Bioorganic & Medicinal Chemistry 20 (2012) 2382–2391

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of macrocyclic bisbibenzyl derivatives and their anticancer effects as anti-tubulin agents

Juan Jiang [a,†], Bin Sun [a,b,†], Yan-yan Wang [a], Min Cui [c], Li Zhang [a], Chang-zhi Cui [a], Yan-feng Wang [a], Xi-gong Liu [a], Hong-xiang Lou [a,*]

[a] School of Pharmaceutical Sciences, Shandong University, Jinan 250012, PR China
[b] National Glycoengineering Research Center, Shandong University, Jinan 250012, PR China
[c] School of Oceanology, Shandong University, Weihai 264209, PR China

ARTICLE INFO

Article history:
Received 22 November 2011
Revised 30 January 2012
Accepted 1 February 2012
Available online 9 February 2012

Keywords:
Bisbibenzyls
Derivatives
Microtubule inhibitor
Anticancer

ABSTRACT

Based on the core skeleton of the total synthesized bisbibenzyl marchantin C, riccardin D and plagiochin E, a series of brominated and aminomethylated derivatives of above three bisbibenzyls have been synthesized and their cytotoxic activity against KB, MCF-7 and PC3 cell lines has been preliminary evaluated. The bio-test results revealed that the brominated derivatives **21**, **22**, **24**, **25** and **28** exhibited excellent antiproliferative activity, with IC_{50} value lower than their parent compounds. As a most potent microtubule depolymerization agent, compound **28** was found to arrest cells at the G_2/M phase of the cell cycle as determined by the flow cytometry assay in PC3 cell line. The remarkable biological profile and novel structure of these bisbibenzyl derivatives make them possible as promising candidates for clinical development as chemotherapeutic agents.

1. Introduction

Microtubules are cytoskeletal protein polymers formed mainly by dynamic assemblies of tubulin heterodimers.[1] They play crucial roles in mitosis and cell division and are recognized as important targets for anticancer therapy. The anticancer agents targeting on microtubules can be divided into two major groups: microtubule stabilizers like taxanes and microtubule destabilizers such as combretastatin A-4, colchicine, and vinca alkaloids.[2] Some of them (taxanes and vinca alkaloids) have been widely used in the clinical treatment of diverse human cancers for decades. However, these potent drugs still exhibit substantial limitations, such as low bioavailability, systemic toxicity, drug resistance, complex syntheses, and isolation procedures,[3] encouraging scientists to develop novel antimitotic agents for cancer therapy.

Bisbibenzyls are a series of phenolic natural products that are found exclusively in bryophytes. These natural products exhibit versatile biological activities,[4–13] including 5-lipoxygenase, cyclooxygenase and calmodulin inhibitory effects, and antifungal, antimicrobial, antioxidative, muscle-relaxing, and cytotoxic activities. Recently, we found that the natural bisbibenzyl compound marchantin C (Fig. 1) was a novel microtubule inhibitor, which can block mitosis progress and induce apoptosis of cancer cell in vivo and in vitro by interfering the microtubule polymerization.[14] Another bisbibenzyl riccardin D (Fig. 1), previously isolated in our group, was also proven to exhibit excellent anti-cancer activity,[15] and the structure similarity of riccardin D and marchantin C on bisbibenzyl skeleton implies that riccardin D might also target on microtubules.

The attractive biological results motivated us to synthesize the bisbibenzyls and their derivatives in order to improve their bioactivities and to discover more potent cytotoxic agents. It has been reported that the antiproliferative effect of some natural compounds can be enhanced obviously by halogenation and aminomethylation.[16–21] In light of this point, we were interested in the effect of the bromine and aminomethyl group on the macrocyclic bisbibenzyl system. Accordingly, some brominated and aminomethylated derivatives were prepared based on the total synthesized marchantin C, riccardin D as well as the structure similarity compound plagiochin E.[22]

Marchantin C has been previously synthesized,[23] and in present study, we report the synthetic details of riccardin D and plagiochin E, which were slightly modified according to previous report, and the preparation of brominated and aminomethylated derivatives of above three bisbibenzyls, as well as their cytotoxic activities against the KB, MCF-7 and PC3 cell lines. Molecular docking analyses were also used to elucidate the potential binding modes of the derivatives to tubulin.[24,25]

* Corresponding author. Tel.: +86 531 88364778; fax: +86 531 88382019.
E-mail address: louhongxiang@sdu.edu.cn (H. Lou).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2012.02.004

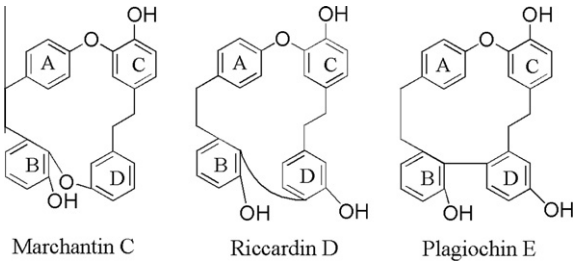
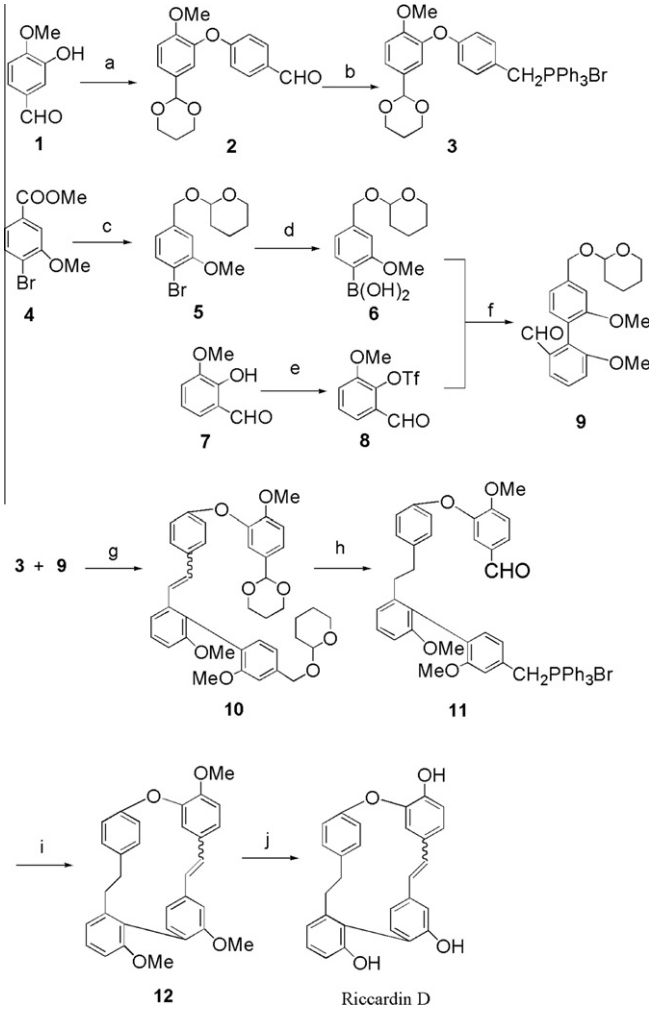


Figure 1. The chemical structures of marchantin C, riccardin D and plagiochin E.



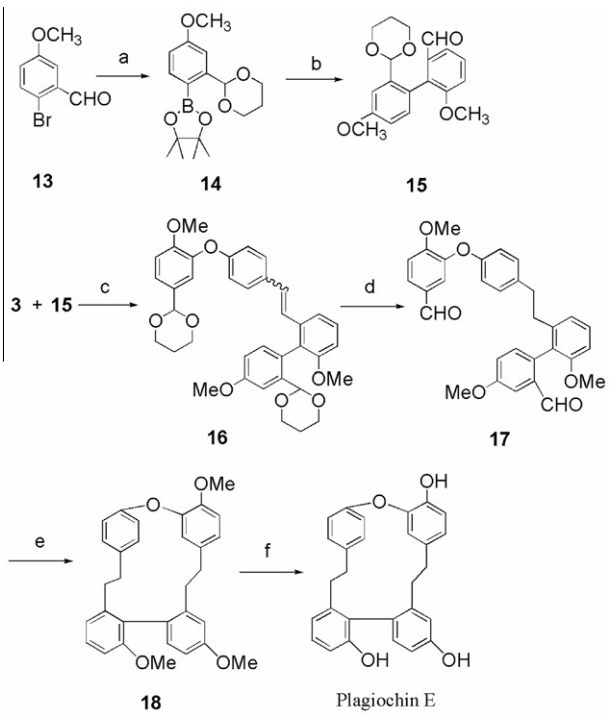
Scheme 1. Synthesis of riccardin D. Reagents and conditions: (a) (i) 1,3-propanediol, DMS–DMF, DCM, rt, 24 h; (ii) 4-iodo-benzaldehyde, Cs_2CO_3, CuBr, TMHD, NMP, 57%; (b) (i) $NaBH_4$, EtOH, rt, 2–3 h; (ii) CBr_4, PPh_3, DCM; (iii) PPh_3, toluene, reflux, 81%; (c) (i) $LiAlH_4$, THF, −40 °C, 0.5–1 h; (ii) 2,3-Dihydropyran, *p*-toluenesulfonic acid, DCM, 0–25 °C, 0.5 h, 77%; (d) *n*-BuLi, $B(OMe)_3$, KH_2PO_4, THF, −40 °C, 0.5 h, rt, 2 h, 78%; (e) Tf_2O, pyridine, DCM, rt, 1–2 h, 95%;(f) $Pd(PPh_3)_4$, toluene, EtOH, 2 mol/L Na_2CO_3, reflux, 10 h, 81%; (g) K_2CO_3, 18-crown-6, DCM, reflux, 24 h, 91%; (h) (i) Pd/C (5%), 3 bar H_2, Et_3N, EtOAc, rt, 24 h; (ii) 2 M HCl/THF (1:1), rt, 12 h; (iii) CBr_4, PPh_3, DCM; (iv) PPh_3, toluene, reflux,67%; (i) NaOMe, DCM, rt, 24 h, 67%; (j) (i) Pd/C (5%), 3 bar H_2, EtOAc, rt, 24 h; (ii) BBr_3, DCM, –40 °C to rt, 12 h, 81%.

2. Results and discussion

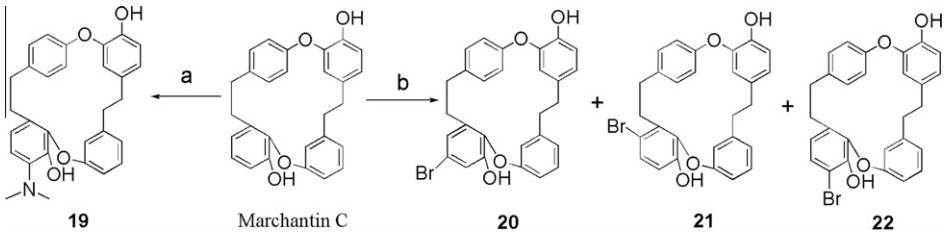
2.1. Chemistry

The synthesis of riccardin D was achieved in 18 steps as shown in Scheme 1. The synthetic route began with the Ullmann coupling of the protected 3-hydroxy-4-methoxyl-benzaldehyde with commercially available 4-iodo-benzaldehyde, resulting in the formation of the diphenyl ether **2**.[26] Compound **2** was then reduced with sodium borohydride to give the benzyl alcohol, followed by bromination and reaction with triphenylphosphine, affording phosphonium salt **3** in three steps. The biphenyl building block **9** was synthesized from compound **6** and **8** by standard Suzuki reaction, following the approach reported by Speicher et al.[27] The building blocks **3** and **9** were coupled by Wittig reaction in the presence of potassium carbonate and 18-crown-6.[28] The stilbene **10** (obtained as *E*/*Z* mixture) was then hydrogenated over Pd/C, and the carboxylic ester group was reduced with lithium aluminium hydride, followed by acidic hydrolysis. After bromination with carbon tetrabromide and subsequent reaction with triphenylphosphine, compound **11** was obtained. Cyclization of **11** by means of an intramolecular Wittig reaction was achieved with sodium methoxide, leading to intermediate **12.** Riccardin D was finally obtained after the hydrogenation and subsequent methyl ether cleavage. The synthesis of plagiochin E was achieved in 15 steps as shown in Scheme 2. The biphenyl moiety **15** was prepared from 2-bromo-5-methoxylbenzaldehyde **13** by the acetal protection, halogen/lithium exchange and subsequent scavenging with trimethyl borate, followed by treatment with pinacol. The building blocks **3** and **15** were connected by intermolecular Wittig reaction and the macrocyclic stilbene **18** was synthesized by intramolecular McMurry reaction, following the procedure depicted by Speicher et al.[22] Plagiochin E was finally obtained after hydrogenation and methyl ether cleavage.[29]

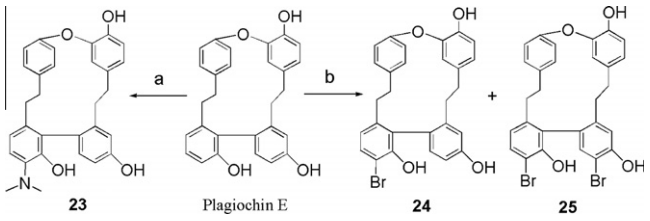
The derivatives **19**, **23** and **26** were then prepared from marchantin C, riccardin D and plagiochin E by Mannich reaction, and the derivatives **20–22**, **24**, **25**, **27** and **28** were prepared by bromination with N-bromosuccinimide (NBS) and were purified by HPLC



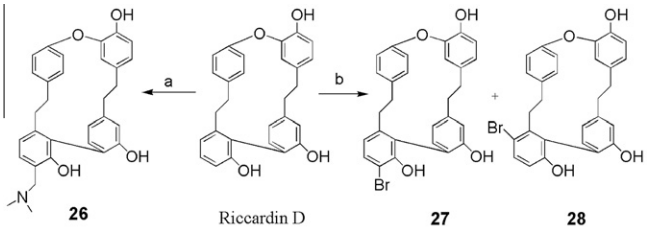
Scheme 2. Synthesis of plagiochin E. Reagents and conditions: (a) (i) 1,3-propanediol, *p*-toluenesul- fonic acid, toluene, reflux, 24 h; (ii) *n*-BuLi, $B(OMe)_3$, KH_2PO_4, THF, −40 °C, 0.5 h, rt, 2 h; (iii) pinacol, $MgSO_4$, toluene, rt, 12 h, 69%; (b) compound **8**, $Pd(PPh_3)_4$, toluene, EtOH, 2 mol/L Na_2CO_3, reflux, 10 h, 80%; (c) K_2CO_3, 18-crown-6, CH_2Cl_2, reflux, 24 h, 91% (d) (i) Pd/C (5%), 3 bar H_2, Et_3N, EtOAc, rt, 24 h; (ii) 2 M HCl/THF (1:1), rt, 12 h, 89%; (e) (i) Zn, $TiCl_4$, THF, reflux, 24 h, 40%; (ii) Pd/C (5%), 3 bar H_2, EtOAc, rt, 24 h; (f) BBr_3, DCM, –40 °C to rt, 12 h, 70%.



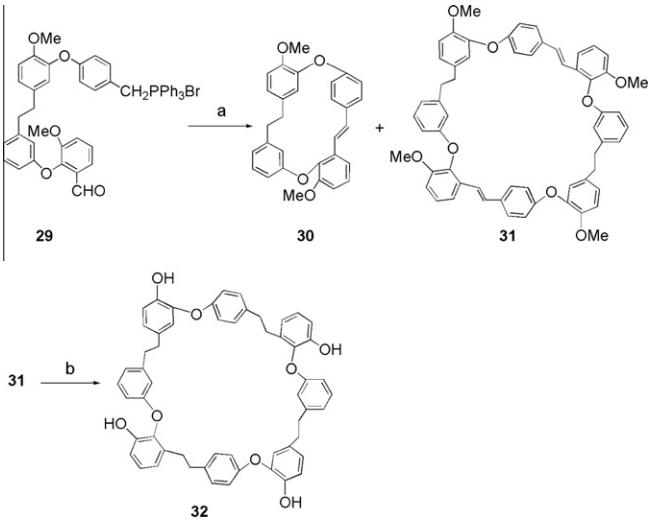
Scheme 3. Synthesis of marchantin C's derivatives. Reagents and conditions: (a) Me_2NH, HCHO, MeOH, reflux, 82%; (b) NBS, CH_3CN, 0 °C, 89% in all.



Scheme 4. Synthesis of plagiochin E's derivatives. Reagents and conditions: (a) Me_2NH, HCHO, MeOH, reflux, 79%; (b) NBS, CH_3CN, 0 °C, 86% in all.



Scheme 5. Synthesis of riccardin D's derivatives. Reagents and conditions: (a) Me_2NH, HCHO, MeOH, reflux, 80%; (b) NBS, CH_3CN, 0 °C, 76% in all.



Scheme 6. Synthesis of marchantin C's dimmer. Reagents and conditions: (a) NaOMe, DCM, 76% in all; (b) (i) H_2, Pd/C, EtOAc; (ii) BBr_3, DCM, 89%.

(Schemes 3–5). In addition, the macrocyclic compound **31** was obtained as byproduct when the synthesis of marchantin C was scaled up, and the dimer of marchantin C **32** was prepared after the hydrogenation and methyl ether cleavage, accordingly (Scheme 6).

2.2. Biological evaluation of bisbibenzyl derivatives

2.2.1. Bisbibenzyl derivatives inhibit cancer cell proliferation in vitro

To evaluate the anticancer effects of bisbibenzyl derivatives in vitro (**19–28**, **32**), MTT assays were performed as described in the Section 4 to examine their proliferative inhibitory activity against three cancer cell lines: KB (oral cancer cell line), MCF-7 (human breast adenocarcinoma cell line) and PC3 (prostate cancer cell line). The inhibition percentage at a concentration of 20 μM and the IC_{50} values of each compounds are reported in Table 1. The data demonstrate that most derivatives exhibit obvious inhibitory activity against three human cancer cell lines, with IC_{50} value ranging from 5.4 to 33.8 μM. Within the marchantin C derivatives, the bromination at either the *para*- or *ortho*-position of hydroxyl group on ring B improves the activity obviously (compounds **21** and **22**); however, bromination at the *meta*-position and aminomethylation at the *ortho*-position of hydroxyl group on ring B impacted the activity slightly, and the dimer compound **32** was less potent than marchantin C. The cytotoxicity of brominated plagiochin E **24** and **25** was greatly improved comparing to the parent compound and the aminomethylated product **23** also exhibited good activity against KB cell line. The riccardin D derivative **28**, with a bromide at the *para*-position of hydroxyl group on ring B, was the most cytotoxic compound against KB, MCF-7 and PC3 cell lines, with IC_{50} values of 5.9, 5.4 and 5.6 μM, respectively. The substitution of aminomethyl group on ring B of riccardin D led to reduced activity (compound **26**), and the bromination at the *ortho*-position of hydroxyl group on ring B made the resulting compound inactive (compound **27**, IC_{50} >50 μM). Overall, the activities of most brominated derivatives (compounds **21**, **22**, **24**, **25**, and **28**) were more potent than their parent compounds and aminomethylated derivatives (compounds **19**, **23** and **26**) hardly exhibited the improved activity.

2.2.2. Bisbibenzyl derivative induces cell cycle arrest at G_2/M phase

After the activity evaluation, we extend our work to the mechanism investigation and the flow cytometry was then used to analyze the effects of bisbibenzyl derivative on the cell growth and division. The most potent antitubulin agent **28** was selected for cell cycle studies in PC3, MCF-7 and KB cell lines by measuring the DNA content. PC3 cells were cultured with 2.5, 5 or 10 μM **28**, respectively, for 24 h and then collected for flow cytometry assay. As shown in Figure 3, in the absence of **28**, there were 7.02% of cells in G_2/M phase. In contrast, significant accumulation of PC3 cells in G_2/M phase were observed after **28** treatment at the concentration of 2.5 (6.15%), 5 (17.05%) and 10 μM (30.10%). Such cells appear to have the capacity to replicate their DNA but are not able to proceed through the cell cycle to cell division. The effects of **28** on cell growth of MCF-7 and KB cell lines have also been evaluated by the same way as used for PC3 cells. As shown in Figure 4 and 5, the cell cycle of both cell lines were arrested in G_2/M phase as well, and increasing concentration of **28** to 10 μM led to essen-

Table 1
In vitro cytotoxicity of three bisbibenzyls and their derivatives in three cancer cell lines

Compound	KB		MCF-7		PC3	
	% Inhib[a]	IC_{50} [b] (μM)	% Inhib[a]	IC_{50} [b] (μM)	% Inhib[a]	IC_{50} [b] (μM)
19	83.75%	16.8 ± 1.20	85.46%	17.5 ± 0.27	69.26%	12.6 ± 0.34
20	81.34%	11.5 ± 0.15	62.55%	15.3 ± 0.41	48.66%	15.7 ± 0.42
21	68.54%	11.0 ± 0.70	58.45%	6.3 ± 0.82	56.80%	15.6 ± 0.23
22	76.76%	11.3 ± 0.22	83.01%	12.1 ± 0.14	64.88%	8.5 ± 0.02
23	2.62%	15.1 ± 0.28	0.75%	33.8 ± 2.37	39.58%	25.1 ± 0.54
24	84.32%	8.2 ± 0.72	62.41%	6.3 ± 0.69	71.74%	9.3 ± 0.05
25	90.71%	9.7 ± 0.48	81.37%	8.4 ± 0.09	91.82%	9.2 ± 0.03
26	91.59%	10.7 ± 0.07	92.83%	13.3 ± 0.21	98.03%	9.7 ± 0.02
27	1.63%	>50	6.76%	>50	2.23%	>50
28	95.26%	5.9 ± 0.30	95.53%	5.4 ± 0.07	97.45%	5.6 ± 0.23
32	87.91%	13.8 ± 0.54	56.85%	16.4 ± 0.24	20.66%	27.2 ± 0.13
Marchantin C	22.30%	15.3 ± 0.32	13.03%	12.8 ± 0.22	17.39%	15.8 ± 0.28
Riccardin D	98.12%	7.1 ± 0.11	59.69%	6.6 ± 0.70	94.87%	10.1 ± 0.09
Plagiochin E	10.27%	40.1 ± 1.52	6.90%	34.9 ± 1.32	8.99%	28.0 ± 1.86

[a] The inhibition percentage was tested at a concentration of 20 μM of each compound.
[b] The IC_{50} values (μM) are the concentrations corresponding to 50% inhibition of each cell line.

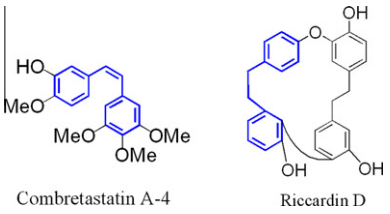


Figure 2. The chemical structures of combretastatin A-4 and riccardin D. The blue bonds emphasize the structure similarity of these two compounds.

tially the same result in MCF-7 and KB cells (20.24% and 39.56% in G_2/M phase, respectively). Overall, these results demonstrated that bisbibenzyl derivative **28** induced cell cycle arrest at G_2/M phase in a dose-dependent manner, which is consistent with those obtained for classical tubulin-targeting drugs.[30]

2.2.3. Immunofluorescence microscopy observation

The significant cell growth inhibitory properties of bisbibenzyl derivatives supported by their obvious G_2/M blocking properties promoted us to investigate the further biological mechanism. Since the bisbibenzyl compound marchantin C has been reported to

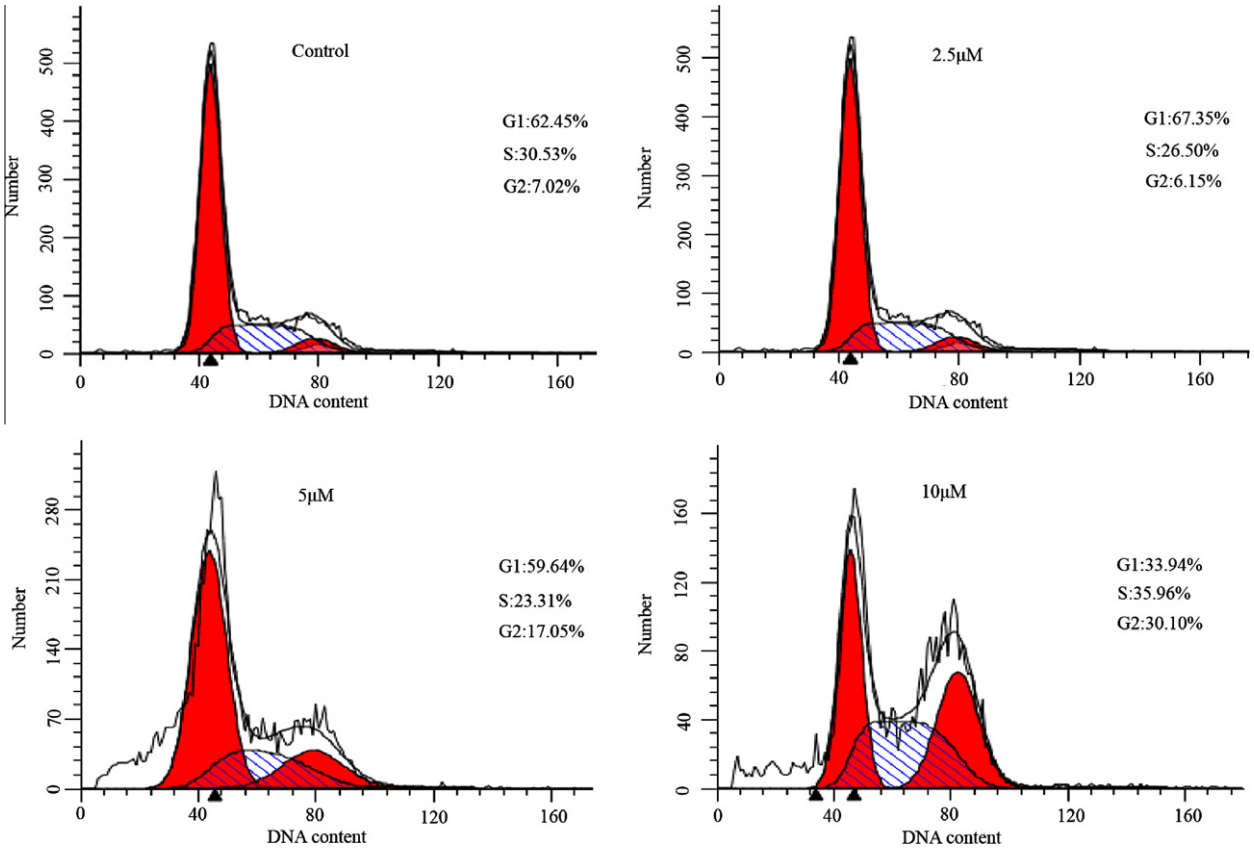


Figure 3. Compound **28** induced PC3 cell cycle arrest at G_2/M phase. PC3 cells were treated with 2.5, 5, 10 μM of **28** for 24 h and then trypsinized, fixed and stained with PI to measure cell cycle profile by flow cytometry. Control cells were treated with DMSO alone. Results are representatives of three independent experiments.

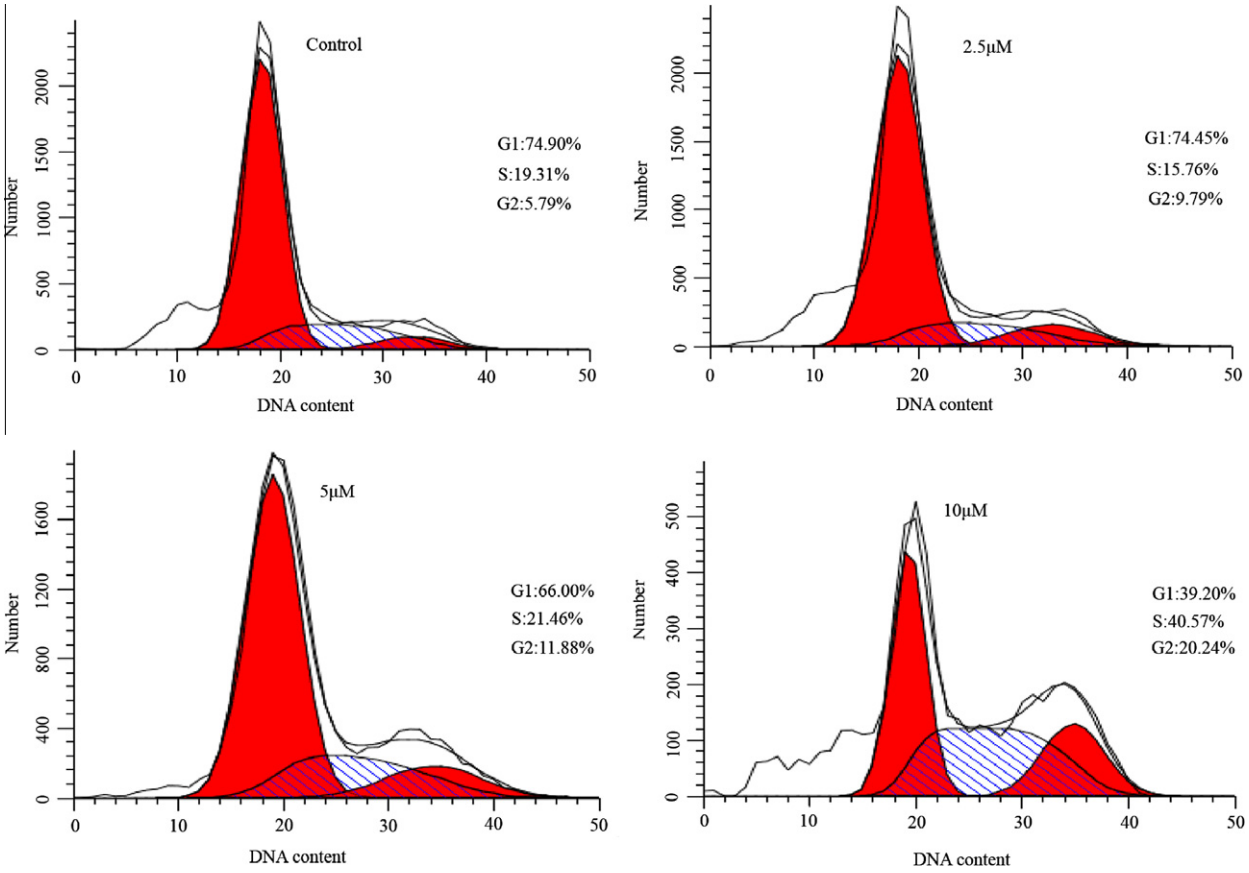


Figure 4. Compound **28** induced MCF-7 cell cycle arrest at G_2/M phase. MCF-7 cells were treated with 2.5, 5, 10 μM of **28** for 24 h and then trypsinized, fixed and stained with PI to measure cell cycle profile by flow cytometry. Control cells were treated with DMSO alone. Results are representatives of three independent experiments.

exhibit the microtubule depolymerization activity, we next examined the effect of selected potent compounds **22**, **24**, **26** and **28** on microtubule by immunofluorescent staining techniques. First, PC3 cells were cultured for 24 h in the presence of compounds **22**, **24**, **26** and **28** at the concentration of 8, 10, 6 and 6 μM, respectively, and then fixed with cold methanol/acetone, followed by immunostaining for α-tubulin and β-actin. The cellular microtubule networks and actins were then visualized by fluorescence microscopy. As shown in Figure 6, intact microtubules arrays could be observed in untreated cells. However, after treatment with compounds **22**, **24**, **26** or **28**, microtubules networks were decreased and short microtubules fragments were observed in the cytoplasm. Such morphological changes are indicative of depolymerized and dispersed tubulin dimmers, which are very similar to the reported cellular changes caused by treatment with marchantin C and colchicine.[14,31]

2.2.4. Molecular modeling

The excellent bioactivity of derivatives encouraged us to investigate the possible mechanism of action at the molecular level, specifically, the binding mode of bisbibenzyls to tubulin.[32,33] It has been reported that there are three ligand binding sites in tubulins: the colchicine, vinca alkaloid, and taxane.[34] The antimitotic agents binding to taxane binding site inhibit the depolymerization of microtubules, while the compounds binding to vinca alkaloid binding site and colchicine binding site inhibit the polymerization of microtubules. Combretastatin A-4 (CA-4) (Fig. 2) is a potent antimitotic agent by inhibiting the polymerization of microtubules which is similar to the mechanism of bisbibenzyl. CA-4 has been proved to binds to colchicine binding site.[14,35–38] The structure and effect similarity between CA-4 and bisbibenzyls implies that bisbibenzyls might also bind to the colchicine binding site. Based on this judgment, the most potent compound **28** was docked into the colchicine binding site of tubulin (PDB code 1SA0) using the GOLD (Genetic Optimization for Ligand Docking) program,[39] and the energy minimized. In the resulting hypothetical structure (Fig. 7 and Fig. 8), the hydroxyl group on ring C may forms hydrogen bonds with carbonyl group of Ser178 and hydroxyl group of Try224. The hydroxyl group on ring B is involved in a potential hydrogen bond with the carbonyl group of Lys254. It is also possible that ring B forms a CH-π interaction with the methyl group of Leu255. We were able to observe that the bromine atom on ring B could fit into the pocket formed by Ala316 and Lys352. These combined CH–π interaction and hydrogen bonds could play a crucial role in tubulin inhibitory activity of the bisbibenzyl compounds. In addition, the introduction of bromine atom to riccardin D could change the electron distribution on ring B, which may create stronger hydrogen bond with Lys254 and CH–π interaction with Leu255, and this might be the reason for the improved potency of compound **28** than riccardin D.

3. Conclusions

As shown in Schemes 1–6, and Table 1, we have achieved the synthesis of riccardin D and plagiochin E, prepared a series of bisbibenzyl derivatives (**19–28** and **32**) with the bromine and aminomethyl groups, and tested their cytotoxic activity against PC3, MCF-7 and KB cell lines. Among the tested compounds, **28** showed the most potent antiproliferative activity toward three cell lines, and compounds **21**, **24**, **25** and **26** also exhibited excellent bioactiv-

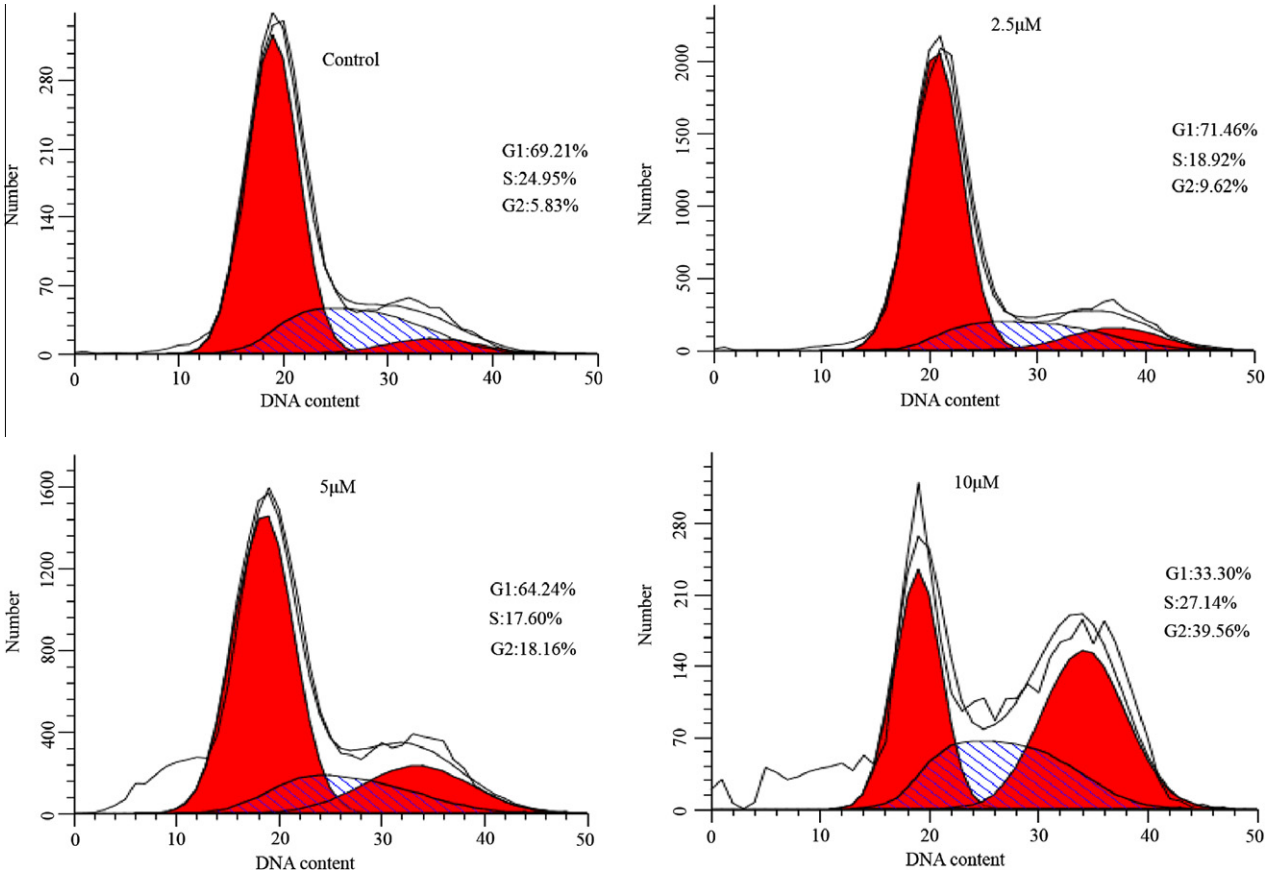


Figure 5. Compound **28** induced KB cell cycle arrest at G_2/M phase. KB cells were treated with 2.5, 5, 10 μM of **28** for 24 h and then trypsinized, fixed and stained with PI to measure cell cycle profile by flow cytometry. Control cells were treated with DMSO alone. Results are representatives of three independent experiments.

ity. In addition, the further mechanism study revealed that bisbibenzyl derivatives could arrest cancer cells at G_2/M phase by destroying the microtubules network. Finally, molecular modeling was also used to elucidate the binding models of the derivatives to tubulin. From the study of a preliminary structure-activity relationship, it was considered that the bisbibenzyl skeleton and phenolic hydroxyl groups played essential roles in the tubulin inhibitory activity. The introduction of bromine into the structure could change the electron distribution on benzene ring, which might improve the strength of hydrogen bond and CH–π interaction between the protein and ligand. This model could be utilized in the further modification of bisbibenzyls for the discovery of novel anticancer agents.

4. Experimental section

4.1. Chemistry

Column chromatography was carried out on silica gel or alumina (200–300 mesh). Reactions were monitored by thin-layer chromatography, using Merck plates with fluorescent indicator. Melting points were determined on an X-6 melting-point apparatus and are uncorrected. The NMR spectra were recorded on a Bruker Spectospin spectrometer at 600 MHz (^{1}H) and 150 MHz (^{13}C), using TMS as an internal standard. The chemical shifts are reported in parts per million (ppm δ) referenced to the residual ^{1}H resonance of the solvent ($CDCl_3$, 7.28 ppm). Abbreviations used in the splitting pattern were as follows: s = singlet, d = doublet, t = triplet, quin = quintet, m = multiplet, and br=broad. All HRMS spectra (ESI) were obtained on a LTQ Orbitrap mass spectrometer. Reagents were used as purchased without further purification. Solvents (THF, DCM, pyridine and toluene) were dried and freshly distilled before use according to procedures reported in the literature.

4.1.1. General procedures

4.1.1.1. General procedure 1 (GP 1) for the bromination of bisbibenzyls. A mixture of bisbibenzyl (riccardin D, plagiochin E or marchantin C, 20 mg, 0.047 mmol) and a catalytic amount of manganese dioxide in anhydrous CH_2Cl_2 (1 mL) was stirred at room temperature. The bromine (2.4 μL, 0.047 mmol)in anhydrous CH_2Cl_2 (0.5 mL) was added dropwise to the solution and the reaction system was stirred for 24 h. The solvent was evaporated and the residue was purified by HPLC (80% methanol in water).

4.1.1.2. General procedure 2 (GP 2) for the bromination of bisbibenzyls. To a solution of bisbibenzyl (riccardin D, plagiochin E or marchantin C, 20 mg, 0.047 mmol) in acetonitrile (1.3 mL) was added NBS (4.2 mg, 0.0235 mmol). The mixture was stirred at 0 °C for 12 h. After rise to room temperature, the solid was filtered off and the solvent was removed in vacuo. The residue was purified by HPLC (78% methanol in water).

4.1.1.3. General procedure 3 (GP 3) for the aminomethylation of bisbibenzyls. Formaldehyde aqueous solution (37%, 5 μL, 0.061 mmol) was added to a solution of bisbibenzyl (riccardin D, plagiochin E or marchantin C, 20 mg, 0.047 mmol) in methanol (1 mL). Dimethylamine was then added to the reaction mixture at 65 °C. The mixture was refluxed for 12 h and after cooling to

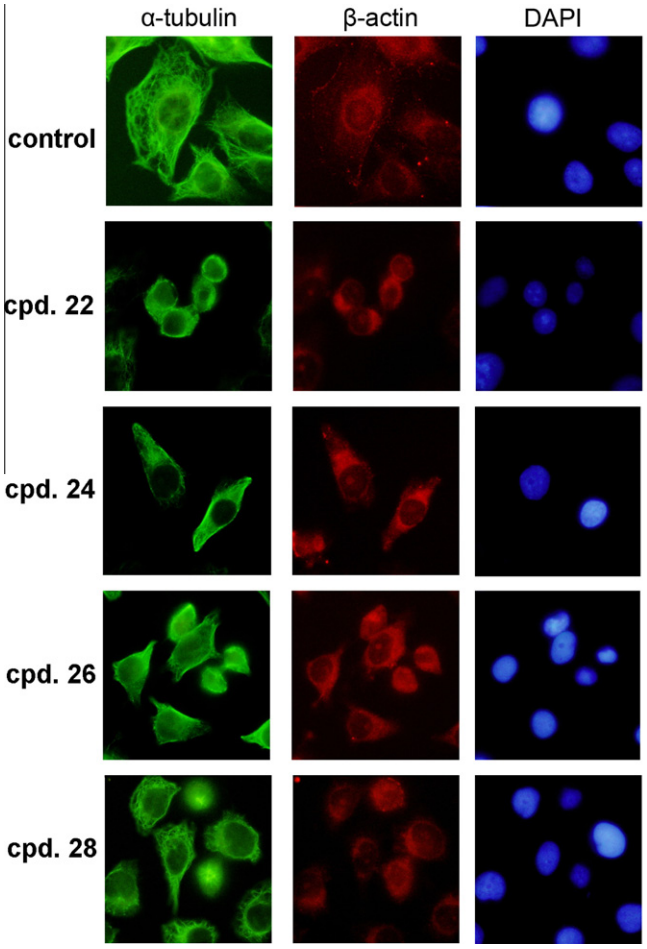


Figure 6. Effects of selective derivatives **22**, **24**, **26** and **28** on the microtubule distribution in PC3 cells. PC3 cells were incubated with **22**, **24**, **26** and **28** at the concentration of 8, 10, 6 and 6 μM, respectively, for 24 h and then fixed and immunostained with monoclonal antibody. DMSO was used as control.

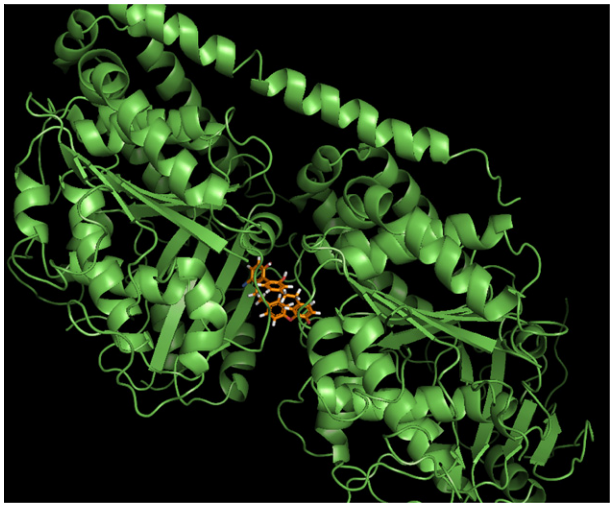


Figure 7. Ribbon diagram illustrating the hypothetical complex structure of tubulin with compound **28** (orange) bound to the colchicine binding site.

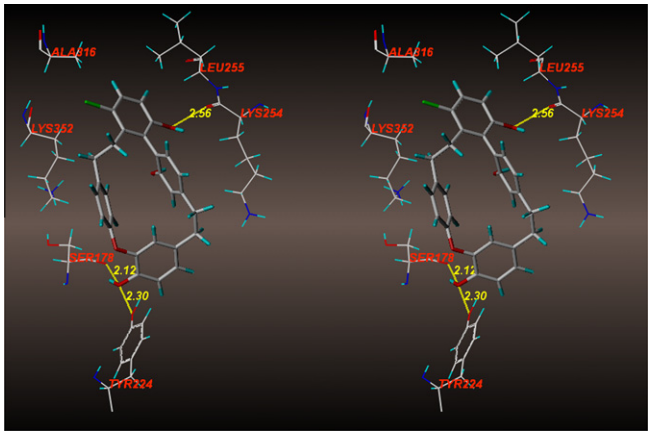


Figure 8. Potential interactions between compound **28** and Ala316, Lys352, Ser178, Tyr224, Leu255 and Lys254 in tubulin colchicine binding site. The diagram is programmed for wall-eyed (relaxed) viewing. The hydrogen bonds are labeled as yellow lines.

room temperature the solvent was evaporated. The crude product was purified by column chromatography on silica gel eluted with acetone: petroleum ether (3:8).

4.2. Synthesis

4.2.1. The data of three parent compounds and their intermediates

4.2.1.1. The dimer of marchantin C (32). Compound **32** was prepared by following the procedure reported in the literature.[23] White solid. Mp 151–152 °C. ^{1}H NMR ($CDCl_3$): δ = 7.28 (s, 12H, Ar-H), 7.19 (t, J = 7.8 Hz, 1H, Ar-H), 7.10 (t, J = 7.8 Hz, 1H, Ar-H), 6.94 (d, J = 8.4 Hz, 2H, Ar-H), 6.92 (d, 2H, Ar-H), 6.81 (m, 5H, Ar-H), 6.72 (d, J = 7.8 Hz, 1H, Ar-H), 6.68 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 5.44 (s, 1H, Ar-H), 5.33 (s, 1H, Ar-H), 2.72 (m, 9H, –CH_2–), 1.27 (m, 7H, –CH_2–); HRMS (ESI) calcd for $C_{56}H_{48}O_8$ 848.9732 found 848.9756; MS (ESI) 849 $(M+H)^+$.

4.2.1.2. 2′,6-Dimethoxy-4′-[(tetrahydro-2*H*-pyran-2-yloxy)methyl]biphenyl-2-carbaldehyde (9). Compound **9** was by following the procedure reported in the literature.[23] Colorless oil. ^{1}H NMR ($CDCl_3$): δ = 9.69 (s, 1H, CHO), 7.63 (d, J = 7.8 Hz, 1H, Ar-H), 7.47 (t, J = 7.8 Hz, 1H, Ar-H), 7.22 (t, J = 7.2 Hz, 2H, Ar-H), 7.07 (m, 1H, Ar-H), 7.04 (d, J = 4.8 Hz, 1H, Ar-H), 4.89 (d, J = 12.44 Hz, 1H, Ph –CH_2–O), 4.79 (m, 1H, O–CH–O), 4.59 (dd, 1H, Ph –CH_2–O), 3.98 (m, 1H, –CH_2–), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.61 (m, 1H, –CH_2–), 1.93 (m, 1H, –CH_2–), 1.82 (m, 1H, –CH_2–), 1.70 (m, 3H, –CH_2–), 1.27 (m, 1H, –CH_2–); HRMS (ESI) calcd for $C_{21}H_{24}O_5$ 356.4127 found 356.4223; MS (ESI) 357 $(M+H)^+$.

4.2.1.3. The Bibenzyl (11). Compound **11** was prepared by following the procedure reported in the literature.[23] Yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.29 (d, J = 3 Hz, 1H, Ar-H), 7.26 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 7.08 (d, J = 7.2 Hz, 2H, Ar-H), 7.05 (d, J = 5.4 Hz, 2H, Ar-H), 6.99 (d, J = 8.4 Hz, 1H, Ar-H), 6.92 (d, J = 7.2 Hz, 1H, Ar-H), 6.86 (d, J = 8.4 Hz, 1H, Ar-H), 6.83 (d, J = 7.8 Hz, 2H, Ar-H), 6.79 (d, J = 8.4 Hz, 2H, Ar-H), 5.41 (s, 1H, Ph–CH–O), 4.89 (dd, J_1 = 12.44 Hz, J_2 = 2.4 Hz, 1H, Ph–CH_2–O), 4.80 (m, 1H, O–CH–O), 4.60 (d, J = 12.44 Hz, 1H, Ph–CH_2–O), 4.23 (dd, J_1 = 11.44 Hz, J_2 = 4.8 Hz, 2H, –CH_2–),3.97 (m, 3H, –CH_2–), 3.85(s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3) 3.60 (m, 1H, –CH_2–), 2.68 (m, 3H, –CH_2–), 2.57 (m, 1H, –CH_2–), 2.20 (m, 1H, –CH_2–), 2.08 (m, 1H, –CH_2–), 1.92 (m, 1H, –CH_2–), 1.79 (m, 1H, –CH_2–), 1.72 (m, 1H, –CH_2–), 1.64 (m, 3H, –CH_2–); HRMS (ESI) calcd for $C_{39}H_{44}O_8$ 640.7620 found 640.7596; MS (ESI) 641 $(M+H)^+$.

4.2.1.4. Riccardin D trimethyl ether (12). Compound **12** was prepared by following the procedure reported in the literature.[22] White solid. Mp 116–117 °C. ^{1}H NMR ($CDCl_3$): δ = 7.39 (t, J = 7.8 Hz, 1 H, Ar-H), 7.12 (d, J = 7.8 Hz, 1H, Ar-H), 6.95 (d, J = 8.4 Hz, 1H, Ar-H), 6.93 (d, J = 8.4 Hz, 1H, Ar-H), 6.91 (d, J = 7.2 Hz, 1 H, Ar-H), 6.88 (d, J = 7.2 Hz, 1H, Ar-H), 6.87 (d, J = 7.8 Hz, 1H, Ar-H), 6.83 (dd, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 2H, Ar-H), 6.79 (d, J = 7.8 Hz, 1H, Ar-H), 6.45 (d, J = 7.8 Hz, 1H, Ar-H), 6.43 (s, 1H, Ar-H), 5.51 (s, 1H, Ar-H), 3.99 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 2.98 (m, 3H, $-CH_2-$), 2.76 (m, 5H, $-CH_2-$); HRMS (ESI) calcd for $C_{31}H_{30}O_4$ 466.5715 found 466.5618; MS (ESI) 467 $(M+H)^+$.

4.2.1.5. Riccardin D. Riccardin D was prepared by following the procedure reported in the literature.[22] White solid. Mp 152–153 °C. ^{1}H NMR ($CDCl_3$): δ = 7.37 (t, J = 7.8 Hz, 1H, Ar-H), 7.09 (d, J = 7.8 Hz, 1H, Ar-H), 6.95 (d, J = 7.8 Hz, 2H, Ar-H), 6.93 (d, J = 8.4 Hz, 1H, Ar-H), 6.88 (d, J = 8.4 Hz, 1H, Ar-H), 6.85 (d, J = 7.8 Hz, 2H, Ar-H), 6.80 (d, J = 8.4 Hz, 1H, Ar-H), 6.77 (d, J = 7.8 Hz, 1H, Ar-H), 5.01 (s, 1H, -OH), 4.98 (s, 1H, -OH), 3.01 (m, 2H, $-CH_2-$), 2.93 (t, 1H, $-CH_2-$), 2.79 (m, 2H, $-CH_2-$), 2.68 (m, 2H, $-CH_2-$), 2.63 (t, 1H, $-CH_2-$); HRMS (ESI) calcd for $C_{28}H_{24}O_4$ 424.4775 found 424.4735; MS (ESI) 465 $(M+H)^+$.

4.2.1.6. 2′-(1,3-Dioxan-2-yl)-4′,6-dimethoxybiphenyl-2-carbaldehyde (15). Compound **15** was prepared by following the procedure reported in the literature.[22] Yellow solid. Mp 105–106 °C. ^{1}H NMR ($CDCl_3$): δ = 9.57 (s, 1H, CHO), 7.64 (d, J = 7.8 Hz, 1H, Ar-H), 7.49 (t, J = 7.8 Hz, 1H, Ar-H), 7.35 (d, J = 1.2 Hz, 1H, Ar-H), 7.20 (d, J = 7.8 Hz, 1H, Ar-H), 7.11 (d, J = 8.4 Hz, 1H, Ar-H), 6.98 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 5.00 (s, 1H, -CH-Ph), 4.18 (m, 1H, $-CH_2-$), 3.93 (m, 1H, $-CH_2-$), 3.92 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.71 (t, 1H, $-CH_2-$), 3.42 (t, 1H, $-CH_2-$), 2.12 (m, 1H, $-CH_2-$), 1.27 (m, 1H, $-CH_2-$); HRMS (ESI) calcd for $C_{19}H_{20}O_5$ 328.3535 found 328.2126; MS (ESI) 329 $(M+H)^+$.

4.2.1.7. The bibenzyl dialdehyde (17). Compound **17** was prepared by following the procedure reported in the literature.[22] Yellow oil. ^{1}H NMR ($CDCl_3$): δ = 9.82 (s, 1H, CHO), 9.60 (s, 1H, CHO), 7.64 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 7.54 (d, J = 2.4 Hz, 1H, Ar-H), 7.41 (d, J = 1.8 Hz, 1H, Ar-H), 7.35 (t, J = 7.8 Hz, 1H, Ar-H), 7.23 (dd, J_1 = 8.4 Hz, J_2 = 3.0 Hz, 1H, Ar-H), 7.11 (d, J = 8.4 Hz, 2H, Ar-H), 6.98 (d, J = 7.8 Hz, 1H, Ar-H), 6.84 (m, 5H, Ar-H), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3) 2.68 (m, 4H, $-CH_2-$); HRMS (ESI) calcd for $C_{31}H_{28}O_6$ 496.2323 found 496.2315; MS (ESI) 497 $(M+H)^+$.

4.2.1.8. Plagiochin E trimethyl ether (18). Compound **18** was prepared by following the procedure reported in the literature.[22] White solid. Mp 103–104 °C. ^{1}H NMR ($CDCl_3$): δ = 7.38 (d, J = 6.0 Hz, 1H, Ar-H), 7.37 (d, J = 7.8 Hz, 1H, Ar-H), 7.05 (d, J = 8.4 Hz, 2H, Ar-H), 6.93 (dd, J_1 = 9.6 Hz, J_2 = 3.0 Hz, 1H, Ar-H), 6.91 (d, J = 7.8 Hz, 1 H, Ar-H), 6.78 (t, J = 7.8 Hz, 1H, Ar-H), 6.76 (d, J = 8.4 Hz, 2H, Ar-H), 6.74 (d, J = 7.8 Hz, 1H, Ar-H), 6.73 (s, 1H, Ar-H), 6.71 (dd, J_1 = 9.6 Hz, J_2 = 3.0 Hz, 1H, Ar-H), 5.26 (s, 1H, Ar-H), 3.95 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.32 (t, 1H, $-CH_2-$), 3.05 (m, 2H, $-CH_2-$), 2.96 (m, 1H, $-CH_2-$), 2.85 (m, 2H, $-CH_2-$), 2.55 (t, 1H, $-CH_2-$), 1.16 (m, 1H, $-CH_2-$); HRMS (ESI) calcd for $C_{31}H_{30}O_4$ 466.5670 found 466.5623; MS (ESI) 467 $(M+H)^+$.

4.2.1.9. Plagiochin E. Plagiochin E was prepared by following the procedure reported in the literature.[22] White solid. Mp 197–198 °C. ^{1}H NMR ($CDCl_3$): δ = 7.29 (d, J = 7.8 Hz, 1H, Ar-H), 7.23 (d, J = 7.8 Hz, 1H, Ar-H), 7.08 (d, J = 8.4 Hz, 2H, Ar-H), 6.85 (d, J = 8.4 Hz, 1H, Ar-H), 6.82 (d, J = 7.8 Hz, 1 H, Ar-H), 6.74 (t, J = 8.4 Hz, 1H, Ar-H), 6.73 (d, J = 7.8 Hz, 2H, Ar-H), 6.69 (d, J = 7.8 Hz, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 6.66 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 5.60 (s, 1H, Ar-H), 3.32 (m, 2H, $-CH_2-$), 3.05 (m, 2H, $-CH_2-$), 2.96 (m, 2H, $-CH_2-$), 2.85 (m, 2H, $-CH_2-$); HRMS (ESI) calcd for $C_{28}H_{24}O_4$ 424.1713 found 424.1610; MS (ESI) 425 $(M+H)^+$.

4.2.2. The data of all the derivatives

4.2.2.1. Brominated derivative of marchantin C (19). Compound **19** was prepared by following the GP3 from marchantin C, yield 80%, White powder, mp 79–80 °C. ^{1}H NMR ($CDCl_3$): δ = 7.01 (d, J = 8.4 Hz, 2H, Ar-H), 6.94 (t, J = 7.8 Hz, 1H, Ar-H), 6.89 (d, J = 4.2 Hz, 1H, Ar-H), 6.88 (d, J = 4.2 Hz, 1H, Ar-H), 6.88 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.74 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 6.62 (d, J = 8.4 Hz, 2H, Ar-H), 6.49 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.29 (s, J = 7.8 Hz, 1H, Ar-H), 5.55 (d, J = 1.8 Hz, 1H, Ar-H), 5.32 (s, 1H, OH), 3.75 (d, J = 7.2 Hz, 1H, Ph $-CH_2-$N), 3.73 (d, J = 7.2 Hz, 1H, Ph $-CH_2-$N), 3.07–3.01 (m, 4H, Ph-CH_2CH_2-Ph), 2.88–2.84 (m, 2H, Ph-CH_2CH_2-Ph), 2.78–2.76 (m, 2H, Ph-CH_2CH_2-Ph), 2.31 (s, 6H, N-CH_3); ^{13}C NMR ($CDCl_3$): δ = 157.86, 152.58, 150.42, 145.95, 143.33, 142.08, 140.02, 139.25, 135.73, 133.00, 129.72, 129.63, 127.94, 124.46, 122.51, 122.24, 121.28, 120.06, 115.57, 115.43, 114.73, 112.06, 58.47, 44.18, 36.40, 36.10, 34.71, 30.36; HRMS (ESI) calcd for $C_{31}H_{32}O_4N$ 482.2323 found 482.2326; MS (ESI) 482 $(M+H)^+$.

4.2.2.2. Brominated derivative of marchantin C (20). Compound **20** was prepared by following the GP1 from marchantin C, yield 70%, White powder, mp 110–111 °C. ^{1}H NMR ($CDCl_3$): δ = 6.99 (d, J = 8.4 Hz, 2H, Ar-H), 6.92 (t, J = 7.8 Hz, Ar-H), 6.89 (d, J = 7.8 Hz, 1H, Ar-H), 6.87 (d, J = 8.4 Hz, 1H, Ar-H), 6.72 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.64 (s, 1H, Ar-H), 6.61 (d, J = 7.8 Hz, 2H, Ar-H), 6.47 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.28 (d, J = 7.2 Hz, 1H, Ar-H), 5.53 (d, J = 1.8 Hz, 1H, Ar-H), 3.06–2.88 (m, 4H, Ph–CH_2CH_2–Ph), 2.84–2.74 (m, 4H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 157.74, 152.60, 145.94, 143.33, 142.19, 140.09, 139.22, 132.96, 129.72, 128.04, 122.63, 122.26, 121.30, 120.30, 115.54, 115.45, 114.73, 112.02, 36.36, 36.03, 34.67, 30.39; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 $(M+NH_4^+)$; MS (ESI) 503, 501 $(M-H)^-$.

4.2.2.3. Brominated derivative of marchantin C (21). Compound **21** was prepared by following the GP2 from marchantin C, yield 40%, White powder, mp 116–117 °C. ^{1}H NMR ($CDCl_3$): δ = 7.41 (d, J = 9 Hz, 1H, Ar-H), 7.03 (d, J = 8.4 Hz, 2H, Ar-H), 6.97 (t, J = 7.8 Hz, 1H, Ar-H), 6.91 (d, J = 8.4 Hz, 1H, Ar-H), 6.81 (d, J = 9 Hz, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.76 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 6.70 (d, J = 7.8 Hz, 2H, Ar-H), 6.45 (d, J = 7.2 Hz, 1H, Ar-H), 6.37 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 5.60 (d, J = 1.2 Hz, 1H, Ar-H), 5.55 (s, 1H, OH), 4.66 (s, 1H, OH), 3.15–3.13 (m, 2H, Ph–CH_2CH_2–Ph), 3.06–3.05 (m, 2H, Ph–CH_2CH_2–Ph), 2.88–2.86 (m, 2H, Ph–CH_2CH_2–Ph), 2.81–2.80 (m, 2H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 156.21, 152.94, 147.76, 146.27, 143.80, 143.61, 139.78, 136.67, 133.01, 130.04, 129.93, 129.06, 124.35, 122.42, 121.54, 116.26, 116.02, 115.80, 114.74, 114.98, 110.78, 110.02, 36.66, 34.93, 34.58, 31.82; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 $(M+NH_4^+)$; MS (ESI) 503, 501 $(M-H)^-$.

4.2.2.4. Aminomethylated derivative of marchantin C (22). Compound **22** was prepared by following the GP2 from marchantin C, yield 40%, White powder, mp 104–105 °C. ^{1}H NMR ($CDCl_3$): δ = 7.39 (d, J = 8.4 Hz, 1H, Ar-H), 6.99 (t, J = 7.8 Hz, 1H, Ar-H), 6.97 (d, J = 8.4 Hz, 2H, Ar-H), 6.97 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.90 (d, J = 8.4 Hz, 1H, Ar-H), 6.76 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.63 (d, J = 8.4 Hz, 2H, Ar-H),

6.52 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.40 (d, J = 7.8 Hz, 1H, Ar-H), 5.56 (d, J = 1.8 Hz, 1H, Ar-H), 5.50 (s, 1H, OH), 5.38 (s, 1H, OH), 3.04–3.01 (m, 4H, Ph–CH_2CH_2–Ph), 2.89–2.87 (m, 2H, Ph–CH_2CH_2–Ph), 2.81–2.79 (m, 2H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 157.52, 150.23, 147.31, 145.50, 140.66, 140.09, 138.98, 136.09, 129.59, 127.00, 124.90, 122.87, 120.30, 119.95, 115.78, 115.15, 114.22, 113.31, 36.77, 36.07, 30.93, 29.70; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 (M+NH_4^+); MS (ESI) 503, 501 $(M-H)^-$.

4.2.2.5. Aminomethylated derivative of plagiochin E (23). Compound **23** was prepared by following the GP3 from plagiochin E, yield 80%, White powder, mp 94–95 °C. ^{1}H NMR ($CDCl_3$): δ = 7.17 (d, J = 7.8 Hz, 1H, Ar-H), 7.05 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 7.02 (d, J = 7.8 Hz, 1H, Ar-H), 6.78 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.76 (d, J = 8.4 Hz, 1H, Ar-H), 6.73 (d, J = 7.8 Hz, 1H, Ar-H), 6.66 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.65 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.60 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.54 (d, J = 2.4 Hz, 1H, Ar-H), 5.29 (d, J = 1.8 Hz, 1H, Ar-H), 3.72 (d, J = 7.8 Hz, 1H, Ph –CH_2–N), 3.54 (d, J = 7.8 Hz, 1H, Ph–CH_2–N), 3.22–3.19 (m, 4H, Ph–CH_2CH_2–Ph), 2.74–2.61 (m, 4H, Ph–CH_2CH_2–Ph), 2.26 (s, 6H, N–CH3); ^{13}C NMR ($CDCl_3$): δ = 156.53, 156.14, 155.78,155.40, 143.43, 142.21, 140.36, 138.47, 132.72, 131.94, 130.06, 129.96, 128.58, 127.58, 126.67, 123.81, 122.21, 121.09, 119.00, 118.64, 115.62, 115.53, 112.74, 110.71, 62.38, 43.40, 35.23, 33.36, 29.66, 29.59; HRMS (ESI) calcd for $C_{31}H_{32}O_4N$ 482.2323 found 482.2326; MS (ESI) 482 $(M+H)^+$.

4.2.2.6. Brominated derivative of plagiochin E (24). Compound **24** was prepared by following the GP2 from plagiochin E, yield 50%, White powder, mp 126–127 °C. ^{1}H NMR ($CDCl_3$): δ = 7.52 (d, J = 8.4 Hz, 1H, Ar-H), 7.20 (d, J = 8.4 Hz, 1H, Ar-H), 7.04 (t, J = 8.4 Hz, 2H, Ar-H), 6.90 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.88 (d, J = 8.4 Hz, 1H, Ar-H), 6.82 (d, J = 7.8 Hz, 1H, Ar-H), 6.73 (dd, J_1 = 9 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.71 (d, J = 9 Hz, 1H, Ar-H), 6.69 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.65 (d, J = 2.4 Hz, 1H, Ar-H), 5.46 (s, 1H, OH), 5.30 (s, 1H, OH), 5.21 (d, J = 2.4 Hz, Ar-H), 4.77 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ = 157.24, 156.83, 154.32, 151.13, 142.66, 142.64, 140.01, 132.96, 132.52, 130.58, 130.36, 129.90, 124.26, 123.54, 122.77, 121.56, 121.38, 119.11, 118.56, 117.56, 115.42, 114.73, 113.88, 112.19, 35.37, 33.18, 30.63, 30.02; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 (M+NH_4^+); MS (ESI) 503, 501 $(M-H)^-$.

4.2.2.7. Brominated derivative of plagiochin E (25). Compound **20** was prepared by following the GP2 from plagiochin E, yield 30%, White powder, mp111–112 °C. ^{1}H NMR ($CDCl_3$): δ = 7.53 (d, J = 9 Hz, 1H, Ar-H), 7.22 (d, J = 8.4 Hz, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.08 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 7.01 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.94 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 6.81 (d, J = 7.8 Hz, 1H, Ar-H), 6.72 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.69 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 5.49 (s, 1H, OH), 5.47 (s, 1H,OH), 5.37 (s, 1H, OH), 5.20 (d, J = 1.8 Hz, 1H, Ar-H), 3.27–3.03 (m, 1H, Ph–CH_2CH_2–Ph), 3.09–3.03 (m, 2H, Ph–CH_2CH_2–Ph), 3.00–2.94 (m, 1H, Ph–CH_2CH_2–Ph), 2.84–2.79 (m, 2H, Ph–CH_2CH_2–Ph), 2.50–2.45 (m, 1H, Ph–CH_2CH_2–Ph), 1.91–1.86 (m, 1H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 155.93, 155.80, 153.04, 151.96, 142.78, 142.76, 142.75, 140.29, 139.17, 133.82, 133.80, 132.87, 130.76, 130.39, 129.89, 127.65, 124.41, 122.76, 121.63, 121.97, 121.42, 115.50, 114.76, 114.36, 35.44, 33.05, 30.85, 30.20; HRMS (ESI) calcd for $C_{28}H_{26}O_4NBr_2$ 600.2803; found 600.2805 (M+NH_4^+); MS (ESI) 580, 582, 584 $(M-H)^-$.

4.2.2.8. Aminomethylated derivative of riccardin D (26). Compound **26** was prepared by following the GP3 from riccardin D, yield 80%, White powder, mp 110–111 °C. ^{1}H NMR ($CDCl_3$): δ = 7.03 (d, J = 7.2 Hz, 1H, Ar-H), 6.94 (d, J = 7.8 Hz, 1H, Ar-H), 6.89 (d, J = 7.8 Hz, 2H, Ar-H), 6.87 (d, J = 7.8 Hz, 1H, Ar-H), 6.85 (d, J = 9.6 Hz, 1H, Ar-H), 6.79 (t, J = 9.6 Hz, 2H, Ar-H), 6.71 (d, J = 7.8 Hz, 1H, Ar-H), 6.52 (d, J = 7.2 Hz, 1H, Ar-H), 6.25 (s, 1H, Ar-H), 5.33 (s, 1H, Ar-H), 5.28 (s, 1H, OH), 3.83 (d, J = 7.8 Hz, 1H, Ph–CH_2–N), 3.61 (d, J = 7.8 Hz, 1H, Ph–CH_2–N), 2.96–2.90 (m, 3H, Ph–CH_2CH_2–Ph), 2.83–2.77 (m, 3H, Ph–CH_2CH_2–Ph), 2.61–2.55 (m, 1H, Ph–CH_2CH_2–Ph), 2.51–2.48 (m, 1H, Ph–CH_2CH_2–Ph), 2.32 (s, 6H, N–CH_3); ^{13}C NMR ($CDCl_3$): δ = 155.00, 153.19, 152.76, 146.70, 143.67, 143.33, 142.06, 140.06, 140.35, 133.29, 132.03, 129.69, 128.87, 128.29, 124.30, 122.49, 122.08, 122.03, 121.85, 121.51, 120.88, 119.17, 118.70, 116.19, 114.87, 58.46, 44.17, 38.10, 37.67, 36.81, 34.50; HRMS (ESI) calcd for $C_{31}H_{32}O_4N$ 482.2323 found 482.2326; MS (ESI) 482 $(M+H)^+$.

4.2.2.9. Brominated derivative of riccardin D (27). Compound **27** was prepared by following the GP2 from riccardin D, yield 40%, White powder, mp 99–100 °C. ^{1}H NMR ($CDCl_3$): δ = 7.58 (d, J = 7.8 Hz, 1H, Ar-H), 7.01 (d, J = 8.4 Hz, 1H, Ar-H), 6.95 (d, J = 8.4 Hz, 1H, Ar-H), 6.94 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 6.88 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 6.85 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 6.84 (d, J = 7.8 Hz, 1H, Ar-H), 6.82 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 6.77 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.47 (d, J = 1.8 Hz, 1H, Ar-H), 6.39 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H, Ar-H), 5.51 (s, 1H, OH), 5.43 (d, J = 2.4 Hz, 1H, Ar-H), 5.61 (s, 1H, OH), 4.75 (s, 1H, OH), 2.97–2.90 (m, 4H, Ph-CH_2CH_2-Ph), 2.79–2.67 (m, 4H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 156.83, 156.79, 152.65, 144.78, 143.58, 143.06, 140.59, 140.10, 139.06, 136.76, 133.09, 132.63, 132.56, 131.53, 129.27, 123.94, 122.28, 122.13, 117.45, 117.31, 116.02, 115.05, 37.70, 37.66, 36.64, 34.78; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 (M+NH_4^+); MS (ESI) 503, 501 $(M-H)^-$.

4.2.2.10. Brominated derivative of riccardin D (28). Compound **28** was prepared by following the GP2 from riccardin D, yield 40%, White powder, mp 129–130 °C. ^{1}H NMR ($CDCl_3$): δ = 7.61 (d, J = 8.4 Hz, 1H, Ar-H), 7.01 (d, J = 8.4 Hz, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 6.95 (dd, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 6.85 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 2H, Ar-H), 6.87 (t, J = 8.4 Hz, 1H, Ar-H), 6.82 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H, Ar-H), 6.76 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, Ar-H), 6.59 (s, 1H, Ar-H), 6.45 (d, J = 7.8 Hz, 1H, Ar-H), 5.64 (s, 1H, OH), 5.41 (d, J = 1.2 Hz, 1H, Ar-H), 4.85 (s, 1H, OH), 4.76 (s, 1H, OH), 3.05–2.83 (m, 4H, Ph–CH_2CH_2–Ph), 2.80–2.67 (m, 4H, Ph–CH_2CH_2–Ph); ^{13}C NMR ($CDCl_3$): δ = 156.98, 155.06, 153.01, 152.93, 144.75, 142.74, 140.65, 135.46, 134.42, 133.31, 131.50, 129.68, 129.67, 122.68, 122.52, 122.36, 117.26, 117.19, 116.40, 115.80, 115.45, 113.61, 37.44, 36.08, 36.01, 35.52; HRMS (ESI) calcd for $C_{28}H_{27}O_4NBr$ 520.1118; found 520.1112 (M+NH_4^+); MS (ESI) 503, 501 $(M-H)^-$.

4.3. Biological evaluation

4.3.1. Cell culture and cytotoxicity assay

The human squamous cell carcinoma KB, human breast adenocarcinoma cell line MCF-7 and human human prostate cancer PC3 cells (The Cell Bank of Chinese Academy of Sciences, Shanghai) were maintained in RPMI 1640 medium (Hyclone, Logan, USA) supplemented with 10% fetal bovine serum (HyClone) and supplemented with 100 U/mL of penicillin and 100 μg/mL of streptomycin. All cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. The cytotoxity of the candidate drug on tumor cells was measured by MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetra- zoliumromide) method as previously described.[35]

Briefly, after treatment of the candidate drug for 24 h, aborrbance of the soluble MTT product was measured at 570 nm. All experiments were measured at least three times.

4.3.2. Cell cycle analysis

For cell-cycle analysis, cells were cultured in the presence of different concentrations of the candidate drugs. On incubation, the cells were harvested and washed in PBS, fixed in 70% cold ethanol overnight at 4 °C, washed again in PBS, and incubated for 1 h in PBS containing 100 μg/ml RNase (Sigma) and then incubated with 50 μg/mL PI (Sigma) at 4 °C for 30 min in the dark. The cellular DNA content was analyzed by FACScan cytometry (FACSCalibur, Becton Dickinson, USA). Data were analyzed using MODFIT and CELLQUEST software (Verity Software House, Topsham, Maine, USA).

4.3.3. Immunocytochemistry

PC3 cells seeded on 6-mm round glass cover-slips and placed at the bottom of 24-well plates. After RN treatment, cells on glass cover-slips were fixed with cold methanol/acetone (1:1) for 5 min followed by immunostaing for α-tubulin and β-actin using mouse anti-α-tubulin (all from Cell Signaling Technology, Boston, MA) antibody and rabbit anti-β-actin antibody as described previously.[14] DNA was counterstained with Hoescht (1 μg/ml) for 15 min at room temperature. The samples were mounted on microscope slides with mounting medium and analyzed by fluorescence microscopy (Olympus IX71; Olympus135 Co., Tokyo, Japan).

4.4. Molecular modeling

The crystal structure of tubulin in complex with colchicine was downloaded from the Protein Data Bank (PDB code 1SA0).[40] Hydrogens were added and minimized using the Amber force field and the Amber charges. Modeled analogues were constructed in SYBYL-X,[41] and energy was minimized with the Amber force field and Amber charges. Docking compound **28** into the binding site of tubulin was performed using the GOLD program. For the genetic algorithm (GA) runs, a maximum number of 100,000 GA operations were performed on a single population of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively, which are the standard default settings recommended by the authors. The maximum distance between hydrogen bond donors and acceptors for hydrogen bonding was set to 3.5 Å. After docking, the best docked conformation of compound **28** was merged into the ligand-free protein. The new ligand–protein complex was subsequently subjected to energy minimization using the Amber force field with Amber charges. During the energy minimization, the structure of the compound **28** and a surrounding 6 Å sphere were allowed to move, while the structures of the remaining protein were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/(mol Å) energy gradient convergence criterion and a distance dependent dielectric function.

Acknowledgments

This work was supported by grant from the *National Natural Science Foundation* (*NNSF*) of China (No.30925038) and Drug Development Fund from Ministry of Science and Technology (ZX09102–127).

References and notes

1. Romagnoli, R.; Baraldi, P. G.; Remusat, V.; Carrion, M. D.; Cara, C. L.; Preti, D.; Fruttarolo, F.; Pavani, M. G.; Tabrizi, M. A.; Tolomeo, M.; Grimaudo, S.; Balzarini, J.; Jordan, M. A.; Hamel, E. *J. Med. Chem.* **2006**, *49*, 6425.
2. Wu, M. J.; Lo, Y. H.; Lin, C. C.; Lin, C. F.; Lin, Y. T.; Duh, T. H.; Hong, Y. R.; Yang, S. H.; Lin, S. R.; Yang, S. C.; Chang, L. S. *J. Med. Chem.* **2008**, *51*, 2682.
3. Alami, M.; Messaoudi, S.; Treguier, B.; Hamze, A.; Provot, O.; Peyrat, J. F.; De Losada, J. R.; Liu, J. M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J.; Brion, J. D. *J. Med. Chem.* **2009**, *52*, 4538.
4. Morita, H.; Tomizawa, Y.; Tsuchiya, T.; Hirasawa, Y.; Hashimoto, T.; Asakawa, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 493.
5. Iwai, Y.; Murakami, K.; Gomi, Y.; Tashimoto, T.; Asakawa, Y.; Okuno, Y.; Ishikawa, T.; Hatakeyama, D.; Echigo, N.; Kuzuhara, T. *PLoS ONE* **2011**, *6*, e19825.
6. Asakawa, Y. *Curr. Pharm. Des.* **2008**, *14*, 3067.
7. Sun, C. C.; Zhang, Y. S.; Xue, X.; Cheng, Y. N.; Liu, H. P.; Zhao, C. R.; Lou, H. X.; Qu, X. J. *Eur. J. Pharmacol.* **2011**, *667*, 136.
8. Shi, Y. Q.; Qu, X. J.; Liao, Y. X.; Xie, C. F.; Cheng, Y. N.; Li, S.; Lou, H. X. *Eur. J. Pharmacol.* **2008**, *584*, 66.
9. Asakawa, Y.; Toyota, M.; Hashimoto, T.; Tori, M.; Nagashima, F.; Harinantenaina, L. *Heterocycles* **2008**, *76*, 99.
10. Speicher, A.; Holz, J. *Tetrahedron Lett.* **2010**, *51*, 2986.
11. Nagashima, F.; Sekiguchi, T.; Takaoka, S.; Asakawa, Y. *Chem. Pharm. Bull* **2004**, *52*, 556.
12. Zinsmeister, H. D. *Bryophytes their chemistry and chemical taxonomy*; Oxford University Press: Oxford, 1990.
13. Herz, W. *Progress in the chemistry of Organic natural products*; Springer Wien: New York, 1995.
14. Shi, Y. Q.; Zhu, C. J.; Yuan, H. Q.; Li, B. Q.; Gao, J.; Qu, X. J.; Sun, B.; Cheng, Y. N.; Li, S.; Li, X.; Lou, H. X. *Cancer Lett.* **2009**, *276*, 160.
15. Qu, X. J.; Sun, C. C.; Zhang, Y. S.; Xue, X.; Cheng, Y. N.; Liu, H. P.; Zhao, C. R.; Lou, H. X. *Eur. J. Pharmacol.* **2011**, *667*, 136.
16. Wang, J.; Zhao, L.; Wang, R.; Lu, M.; Chen, D.; Jing, Y. *Bioorg. Med. Chem.* **2005**, *13*, 1285.
17. Takahashi, T.; Kanazawa, J.; Akinaga, S.; Tamaoki, T.; Okabe, M. *Cancer Chemother. Pharmacol.* **1999**, *43*, 233.
18. Parker, W. B.; Allan, P. W.; Hassan, A. E.; Secrist, J. A.; Sorscher, E. J., 3rd; Waud, W. R. *Cancer Gene Ther.* **2003**, *10*, 23.
19. Speicher, A. *J. Prak. Chem.-Chem. Ztg.* **2000**, *342*, 162.
20. De Ines, C.; Argandona, V. H.; Rovirosa, J.; San-Martin, A.; Diaz-Marrero, A. R.; Cueto, M.; Gonzalez-Coloma, A. Z. *Naturforsch. C.* **2004**, *59*, 339.
21. Speicher, A.; Kolz, J.; Sambanje, R. P. *Synthesis-Stuttgart* **2002**, *16*, 2503.
22. Speicher, A.; Groh, M.; Zapp, J.; Schaumloffel, A.; Knauer, M.; Bringmann, G. *Synlett* **1852**, *2009*.
23. Xi, G. M.; Sun, B.; Jiang, H. H.; Kong, F.; Yuan, H. Q.; Lou, H. X. *Bioorg. Med. Chem.* **2010**, *18*, 6725.
24. György, M. K.; Mihály, N. *Bioorg. Med. Chem.* **1995**, *3*, 1511.
25. György, M. K.; Gabriella, M.; Mihály, N.; Borbála, V.; Mária, K. *Tetrahedron* **1992**, *48*, 913.
26. Chattopadhyay, S. K.; Bandyopadhyay, A.; Pal, B. K. *Tetrahedron Lett.* **2007**, *48*, 3655.
27. Speicher, A.; Groh, M.; Hennrich, M.; Huynh, A. M. *Eur. J. Org. Chem.* **2010**, 6760.
28. Ma, Y. D.; Sun, B.; Yuan, H. Q.; Xi, G. M.; Lou, H. X. *Bioorg. Med. Chem.* **2009**, *17*, 4981.
29. Speicher, A.; Holz, J.; Hoffmann, A. *Nat. Prod. Commun.* **2011**, *6*, 393.
30. Hadfield, J. A.; Ducki, S.; Hirst, N.; McGown, A. T. *Prog. Cell. Cycle. Res.* **2003**, *5*, 309.
31. Nogales, E.; Wang, H. W.; Niederstrasser, H. *Curr. Opin. Struc. Biol.* **2003**, *13*, 256.
32. Keseru, G. M.; Nogradi, M. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 1511.
33. Keseru, G. M. *J. Mol. Recogn.* **1996**, *9*, 133.
34. Zhang, Q.; Peng, Y. Y.; Wang, X. I.; Keenan, S. M.; Arora, S.; Welsh, W. J. *J. Med. Chem.* **2007**, *50*, 749.
35. Flynn, B. L.; Flynn, G. P.; Hamel, E.; Jung, M. K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2341.
36. Bellina, F.; Cauteruccio, S.; Monti, S.; Rossi, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5757.
37. Pandit, B.; Sun, Y. J.; Chen, P.; Sackett, D. L.; Hu, Z. G.; Rich, W.; Li, C. L.; Lewis, A.; Schaefer, K.; Li, P. K. *Bioorg. Med. Chem.* **2006**, *14*, 6492.
38. Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231.
39. Sun, B.; Hoshino, J.; Jermihov, K.; Marler, L.; Pezzuto, J. M.; Mesecar, A. D.; Cushman, M. *Bioorg. Med. Chem.* **2010**, *18*, 5352.
40. www.rcsb.org.
41. SYBYL-X 1.1 for windows, Tripos, Inc.: 1699 South Hanley Rd., St. Louis, MO. 63144–2917, USA. 31-28.