Total Synthesis of Arctigenin Derivatives as Potential Anticancer Agents

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Abstract: Mitotic spindle dynamics represents an important target of anticancer drugs. Many small natural and semi-synthetic drugs influence tubulin assembly and cause apoptosis of excessively dividing cells. Molecules attacking the colchicine domain of tubulin block its polymerization. Despite this there are colchicine site inhibitors (CSIs) not used in cancer treatment due to their high toxicity, low activity or poor solubility. On the basis of the structure-activity relationships, aryltetralin and dibenzylbutyrolactone lignan-like molecules were designed in an attempt to obtain active drugs. Structure modifications embraced the elimination of typically toxic groups. Two new dibenzylbutyrolactone-type compounds were prepared by total synthesis from substituted benzaldehyde, benzyl bromide and dimethyl succinate. The Stobbe condensation of the substituted benzaldehyde with dimethyl succinate followed with the selective hydrogenation of the double bond and the selective reduction of the ester in the presence of carboxyl led to the β-benzylbutyrolactone. Alkylation of butyrolactone enolate with a substituted benzyl bromide provided the final 2-hydroxy-5'-methoxy-4'-*O*-methyl arctigenin after phenolic group deprotection. The target 2,5'-dimethoxy-4'-*O*-methyl arctigenin was obtained by means of one-step methylation of dibenzylbutyrolactone derivative.

Keywords: dibenzylbutyrolactones; microtubule polymerization inhibitors; Stobbe condensation; catalytic hydrogenation; selective reduction

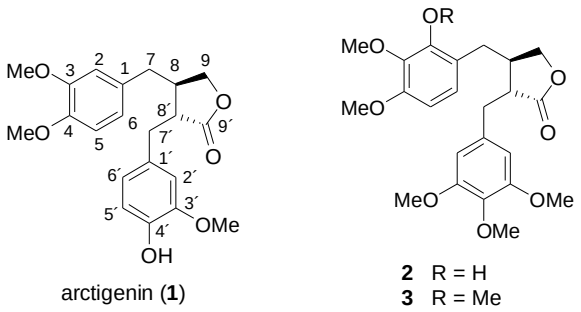
INTRODUCTION

Tubulin is a polymeric protein formed by structural subunits called α- and β-heterodimers. It creates microtubules that are arranged into a more complex structure of the cytoskeleton. Microtubules of mitotic spindle have uniquely rapid dynamics, which are necessary for correct cell division. Thus, tubulin became one of the principal targets of several drugs and inhibitors of tubulin dynamics [1].

There are three main binding sites on the tubulin known as the colchicine binding site, the *Vinca* domain and the taxane site (sometimes also named as the paclitaxel site) [2]. While drugs that bind to the *Vinca* and taxane sites have well-established roles in the treatment of human cancers, the therapeutic potential of the colchicine site has yet to be put into effect [3]. Large molecular diversities among colchicine site inhibitors are favourable for drug design and offer an area for structural modifications leading to desired properties [4].

The two derivatives of natural non-toxic compound arctigenin (**1**), 2-hydroxy-5'-methoxy-4'-*O*-methyl arctigenin (**2**) and 2,5'-dimethoxy-4'-*O*-methyl arctigenin (**3**), were rationally designed in accordance with knowledge of structure-activity relationships (SAR), see Fig. 1, and in attempt to introduce molecular features which are supposed to enable interaction with

the colchicine binding site of tubulin and/or to help to reveal the unclear influence of some structure changes on the activity and toxicity [2].
A modified procedure for the preparation of lignans *via* the Stobbe condensation and alkylation of lactone enolate was successfully applied in the total synthesis of the designed dibenzylbutyrolactones [2].



arctigenin (**1**)

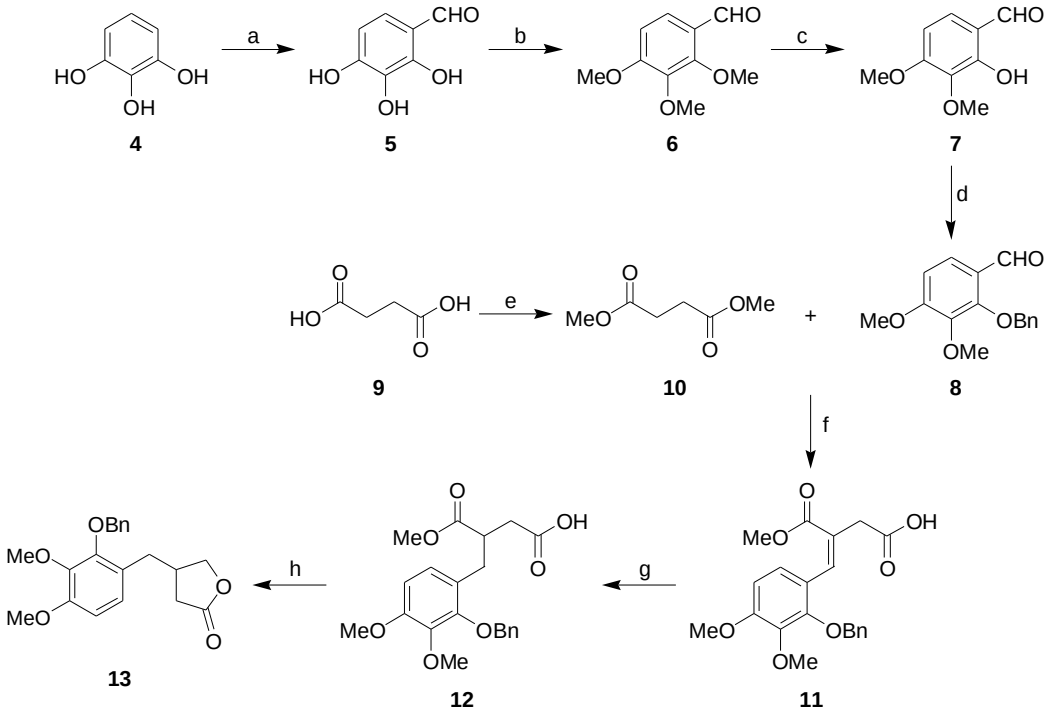
2 R = H
3 R = Me

Figure 1: Structures of arctigenin (**1**), 2-hydroxy-5'-methoxy-4'-*O*-methyl arctigenin (**2**) and 2,5'-dimethoxy-4'-*O*-methyl arctigenin (**3**).

RESULTS AND DISCUSSION

Several possible synthetic pathways for the preparation of dibenzylbutyrolactone structures can be found in literature [5]. In this case an optimized/modified approach used for total synthesis of natural lignans secoisolariciresinol or matairesinol was chosen [2]. This synthetic route is based on the Stobbe condensation of substituted benzaldehyde with dimethyl succinate leading to the monomethylester of substituted itaconic acid. After hydrogenation of the double bond and selective reduction of the ester group connected with formation of β-substituted γ-lactone, the enolate is generated and alkylated with substituted benzyl bromide [6], see Schemes 1 and 2. The final products were generated as racemic mixtures. Optimized synthetic pathways for the preparation of the required benzaldehyde and benzyl bromide were proposed [2].
First, pyrogallol **4** was formylated using triethyl orthoformate [7] and the prepared 2,3,4-trihydroxybenzaldehyde (**5**) was subsequently methylated with MeI to 2,3,4-trimethoxybenzaldehyde (**6**) [2]. Consequent demethylation of aldehyde **6** in the *o*-position using $AlCl_3$ as a Lewis acid [8] yielded 2-hydroxy-3,4-dimethoxybenzaldehyde (**7**) together with undesirable by-product 2,3-dihydroxy-4-methoxybenzaldehyde. By optimizing reaction time to six hours the undesirable di-demethylated product was eliminated [5].
The Stobbe condensation of benzaldehyde **7** with dimethyl succinate **10**, the ester of the starting succinic acid **9**, did not afford any product. In the series of experiments it was found that protection/masking of the *o*-phenolic group is crucial for reactivity. Due to electron-donating effect of *o*-phenolic moiety, the electrophilicity of carbonyl carbon decreases. Thus 2-benzyloxy-3,4-dimethoxybenzaldehyde (**8**) was prepared from compound **7** and benzyl chloride or benzyl bromide. Subsequently the mixture of MeOH, MeOLi and THF was used as a solvent medium in the Stobbe condensation due to the poor solubility of aldehyde **8** in the mixture of MeOH and MeOLi. The condensation of compound **8** with compound **10** required longer reaction time and vigorous stirring probably as a consequence of steric hindrance in the structure of substituted benzaldehyde **8** and the high viscosity of the reaction mixture, nevertheless 4-[2-(benzyloxy)-3,4-dimethoxyphenyl]-3-(methoxycarbonyl)but-3-enoic acid (**11**) was obtained in the yield of 92% [5]. The reaction product extraction described in

literature [6] led to the hydrolysis to the monomethyl ester of substituted itaconic acid **11** that was used for the next reaction step without further purification [2].
The double bond in compound **11** was hydrogenated to compound **12** under heterogeneous conditions using Pd/C. 2,2'-Bipyridine was chosen as a poison due to described high performance and compatibility with the system [9]. The catalytic poison was used to prevent deprotection of the benzyloxy group in itaconic acid derivate **11**. The hydrogenation proceeded selectively, but extremely slowly. Catalytic poison was removed, but the reaction was still very slow and selective. Interestingly, even hydrogenation of the new substrate **11** that was never in contact with poison was slow and selective. Thus, substrate **11** itself or residual dimethyl succinate used in excess in the Stobbe condensation can also be probably a catalytic poison and led to hydrogenation selectivity [2].
The selective reduction of the ester group in 4-[2-(benzyloxy)-3,4-dimethoxyphenyl]-3-(methoxycarbonyl)butanoic acid (**12**) and subsequent formation of β-substituted γ-butyrolactone **13** were performed with $NaBH_4$ in the presence of $CaCl_2$ and LiOH [2].

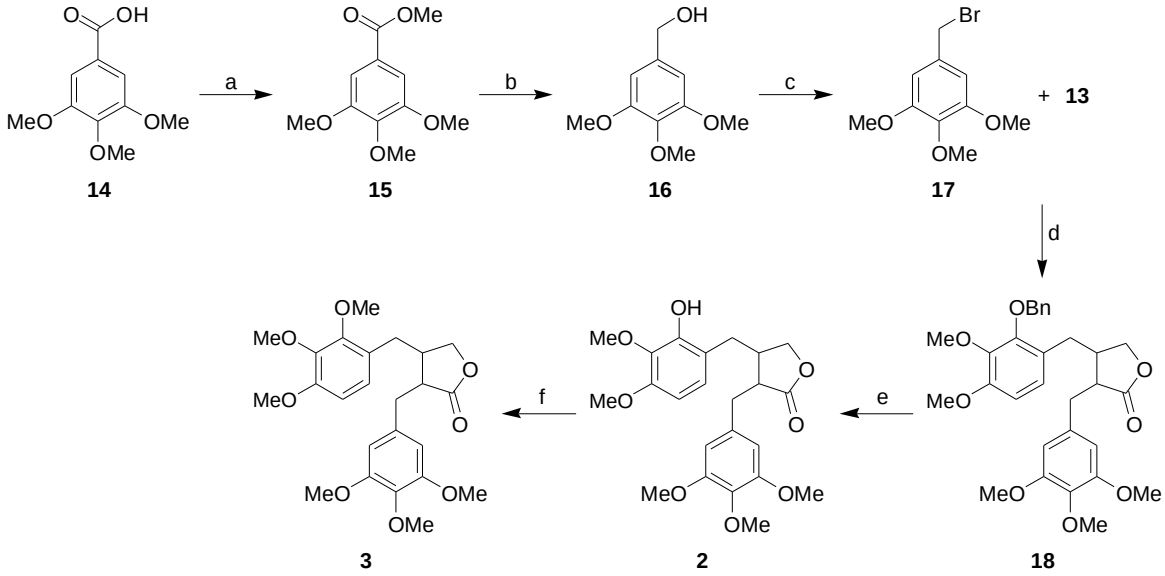


Scheme 1: Preparation of benzylbutyrolactone **13.** Reagents and conditions: (a) $AlCl_3$, $HC(OEt)_3$, EtOAc, ≤ 35 °C to RT, 0.5 h; (b) K_2CO_3, MeI, acetone, RT, 3 h, reflux, 3 h; (c) 1. $AlCl_3$, benzene, reflux, 6 h; 2. H_2O, HCl, 0 °C, 0.5 h; (d) K_2CO_3, BnBr, NaI, acetone, reflux, 24 h; (e) *p*-TsOH·H_2O, 2,2-dimethoxypropan, dry MeOH, reflux, 4.5 h; (f) 1. MeOH, MeOLi, THF, RT, 24 h; 2. HCl, ice; (g) H_2, 10% Pd/C, methanol, RT, 7 days; (h) 1. $NaBH_4$, LiOH, $CaCl_2$, EtOH, 0 °C to RT, 17 h; 2. brine, HCl.

A crucial step of total synthesis was the alkylation of the enolate anion formed from lactone **13** with 3,4,5-trimethoxybenzyl bromide (**17**). Bromide **17** was prepared from 3,4,5-trimethoxybenzoic acid (**14**) that was esterified to give product **15,** which was subsequently reduced with $LiAlH_4$ to yield benzyl alcohol **16**. Benzyl alcohol **16** was converted using an Appel-type reaction to benzyl bromide **17**.
Coupling of lactone **13** with benzyl bromide **17** was performed under the optimized Daugan and Brown [6] procedure conditions. Lithium bis(trimethylsilyl)amide (lithium

hexamethyldisilazide, LiHMDS) was used as a base that was generated *in situ* from bis(trimethylsilyl)amine (hexamethyldisilazane, HMDS) added to the solution of *n*-BuLi in THF and hexane. The steric demand of LiHMDS protects it from acting as a nucleophile. The reaction was carried out with optimization of the period between addition of lactone and benzyl bromide and the reaction time and temperature, and reaction afforded 2-benzyloxy-5'-methoxy-4'-*O*-methyl arctigenin (**18**) [2].

The last step of the synthesis of compound **2** was the deprotection of the phenolic group. The benzyl moiety was removed by the standard catalytic hydrogenation using Pd/C. The target compound **2** was obtained along the 13-step synthetic pathway. Methylation of derivative **2** with MeI yielded the final compound **3** along the 14-step synthetic pathway [2].



Scheme 2: Preparation of designed derivatives **2** and **3**. Reagents and conditions: (a) 1. MeOH, H_2SO_4, reflux, 4 h; 2. Na_2SO_4, H_2O; (b) 1. $LiAlH_4$, THF, RT, 45 min; 2. EtOAc, tartaric acid; (c) CBr_4, PPh_3, acetone, 0 °C, 12 h; (d) 1. *n*-BuLi, HMDS, THF/hexane, -78 °C to RT, 20 h; 2. HCl; (e) H_2, 5% Pd/C, MeOH, RT, 2 days; (f) MeI, K_2CO_3, acetone, RT, 2h, reflux, 2h.

EXPERIMENTAL

General

Chemicals were purchased form Sigma-Aldrich. Solvents (analytical grade) were purchased from Penta. The reactions were monitored and the purity of the products was checked by thin-layer chromatography (TLC) and gas chromatography (GC). TLC plates were coated with 0.2 mm silica gel 60 F_{254} (Merck, Darmstadt, Germany) and were visualized by UV irradiation (254 nm). For UV-invisible dimethyl succinate visualization was achieved with 10% phosphomolybdic acid in ethanol. GC/MS spectra were recorded on a Hawlett-Packard GCMS 5890/5971 (USA) equipped with a column J&W DB-5MS (59 m×0.25 mm; stationary phase 0.25 μm (5 % fenylmethylpolysiloxane) and quadrupole ionization detector HP 597 (ionization 70 eV). Gas chromatography was performed on a Shimadzu GC 2010 (Schimadzu, Japan) gas chromatograph equipped with a 14-m column (5% diphenyldimethylsiloxane) and a flame ionization detector. Melting points of final products **2** and **3** were determined on a Melting Point Apparatus Franz Künster Nacht. KG (Franz Künster Nacht. KG, Germany) and are uncorrected. The NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solutions at ambient

temperature on a Bruker Avance 300 MHz spectrometer (Bruker, Karlsruhe, Germany, 300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemicals shifts are reported in ppm (δ) to internal $Si(CH_3)_4$, when diffused easily exchangeable signals are omitted. Mass spectra of final products **2** and **3** were measured on a mass spectrometr Schimadzu GC-MS QP 2010 (Shimadzu, Japan) with direct sample inlet unit DI-2010.

Synthesis

2,3,4-Trihydroxybenzaldehyde (5) [7]. Anhydrous $AlCl_3$ (21.15 g, 0.158 mol) was dissolved in EtOAc (317 mL) under argon. After cooling of reaction mixture to 15 °C pyrogallol (**4**) (10.00 g, 0.079 mol) and subsequently $HC(OEt)_3$ (39.6 mL, 0.238 mol) were added. The mixture was stirred for 0.5 h, cooled to 10 °C and cooling 10% HCl (200 mL) was added to dissolve $AlCl_3$. The organic layer was separated and the aqueous layer was washed with Et_2O (3×200 mL). Combined organic layers were washed 3 times with brine, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The product was dried for 24 h at 30 °C under reduce pressure. This provided a light beige crystalline compound. Yield: 8.80 g (72%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 6.09 (d, *J*=8.53 Hz, 1H), 6.56 (d, *J*=8.52 Hz, 1H), 9.22 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 193.77, 152.74, 151.08, 131.86, 125.04, 114.52, 108.05.

2,3,4-Trimethoxybenzaldehyde (6) [5]. Aldehyde **5** (12.74 g, 0.083 mol) and dry K_2CO_3 (45.00 g, 0.326 mol) were stirred in acetone (105 mL) under argon, and then MeI (45.00 mL, 0.772 mol) was added. The mixture was vigorously stirred at room temperature for 3 h and then refluxed for additional 3 h. The suspension of K_2CO_3 was sonified every hour for 5 min. The residual MeI and acetone were removed under reduced pressure, and the product was extracted with Et_2O (3×75 mL). Combined organic layers were washed with 1M NaOH (1×75 mL) and then with brine (2×75 mL), dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. This provided a light beige crystalline compound. Yield: 13.07 g (80%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.75 (s, 3H), 3.81 (s, 3H), 3.90 (s, 3H), 6.64 (d, *J*=8.82, 1H), 7.45 (d, *J*=8.69, 1H), 10.09 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 188.65, 159.25, 156.73, 141.42, 124.06, 123.06, 107.35, 62.14, 60.75, 56.07.

2-Hydroxy-3,4-dimethoxybenzaldehyde (7) [2,8]. An anhydrous $AlCl_3$ (10.50 g, 0.079 mol) and benzaldehyde **6** (15.00 g, 0.076 mol) were dissolved in dry benzene (150 mL) and vigorously stirred under argon. The mixture was refluxed for 7 h and then cooling HCl was added. The mixture was extracted with Et_2O (2×400 mL). The combined organic layers were washed twice with water. To remove the residual starting compound, the solution was washed with 1M NaOH (100 mL). The basic solution was immediately cooled and acidified with 17% HCl to pH 1 and washed with Et_2O (3×300 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated under reduced pressure. This provided a light brown crystalline compound. Yield: 10.90 g (78%). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 3.73 (s, 3H), 3.90 (s, 3H), 6.77 (d, *J*=8.82, 1H), 7.48 (d, *J*=8.80, 1H), 10.03 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 191.86, 158.66, 154.34, 135.69, 126.92, 117.14, 104.44, 60.10, 56.09.

2-Benzyloxy-3,4-dimethoxybenzaldehyde (8). Aldehyde **7** (10.72 g, 0.059 mol), K_2CO_3 (8.37 g, 0.061 mol), benzyl bromide (7.14 mL, 0.060 mol) and NaI (0.66 g, 0.004 mol) were refluxed in acetone (60 mL) and vigorously stirred under argon for 24 h. Then solvent was removed under reduced pressure, and the mixture was extracted with Et_2O (3×60 mL). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. This provided a pink oil. Final product **8** was crystallized (petroleum ether/EtOAc 2:1). The procedure gave product **8** as a yellow crystalline

compound. Yield: 15.57 g (97%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.68 (s, 3H), 3.71 (s, 3H), 5.01 (s, 2H), 6.54 (d, *J*=8.77, 1H), 7.13-7.39 (m, 6H), 9.94 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 187.63, 158.69, 154.79, 141.31, 136.17, 127.93, 127.80, 123.26, 107.31, 75.91, 60.17, 55.43.

Dimethyl succinate (10). Succinic acid (**9**) (47.00 g, 0.398 mol) and *p*-toluensulphonic acid monohydrate (0.60 g) were dissolved in anhydrous MeOH (60 mL) and 2,2-dimethoxypropan (83.20 g, 0.799 mol). The mixture was refluxed under argon for 4.5 h. Organic solvents were evaporated under reduced pressure. The product was dissolved in CH_2Cl_2 (50 mL) and washed twice with saturated aqueous $NaHCO_3$. Organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. This provided a colourless oil. Yield: 52.50 g (90%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.70 (s, 6H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 172.67, 51.71, 28.76.

4-[2-(Benzyloxy)-3,4-dimethoxyphenyl]-3-(methoxycarbonyl)but-3-enoic acid (11) [5,6]. In a flask equipped with condenser and filled with argon, the Li (1.131 g, 0.163 mol) was vigorously stirred and MeOH (39 mL) was dropwise added. If necessary (larger amounts), the mixture was cooled. After complete formation of MeOLi, 2-benzyloxy-3,4-dimethoxybenzaldehyde (**8**) (16.874 g, 0.062 mol) dissolved in dimethyl succinate (**10**) (13.323 g, 0.091 mol) and diluted with dry THF (10 mL) was slowly added. The mixture was stirred at room temperature for three days. Cooling 17% HCl (100 mL) was slowly added and mixture was extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were washed with brine, acidified with a drop of H_2SO_4, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was obtained as an orange-red viscous oil, contained 22.14 g (92%) of compound **11** and was used directly in the next step. ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.54, 175.77, 167.71, 154.68, 151.02, 142.40, 138.41, 136.84, 128.56, 128.23, 128.12, 124.27, 124.00, 122.23, 107.53, 75.66, 60.70, 55.81, 51.89, 33.49.

3-(2-Benzyloxy-3,4-dimethoxybenzyl)-4-methoxy-4-oxobutanoic acid (12) [2]. The crude mixture of acid **11** and dimethyl succinate (15.00 g of mixture, calc. 12.73 g, 33 mmol of acid **11**) was stirred with 10% Pd/C (1.85 g) in MeOH (450 mL) under hydrogen atmosphere for 7 days. The mixture was filtrated and MeOH was evaporated under reduced pressure. The procedure gave mixture of acid **12** and dimethyl succinate (14.58 g, 97%) as a yellow-brown oil. Part of crude acid (3.30 g) **12** was purified by column chromatography on silica gel (EtOAc/hexan/AcOH 1:1:0.15). In this mobile phase compound **12** was separated from traces of hydrogenation by-products and starting acid **11**. Dimethyl succinate was removed on silica gel (EtOAc/hexan 1:3), and finally the product was rinsed from the column (EtOAc/hexane/acetic acid 1:1:0.15). This provided a light beige viscous oil. Yield: 2.01 g (71%). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.69, 174.39, 152.48, 150.52, 141.99, 137.28, 127.96, 127.79, 127.55, 124.46, 123.89, 107.15, 74.68, 60.22, 55.42, 51.29, 41.45, 34.47, 31.87.

4-[2-(Benzyloxy)-3,4-dimethoxybenzyl]dihydrofuran-2(3*H*)-one (13) [2]. Acid **12** (1.96 g, 5.05 mmol) was dissolved in EtOH (18 mL). $CaCl_2$ (0.616 g, 5.6 mmol) and subsequently LiOH (0.121 g, 5.05 mmol) were added. The mixture was cooled and $NaBH_4$ (0.76 g, 20.2 mmol) was added slowly. The mixture was vigorously stirred at RT for 17 h. Most of EtOH was removed under reduced pressure, and brine (13 mL) was slowly added followed by dropwise addition of concentrated HCl (9 mL). The mixture was extracted with CH_2Cl_2 (3×20 mL), dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure.

Pure lactone **13** was obtained as beige oil and used without purification in next step. Yield: 1.57 g (91%). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 176.97, 152.36, 150.23, 142.00, 137.30, 128.02, 127.75, 127.57, 124.23, 123.90, 107.25, 74.64, 72.36, 60.22, 55.43, 35.79, 33.63, 32.54.

Methyl-3,4,5-trimethoxybenzoate (15) [10]. 3,4,5-Trimethoxybenzoic acid (**14**) (20.00 g, 0.094 mol) was dissolved in anhydrous MeOH (70 mL), H_2SO_4 (1.8 mL, 0.035 mol) was added, and the mixture was refluxed under argon atmosphere for 4 h. The acid was neutralized with Na_2CO_3, MeOH was evaporated under reduced pressure and water was added. The mixture was extracted with Et_2O (2×70 mL) and organic extract was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. This provided a yellowish crystalline compound. Yield: 20.20 g (95%). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 3.77 (s, 3H), 3.88 (s, 6H), 3.89 (s, 3H), 7.28 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 165.75, 152.68, 141.71, 124.62, 106.41, 60.05, 55.92, 52.08.

3,4,5-Trimethoxybenzyl alcohol (16) [5]. Ester **15** (16.00 g, 0.071 mol) was dissolved in dry THF (42 mL) and dropwise added to the vigorously stirred suspension of $LiAlH_4$ (1.48 g, 0.028 mol) in dry THF (45 mL). The mixture was stirred for 45 min. Excess $LiAlH_4$ was eliminated by careful addition of EtOAc (10 mL). Most of solvents were evaporated and saturated solution of tartaric acid was added to dissolve aluminium salts. The mixture stayed overnight, and the product was then extracted with CH_2Cl_2 (3×40 mL). Organic extract was twice washed with water and evaporated under reduced pressure. This provided an orange oil. Yield: 13.75 g (98%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.72 (s, 3H), 3.73 (s, 6H), 4.50 (s, 2H), 6.48 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ:153.25, 137.15, 136.92, 103.85, 65.15, 60.82, 56.05.

3,4,5-Trimethoxybenzyl bromide (17) [11]. Alcohol **16** (11.54 g, 0.058 mol) was dissolved in acetone (206 mL). The solution was cooled to 0 °C, and CBr_4 (26.59 g, 0.080 mol) and triphenylphosphine (20.00 g, 0.076 mol) were added. The mixture was vigorously stirred and cooled for 12 h. Most of acetone was removed at laboratory temperature under reduced pressure. The mixture was frozen, and the necessary amount of the formed unstable 3,4,5-trimethoxybenzyl bromide (**17**) was purified by flash chromatography (CH_2Cl_2/hexan 2:1) directly before its use in the reactions. This provided a white crystalline compound. Yield of purifications: >90%. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.84 (s, 3H), 3.87 (s, 6H), 4.59 (s, 2H), 6.62 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 153.52, 138.48, 133.36, 106.43, 61.04, 56.36, 34.42.

2-Benzyloxy-5'-methoxy-4'-*O*-methyl arctigenin (18) [2,6]. Solution of *n*-BuLi in hexane (2.5 M, 2.39 mL, 6.0 mmol) was mixed under argon with dry THF (4 mL). The mixture was stirred and cooled to -78 °C, while HMDS (0.96 mL, 4.6 mmol) was slowly added (without immersing of syringe to the cooled solution of THF, since there is risk of freeze of solution inside the syringe). The reaction mixture was stirred for 5 min at -40 °C. After restoration of temperature to -78 °C substituted γ-butyrolactone **13** (1.57 g, 4.6 mmol) in dry THF (1.5 mL) was added, and the mixture was stirred for 10 min. Bromide **17** (1.20 g, 4.6 mmol) in dry THF (1.5 mL) was added, and the mixture was stirred at room temperature overnight. 1M HCl (12 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×17 mL), washed with distilled water, dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. Conversion of starting lactone **13** to product **18** was 50% according to GC-MS, and the crude product (2.18 g) as a brown oil was used in the next step without purification. ^{13}C NMR (75 MHz, $CDCl_3$), δ: 178.45, 152.83, 152.70, 150.27, 142.02, 137.15, 136.39, 132.88, 128.05,

127.95, 127.77, 124.24, 123.96, 107.18, 105.95, 74.78, 70.86, 60.26, 60.18, 55.59, 55.54, 46.18, 39.53, 33.89, 32.16.

2-Hydroxy-5'-methoxy-4'-*O*-methyl arctigenin (2) [2]. Crude mixture (2.18 g) containing dibenzylbutyrolactone **18** was stirred with 5% Pd/C (0.22 g, 10% m/m) in MeOH (40 mL) under hydrogen atmosphere for 2 days. After the conversion was complete, the mixture was filtered, and MeOH was evaporated under reduced pressure. Final product **2** was crystallized (EtOAc/hexane 1:1). The procedure gave 2-hydroxy-5'-methoxy-4'-*O*-methyl arctigenin (**2**) as white crystals. Yield: 0.77 g, (76%). Mp 175-177 °C. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.40-2.96 (m, 6H), 3.69-3.81 (m, 12H), 3.82 (s, 3H), 3.96-4.13 (m, 2H), 6.33 (m, 3H), 6.53 (d, *J*=8.00 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 179.07, 153.34, 151.39, 147.60, 137.02, 135.73, 133.74, 124.87, 117.30, 106.77, 103.84, 71.56, 61.11, 61.01, 56.29, 56.00, 46.89, 39.90, 35.12, 32.29. MS for $C_{23}H_{28}O_8$ (EI, 70 eV): *m/z* 432, 265, 233, 219, 189, 181, 167, 151, 136, 106, 91, 77, 55.

2,5'-Dimethoxy-4'-*O*-methyl arctigenin (3) [2]. A solution of dibenzylbutyrolactone **2** (9.7 mg, 0.022 mmol) in acetone (1 mL) was stirred with MeI (120 μl, excess) in the presence of K_2CO_3 (5.7 mg, 0.041 mmol) for 2 h, and the mixture was further refluxed with an additional MeI (120 μl, excess) for 2 h. The solvent and residual MeI were evaporated under reduced pressure, and $CHCl_3$ was added to the solid residue. The organic phase was washed with water, dried over anhydrous $MgSO_4$ and filtered. The organic extract was evaporated under reduced pressure. This provided a yellowish oil. Yield: 9.5 mg (95%) The dibenzylbutyrolactone **3** was dissolved in EtOAc/hexane (3:2) when heated. The solution was cooled and hexane was added. This provided a white crystalline compound. Yield: 5.6 mg (59%). Mp. 153-156 °C $^{.1}$H NMR (200 MHz, $CDCl_3$), δ: 2.43-3.00 (m, 6H), 3.79-3.86 (m, 15H), 3.89 (s, 3H) 4.07-4.22 (m, 2H), 6.40 (m, 3H), 6.61 (d, *J*=8.64 Hz, 1H). MS for $C_{24}H_{30}O_8$ (EI, 70 eV): *m/z* 446, 432, 265, 251, 219, 207, 181, 167, 151, 136, 106, 91, 77, 53.

CONCLUSION

The designed structures 2-hydroxy-5'-methoxy-4'-*O*-methyl arctigenin (**2**) and 2,5'-dimethoxy-4'-*O*-methyl arctigenin (**3**) were prepared along the 13-step and 14-step synthetic pathway respectively from pyrogallol, succinic acid and 3,4,5-trimethoxybenzoic acid. Their activity as inhibitors of microtubule polymerization was evaluated in a preliminary *in vitro* study. The compounds will be further investigated.

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