s, 3H), 3.75 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.72, 153.28, 142.74, 136.88, 135.84, 134.49, 128.61, 128.06, 125.37, 122.30, 120.01, 119.60, 114.43, 114.27, 111.00, 103.67, 85.29, 60.87, 56.22, 55.35, 47.16; HRMS (ESI+) calculated for $C_{27}H_{27}NO_5$ [M+Na] 468.1781, actual 468.1783. HPLC (Method B): retention time 15.6 min.

4.1.2.11. 2-(*p*-Tolyl)-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5j): To a well-stirred solution of indole **4j** (0.50 g, 2.4 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.40 mL, 3.6 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then

3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.64 g, 2.7 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5j** (0.21 g, 0.65 mmol, 27%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.46 (d, *J* = 8.2, 1H), 7.24 (ddd, *J* = 8.2, 6.9, 1.3 Hz, 1H), 7.18 (s, 4H), 7.07 (ddd, *J* = 8.0, 6.9, 1.0 Hz, 1H), 7.03 (dd, *J* = 7.9, 1.1 Hz, 1H), 6.96 (s, 2H), 5.18 (d, *J* = 5.9 Hz, 2H), 4.93 (d, *J* = 6.0 Hz, 2H), 3.91 (s, 3H), 3.75 (s, 6H), 2.39 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.28, 142.70, 138.34, 136.87, 135.91, 134.66, 130.04, 129.68, 128.03, 127.19, 122.42, 120.01, 119.70, 114.69, 111.08, 103.68, 85.33, 60.87, 56.21, 47.18, 21.28; HRMS (ESI+) calculated for $C_{27}H_{27}NO_4$ [M+Na] 452.1832, actual 452.1837. HPLC (Method B): retention time 17.1 min.

4.1.2.12. 6-Chloro-2-phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5k): To a well-stirred solution of indole **4k** (0.50 g, 2.2 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.41 mL, 3.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.58 g, 2.4 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 50% ethyl acetate in hexanes) to afford oxetane analogue **5k** (0.11 g, 0.24 mmol, 11%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 – 8.20 (m, 1H), 7.48 – 7.45 (m, 1H), 7.41 – 7.35 (m, 3H), 7.30 – 7.26 (m, 2H), 7.05 (dd, J = 8.4, 2.0 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.92 (s, 2H), 5.12 (d, J = 5.8 Hz, 2H), 4.91 (d, J = 6.0 Hz, 2H), 3.92 (s, 3H), 3.76 (s, 6H).; ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.37, 142.28, 137.01, 136.33, 135.29, 132.46, 129.07, 128.63, 128.47, 127.29, 126.55, 120.90, 120.60, 115.19, 111.18, 103.59, 85.20, 60.89, 56.23, 46.97; HRMS (ESI+) calculated for $C_{26}H_{24}ClNO_4$ [M+Na] 472.1286, actual 472.1288. HPLC (Method B): retention time 5.9 min.

4.1.2.13. 5-Methoxy-2-phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5l): To a well-stirred solution of indole **4l** (0.80 g, 3.6 mmol) in CH_2Cl_2 (10 mL) under N_2 at 0 °C, $SnCl_4$ (0.60 mL, 5.4 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.95 g, 4.0 mmol) was added in small portions to the suspension, followed by nitromethane (10 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (30 mL), the organic material was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column (0 to 60% ethyl acetate in hexanes) to afford oxetane analogue **5l** (0.35 g, 0.80 mmol, 22%) as a colorless

solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.41 – 7.32 (m, 4H), 7.32 – 7.23 (m, 2H), 6.97 (s, 2H), 6.92 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 5.16 (d, *J* = 5.8 Hz, 2H), 4.92 (d, *J* = 5.9 Hz, 2H), 3.92 (s, 3H), 3.78 (s, 3H), 3.76 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.24, 153.32, 142.49, 136.92, 135.40, 133.04, 131.23, 128.96, 128.51, 128.27, 127.24, 114.92, 112.29, 111.91, 103.67, 101.92, 85.27, 60.88, 56.23, 55.99, 47.10; HRMS (ESI+) calculated for $C_{27}H_{27}NO_5$ [M+Na] 468.1781, actual 468.1795. HPLC (Method A): retention time 19.8 min.

4.1.2.14. 2-Methoxy-5-(6-methoxy-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indol-2-yl)phenol (5m): To a well-stirred solution of indole **4m** (0.10 g, 0.26 mmol) in CH_2Cl_2 (5 mL) under N_2 at 0 °C, $SnCl_4$ (0.05 mL, 0.4 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (69 mg, 0.29 mmol) was added in small portions to the suspension, followed by nitromethane (5 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (10 mL), the organic material was extracted with CH_2Cl_2 (3×10 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent. The crude reaction product was added to THF (5 mL) and TBAF (5 mL), and the reaction solution was stirred at room temperature for 30 min. After the reaction was quenched with ice and water (5 mL), the organic material was extracted with CH_2Cl_2 (3×5 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product purified by flash chromatography using a prepacked 25 g silica column (0 to 60 % ethyl acetate in hexanes) to afford oxetane analogue **5m** (15 mg, 0.03 mmol, 12%) as a colorless solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (s, 1H), 6.85 (m, 3H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 6.72 (d, *J* = 8.3 Hz, 1H), 6.63 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.56 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.60 (s, 1H), 5.07 (d, *J* = 5.6 Hz, 2H), 4.83 (d, *J* = 5.6 Hz, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.66 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.65, 153.26, 146.52, 145.88, 142.65, 136.85, 136.65, 133.19, 126.49, 122.40, 120.28, 119.37, 114.37, 113.08, 110.87, 109.76, 103.65, 94.62, 85.25, 60.86, 56.21, 55.99, 55.66, 47.14. HRMS (ESI+) calculated for $C_{28}H_{29}NO_7$ [M+Na] 514.1836, actual 514.1833. HPLC (Method B): retention time 11.5 min.

4.1.2.15. 5-Bromo-2-phenyl-3-(3-(3,4,5-trimethoxyphenyl)oxetan-3-yl)-1*H*-indole (5n): To a well-stirred solution of indole **4n** (0.60 g, 2.2 mmol) in CH_2Cl_2 (5 mL) under N_2 at 0 °C, $SnCl_4$ (0.45 mL, 3.3 mmol) was added in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then 3-(3,4,5-trimethoxyphenyl)oxetan-3-ol (0.60 g, 2.4 mmol) was added in small portions to the suspension, followed by nitromethane (5 mL). The mixture was stirred for 8 h at room temperature. After the reaction was quenched with ice and water (10 mL), the organic material was extracted with CH_2Cl_2 (3×10 mL). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure to remove the solvent, and the crude reaction product was purified by flash chromatography using a prepacked 50 g silica column (0 to 60 % ethyl acetate in hexanes) to afford oxetane analogue **5n** (0.15 g, 0.31 mmol, 14%) as a yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.39 – 7.36 (m, 3H), 7.34 – 7.32 (m, 2H), 7.30 – 7.25 (m, 2H), 7.16 – 7.13 (m, 1H), 6.92 (s, 2H), 5.12 (d, *J* = 5.7 Hz, 2H), 4.91

(d, J = 5.8 Hz, 2H), 3.93 (s, 3H), 3.76 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.39, 142.19, 137.07, 135.93, 134.64, 132.40, 129.57, 129.06, 128.71, 127.34, 125.52, 121.98, 114.62, 113.23, 112.75, 103.67, 85.21, 60.90, 56.26, 46.90; HRMS (ESI+) calculated for $C_{26}H_{24}BrNO_4$ [M+Na] 516.0781, actual 516.0784. HPLC (Method B): retention time 5.7 min.

4.2 Biological Evaluation

4.2.1. Cell Lines and Sulforhodamine B (SRB) Assay.—The SRB assay was used to assess growth inhibition of human cancer cells, as previously described.[43–45] The MDA-MB-231 and PANC-1 cancer cell lines (obtained from ATCC) were plated in triplicate at 12,000 cells/well into 96-well plates (Corning) and incubated for 24 h at 37 °C in a humidified incubator in a 5% CO_2 atmosphere using high glucose DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco) and 30 μg/mL gentamicin sulfate. Compounds to be tested were dissolved in DMSO to generate 10 mg/mL stock solutions. Serial dilutions were made in media and added to the plates. Paclitaxel (Tokyo Chemical) and CA4 were used as positive controls. After a 48 h drug treatment, the cells were fixed with 6% final concentration trichloroacetic acid (Acros), washed with deionized water, air-dried, stained with 100 μL 7.1 mM SRB dye, washed with 1% acetic acid to remove excess dye, and air-dried. SRB was solubilized with 10 mM Tris-base, and absorbances were measured at 530 nm using a Varioskan LUX Multimode Microplate Reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing 50% reduction in net protein increase) relative to control was calculated from the absorbance data using GraphPad. A minimum of three independent experiments were performed. The MCF-7 breast cancer cells were obtained from the NCI Cell Screen, and the procedure used was that described by Monks et al.[45] The GI_{50} values were obtained following 96 h of growth in varying drug concentrations, essentially as described above for the MDA-MB-231 and PANC-1 cells. CA4 was used as a positive control.

4.2.2. Wound Healing Assay—PANC-1 cells (ATCC CRL-1469) were cultured in T75 (Corning U420720U) flasks with Dulbecco's modified eagle media (DMEM) supplemented with 10% heat inactivated FBS and 30 μg/mL gentamicin sulfate. The cells were grown in a humidified incubator supplemented with 5% CO_2 at 37 °C. Cells were detached with Tryple, plated at a seeding density of approximately 1×10^5 cells in a 6-well plate (sterile tissue culture treated Corning 3516) and allowed to reach 70–80% confluency before treatment. A scratch was made with a sterile 10 μL pipette tip in each well before removing the media and any detached cells. Two wells of PANC-1 cells were treated with either 1 μM or 0.5 μM compound **5c** or **5h**, with two wells as controls with the final DMSO being no greater than 0.2% and the final media volume being 3 mL in each well. Wound status was monitored with an automated Biotek Lionheart ELx800 microscope with a 4X objective for 48 h. We utilized the supplemented Gen5 software to perform a cellular and statistical analysis on the wound closure data.

4.2.3. Inhibition of Tubulin Polymerization.[46]—Tubulin polymerization was evaluated in 0.25 mL reaction mixtures (final volume) containing 1 mg/mL (10 μM) purified bovine brain tubulin, 0.8 M monosodium glutamate (pH 6.6), 4% (v/v) dimethyl sulfoxide,

0.1 mM GTP, and different compound concentrations. All components except GTP were preincubated for 15 min at 30 °C in 0.24 mL. The assay mixtures were cooled to 0 °C, and 10 μL of 0.0025 M GTP was added to each sample. Reaction mixtures were transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was increased to 30 °C, over about 30 s, and polymerization was followed turbidimetrically at 350 nm for 20 min. The IC_{50} was defined as the compound concentration inhibiting extent of polymerization by 50% after 20 min.

Supplementary Material

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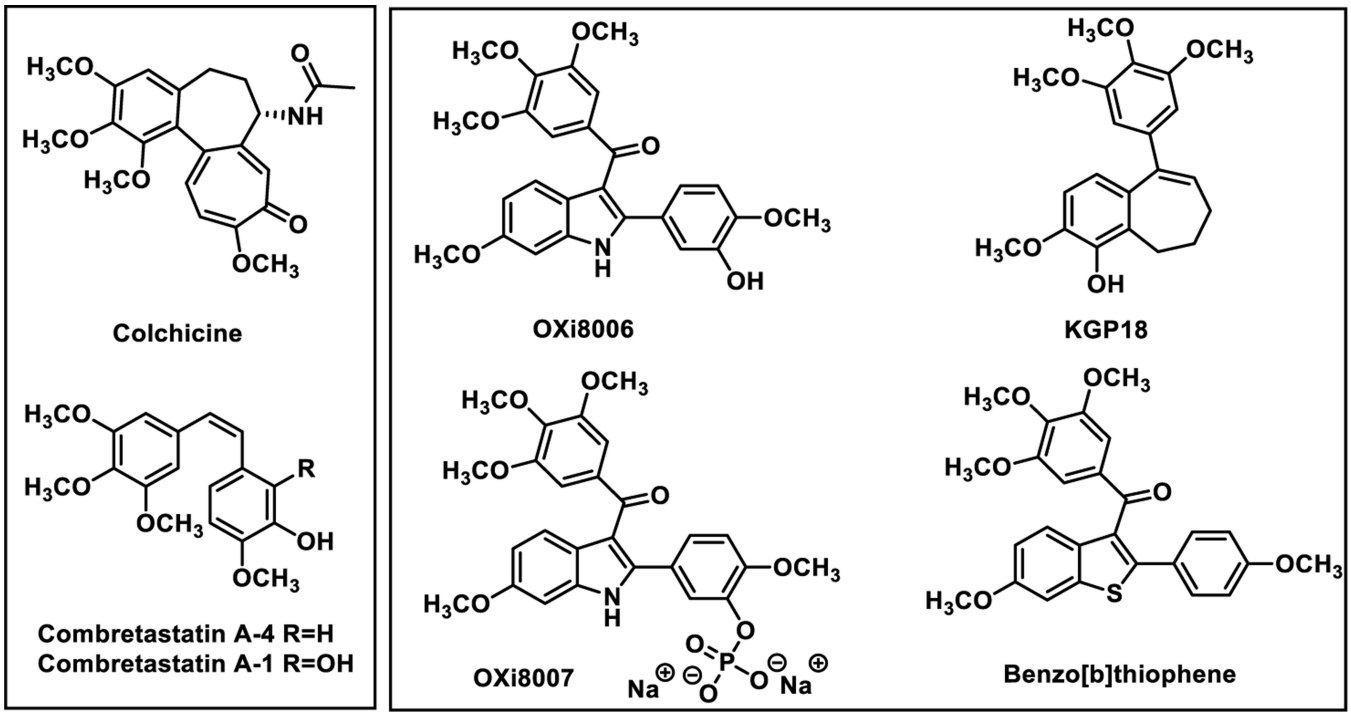


Figure 1.

Natural Products Colchicine,[1] CA-4[2], CA-1[3] and Selected Synthetic Small-Molecule Analogues Designed and Synthesized by the Pinney Group[4,6–11,14–18] as Inhibitors of Tubulin Polymerization.

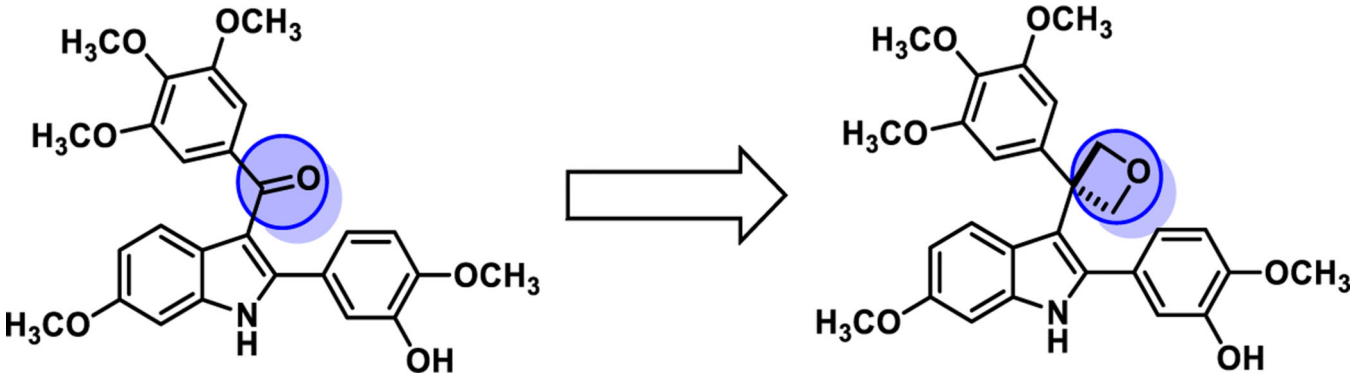


Figure 2.
Molecular Design Paradigm: Oxetane Moiety as Ketone Surrogate in OXi8006

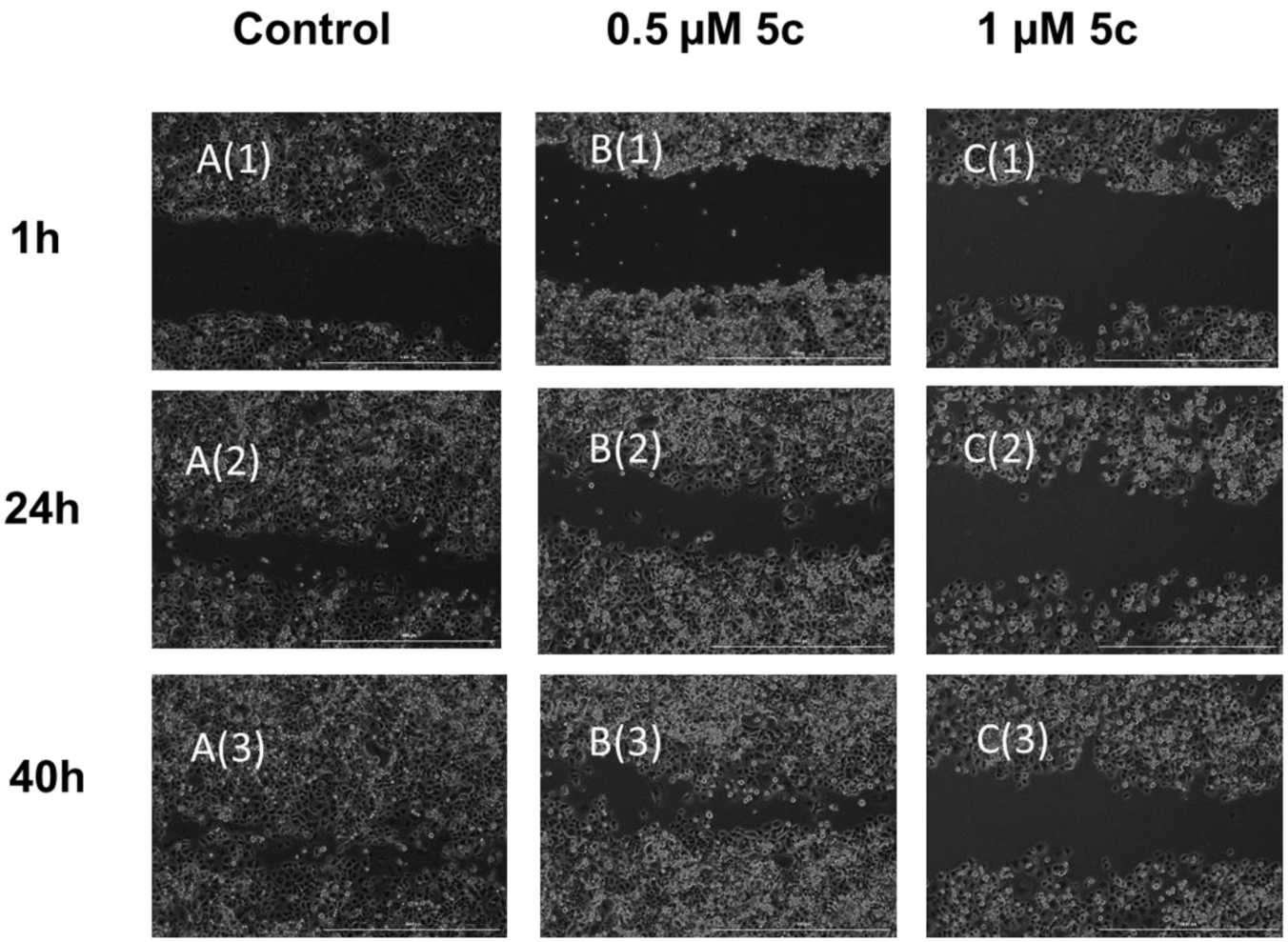


Figure 3: Scratch Assay for PANC-1 Cells Treated with Compound 5c

Representative images of PANC-1 cells at 1, 24, and 40 h after making scratches. Cells were incubated at 37 °C with 5% CO_2. A(1) A(2) and A(3): control wells; scratch gap closed after 40 h; B(1), B(2) and B(3): 0.5 μM **5c** treated cells; cells slowly move towards the center of the gap, but gap is not completely closed after 40 h; C(1), C(2) and C(3): 1 μM **5c** treated cells; gap remains open after 40 h.

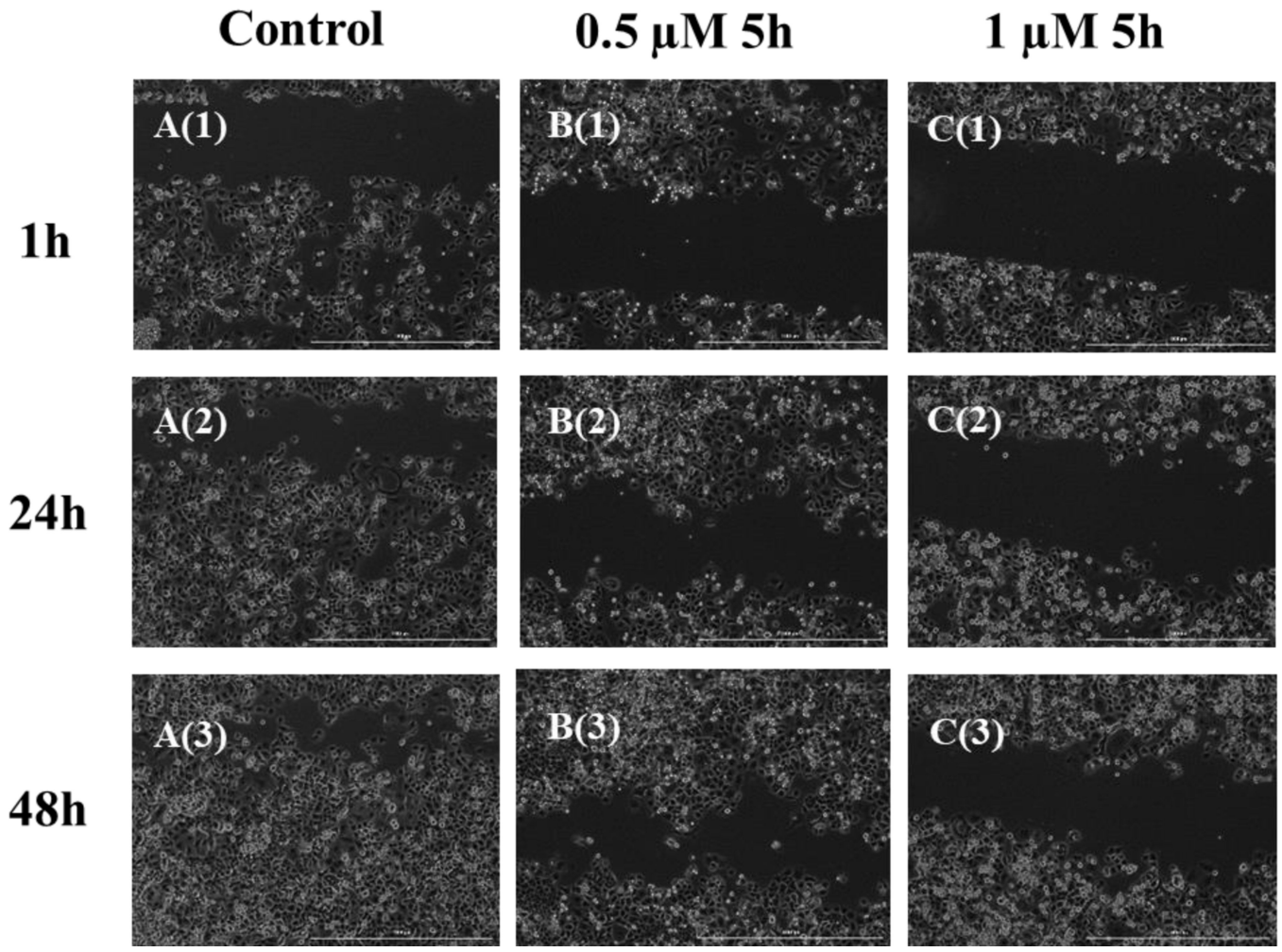


Figure 4: Scratch Assay for PANC-1 Cells Treated with Compound 5h

Representative images of PANC-1 cells at time 1, 24, and 48 h after making scratches. Cells were incubated at 37 °C with 5% CO_2. A(1) A(2) and A(3): control wells; scratch gap closed after 48 h; B(1), B(2) and B(3): 0.5 μM **5h** treated cells; cells slowly move towards the center of the gap, but gap is not closed after 48 h; C(1), C(2) and C(3): 1 μM **5h** treated cells; gap remains open after 48 h.

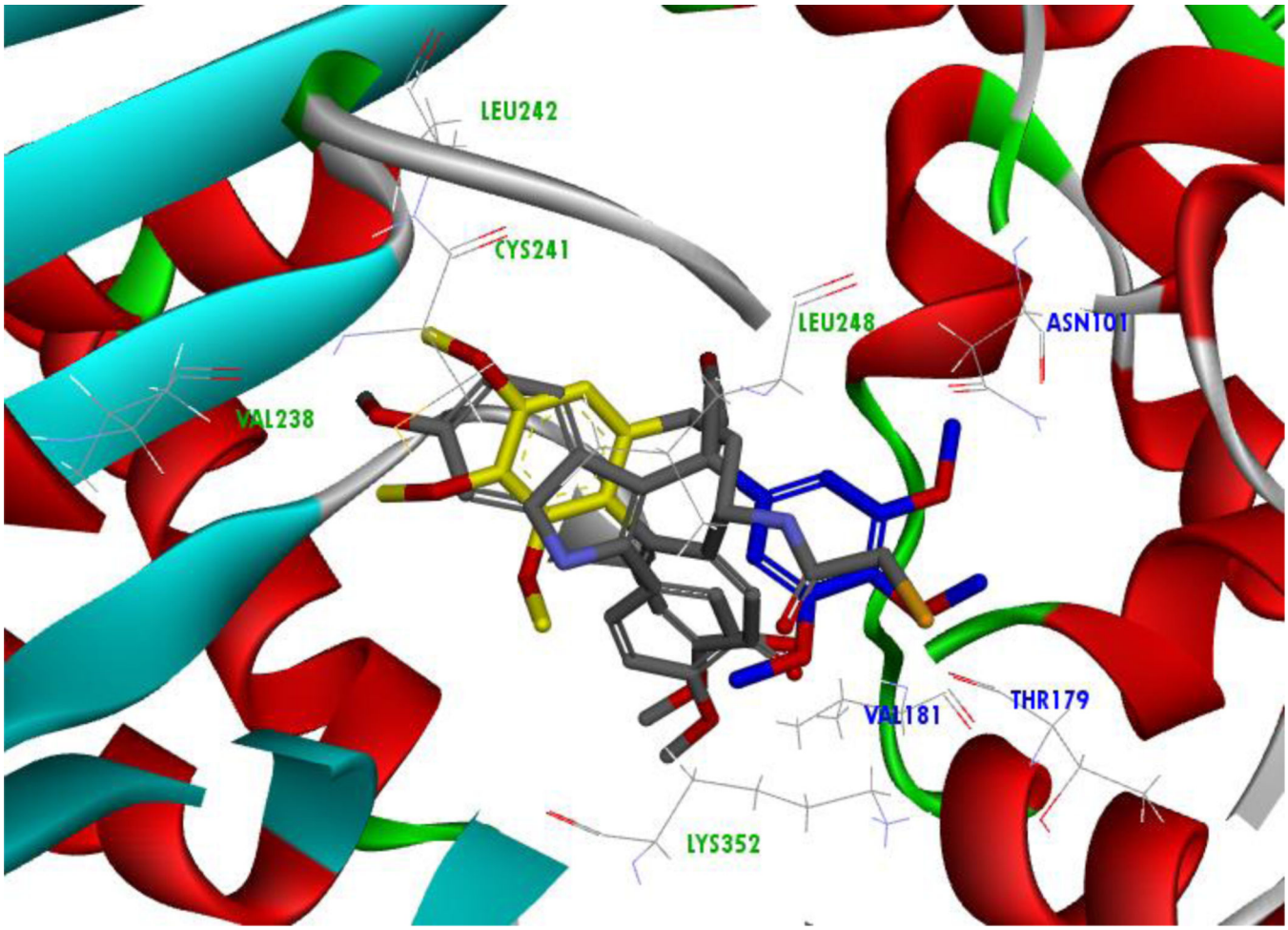


Figure 5: Compound 5m Trimethoxy Ring (blue) Interaction with the α-Subunit Residues (Blue).
The DAMA-colchicine (co-crystalized structure with tubulin) trimethoxy ring indicated in yellow interaction with β-tubulin residues (green).

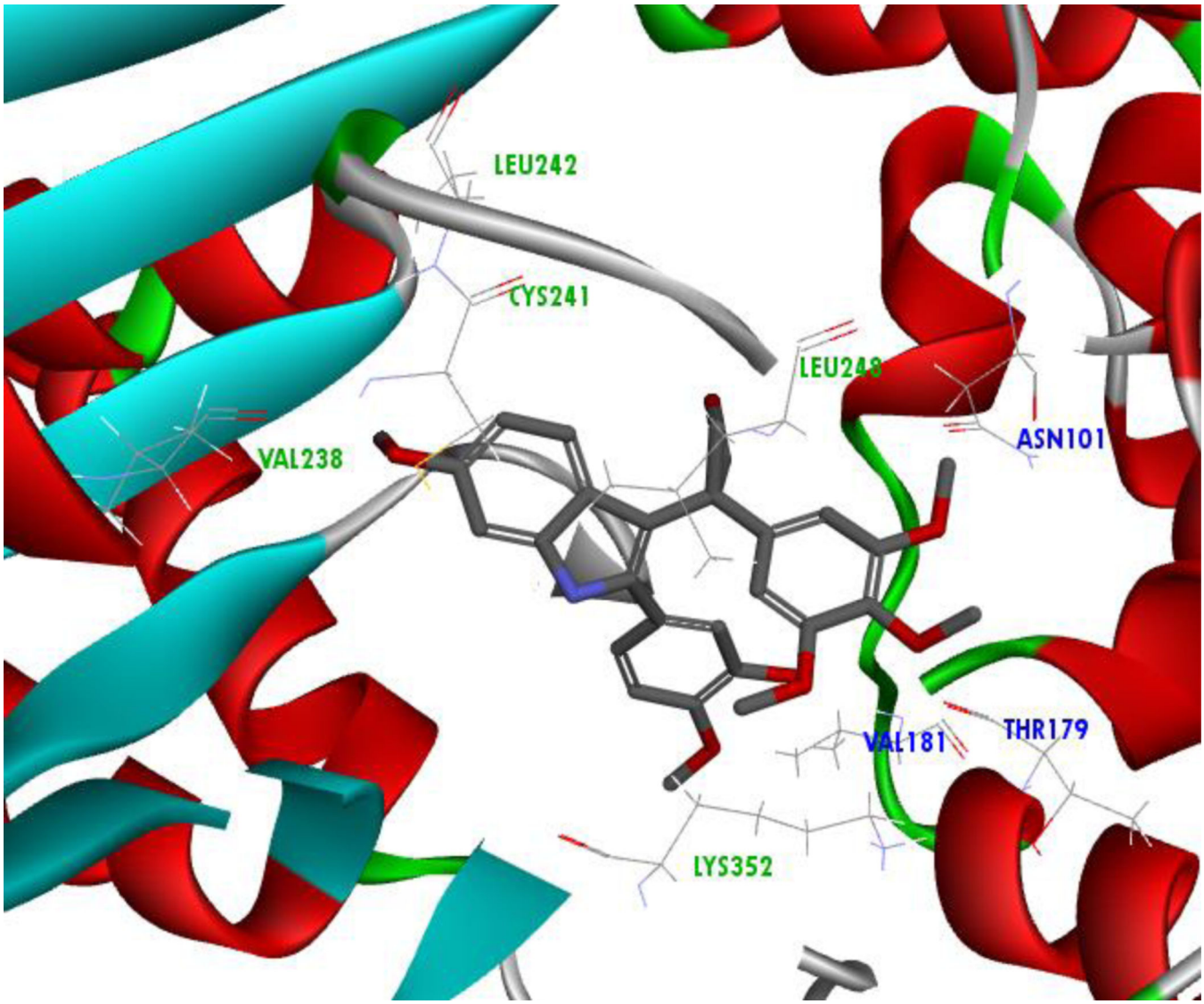
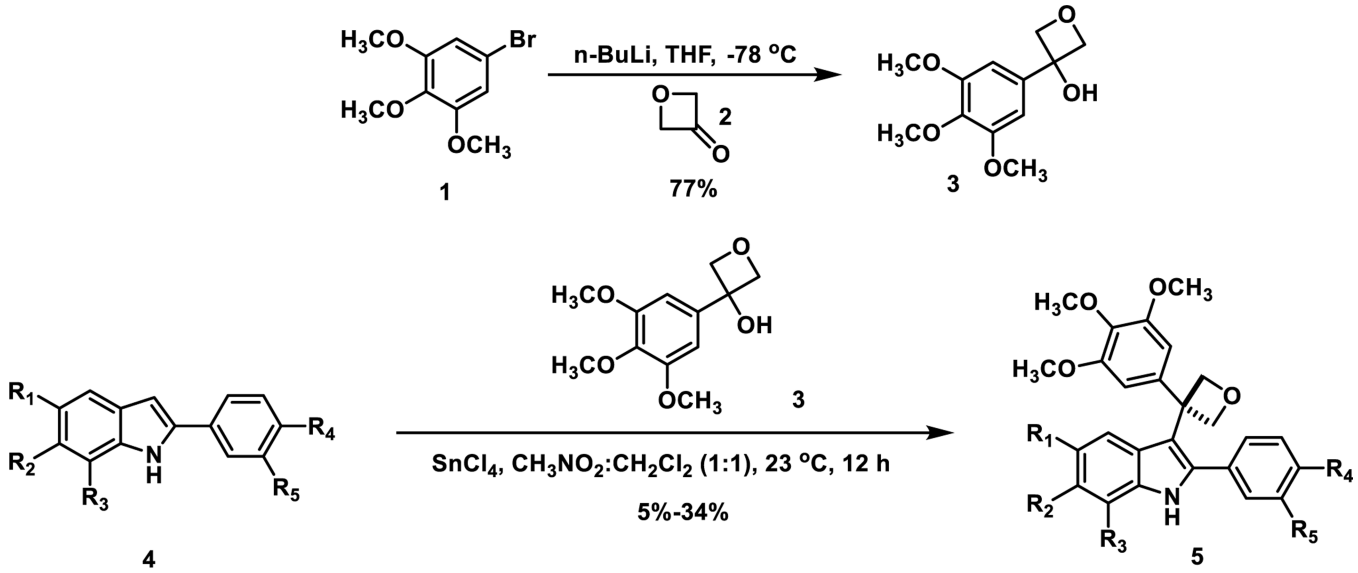
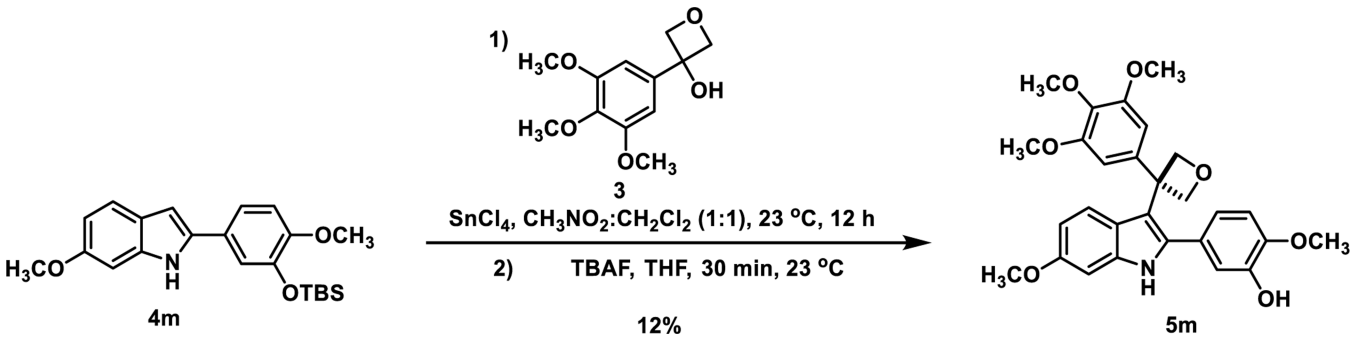


Figure 6:
Molecular Docking of Compound **5m**, α-Subunit Residues (Blue); All Other Residues are Located on the β-Subunit (Green).



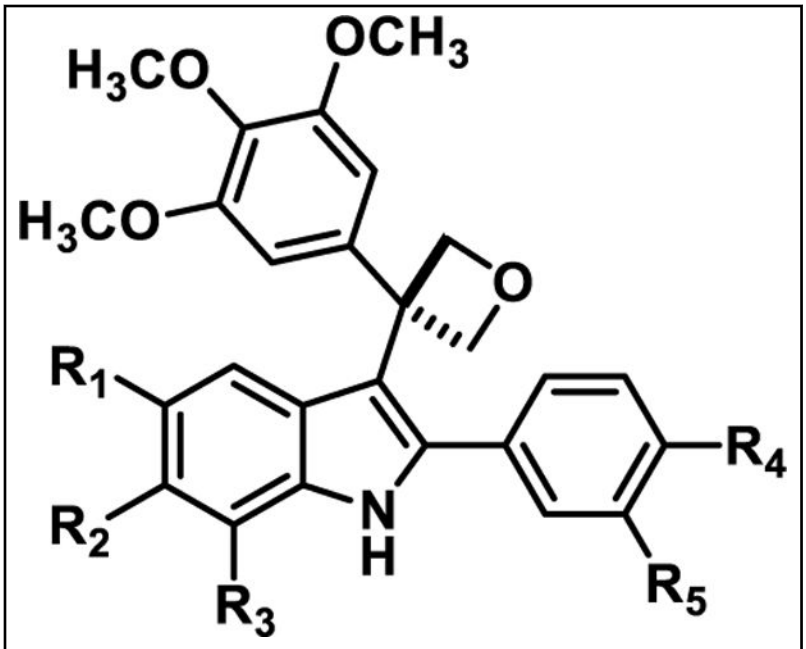
Scheme 1.
Synthesis of Indole-Based Oxetane-Containing Analogues



Scheme 2.
Synthesis of Oxetane-Substituted OXi8006

Table 1.

Oxetane-Containing Indole-based Analogues



Compound	R_1	R_2	R_3	R_4	R_5	Yield
5a	H	H	H	H	H	23%
5b	H	Cl	H	CH_3	H	31%
5c	H	Br	H	H	H	14%
5d	OCH_3	H	H	CH_3	H	5%
5e	H	Cl	H	OCH_3	H	17%
5f	H	Br	H	CH_3	H	34%
5g	H	H	H	H	OCH_3	30%
5h	H	H	OCH_3	H	H	13%
5i	H	H	H	OCH_3	H	10%
5j	H	H	H	CH_3	H	27%
5k	H	Cl	H	H	H	11%
5l	OCH_3	H	H	H	H	22%
5m	H	OCH_3	H	OCH_3	OH	12%
5n	Br	H	H	H	H	14%

Table 2.

Cytotoxicity against the MCF-7 Human Breast Cancer Cell Line

Compound	IC_{50} (μM) SRB assay MCF-7[a]
OXi8006 [b]	0.048 ± 0.01
5a	>5
5b	2.7 ± 0.2
5c	0.47 ± 0.02
5d	>5
5e	3.7 ± 0.07
5f	1.7 ± 0.2
5g	4.8 ± 0.3
5h	0.78 ± 0.2
5i	>5
5j	>5
5k	0.84 ± 0.09
5l	3.2 ± 1.0
5m	1.6 ± 0.4
5n	2.2 ± 0.5

[a] Average of n ≥ 3 independent determinations

[b] See ref[16] for additional OXi8006 data

Table 3.

Cytotoxicity Evaluation Against the MDA-MB-231 Human Breast Cancer and Pancreatic Cancer PANC-1 Cell Lines in Comparison with the MCF-7 Data

Compound	IC_{50} (μM) SRB assay MCF-7[a]	IC_{50} (μM) SRB assay MDA-MB-231[a]	IC_{50} (μM) SRB assay PANC-1[a]
OXi8006	0.048 ± 0.01	0.044 ± 0.008	ND[b]
5c	0.47 ± 0.02	1.9 ± 0.1	0.43 ± 0.1
5h	0.78 ± 0.2	3.0 ± 1.0	0.44 ± 0.01
5k	0.84 ± 0.09	2.2 ± 0.2	1.1 ± 0.5

[a] Average of n ≥ 3 independent determinations

[b] ND: not determined