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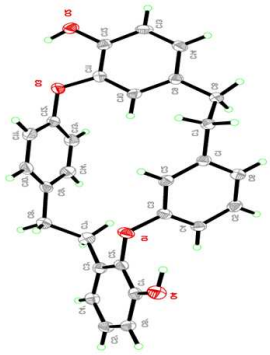
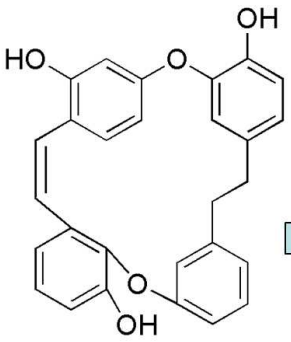


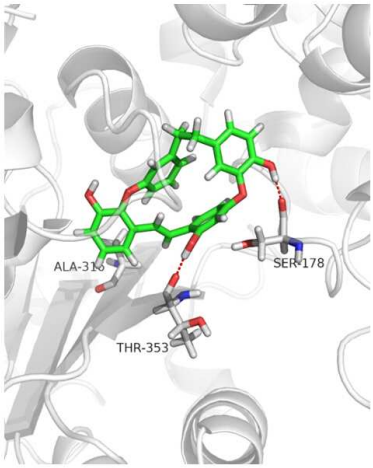
**Marchantin C**

$IC_{50}$ = 20.20 μM (Hela)
= 11.96 μM (k562)
= 12.30 μM (HCC1428)
= 11.28 μM (HT29)
= 12.00 μM (PC-3/Doc)




**Cpd 88**

$IC_{50}$ = 2.77 μM (Hela)
= 3.36 μM (k562)
= 1.26 μM (HCC1428)
= 2.66 μM (HT29)
= 3.56 μM (PC-3/Doc)

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# Design, synthesis and biological evaluation of novel macrocyclic bisbibenzyl analogues as tubulin polymerization inhibitors

Bin Sun[a,b], Lin Li [b], Qing-wen Hu [b], Fei Xie [b], Hong-bo Zheng [b], Huan-min Niu [c], Hui-qing Yuan [c], and Hong-xiang Lou [a,b] *

[a] *National Glycoengineering Research Center, Shandong University, Jinan 250012, P. R.China*

[b] *Key Laboratory of Natural Products & Chemical Biology, Ministry of Education, Shandong University, Jinan, 250012, P.R. China*

[c] *School of Medicine, Shandong University, Jinan 250012, P.R. China*

ABSTRACT

A series of novel macrocyclic bisbibenzyl analogues was designed, synthesized, and evaluated for their antiproliferative activity *in vitro*. All of the compounds were tested in five anthropic cancer cell lines, including a multidrug-resistant phenotype. Among these novel molecules, compounds **88**, **92** and **94** displayed excellent anticancer activity against Hela, k562, HCC1428, HT29, and PC-3/Doc cell lines, with average $IC_{50}$ values ranging from 2.23 μM to 3.86 μM, and were more potent than the parental compound marchantin C and much more potent than the positive control Adriamycin. In addition, the mechanism of action of compound **88** was investigated by cell cycle analysis and a tubulin polymerization assay in HCC1482 cells. The binding mode of compound **88** to tubulin was also investigated utilizing a molecular docking study. In conclusion, the present study improves our understanding of the action of bisbibenzyl-based tubulin polymerization inhibitors and provides a new molecular scaffold for the further development of antitumor agents that target tubulin.

**Keywords**: Marchantin C; Bisbibenzyls; Analogues; Tubulin polymerization inhibitors; Anticancer

* Corresponding authors: Hong Xiang Lou, Ph.D.

Tel: +86-531-88382051; Fax: +86-531-88382019.

E-mail address: louhongxiang@sdu.edu.cn (H. Lou)

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## 1. Introduction

Microtubules play crucial roles in the growth and functions of eukaryotic cells, such as division, shape maintenance and intracellular transport and are recognized as one of the most important molecular targets for cancer chemotherapy [1-4]. Microtubules are formed by a dynamic process that involves the polymerization and depolymerization of α and β-tubulin heterodimers. Drugs interfering with this dynamic equilibrium could lead to arrested cell division and eventually to apoptosis [5-8]. The inhibitors of tubulin can be mainly divided into two groups: microtubule destabilizers, such as combretastatin A-4, colchicine and vinca alkaloids, and microtubule stabilizers, such as taxanes [9-11]. Some of them (taxanes and vinca alkaloids) have been widely used in the clinical treatment of diverse human cancers for decades [12-14]. However, the clinical use of these anti-tubulin drugs is associated with problems of drug resistance and complex synthesis and isolation procedures [15-18]. Because of these undesirable limitations, there is an urgent need to develop novel antimitotic agents for cancer therapy.

Bisbibenzyls are a series of phenolic natural products that are mainly found in bryophytes. These natural products exhibit versatile and excellent biological activities, including antimicrobial, antifungal, antioxidative, muscle-relaxing, cytotoxic and multidrug resistance reversal activities [19-26]. The remarkable biological profile and interesting macrocyclic structure of bisbibenzyls make them promising resources for new drug discovery. Marchantin C, a natural macrocyclic bisbibenzyl, was first discovered in the liverwort Marchantia tosana by Asakawa and co-workers, and now has been discovered from more than 10 liverwort species [19, 27-32]. It was found to be an excellent cytotoxic agent against KB and P388 leukemia cell lines, with $IC_{50}$ values 10 and 8.5 μM, respectively. We previously discovered that marchantin C could arrest the cell cycle in the G2/M phase by inhibiting cellular microtubule assembly, specifically promoting cellular microtubule depolymerization, and this antiproliferative mechanism is similar to that of the anti-tubulin agents colchicine and combretastatin A-4 [33]. Morita et al. has also reported the antimitotic activity of isoplagiochin A and isoplagiochin B, which have the similar bisbibenzyl skeleton with marchantin C [19]. Recently, we discovered that marchantin C and its analogues also exhibit multidrug resistance-reversing activity by inhibiting the P-glycoprotein [34]. From the study mentioned above, marchantin C shows considerable promise as a lead compound, and its further structural optimization provides the potential for the discovery of more effective anti-tubulin agents.

In the present study, a series of novel marchantin C analogues were designed, synthesized, and evaluated for cytotoxic activity by the MTT assay. Among these analogues, **88**, **92** and **94** display excellent anticancer activity. The mechanism of action of the potent compound **88** was investigated using cell cycle analysis, a tubulin polymerization assay and molecular modeling. These studies indicate that the antiproliferative activity of marchantin C analogues was achieved through an antimitotic effect by inhibiting tubulin polymerization.

## 2. Results and Discussion

### *2.1. Design of marchantin C analogues*

Our modification strategy of marchantin C was first based on the understanding of the interaction between marchantin C and tubulin. Dr Iwai et al. have reported the docking research of marchantin E with influenza PA endonuclease, which provided a very good precedent for the molecular modeling research of marchantins [20]. In a previous study, the macrocyclic bisbibenzyl compound was found to be a colchicine site binder on tubulin [35]. We anticipate that marchantin C should also target the colchicine-binding site in tubulin, due to its bisbibenzyl skeleton and microtubule-depolymerizing activity. To obtain an exact stereochemical structure of marchantin C for the molecular modeling study, its crystal structure was determined by X-ray diffraction analysis, and the crystal data are presented in the supporting information. Marchantin C was then docked into the colchicine-binding site of tubulin (PDB code 1SA0) using the GOLD (Genetic Optimization for Ligand Docking) program [36]. In the resulting binding structure (Fig. 1), marchantin C fit well to the binding site of colchicine on tubulin, and the hydroxyl group on the arene *C* might form a hydrogen bond with the carbonyl group of Ser 178. Beside the ligand-protein potential interactions mentioned above, we were also able to observe that the carbonyl group of Thr 353 was closed to the arene *A* of marchantin C. We then predicted that the introduction of a hydrogen bond donor, such as a hydroxyl group, to the meta-position of the oxygen atom on arene *A* might facilitate the formation of a strong hydrogen bond between the ligand and protein. Accordingly, this modification might lead to a cytotoxic potency enhancement against tumor cell lines.

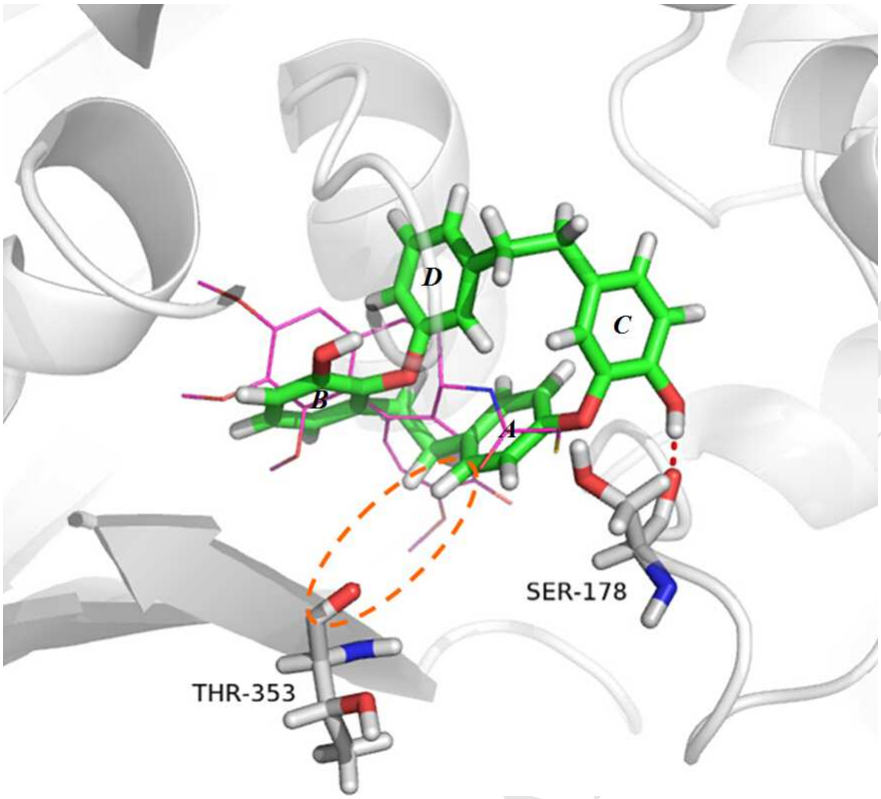


**Fig. 1.** Proposed binding mode of marchantin C as green stick model in the colchicine binding site (PDB code 1SA0). The native ligand, DAMA-colchicine, is shown in magenta thin wire model. Hydrogen bonds are shown as dotted red lines, and the distance between ligand and protein is less than 3 Å. Molecular modeling was perform by GLOD software.

[Figure 1 here]

Moreover, to define the structure-activity relationship of bisbibenzyl analogues and to discover more potent tubulin inhibitors, a series of novel marchantin C analogues were then designed. According to the strategy shown in Fig. 2, we focused on modifications of the four phenyl rings by introducing a hydroxyl group or bromine atom to arenes *A*-*D*, while maintaining the bisbibenzyl skeleton, which was regarded as a fundamental structural core for the antitumor activity. We also changed the ethylene bridge to a double bond and determined the effect of this stereochemical structural change on the cytotoxic activity.

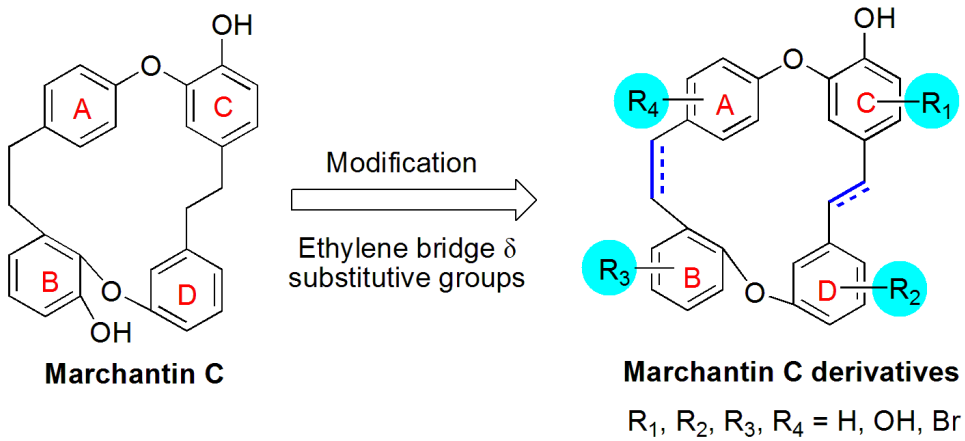


**Fig. 2.** Modification strategy for marchantin C analogues

[Figure 2 here]

*2.2. Chemistry*

The general synthesis of the marchantin C analogues is outlined in Schemes 1-4. The synthetic route of analogues **53**-**57** began with the Ullmann coupling of the appropriately substituted methyl 4-bromobenzoates **1**-**3** with 3-hydroxy-4-methoxybenzaldehyde (**4**), resulting in the formation of diphenyl ethers **5**-**7** [34]. Compounds **5**-**7** were then reduced with sodium borohydride to give the benzyl alcohols **8**-**10**, followed by the reaction with triphenylphosphonium bromide, affording **11**-**13** in two steps [34]. Compounds **19**-**22** were prepared by the Ullmann coupling of the protected 2-hydroxy-benzaldehyde (**14**) or 2-hydroxy-3-methoxybenzaldehyde (**15**) with the appropriate 3-bromo-benzaldehydes **16**-**18** in good yield. The building blocks **11**-**13** and **19**-**22** were combined by an intermolecular Wittig reaction in the presence of potassium carbonate and 18-crown-6 and followed by hydrogenation over Pd/C to give the bibenzyls **23**-**27**. The carboxylic ester group of **23**-**27** was then reduced with lithium aluminum hydride and subsequently deprotected with $HCl/H_2O$ to give compounds **33**-**37**. The reaction of **33**-**37** with triphenylphosphonium bromide afforded compounds **38**-**42**. The cyclization of **38**-**42** by means of an intramolecular Wittig reaction was achieved with sodium methoxide, leading to key macrocyclic intermediates **43**-**47**. Finally, **43**-**47** could be hydrogenated to give compounds **48**-**52**, followed by demethylation to afford compounds **53**-**57** (Scheme 1).

However, the Ullmann coupling of the protected 2-hydroxy-4-methoxybenzaldehyde (**58**) or 2-hydroxy-5-methoxybenzaldehyde (**59**) with 3-bromo-benzaldehyde (**60**) failed, and this might be because the phenolic group of compounds **58** was deactivated by the methoxyl group, and compound

**59** was deactivated by the electronic effect. 2-Bromo-4-methoxybenzaldehyde (**63**), 2-bromo-5-methoxybenzaldehyde (**64**) and the protected 2-hydroxybenzaldehyde (**65**) were then used as starting materials for the Ullmann coupling, and diphenyl esters **66** and **67** were obtained, accordingly. Compounds **66** and **67** were then reduced with sodium borohydride to give the benzyl alcohols **68**-**69**, followed by reaction with triphenylphosphonium bromide, affording **70**-**71** in two steps. The diphenyl ether **75**, as the second building block, was synthesized from 2-methoxy-5-((tetrahydro-2H-pyran-2-yloxy)methyl)phenol (**73**) and 4-bromo-benzaldehyde (**74**) by the Ullmann reaction. The building blocks **70**-**71** and **75** were combined by an intermolecular Wittig reaction in the presence of potassium carbonate and 18-crown-6, followed by hydrogenation over Pd/C to give bibenzyls **76** and **77**. Compounds **76** and **77** were deprotected by $HCl/H_2O$ to give compounds **78** and **79**, and the analogues **86** and **87** were finally obtained by following the protocol described for analogues **53**-**57** (Scheme 2). In addition, during the preparation of analogues **54** and **86**, two important macrocyclic intermediates **44** and **82** were prepared in large amounts. The methyl ether cleavage of **44** and **82** was achieved by aluminum iodide, providing analogues **88** and **89**, respectively (Scheme 3).

The preparation method for the brominated analogues is shown in Scheme 4. We have previously synthesized three brominated analogues of marchantin C with N-bromosuccinimide (NBS) and evaluated their anticancer activity [31]. In this paper, a more convenient and efficient method was applied for the bromination. Marchantin C was treated with HBr in the presence of DMSO in ethyl acetate [37], and analogues **90**-**95** were provided by HPLC separation.

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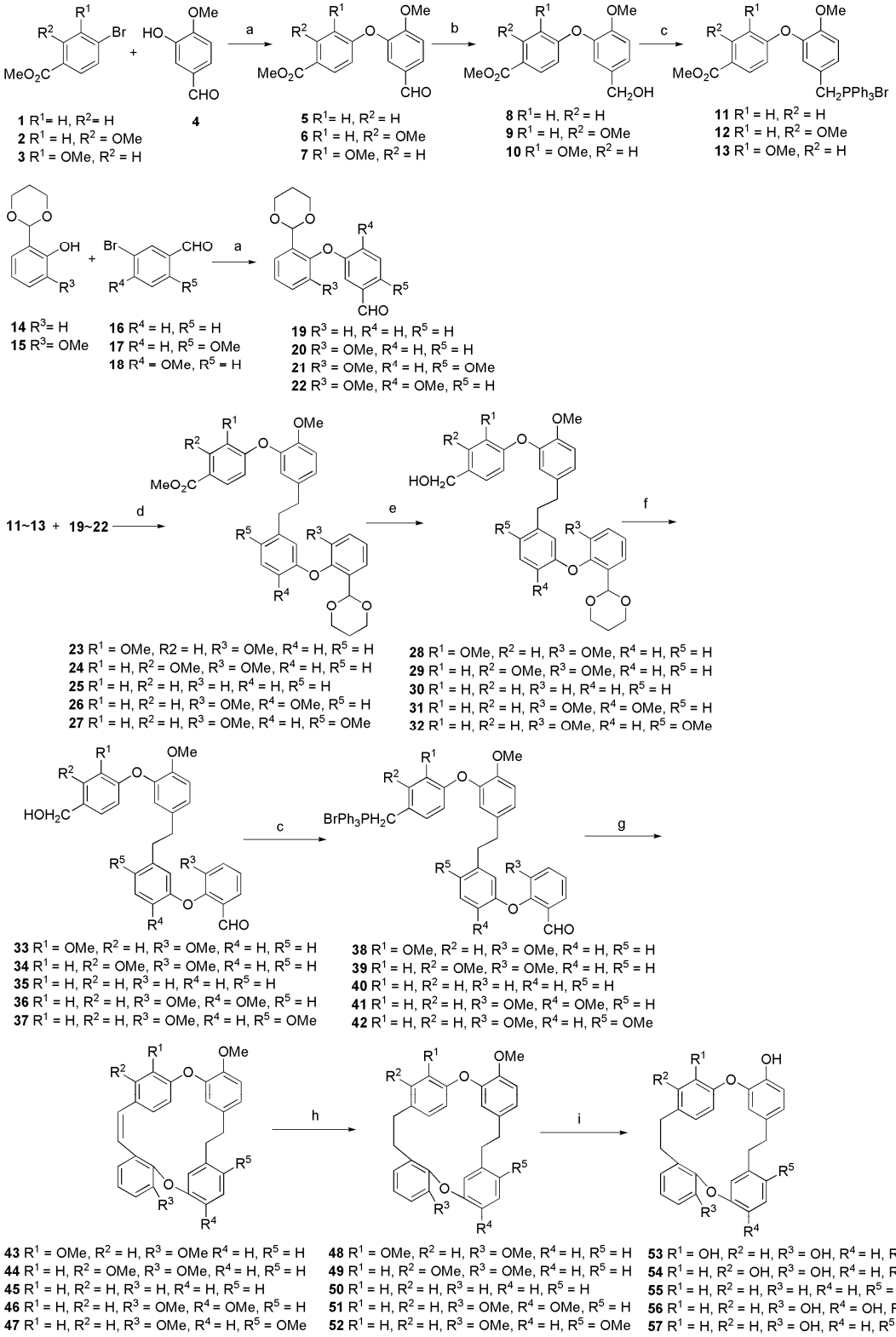
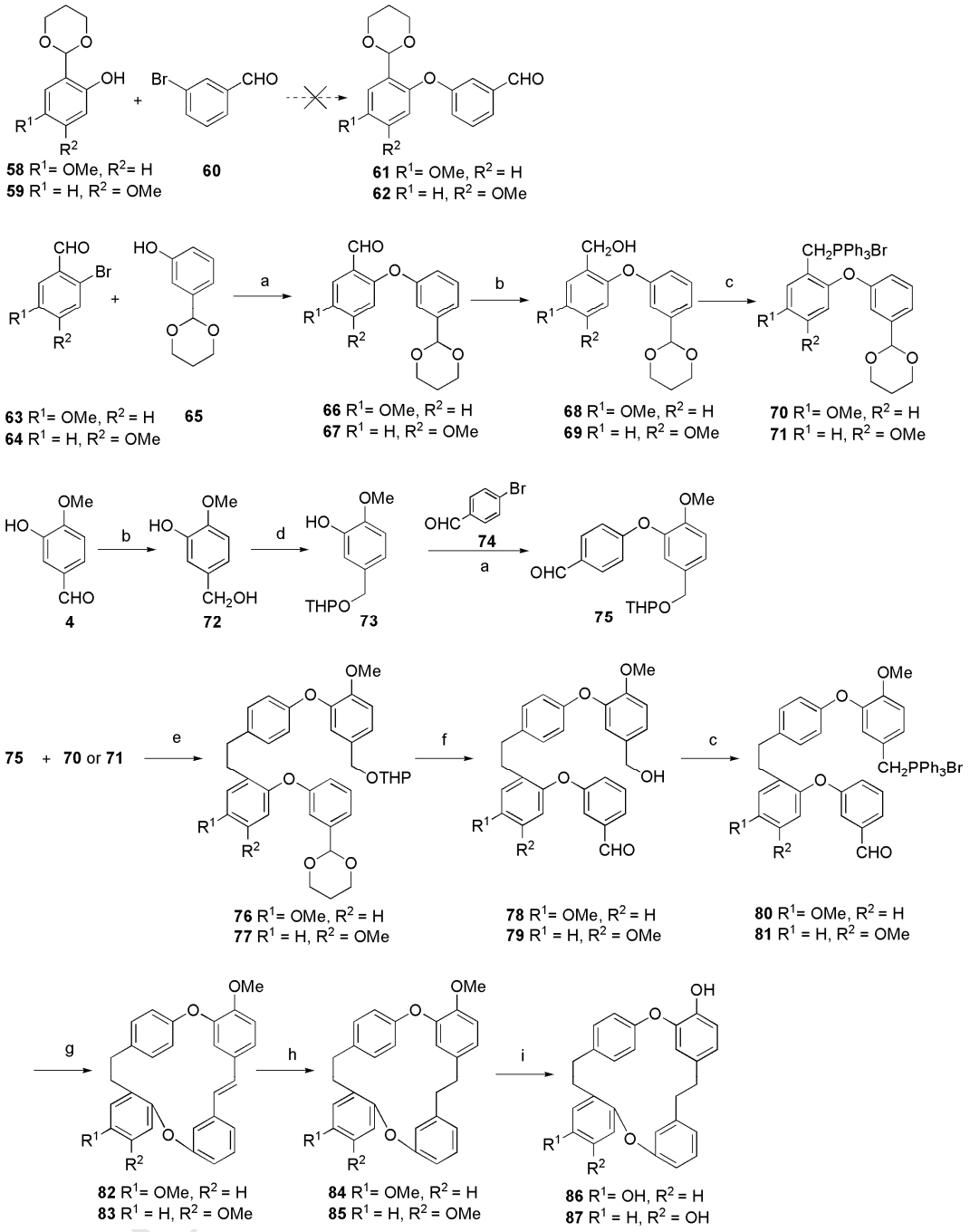


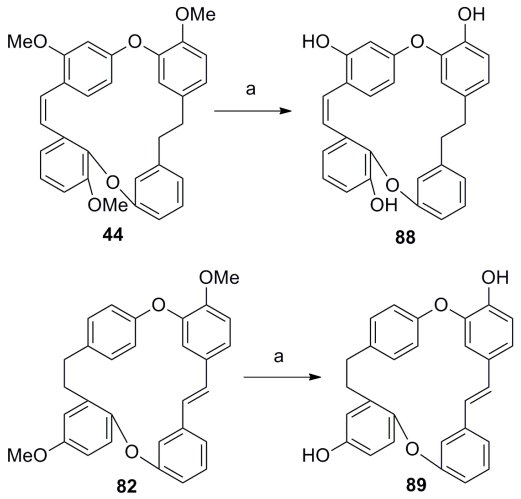

**Scheme 1.** Synthesis of compounds **53**-**57**. *Reagents and conditions*: (a) CuO, $K_2CO_3$, Py, reflux, (yields 65%-71%); (b) $NaBH_4$, THF, 0 ℃ to r.t. (yields 88%-91%); (c) $PPh_3HBr$, MeCN, reflux, (yields 86%-90%); (d) i. $K_2CO_3$, 18-crown-6, DCM, reflux; ii. Pd/C (10%), $H_2$, $Et_3N$, EtOAc, r.t. (yields 74%-82%, two steps); (e) $LiAlH_4$, THF, -40 ℃ to rt. (yields 83%-88%); (f) HCl/THF (1:1), r.t. (yields 87%-91%); (g) NaOMe, DCM, r.t. (yields 85%-92%); (h) Pd/C (10%), $H_2$, EtOAc, r.t. (yields 94%-98%); (i) $BBr_3$, DCM, -40 ℃ to r.t. (yields 86%-91%).

[Scheme 1 here]



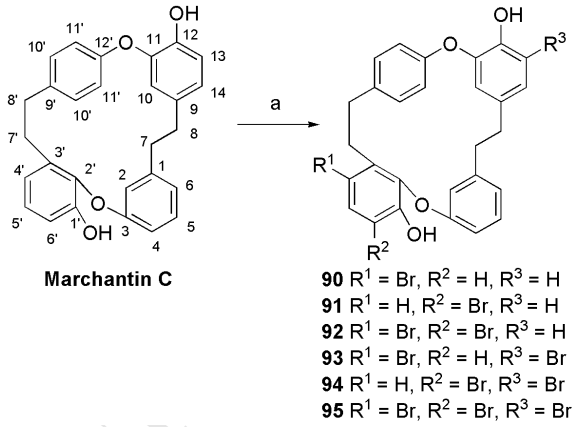

**Scheme 2.** Synthesis of compounds **86**-**87**. *Reagents and conditions*: (a) CuO, $K_2CO_3$, Py, reflux, (yields 70%-74%); (b) $NaBH_4$, THF, 0 ℃ to r.t. (yields 90%-96%); (c) $PPh_3HBr$, MeCN, reflux, (yields 86%-90%); (d) 2,3-Dihydropyran, *p*-toluenesulfonic acid, DCM, (yield 89%); (e) i. $K_2CO_3$, 18-crown-6, DCM, reflux; ii. Pd/C (10%), $H_2$, $Et_3N$, EtOAc, r.t. (yields 85%-88%, two steps); (f) HCl/THF (1:1), r.t.; (g) NaOMe, DCM, r.t. (yields 80%-81%); (h) Pd/C (10%), $H_2$, EtOAc, r.t. (yields 90%-93%); (i) $BBr_3$, DCM, -40 ℃ to r.t. (yields 71%-76%).

[Scheme 2 here]




**Scheme 3.** Synthesis of compounds **88**-**89**. *Reagents and conditions*: (a) $BBr_3$, DCM, –78 ℃ to r.t. (yields 16%-34%).

[Scheme 3 here]




**Scheme 4.** Synthesis of compounds **90**-**95**. *Reagents and conditions*: (a) HBr, DMSO, EtOAc, (overall yield 84%).

[Scheme 4 here]

*2.3. Biological evaluation*

*2.3.1. In vitro antiproliferative activity and structure-activity relationship (SAR) analysis*

The cytotoxic activity of the synthesized analogues (**53**-**57** and **86**-**95**) was evaluated in four human cancer cell lines (Hela, k562, HCC1428, and HT29) and one paclitaxel-resistant cell line (PC-3/Doc). Adriamycin (ADR) was used as a positive control. The cells were treated with each compound for 48 h, and the cell viability was assessed using the MTT method. The results are summarized in Table 1. All of the synthesized analogues consistently exhibited anticancer activity. Compounds **88**, **92** and **94** displayed excellent cytotoxic activity, with average $IC_{50}$ values ranging from

2.23 μM to 3.86 μM, indicating them to be more potent than ADR (average $IC_{50}$ = 3.94 μM) and marchantin C (average $IC_{50}$ = 13.51 μM). Surprisingly, the cytotoxicities of those three compounds against the human breast cancer HCC1428 cell line ($IC_{50}$ = 1.26, 1.09 and 1.40 μM for **88**, **92** and **94**, respectively) were comparable to or even better than that of the reference drug ADR ($IC_{50}$ = 1.56 μM). These compounds also showed obvious anticancer activity against the paclitaxel-resistant cell line, with $IC_{50}$ values of 3.56, 5.75 and 4.95 μM, respectively, and were much more potent than the reference marchantin C ($IC_{50}$ = 12.00 μM) and ADR ($IC_{50}$ = 15.17 μM).

The structure-activity relationships of these novel compounds were analyzed as follows:

Effects of hydroxyl substitutions on arene *A*, *B* and *D*: As shown in Table 1, the introduction of a hydroxyl group at the C-10' of arene *A* led to potent compound **54** (average $IC_{50}$ = 8.45 μM), which showed improved anticancer activity in four cancer cell lines compared with marchantin C (average $IC_{50}$ = 13.51 μM), and this result is consistent with our anticipation mentioned above. However, a shift of the hydroxyl group from C-10' to C-11' caused decreased activity (average $IC_{50}$ = 17.36 μM for compound **53**). Compounds **55**, **86** and **87**, with different hydroxyl substitution patterns on arene *B*, showed a comparable antitumor activity to marchantin C (average $IC_{50}$ = 11.37, 11.36 and 12.16 μM for **55**, **86** and **87**, respectively), indicating that the removal or introduction of a hydroxyl group on arene *B* does not provide any obvious effect on the activity. When the hydroxyl group was introduced to the C-4 or C-6 of arene *D* (compounds **56** and **57**), the activity was slightly diminished. All of these suggest that no potential hydrogen bond acceptor is present close to arenes *B* or *D*, and this finding is consistent with results of molecular modeling studies of marchantin C to tubulin.

**Table 1**

In Vitro Cytotoxicity of marchantin C analogues in five cancer cell lines

| Compd | $IC_{50}$ (μM) [a] | | | | | |
|---|---|---|---|---|---|---|
| | Hela | k562 | HCC1428 | HT29 | PC-3/Doc | Average |
| **53** | 16.85±3.92 | 11.41±2.15 | 24.98±4.25 | 16.23±1.20 | N/T[b] | 17.36 |
| **54** | 12.52±4.53 | 9.86±1.01 | 3.51±0.44 | 7.92±1.65 | N/T[b] | 8.45 |
| **55** | 12.87±1.02 | 13.48±1.33 | 9.64±2.05 | 9.50±1.91 | N/T[b] | 11.37 |
| **56** | 17.77±4.56 | 20.12±2.11 | 11.31±1.02 | 20.00±5.44 | N/T[b] | 17.30 |
| **57** | 18.08±1.36 | 13.69±3.99 | 16.44±2.96 | 15.56±1.04 | N/T[b] | 15.94 |
| **86** | 12.54±2.47 | 13.96±2.54 | 5.52±1.09 | 13.46±2.21 | N/T[b] | 11.36 |
| **87** | 14.60±1.02 | 15.63±1.73 | 10.04±2.21 | 8.35±1.56 | N/T[b] | 12.16 |
| **88** | 2.77±0.52 | 3.36±0.54 | 1.26±0.16 | 2.66±0.42 | 3.56±0.58 | 2.72 |
| **89** | 16.68±1.98 | 19.54±2.67 | 13.29±2.66 | 17.22±3.33 | N/T[b] | 16.68 |
| **90** | 12.62±1.75 | 13.32±1.42 | 14.24±1.25 | 11.42±2.01 | 5.51±1.93 | 11.42 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **91** | 10.35±1.59 | 5.78±0.74 | 2.56±0.41 | 6.80±1.63 | 6.02±0.68 | 6.30 |
| **92** | 5.57±0.73 | 2.86±0.88 | 1.09±0.39 | 4.01±1.01 | 5.75±1.04 | 3.86 |
| **93** | 16.39±3.89 | 19.44±3.35 | 21.88±6.98 | 13.97±2.20 | 14.60±2.35 | 17.26 |
| **94** | 1.64±0.14 | 1.32±0.09 | 1.40±0.24 | 1.86±0.99 | 4.95±1.11 | 2.23 |
| **95** | 15.31±1.02 | 10.84±1.96 | 7.02±1.49 | 13.49±3.39 | 11.10±2.47 | 11.55 |
| Marchantin C | 20.2±3.20 | 11.96±1.77 | 12.30±4.32 | 11.28±1.88 | 12.00±2.86 | 13.51 |
| Adriamycin | 1.06±0.21 | 0.47±0.09 | 1.56±0.15 | 1.46±0.29 | 15.17±1.21 | 3.94 |

[a] The $IC_{50}$ values (μM) are the concentrations corresponding to 50% inhibition of each cell line; Mean values based on three independent experiments; [b] N/T means not tested.

[Table 1 here]

Effects of the double bond: As shown in Table 1, the double bond impacted the cytotoxic activity positively (**54** vs **88**) or negatively (**86** vs **89**) to some extent. Compound **88**, with a double bond at C-7' (8'), displayed potent anticancer activity, with an average $IC_{50}$ value of 2.72 μM, being more effective than **54** and five-fold better than the parent marchantin C. As shown in the molecular modeling studies below (Fig. 3), the double bond changed the stereochemical structure of the bisbibenzyl skeleton and made the hydroxyl group on arene *A* closer to the carbonyl group of Thr 353, which might facilitate the formation of a hydrogen bond between the ligand and the protein. The double bond at C-7 (8) caused the diminished activity of compound **89** compared with **86**, and the resulting stereochemical skeleton might exhibit weakened fitness as a tubulin-binding pocket.

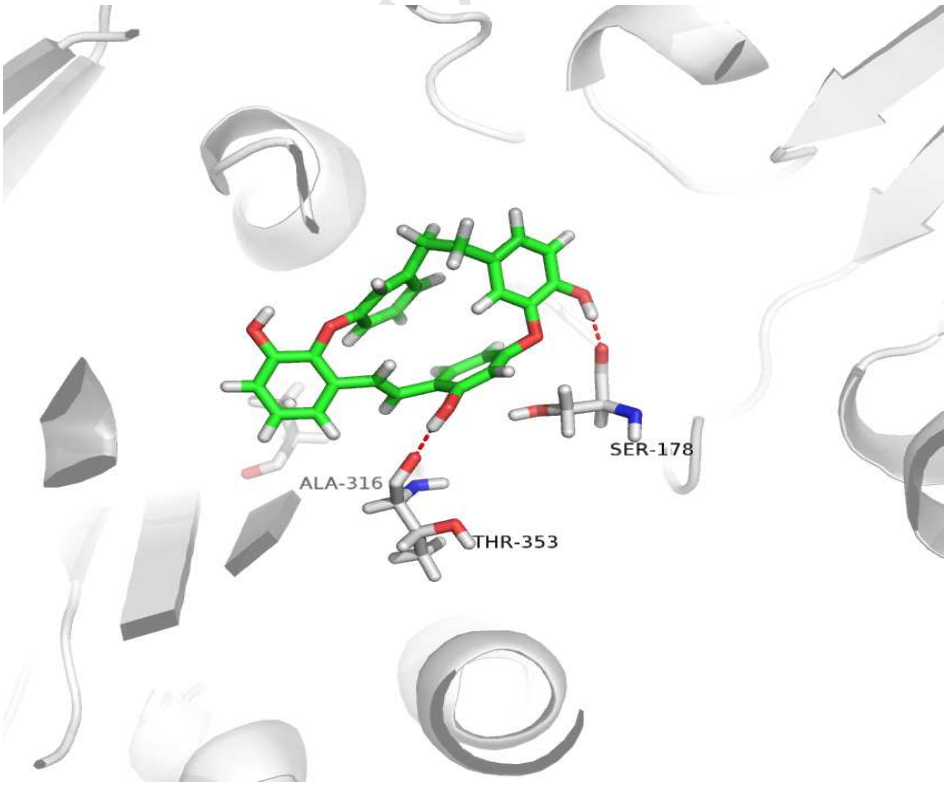



**Fig. 3.** Proposed binding mode of compound **88** as green stick model in the colchicine binding site

(PDB code 1SA0). Hydrogen bonds are shown as dotted red lines, and the distance between ligand and protein is less than 3 Å.

[Figure 3 here]

Effects of bromine atom substitutions: A moderate cytotoxicity was observed when arene *B* was substituted at the C-4' position with a bromine atom (average $IC_{50}$ = 11.42 μM for compound **90**). A two-fold increase in the activity was observed in compound **91** (average $IC_{50}$ = 6.30 μM) when the bromine atom on arene *B* was relocated to the C-6' position. In addition, compound **92**, with two bromine atoms at C-4' and C-6' of arene *B*, exhibited excellent anticancer activity, with an average $IC_{50}$ value of 3.86 μM, three-fold better than the that of the parent marchantin C. To our surprise, the introduction of bromine atoms at the C-6' position on arene *B* and the C-13 position on arene *C* led to the most potent compound **94** (average $IC_{50}$ = 2.23 μM), which demonstrated a six-fold improved cytotoxic activity compared to marchantin C. Interestingly, the substitution position of the bromine atom on arene *B* was critical for the cytotoxicity because a shift from 6'-bromo to 4'-bromo resulted in an obvious decrease in the activity (average $IC_{50}$ = 17.26 μM for **93** compared with 2.23 μM for **94**). Unexpectedly, the tri-bromination of marchantin C did not significantly enhance the activity, and the $IC_{50}$ value of **95** was 11.55 μM, which was comparable to that of marchantin C. All these suggest that the addition of bromine at the 6'-position of arene *B* is critical for the enhancement of the antitumor activity, but the tri-bromination or multi-bromination might decrease this contribution.

The further antiproliferative activity of compound **88** in HCC1428 cells was detected by the xCELLigence system, and vincristine (VCR) was used as a positive control. As shown in Fig. 4, compound **88** inhibited HCC1428 cell proliferation within 5 h and decreased the number of cells markedly within 20 h, showing a similar role to VCR. After treatment for approximately 60 h, the effect of **88** was close to that of VCR. The cell growth curve showed that compound **88** could inhibit HCC1428 cell proliferation and subsequently induce cell death.

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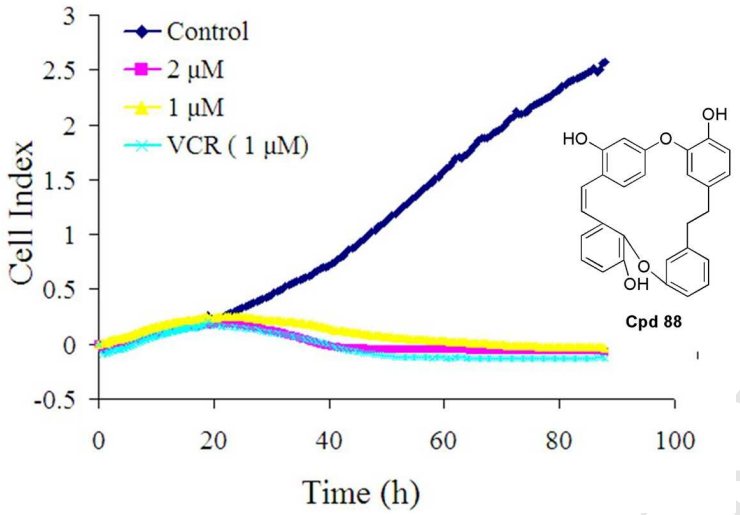


**Fig. 4.** HCC1428 cells were treated with compound **88** as indicated. Cells were then plated 2000 cells/well into E-plate 16 and analyzed using a xCELLigence RTCA DP instrument. Results are representatives of three independent experiments.

[Figure 4 here]

*2.3.2. Cell cycle analysis*

After the antiproliferative activity evaluation, we extended our work to the mechanism study. Flow cytometry was used to analyze the effects of the marchantin C analogues on the cell growth and division. HCC1428 cells was treated with compound **88** for 24 h. DMSO was used as a vehicle control, and VCR was used as a positive control. As shown in Fig. 5, in the vehicle group, 9.17% of the HCC1428 cells were in the $G_2$/M phase. Compound **88** increased the proportion of cells in the $G_2$/M phase, and approximately 64.93% were found in the $G_2$/M phase when treated with 3 μM compound **88** for 24 h. However, a decline in the $G_2$/M population (34.67%) occurred after treatment with 6 μM **88**, concomitant with a dramatic increase in the sub-$G_1$ population, indicating increased cell death. These results demonstrate that bisbibenzyl derivative **88** could induce cell cycle arrest at the $G_2$/M phase, which is consistent with the results obtained for classical tubulin-targeting drugs.

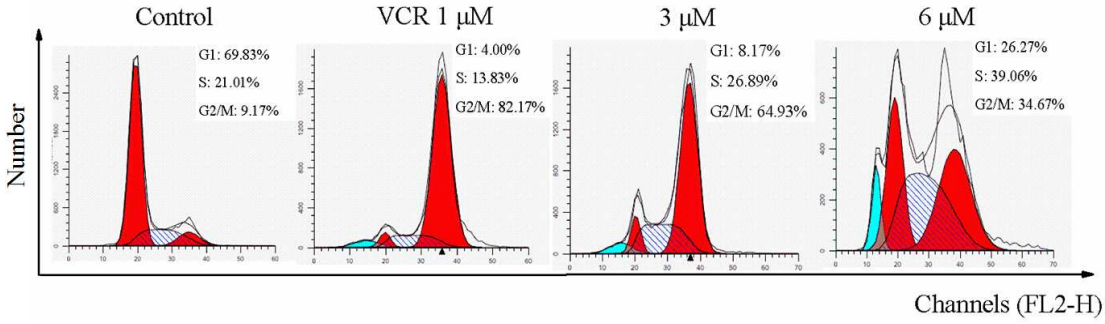


**Fig. 5.** Compound **88** induced HCC1428 cells cycle arrest at $G_2$/M phase. HCC1428 cells were treated with 3μM and 6μM of compound **88** for 24 h, and then trypsinized, fixed and stained with PI to

measure cell cycle profile by flow cytometry. Control cells were treated with DMSO alone. Results are representatives of three independent experiments.

[Figure 5 here]

*2.3.3. Immunofluorescence staining*

The significant cell growth inhibitory properties of marchantin C analogues supported by their obvious $G_2$/M phase arresting properties encouraged us to investigate the further biological mechanism. Given the microtubule-depolymerization activity of marchantin C in culture cells, we next examined the effect of compound **88** on the cytoskeleton network by immunofluorescent staining techniques. The cellular microtubule networks were visualized by confocal microscopy. As shown in Fig. 6, intact microtubules arrays could be observed in untreated cells. However, during treatment with increasing dosages of **88**, the microtubule networks were decreased and dispersed in the cytoplasm, which was similar to the result observed with 1 μM VCR treatment. These results indicate that **88** could affect the cellular microtubule dynamics.

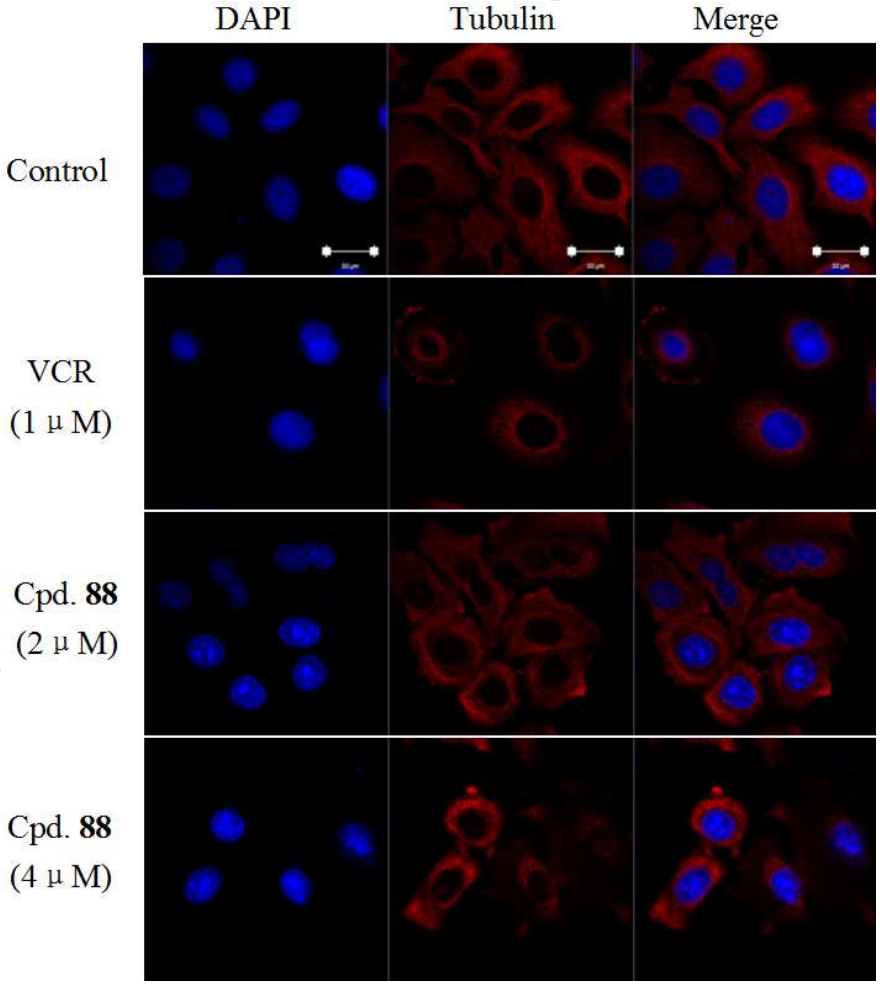


**Fig. 6.** HCC1428 cells were treated with compound **88** as indicated for 24 h and then fixed and immunostained with monoclonal anti-α-tubulin antibody (red) and DAPI (blue). One micromolar of

Vincristine (VCR) and same amount of DMSO were used as controls. Results are representatives of three independent experiments. Bar = 10 μm.

[Figure 6 here]

*2.3.4. Tubulin polymerization inhibition*

We next investigated the inhibition of tubulin polymerization by the selected potent compound **88** and compared it with the results using a positive control VCR and negative control taxol. DMSO was used as a blank control. Bovine brain tubulin was incubated with compound **88** at concentrations of 5 and 10 μM. VCR and taxol were both used at a concentration of 5 μM. As shown in Fig. 7, paclitaxel increased the absorbance obviously and immediately, indicating that it enhanced tubulin polymerization. In contrast, VCR and **88** decreased the absorbance, indicating that the tubulin polymerization was inhibited. Compound **88** showed stronger inhibition than VCR at the two tested concentrations. These results clearly indicate that compound **88** significantly inhibits the polymerization of tubulin *in vitro*.

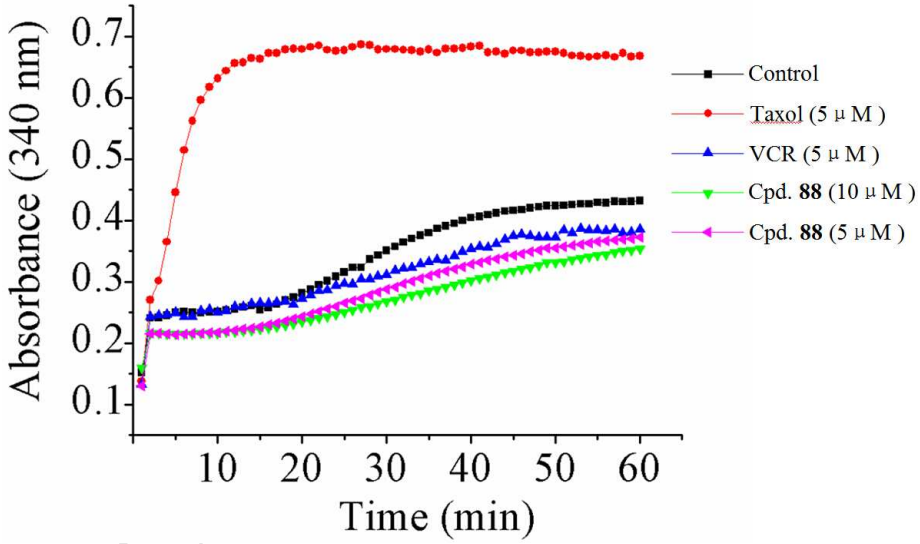


**Fig. 7.** Effect of compound **88** on tubulin polymerization *in vitro*. Purified bovine brain tubulin was incubated in the presence of Taxol, VCR, DMSO (control), and compound **88** under the indicated concentrations at 37℃, and absorbance readings were recorded every minute for 1 h.

[Figure 7 here]

*2.4. Molecular modeling*

The binding mode of active compound **88** was determined at the colchicine binding site on tubulin using *in silico* molecular docking protocols. Docking was performed using the GOLD (Genetic Optimization for Ligand Docking) program. In the resulting hypothetical structure (Fig. 3), the hydroxyl group on arene *C* may form a hydrogen bond with the carbonyl group of Ser 178. The

hydroxyl group on arene *A* is involved in a potential hydrogen bond with the carbonyl group of Thr 353. It is also possible that arene *B* forms a CH-π interaction with the methyl group of Ala 316. The combination of the CH-π interaction and hydrogen bonds could play a crucial role in the improved tubulin inhibitory activity of derivative **88**. In addition, the introduction of a bromine atom to marchantin C could change the electron distribution on the phenyl rings, which may create a stronger hydrogen bond with Ser 178 and enable CH-π interaction with Ala 316, and this might be the reason for the improved potency of the brominated analogues.

## 3. Conclusions

In summary, a series of novel marchantin C analogues was designed, synthesized and evaluated for their antiproliferative activity against five anthropic cancer cell lines. The structure-activity relationships were investigated by introducing hydroxyl groups and/or bromine atoms into arenes *A*-*D*. Several novel analogues showed excellent antiproliferative activity comparable to that of the positive control ADR. Those compounds were also effective against the paclitaxel-resistant cancer cell line. The cell cycle analysis and immunofluorescence microscopy observations revealed that marchantin C derivative **88** could arrest cancer cells at the $G_2$/M phase by destroying the microtubule networks. Further mechanism of action studies confirmed that marchantin C analogues maintained their ability by inhibiting tubulin polymerization at the colchicine-binding site. Molecular modeling provided insights into the binding mode of marchantin C analogues in tubulin. Marchantin C analogues represent a potent, new class of tubulin inhibitors, and compound **88** could serve as lead compounds in the further optimization for the discovery of novel anticancer agents.

## 4. Experimental section

### *4.1. Chemistry*

Chemicals were commercially available and used as received without further purification. Solvents (THF, DCM and $CH_3CN$) were dried and freshly distilled before use according to procedures reported in the literature. Reactions were monitored by thin-layer chromatography, using Merck plates with fluorescent indicator. Column chromatography was carried out on silica gel or alumina (200-300 mesh). The NMR spectra were recorded on a Bruker Spectospin spectrometer at 600 MHz ($^{13}$C NMR at 150 MHz), using TMS as an internal standard. The chemical shifts are reported in parts per million (ppm $\delta$) referenced to the residual $^1$H resonance of the solvent ($CDCl_3$, 7.28 ppm). Semi-prep. HPLC: *Agilent 1100-G1310A* isopump equipped with a *G1322A* degasser, a *G1314A VWD* detector (210 nm)

and a *ZORBAX SB-$C_{18}$* column (9.4 × 250 mm, 5-μm). Abbreviations used in the splitting pattern were as follows: s=singlet, d=doublet, t=triplet, quin=quintet, m=multiplet, and br=broad. Mass spectral analyses were performed at the Shandong University-Chemical Analysis Center. All HRMS spectra (ESI) were obtained on a LTQ Orbitrap mass spectrometer.

*4.1.1. Methyl 4-(5-formyl-2-methoxyphenoxy)-2-methoxy benzoate (**6**)*

This compound was prepared from methyl 2-methoxyl-4-bromobenzoate **2** (8.55 g, 35.01 mmol) and 3-hydroxy-4-methoxybenzaldehyde **4** (5.31 g, 35.01 mmol) by means of a procedure similar to that used for **5**. After concentration of the solution, the residue was purified by flash column chromatography ($SiO_2$), eluting with a 3:2 solution of petroleum ether-DCM to afford **6** (7.85 g, 71%) as an orange solid; mp 125-126 °C. $^1$H NMR ($CDCl_3$) δ 9.87 (s, 1H), 7.80 (d, *J* = 8.7 Hz, 1H), 7.76 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.60 (d, *J* = 1.9 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 2.1 Hz, 1H), 6.38 (dd, *J* = 8.7, 2.2 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H); MS (ESI) 317 $(M+H)^+$.

*4.1.2. Methyl 4-(5-formyl-2-methoxyphenoxy)-3-methoxy benzoate (**7**)*

This compound was prepared from 3-hydroxy-4-methoxybenzaldehyde (**4**) and methyl 4-bromo-3-methoxybenzoate (**3**) in 69% yield by following the procedure described for **6**. Yellow solid; mp 121-122 °C. $^1$H NMR ($CDCl_3$) δ 9.83 (s, 1 H), 7.70 (d, *J* = 1.8 Hz, 1 H), 7.69 (s, 1 H), 7.61 (dd, *J* = 1.8 Hz, 8.4 Hz, 1 H), 7.42 (d, *J* = 1.8 Hz, 1 H), 7.13 (d, *J* = 8.4 Hz, 1 H), 6.81 (d, *J* = 8.4 Hz, 1 H), 3.95 (s, 3 H), 3.94 (s, 3 H), 3.93 (s, 3 H); MS (ESI) 317 $(M+H)^+$.

*4.1.3. Methyl 4-(5-(hydroxymethyl)-2-methoxyphenoxy)-2-methoxybenzoate (**9**)*

This compound was prepared from compound **6** by following the procedure described for **8**, yield 90%, white solid; mp 85-86 °C.$^1$H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.7 Hz, 1H), 7.22 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 2.3 Hz, 1H), 6.38 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.65 (s, 2H), 3.87 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H); MS (ESI) 319 $(M+H)^+$.

*4.1.4. Methyl 4-(5-((bromotriphenylphosphoranyl)methyl)-2-methoxyphenoxy)-2-methoxybenzoate (**12**)*

This compound was prepared from compound **9** by following the procedure described for **11**, yield 91%, white solid; mp 233-234 °C. $^1$H NMR ($CDCl_3$) δ 7.77-7.76 (m, 15 H), 7.34-7.32 (m, 2 H), 6.84 (d, *J* = 7.2 Hz, 1 H ), 6.50 (s, 1 H), 6.46 (s, 1 H), 6.11 (d, *J* = 8.4 Hz, 1 H), 5.44 (d, *J* = 14.4 Hz, 2 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.75 (s, 3 H); MS (ESI) 563 $(M-Br)^+$.

*4.1.5. (4-Methoxy-3-(2-methoxyl-4-(methoxycarbonyl)phenoxy)benzyl)triphenylphos-phonium Bromide (**13**)*

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This compound was prepared from compound **10** by following the procedure described for **11**, yield 87%, white solid; mp 215-216 °C. $^1$H NMR ($CDCl_3$) δ 7.83-7.70 (m, 9 H), 7.67-7.60 (m, 6 H), 7.57 (d, *J* = 1.8 Hz, 1 H), 7.36 (s, 1 H), 7.03 (d, *J* = 1.8 Hz, 1 H), 6.91 (dd, *J* = 1.8 Hz, 8.4 Hz, 1 H), 6.87 (d, *J* = 8.4 Hz, 1 H), 6.79 (d, *J* = 8.4 Hz, 1 H), 5.40 (d, *J* = 14 Hz, 2 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.83 (s, 3 H); MS (ESI) 563 $(M\text{-}Br)^+$.

*4.1.6. 3-(2-(1,3-Dioxan-2-yl)phenoxy)benzaldehyde (**19**)*

A mixture of 2-(1,3-dioxan-2-yl)phenol (**14,** 10 g, 0.05 mol) [38], 3-bromo-benzaldehyde (**16**, 8.8 g, 0.05 mmol), potassium carbonate (13.5 g, 0.09 mol) and cupric oxide (0.95 g, 5.9 mmol) in pyridine (50 mL) was stirred under reflux for 12 h. The pyridine was distilled off in vacuo and the residue was extracted with ethyl acetate (200 mL). The solution was concentrated and the residue was purified by flash column chromatograph ($Al_2O_3$), eluting with a 2:1 solution of petroleum ether-DCM to afford **19** (12.6 g, 81%) as a yellow solid; white solid; mp 129-130 °C. $^1$H NMR ($CDCl_3$) δ 9.98 (s, 1H), 7.78 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.66-7.60 (m, 1H), 7.51-7.49 (m, 2H), 7.37-7.34 (m, 1H), 7.28-7.24 (m, 2H), 6.92 (dd, *J* = 8.2, 1.0 Hz, 1H), 5.82 (s, 1H), 4.22 (ddd, *J* = 11.9, 4.9, 1.1 Hz, 2H), 3.93 (ddd, *J* = 12.4, 3.9, 2.5 Hz, 2H), 2.28-2.19 (m, 1H), 1.44-1.40 (m, 1H) ; MS (ESI) 285 $(M+H)^+$.

*4.1.7. 3-(2-(1,3-Dioxan-2-yl)-6-methoxyphenoxy)benzaldehyde (**20**)*

This compound was prepared from 2-(1,3-dioxan-2-yl)-6-methoxyphenol (**15**) and 3-bromo-benzaldehyde (**16**) by following the procedure described for **19**, yield 86%, white solid; mp 127-128 °C. $^1$H NMR ($CDCl_3$) δ 9.95 (s, 1 H), 7.55 (d, *J* = 7.2 Hz, 1 H), 7.43 (t, *J* = 7.8 Hz, 1 H), 7.37 (d, *J* = 8.4 Hz, 2 H), 7.30 (t, *J* = 7.8 Hz, 1 H), 7.15 (d, *J* = 5.4 Hz, 1 H), 7.02 (d, *J* = 7.8 Hz, 1 H), 5.69 (s, 1 H), 4.14 (d, *J* = 7.8 Hz, 2 H), 3.85 (t, *J* = 12.0 Hz, 2 H), 3.74 (s, 3 H), 2.21-2.16 (m, 1 H), 1.37 (d, *J* = 13.2 Hz, 1 H); MS (ESI) 315 $(M+H)^+$.

*4.1.8. 5-(2-(1,3-Dioxan-2-yl)-6-methoxyphenoxy)-2-methoxybenzaldehyde (**21**)*

This compound was prepared from 2-(1,3-dioxan-2-yl)-6-methoxyphenol (**15**) and 5-bromo-2-methoxybenzaldehyde (**17**) by following the procedure described for **19**, yield 80%, white solid; mp 119-120 °C. $^1$H NMR ($CDCl_3$) δ 10.42 (s, 1 H), 7.37 (s, 1 H), 7.34 (d, *J* = 7.5 Hz, 1 H), 7.25 (t, *J* = 7.9 Hz, 1 H), 7.06 (d, *J* = 8.7 Hz, 1 H), 6.99 (d, *J* = 8.0 Hz, 1 H), 6.89 (d, *J* = 8.9 Hz, 1 H), 5.68 (s, 1 H), 4.16-4.14 (m, 2 H), 3.90 (s, 3 H), 3.85 (t, *J* = 11.9 Hz, 2 H), 3.72 (s, 3 H), 2.25-2.14 (m, 1H), 1.36 (d, *J* = 13.6 Hz, 1 H); MS (ESI) 345 $(M+H)^+$.

*4.1.9. 3-(2-(1,3-Dioxan-2-yl)-6-methoxyphenoxy)-4-methoxybenzaldehyde (**22**)*

This compound was prepared from 2-(1,3-dioxan-2-yl)-6-methoxyphenol (**15**) and 5-bromo-4-methoxybenzaldehyde (**18**) by following the procedure described for **19**, yield 79%, white solid; mp 127-128 °C. $^1$H NMR ($CDCl_3$) δ 9.71 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 7.4 Hz, 1H), 7.28-7.25 (m, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 7.01-6.99 (m, 2H), 5.71 (s, 1H), 4.12 (d, *J* = 10.8 Hz, 2H), 4.06 (s, 3H), 3.82 (t, *J* = 11.7 Hz, 2H), 3.71 (s, 3H), 2.26-2.09 (m, 1H), 1.34 (d, *J* = 13.3 Hz, 1H); MS (ESI) 345 $(M+H)^+$.

*4.1.10. Methyl 4-(5-(3-(2-(1,3-dioxan-2-yl)-6-methoxyphenoxy)phenethyl)-2-methoxy-phenoxy)-3-methoxybenzoate (**23**)*

Potassium carbonate (4.45 g, 32.22 mmol) and a trace of 18-crown-6 were added to a solution of **13** (10.34 g, 16.11 mmol) and **20** (5.06 g, 16.11 mmol) in anhydrous DCM (50 mL), the resulting mixture was stirred under reflux for 24 h. The insoluble material was then filtered off and the filtrate was concentrated to provide the orange oil that was purified by flash column chromatography ($Al_2O_3$), eluting with a 3:1 solution of hexane-dichloromethane to afford a mixture of *Z/E* isomers (8.57 g, 89%) as a yellow oil. Pd/C 10% (0.7 g) and triethylamine (16 mL) were then added to a solution of isomers (7.89 g, 13.20 mmol) in ethyl acetate (100 mL). The suspension was stirred under $H_2$ for 24 h at room temperature. The mixture was filtered, and concentrated to afford **23** (7.54 g, 95%) as a yellow solid; mp 100-101 °C. $^1$H NMR ($CDCl_3$) δ 7.66 (s, 1 H), 7.56 (d, *J* = 8.4 Hz, 1 H ), 7.35 (d, *J* = 7.8 Hz, 1 H), 7.26 (t, d, *J* = 7.8 Hz, 1 H), 6.99 (d, *J* = 8.4 Hz, 2 H), 6.91 (d, *J* = 8.4 Hz, 1 H), 6.84 (s, 1 H), 6.76-6.74 (m, 2 H), 6.68 (d, *J* = 7.8 Hz, 1 H), 6.61 (d, *J* = 8.4 Hz, 1 H), 5.68 (s, 1 H), 4.17-4.14 (m, 2 H), 3.99 (s, 3 H), 3.92 (s, 3 H), 3.86-3.82 (m, 2 H), 3.79 (s, 3 H), 3.71 (s, 3 H), 2.86-2.83 (m, 4 H), 2.21-2.19 (m, 1 H), 1.36 (d, *J* = 13.8 Hz, 1 H); MS (ESI) 623 $(M+Na)^+$.

*4.1.11. Methyl 4-(5-(3-(2-(1,3-dioxan-2-yl)-6-methoxyphenoxy)phenethyl)-2-methoxyphenoxy)-2-methoxybenzoate (**24**)*

This compound was prepared from **12** and **20** by following the procedure described for **23**, yield 82%, white solid; mp 95-96 °C. $^1$H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.8 Hz, 1H ), 7.35 (d, *J* = 7.2 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.03-6.97 (m, 2H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.87 (s, 1 H), 6.76-6.74 (m, 2H), 6.68 (d, *J* = 4.8 Hz, 1H), 6.60 (s, 1H), 6.32 (d, *J* = 7.8 Hz, 1H), 5.67 (s, 1H), 4.12 (m, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.81-3.80 (m, 2H), 3.76 (s, 3H), 3.68 (s, 3H), 2.84 (s, 4H), 2.18-2.16 (m, 1H), 1.33-1.29 (m, 2H); MS (ESI) 623 $(M+Na)^+$.

*4.1.12. Methyl 4-(5-(3-(2-(1,3-dioxan-2-yl)phenoxy)phenethyl)-2-methoxyphenoxy)benzoate (**25**)*

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This compound was prepared from **11** and **19** by following the procedure described for **23**, yield 75%, white solid; mp 104-105 °C. $^1$H NMR ($CDCl_3$) δ 8.02-7.96 (m, 2H), 7.74 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.28-7.25 (m, 1H), 7.23 (t, *J* = 7.8 Hz, 1H), 7.17 (td, *J* = 7.6, 0.9 Hz, 1H), 7.00 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.90-6.87 (m, 3H), 6.87-6.85 (m, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.81 (t, *J* = 1.8 Hz, 1H), 6.79 (dd, *J* = 8.2, 0.9 Hz, 1H), 5.87 (s, 1H), 4.27-4.20 (m, 2H), 3.95 (td, *J* = 12.4, 2.5 Hz, 2H), 3.91 (s, 3H), 3.79 (s, 3H), 2.91-2.83 (m, 4H), 2.29-2.21 (m, 1H), 1.45-1.38 (m, 1H); MS (ESI) 563 $(M+Na)^+$.

*4.1.13. Methyl 4-(5-(3-(2-(1,3-dioxan-2-yl)-6-methoxyphenoxy)-4-methoxyphenethyl)-2-methoxy-phenoxy)benzoate (**26**)*

This compound was prepared from **11** and **22** by following the procedure described for **23**, yield 80%, white solid; mp 118-119 °C. $^1$H NMR ($CDCl_3$) δ 7.97 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 6.92 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 8.2 Hz, 3H), 6.73 (d, *J* = 1.7 Hz, 1H), 6.64 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.38 (d, *J* = 1.7 Hz, 1H), 5.74 (s, 1H), 4.11 (dd, *J* = 11.2, 4.6 Hz, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.80 (t, *J* = 11.2 Hz, 2H), 3.73 (s, 3H), 3.66 (s, 3H), 2.68 (s, 4H), 2.22-2.11 (m, 1H), 1.31 (d, *J* = 13.3 Hz, 1H); MS (ESI) 623 $(M+Na)^+$.

*4.1.14. Methyl 4-(5-(5-(2-(1,3-dioxan-2-yl)-6-methoxyphenoxy)-2-methoxyphenethyl)-2-methoxy-phenoxy)benzoate (**27**)*

This compound was prepared from **11** and **21** by following the procedure described for **23**, yield 74%, white solid; mp 99-100 °C. $^1$H NMR ($CDCl_3$) δ 7.98 (d, *J* = 7.0 Hz, 2H), 7.34 (d, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 6.0 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.94-6.89 (m, 4H), 6.74 (s, 1H), 6.70 (d, *J* = 8.6 Hz, 1H), 6.63 (d, *J* = 5.7 Hz, 1H), 5.69 (s, 1H), 4.25-4.08 (m, 2H), 3.90 (s, 3H), 3.85 (t, *J* = 12.2 Hz, 2H), 3.78 (s, 3H), 3.75 (s, 3H), 3.70 (s, 3H), 2.85-2.81 (m, 4H), 2.27-2.13 (m, 1H), 1.36 (d, *J* = 13.1 Hz, 1H) ; MS (ESI) 623 $(M+Na)^+$.

*4.1.15. (4-(5-(3-(2-(1,3-Dioxan-2-yl)-6-methoxyphenoxy)phenethyl)-2-methoxyphenoxy)-2-methoxy-phenyl)methanol (**29**)*

A solution of **24** (13.11 g, 23.72 mmol) in anhydrous THF (25 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (1.75 g, 46.07 mmol) in anhydrous THF (30 mL). The resulting mixture was stirred at room temperature for 2.5 h and carefully hydrolysed with sat aq ammonium chloride (10 mL). THF was removed in vacuo and the resulting mixture was diluted with

DCM (100 mL), washed with saturated aqueous NaCl (20 mL × 3) and dried over sodium sulfate. The solvent was removed in vacuo and the residue was purified by flash chromatography ($SiO_2$) eluting with DCM to yield **29** (12.30g, 88%), colorless oil; $^1$H NMR ($CDCl_3$) δ 7.36 (d, *J* = 6.9 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.94 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.83 (d, *J* = 1.7 Hz, 1H), 6.79 – 6.72 (m, 2H), 6.68 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.61 (d, *J* = 2.0 Hz, 1H), 6.35 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.68 (s, 1H), 4.61 (s, 2H), 4.11 (dd, *J* = 11.3, 4.4 Hz, 2H), 3.81-3.79 (m, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 3.67 (s, 3H), 2.83 (s, 4H), 2.17-2.14 (m, 1H), 1.59 -1.30 (m, 1H); MS (ESI) 573 $(M+H)^+$.

*4.1.16. (4-(5-(3-(2-(1,3-Dioxan-2-yl)phenoxy)phenethyl)-2-methoxyphenoxy)phenyl)methanol (**30**)*

This compound was prepared from **25** by following the procedure described for **29**, yield 83%, colorless oil; $^1$H NMR ($CDCl_3$) δ 7.74 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.30-7.28 (m, 3H), 7.28-7.25 (m, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.16 (td, *J* = 7.6, 0.9 Hz, 1H), 6.92 (d, *J* = 1.1 Hz, 2H), 6.90-6.88 (m, 2H), 6.86-6.82 (m, 1H), 6.81-6.78 (m, 1H), 6.77 (dd, *J* = 9.7, 1.1 Hz, 2H), 5.87 (s, 1H), 4.65 (s, 2H), 4.26-4.19 (m, 2H), 3.95 (td, *J* = 12.4, 2.4 Hz, 2H), 3.83 (s, 3H), 2.90-2.76 (m, 4H), 2.31-2.16 (m, 1H), 1.45-1.39 (m, 1H); MS (ESI) 513 $(M+H)^+$.

*4.1.17. (4-(5-(5-(2-(1,3-Dioxan-2-yl)-6-methoxyphenoxy)-2-methoxyphenethyl)-2-methoxyphenoxy) phenyl)methanol (**32**)*

This compound was prepared from **27** by following the procedure described for **29**, yield 79%, colorless oil; $^1$H NMR ($CDCl_3$) δ 7.34-7.28 (m, 3H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 5.9 Hz, 2H), 6.92-6.90 (m, 3H), 6.83 (s, 1H), 6.73 (s, 1H), 6.69 (d, *J* = 8.7 Hz, 1H), 6.60 (d, *J* = 8.5 Hz, 1H), 5.67 (s, 1H), 4.63 (s, 2H), 4.14 (d, *J* = 10.1 Hz, 2H), 3.84-3.82 (m, 2H), 3.81 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 2.81-2.79 (m, 4H), 2.25-2.10 (m, 1H), 1.35 (d, *J* = 12.8 Hz, 1H); MS (ESI) 573 $(M+H)^+$.

*4.1.18. 2-(3-(3-(4-(Hydroxymethyl)-3-methoxyphenoxy)-4-methoxyphenethyl)phenoxy)-3-methoxy-benzaldehyde (**34**)*

Compound **29** (12.33g, 21.5mmol) was dissolved in a solution of ethanol (100 mL) and 10 % aq HCl (20 mL). The resulting mixture was then stirred at room temperature for 12 h. Sat aq sodium bicarbonate (150 mL) was added and the ethnol was removed in vacuo. The resulting mixture was extracted with $CH_2Cl_2$ (200 mL) , washed with saturated aqueous NaCl (25 mL × 3) and dried over sodium sulfate. The solution was concentrated to yield **34** (9.64 g, 87%) as white solid; mp 86-87 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ 10.26 (d, *J* = 0.8 Hz, 1H), 7.57 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.33 (td, *J* =

8.0, 0.7 Hz, 1H), 7.28-7.23 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.88 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.84 (d, *J* = 7.7 Hz, 1H), 6.82 (d, *J* = 2.0 Hz, 1H), 6.70 (t, *J* = 2.4 Hz, 1H), 6.68-6.64 (m, 1H), 6.62 (d, *J* = 2.3 Hz, 1H), 6.39 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.51 (s, 2H), 3.85 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 2.8-2.79 (m, 4H); MS (ESI) 515 $(M+H)^+$.

*4.1.19. 2-(3-(3-(4-(Hydroxymethyl)phenoxy)-4-methoxyphenethyl)phenoxy)benzaldehyde (**35**)*

This compound was prepared from **30** by following the procedure described for **34**, yield 89%, white solid; mp 90-91 °C. $^1$H NMR ($CDCl_3$) δ 10.50 (s, *J* = 2.3 Hz, 1H), 7.94 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.51 (ddd, *J* = 8.4, 7.3, 1.8 Hz, 1H), 7.30-7.28 (m, 3H), 7.23-7.15 (m, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.92 (s, 2H), 6.91-6.87 (m, 3H), 6.84-6.79 (m, 2H), 6.75 (s, 1H), 4.66 (s, 2H), 3.83 (s, 3H), 2.91-2.82 (m, 4H); MS (ESI) 455 $(M+H)^+$.

*4.1.20. 2-(3-(3-(4-(hydroxymethyl)phenoxy)-4-methoxyphenethyl)-4-methoxyphenoxy)-3-methoxy-benzaldehyde (**37**)*

This compound was prepared from **32** by following the procedure described for **34**, yield 92%, white solid; mp 94-95 °C. $^1$H NMR (DMSO-$d_6$) δ 10.23 (s, 1 H), 7.54 (d, *J* = 7.2 Hz, 1 H), 7.30-7.27 (m, 4 H), 7.23 (d, *J* = 7.8 Hz, 1 H), 6.91-6.90 (m, 3 H), 6.81 (s, 1 H), 6.67 (d, *J* = 8.4 Hz, 1 H), 6.65 (s, 1 H), 6.60-6.58 (m, 1 H), 4.65 (s, 2 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.78 (s, 3 H), 2.82-2.78 (m, 4 H); MS (ESI) 515 $(M+H)^+$.

*4.1.21. (4-(5-(3-(2-Formyl-6-methoxyphenoxy)phenethyl)-2-methoxyphenoxy)-3-methoxybenzyl) triphenylphosphonium bromide (**38**)*

Compound **33** (5.32 g, 10.35 mmol) and triphenylphosphonium bromide (3.72 g, 10.86 mmol) was dissolved in anhydrous $CH_3CN$ (50 mL), the resulting mixture was then refluxed for 3 h, the solvent was removed in vacuo and the residue was purified by silica gel column chromatography, eluting with 5% ethanol in dichloromethane, to yield **38** as white solid (8.12 g, 89%); mp 235-236 °C. $^1$H NMR ($CDCl_3$) δ 7.77-7.63 (m, 15 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.31 (t, *J* = 7.8 Hz, 1 H), 7.27 (t, *J* = 7.8 Hz, 1 H), 7.11 (t, *J* = 7.8 Hz, 1 H), 7.02 (s, 1 H), 6.83 (d, *J* = 8.4 Hz, 1 H), 6.80 (d, *J* = 8.4 Hz, 2 H), 6.65 (s, 1 H), 6.61-6.59 (m, 2 H), 6.51 (s, 2 H), 5.41 (d, *J* = 7.8 Hz, 2 H), 3.81 (s, 3 H), 3.76 (s, 3 H), 2.77 (s, 4 H); MS (ESI) 759 $(M-Br)^+$.

*4.1.22. 2-(3-(3-(4-((Bromotriphenylphosphoranyl)methyl)-3-methoxyphenoxy)-4-methoxyphenethyl) phenoxy)-3-methoxybenzaldehyde (**39**)*

This compound was prepared from **34** by following the procedure described for **38**, yield 90%,

white solid; mp 221-222 °C. $^1$H NMR ($CDCl_3$) δ 10.23 (s, 1H), 7.80-7.71 (m, 9H), 7.67-7.63 (m, 6H), 7.54 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.31-7.28 (m, 2H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 1.1 Hz, 2H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.74 (s, 1H), 6.66-6.64 (m, 1H), 6.62 (dd, *J* = 8.5, 2.9 Hz, 1H), 6.36 (d, *J* = 2.2 Hz, 1H), 6.21 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.25 (d, *J* = 13.4 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.17 (s, 3H), 2.87-2.76 (m, 4H); MS (ESI) 759 $(M\text{-}Br)^+$.

*4.1.23. 2-(3-(3-(4-((Bromotriphenylphosphoranyl)methyl)phenoxy)-4-methoxyphenethyl)phenoxy) benzaldehyde (**40**)*

This compound was prepared from **35** by following the procedure described for **38**, yield 85%, white solid; mp 230-231 °C. $^1$H NMR ($CDCl_3$) δ 10.49 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.79-7.74 (m, 9H), 7.67-7.63 (m, 6H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.52-7.48 (m, 3H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.04 (dd, *J* = 8.7, 2.5 Hz, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.91-6.84 (m, 3H), 6.81-6.76 (m, 2H), 5.40 (d, *J* = 13.8 Hz, 2H), 3.80 (s, 3H), 2.88-2.80 (m, 4H); MS (ESI) 699 $(M\text{-}Br)^+$.

*4.1.24. 2-(3-(3-(4-((Bromotriphenylphosphoranyl)methyl)phenoxy)-4-methoxyphenethyl)-4-methoxy-phenoxy)-3-methoxybenzaldehyde (**42**)*

This compound was prepared from **37** by following the procedure described for **38**, yield 92%, white solid; mp 244-245 °C. $^1$H NMR ($CDCl_3$) δ 9.95 (s, 1H), 7.75-7.69 (m, 9H), 7.60-7.58 (m, 6H), 7.55 (dt, *J* = 7.5, 1.2 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.33 (dd, *J* = 2.5, 1.4 Hz, 1H), 7.22 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.96-6.91 (m, 2H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.80-6.74 (m, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 6.26 (t, *J* = 2.1 Hz, 1H), 5.29 (d, *J* = 13.8 Hz, 2H), 3.81 (s, 3H), 3.78 (s, 3 H), 3.79 (s, 3H), 2.87-2.79 (m, 4H); MS (ESI) 759 $(M\text{-}Br)^+$.

*4.1.25. Macrocycle (stilbene bridge) (**43**)*

A solution of phosphonium salt **38** (2.58 g, 3.07 mmol) in anhydrous dichloromethane (150 mL/mmol) was added dropwise (5 h/mmol) to a suspension of sodium methoxide (1.35 g, 24.6 mmol) in anhydrous dichloromethane (50 mL/mmol). The reaction mixture was stirred for 15 h at room temperature. Insoluble material was filtered off, the solvent was removed in vacuo and the residue was purified by silica gel column chromatography, eluting with dichloromethane, to provide the compound **43** as white solid (1.19 g, 81%); mp 155-156 °C. $^1$H NMR ($CDCl_3$) δ 7.23 (t, *J* = 7.8 Hz, 1 H), 7.11 (d, *J* = 7.2 Hz, 1 H), 7.00 (d, *J* = 7.2 Hz, 1 H), 6.94 (d, *J* = 8.4 Hz, 2 H), 6.88 (d, *J* = 7.2 Hz, 2 H), 6.81-6.79 (m, 2 H), 6.75 (d, *J* = 7.8 Hz, 1 H), 6.65 (d, *J* = 16.2 Hz, 1 H), 6.57-6.55 (m, 2 H), 6.15 (d, *J* = 7.8 Hz, 1 H), 5.72 (s, 1 H), 3.98 (s, 3 H), 3.92 (s, 3 H), 3.74 (s, 3 H), 3.04-2.83 (m, 4 H); MS (ESI)

503 $(M+Na)^+$.

*4.1.26. Macrocycle (stilbene bridge) (**44**)*

This compound was prepared from **39** by following the procedure described for **43**, yield 83%, white solid; mp 149-150 $^oC$. $^1H$ NMR ($CDCl_3$) δ 7.25 (d, *J* = 7.6 Hz, 1 H), 7.22 (t, *J* = 7.6 Hz, 1 H ), 7.15 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1 H), 6.97 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1 H), 6.95-6.92 (m, 2 H ), 6.88 (d, *J* = 8.0 Hz, 1 H), 6.84 (d, *J* = 8.0 Hz, 1 H), 6.80 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1 H), 6.59 (dd, *J* = 2.0 Hz, *J* = 8.4 Hz, 1 H), 6.53 (d, *J* = 7.2 Hz, 1 H), 6.51-6.47 (m, 2H), 6.19 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1 H), 5.72 (d, *J* = 2.0 Hz, 1 H), 3.98 (s, 3H), 3.91 (s, 3H), 3.69 (s, 3H), 3.11-2.81 (m, 4H). $^{13}C$ NMR ($CDCl_3$) δ 158.5, 157.9, 156.0, 152.7, 150.3, 146.7, 142.9, 141.5, 135.2, 132.8, 131.2, 128.4, 127.9, 126.6, 126.2, 125.4, 121.9, 119.6, 118.2, 116.9, 115.4, 114.7, 113.0, 111.7, 111.6, 105.4, 56.3, 56.2, 55.5, 34.4, 31.3. MS (ESI) 481 $(M+H)^+$.

*4.1.27. Macrocycle (stilbene bridge) (**45**)*

This compound was prepared from **40** by following the procedure described for **43**, yield 80%, white solid; mp 180-181 °C. $^1H$ NMR ($CDCl_3$) δ 7.48 (dd, *J* = 1.2 Hz, *J* = 3.2 Hz, 1 H ),7.34 (td, *J* = 1.2 Hz, *J* = 7.6 Hz, 1 H), 7.27-7.23 (m, 1 H), 7.20 (dd, *J* = 1.2 Hz, *J* = 8.0 Hz, 1 H), 7.15 (d, *J* = 8.4 Hz, 2 H), 6.91 (d, *J* = 8.4 Hz, 2 H), 6.87-6.83 (m, 3 H), 6.78 (dd, *J* = 2.0 Hz, *J* = 8.4 Hz, 1 H), 6.63 (d, *J* = 16 Hz, 1 H), 6.51 (d, *J* = 7.6 Hz, 1 H), 6.42 (d, *J* = 16 Hz, 1 H), 6.09 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1 H), 5.68 (d, *J* = 2.0 Hz, 1 H), 3.96 (s, 3 H), 2.99-2.88 (m, 4 H); $^{13}C$ NMR ($CDCl_3$) δ 158.9, 155.2, 152.7, 150.4, 146.8, 143.0, 137.5, 135.1, 131.9, 131.5, 131.2, 130.9, 128.8, 128.6, 126.9, 126.4, 125.3, 122.8, 122.5, 121.9, 119.4, 116.8, 114.6, 113.1, 111.7, 56.2, 34.0, 30.7; MS (ESI) 421 $(M+H)^+$.

*4.1.28. Macrocycle (stilbene bridge) (**46**)*

This compound was prepared from **41** by following the procedure described for **43**, yield 86%, white solid; mp 174-175 $^oC$. $^1H$ NMR ($CDCl_3$) δ 7.16 (d, *J* = 8.5 Hz, 2H), 7.11 (t, *J* = 7.9 Hz, 1H), 7.02 (dd, *J* = 7.7, 1.3 Hz, 1H), 6.96-6.91 (m, 3H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.81 (dd, *J* = 8.2, 2.0 Hz, 2H), 6.64 (d, *J* = 15.9 Hz, 1H), 6.56-6.53 (m, 2H), 6.46-6.44 (m, 1H), 5.70 (d, *J* = 2.0 Hz, 1H), 3.99 (s, 3H), 3.93 (s, 3H), 3.53 (s, 3H), 3.03-2.95 (m, 2H), 2.86-2.79 (m, 2H); $^{13}C$ NMR (151 MHz, $CDCl_3$) δ 155.1, 151.0, 150.6, 147.3, 147.2, 146.3, 143.7, 137.2, 134.6, 133.9, 131.3, 130.1, 130.0, 127.0, 123.7, 123.0, 121.7, 119.7, 119.2, 117.9, 114.0, 113.0, 111.6, 111.4, 56.6, 56.4, 56.2, 31.3, 29.2; MS (ESI) 481 $(M+H)^+$.

*4.1.29. Macrocycle (stilbene bridge) (**47**)*

This compound was prepared from **42** by following the procedure described for **43**, yield 76%, white solid; mp 203-204 °C. $^{1}$H NMR ($CDCl_3$) δ 7.39 (d, *J* = 8.4 Hz, 2 H ), 7.22-7.20 (m, 2 H), 7.05 (d, *J* = 8.4 Hz, 2 H), 7.01-6.97 (m, 2 H), 6.89-6.86 (m, 2 H), 6.83 (d, *J* = 7.8 Hz, 1 H), 6.75 (dd, *J* = 1.8 Hz, *J* = 7.8 Hz, 1 H), 6.55 (d, *J* = 9.0 Hz, 1 H), 6.46 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1 H), 5.36 (d, *J* = 1.8 Hz, 1 H), 3.97 (s, 3 H), 3.93 (s, 3 H), 3.57 (s, 3 H), 2.69-2.58 (m, 4 H); $^{13}$C NMR ($CDCl_3$) δ 155.5, 152.6, 125.5, 151.8, 150.4, 146.0, 142.7, 137.7, 136.0, 132.2, 132.1, 130.5, 130.0, 127.0, 125.0, 122.8, 120.6, 118.8, 116.9, 115.6, 113.9, 111.1, 110.9, 110.8, 55.6, 55.4, 52.9, 35.0, 33.1; MS (ESI) 481 $(M+H)^+$.

*4.1.30. Macrocyclic derivative (**48**)*

Palladium on activated carbon (10% Pd, 480 mg) were added to a solution of compound **43** (4.80 g, 10 mmol) in ethyl acetate (150 mL). The suspension was stirred under $H_2$ for 24 h at room temperature. The reaction mixture was filtered, and the solution was concentrated to provide the compound **48** as a white solid (4.70 g, 95%); mp 166-167 °C. $^{1}$H NMR ($CDCl_3$) δ 7.24 (t, *J* = 7.8 Hz, 1 H), 7.11 (d, *J* = 7.2 Hz, 1 H), 6.90 (t, *J* = 7.2 Hz, 1 H), 6.85 (d, *J* = 7.8 Hz, 2 H), 6.78 (d, *J* = 7.8 Hz, 1 H), 6.62 (s, 2 H), 6.59 (d, *J* = 7.8 Hz, 1 H), 6.50 (d, *J* = 7.2 Hz, 1 H), 6.43 (d, *J* = 7.8 Hz, 1 H), 6.29 (d, *J* = 7.8 Hz, 1 H), 5.47 (s, 1 H), 3.92 (s, 3 H), 3.69 (s, 3 H), 3.61 (s, 3 H), 3.07-3.05 (m, 4 H), 2.86 (s, 2 H), 2.78 (s, 2 H); $^{13}$C NMR ($CDCl_3$) δ 158.0, 152.3, 151.4, 147.6, 146.4, 142.0, 141.1, 140.9, 140.1, 136.6, 133.4, 127.9, 125.3, 123.2, 122.4, 122.3, 121.2, 120.8, 115.0, 114.8, 112.8, 111.8, 111.4, 110.1, 56.0, 55.7, 55.6, 36.5, 36.4, 34.6, 30.2; MS (ESI) 505 $(M+Na)^+$.

*4.1.31. Macrocyclic derivative (**49**)*

This compound was prepared from **44** by following the procedure described for **48**, yield 96%, white solid; mp 155-156 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.21 (t, *J* = 8.0 Hz, 1H), 7.11 (dd, *J* = 7.9, 1.4 Hz, 1H), 6.92 (t, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.84 (dd, *J* = 8.1, 2.1 Hz, 2H), 6.80 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.63 (t, *J* = 2.2 Hz, 1H), 6.43 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.38-6.30 (m, 2H), 6.19 (dd, *J* = 8.0, 2.2 Hz, 1H), 5.64 (d, *J* = 2.0 Hz, 1H), 3.93 (s, 3H), 3.69 (s, 3H), 3.64 (s, 3H), 3.02 (s, 4H), 2.91-2.89 (m, 2H), 2.80-2.78 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 158.3, 157.6, 153.9, 152.3, 148.3, 146.7, 141.9, 141.1, 137.5, 133.7, 130.6, 128.1, 127.1, 125.2, 122.6, 122.0, 121.6, 116.0, 115.2, 113.1, 112.0, 111.6, 110.0, 104.6, 56.1, 55.8, 55.2, 36.1, 34.3, 30.3, 28.9; MS (ESI) 505 $(M+Na)^+$.

*4.1.32. Macrocyclic derivative (**50**)*

This compound was prepared from **45** by following the procedure described for **48**, yield 95%, white solid; mp 178-179 °C. $^{1}$H NMR ($CDCl_3$) δ 7.43-7.38 (m, 1H), 7.12 (t, *J* = 7.9 Hz, 1H), 7.10-7.07

(m, 2H), 7.05-7.02 (m, 2H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.81-6.78 (m, 1H), 6.78-6.73 (m, 2H), 6.72-6.66 (m, 3H), 6.55-6.51 (m, 1H), 6.06 (d, *J* = 2.0 Hz, 1H), 5.54 (s, 1H), 3.90 (s, 3H), 3.19-3.13 (m, 2H), 3.06-3.00 (m, 2H), 2.95-2.91 (m, 2H), 2.87-2.79 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 155.9, 154.8, 153.8, 145.4, 144.3, 143.0, 137.9, 133.3, 131.9, 131.4, 130.0, 129.3, 126.7, 123.6, 123.4, 122.7, 120.1, 118.9, 116.6, 116.5, 116.4, 115.0, 37.1, 35.6, 34.5, 30.1; MS (ESI) 445 $(M+Na)^+$.

*4.1.33. Macrocyclic derivative (**51**)*

This compound was prepared from **46** by following the procedure described for **48**, yield 98%, white solid; mp 145-146 °C. $^1$H NMR ($CDCl_3$) δ 7.19 (t, *J* = 7.9 Hz, 1H), 7.15 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.94 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.82 (ddd, *J* = 10.2, 8.1, 1.7 Hz, 2H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.53 (d, *J* = 8.4 Hz, 2H), 6.47 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.18 (d, *J* = 2.0 Hz, 1H), 5.87 (d, *J* = 1.9 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.75 (s, 3H), 3.09-2.90 (m, 4H), 2.89-2.47 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 153.4, 152.7, 148.0, 146.9, 146.8, 146.7, 142.6, 136.6, 135.1, 133.2, 132.2, 129.4, 125.0, 121.9, 121.5, 120.6, 120.2, 116.1, 115.7, 111.9, 111.5, 110.3, 56.3, 56.1, 55.8, 34.0, 33.0, 31.0, 27.9; MS (ESI) 505 $(M+Na)^+$.

*4.1.34. Macrocyclic derivative (**52**)*

This compound was prepared from **47** by following the procedure described for **48**, yield 97%, white solid; mp 156-157 °C. $^1$H NMR ($CDCl_3$) δ 7.21 (t, *J* = 8.0 Hz, 1H), 7.08 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.85 (dd, *J* = 11.7, 4.7 Hz, 2H), 6.81 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 3.1 Hz, 1H), 6.45 (d, *J* = 8.9 Hz, 1H), 6.24 (dd, *J* = 8.9, 3.1 Hz, 1H), 5.49 (d, *J* = 1.9 Hz, 1H), 3.93 (s, 3H), 3.67 (s, 3H), 3.26 (s, 3H), 3.07-2.98 (m, 4H), 2.83-2.69 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 152.7, 152.5, 152.4, 151.9, 148.4, 146.6, 141.5, 139.4, 137.0, 134.6, 131.0, 129.6, 125.1, 122.7, 122.0, 121.6, 117.1, 115.7, 111.6, 111.5, 110.9, 110.4, 56.2, 55.9, 55.8, 36.4, 36.1, 31.0, 30.6; MS (ESI) 505 $(M+Na)^+$.

*4.1.35. Macrocyclic derivative (**53**)*

A solution of boron tribromide (6.31 g, 25.22 mmol) in anhydrous dichloromethane (20 mL) was added dropwise to a stirred solution of compound **48** (1.52 g, 3.15 mmol) in anhydrous dichloromethane (20 mL) at -78 ˚C. The reaction mixture was stirred at -78 ˚C for 3 h, and then was allowed to warm up to room temperature within 12 h. The ice-cold water was added, and the reaction mixture was stirred vigorously for 1 h. The solution was then diluted with dichloromethane (50 mL), washed with saturated aqueous NaCl (20 mL × 3) and dried over sodium sulfate. The solution was

concentrated, and the residue was purified by silica gel column chromatography, eluating with dichloromethane, to provide compound **53** as white solid (1.26 g, 91%); mp 161-162 °C. $^{1}$H NMR ($CDCl_3$) δ 7.18 (t, *J* = 7.9 Hz, 1H), 7.03 (d, *J* = 6.5 Hz, 1H), 6.98 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.80 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.71 (s, 1H), 6.66 (s, 1H), 6.55-6.45 (m, 3H), 6.29 (d, *J* = 7.5 Hz, 1H), 5.50 (d, *J* = 1.7 Hz, 1H), 5.05 (s, 1H), 4.82 (s, 1H), 3.00 (s, 4H), 2.86-2.78 (m, 4H); $^{13}$C NMR ($CDCl_3$) δ 156.8, 148.6, 147.4, 144.8, 143.2, 142.9, 141.2, 139.6, 139.0, 136.2, 133.2, 128.9, 126.1, 123.7, 123.2, 122.1, 121.8, 121.0, 116.7, 115.5, 115.4, 115.0, 114.4, 111.8, 36.3, 35.9, 34.7, 30.4; MS (ESI) 463 $(M+Na)^+$; HRMS calcd for $C_{28}H_{24}O_5Na$ 463.1516, found: 463.1510 $(M+Na)^+$.

*4.1.36. Macrocyclic derivative (**54**)*

This compound was prepared from **49** by following the procedure described for **53**, yield 88%, white solid; mp 140-141 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.19 (t, *J* = 7.9 Hz, 1H), 7.08 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.97 (t, *J* = 7.9 Hz, 1H), 6.90 (dd, *J* = 8.1, 2.8 Hz, 3H), 6.76 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.72 (s, 1H), 6.53 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.38 (d, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 8.1 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 5.56 (d, *J* = 2.1 Hz, 1H), 5.56 (s, 1H), 4.96 (s, 1 H), 4.90 (s, 1 H), 3.12-2.94 (m, 4H), 2.90-2.86 (m, 2H), 2.82-2.69 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.8, 153.8, 153.5, 148.7, 145.9, 143.3, 143.1, 139.7, 136.6, 132.8, 131.3, 128.9, 126.3, 125.7, 123.6, 122.5, 121.9, 115.7, 115.5, 115.0, 114.7, 113.9, 111.6, 109.2, 36.1, 34.5, 30.3, 29.4; MS (ESI) 463 $(M+Na)^+$; HRMS calcd for $C_{28}H_{24}O_5Na$ 463.1516, found: 463.1510 $(M+Na)^+$.

*4.1.37. Macrocyclic derivative (**55**)*

This compound was prepared from **50** by following the procedure described for **53**, yield 86%, white solid; mp 139-140 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.44-7.39 (m, 1H), 7.14-7.07 (m, 3H), 7.02 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.84-6.79 (m, 2H), 6.77 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.70 (d, *J* = 8.3 Hz, 2H), 6.63 (d, *J* = 7.6 Hz, 1H), 6.57 (d, *J* = 1.7 Hz, 1H), 6.02 (d, *J* = 2.0 Hz, 1H), 3.17-3.11 (m, 2H), 3.05-2.98 (m, 2H), 2.96-2.90 (m, 2H), 2.86-2.79 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.2, 154.6, 154.1, 147.8, 147.7, 142.9, 137.3, 133.8, 132.3, 131.4, 129.8, 129.1, 126.7, 123.6, 122.9, 122.5, 120.4, 118.6, 117.4, 116.9, 116.1, 112.0, 56.1, 37.2, 35.8, 34.6, 30.2; MS (ESI) 409 $(M+H)^+$; HRMS calcd for $C_{28}H_{28}O_3N_1$ 426.2068 found: 426.2064 $(M+NH_4)^+$**.**

*4.1.38. Macrocyclic derivative (**56**)*

This compound was prepared from **51** by following the procedure described for **53**, yield 90%,

white solid; mp 225-226 $^{o}$C .$^{1}$H NMR ($CDCl_3$) δ 7.21 (t, *J* = 7.9 Hz, 1H), 7.13 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.92 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.78 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.55 (dd, *J* = 8.5, 2.0 Hz, 3H), 6.25 (d, *J* = 1.8 Hz, 1H), 5.85 (d, *J* = 1.9 Hz, 1H), 5.73 (s, 1H), 5.46 (s, 1H), 5.04 (s, 1H), 3.16-3.09 (m, 4H), 2.98-2.96 (m, 2H), 2.76-2.74 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 153.4, 148.9, 145.6, 143.5, 143.4, 143.2, 140.5, 136.6, 133.7, 132.7, 132.3, 129.5, 126.3, 123.0, 122.0, 121.0, 120.3, 115.3, 115.2, 115.1, 114.4, 114.3, 33.2, 32.4, 30.3, 28.6; MS (ESI) 463 $(M+Na)^+$; HRMS calcd for $C_{28}H_{24}O_5Na$ 463.1516, found: 463.1510 $(M+Na)^+$.

*4.1.39. Macrocyclic derivative (**57**)*

This compound was prepared from **51** by following the procedure described for **53**, yield 90%, white solid; mp 124-125 $^{o}$C . $^{1}$H NMR ($CDCl_3$) δ 7.18 (t, *J* = 7.9 Hz, 1H), 7.04 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 6.90 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.82 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.75 (d, *J* = 3.1 Hz, 1H), 6.70 (d, *J* = 8.2 Hz, 2H), 6.50 (d, *J* = 8.7 Hz, 1H), 6.31 (dd, *J* = 8.7, 3.1 Hz, 1H), 5.62 (s, 1H), 5.41 (d, *J* = 1.9 Hz, 1H), 4.96 (s, 1H), 3.54 (s, 1H), 3.08-3.06 (m, 2H), 3.00-2.92 (m, 2H), 2.80-2.69 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 152.2, 150.9, 149.4, 148.8, 146.7, 143.9, 140.1, 139.8, 136.5, 132.4, 129.8, 129.4, 126.1, 122.2, 122.0, 121.4, 117.4, 116.8, 115.5, 115.4, 114.3, 112.3, 36.1, 35.9, 31.6, 30.8; MS (ESI) 463 $(M+Na)^+$; HRMS calcd for $C_{28}H_{24}O_5Na$ 463.1516, found: 463.1510 $(M+Na)^+$.

*4.1.40. 2-(3-(1,3-Dioxan-2-yl)phenoxy)-5-methoxybenzaldehyde (**66**)*

This compound was prepared from 2-bromo-5-methoxybenzaldehyde (**63**) and 3-(1,3-dioxan-2-yl)phenol (**65**) in 74% yield by following the procedure described for **6**, white solid; mp 123-124 °C. $^{1}$H NMR ($CDCl_3$) δ 10.40 (s, 1H), 7.41 (s, 1H), 7.35 (d, *J* = 7.4 Hz, 1H), 7.18 (s, 1H), 7.13 (d, *J* = 5.9 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.86 (s, 1H), 5.49 (s, 1H), 4.26 (d, *J* = 10.6 Hz, 2H), 3.99 (t, *J* = 11.9 Hz, 2H), 3.86 (s, 3H), 2.26-2.20 (m, 1H), 1.45 (d, *J* = 13.4 Hz, 1H); MS (ESI) 315 $(M+H)^+$.

*4.1.41. 2-(3-(1,3-Dioxan-2-yl)phenoxy)-4-methoxybenzaldehyde (**67**)*

This compound was prepared from 2-bromo-4-methoxybenzaldehyde (**64**) and 3-(1,3-dioxan-2-yl) phenol (**65**) in 70% yield by following the procedure described for **6**, white solid; mp 111-112 °C. $^{1}$H NMR ($CDCl_3$) δ 10.36 (s, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 6.7 Hz, 1H), 7.28 (s, 1H), 7.05 (d, *J* = 7.1 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.34 (s, 1H), 5.51 (s, 1H), 4.28 (d,

*J* = 10.6 Hz, 2H), 4.00 (t, *J* = 11.9 Hz, 2H), 3.76 (s, 3H), 2.32-2.16 (m, 1H), 1.47 (d, *J* = 13.4 Hz, 1H); MS (ESI) 315 $(M+H)^+$.

*4.1.42. (2-(3-(1,3-Dioxan-2-yl)phenoxy)-5-methoxyphenyl)methanol (**68**)*

This compound was prepared from compound **66** by following the procedure described for **8**, yield 96%, colorless oil. $^1$H NMR ($CDCl_3$) δ 7.29 (t, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.08 (s, 1H), 7.05 (d, *J* = 2.7 Hz, 1H), 6.89-6.86 (m, 2H), 6.81 (dd, *J* = 8.4 Hz, *J* = 3.0 Hz, 1H), 5.46 (s, 1H), 4.65 (d, *J* = 6.0 Hz, 2H), 4.25 (dd, *J* = 11.0, 4.7 Hz, 2H), 3.97 (t, *J* = 11.4 Hz, 2H), 3.83 (s, 3H), 2.27-2.17 (m, 1H), 1.44 (d, *J* = 13.5 Hz, 1H); MS (ESI) 317 $(M+H)^+$.

*4.1.43 (2-(3-(1,3-Dioxan-2-yl)phenoxy)-5-methoxybenzyl)bromotriphenylphosphorane (**70**)*

This compound was prepared from compound **68** by following the procedure described for **11**, yield 86%, white solid; mp 220-221 °C. $^1$H NMR ($CDCl_3$) δ 7.80-7.72 (m, 9H), 7.67-7.63 (m, 6H), 7.22-7.17 (m, 3H), 6.73 (dt, *J* = 9.0, 2.8 Hz, 1H), 6.68 – 6.63 (m, 1H), 6.54 (d, *J* = 9.0 Hz, 1H), 6.44 (ddd, *J* = 8.0, 2.5, 1.1 Hz, 1H), 5.41 (s, 1H), 5.25 (d, *J* = 14.2 Hz, 2H), 4.29-4.21 (m, 2H), 4.02-3.94 (m, 2H), 3.60 (s, 3H), 2.25-2.18 (m, 1H), 1.47 (dtt, *J* = 13.5, 2.5, 1.3 Hz, 1H); MS (ESI) 561 $(M-Br)^+$.

*4.1.44. (2-(3-(1,3-Dioxan-2-yl)phenoxy)-4-methoxybenzyl)bromotriphenylphosphorane (**71**)*

This compound was prepared from compound **69** by following the procedure described for **11**, yield 90%, white solid; mp 204-205 °C. $^1$H NMR ($CDCl_3$) δ 7.82-7.72 (m, 9H), 7.69-7.65 (m, 6H), 7.50 (t, *J* = 2.8 Hz, 1H), 7.26-7.22 (m, 2H), 6.61 (d, *J* = 1.0 Hz, 1H), 6.51 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.44 (dt, *J* = 6.6, 2.6 Hz, 1H), 6.05 (d, *J* = 2.2 Hz, 1H), 5.43 (s, 1H), 5.39 (d, *J* = 12.8 Hz, 2H), 4.28 (dd, *J* = 10.7, 5.0 Hz, 2H), 4.00 (td, *J* = 12.3, 2.5 Hz, 2H), 3.64 (s, 3H), 2.30-2.19 (m, 1H), 1.50-1.46 (m, 1H); MS (ESI) 561 $(M-Br)^+$.

*4.1.45. 5-(Hydroxymethyl)-2-methoxyphenol (**72**)*

This compound was prepared from compound **4** by following the procedure described for **8**, yield 95%, colorless oil; $^1$H NMR ($CDCl_3$) δ 6.97 (s, 1 H), 6.93-6.80 (m, 2 H), 5.64 (s, 1 H), 4.61 (d, *J* = 5.8 Hz, 2 H), 3.91 (s, 3 H); MS (ESI) 155 $(M+H)^+$.

*4.1.46. 2-Methoxy-5-((tetrahydro-2H-pyran-2-yloxy)methyl)phenol (**73**)*

The compound **72** (5.36 g, 34.80 mmol) was dissolved in anhydrous DCM. After addition of 3,4-dihydro-2*H*-pyran (8.77 g, 104.44 mmol) and *p*-TosOH (280 mg, 1.74 mmol) the mixture was stirred for 24 h at room temperature, the solvent was concentrated and the crude material was purified by flash column chromatography (SiO2) eluting with n-hexane/DCM 1:1, to provide **73** as colorless oil

(7.37 g, 89%); $^1$H NMR ($CDCl_3$) δ 6.95 (s, 1 H), 6.89-6.80 (m, 1 H), 6.78 (d, *J* = 8.2 Hz, 1 H), 6.24 (d, *J* = 5.3 Hz, 1 H), 4.81-4.70 (m, 1 H), 4.68 (d, *J* = 11.7 Hz, 1 H), 4.40 (d, *J* = 11.7 Hz, 1 H), 3.91 (dd, *J* = 14.5, 5.7 Hz, 1 H), 3.81 (s, 3 H), 3.62-3.50 (m, 1 H), 1.93-1.79 (m, 1 H), 1.76-1.68 (m, 1 H), 1.68-1.46 (m, 4 H); MS (ESI) 239 $(M+H)^+$.

*4.1.47. 4-(2-Methoxy-5-((tetrahydro-2H-pyran-2-yloxy)methyl)phenoxy)benzaldehyde (**75**)*

This compound was prepared from 4-bromo-benzaldehyde (**74**) and compound **73** in 70% yield by following the procedure described for **6**, white solid; mp 100-101 °C. $^1$H NMR ($CDCl_3$) δ 9.82 (s, 1 H), 7.74 (d, *J* = 8.7 Hz, 2 H), 7.18 (d, *J* = 8.4 Hz, 1 H), 7.09 (d, *J* = 1.9 Hz, 1 H), 6.96-6.92 (m, 3 H), 4.68 (d, *J* = 11.9 Hz, 1 H), 4.66-4.62 (m, 1 H), 4.39 (d, *J* = 11.9 Hz, 1 H), 3.91-3.78 (m, 1 H), 3.70 (s, 3 H), 3.53-3.43 (m, 1 H), 1.85-1.74 (m, 1 H), 1.67 (dd, *J* = 13.0, 9.7 Hz, 1 H), 1.62-1.42 (m, 4 H); MS (ESI) 343 $(M+H)^+$.

*4.1.48. 2-(3-(2-(4-(2-Methoxy-5-((tetrahydro-2H-pyran-2-yloxy)methyl)phenoxy)phenethyl)-4-methoxy phenoxy) phenyl)-1,3-dioxane (**76**)*

This compound was prepared from compound **70** and compound **75** in 85% yield by following the procedure described for **23**, white solid; mp 154-155 °C. $^1$H NMR ($CDCl_3$) δ 7.29 (t, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.12 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.08 (s, 1H), 7.06 (d, *J* = 2.6 Hz, 1H), 7.05 (d, *J* = 2.6 Hz, 1H), 7.00-6.95 (m, 2H), 6.92-6.91 (m, 2H), 6.87-6.68 (m, 3 H), 6.75-6.73 (m, 4 H), 5.47 (s, 1H), 4.69 (d, *J* = 11.9 Hz, 1H), 4.67 (t, *J* = 2.8 Hz, 1H), 4.40 (d, *J* = 11.8 Hz, 1H), 4.27-4.25 (m, 2H), 3.98 (ddd, *J* = 12.3, 4.0, 2.5 Hz, 2H), 3.91-3.89 (m, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.54-3.48 (m, 1H), 2.26-2.18 (m, 1H), 1.88-1.81 (m, 1H), 1.77-1.69 (m, 1H), 1.63-1.52 (m, 4H), 1.46-1.43 (m, 1H); MS (ESI) 627 $(M+H)^+$.

*4.1.49. 2-(3-(2-(4-(2-Methoxy-5-((tetrahydro-2H-pyran-2-yloxy)methyl)phenoxy)phenethyl)-5-methoxy phenoxy)phenyl)-1,3-dioxane (**77**)*

This compound was prepared from compound **71** and compound **75** in 88% yield by following the procedure described for **23**, white solid; mp 131-132 °C. $^1$H NMR ($CDCl_3$) δ 7.27 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.13 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.11-7.10 (m, 3H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 8.6 Hz, 1H), 6.99 (d, *J* = 2.2 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.91-6.88 (m, 4H), 5.42 (s, 1H), 4.69 (d, *J* = 11.9 Hz, 1H), 4.68 (t, *J* = 2.8 Hz, 1H), 4.41 (d, *J* = 11.8 Hz, 1H), 4.23 (ddd, *J* = 11.9, 4.9, 1.2 Hz, 2H), 3.95 (ddd, *J* = 12.3, 4.0, 2.5 Hz, 2H), 3.91-3.87 (m, 1H), 3.86 (s, 6H), 3.55-3.49 (m, 1H), 2.25-2.15 (m,

1H), 1.88-1.81 (m, 1H), 1.75-1.70 (m, 1H), 1.63-1.51 (m, 4H), 1.45-1.41 (m, 1H); MS (ESI) 627 $(M+H)^+$.

*4.1.50. 3-(2-(4-(5-(Hydroxymethyl)-2-methoxyphenoxy)phenethyl)-4-methoxyphenoxy)benzaldehyde (**78**)*

This compound was prepared from **76** by following the procedure described for **33**, yield 90%, colorless oil. $^1$H NMR ($CDCl_3$) δ 9.95 (s, 1H), 7.55 (dt, *J* = 7.5, 1.1 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.32 (dd, *J* = 2.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H), 7.11 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.04-7.00 (m, 2H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.94-6.93 (m, 2H), 6.87-6.82 (m, 2H), 6.81-6.74 (m, 2H), 4.58 (s, 2H), 3.86 (s, 3H), 3.82 (s, 3H), 2.88-2.80 (m, 4H); MS (ESI) 485 $(M+H)^+$.

*4.1.51. 3-(2-(4-(5-(Hydroxymethyl)-2-methoxyphenoxy)phenethyl)-5-methoxyphenoxy)benzaldehyde (**79**)*

This compound was prepared from **77** by following the procedure described for **33**, yield 92%, colorless oil. $^1$H NMR ($CDCl_3$) δ 9.96 (s, 1H), 7.62-7.55 (m, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.40 (dd, *J* = 2.4, 1.4 Hz, 1H), 7.24 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.11 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.05-7.01 (m, 2H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.94 (d, *J* = 2.1 Hz, 1H), 6.87-6.81 (m, 2H), 6.71 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.49 (d, *J* = 2.6 Hz, 1H), 4.57 (s, 2H), 3.86 (s, 3H), 3.76 (s, 3H), 2.83 (s, 4H); MS (ESI) 485 $(M+H)^+$.

*4.1.52. 3-(2-(4-(5-((Bromotriphenylphosphoranyl)methyl)-2-methoxyphenoxy)phenethyl)-4-methoxyphenoxy)benzaldehyde (**80**)*

This compound was prepared from **78** by following the procedure described for **38**, yield 92%, white solid; mp 219-220 °C. $^1$H NMR ($CDCl_3$) δ 9.95 (s, 1H), 7.75-7.69 (m, 9H), 7.60-7.59 (m, 6H), 7.55 (dt, *J* = 7.5, 1.2 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.33 (dd, *J* = 2.5, 1.4 Hz, 1H), 7.22 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.96-6.91 (m, 3H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.80-6.74 (m, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 6.26 (t, *J* = 2.1 Hz, 1H), 5.29 (d, *J* = 13.8 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 2.87-2.79 (m, 4H); MS (ESI) 729 $(M-Br)^+$.

*4.1.53. 3-(2-(4-(5-((Bromotriphenylphosphoranyl)methyl)-2-methoxyphenoxy)phenethyl)-5-methoxyphenoxy)benzaldehyde (**81**)*

This compound was prepared from **79** by following the procedure described for **38**, yield 89%, white solid; mp 241-242 °C. $^1$H NMR ($CDCl_3$) δ 9.97 (s, 1H), 7.74-7.69 (m, 9H), 7.62-7.58 (m, 6H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.50-7.48 (m, 1H), 7.40 (dd, *J* = 2.4, 1.4 Hz, 1H), 7.25 (ddd, *J* = 8.1, 2.6, 1.0

Hz, 1H), 7.20 (dt, *J* = 8.5, 2.5 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.71 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.59 (d, *J* = 8.6 Hz, 2H), 6.49 (d, *J* = 2.5 Hz, 1H), 6.27 (t, *J* = 2.3 Hz, 1H), 5.31 (d, *J* = 13.8 Hz, 2H), 3.79 (s, 3H), 3.75 (s, 3H), 2.83 (s, 4H); MS (ESI) 729 $(M\text{-}Br)^+$.

*4.1.54. Macrocycle (stilbene bridge) (**82**)*

This compound was prepared from **80** by following the procedure described for **43**, yield 81%, white solid; mp 124-125 $^oC$. $^1H$ NMR ($CDCl_3$) δ 7.00 (d, *J* = 8.0 Hz, 1 H ), 6.98-6.95 (m, 2 H), 6.92 (d, *J* = 8.4 Hz, 2 H), 6.85 (d, *J* = 8.0 Hz, 1 H), 6.83 (d, *J* = 2.0 Hz, 1 H), 6.75-6.72 (m, 2 H), 6.63-6.59 (m, 4 H), 6.48 (d, *J* = 12.0 Hz, 1 H), 6.42 (d, *J* = 12.0 Hz, 1 H), 6.21 (d, *J* = 3.2 Hz, 1 H), 3.91 (s, 3 H), 3.85 (s, 3 H), 3.09-3.06 (m, 2 H), 2.99-2.96 (m, 2 H); $^{13}C$ NMR ($CDCl_3$) δ 158.5, 156.1, 153.0, 148.4, 148.0, 146.6, 139.7, 137.9, 135.2, 130.0, 129.8, 129.4, 129.1, 128.7, 123.6, 122.6, 121.4, 121.2, 116.1, 116.0, 115.5, 114.4, 112.0, 111.4, 56.0, 55.6, 36.5, 30.5; MS (ESI) 451 $(M+H)^+$.

*4.1.55. Macrocycle (stilbene bridge) (**83**)*

This compound was prepared from **81** by following the procedure described for **43**, yield 80%, white solid; mp 183-184 $^oC$. $^1H$ NMR ($CDCl_3$) δ 7.74 (d, *J* = 8.8 Hz, 1H), 7.40-7.34 (m, 2H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 7.06-6.99 (m, 3H), 6.94 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.87 (d, *J* = 16.1 Hz, 1H), 6.83 (dd, *J* = 7.9, 5.0 Hz, 2H), 6.60 (d, *J* = 2.7 Hz, 1H), 6.0-6.04 (m, 1H), 6.01 (d, *J* = 16.1 Hz, 1H), 5.98 (d, *J* = 1.6 Hz, 1H), 3.99 (s, 3H), 3.79 (s, 3H), 3.28-3.26 (m, 2H), 3.07-3.00 (m, 2H); $^{13}C$ NMR (151 MHz, $CDCl_3$) δ 159.4, 158.9, 155.7, 154.4, 150.4, 147.7, 140.4, 139.0, 135.5, 133.0, 130.9, 130.0, 127.8, 127.4, 125.4, 123.6, 119.8, 118.9, 118.0, 116.5, 116.1, 111.4, 111.3, 108.8, 56.2, 55.5, 29.9, 29.7; MS (ESI) 451 $(M+H)^+$.

*4.1.56. Macrocyclic derivative (**84**)*

This compound was prepared from **82** by following the procedure described for **48**, yield 90%, white solid; mp 100-101 °C. $^1H$ NMR ($CDCl_3$) δ 7.02 (t, *J* = 8.0 Hz, 1 H ), 6.98 (d, *J* = 8.4 Hz, 2 H), 6.94 (d, *J* = 3.2 Hz, 1 H), 6.83 (t, *J* = 8.0 Hz, 2 H), 6.77 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1 H), 6.71-6.65 (m, 2 H), 6.63 (d, *J* = 8.4 Hz, 2 H), 6.56 (s, 1 H), 6.48 (d, *J* = 7.6 Hz, 1 H), 5.83 (d, *J* = 1.6 Hz, 1 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.09-2.98 (m, 4 H), 2.89-2.77 (m, 4 H); $^{13}C$ NMR ($CDCl_3$) δ 157.3, 155.4, 153.8, 147.9, 147.8, 147.5, 142.6, 137.5, 134.0, 133.7, 129.7, 128.8, 123.0, 122.2, 120.7, 119.1, 117.1, 116.1, 115.1, 112.0, 111.9, 56.1, 55.6, 36.8, 36.0, 34.5, 30.4; MS (ESI) 453 $(M+H)^+$.

*4.1.57. Macrocyclic derivative (**85**)*

This compound was prepared from **83** by following the procedure described for **48**, yield 93%, white solid; mp 115-116 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.30 (d, *J* = 8.5 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.05-6.98 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.81 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.78 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.73-6.67 (m, 2H), 6.67-6.63 (m, 2H), 6.59-6.54 (m, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 6.04 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.71 (s, 3H), 3.10-3.03 (m, 2H), 3.01-2.94 (m, 2H), 2.94-2.89 (m, 2H), 2.86-2.80 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 158.3, 155.9, 155.3, 154.1, 147.8, 147.7, 142.9, 137.4, 133.9, 131.7, 129.8, 129.1, 124.4, 123.8, 122.6, 120.3, 118.9, 117.5, 116.3, 112.0, 108.2, 103.0, 56.1, 55.4, 37.4, 35.8, 34.7, 29.7; MS (ESI) 453 $(M+H)^+$.

*4.1.58. Macrocyclic derivative (**86**)*

This compound was prepared from **84** by following the procedure described for **53**, yield 76%, white solid; mp 111-112 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.06 (d, *J* = 8.0 Hz, 1 H ),7.01 (d, *J* = 8.4 Hz, 2 H), 6.88 (d, *J* = 1.6 Hz, 1 H), 6.87 (d, *J* = 2.8 Hz, 1 H), 6.73 (d, *J* = 8.4 Hz, 2 H), 6.69 (dd, *J* = 1.2 Hz, *J* = 8.8 Hz, 1 H), 6.63 (d, *J* = 8.4 Hz, 2 H), 6.58 (dd, *J* = 2.8 Hz, *J* = 8.8 Hz, 1 H), 6.55-6.53 (m, 2 H), 5.89 (s, 1 H), 3.07-2.97 (m, 4 H), 3.91-2.77 (m, 4 H); $^{13}$C NMR ($CDCl_3$) δ 157.0, 153.5, 151.1, 148.1, 145.5, 144.0, 142.8, 137.9, 133.9, 133.2, 129.9, 129.0, 123.1, 123.0, 120.4, 118.9, 117.7, 117.4, 116.2, 115.3, 115.0, 113.6, 36.7, 35.7, 34.4, 30.2; MS (ESI) 425 $(M+H)^+$; HRMS calcd for $C_{28}H_{24}O_4Na$ 447.1567, found: 447.1554 $(M+Na)^+$.

*4.1.59. Macrocyclic derivative (**87**)*

This compound was prepared from **85** by following the procedure described for **53**, yield 71%, white solid; mp 158-159 $^{o}$C. $^{1}$H NMR ($CDCl_3$) δ 7.23 (d, *J* = 8.3 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.07-7.03 (m, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.77 (dd, *J* = 8.2, 2.0 Hz, 2H), 6.74 (d, *J* = 7.8 Hz, 1H), 6.73-6.70 (m, 2H), 6.55 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.52-6.47 (m, 1H), 6.23 (d, *J* = 2.5 Hz, 1H), 6.15 (d, *J* = 2.0 Hz, 1H), 5.56 (s, 1H), 4.73 (s, 1H), 3.14-3.04 (m, 2H), 2.99-2.90 (m, 4H), 2.88-2.80 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 155.9, 155.4, 154.2, 154.0, 145.3, 144.5, 143.2, 137.8, 133.5, 131.9, 130.0, 129.4, 124.0, 123.7, 123.6, 119.9, 119.6, 117.0, 116.7, 115.1, 109.6, 103.3, 37.4, 35.5, 34.6, 29.6; MS (ESI) 425 $(M+H)^+$; HRMS calcd for $C_{28}H_{24}O_4Na$ 447.1567, found: 447.1554 $(M+Na)^+$.

*4.1.60. Macrocyclic derivative (**88**)*

This compound was prepared from **44** by following the procedure reported in the literature [35], compound **88** was further purified by semi-prep. HPLC. HPLC: *Agilent 1100-G1310A* isopump equipped with a *G1322A* degasser, a *G1314A VWD* detector (210 nm) and a *Eclipse XDB-$C_{18}$* column

(9.4 × 250 mm, 5-μm, 1.5 mL/min, 78% MeOH/$H_2O$, over 30 minutes), yield 16%, white solid; mp 159-160 °C. $^1$H NMR ($CDCl_3$) δ 7.14 (t, *J* = 8.2 Hz, 1 H), 7.01 (d, *J* = 2.8 Hz, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.92 (s, 1H), 6.91(d, *J* = 8.1 Hz, 1H), 6.87 (t, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 1.9 Hz, 1H), 6.80 (s, dd, *J* = 8.3, 1.9 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.65 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.59 (d, *J* = 16.0 Hz, 1H), 6.51 (d, *J* = 7.5 Hz, 1H), 6.36 (d, *J* = 16.0 Hz, 1H), 6.16 (dd, *J* = 8.3, 1.9 Hz, 1H), 5.75 (d, *J* = 1.8 Hz, 1H), 5.68 (s, 1H), 5.58 (s, 1H), 5.28 (s, 1H), 5.23 (s, 1H), 3.03-2.94 (m, 3H), 2.90 (s, 1H); $^{13}$C NMR ($CDCl_3$) δ 157.8, 148.8, 148.6, 146.2, 143.1, 142.9, 141.0, 139.5, 134.5, 131.9, 129.9, 129.8, 128.8, 125.8, 123.7, 122.6, 120.4, 119.1, 117.5, 117.0, 115.5, 115.1, 113.4, 113.3, 112.9, 35.9, 31.9; MS (ESI) 481 $(M+H)^+$. MS (ESI) 439 $(M+H)^+$; HRMS calcd for $C_{28}H_{23}O_5$ 439.1540, found: 439.1541 $(M+H)^+$.

*4.1.61. Macrocyclic derivative (**89**)*

This compound was prepared from **82** by following the procedure described for **88**, yield 34%, white solid; mp 137-138 °C. $^1$H NMR ($CDCl_3$) δ 7.01 (t, *J* = 7.9 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 2H), 6.90 (d, *J* = 3.3 Hz, 1H), 6.89 (d, *J* = 1.4 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.79 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.74 (s, 1H), 6.67-6.64 (m, 1H), 6.63 (d, *J* = 8.1 Hz, 2H), 6.61 (s, 1H), 6.59 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.46 (d, *J* = 12.0 Hz, 1H), 6.41 (d, *J* = 12.0 Hz, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 5.66 (s, 1H), 4.72 (s, 1H), 3.08-3.02 (m, 2H), 3.00-2.92 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 158.2, 152.6, 151.8, 146.9, 145.8, 145.0, 139.8, 138.5, 130.2, 130.0, 129.0, 128.4, 124.5, 122.9, 121.2, 120.9, 117.4, 115.8, 115.2, 114.8, 114.6, 113.7, 36.5, 30.6; MS (ESI) 423 $(M+H)^+$; HRMS calcd for $C_{28}H_{23}O_4$ 423.1591, found: 423.1590 $(M+H)^+$.

*4.1.62. General method for synthesis of brominated marchantin C analogues **90-95***

Marchantin C (212 mg, 0.5 mmol) and DMSO (0.55mmol) in ethyl acetate (2 mL) were added hydrobromic acid (48%, 0.8 mmol) at 60 □ under air. The reaction mixture were stirred for 12 h. The solvent was then removed *in vacuum*, the residue was further purified by semi-preparative HPLC to yield compounds **90**-**95**. HPLC: *Agilent 1100-G1310A* isopump equipped with a *G1322A* degasser, a *G1314A VWD* detector (210 nm) and a *Eclipse XDB-$C_{18}$* column (9.4 × 250 mm, 5-μm, 1.5 mL/min, 70% MeOH/$H_2O$, over 70 minutes).

*4.1.62.1. 4'-Bromo marchantin C (**90**)*

White solid, mp 116-117 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.7 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.97 (t, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 6.78 (t, *J* = 2.2 Hz, 1H), 6.76 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 2H), 6.44 (d, *J* = 7.7 Hz, 1H), 6.37 (dd, *J* =

8.2, 2.0 Hz, 1H), 5.59 (d, *J* = 2.0 Hz, 1H), 5.56 (s, 1H), 4.74 (s, 1H), 3.17-3.11 (m, 2H), 3.07-3.04 (m, 2H), 2.88-2.79 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.2, 152.8, 147.7, 146.2, 143.7, 143.5, 140.0, 139.8, 136.6, 133.0, 130.0, 129.9, 129.0, 124.4, 122.4, 121.6, 116.3, 116.0, 115.7, 115.0, 110.8, 36.7, 35.0, 34.6, 31.8; MS (ESI) 503 $(M+H)^+$; HRMS calcd for $C_{28}H_{24}O_4Br$ 503.0852, found: 503.0841 $(M+H)^+$.

*4.1.62.2. 6'-Bromo marchantin C (**91**)*

White solid, mp 104-105 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.5 Hz, 1H), 6.99 (t, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.76 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 6.62 (t, *J* = 2.2 Hz, 1H), 6.52 (dd, *J* = 7.9, 2.3 Hz, 1H), 6.39 (d, *J* = 7.6 Hz, 1H), 5.55 (d, *J* = 2.0 Hz, 1H), 5.51 (s, 1H), 3.05-2.99 (m, 4H), 2.88-2.79 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.8, 152.9, 145.9, 145.8, 143.4, 142.8, 140.3, 138.7, 136.2, 132.7, 129.7, 128.6, 128.5, 123.4, 122.6, 122.5, 121.3, 115.6, 115.5, 114.9, 112.2, 107.7, 35.9, 35.4, 34.2, 30.3; MS (ESI) 503 $(M+H)^+$; HRMS calcd for $C_{28}H_{24}O_4Br$ 503.0852, found: 503.0841 $(M+H)^+$.

*4.1.62.3. 4', 6'-Dibromo marchantin C (**92**)*

White solid, mp 120-121 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.02 (d, *J* = 7.9 Hz, 2H), 6.95 (t, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.76 (s, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.69 (d, *J* = 7.7 Hz, 2H), 6.41 (d, *J* = 7.5 Hz, 1H), 6.33 (d, *J* = 8.2 Hz, 1H), 5.58 (s, 1H), 5.54 (s, 1H), 5.40-5.11 (br, 1H), 3.20-3.10 (m, 2H), 3.04-3.02 (m, 2H), 2.90 – 2.75 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.6, 152.9, 146.0, 145.0, 143.5, 143.3, 140.5, 139.3, 136.6, 132.9, 131.6, 129.9, 128.5, 124.2, 122.4, 121.6, 115.9, 115.7, 114.9, 111.2, 108.8, 36.7, 35.1, 34.4, 31.8; MS (ESI) 581 $(M+H)^+$; HRMS calcd for $C_{28}H_{23}O_4Br_2$ 580.9958, found: 580.9959 $(M+H)^+$.

*4.1.62.4. 4', 13-Dibromo marchantin C (**93**)*

White solid, mp 115-116 °C. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 1.9 Hz, 1H), 6.97 (t, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 6.78 (s, 1H), 6.68 (d, *J* = 7.8 Hz, 2H), 6.42 (d, *J* = 7.6 Hz, 1H), 6.38 (dd, *J* = 7.9, 2.3 Hz, 1H), 5.86 (s, 1H), 5.48 (d, *J* = 1.9 Hz, 1H), 4.70 (s, 1H), 3.17-3.10 (m, 2H), 3.07-3.03 (m, 2H), 2.85-2.78 (m, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.3, 152.4, 147.7, 146.9, 143.2, 141.0, 140.1, 140.0, 136.6, 133.6, 130.1, 130.0, 129.1, 125.3, 124.5, 121.5, 116.3, 115.9, 114.9, 111.0, 108.4, 36.6, 34.9, 34.6, 31.7; MS (ESI) 581 $(M+H)^+$; HRMS calcd for $C_{28}H_{23}O_4Br_2$ 580.9958, found: 580.9959 $(M+H)^+$.

*4.1.62.5. 6', 13-Dibromo marchantin C (**94**)*

White solid, mp 109-110 °C. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.4 Hz, 1H), 7.01 (s, 1H), 6.99 (t, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.63 (s, 1H), 6.62 (d, *J* = 8.4 Hz, 2H), 6.52 (d, *J* = 8.2 Hz, 1H), 6.37 (d, *J* = 7.5 Hz, 1H), 5.80 (s, 1H), 5.45 (s, 1H), 5.37 (s, 1H), 3.07-2.98 (m, 4H), 2.86-2.81 (m, 2H), 2.80-2.76 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.9, 152.5, 146.6, 145.7, 142.3, 140.8, 140.2, 139.1, 136.1, 133.4, 129.7, 128.6, 128.5, 125.3, 123.4, 122.6, 121.4, 115.5, 114.8, 112.3, 108.4, 107.8, 35.9, 35.5, 34.2, 30.3; MS (ESI) 581 $(M+H)^+$; HRMS calcd for $C_{28}H_{23}O_4Br_2$ 580.9958, found: 580.9959 $(M+H)^+$.

*4.1.62.6. 4', 6', 13-Tribromo marchantin C (**95**)*

White solid, mp 125-126 °C. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.00 (s, 1H), 6.95 (s, 1H), 6.76 (s, 1H), 6.67 (d, *J* = 7.9 Hz, 2H), 6.39 (d, *J* = 7.5 Hz, 1H), 6.34 (dd, *J* = 8.2, 2.3 Hz, 1H), 5.82 (s, 1H), 5.46 (d, *J* = 1.3 Hz, 1H), 5.25 (s, 1H), 3.22-3.11 (m, 2H), 3.05-3.03 (m, 2H), 2.83-2.75 (d, *J* = 4.3 Hz, 4H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 156.7, 152.4, 146.7, 144.9, 142.8, 140.9, 140.4, 139.7, 136.5, 133.6, 131.6, 130.0, 128.6, 125.3, 124.3, 121.6, 115.9, 115.8, 115.0, 111.4, 108.9, 108.3, 36.6, 35.1, 34.5, 31.7; MS (ESI) 676 $(M+NH_4)^+$; HRMS calcd for $C_{28}H_{25}O_4N_1Br_3$ 675.9328, found: 675.9334 $(M+NH_4)^+$.

*4.2. Biological studies*

*4.2.1. Antiproliferative studies*

Human breast adenocarcinoma cell line HCC1428 and human colonic cancer cell line HT29 were purchased from the Shanghai Institute for Biological Sciences (SIBS), China Academy of Sciences (China). Human myelogenous leukemia k562 cell line was purchased from the Department of Pharmacology, Institute of Hematology of the Chinese Academy of Medical Sciences, Tianjin (China). They were cultured in RPMI-1640 (Hyclone) medium containing 10% FBS (Sijiqing Company, Ltd.), 100 units/mL of penicillin G, and 100 μg/mL of streptomycin in a stable environment with 37 □ and 5% $CO_2$. HeLa (cervical carcinoma) was obtained from the American Type Culture Collection (ATCC) and cultured at 37 □ and 5% $CO_2$ in DMEM medium supplemented as described above. The PC-3/Doc cell line was obtained from Yuan's group at Shandong University. The antiproliferative activity of the marchantin C analogues on the five tumor cell lines was measured by the MTT method, as previously described [39]. Briefly, after treatment with the candidate drug for 48 h, the absorbance of the soluble MTT product was measured at 570 nm. All experiments were measured at least three times.

*4.2.2. Cell growth curve*

The cell growth curves of HCC1428 cells treated by the indicated concentrations of compound **88** for 84 h were detected by the xCELLigence system from Roche according to the manufacturer's protocol. The cells were incubated in RPMI-1640 medium containing 2% FBS at 37 □ and 5% $CO_2$ throughout.

*4.2.3. Cell cycle analysis*

The cell cycle distribution was measured by flow cytometric analysis. HCC1428 cells were seeded into 6-well plates and then treated with varying concentrations of compound **88**. Adherent cells were detached with trypsin and collected by centrifugation, followed by washing, fixation, and PI staining. The cell cycle distribution was examined by a FACScan flow cytometer (Becton–Dickinson, USA), and the data were analyzed using the Modfit program (Becton–Dickinson, USA)

*4.2.4. Immunofluorescence*

For the immunofluorescence studies, HCC1428 cells were seeded on 12-mm round glass cover slips and placed at the bottom of 24-well plates. After the experimental treatment, the cells were fixed with cold methanol/acetone (1:1) for 5 min followed by incubating with 3% goat serum (in 0.1% Triton X-100) for 20 min to prevent nonspecific antibody binding. Then, they were immunostained for α-tubulin using mouse anti-α-tubulin antibody followed by FITC-conjugated goat anti-rabbit secondary antibody. The DNA was counterstained with DAPI (4 μg/ml) for 15 min at room temperature. The samples were mounted on microscope slides with mounting medium and analyzed by confocal microscopy.

*4.2.5. Tubulin polymerization assay*

An *in vitro* assay for monitoring the time-dependent polymerization of tubulin to microtubules was performed. Bovine brain tubulin (>97% pure tubulin) was suspended with 10 ml of G-PEM buffer (80 mM PIPES, 2 mg $MgCl_2$, 0.5 mM EGTA, 1.0 mM GTP, pH 6.9) in 0.1% DMSO at 4 □, with and without test compound, using the HTS-Tubulin Polymerization Assay Kit (Cat. BK004P), according to the manufacturer's protocol (Cytoskeleton, Inc., Denver, CO, USA). The polymerization of tubulin was measured by the change in absorbance at 340 nm every 1 min for 1 h using a spectrophotometer (Thermo Fisher Scientific, Inc., USA) in a stable environment at 37 □.

*4.3. 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, and the energy was minimized with the Amber force field and Amber charges [41]. Docking marchantin C into the colchicine 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. The 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 marchantin C 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 marchantin C and a surrounding 6 Å sphere were allowed to move, while the structures of the remaining proteins 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. The molecular modeling of compound **88** was performed in the same way as described for marchantin C.

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**List of captions:**

**Fig. 1.** Proposed binding mode of marchantin C as green stick model in the colchicine binding site (PDB code 1SA0). The native ligand, DAMA-colchicine, is shown in magenta thin wire model. Hydrogen bonds are shown as dotted red lines, and the distance between ligand and protein is less than 3 Å. Molecular modeling was perform by GLOD software.

**Fig. 2.** Modification strategy for marchantin C analogues.

**Fig. 3.** Proposed binding mode of compound **88** as green stick model in the colchicine binding site (PDB code 1SA0). Hydrogen bonds are shown as dotted red lines, and the distance between ligand and protein is less than 3 Å.

**Fig. 4.** HCC1428 cells were treated with compound **88** as indicated. Cells were then plated 2000 cells/well into E-plate 16 and analyzed using a xCELLigence RTCA DP instrument. Results are representatives of three independent experiments.

**Fig. 5.** Compound **88** induced HCC1428 cells cycle arrest at $G_2$/M phase. HCC1428 cells were treated with 3μM and 6μM of compound **88** 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.

**Fig. 6.** HCC1428 cells were treated with compound **88** as indicated for 24 h and then fixed and immunostained with monoclonal anti-α-tubulin antibody (red) and DAPI (blue). One micromolar of Vincristine (VCR) and same amount of DMSO were used as controls. Results are representatives of three independent experiments. Bar = 10 μm.

**Fig. 7.** Effect of compound **88** on tubulin polymerization *in vitro*. Purified bovine brain tubulin was incubated in the presence of Taxol, VCR, DMSO (control), and compound **88** under the indicated concentrations at 37℃, and absorbance readings were recorded every minute for 1 h.

**Scheme 1.** Synthesis of compounds **53**-**57**. *Reagents and conditions*: (a) CuO, $K_2CO_3$, Py, reflux, (yields 65%-71%); (b) $NaBH_4$, THF, 0 ℃ to r.t. (yields 88%-91%); (c) $PPh_3HBr$, MeCN, reflux, (yields 86%-90%); (d) i. $K_2CO_3$, 18-crown-6, DCM, reflux; ii. Pd/C (10%), $H_2$, $Et_3N$, EtOAc, r.t. (yields 74%-82%, two steps); (e) $LiAlH_4$, THF, -40 ℃ to rt. (yields 83%-88%); (f) HCl/THF (1:1), r.t. (yields 87%-91%); (g) NaOMe, DCM, r.t. (yields 85%-92%); (h) Pd/C (10%), $H_2$, EtOAc, r.t. (yields 94%-98%); (i) $BBr_3$, DCM, -40 ℃ to r.t. (yields 86%-91%).

**Scheme 2.** Synthesis of compounds **86**-**87**. *Reagents and conditions*: (a) CuO, $K_2CO_3$, Py, reflux, (yields 70%-74%); (b) $NaBH_4$, THF, 0 ℃ to r.t. (yields 90%-96%); (c) $PPh_3HBr$, MeCN, reflux, (yields 86%-90%); (d) 2,3-Dihydropyran, *p*-toluenesulfonic acid, DCM, (yield 89%); (e) i. $K_2CO_3$, 18-crown-6, DCM, reflux; ii. Pd/C (10%), $H_2$, $Et_3N$, EtOAc, r.t. (yields 85%-88%, two steps); (f) HCl/THF (1:1), r.t.; (g) NaOMe, DCM, r.t. (yields 80%-81%); (h) Pd/C (10%), $H_2$, EtOAc, r.t. (yields 90%-93%); (i) $BBr_3$, DCM, -40 ℃ to r.t. (yields 71%-76%).

**Scheme 3.** Synthesis of compounds **88**-**89**. *Reagents and conditions*: (a) $BBr_3$, DCM, –78 ℃ to r.t. (yields 16%-34%).

**Scheme 4.** Synthesis of compounds **90**-**95**. *Reagents and conditions*: (a) HBr, DMSO, EtOAc, (overall yield 84%).

**Table 1**

In Vitro Cytotoxicity of marchantin C analogues in five cancer cell lines

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**Highlights**

- Novel marchantin C derivatives were synthesized and evaluated as anticancer agents
- Derivatives showed improved anticancer activity compared to positive controls
- Derivatives were also effective in multidrug-resistant cancer cell line
- Structure-activity relationship was discussed
- The anticancer mechanism could be attributed to the inhibition of tubulin polymerization

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