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# Synthesis, biological evaluation and molecular docking studies of resveratrol derivatives possessing curcumin moiety as potent antitubulin agents

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ABSTRACT

A series of resveratrol derivatives possessing curcumin moiety were synthesized and evaluated for their antiproliferative activity against three cancer cell lines including murine melanoma B16-F10, human hepatoma HepG2 and human lung carcinoma A549. Among them, compound **C5** displayed the most potent in vitro antiproliferative activity against B16-F10 with $IC_{50}$ value of 0.71 μg/mL. Compound **C5** also exhibited good tubulin polymerization inhibitory activity with $IC_{50}$ value of 1.45 μg/mL. Furthermore, docking simulation was carried out to position **C5** into the tubulin-colchicine binding site to determine the probable binding mode.



## 1. Introduction

α,β-Tubulin heterodimers are components of both microtubules and cytoskeleton, which are involved in crucial biological process such as cell mobility, cell to cell interactions and mitosis.[1] Therefore, microtubule system has been recognized as an established target of many anticancer drugs. A number of naturally occurring compounds, such as colchicine, paclitaxel,[2] epothilone A,[3] vinblastine,[4] combretastatin A-4 (CA4),[5] and podophyllotoxin,[6] all exhibit their anticancer properties by interfering with the dynamics of tubulin polymerization or depolymerization, resulting in mitotic arrest.

However, in most cases, the clinical use of antitubulin drugs is associated with problems of significant toxicity, drug resistance, bioavailability and other side effects.[7] For this reason, scientists have been actively exploring new potent antitubulin agents. Interestingly, there is compelling evidence from epidemiological and other experimental studies that highlight the importance of many dietary agents such as resveratrol,[8] genistein,[9] napthoquinone,[10] plumbagin,[11] and curcumin[12] reducing the risk of cancer and inhibition of the development and spread of tumors in experimental animals. The advantage of using such compounds for cancer treatment is their relatively nontoxic nature and availability in an ingestive form.[13]

Curcumin (**1**, Fig. 1), originally isolated from the rhizomes of *Curcuma longa*,[14] is the active ingredient in the traditional herbal remedy and dietary Indian spice turmeric. Besides its wide range of medicinal properties like antibacterial, antifungal, antiviral, antioxidative, anti-inflammatory and antiproliferative,[15] curcumin is also known for its strong cancer preventive activity against a wide range of tumor cells and prevention of tumor initiation, promotion, metastasis, and angiogenesis in experimental animal systems.[16–21] Curcumin is currently in phase II clinical trials in patients with advanced pancreatic cancer.[22]

One of the predominant targets of curcumin is the microtubule system. Recently, Gupta et al. had shown that curcumin inhibited HeLa and MCF-7 cell proliferation, disrupted microtubule assembly in vitro, reduced GTPase activity, and partially inhibited colchicine's binding activity. They also reported that colchicine and podophyllotoxin partly inhibited the binding of curcumin to tubulin, while vinblastine had no effect on the curcumin–tubulin interaction, strongly suggesting that curcumin interacts with dimeric tubulin or microtubules.[23] More recently, Banerjee et al. from the same laboratory reported that curcumin suppressed the dynamic instability of microtubules in MCF-7 cells.[24]

Unfortunately, the clinical potential of curcumin remains limited because of its relatively low potency and poor bioavailability.[25] Attempts have been made by others to chemically modify curcumin in order to increase its activity against cancer,[26–31] see Figure 1. To continue our study on resveratrol,[32] another famous natural product that is well-known for its cancer chemopreventive activity,[8,33] we report herein the synthesis and bioactivities of a series of resveratrol

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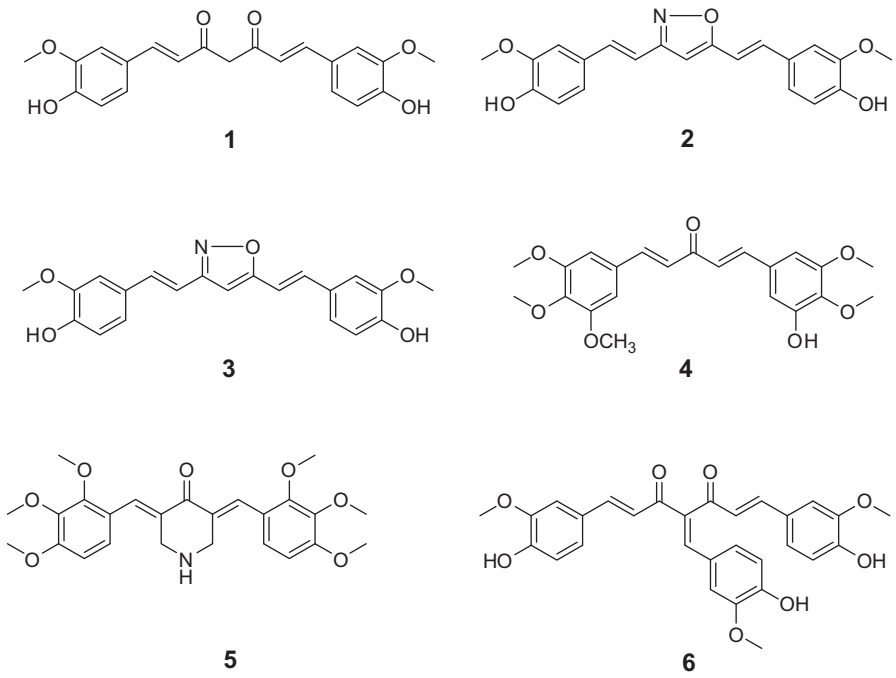



**Figure 1.** Curcumin and its analogues.

derivatives possessing curcumin moiety as potent antimitotic agents. Their antiproliferative and inhibition of tubulin polymerization activities were evaluated. Molecular modeling studies were consequently performed to understand the tubulin-inhibitor interaction.

## 2. Results and discussion

### 2.1. Synthesis

The structures of these resveratrol derivatives possessing curcumin moiety were listed in Table 1. General synthesis of them was outlined in Scheme 1. Compound **A** was prepared as previously reported.[34] Then, **C1**–**C34** were synthesized by two steps of base-catalyzed Claisen condensation reaction as previously reported with some modification.[35] The first step consisted of compound **A** with excess acetone in the presence of NaOH (2 M) to afford the intermediate **B**. Then, the target compounds (**C1**–**C34**) were prepared by sodium ethylate-catalyzed Claisen condensation of **B** and suitably substituted aromatic aldehydes or pyridinecarbaldehydes in good yield, respectively. All the synthetic compounds were purified by column chromatography or recrystallization and characterized by $^1$H NMR, elemental analysis and mass spectrum, which were in full accordance with their depicted structures.

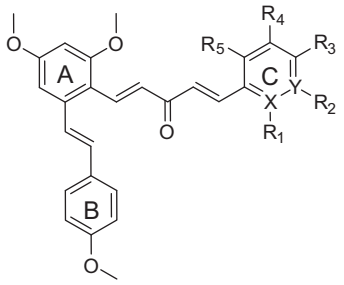
### 2.2. Biological evaluation

The antiproliferative activity of **C1**–**C34** against B16-F10, HepG2 and A549 cell lines was evaluated by using the MTT assay. The results were summarized in Table 2. All the compounds exhibited fairly good antiproliferative activity with the $IC_{50}$ values within 0.71–15.23 μg/mL, which were much better than resveratrol. Among them, the 3,5-dimethoxy derivatives (**C5**) displayed the most potent antiproliferative activity against B16-F10 cell line with the $IC_{50}$ value of 0.71 μg/mL, which was comparable to the positive control.

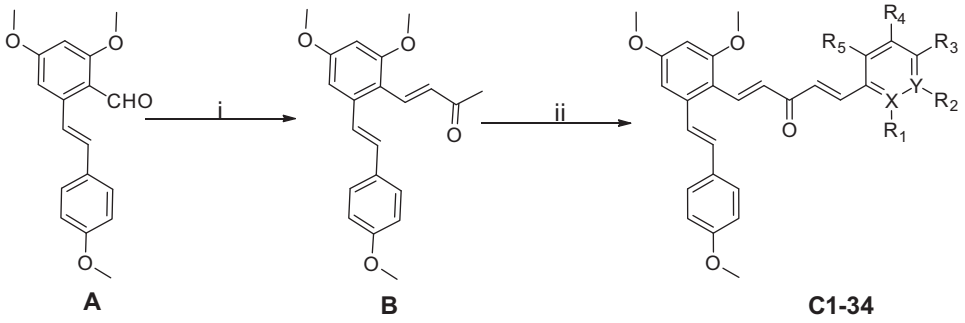
Subsequently SAR studies were performed to determine how the substituents on the C-ring affected the antiproliferative activity. Compound **C1** without any substituent on C-ring showed moderate antiproliferative activity with the mean $IC_{50}$ values of 2.66, 4.79 and 5.25 μg/mL when tested against B16-F10, HepG2 and A549, respectively. Then, we evaluated the effect of introducing different substituent groups on C-ring in **C1**. Introducing methyl or methoxy group(s) on the C-ring in **C1** to form a class of compounds greatly increased the antiproliferative activity against B16-F10 (e.g., **C5**, $IC_{50}$ = 0.71 μg/mL; **C8**, $IC_{50}$ = 1.08 μg/mL), especially for **C5** with two methoxy groups ($R_2 = R_4 = OCH_3$) whose antiproliferative activity increased about 50-fold compared to resveratrol. It can be seen that the number and the position of the methoxy group may play an important role in affecting the antiproliferative activity. A comparable increase in antiproliferative activity was also observed on introducing F and methyl groups on C-ring in **C26** ($R_1 = CH_3$, $R_4$ = F) with $IC_{50}$ value of 0.75 μg/mL against B16-F10. However, a comparable decrease was observed on introducing *ortho*- halogen groups on the C-ring. When tested against A549 cell line, the $IC_{50}$ values of compounds **C10**–**C12** were 8.01, 15.23 and 9.37 μg/mL, respectively. This decrease was also observed in **C22** ($R_1 = R_6 = Cl$). These results demonstrated that hydrophobic and electron-donating methoxy or methyl group might increase the antiproliferative activity to some great extent; however, electron-withdrawing halogen groups had slightly promotion function on the antiproliferative activity of the target compounds.

To investigate whether this series of compounds were exerting their antiproliferative activity by interacting with microtubule system, the selected compounds (**C1**–**C9**, **C26**) were evaluated for antitubulin activity in a cell-free in vitro assay. The $IC_{50}$ values of the inhibition of tubulin polymerization by these selected compounds were also shown in Table 2. Cohchicine (Col) and combretastatin A4 (CA4), two known tubulin polymerization inhibitors, were used as references. Consistent with its significant antiproliferative activity, **C5** was found to exhibit the most potent tubulin polymerization inhibitory effect with $IC_{50}$ value of 1.45 μg/mL, which was almost the similar to colchicine. This suggests that the two methoxy groups on C-ring in **C5** are playing an important role in the inhibition of tubulin polymerization.

**Table 1**
Chemical structures of compounds **C1–C34**




| Compd No. | X | Y | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Compd No. | X | Y | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C1** | C | C | H | H | H | H | H | **C18** | C | C | H | H | Br | H | H |
| **C2** | C | C | OMe | H | H | H | H | **C19** | C | C | Cl | H | Cl | H | H |
| **C3** | C | C | H | OMe | H | H | H | **C20** | C | C | H | Cl | Cl | H | H |
| **C4** | C | C | H | H | OMe | H | H | **C21** | C | C | H | Cl | H | Cl | H |
| **C5** | C | C | H | OMe | H | OMe | H | **C22** | C | C | Cl | H | H | H | Cl |
| **C6** | C | C | OMe | H | H | OMe | H | **C23** | C | C | H | Cl | F | H | H |
| **C7** | C | C | H | Me | H | H | H | **C24** | C | C | H | F | F | H | H |
| **C8** | C | C | H | H | Me | H | H | **C25** | C | C | Me | F | H | H | H |
| **C9** | C | C | H | Me | Me | H | H | **C26** | C | C | Me | H | H | F | H |
| **C10** | C | C | F | H | H | H | H | **C27** | C | C | H | $CF_3$ | H | H | H |
| **C11** | C | C | Cl | H | H | H | H | **C28** | C | C | Cl | H | H | $CF_3$ | H |
| **C12** | C | C | Br | H | H | H | H | **C29** | C | C | $NO_3$ | H | H | H | H |
| **C13** | C | C | H | F | H | H | H | **C30** | C | C | H | $NO_3$ | H | H | H |
| **C14** | C | C | H | Cl | H | H | H | **C31** | C | C | H | H | $NO_3$ | H | H |
| **C15** | C | C | H | Br | H | H | H | **C32** | C | C | H | H | $(CH_3)_2$ | H | H |
| **C16** | C | C | H | H | F | H | H | **C33** | N | C | \ | H | H | H | H |
| **C17** | C | C | H | H | Cl | H | H | **C34** | C | N | H | \ | H | H | H |




**Scheme 1.** Synthesis of compounds **C1–C34**. Reagents and conditions: (i) NaOH (2 M), acetone, room temperature; (ii) NaOEt, EtOH, substituted benzaldehydes or pyridinecarbaldehydes, room temperature.

Moreover, cell cycle analysis of **C5** was performed using flow cytometry, see Figure 2. It can be seen that **C5** induced G2/M arrest in B16-F10 cells to some extent. About 52% of the cells were arrested in G2/M phase in the presence of 1.0 μg/mL **C5**. At the same concentration, 85% of the cells were arrested in G2/M phase induced by colchicine. These findings indicated a continuing impairment of cell division and confirmed **C5** was a potent antitubulin agent.

### 2.3. Binding mode of compound C5 into tubulin-colchicine site

To gain better understanding on the potency of the studied compounds and guide further SAR studies, we proceeded to examine the interactions of **C5** with tubulin-colchicine binding site of β-tubulin (PDB code: 1SA0). All docking runs were applied the Lamarckian genetic algorithm of Auto-Dock 4.0. The binding mode of **C5** and tubulin-colchicine site was depicted in Figure 3. All the amino acid residues which had interactions with tubulin-colchicine binding site were exhibited. Our aim was to vary the substitutions on the C-ring so that they selectively inhibit formation of the α,β-dimer. In the binding mode, **C5** is nicely bound to the tubulin-colchicine binding site via hydrogen bonds and hydrophobic interactions. The oxygen atom of the monocarbonyl curcumin system formed one hydrogen bond with the amino hydrogen of GLN11 (bond length: GLN11N–H···O = 2.193 Å; bond angle: GLN11N–H···O = 140.1°). The oxygen atom of one of the methoxyl groups on C-ring in **C5** formed another hydrogen bond with the amino hydrogen of ASN101 (bond length: ASN101N–H···O = 1.888 Å; bond angle: ASN101N–H···O = 151.3°). The hydrophobic interactions may play an auxiliary role in stabilizing the interaction between **C5** and the tubulin-colchicine binding site.

The 3D model of the interaction between **C5** and the tubulin-colchicine binding site was depicted in Figure 4, showing well binding affinity to the target. We can clearly see that the hydrophobic pockets of the tubulin-colchicine binding site are occupied by a methoxy group on C-ring and the monocarboxyl group of the curcumin moiety. The two methoxyl groups on C-ring may play a

**Table 2**
Antiproliferative activity ($IC_{50}$) against B16-F10, HepG2 and A549 cell lines of compounds **C1–C34** and antitubulin activity ($IC_{50}$) of the selected compounds

| Compd No. | $IC_{50}$ ± SD (μg/mL) | | | |
|---|---|---|---|---|
| | B16-F10[a] | HepG2[a] | A549[a] | Tubulin[b] |
| **C1** | 2.66 ± 0.71 | 4.79 ± 1.30 | 5.25 ± 0.91 | 6.28 ± 0.85 |
| **C2** | 2.13 ± 0.25 | 5.82 ± 0.53 | 6.49 ± 1.22 | 5.99 ± 0.62 |
| **C3** | 2.72 ± 0.53 | 8.52 ± 2.10 | 7.66 ± 1.47 | 8.05 ± 1.24 |
| **C4** | 2.40 ± 0.46 | 6.99 ± 1.44 | 7.65 ± 1.63 | 2.89 ± 0.38 |
| **C5** | 0.71 ± 0.11 | 1.60 ± 0.12 | 2.10 ± 0.53 | 1.45 ± 0.22 |
| **C6** | 1.30 ± 0.24 | 2.78 ± 0.64 | 4.09 ± 0.94 | 2.11 ± 0.35 |
| **C7** | 1.67 ± 0.26 | 4.58 ± 1.36 | 4.08 ± 0.42 | 3.08 ± 0.21 |
| **C8** | 1.08 ± 0.13 | 2.37 ± 0.42 | 2.69 ± 0.44 | 2.68 ± 0.42 |
| **C9** | 1.57 ± 0.12 | 4.12 ± 1.13 | 5.33 ± 0.32 | 3.57 ± 0.54 |
| **C10** | 2.51 ± 0.28 | 4.85 ± 1.58 | 8.01 ± 0.80 | –[d] |
| **C11** | 5.94 ± 0.62 | 12.45 ± 2.43 | 15.23 ± 2.52 | – |
| **C12** | 3.09 ± 0.30 | 10.24 ± 4.21 | 9.37 ± 1.26 | – |
| **C13** | 1.67 ± 0.22 | 2.65 ± 0.24 | 4.47 ± 0.82 | – |
| **C14** | 2.99 ± 0.45 | 6.94 ± 2.11 | 8.23 ± 1.84 | – |
| **C15** | 1.97 ± 0.23 | 5.52 ± 0.97 | 4.75 ± 1.01 | – |
| **C16** | 1.81 ± 0.31 | 4.35 ± 0.73 | 5.83 ± 1.63 | – |
| **C17** | 3.17 ± 0.42 | 8.29 ± 1.62 | 11.15 ± 4.65 | – |
| **C18** | 2.44 ± 0.26 | 7.75 ± 0.97 | 7.89 ± 2.15 | – |
| **C19** | 2.11 ± 0.52 | 4.67 ± 0.53 | 5.05 ± 0.93 | – |
| **C20** | 2.73 ± 0.47 | 9.49 ± 1.29 | 6.99 ± 1.68 | – |
| **C21** | 2.08 ± 0.34 | 3.28 ± 0.42 | 6.18 ± 1.32 | – |
| **C22** | 3.07 ± 0.64 | 9.31 ± 0.80 | 8.93 ± 1.94 | – |
| **C23** | 1.79 ± 0.28 | 9.07 ± 1.82 | 4.65 ± 0.85 | – |
| **C24** | 2.23 ± 0.22 | 5.97 ± 1.23 | 6.23 ± 1.30 | – |
| **C25** | 1.80 ± 0.16 | 2.74 ± 0.32 | 3.98 ± 0.63 | – |
| **C26** | 0.75 ± 0.12 | 1.96 ± 0.24 | 2.32 ± 0.21 | 1.81 ± 0.43 |
| **C27** | 3.10 ± 0.27 | 5.38 ± 0.72 | 4.97 ± 0.41 | – |
| **C28** | 1.85 ± 0.42 | 6.79 ± 0.96 | 5.96 ± 1.96 | – |
| **C29** | 3.43 ± 0.67 | 6.75 ± 0.81 | 11.65 ± 3.43 | – |
| **C30** | 1.47 ± 0.34 | 2.44 ± 0.32 | 3.09 ± 0.68 | – |
| **C31** | 2.35 ± 0.48 | 6.95 ± 0.65 | 6.69 ± 1.20 | – |
| **C32** | 2.24 ± 0.37 | 4.43 ± 1.17 | 6.06 ± 1.12 | – |
| **C33** | 3.12 ± 0.21 | 9.17 ± 2.32 | 9.16 ± 2.14 | – |
| **C34** | 1.48 ± 0.13 | 3.50 ± 0.97 | 3.48 ± 0.92 | – |
| **B** | 2.88 ± 0.36 | 8.41 ± 1.78 | 6.97 ± 0.76 | – |
| RES[c] | 37.3 ± 3.0 | 38.9 ± 6.0 | 30.4 ± 4.0 | – |
| Col[c] | 0.46 ± 0.04 | 0.21 ± 0.02 | 0.11 ± 0.06 | 1.28 ± 0.4 |
| CA4[c] | 0.38 ± 0.15 | 0.18 ± 0.03 | 0.13 ± 0.02 | 0.69 ± 0.2 |

[a] Inhibition the growth of tumor cell lines.
[b] Inhibition of tubulin polymerization.
[c] Data from Ref. 32.
[d] Not measured.

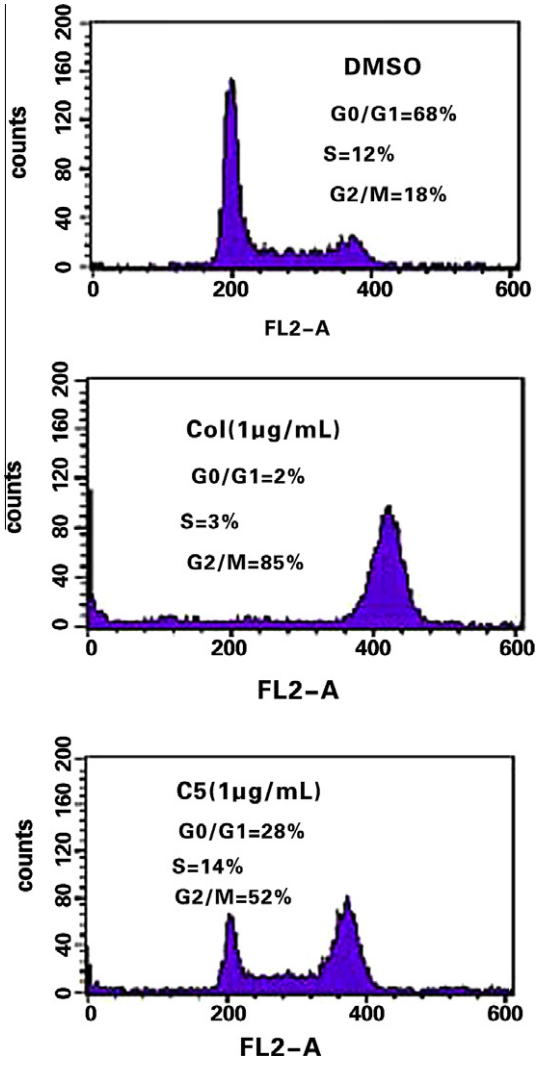



**Figure 2.** Effects of compound **C5** on cell cycle progression of B16-F10 cells were determined by flow cytometry analysis. B16-F10 cells were treated with **C5** at the concentration of 1 μg/mL for 24 h. The percentage of cells in each cycle phase was indicated.

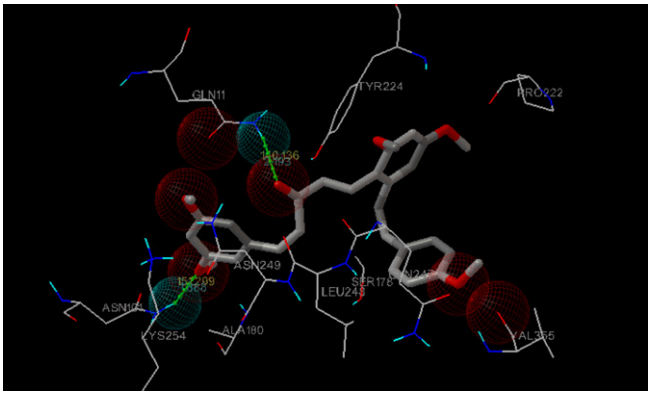



**Figure 3.** Binding mode of compound **C5** with tubulin-colchicine site. The molecules are colored by the atoms (carbons, gray; nitrogen, blue; oxygen, red). The green dotted lines show the hydrogen bonds. Hydrophobic interactions were shown as red ball.

role as anchor to bind with tubulin. The methoxyl group on the B-ring in the stilbene moiety also exhibited good affinity towards tubulin via hydrophobic interactions. The results of the molecular docking study indicated that the resveratrol skeleton and the monocarbonyl curcumin moiety could act synergistically to interact with the tubulin-colchicine binding site, suggesting that **C5** is a potential inhibitor of tubulin.

## 3. Conclusions

In summary, a series of resveratrol derivatives possessing curcumin moiety were prepared and evaluated for antiproliferative and tubulin polymerization inhibition activities. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(3,5-dimethoxyphenyl) penta-1,4-dien-3-one (**C5**) showed the most potent antiproliferative activity against B16-F10 cell line with the $IC_{50}$ value of 0.71 μg/mL. Correlated with its antiproliferative activity, **C5** also exhibited the most potent antitubulin activity to the same extent as colchicine with $IC_{50}$ value of 1.45 μg/mL. Molecular docking analysis of the binding conformation of **C5** into the tubulin-colchicine binding site demonstrated that hydrogen bonds and hydrophobic interactions with the protein residues might lead to the antitubulin polymerization and antiproliferative activities. The results of this work might be helpful for discovering novel class of potent antimitomic agents.

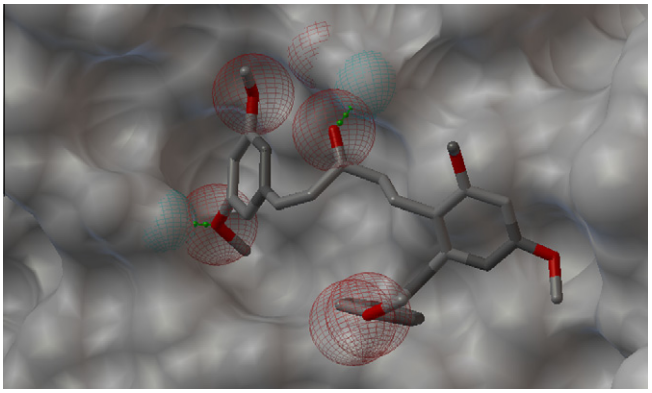


**Figure 4.** 3D mode of the interaction between compound **C5** and the tubulin-colchicine site. The protein is represented by surface. **C5** is depicted by sticks and balls.

## 4. Experimental

### 4.1. General

All the chemicals used were commercial products employed without further purification. The $^1$H NMR spectra were recorded on a Bruker DRX 400 model spectrometer in $CDCl_3$ solutions at room temperature with TMS as an internal standard. Chemical shifts ($\delta$) for $^1$H NMR spectra were reported in parts per million to residual solvent protons. Melting points were measured on a Boetius micro melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Carbon, hydrogen and nitrogen assays were carried out with a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values. TLC was run on the silica gel coated aluminum sheets (Silica Gel 60 GF254, E. Merk, Germany) and visualized in UV light (254 nm).

### 4.2. Synthesis method for (*E*)-4-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)but-3-en-2-one (B)

To a solution of (*E*)-2,4-dimethoxy-6-(4-methoxystyryl)benzaldehyde (**A**) (5.96 g, 20 mmol) in 100 mL of acetone was added 2 mL of NaOH (2 M), and the reaction mixture was stirred for 12 h at room temperature. Then, the solvent was concentrated, 100 mL of water was added, and the yellow solid that formed was collected by filtration, washed with water and cold hexane, and then dried under vacuum to afford 4.92 g **B**. Yield: 72.8%. Mp: 125–127 °C. $^1$H NMR ($CDCl_3$): 2.35 (s, 3H), 3.83 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 6.41 (s, 1H), 6.69 (s, 1H), 6.72 (d, 1H, $J$ = 12.4 Hz), 6.89–6.93 (dd, 3H, $J$ = 4.0, 12.8 Hz), 7.21 (d, 1H, $J$ = 16.4 Hz), 7.44 (d, 2H, $J$ = 8.8 Hz), 7.87(d, 1H, $J$ = 16.4 Hz). MS (ESI): 339.4 ($C_{21}H_{22}O_4$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55. Found: C, 74.42; H, 6.57.

### 4.3. General procedure: preparation of compounds C1–34

To a solution of **B** (0.338 g, 1 mmol) and substituted aromatic aldehydes (1 mmol) in 20 mL of ethanol was added EtONa (0.136 g, 2 mmol). The reaction mixture was stirred for 12 h at room temperature. The precipitate was collected by filtration, washed with water, and recrystallized from methanol or purified by silica gel chromatography to afford the target products **C1**–**C32**.

Compounds **C33** and **C34** were obtained when the intermediate **B** was reacted with 2-pyridinecarbaldehyde or 3-pyridinecarbaldehyde by running the same procedure as mentioned above, respectively.

#### 4.3.1. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-phenylpenta-1,4-dien-3-one (C1)

Yellow solid (0.346 g, 81.2% yield). Mp 94–95 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.73 (d, 1H, $J$ = 2.4 Hz), 6.70 (d, 2H, $J$ = 8.8 Hz), 6.94 (d, 1H, $J$ = 16.4), 7.03 (d, 1H, $J$ = 16.0 Hz), 7.10 (d, 1H, $J$ = 16.0 Hz), 7.31 (d, 1H, $J$ = 16.0 Hz), 7.36–7.37 (m, 3H), 7.47–7.52 (m, 4H), 7.65 (d, 1H, $J$ = 16.0 Hz), 8.12 (d, 1H, $J$ = 15.6 Hz). MS (ESI): 427.5 ($C_{28}H_{26}O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{26}O_4$: C, 78.85; H, 6.14. Found: C, 78.73; H, 6.17.

#### 4.3.2. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(2-methoxyphenyl)penta-1,4-dien-3-one (C2)

Yellow solid (0.368 g, 80.6% yield). Mp 103–105 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.81 (s, 3H), 3.82 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.73 (d, 1H, $J$ = 2.0 Hz), 6.88–6.97 (m, 5H), 7.10 (d, 1H, $J$ = 7.2 Hz), 7.14 (d, 1H, $J$ = 6.4 Hz), 7.33 (d, 2H, $J$ = 16.0 Hz), 7.47 (d, 2H, $J$ = 8.4 Hz), 7.54 (dd, 1H, $J$ = 1.2, 7.6 Hz), 8.01 (d, 1H, $J$ = 16.0 Hz), 8.09 (d, 1H, $J$ = 15.6 Hz). MS (ESI): 457.2 ($C_{29}H_{28}O_5$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{28}O_5$: C, 76.30; H, 6.18. Found: C, 76.13; H, 6.20.

#### 4.3.3. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(3-methoxyphenyl)penta-1,4-dien-3-one (C3)

Yellow solid (0.385 g, 84.3% yield). Mp 121–123 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.81 (s, 3H), 3.83 (s, 3H), 3.91 (s, 6H), 6.44 (d, 1H, $J$ = 2.1 Hz), 6.72 (d, 1H, $J$ = 2.4 Hz), 6.89–6.94 (m, 4H), 6.97 (d, 1H, $J$ = 2.7 Hz), 7.03–7.12 (m, 3H), 7.25–7.34 (m, 2H), 7.47–7.49 (m, 2H), 7.61 (d, 1H, $J$ = 16.2 Hz), 8.12 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 457.5 ($C_{29}H_{28}O_5$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{28}O_5$: C, 76.30; H, 6.18. Found: C, 76.49; H, 6.20.

#### 4.3.4. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(4-methoxyphenyl)penta-1,4-dien-3-one (C4)

Yellow solid (0.419 g, 91.7% yield). Mp 150–152 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.84 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.72 (d, 1H, $J$ = 2.0 Hz), 6.87–6.92 (m, 5H), 6.94 (d, 1H, $J$ = 16.6 Hz), 7.07 (d, 1H, $J$ = 16.0 Hz), 7.32 (d, 1H, $J$ = 16.0 Hz), 7.45–7.49 (m, 4H), 7.62 (d, 1H, $J$ = 15.6 Hz), 8.09 (d, 1H, $J$ = 16.0 Hz).

MS (ESI): 457.3 ($C_{29}H_{28}O_5$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{28}O_5$: C, 76.30; H, 6.18. Found: C, 76.52; H, 6.16.

#### 4.3.5. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(3,5-dimethoxyphenyl)penta-1,4-dien-3-one (C5)

Yellow solid (0.372 g, 76.4% yield). Mp 109–111 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.79 (s, 6H), 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.1 Hz), 6.49 (t, 1H, $J$ = 2.1 Hz), 6.66–6.67 (m, 2H), 6.72 (d, 1H, $J$ = 2.1 Hz), 6.90 (m, 2H), 6.93 (d, 1H, $J$ = 10.8), 6.98 (d, 1H, $J$ = 11.1), 7.09 (d, 1H, $J$ = 15.9 Hz), 7.31 (d, 1H, $J$ = 16.2 Hz), 7.46–7.49 (m, 2H), 7.57 (d, 1H, $J$ = 15.9 Hz), 8.11 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 487.5 ($C_{30}H_{30}O_6$, $[M+H]^+$). Anal. Calcd for $C_{30}H_{30}O_6$: C, 74.06; H, 6.21. Found: C, 74.26; H, 6.20.

#### 4.3.6. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)-5-(2,5-dimethoxyphenyl)penta-1,4-dien-3-one (C6)

Yellow solid (0.385 g, 79.1% yield). Mp 90–91 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.76 (s, 3H), 3.78 (s, 3H), 3.82 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.73 (d, 1H, $J$ = 2.4 Hz), 6.81–6.92 (m, 4H), 6.97–7.04 (m, 1H), 7.10–7.16 (m, 3H), 7.33 (d, 1H, $J$ = 16.2 Hz), 7.45–7.48 (m, 2H), 7.99 (d, 1H, $J$ = 16.2 Hz), 8.09 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 487.6 ($C_{30}H_{30}O_6$, $[M+H]^+$). Anal. Calcd for $C_{30}H_{30}O_6$: C, 74.06; H, 6.21. Found: C, 73.94; H, 6.24.

#### 4.3.7. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl) -5-(m-tolyl)penta-1,4-dien-3-one (C7)

Yellow solid (0.326 g, 74.1% yield). Mp 99–101 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 2.36 (s, 3H), 3.83 (s, 3H), 3.91 (s, 6H), 6.44 (d, 1H, $J$ = 2.8 Hz), 6.72 (d, 1H, $J$ = 2.8 Hz), 6.88–6.98 (m, 4H), 7.06 (d, 1H, $J$ = 15.6 Hz), 7.04–7.23 (m, 2H), 7.28–7.35 (m, 3H), 7.46–7.50 (m, 2H), 7.62 (d, 1H, $J$ = 16.2 Hz), 8.12 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 441.5 ($C_{29}H_{28}O_4$ $[M+H]^+$). Anal. Calcd for $C_{29}H_{28}O_4$: C, 79.07; H, 6.41. Found: C, 78.90; H, 6.42.

#### 4.3.8. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl) -5-(*p*-tolyl)penta-1,4-dien-3-one (C8)

Yellow solid (0.332 g, 75.4% yield). Mp 114–115 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 2.40 (s, 3H), 3.86 (s, 3H), 3.92 (s, 6H), 6.46 (d, 1H, $J$ = 2.0 Hz), 6.74 (d, 1H, $J$ = 2.4 Hz), 6.92–6.95 (m, 2H), 7.01 (d, 2H, $J$ = 15.6 Hz), 7.11 (d, 1H, $J$ = 16.0 Hz), 7.19 (d, 2H, $J$ = 8.0 Hz), 7.34 (d, 1H, $J$ = 16.4 Hz), 7.43 (d, 2H, $J$ = 8.0 Hz), 7.50 (d, 2H, $J$ = 8.8 Hz), 7.65 (d, 1H, $J$ = 16.0 Hz), 8.13 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 441.4 ($C_{29}H_{28}O_4$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{28}O_4$: C, 79.07; H, 6.41. Found: C, 78.27; H, 6.39.

#### 4.3.9. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl)phenyl) -5-(3,4-dimethylphenyl)penta-1,4-dien-3-one (C9)

Yellow solid (0.311 g, 68.5% yield). Mp 119–121 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 2.26 (s, 3H), 2.28 (s, 3H), 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.8 Hz), 6.72 (d, 1H, $J$ = 2.8 Hz), 6.89–6.95 (m, 3H), 6.98 (d, 1H, $J$ = 8.7 Hz), 7.06–7.14 (m, 2H,), 7.25–7.27 (m, 2H), 7.32 (d, 1H, $J$ = 15.9 Hz), 7.47–7.50 (m, 2H), 7.61 (d, 1H, $J$ = 15.9 Hz), 8.11 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 455.6 ($C_{30}H_{30}O_4$, $[M+H]^+$). Anal. Calcd for $C_{30}H_{30}O_4$: C, 79.27; H, 6.65. Found: C, 79.10; H, 6.67.

#### 4.3.10. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(2-fluorophenyl)penta-1,4-dien-3-one (C10)

Yellow solid (0.325 g, 73.2% yield). Mp 95–96 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.73 (d, 1H, $J$ = 2.4 Hz), 6.90 (d, 2H, $J$ = 8.7), 6.94 (d, 1H, $J$ = 16.0 Hz), 7.07–7.17 (m, 4H), 7.32 (d, 2H, $J$ = 16.0 Hz), 7.48 (d, 2H, $J$ = 8.8 Hz), 7.54–7.58 (m, 1H), 7.80 (d, 1H, $J$ = 16.0 Hz), 8.12 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 445.2 ($C_{28}H_{25}FO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}FO_4$: C, 75.66; H, 5.67. Found: C, 75.88; H, 5.65.

#### 4.3.11. (1*E*,4*E*)-1-(2-Chlorophenyl)-5-(2,4-dimethoxy-6-((*E*)- 4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C11)

Yellow solid (0.352 g, 76.4% yield). Mp 110–112 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.73 (d, 1H, $J$ = 2.4 Hz), 6.89 (d, 2H, $J$ = 8.8 Hz), 6.94 (d, 1H, $J$ = 16.0 Hz), 6.99 (d, 1H, $J$ = 16.0 Hz), 7.17 (d, 1H, $J$ = 15.6 Hz), 7.27 (d, 1H, $J$ = 2.0 Hz), 7.31 (d, 2H, $J$ = 16.0 Hz), 7.41 (dd, 1H, $J$ = 1.6, 8.0 Hz), 7.45–7.48 (m, 2H), 7.66 (dd, 1H, $J$ = 2.0, 7.2 Hz), 8.06 (d, 1H, $J$ = 16.0 Hz), 8.12 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 461.9 ($C_{28}H_{25}ClO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}ClO_4$: C, 72.96; H, 5.47. Found: C, 72.85; H, 5.49.

#### 4.3.12. (1*E*,4*E*)-1-(2-Bromophenyl)-5-(2,4-dimethoxy-6-((*E*)- 4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C12)

Yellow solid (0.395 g, 78.2% yield). Mp 118–120 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.73 (d, 1H, $J$ = 2.0 Hz), 6.89–6.92 (m, 3H), 6.96 (d, 1H, $J$ = 4 Hz), 7.17–7.23 (m, 2H), 7.29–7.33 (m, 2H), 7.46 (d, 2H, $J$ = 8.8 Hz), 7.60 (d, 1H, $J$ = 8.0 Hz), 7.65 (d, 1H, $J$ = 7.6 Hz), 8.01 (d, 1H, $J$ = 16.0 Hz), 8.13 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 506.4 ($C_{28}H_{25}BrO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}BrO_4$: C, 66.54; H, 4.99. Found: C, 66.77; H, 4.50.

#### 4.3.13. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(3-fluorophenyl)penta-1,4-dien-3-one (C13)

Yellow solid (0.356 g, 80.1% yield). Mp 99–100 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, $J$ = 2.8 Hz), 6.72 (d, 1H, $J$ = 2.1 Hz), 6.89–7.11 (m, 6H), 7.18–7.22 (m, 1H), 7.28–7.34 (m, 3H), 7.47–7.50 (m, 2H), 7.58 (d, 1H, $J$ = 15.9 Hz), 8.13 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 445.5 ($C_{28}H_{25}FO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}FO_4$: C, 75.66; H, 5.67. Found: C, 75.87; H, 5.68.

#### 4.3.14. (1*E*,4*E*)-1-(3-Chlorophenyl)-5-(2,4-dimethoxy-6-((*E*)- 4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C14)

Yellow solid (0.390 g, 84.5% yield). Mp 98–99 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.91 (s, 6H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.72 (d, 1H, $J$ = 2.0 Hz), 6.90–7.11 (m, 5H), 7.26–7.39 (m, 4H), 7.47–7.49 (m, 3H), 7.57 (d, 1H, $J$ = 16.0 Hz), 8.13 (d, 1H, $J$ = 15.6 Hz). MS (ESI): 461.9 ($C_{28}H_{25}ClO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}ClO_4$: C, 72.96; H, 5.47. Found: C, 72.84; H, 5.48.

#### 4.3.15. (1*E*,4*E*)-1-(3-Bromophenyl)-5-(2,4-dimethoxy-6-((*E*)- 4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C15)

Yellow solid (0.424 g, 83.9% yield). Mp 102–104 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.91 (s, 6H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.72 (d, 1H, $J$ = 2.40 Hz), 6.90–7.02 (m, 4H), 7.09 (d, 1H, $J$ = 15.6 Hz), 7.22 (d, 1H, $J$ = 7.6 Hz), 7.30 (d, 1H, $J$ = 16.0 Hz), 7.41–7.49 (m, 4H), 7.55 (d, 1H, $J$ = 16.0 Hz), 7.66 (s, 1H), 8.12 (d, 1H, $J$ = 15.6 Hz). MS (ESI): 506.4 ($C_{28}H_{25}BrO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}BrO_4$: C, 66.54; H, 4.99. Found: C, 66.40; H, 5.00.

#### 4.3.16. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(4-fluorophenyl)penta-1,4-dien-3-one (C16)

Yellow solid (0.380 g, 85.6% yield). Mp 106–108 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.72 (d, 1H, $J$ = 2.0 Hz), 6.89–6.96 (m, 4H), 7.03–7.09 (m, 3H), 7.30 (d, 1H, $J$ = 16.4 Hz), 7.46–7.49 (m, 4H), 7.60 (d, 1H, $J$ = 16.0 Hz), 8.11 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 445.2 ($C_{28}H_{25}FO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}FO_4$: C, 75.66; H, 5.67. Found: C, 75.54; H, 5.68.

#### 4.3.17. (1*E*,4*E*)-1-(4-Chlorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4- methoxystyryl)phenyl)penta-1,4-dien-3-one (C17)

Yellow solid (0.407 g, 88.3% yield). Mp 137–139 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.72 (d, 1H, $J$ = 2.4 Hz), 6.89–6.96 (m, 3H), 6.98 (d, 1H, $J$ = 16.0 Hz), 7.08 (d, 1H, $J$ = 15.6 Hz), 7.28–7.34 (m, 3H), 7.43 (d, 2H, $J$ = 8.4 Hz), 7.48 (d, 2H, $J$ = 8.8 Hz), 7.58 (d, 1H, $J$ = 16.0 Hz), 8.12 (d, 1H, $J$ = 16. 0 Hz). MS (ESI): 461.7 ($C_{28}H_{25}ClO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}ClO_4$: C, 72.96; H, 5.47. Found: C, 72.84; H, 5.48.

#### 4.3.18. (1*E*,4*E*)-1-(4-Bromophenyl)-5-(2,4-dimethoxy-6-((*E*)-4- methoxystyryl)phenyl)penta-1,4-dien-3-one (C18)

Yellow solid (0.441 g, 87.3% yield). Mp 139–141 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.0 Hz), 6.72 (d, 1H, $J$ = 2.0 Hz), 6.89–6.96 (m, 3H), 6.99 (d, 1H, $J$ = 16.0 Hz), 7.08 (d, 1H, $J$ = 16.0 Hz), 7.30 (d, 1H, $J$ = 16.0 Hz), 7.36 (d, 2H, $J$ = 8.4 Hz), 7.46–7.50 (m, 4H), 7.56 (d, 1H, $J$ = 16.0 Hz), 8.12 (d, 1H, $J$ = 16.0 Hz). MS (ESI): 505.4 ($C_{28}H_{25}BrO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}BrO_4$: C, 66.54; H, 4.99. Found: C, 66.73; H, 5.00.

#### 4.3.19. (1*E*,4*E*)-1-(2,4-Dichlorophenyl)-5-(2,4-dimethoxy-6-((*E*)- 4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C19)

Yellow solid (0.335 g, 67.6% yield). Mp 117–119 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.86 (s, 3H), 3.92 (s, 3H), 3.93 (s, 3H), 6.44 (d, 1H, $J$ = 2.4 Hz), 6.74 (d, 1H, $J$ = 2.4 Hz), 6.92 (d, 2H, $J$ = 8.8 Hz), 6.96 (d, 1H, $J$ = 16.0 Hz), 6.99 (d, 1H, $J$ = 16.0 Hz), 7.17

(d, 1H, *J* = 15.6 Hz), 7.23–7.26 (m, 1H), 7.33 (d, 1H, *J* = 16.0 Hz), 7.43–7.48 (m, 3H), 7.61 (d, 1H, *J* = 8.4 Hz), 8.00 (d, 1H, *J* = 16 Hz), 8.16 (d, 1H, *J* = 16.0 Hz). MS (ESI): 496.4 ($C_{28}H_{24}Cl_2O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}Cl_2O_4$: C, 67.89; H, 4.88. Found: C, 67.78; H, 4.90.

#### 4.3.20. (1*E*,4*E*)-1-(3,4-Dichlorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C20)

Yellow solid (0.343 g, 69.2% yield). Mp 116–117 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.86 (s, 3H), 3.93 (s, 6H), 6.46 (d, 1H, *J* = 2.0 Hz), 6.72 (d, 1H, *J* = 2.0 Hz), 6.92–6.98 (m, 4H), 7.09 (d, 1H, *J* = 16.0 Hz), 7.11–7.25 (m, 2H), 7.30–7.34 (m, 2H), 7.50 (d, 2H, *J* = 8.8 Hz), 7.55 (d, 1H, *J* = 16.0 Hz), 8.15 (d, 1H, *J* = 16.0 Hz). MS (ESI): 416.18 ($C_{28}H_{24}Cl_2O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}Cl_2O_4$: C, 67.89; H, 4.88. Found: C, 68.06; H, 4.87.

#### 4.3.21. (1*E*,4*E*)-1-(3,5-Dichlorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C21)

Yellow solid (0.361 g, 72.8% yield). Mp 161–1630 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.43 (d, 1H, *J* = 1.8 Hz), 6.71 (d, 1H, *J* = 2.8 Hz), 6.90–6.96 (m, 3H), 6.99 (d, 1H, *J* = 15.9 Hz), 7.08 (d, 1H, *J* = 15.9 Hz), 7.30 (d, 1H, *J* = 15.9 Hz), 7.34–7.37 (m, 3H), 7.46–7.51 (m, 3H), 8.13 (d, 1H, *J* = 15.9 Hz). MS (ESI): 496.3 ($C_{28}H_{24}Cl_2O_4$ $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}Cl_2O_4$: C, 67.89; H, 4.88. Found: C, 67.74; H, 4.90.

#### 4.3.22. (1*E*,4*E*)-1-(2,6-Dichlorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C22)

Yellow solid (0.326 g, 65.9% yield). Mp 97–98 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, *J* = 2.4 Hz), 6.72 (d, 1H, *J* = 2.0 Hz), 6.89 (d, 2H, *J* = 8.4 Hz), 6.93 (d, 1H, *J* = 16.4 Hz), 7.11 (d, 1H, *J* = 16.0 Hz), 7.14–7.19 (m, 2H), 7.30 (d, 1H, *J* = 16.0 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.46 (d, 2H, *J* = 8.8 Hz), 7.73 (d, 1H, *J* = 16.4 Hz), 8.13 (d, 1H, *J* = 15.6 Hz). MS (ESI): 496.5 ($C_{28}H_{24}Cl_2O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}Cl_2O_4$: C, 67.89; H, 4.88. Found: C,67.80; H, 4.89.

#### 4.3.23. (1*E*,4*E*)-1-(3-chloro-4-fluorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C23)

Yellow solid (0.360 g, 75.1% yield). Mp 98–100 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, *J* = 2.0 Hz), 6.72 (d, 1H, *J* = 2.0 Hz), 6.90–6.96 (m, 4H), 7.07 (d, 1H, *J* = 16.0 Hz), 7.09–7.15 (m, 1H), 7.30 (d, 1H, *J* = 16.0 Hz), 7.35–7.38 (m, 1H), 7.47–7.56 (m, 4H), 8.12 (d, 1H, *J* = 16.0 Hz). MS (ESI): 479.9 ($C_{28}H_{24}ClFO_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}ClFO_4$: C, 70.22; H, 5.05. Found: C, 70.08; H, 5.07.

#### 4.3.24. (1*E*,4*E*)-1-(3,4-Difluorophenyl)-5-(2,4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C24)

Yellow solid (0.357 g, 77.2% yield). Mp 120–122 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.44 (d, 1H, *J* = 2.0 Hz), 6.72 (d, 1H, *J* = 2.4 Hz), 6.90–6.96 (m, 3H), 6.98 (d, 1H, *J* = 16.0 Hz), 7.08 (d, 1H, *J* = 15.6 Hz), 7.28–7.33 (m, 2H), 7.42–7.49 (m, 3H), 7.52 (d, 1H, *J* = 16.0 Hz), 7.58 (d, 1H, *J* = 1.6 Hz), 8.13 (d, 1H, *J* = 16.0 Hz). MS (ESI): 463.5 ($C_{28}H_{24}F_2O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{24}F_2O_4$: C, 72.72; H, 5.23. Found: C, 72.56; H, 5.24.

#### 4.3.25. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(3-fluoro-2-methylphenyl)penta-1,4-dien-3-one (C25)

Yellow solid (0.301 g, 65.6% yield). Mp 101–103 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 2.34 (s, 3H), 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, *J* = 2.8 Hz), 6.72 (d, 1H, *J* = 2.1 Hz), 6.88–6.96 (m, 5H), 7.08 (d, 1H, *J* = 15.9 Hz), 7.12–7.17 (m, 1H), 7.27–7.34 (m, 2H), 7.45–7.48 (m, 2H), 7.88 (dd, 1H, *J* = 1.5, 15.6 Hz), 8.13 (d, 1H, *J* = 15.9 Hz). MS (ESI): 459.5 ($C_{29}H_{27}FO_4$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{27}FO_4$: C, 75.96; H, 5.94. Found: C, 75.78; H, 5.957.

#### 4.3.26. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(5-fluoro-2-methylphenyl)penta-1,4-dien-3-one (C26)

Yellow solid (0.314 g, 68.4% yield). Mp 92–93 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 2.28 (d, 3H, *J* = 2.1 Hz), 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, *J* = 2.1 Hz), 6.72 (d, 1H, *J* = 2.8 Hz), 6.87–6.91 (m, 2H), 6.95 (d, 1H, *J* = 15.6 Hz), 6.98–7.19 (m, 4H), 7.31 (d, 1H, *J* = 16.2 Hz), 7.38 (d, 1H, *J* = 7.5 Hz), 7.46 (m, 2H), 7.91 (d, 1H, *J* = 15.6 Hz), 8.13 (d, 1H, *J* = 15.9 Hz). MS (ESI): 459.4 ($C_{29}H_{27}FO_4$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{27}FO_4$: C, 75.96; H, 5.94. Found: C, 75.75; H, 5.96.

#### 4.3.27. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(3-(trifluoromethyl)phenyl)penta-1,4-dien-3-one (C27)

Yellow solid (0.363 g, 73.5% yield). Mp 147–149 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.85 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 6.47 (d, 1H, *J* = 2.4 Hz), 6.75 (d, 1H, *J* = 2.4 Hz), 6.93 (d, 2H, *J* = 8.8 Hz), 6.97 (d, 1H, *J* = 16.4 Hz), 7.09 (d, 1H, *J* = 16.4 Hz), 7.14 (d, 1H, *J* = 16.0 Hz), 7.34 (d, 1H, *J* = 16.0 Hz), 7.49–7.57 (m, 3H), 7.63–7.70 (m, 3H), 7.80 (s, 1H), 8.17 (d, 1H, *J* = 15.6 Hz). MS (ESI): 495.5 ($C_{29}H_{25}F_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{25}F_3O_4$: C, 70.44; H, 5.10. Found: C, 70.61; H, 5.08.

#### 4.3.28. (1*E*,4*E*)-1-(2-Chloro-5-(trifluoromethyl)phenyl)-5-(2, 4-dimethoxy-6-((*E*)-4-methoxystyryl)phenyl)penta-1,4-dien-3-one (C28)

Yellow solid (0.329 g, 62.2% yield). Mp 120–122 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.91 (s, 3H), 3.92 (s, 3H), 6.44 (d, 1H, *J* = 2.8 Hz), 6.73 (d, 1H, *J* = 2.8 Hz), 6.89–6.92 (m, 2H), 6.95 (d, 1H, *J* = 15.9 Hz), 7.04 (d, 1H, *J* = 15.9 Hz), 7.17 (d, 1H, *J* = 15.9 Hz), 7.32 (d, 1H, *J* = 15.9 Hz), 7.46–7.50 (m, 2H), 7.53–7.54 (m, 2H), 7.91 (s, 1H), 8.03 (d, 1H, *J* = 15.9 Hz), 8.16 (d, 1H, *J* = 15.6 Hz). MS (ESI): 529.9 ($C_{29}H_{24}ClF_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{29}H_{24}ClF_3O_4$: C, 65.85; H, 4.57. Found: C, 65.75; H, 4.59.

#### 4.3.29. 2-((1*E*,4*E*)-5-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl ) phenyl)-3-oxopenta-1,4-dien-1-yl)phenyl nitrate (C29)

Brown solid (0.426 g, 87.4% yield). Mp 130–132 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 6.45 (d, 1H, *J* = 2.4 Hz), 6.73 (d, 1H, *J* = 2.0 Hz), 6.87–6.91 (m, 3H), 6.93 (d, 1H, *J* = 16.0 Hz), 7.23 (d, 1H, *J* = 16.0 Hz), 7.32 (d, 1H, *J* = 16.0 Hz), 7.47 (d, 2H, *J* = 8.8 Hz), 7.51–7.55 (m, 1H), 7.61–7.70 (m, 2H), 8.04 (d, 1H, *J* = 8.0 Hz), 8.08 (d, 1H, *J* = 16.0 Hz), 8.13 (d, 1H, *J* = 15.6 Hz). MS (ESI): 488.5 ($C_{28}H_{25}NO_7$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}NO_7$: C, 68.98; H, 5.17, N, 2.87. Found: C, 68.90; H, 5.15, N, 2.88.

#### 4.3.30. 3-((1*E*,4*E*)-5-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-3-oxopenta-1,4-dien-1-yl)phenyl nitrate (C30)

Yellow solid (0.434 g, 89.1% yield). Mp 137–139 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.44 (d, 1H, *J* = 2.8 Hz), 6.72 (d, 1H, *J* = 2.8 Hz), 6.89–6.97 (m, 3H), 7.10 (d, 1H, *J* = 2.1 Hz), 7.15 (d, 1H, *J* = 2.1 Hz), 7.31 (d, 1H, *J* = 16.2 Hz), 7.46–7.50 (m, 2H), 7.55 (t, 1H, *J* = 8.1 Hz), 7.66 (d, 1H, *J* = 15.9 Hz), 7.79 (d, 1H, *J* = 7.8 Hz), 8.13–8.23 (m, 2H), 8.38 (s, 1H). MS (ESI): 488.4 ($C_{28}H_{25}NO_7$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}NO_7$: C, 68.98; H, 5.17; N, 2.87. Found: C, 68.88; H, 5.18; N, 2.86.

#### 4.3.31. 4-((1*E*,4*E*)-5-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-3-oxopenta-1,4-dien-1-yl)phenyl nitrate (C31)

Brown solid (0.439 g, 90.1% yield). Mp 191–193 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.84 (s, 3H), 3.91 (s, 3H), 3.92 (s, 3H), 6.44 (d, 1H, *J* = 2.4 Hz), 6.72 (d, 1H, *J* = 2.4 Hz), 6.90–6.96 (m, 3H), 7.09 (d, 1H, *J* = 5.2 Hz), 7.13 (d, 1H, *J* = 5.2 Hz), 7.29 (d, 1H, *J* = 16.0 Hz), 7.48 (d, 2H, *J* = 8.8 Hz), 7.63 (d, 2H, *J* = 8.4 Hz), 7.66 (s, 1H), 8.16 (d, 1H, *J* = 16.0 Hz), 8.21 (d, 2H, *J* = 8.8 Hz). MS (ESI): 488.5 ($C_{28}H_{25}NO_7$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{25}NO_7$: C, 68.98; H, 5.17; N, 2.87. Found: C, 69.18; H, 5.18; N, 2.86.

#### 4.3.32. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(4-isopropylphenyl)penta-1,4-dien-3-one (C32)

Yellow solid (0.404 g, 86.2% yield). Mp 74–75 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 1.25 (s, 3H), 1.27 (s, 3H), 2.88–2.97 (m, 1H), 3.83 (s, 3H), 3.90 (s, 6H), 6.44 (d, 1H, $J$ = 2.8 Hz), 6.72 (d, 1H, $J$ = 2.8 Hz), 6.89–6.96 (m, 3H), 6.99 (d, 1H, $J$ = 11.7 Hz), 7.08 (d, 1H, $J$ = 15.9 Hz), 7.23 (d, 2H, $J$ = 8.1 Hz), 7.32 (d, 1H, $J$ = 16.2 Hz), 7.43–7.50 (m, 4H), 7.64 (d, 1H, $J$ = 16.2 Hz), 8.11 (d, 1H, $J$ = 15.9 Hz). MS (ESI): 469.2 ($C_{31}H_{32}O_4$, $[M+H]^+$). Anal. Calcd for $C_{31}H_{32}O_4$: C, 79.46; H, 6.88. Found: C, 79.66; H, 6.90.

#### 4.3.33. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(pyridin-2-yl)penta-1,4-dien-3-one (C33)

Yellow solid (0.223 g, 52.1% yield). Mp 91–92 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.43 (d, 1H, $J$ = 2.0 Hz), 6.73 (d, 1H, $J$ = 2.4 Hz), 6.90 (d, 2H, $J$ = 8.4 Hz), 6.94 (d, 1H, $J$ = 16.4 Hz), 7.15 (d, 1H, $J$ = 16.0 Hz), 7.26 (d, 1H, $J$ = 12.0 Hz), 7.32 (d, 1H, $J$ = 16.0 Hz), 7.41 (d, 1H, $J$ = 7.6 Hz), 7.48 (d, 2H, $J$ = 8.8 Hz), 7.55 (d, 1H, $J$ = 15.6 Hz), 7.64–7.73 (m, 2H), 8.16 (d, 1H, $J$ = 16.0 Hz), 8.65 (d, 1H, $J$ = 5.2 Hz). MS (ESI): 428.5 ($C_{27}H_{25}NO_4$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}NO_4$: C, 75.86; H, 5.89, N, 3.28. Found: C, 75.72; H, 5.91; N, 3.29.

#### 4.3.34. (1*E*,4*E*)-1-(2,4-Dimethoxy-6-((*E*)-4-methoxystyryl) phenyl)-5-(pyridin-3-yl)penta-1,4-dien-3-one (C34)

Yellow solid (0.250 g, 58.4% yield). Mp 67–68 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 3.83 (s, 3H), 3.89 (s, 3H), 3.90 (s, 3H), 6.43 (d, 1H, $J$ = 2.4 Hz), 6.71 (d, 1H, $J$ = 2.0 Hz), 6.90 (d, 2H, $J$ = 8.8 Hz), 6.93 (d, 1H, $J$ = 16.4 Hz), 7.06 (d, 1H, $J$ = 5.6 Hz), 7.10 (d, 1H, $J$ = 5.6 Hz), 7.27–7.31 (m, 2H), 7.45–7.48 (m, 2H), 7.62 (d, 1H, $J$ = 16.0 Hz), 7.81–7.83 (m, 1H), 8.14 (d, 1H, $J$ = 16.0 Hz), 8.58 (dd, 1H, $J$ = 1.2, 4.4 Hz), 8.73 (d, 1H, $J$ = 2.0 Hz). MS (ESI): 428.6 ($C_{27}H_{25}NO_4$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}NO_4$: C, 75.86; H, 5.89, N, 3.28. Found: C, 76.12; H, 5.88; N, 3.28.

### 4.4. Antiproliferation assay

The antiproliferative activity of the prepared compounds against B16-F10, HepG2 and A549 cell lines were evaluated as described elsewhere with some modifications.[36] Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to $2 \times 10^4$ cells mL$^{-1}$ with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5% $CO_2$ atmosphere for 24 h before the cytotoxicity assessments. Tested samples at pre-set concentrations were added to 6 wells with colchicine and CA-4 coassayed as positive reference. After 48 h exposure period, 40 μL of PBS containing 2.5 mg mL$^{-1}$ of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) was added to each well. 4 h later, 100 μL extraction solution (10% SDS–5% isobutyl alcohol–0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times. The results were summarized in Table 2.

### 4.5. Effects on tubulin polymerization

Bovine brain tubulin was purified as described previously.[37] To evaluate the effect of the compounds on tubulin assembly in vitro,[38] varying concentrations were preincubated with 10 μM tubulin in glutamate buffer at 30 °C and then cooled to 0 °C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed-up to 30 °C and the assembly of tubulin was observed turbidimetrically. The $IC_{50}$ was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min incubation.

### 4.6. Assessment of cell cycle distribution

The cell cycle distribution of B16-F10 cells was determined by flow cytometry analysis based on the previously described method.[39] Briefly, the B16-F10 cells were plated at a density of $5 \times 10^5$ cells/mL. After treatments, both the adherent and detached cells were collected, washed twice with ice-cold PBS buffer (pH 7.4), fixed with 70% alcohol overnight, and then stained with propidium iodide (1 mg/ml) in the presence of 1% RNAase A for at least 30 min before analysis by flow cytometry (Becton Dickinson, Franklin Lakes, NJ). Ten thousand cells were counted per sample. The data were analyzed with CellQuest software (Becton Dickinson).

### 4.7. Docking simulations

Molecular docking of compound **C5** into the three-dimensional X-ray structure of tubulin-colchicine site (PDB code: 1SA0) was carried out using the Auto-Dock software (Version 4.0) as implemented through the graphic user interface Auto-Dock Tool Kit (ADT 1.4.6).[40]

The graphical user interface AUTODOCKTOOLS was employed to set-up the enzymes: all hydrogens were added, Gasteiger charges were calculated and nonpolar hydrogens were merged to carbon atoms. The Ni initial parameters are set as $r$ = 1.170 Å, $q$ = +2.0, and van der Waals well depth of 0.100 kcal/mol.[41] For macromolecules, generated pdbqt files were saved.

The 3D structures of ligand molecules were built, optimized (PM3) level, and saved in Mol2 format with the aid of the molecular modeling program SPARTAN (Wavefunction Inc.). These partial charges of Mol2 files were further modified by using the ADT package (version 1.4.6) so that the charges of the nonpolar hydrogens atoms assigned to the atom to which the hydrogen is attached. The resulting files were saved as pdbqt files.

AUTODOCK 4.0 was employed for all docking calculations. The AUTODOCKTOOLS program was used to generate the docking input files. In all docking a grid box size of $60 \times 60 \times 60$ points in $x$, $y$, and $z$ directions was built and the coordinate of $X$, $y$, and $Z$ is 121.952, 90.314, 5.066. The maps were centered on N1 atom of the Kcx 219 in the catalytic site of the protein. A grid spacing of 0.375 Å (approximately one fourth of the length of carbon–carbon covalent bond) and a distances-dependent function of the dielectric constant were used for the calculation of the energetic map. Ten runs were generated by using Lamarckian genetic algorithm searches. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of $2.5 \times 10^6$ energy evaluations, and a maximum number of $2.7 \times 10^4$ generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

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