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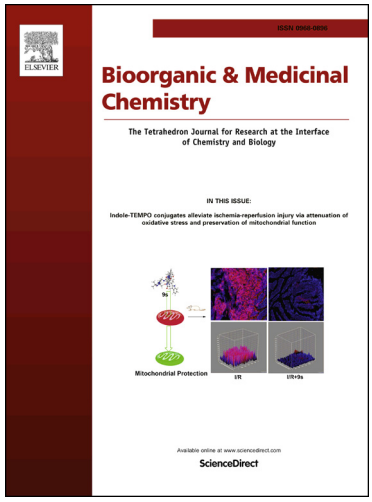

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The synthesis and evaluation of new butadiene derivatives as tubulin polymerization inhibitors

Yanqing Pang, Jun Yan, Baijiao An, Ling Huang and Xingshu Li*[a]

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China.

ABSTRACT

A series of new butadiene derivatives was synthesized and evaluated as tubulin polymerization inhibitors for the treatment of cancer. The optimal butadiene derivative, **9a**, exhibited IC_{50} values of 0.056 – 0.089 μM for five human cancer cell lines. This paper included a mechanistic study of the antiproliferative activity, including a tubulin polymerization assay, an examination of morphological alterations of cancer cells, an analysis of cell cycle arrest and an apoptosis assay.

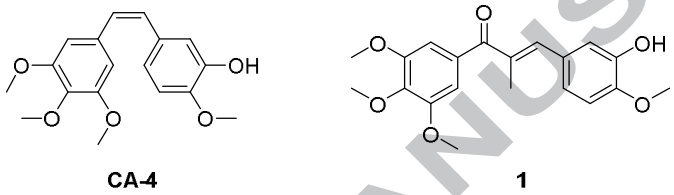
Keywords: Butadiene derivatives, tubulin polymerization inhibitors, antiproliferative, cell cycle arrest, apoptosis, morphological alterations

1. Introduction

Because traditional anticancer drugs have difficulty in treating cancer, serious side effects and rapidly lead to drug resistance, pharmaceutical chemists constantly search for more effective anticancer drugs.[1] In the past decades, many anticancer agents aimed at various targets have been developed.[2-6] Some of these agents target tubulin polymerization, which has attracted much attention because it is crucial for a wide range of cellular processes and represents a prominent cancer drug target; several drugs have been used in clinics (e.g., paclitaxel and vinblastine).[7] Combretastatin A-4 (CA-4) (Figure 1), a natural cis-stilbene derivative was first isolated from the bark of the African willow tree (Combretum caffrum) in 1982, is another typical tubulin polymerization inhibitor.[8] CA-4 strongly inhibits tubulin polymerization by binding to the colchicine-binding site; the subsequent disruption of tubulin assembly results in mitotic arrest and eventually cancer cell death. Currently, the disodium phosphate form of CA-4 (CA-4P) has been approved for clinical trials.[9-11] The disadvantage of CA-4 is its cis double bond structure, which has the tendency to isomerize to the more thermodynamically stable but less bioactive trans

[a] *E-mail : lixsh@mail.sysu.edu.cn

isomers.[12,13] Thus, modifying the olefinic bridge with a ring or carbonyl to stabilize the conformation become a hotspot in recent years.[14-18] Among them, chalcone analogue **1** (Figure 1), developed by Ducki S. et al, which replaced the olefinic bridge by α,β-unsaturated ketone, exhibited very potent antiproliferative activity.[19] In our study to search for new structural compounds as anticancer agents, we have developed several series of ortho-(3,4,5-trimethoxybenzoyl)-acetanilides and chalcone analogues as novel anti-cancer agents.[20,21] Herein, inspired by the work of Ducki S. et al, we reported our preliminary study that replaced the enone system of chalcone derivatives by a conjugated diene to obtain a new series of butadiene derivatives as tubulin polymerization inhibitors.



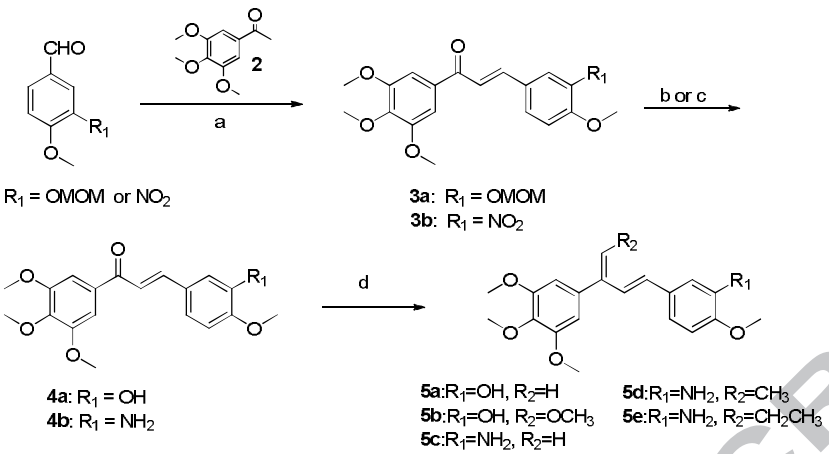
CA-4 **1**

Figure 1. The structure of **CA-4** and **1**.

2. Results and discussion

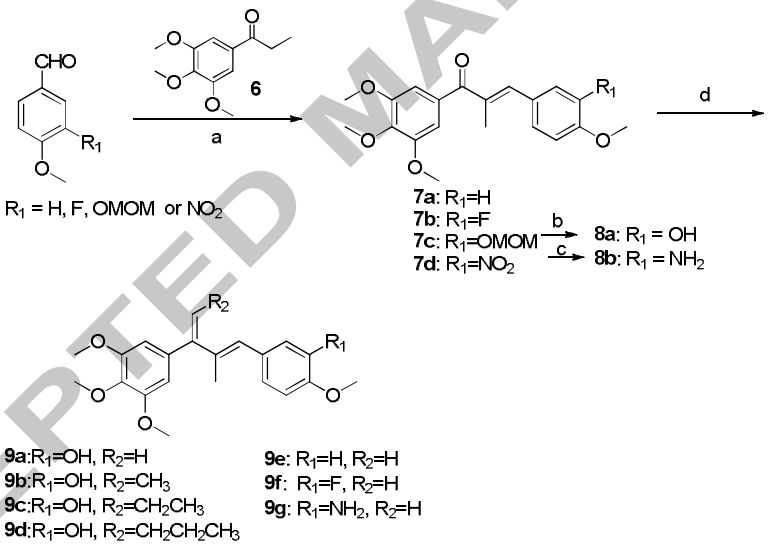
2.1. Chemistry

The synthesis of new butadiene derivatives 5a-e is shown in scheme 1. The commercially available compounds 4-methoxy-3-nitrobenzaldehyde or 4-methoxy-3-MOM-protected hydroxyl benzaldehyde were first reacted with 3,4,5-trimethoxyacetophenone in the presence of NaOH at room temperature for 12 h and subsequently reacted with HCl/MeOH or Fe/AcOH to provide hydroxy or amino substituted chalcone analogues, which were reacted with different Wittig reagents to provide the target compounds **5a–e**.[22-25]



Scheme 1. Synthesis of compounds 5a-e. Reagents and reaction conditions: a) NaOH, MeOH, rt, 12h; b) HCl, MeOH, rt ,3h; c) Fe, AcOH, EtOH, CH_2Cl_2, d) Witting reagents, n-BuLi, anhydrous THF.

Compounds **9a-g** were synthesized using the same procedure as **5a-e** apart from the use of 3,4,5-trimethoxypropiophenone and different aldehydes as the starting materials (scheme 2).



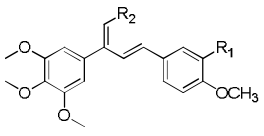
Scheme 2. Synthesis of compounds **9a-e**. Reagents and reaction conditions: a) NaOH, MeOH, rt, 12h; b) HCl, MeOH, rt ,3h; c) Fe, AcOH, EtOH, CH_2Cl_2, d) Witting reagents, n-BuLi, anhydrous THF.

2.2. In vitro cell growth inhibition

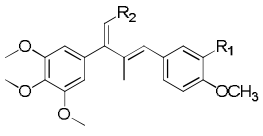
To evaluate the antiproliferative activities of the synthesized butadiene derivatives, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed with five human cancer cell lines: A549 (non-small cell lung carcinoma), HeLa (human epithelial cervical cancer cell line), PC-3 (human prostate cancer line), MCF-7 (human breast cancer cell line), and A2780 (human ovarian cancer cell line). The results listed in Table 1 indicated that most of the butadiene

derivatives exhibited moderate to good antiproliferative activity. In particular, compound **9a**, the target compound derived from 3,4,5-trimethoxy propiophenone and 3-hydroxyl-4-methoxy benzaldehyde, provided the best antiproliferative activity (entry 6, with a IC_{50} value of 56 nM for A549; 60 nM for HeLa; 61 nM for PC-3; 89 nM for MCF-7 and 64 nM for A2780). The methyl in the 3,4,5-trimethoxy propiophenone moiety plays a vital role, as indicated by a comparison of the anti-proliferative activities of **9a** with **5a**, the butadiene derivatives of 3,4,5-trimethoxyacetophenone and 3-hydroxyl-4-methoxy benzaldehyde, respectively. A simple structure–activity relationship also indicated that the antiproliferative activity was related to the substituted groups R_1 and R_2. For example, with the hydroxy group at R_1 and the H at the R_2 position, **5a** had IC_{50} values of 0.286-0.569 μM for the anti-proliferative activity. However, its analogue, **5b**, which had the same group at the R_1 position but a methoxy group at the R_2 position, exhibited very low activities with IC_{50} values of 1.601 to 5.634 μM. In contrast, with an amino group at the R_1 position, R_2 position did not have a clear effect on the antiproliferative activity. Compounds **5c**, **5d** and **5e** (with an amino group at the R_1 position but hydrogen, methyl, and ethyl groups in the R_2 position, respectively) exhibited almost the same activities (Table 1, entries 3-5). However, this tendency was not found in the series of butadiene derivatives synthesized from 3,4,5-trimethoxy propiophenone. Compounds **9a-d**, which had a hydroxy group at the R_1 position and hydrogen, methyl, and ethyl and n-Pr groups at the R_2 position, respectively, exhibited a tendency for lower activity as the the size of the substituted groups increased (Table 1, entries 6-9). In addition, compounds **9e-g**, the hydroxy group replaced by other groups (hydrogen, fluorine, and amino group), exhibited lower activity comparing with **9a** (Table 1, entries 10-12).

Table 1. The anti-proliferative activities of compounds **5a-e**, **9a-g** against five human cancer cell lines[a].



5a-e



9a-g

Entry	Comp.	R1	R2	IC50 (μM)[b]				
				A549	Hela	PC-3	MCF-7	A2780
1	**5a**	OH	H	0.286±0.015	0.320±0.217	0.423±0.031	0.569±0.012	0.457±0.275
2	**5b**	OH	OCH_3	1.601±0.257	5.634±0.436	5.325±0.397	3.256±1.597	2.224±0.987

3	**5c**	NH_2	H	0.137±0.021	0.234±0.178	0.560±0.328	0.786±0.024	0.582±0.369
4	**5d**	NH_2	CH_3	0.247±0.057	0.217±0.091	0.319 ±0.171	0.247±0.174	0.137±0.094
5	**5e**	NH_2	CH_2CH_3	0.398±0.157	0.314±0.137	0.285 ±0.021	0.486±0.028	0.248±0.181
6	**9a**	OH	H	0.056±0.012	0.060±0.013	0.061±0.021	0.089±0.019	0.064±0.029
7	**9b**	OH	CH_3	0.637±0.072	0.563±0.315	0.789±0.312	0.604±0.214	0.505±0.237
8	**9c**	OH	CH_2CH_3	2.020±0.182	3.125±0.178	4.230±0.392	8.245±2.590	5.294±2.198
9	**9d**	OH	n-Pr	2.455±0.247	3.125±0.369	4.512±0.348	3.974±2.248	2.932±0.252
10	**9e**	H	H	0.356±0.123	0.495±0.047	0.487±0.149	0.965±0.347	0.839±0.193
11	**9f**	F	H	0.219±0.079	0.483±0.296	0.354±0.132	0.412±0.148	0.561±0.315
12	**9g**	NH_2	H	0.269±0.079	0.286±0.017	0.457±0.219	0.356±0.139	0.279±0.238
13	**1**			0.004±0.001	0.005±0.001	0.009±0.003	0.005±0.002	0.003±0.001

[a]Cell lines were treated with compounds for 48 h. Cell viability was measured by MTT assay as described in the Experimental Section. [b]IC_{50} values are indicated as the mean ± SD (standard error) of at least three independent experiments.

2.3. Inhibition of tubulin polymerization

Compound **9a**, which exhibited the highest antiproliferative activity, was selected for the tubulin polymerization assay using the method described by Bonne, D. et al. with a moderate modification, and CA-4 was used as the reference.[26,27] The raw data for the polymerization assay of compound **9a** are shown in Figure 2, which shows that compound **9a** was a potent tubulin polymerization inhibitor with an IC_{50} of 1.01 ± 0.15 μM and was slightly stronger than CA-4 (IC_{50}: 1.20±0.04 μM) (Figure 2).

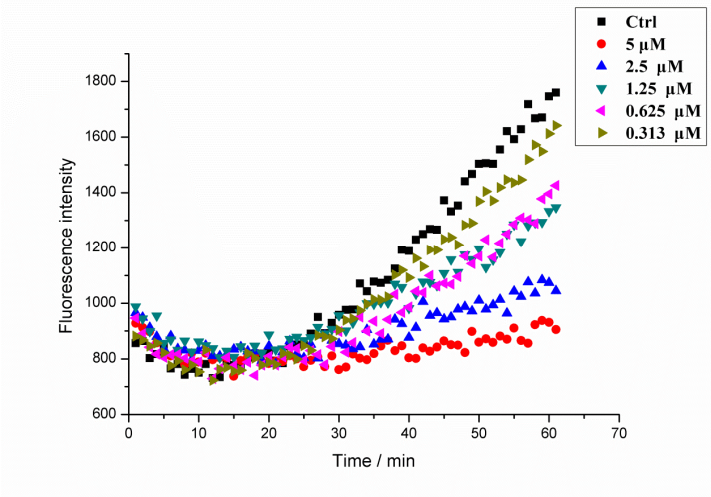


Figure 2. Tubulin polymerization inhibition activity of **9a**. Purified tubulin protein at 10 μM in a reaction buffer was incubated at 37 °C in the absence (control) or presence of **9a** at the

indicated concentrations. Polymerizations are followed by an increase in fluorescence emission at 410 nM over a 60 min period at 37 °C (excitation wavelength was 340 nM).

2.4. Morphological alterations of HeLa cells caused by ***9a***

Compound **9a** was used in further biological studies, such as studies of the morphological alterations of cancer cells. When HeLa cells were exposed to different concentrations of **9a** (25, 50 and 100 nM) for 24 h, morphological alterations, such as microtubule shrinkage, membrane blebbing and chromatin condensation, that are normally associated with the occurrence of apoptosis were observed under a laser scanning confocal microscope (Figure 3).

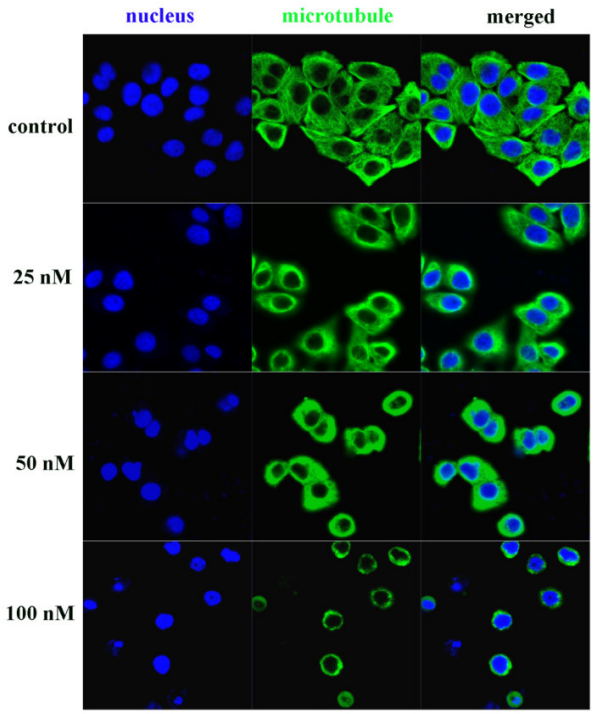


Figure 3. **9a** disrupted the organization of the cellular microtubule network. Hela cells were plated in confocal dishes and incubated with **9a** at the indicated concentrations for 24 h, followed by direct microscopy. The detection of the fixed and stained cells was performed with an LSM 570 laser confocal microscope (Carl Zeiss, Germany). The experiments were performed three times, and the results of the representative experiments were shown.

2.5. Apoptosis assay

To evaluate the mode of cell death induced by **9a**, flow cytometry was performed using propidium iodide (PI), which only stains DNA and only enters dead cells, and the fluorescence probe annexin-V, which selectively binds to the early apoptotic cells that expose phospholipid phosphatidylserine on their surface. As shown in Figure 4,

when HeLa cells were treated for 24 h with **9a** at different concentrations (25, 50, and 100 nM) or with DMSO (0.01%) for the control cells, annexin-V positive cells accumulated, indicating that the number of apoptotic cells increased. These data show that **9a** induced cell apoptosis in a concentration-dependent manner (Figure. 4).

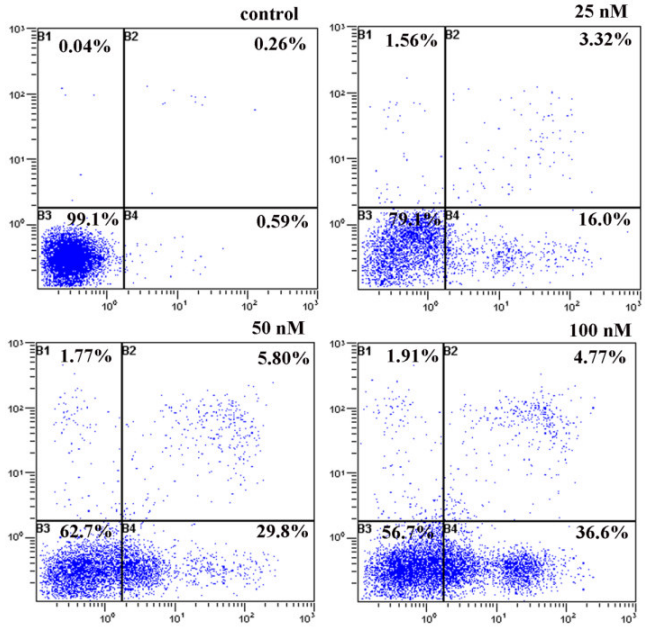


Figure 4. **9a** induced the apoptosis of Hela cells. Hela cells were treated with **9a** at the indicated concentrations for 24 h. Then, the cells were trypsinized, harvested and stained with Annexin V-FITC and PI solution for flow cytometry. The percentages of cells in each stage of apoptosis were quantitated by flow cytometry. (B1, upper left quadrant) necrotic cells; (B2, upper right quadrant) late apoptotic cells; (B3, bottom left quadrant) live cells; and (B4, bottom right quadrant) early apoptotic cells. Representative images from five independent experiments were shown.

2.6. Analysis of cell cycle arrest

To evaluate the cell cycle arrest effect of compound **9a**, HeLa cells were treated with DMSO (0.01%) or compound **9a** at various concentrations (25, 50, and 100 nM) for 24 h. Figure 5 shows the flow cytometry analysis of harvested cells; this assay showed that **9a** resulted in significant cell-cycle arrest at the G_2/M phase in a dose-dependent manner. For the DMSO-treated group, 19.0% of the cells were at the G_2/M phase 24 h after the treatment, whereas for the 9a treatment, 30.0% of the cells were in this phase at the same time after treatment When the concentration of **9a** was increased to 50 and 100 nM for 24 h, the percentage of cells at the G_2/M phase correspondingly increased to 43% and 72%, respectively. These results revealed that

compound **9a** might have the same fashion as CA-4, which arrests cell-cycle progression at the G_2/M phase due to microtubule depolymerization and cytoskeletal disruption (Figure 5).

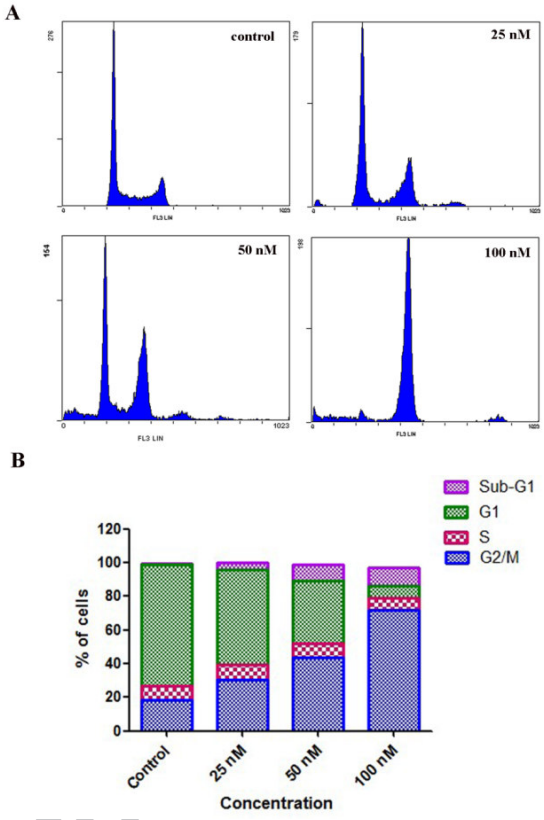


Figure 5. Cell cycle arrest effect of **9a**. Hela cells were treated with **9a** at the indicated concentrations for 24 h. Then, the cells were trypsinized and harvested for PI-stained DNA content by flow cytometry. Quantitative analysis of the percentage of cells in each cell cycle phase by EXPO32 ADC analysis software. The experiments were performed at least three times, and the results of the representative experiments are shown.

3.Conclusions

In summary, to search for new structural compounds that can serve as anticancer agents, we have developed a series of butadiene derivatives as tubulin polymerization inhibitors. Among them, **9a**, which is 2-methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)buta-1,3-dien-1-yl) phenol, exhibited the highest antiproliferative activity. Our mechanistic study showed that 9a is a good tubulin polymerization inhibitor with an IC_{50} of 1.01 μM, and it induces cell apoptosis in a concentration-dependent manner and arrests cell-cycle progression at the G_2/M phase due to microtubule depolymerization and cytoskeletal disruption. Morphological alterations, such as microtubule shrinkage, membrane blebbing and chromatin condensation, were

observed for HeLa cells. Overall, the current study demonstrates that the butadiene derivatives deserve further research as new tubulin binding agents that may become to anticancer drugs.

4.Experimental

4.1. Chemistry.

The ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard on a Bruker BioSpin GmbH spectrometer at 400 and 101 MHz, respectively, and the coupling constants are reported in hertz. The high-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. Reagents used in the synthesis were obtained commercially and used without further purification, unless otherwise specified. The reactions were monitored by thin layer chromatography (TLC) on glass-packed precoated silica gel plates and visualized in an iodine chamber or with a UV lamp. Flash column chromatography was performed using silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purity of the samples was determined by high-performance liquid chromatography (HPLC), conducted on a Shimadzu LC-20AT series system with a TC-C18 column (4.6 mm × 250 mm, 5 μm), and the samples were eluted with a 40:60 acetonitrile/H_2O mixture, at a flow rate of 0.5 mL/min.

*4.1.1. General procedure for the preparation of chalcones (**3**)*

NaOH (2.0 mmol) was added to a solution of 3,4,5-trimethoxyacetophenone (1.2 mmol) in methanol. After the mixture was stirred for 15 min, substituted benzaldehyde (1.0 mmol) was added. The reaction mixture was stirred for 10 h and filtered to give the title compounds.

*4.1.1.1. 3-(4-Methoxy-3-(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3a**)*

White solid. yield: 74%. m.p:109.4-110.6℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 15.6 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.26 (s, 2H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.29 (s, 2H), 3.95 (s, 6H), 3.94 (s, 6H), 3.55 (s, 3H).

*4.1.1.2. 3-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3b**)*

Yellow solid. yield: 76%. m.p:143.5-144.7℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 2.2 Hz ,1H), 7.79 (dd, *J* =2.2; 8.7 Hz ,1H) 7.67 (d, *J* = 15.5 Hz ,1H), 7.32

(d, *J* =15.5 Hz ,1H), 7.28 (s, 2H), 7.15 (d, *J* =8.7 Hz ,1H), 4.03 (s, 3H), 3.97 (s, 6H),3.94 (s, 3H).

*4.1.2. General procedure for the preparation of **4a** and **8a***

Hydrochloric acid (37%, 5.0 mmol) was added dropwise to a solution of **3a** or **7c** (1.0 mmol) in methanol. The reaction mixture was stirred for 3 h at room temperature and then neutralized with a saturated $NaHCO_3$ solution. Water was added to the mixture, and then, the compound was extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and subsequently evaporated to give a pale yellow oil. The crude products were purified by flash column chromatography on silica gel to give the product.

*4.1.2.1. 3-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2- en-1-one (**4a**)*

White solid. yield: 96%. m.p:144.1-145.3℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, 1H, *J* = 16.1 Hz), 7.42 (d, 1H, *J* = 16.1 Hz), 7.25–7.32 (m, 3H), 7.07 (dd, 1H, *J* = 2.1;8.7 Hz), 6.89 (d, 1H, *J* = 8.7 Hz), 5.75 (bs, 1H), 3.96 (s, 9H), 3.94 (s, 3H).

*4.1.2.2. 3-(3-Hydroxy-4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**8a**)*

White solid. yield: 96%. m.p:111.8-113.2℃. 1H NMR (400 MHz, CDCl3) δ 7.20 (q, *J* = 1.2 Hz, 1H), 7.18 (s, 2H), 6.89 (s, 2H), 6.85 (d, *J* = 7.9 Hz, 1H), 5.75 (s, 1H), 3.90 (s, 6H), 3.85 (s, 3H), 3.84 (s, 3H), 2.04 (d, *J* = 1.1 Hz, 3H).

*4.1.3. General procedure for the preparation of **4b** and **8b***

Iron powder (5.0 mmol) and AcOH (2 mL) were added to a solution of nitro-compound (1.0 mmol) in ethanol (5 mL) and CH_2Cl_2 (5 mL). After being stirred at room temperature for 4 h, the mixture was filtered, and the solvent was evaporated in vacuum. The residue was dissolved in ethyl acetate, washed with water, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel to give the product.

*4.1.3.1. 2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)buta-1,3-dienyl)aniline (**4b**)*

Yellow solid； yield: 73%. m.p:89.9-90.6℃. ^{1}H NMR (400 MHz, CDCl3) δ 7.73 (d, 1H, *J* = 15.4 Hz), 7.32 (d, 1H, *J*= 15.4 Hz), 7.28 (s, 2H), 7.06 (bs, 1H), 7.03 (d, 1H, *J* =8.2 Hz), 6.80 (d, 1H, *J* =8.2 Hz), 3.95 (m, 2H), 3.94 (s, 9H),3.89 (s, 3H).

*4.1.3.2. 3-(3-Amino-4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**8b**)*

Yellow solid; yield: 73%. m.p:67.3-68.9℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 1.5 Hz, 1H), 6.98 (s, 2H), 6.89 – 6.84 (m, 2H), 6.82 (d, *J* = 8.1 Hz, 1H), 3.92 (s, 3H), 3.89 (d, *J* = 2.0 Hz, 9H), 2.28 (d, *J* = 1.4 Hz, 3H).

*4.1.4 General procedure for the preparation of **5***

Under an argon atmosphere, triphenylphosphonium bromide (2.0 mmol) with various substitutions was dissolved in dry THF (30 mL) and then cooled to -78 °C. The mixture was slowly added to n-BuLi (2.0 mmol), then warmed to room temperature and stirred for 0.5 h. Afterward, the mixture was cooled to -78 °C again, and compound 4 (1.0 mmol) in THF solution was added. After being stirred for another 0.5 h, the resulting solution was warmed to room temperature and held at that temperature for 12 h. The reaction was quenched by the addition of saturated ammonium chloride solution, and extraction was performed using ethyl acetate. The organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel to give the target compound 5.

*4.1.4.1. 2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)buta-1,3-dienyl)phenol (**5a**)*

White solid. yield: 84%. m.p:64.3-64.7 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 2.0 Hz, 1H), 6.93 – 6.83 (m, 2H), 6.80 (d, *J* = 8.3 Hz, 1H), 6.59 (s, 2H), 6.47 (d, *J* = 16.0 Hz, 1H), 5.35 (d, *J* = 1.6 Hz, 1H), 5.19 (d, *J* = 1.7 Hz, 1H), 3.89 (d, *J* = 1.3 Hz, 6H), 3.87 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.94, 148.21, 146.52, 145.74, 137.37, 136.08, 131.65, 130.86, 128.62, 119.54, 116.25, 111.75, 110.58, 105.54, 60.95, 56.16, 56.00. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{20}H_{22}O_5$, 343.1540; found, 343.1550. Purity: 99.0% (by HPLC).

*4.1.4.2. 2-Methoxy-5-(-3-(3,4,5-trimethoxyphenyl)penta-1,3-dienyl)phenol (**5b**)*

Yellow solid; yield: 63%. m.p:76.4-76.9 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (dd, *J* = 16.3, 0.8 Hz, 1H), 7.09 (d, *J* = 2.1 Hz, 1H), 6.84 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.34 (d, *J* = 16.3 Hz, 1H), 6.13 (s, 1H), 5.55 (s, 1H), 3.88 (d, *J* = 1.9 Hz, 6H), 3.86 (s, 6H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.95, 146.89, 146.00, 145.66, 136.93, 133.49, 131.92, 128.37, 121.72, 120.68, 119.02,

111.56, 110.57, 106.64, 60.94, 60.41, 56.13, 55.99. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{24}O_6$, 342.1700; found, 342.1725. Purity: 96.7% (by HPLC).

*4.1.4.3. 2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)buta-1,3-dienyl)aniline (**5c**)*

Yellow solid; yield: 65%. m.p:184.3-184.9 ℃. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.52 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 16.1 Hz, 1H), 6.62 (s, 2H), 6.47 (d, *J* = 16.1 Hz, 1H), 5.43 (d, *J* = 1.5 Hz, 1H), 5.24 (d, *J* = 1.6 Hz, 1H), 3.98 (s, 3H), 3.84 (s, 6H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 152.96, 152.10, 148.18, 137.28, 136.08, 130.80, 130.00, 129.59, 128.31, 121.18, 119.60, 116.59, 112.17, 105.47, 59.77, 55.56, 55.25. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{20}H_{23}NO_4$, 342.1700; found, 342.1725. Purity: 95.8% (by HPLC).

*4.1.4.4. (Z/E)-2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)penta-1,3-dienyl)aniline (**5d**)*

Yellow solid; yield: 65%. m.p:71.1-71.8 ℃. Major isomer: ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.52 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.35 – 7.31 (m, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 6.54 (s, 2H), 6.35 (d, *J* = 16.0 Hz, 1H), 5.70 (q, *J* = 7.2 Hz, 1H), 3.98 (s, 3H), 3.82 (s, 6H), 3.79 (s, 3H), 1.99 (d, *J* = 7.2 Hz, 3H). All isomer: ^{13}C NMR (101 MHz, DMSO) δ 153.32, 153.01, 151.99, 151.65, 141.98, 140.15, 137.51, 136.94, 136.72, 133.23, 132.71, 130.75, 130.67, 130.18, 129.28, 127.85, 127.66, 127.25, 127.01, 124.68, 121.82, 121.52, 121.39, 113.04, 106.78, 106.22, 60.52, 60.49, 56.71, 56.66, 56.34, 56.28, 15.63, 14.34. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{25}NO_4$, 356.1856; found, 356.1871. Purity: 97.9% (by HPLC).

*4.1.4.5. (Z/E)-2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)hexa-1,3-dienyl)aniline (**5e**)*

Yellow solid; yield: 65%. m.p:65.2-65.8 ℃. Major isomer: ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.52 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.34 – 7.27 (m, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.55 (s, 2H), 6.34 (d, *J* = 16.0 Hz, 1H), 5.62 (t, *J* = 7.6 Hz, 1H), 3.98 (s, 3H), 3.82 (s, 6H), 3.79 (s, 3H), 2.44 (p, *J* = 7.5 Hz, 2H), 1.14 (t, *J* = 7.5 Hz, 3H). All isomer: ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.07, 152.83, 151.76, 151.43, 140.65, 138.92, 137.74, 136.99, 136.73, 136.60, 135.22, 133.01, 131.38, 130.01, 127.51, 127.22, 125.47, 122.30, 121.84, 119.91, 119.72, 111.99, 111.93, 106.37, 106.04, 60.88, 56.35, 56.31, 56.13, 56.10, 22.77, 21.61, 14.32. HRMS (ESI)

(m /z) $[M+H]^+$ calcd for $C_{21}H_{25}NO_4$, 370.2013; found, 370.2031. Purity: 98.4% (by HPLC).

4.1.5. General procedure for the preparation of ***7***

NaOH (2.0 mmol) was added to a solution of 1-(3,4,5-trimethoxyphenyl)propan-1-one (1.2 mmol) in methanol. After the mixture was stirred for 15 min, a substituted benzaldehyde (1.0 mmol) was added. The reaction mixture was stirred for 24 h, and extraction was performed using ethyl acetate. The organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel to give the compound **7**.

*4.1.5.1. 3-(4-Methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7a**)*

White solid. yield: 62%. m.p:94.3-95.7℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.42 (m, 1H), 7.33 (s, 1H), 7.24 – 7.18 (m, 1H), 3.90 (s, 3H), 3.82 (d, *J* = 20.0 Hz, 3H), 2.03 (d, *J* = 1.1 Hz, 2H).

*4.1.5.2. 3-(3-Fluoro-4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7b**)*

White solid. yield: 61%. m.p:96.4-97.3℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 (s, 2H), 7.22 (q, *J* = 1.2 Hz, 1H), 7.16 (ddd, *J* = 7.6, 2.0, 1.0 Hz, 1H), 7.12 – 7.01 (m, 2H), 3.90 (s, 6H), 3.84 (s, 6H), 2.03 (d, *J* = 1.1 Hz, 3H).

*4.1.5.3. 3-(4-Methoxy-3-(methoxymethoxy)phenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7c**)*

Yellow solid; yield: 65%. m.p:100.1-101.6℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.21 (s, 1H), 7.19 – 7.10 (m, 1H), 6.02 (s, 1H), 3.92 – 3.82 (m, 5H), 3.25 (s, 1H), 2.04 (d, *J* = 1.0 Hz, 1H).

*4.1.5.4. 3-(4-Methoxy-3-nitrophenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7d**)*

Yellow solid; yield: 61%. m.p:111.8-113.2℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.92 (dd, *J* = 2.0, 1.0 Hz, 1H), 7.73 (ddd, *J* = 7.6, 2.0, 1.1 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.30 (s, 2H), 7.29 – 7.26 (m, 1H), 4.02 (s, 3H), 3.90 (s, 6H), 3.84 (s, 3H), 2.04 (d, *J* = 1.1 Hz, 3H).

4.1.6. General procedure for the preparation of ***9***.

The General procedure was same as the synthesis of **5**

*4.1.6.1. 2-Methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)buta-1,3-dienyl)phenol (**9a**)*

White solid; yield: 78%. m.p: 76.8-77.2 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, *J* = 1.9 Hz, 1H), 6.88 – 6.74 (m, 2H), 6.57 (s, 1H), 6.44 (s, 1H), 5.34 (s, 1H), 5.18 (s, 1H), 3.89 (d, *J* = 3.6 Hz, 6H), 3.86 (s, 6H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.30, 152.78, 145.41, 145.13, 137.35, 137.22, 136.38, 131.49, 130.51, 121.48, 115.33, 113.08, 110.31, 105.77, 60.94, 56.13, 55.97, 16.63. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{24}O_5$, 357.1697; found, 357.1714. Purity: 99.6% (by HPLC).

*4.1.6.2. (Z/E)-2-methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)penta-1,3-dienyl)phenol (**9b**)*

White oil; yield: 68%. Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (d, *J* = 2.3 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.36 (s, 2H), 6.09 (s, 1H), 5.95 (q, *J* = 6.9 Hz, 1H), 5.56 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.84 (s, 6H), 2.07 (d, *J* = 1.1 Hz, 3H), 1.63 (d, *J* = 7.0 Hz, 3H). All isomer: ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.99, 152.94, 146.21, 145.81, 145.27, 145.05, 145.00, 137.45, 136.86, 136.44, 135.48, 134.57, 132.07, 131.49, 129.99, 128.93, 122.84, 121.96, 121.36, 121.05, 115.38, 115.03, 110.45, 110.22, 106.90, 103.81, 60.96, 60.91, 56.11, 56.08, 56.04, 55.96, 18.31, 15.75, 15.68, 15.46. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{22}H_{26}O_5$, 371.1853; found, 371.1870. Purity: 99.0% (by HPLC).

*4.1.6.3. (Z/E)-2-methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)hexa-1,3-dienyl)phenol (**9c**)*

White oil; yield: 68%. Major isomer: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.02 (d, *J* = 1.4 Hz, 1H), 6.88 (d, *J* = 1.3 Hz, 1H), 6.62 (s, 2H), 6.35 (d, *J* = 1.6 Hz, 1H), 5.79 (t, *J* = 7.4 Hz, 1H), 5.64 (s, 1H), 3.92 (s, 3H), 3.85 (d, *J* = 1.7 Hz, 9H), 2.29 (p, *J* = 7.5 Hz, 2H), 1.93 (s, 3H), 1.07 (t, *J* = 7.5 Hz, 3H). All isomer: ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.00, 152.85, 145.30, 145.27, 145.05, 144.99, 144.82, 144.29, 137.36, 137.25, 136.66, 135.67, 134.93, 132.08, 131.46, 130.47, 129.52, 129.45, 129.24, 121.36, 121.07, 115.37, 115.07, 110.44, 110.21, 106.81, 103.81, 60.96, 60.92, 56.10, 56.04, 55.96, 23.25, 23.11, 18.53, 15.76, 14.85, 14.63. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{23}H_{28}O_5$, 385.2017; found, 385.2010. Purity: 99.0% (by HPLC).

*4.1.6.4. (Z/E)-2-methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)hepta-1,3-dienyl)phenol (**9d**)*

White oil; yield: 68%. Major isomer: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.94 (s, 1H), 6.81 (s, 1H), 6.55 (s, 2H), 6.27 (d, *J* = 1.6 Hz, 1H), 5.72 (t, *J* = 7.4 Hz, 1H), 5.58 (s, 1H), 3.85 (s, 3H), 3.78 (d, *J* = 1.8 Hz, 9H), 2.19 (q, *J* = 7.4 Hz, 2H), 1.84 (s, 3H), 1.42 (q, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). All isomer: ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.94, 151.77, 144.34, 144.24, 144.21, 143.99, 143.93, 143.78, 136.39, 136.18, 135.67, 135.31, 134.71, 133.92, 131.03, 130.43, 128.49, 128.13, 127.83, 126.81, 120.32, 120.00, 114.32, 113.99, 109.39, 109.15, 105.82, 102.74, 59.93, 59.88, 55.05, 54.99, 54.91, 30.85, 30.76, 22.33, 22.15, 17.49, 14.72, 13.06, 12.90. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{24}H_{30}O_5$, 399.2174; found, 399.2166. Purity: 99.0% (by HPLC).

*4.1.6.5. 1,2,3-Trimethoxy-5-(4-(4-methoxyphenyl)-3-methylbuta-1,3-dien-2-yl)benzene (**9e**)*

White solid; yield: 85%. m.p:68.5-68.9℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.18 (m, 2H), 6.94 – 6.83 (m, 2H), 6.57 (d, *J* = 1.5 Hz, 2H), 6.48 (s, 1H), 5.34 (s, 1H), 5.18 (s, 1H), 3.88 (d, *J* = 1.6 Hz, 2H), 3.86 (d, *J* = 1.6 Hz, 6H), 3.82 (d, *J* = 1.6 Hz, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.35, 153.35, 152.83, 137.40, 137.35, 135.97, 130.55, 130.50, 113.59, 112.96, 105.90, 60.91, 56.14, 55.25, 16.56. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{24}O_4$, 341.1747; found, 342.1764. Purity: 99.0% (by HPLC).

*4.1.6.6. 5-(4-(3-Fluoro-4-methoxyphenyl)-3-methylbuta-1,3-dien-2-yl)-1,2,3-trimethoxybenzene (**9f**)*

White solid; yield: 85%. 73.8-74.3℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (dd, *J* = 12.7, 2.0 Hz, 1H), 7.01 – 6.89 (m, 2H), 6.55 (s, 2H), 6.42 (s, 1H), 5.35 (d, *J* = 1.4 Hz, 1H), 5.20 (d, *J* = 1.4 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.86 (s, 6H), 2.08 (d, *J* = 1.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.05, 152.85, 150.69, 146.24, 137.40, 137.20, 131.26, 129.44, 125.36, 116.83, 116.64, 113.45, 113.01, 105.83, 60.92, 56.28, 56.15, 16.54. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{23}O_4F$, 359.1653; found, 359.1668. Purity: 98.6% (by HPLC).

*4.1.6.7. 2-Methoxy-5-(2-methyl-3-(3,4,5-trimethoxyphenyl)buta-1,3-dienyl)aniline (**9g**)*

Yellow solid; yield: 76%. m.p:73.7-74.5 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, *J* = 8.8 Hz, 1H), 6.69 (d, *J* = 6.5 Hz, 2H), 6.56 (s, 2H), 6.41 (s, 1H), 5.32 (s, 1H), 5.16 (s, 1H), 3.88 (s, 3H), 3.86 (s, 9H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

153.46, 152.76, 146.29, 137.47, 137.18, 135.76, 135.66, 130.98, 130.96, 119.85, 115.91, 112.82, 110.00, 105.78, 60.94, 56.13, 55.54, 16.66. HRMS (ESI) (m /z) $[M+H]^+$ calcd for $C_{21}H_{25}NO_4$, 356.1856; found, 356.1868. Purity: 99.3% (by HPLC).

4.2. Cell Culture.

HeLa human epithelial cervical cancer cells, A549 non-small-cell-lung cancer cells, PC-3 human prostate cancer cells, A2780 human ovarian cancer cells and MCF-7 human breast carcinoma cells were cultivated in DMEM containing 10% (v/v) heat-inactivated foetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin, respectively. The cells were incubated at 37 °C under a 5% CO_2 and 90% relative humidity (RH) atmosphere.

4.3. MTT assay.

Cells grown to the logarithmic phase were seeded into 96-well plates (5×10^3 cells/well) for 24 h, and then they were exposed to different concentrations of the test compounds for 48 h. After attached cells were incubated with 5 mg/mL MTT (Sigma, USA) for another 4 h, the suspension was discarded, and subsequently the dark blue crystals (formazan) were solubilized in dimethyl sulfoxide (DMSO). The absorbance of the solution at 570 nm was measured using a multifunction microplate reader (Molecular Devices, Flex Station 3), and each experiment was performed at least in triplicate. IC_{50} values, which represent the drug concentrations required to cause 50% cancer cell growth inhibition, were used to express the cytotoxic effects of each compound and were calculated with GraphPad Prism Software version 5.02 (GraphPad Inc., La Jolla, CA, USA).

4.4. In vitro tubulin polymerization assay.

A tubulin polymerization assay was performed by measuring the increase in fluorescence intensity, which can be easily recorded due to the incorporation of a fluorescent reporter, DAPI (4',6-diamidino-2-phenylindole), a fluorophore that is known to be a DNA intercalator. In our experiment, a commercial kit (cytoskeleton, cat. #BK011P) purchased from Cytoskeleton (Danvers, MA, USA) was used for the tubulin polymerization. The final buffer used for tubulin polymerization contained 80.0 mM piperazine-N, N′-bis(2-ethanesulfonic acid) sequisodium salt (pH 6.9), 2.0 mM $MgCl_2$, 0.5 mM EGTA, 1.0 mM GTP, and 10.2% glycerol. First, 5 μL of the tested compounds at the indicated concentrations was added, and the mixture was

warmed to 37 °C for 1 min; then, the reaction was initiated by the addition of 55 μL of the tubulin solution. The fluorescence intensity enhancement was recorded every 60 sec for 90 min in a multifunction microplate reader (Molecular Devices, Flex Station 3) (emission wavelength of 410 nm, excitation wavelength of 340 nm). The area under the curve was used to determine the concentration that inhibited tubulin polymerization by 50% (IC_{50}) and was calculated using GraphPad Prism Software version 5.02 (GraphPad Inc., La Jolla, CA, USA).

4.5. Immunofluorescence microscopy.

In a 10 mm confocal culture dish, 3×10^4 cells were grown for 24 h and then incubated in the presence/absence of compound 9a at the indicated concentrations for another 12 h. After being washed with phosphate-buffered solution (PBS) and fixed in 4% pre-warmed (37 °C) paraformaldehyde for 15 min, the cells were permeabilized with 0.5% Triton X-100 for 15 min and blocked for 30 min in 10% goat serum. Then, the cells were incubated with mouse anti-tubulin antibody (CST, USA) at 4 °C overnight, washed with PBS three times, and incubated with goat anti-mouse IgG/Alexa-Fluor 488 antibody (Invitrogen, USA) for 1 h. The samples were immediately visualized on a Zeiss LSM 570 laser scanning confocal microscope (Carl Zeiss, Germany) after the nuclei were stained with Hoechst 33342 (Sigma, USA) in the dark at room temperature for 30 min.

4.6. Cell cycle analysis.

HeLa cells were seeded in 6-well plates (3×10^5 cells/well), incubated in the presence/absence of compound 9a at the indicated concentrations for 24 h, harvested by centrifugation, and then fixed in ice-cold 70% ethanol overnight. After the ethanol was removed the next day, the cells were resuspended in ice-cold PBS, treated with RNAse A (Keygen Biotech, China) at 37 °C for 30 min, and then incubated with the DNA staining solution propidium iodide (PI, Keygen Biotech, China) at 4 °C for 30 min. Approximately 10,000 events were detected by flow cytometry (Beckman Coulter, Epics XL) at 488 nm. The data regarding the number of cells in different phases of the cell cycle were analysed using EXPO32 ADC analysis software.

4.7. Apoptosis analysis.

The preparation of the HeLa cell sample was the same as for the cell cycle analysis. After incubation, cells were harvested and incubated with 5 μL of Annexin-

V/FITC (Keygen Biotech, China) in binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$ at pH 7.4) at room temperature for 15 min. PI solution was then added to the medium for another 10 min-incubation. Almost 10,000 events were collected for each sample and analysed by flow cytometry (Beckman Coulter, Epics XL). The percentage of apoptotic cells was calculated using EXPO32 ADC Analysis software.

Declaration instersted

The authors confirm that this article content has no conflict of interest.

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Abbreviation

CA-4, Combretastatin A-4; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; PI, propidium iodide; FBS, foetal bovine serum; RH, relative humidity; TLC, layer chromatography; HPLC, high-performance liquid chromatography; DAPI ,4',6-diamidino-2-phenylindole; PBS ,phosphate-buffered solution.

Figure captions

Figure 1. The structere of **1**.

Figure 2. Tubulin polymerization inhibition activity of **9a**. Purified tubulin protein at 10 μM in a reaction buffer was incubated at 37 °C in the absence (control) or presence of **9a** at the indicated concentrations. Polymerizations are followed by an increase in fluorescence emission at 410 nM over a 60 min period at 37 °C (excitation wavelength was 340 nM).

Figure 3. **9a** disrupted the organization of the cellular microtubule network. Hela cells were plated in confocal dishes and incubated with **9a** at the indicated concentrations for 24 h, followed by direct microscopy. The detection of the fixed and stained cells was performed with an LSM 570 laser confocal microscope (Carl Zeiss, Germany). The experiments were performed three times, and the results of the representative experiments were shown.

Figure 4. **9a** induced the apoptosis of Hela cells. Hela cells were treated with **9a** at the indicated concentrations for 24 h. Then, the cells were trypsinized, harvested and stained with Annexin V-FITC and PI solution for flow cytometry. The percentages of cells in each stage of apoptosis were quantitated by flow cytometry. (B1, upper left quadrant) necrotic cells; (B2, upper right quadrant) late apoptotic cells; (B3, bottom left quadrant) live cells; and (B4, bottom right quadrant) early apoptotic cells. Representative images from five independent experiments were shown.

Figure 5. Cell cycle arrest effect of **9a**. Hela cells were treated with **9a** at the indicated concentrations for 24 h. Then, the cells were trypsinized and harvested for PI-stained DNA content by flow cytometry. Quantitative analysis of the percentage of cells in each cell cycle phase by EXPO32 ADC analysis software. The experiments were performed at least three times, and the results of the representative experiments are shown.

Table captions

Table 1. The anti-proliferative activities of compounds **5a-e**, **9a-f** against five human cancer cell lines[a].

Scheme captions

Scheme 1. Synthesis of compounds 5a-e. Reagents and reaction conditions: a) NaOH, MeOH, rt, 12h; b) HCl, MeOH, rt ,3h; c) Fe, AcOH, EtOH, CH_2Cl_2, d) Witting reagents, n-BuLi, anhydrous THF.

Scheme 2. Synthesis of compounds 9a-e. Reagents and reaction conditions: a) NaOH, MeOH, rt, 12h; b) HCl, MeOH, rt ,3h; c) Fe, AcOH, EtOH, CH_2Cl_2, d) Witting reagents, n-BuLi, anhydrous THF.

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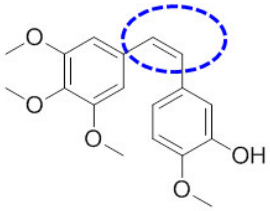

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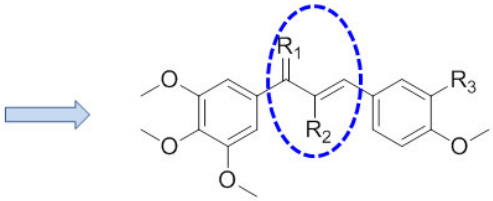
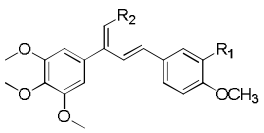


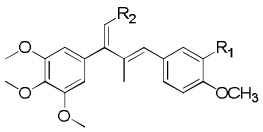
9a:R_1=-CH_2, R_2=-CH_3, R_3=-OH

a. Antiproliferative activity: IC_{50} = 0.056 – 0.089µM;
b. Tubulin polymerisation inhibition : IC_{50} = 1.01µM;
c. G_2/M phase arrest;
d. Multipolar mitosis and apoptosis.

Table 1. The anti-proliferative activities of compounds **5a-e**, **9a-g** against five human cancer cell lines[a].



5a-e



9a-g

Entry	Comp.	R1	R2	IC50 (μM)[b]				
				A549	Hela	PC-3	MCF-7	A2780
1	**5a**	OH	H	0.286±0.015	0.320±0.217	0.423±0.031	0.569±0.012	0.457±0.275
2	**5b**	OH	OCH_3	1.601±0.257	5.634±0.436	5.325±0.397	3.256±1.597	2.224±0.987

3	**5c**	NH_2	H	0.137±0.021	0.234±0.178	0.560±0.328	0.786±0.024	0.582±0.369
4	**5d**	NH_2	CH_3	0.247±0.057	0.217±0.091	0.319 ±0.171	0.247±0.174	0.137±0.094
5	**5e**	NH_2	CH_2CH_3	0.398±0.157	0.314±0.137	0.285 ±0.021	0.486±0.028	0.248±0.181
6	**9a**	OH	H	0.056±0.012	0.060±0.013	0.061±0.021	0.089±0.019	0.064±0.029
7	**9b**	OH	CH_3	0.637±0.072	0.563±0.315	0.789±0.312	0.604±0.214	0.505±0.237
8	**9c**	OH	CH_2CH_3	2.020±0.182	3.125±0.178	4.230±0.392	8.245±2.590	5.294±2.198
9	**9d**	OH	n-Pr	2.455±0.247	3.125±0.369	4.512±0.348	3.974±2.248	2.932±0.252
10	**9e**	H	H	0.356±0.123	0.495±0.047	0.487±0.149	0.965±0.347	0.839±0.193
11	**9f**	F	H	0.219±0.079	0.483±0.296	0.354±0.132	0.412±0.148	0.561±0.315
12	**9g**	NH_2	H	0.269±0.079	0.286±0.017	0.457±0.219	0.356±0.139	0.279±0.238
13	**1**			0.004±0.001	0.005±0.001	0.009±0.003	0.005±0.002	0.003±0.001

[a]Cell lines were treated with compounds for 48 h. Cell viability was measured by MTT assay as described in the Experimental Section. [b]IC_{50} values are indicated as the mean ± SD (standard error) of at least three independent experiments.