Tetrahedron 67 (2011) 6166–6172

Contents lists available at ScienceDirect

Tetrahedron

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

Total synthesis of moniliformediquinone and calanquinone A as potent inhibitors for breast cancer

Shankar Thangaraj [a], Wen-Shing Tsao [a], Yi-Wei Luo [a], Yean-Jang Lee [a,*], Chia-Fu Chang [b], Chun-Cheng Lin [b], Biing-Jiun Uang [b], Chia-Chun Yu [c], Jih-Hwa Guh [c], Che-Ming Teng [d]

[a] Department of Chemistry, National Changhua University of Education, 1 Ching-Der Rd., Changhua 50058, Taiwan
[b] Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan
[c] School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan
[d] Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 17 June 2011
Accepted 21 June 2011
Available online 25 June 2011

Dedicated to my father Ching-Chi Lee on the occasion of his 92nd birthday

Keywords:
Moniliformediquinone
Calanquinone A
Denbinobin
Moscatilin
$TiCl_4$-mediated

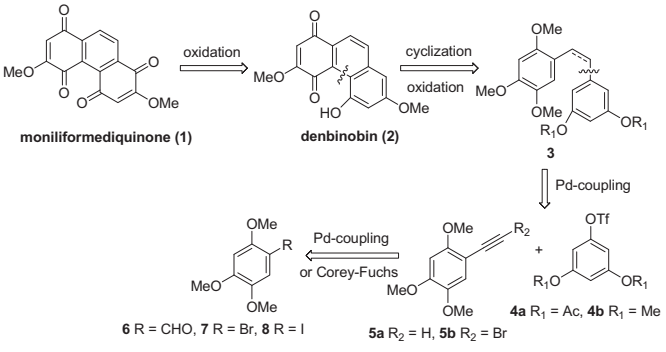
ABSTRACT

The first synthesis of moniliformediquinone has been achieved in which the longest linear sequence is only nine steps. The synthesis proceeds in 23% overall yield from commercially available 2,4,5-trimethoxybenzaldehyde. The key transformations include a Pd-catalyzed coupling between a phenyl triflate and an acetylene, and a $TiCl_4$-mediated cyclization of a benzoquinone intermediate. In addition, in vitro inhibitory effects of moniliformediquinone, denbinobin, moscatilin, and calanquinone A were determined to have IC_{50} values of 0.7, 1.6, 2.5, and 1.5 μM, respectively.

1. Introduction

Plants of the *Dendrobium* genus (Orchidaceae) are used in traditional herbal medicines in Asia, and studies have shown that they contain a wide variety of medicinal properties.[1] In 2001, Chang et al. collected *Dendrobium moniliforme* (L.) Sw. in Taiwan and isolated moniliformediquinone (**1**) and denbinobin (**2**) from the stems.[1a] The structures of moniliformediquinone and denbinobin were determined to be **1** and **2** by examination of IR, UV, NMR, and high-resolution mass spectra (Scheme 1). Recently, denbinobins' biological functions have been intensively studied and found to involve anticancer, anti-HIV type-1 replication, anti-inflammatory, and antioxidant effects.[2]

Perhaps the most direct route to denbinobin (**2**) would use a Diels–Alder reaction between an appropriate styrene and benzoquinone to construct the phenanthraquinone core.[3] Unfortunately, this approach was not successful. Kraus et al.[4] have employed a variety of methodologies to synthesize the denbinobins. For instance, the phosphazene base P_4-^{t}Bu-mediated cyclization of aldehydes and toluenes was achieved to give the key phenanthrenes. A concise synthesis of denbinobin was accomplished by Liou et al.[5] via an intramolecular free radical cyclization of a stilbene intermediate. However, the starting materials were not readily available, which complicated synthetic approaches. A synthesis of



Scheme 1. Retrosynthetic analysis of moniliformediquinone (**1**) and denbinobin (**2**).

* Corresponding author. Tel.: +886 4 7232105x3511; fax: +886 4 7211190; e-mail address: leeyj@cc.ncue.edu.tw (Y.-J. Lee).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.06.054

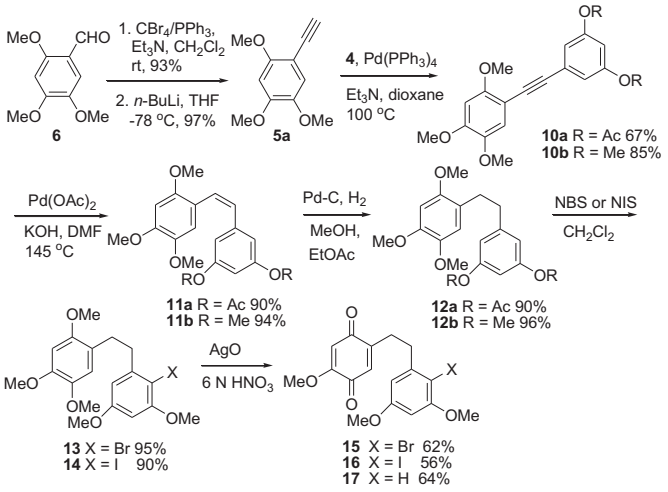
the related calanquinone has also been described,[6] but a synthesis of moniliformediquinone has not been reported. Consequently, there is no information available about moniliformediquinone's behavior in vitro and in vivo. Since moniliformediquinone is the first natural product proposed to possess a phenanthradiquinone core, we undertook the synthesis of moniliformediquinone to confirm its overall structural assignment and to investigate its biological activity. We also sought to examine the in vitro/in vivo anti-tumor activity of denbinobin. In addition, we wanted to examine the binding affinity of protein–ligand complexes by using an automated docking method[7] that has been used for drug lead discovery.

Retrosynthetic analysis (Scheme 1) suggested that the phenanthradiquinone moiety of moniliformediquinone (**1**) could come from the free hydroxyl group induced *para*-oxidation of denbinobin (**2**). The phenanthraquinone core structure of **2** was envisioned to arise from either the sequential intramolecular-cyclization/oxidation or oxidation/cyclization of the corresponding *cis*-stilbene **3**, which could potentially be attained from a Pd-catalyzed cross coupling reaction between aryl acetylene **5** and phenyl triflate **4**. Alternatively, the phenanthracenes can be obtained with photocyclization and the oxidation of *cis*-stilbene **3**.[3,8] In contrast to photocyclizing stilbene **3** in the presence of an oxidant, the 9,10-dihydrophenanthracene moiety can also be formed by direct intramolecular arylation from a brominated dihydrostilbene derivative.[9] Although stilbene **3** has been synthesized by Eling[10] and Kim,[11] the synthesis was time-consuming and complicated. To overcome these technical difficulties and the need for expensive starting materials we report our study on the synthesis of the key stilbene **3**, which was accessed by a Pd-catalyzed coupling between the readily available phenyl triflate **4** and substituted benzenes **6**–**8** (Scheme 1).

2. Results and discussion

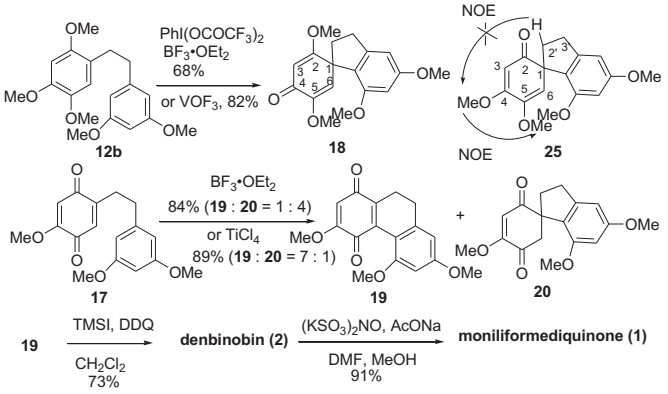
The commercial availability of phloroglucinol prompted us to prepare the substituted phenyl triflate **4** from this compound. It was anticipated that **11** could smoothly be prepared as shown in Scheme 2. A Corey–Fuchs reaction converted benzaldehyde **6** to its corresponding phenylacetylene **5a** in 90% yield. In the case of **4** and **5a**, a Sonogashira coupling with $Pd(PPh_3)_4$ in Et_3N and dioxane at 100 °C under N_2 pressure gave acetylene **10b** in 85% yield. It is worth noting that with $Pd(PPh_3)_4$ 2 equiv of **5a** were required to yield the desired cross-coupled product **10b** and to avoid forming the homocoupled diphenylbutadiyne. With **10b** in hand, reduction with Lindlar's catalyst[12] in MeOH/EtOAc under 50 psi H_2 generated the partially reduced *cis*-stilbene **11b**, albeit with low conversion. As an alternative, we used a Pd-catalyzed semihydrogenation reduction[13] in DMF/KOH to effect the highly chemo- and stereoselective formation of *cis*-stilbene **11b** in 94% yield. At this point, the key step of the synthesis was the cyclization reaction of **11b**; however, treatment with $h\nu/I_2$,[3,8] $PhI(OCOCF_3)_2/BF_3\cdot OEt_2$,[14] or VOF_3[15] did not successfully form the desired phenanthrene product. Instead, the major product was *trans*-stilbene, which arose from *cis-trans* isomerization to form the more stable product. Consequently, we sought to manipulate the reduction of **11b** in a separate step. We found that **11a** and **11b** reacted smoothly with $Pd-C/H_2$ to give 90% and 96% yields of **12a** and **12b**, respectively. In addition, dihydrostilbenes **13** and **14** and benzoquinones **15**–**17** were synthesized to optimize the arylation reactions with Pd-catalysis or $AIBN/Bu_4SnH$ (Scheme 2).

With **12b** in hand, we next followed the procedures of Kita[14] and Wang.[15a] The substrate underwent an oxidative biaryl coupling reaction to give spirodienone **18** in 68% yield. The reaction of an electron-rich aromatic ring with the reagent $PhI(OCOCF_3)_2/BF_3\cdot EtO_2$ results in the formation of a cation radical intermediate.[14] The subsequent nucleophilic attack by the other aromatic ring on



Scheme 2. Preparation of *cis*-stilbenes, dihydrostilbenes, and phenethylbenzoquinones.

the cation radical occurs to provide spiro **18** instead of the fused product. In addition, **18** was determined to be the *para*-spirodienone not the *ortho*-spirodienone **25**. This assignment was made from the significant NOE observed between H-2′ and CH_3-2 in the NOESY spectrum of **18**. In contrast, no NOE was observed between CH_3-2 and CH_3-5, whereas it was observed in the *ortho*-spirodienone **25**. In the case of **13**–**16**, we attributed our failure to achieve the desired fused-cyclization product with Pd-catalysis[9] or $AIBN/Bu_4SnH$[5] due to the 2- and 4-methoxy groups and the electron-withdrawing nature of the conjugated ketones. We believe that these substituents reduced the reactivity of both the Heck and free radical reactions. In all cases, these reactions led to the isolation of only the debrominated product and recovered starting material. Inspired by work from Kraus et al.,[4a] we investigated the possibility of using $SnCl_4$ to effect the cyclization (Scheme 3).



Scheme 3. Synthesis of moniliformediquinone and denbinobin.

We evaluated the intramolecular Lewis acid-mediated cyclization with **17** and $SnCl_4$ under a range of reaction conditions; however, these conditions only resulted in decomposition and the isolation of recovered starting material. When **17** was treated with $BF_3\cdot OEt_2$, it was converted to 1,4-phenanthraquinone **19** and spirodiketone **20** in 17% and 67% yield, respectively. It is interesting to note that the Michael addition to **20** was regioselective. The regiochemical assignments of **20** were based on characteristic spectroscopic signatures and X-ray crystallographic analysis.[16] To our delight, **17** underwent a reaction with $TiCl_4$ to provide the desired product **19** in 78% yield and **20** in 11% yield, respectively. We

propose that this reaction involves the kinetic chelation of $TiCl_4$ by the carbonyl group, followed by Michael addition to the electron-deficient carbon center in benzoquinone **17**, based upon the yield of **19** was decreased at high concentration and temperature. It is notable that we could not achieve the cyclization of **17** to **19** with many other Lewis acids, including $SnCl_2$, BCl_3, $Sc(OTf)_3$, $ZrCl_4$, $AlCl_3$, $CeCl_3$, and $ZnCl_2$. At this point, we do not know the detailed mechanism of the cyclization reaction with $BF_3\cdot OEt_2$ and $TiCl_4$. The selective demethylation[3] and subsequent oxidation of **19** with TMSI and DDQ afforded denbinobin (**2**) in 73% yield, which was then oxidized with Fremy's salt to afford the natural product, moniliformediquinone (**1**), in 91% yield. HRMS, ^1H, and ^{13}C NMR spectra of the synthesized products **1** and **2** are in agreement with those reported for the naturally derived materials.[1a] It is worth noting that our synthetic strategy can also be employed in the syntheses of the naturally occurring moscatilin (**21**) and calanquinone A (**22**) in 51% and 14% overall yield, respectively, from the corresponding benzaldehydes (Fig. 1).

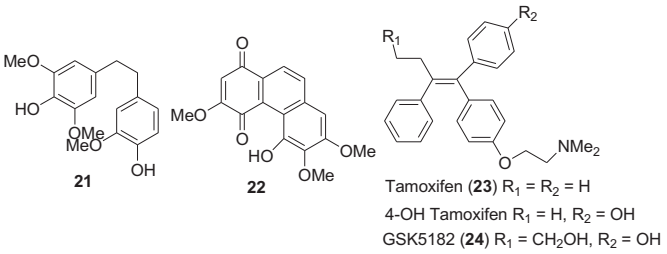


Fig. 1. The structures of moscatilin (**21**), calanquinone A (**22**), and tamoxifens.

Tamoxifen (**23**) has been used for more than 30 years to treat breast cancer in women and men, but clinical trials have shown that it has serious side effects, including blood clots, strokes, uterine cancer, and cataracts.[17] Zuercher et al.[18] have reported that the tamoxifen analogue GSK5182 (**24**) can selectively bind to the orphan estrogen-related receptor ERRγ. We sought to probe the accessibility of the ERRγ receptors in MDA-MB-231 and MCF breast tumor cells. To this end, the binding affinity of protein–ligand complexes was first examined by an automated docking method[7] (Table 1). The results showed that **1** and **2** bind to the receptor ERRγ, thus, these compounds could be potential inhibitors of breast cancer similar to GSK5812 (**24**). Accordingly, the in vitro inhibitory effects of moniliformediquinone (**1**), denbinobin (**2**), moscatilin (**21**), and calanquinone A (**22**) were examined with the SRB method, and IC_{50} values of 0.7, 1.6, 2.5, and 1.5 μM were obtained, respectively. In addition, Wu and Lee have reported[6] that calanquinone A (**22**), which was isolated from *Calanthe arissanensis*, showed potent cytotoxicity ($EC_{50}<0.5$ μg/mL) against prostate (PC-3 and DU145) and breast (MCF7) cancer cell lines. It also showed an improved drug resistance profile compared with paclitaxel.

3. Conclusion

In summary, the first total synthesis of moniliformediquinone (**1**) has been achieved; the longest linear sequence is only nine steps. The synthesis proceeds in 23% overall yield from commercially available trimethoxybenzaldehyde. Although the rationale for the intramolecular Lewis acid-mediated cyclization is unclear, a concise route to three natural products (**2**, **21**, and **22**) has been accomplished in 25%, 51%, and 14% overall yield, respectively. Moreover, our results demonstrate the usefulness of automated docking methods for the discovery of drug leads. Consequently, the rationally optimized and designed denbinobin dimer (Binding energy: K_i −9.47 kcal/mol: 113 nM) was identified as a potentially important inhibitor of the ERRγ receptor. Furthermore, **1**, **2**, **21**, and **22** exhibited strong anti-tumor effects in cancer cells. Large-scale preparations of these compounds are currently underway, and their biological activities will be investigated so that their efficacy as anti-tumor agents can be evaluated.

4. Experimental section

4.1. General details

Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets with an FTIR spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained using a 300 MHz spectrometer. COSY, HMQC, and NOE spectra were provided by a 600 MHz spectrometer. Chemical shifts are reported in parts per million (δ, ppm) using $CHCl_3$ (δ_H 7.26) as an internal standard. Solvents were freshly distilled prior to use from phosphorus pentoxide or CaH_2; THF was distilled from sodium diphenyl ketyl. All reactions were carried out under a nitrogen atmosphere unless otherwise stated. Silica gel (230–400 mesh) was used for chromatography. Organic extracts were dried over anhydrous $MgSO_4$.

*4.1.1. 3,5-Dimethoxyphenyl triflate (**4**)[19].* A mixture of 3,5-dimethoxyphenol (1.00 g, 6.5 mmol) and Et_3N (10.0 mL) was dissolved in CH_2Cl_2 (20.0 mL) for 5 min at room temperature, and followed by addition of $(CF_3SO_2)_2O$ (1.1 mL, 6.5 mmol) dropwise at −78 °C. The solution was stirred at −78 °C for 10 min, and then the solution was warmed to room temperature. After stirring for 20 min the reaction was quenched with water (10.0 mL), the solution was poured into ice water (50.0 mL) and extracted with CH_2Cl_2 (3×50.0 mL); the combined extracts were washed with saturated aqueous $NaHCO_3$ (5.0 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/EtOAc 17/1) to yield 3,5-dimethoxyphenyl triflate (**4**) (1.73 g, 93%) as a colorless liquid. ^1H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 6H), 6.41 (s, 2H), 6.44 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6, 99.9, 100.2, 118.7 (q, J=318.8 Hz, CF_3),

Table 1
Compound K_i values and the lowest binding energies from AutoDock Lamarckian Genetic Algorithm

Ligand	Protein (PDB entry) binding energy (kcal/mol)/K_i (μM)			
	Transcription (ERRγ) (2EWP)	Nuclear receptor (ERα) (3ERT)	Gene regulation (1KV6)	Mg^{2+} transport (Tubulin) (2HXF)
Taxol	23.21/NA	164.73/NA	189.74/NA	109.13/NA
Morusin	−7.63/2.57	−4.68/371.1	−4.05/1070	−5.55/85.66
1	−7.48/3.28	−5.98/41.3	−6.90/8.77	−6.23/27.24
2	−6.93/8.38	−6.36/21.89	−7.11/6.16	−6.74/11.46
21	−7.07/6.52	−4.72/344.4	−5.26/140	−6.58/15.14
22	−7.58/2.77	−6.52/16.56	−6.75/11.34	−7.28/4.28
GSK5182	−7.04/6.88	−2.9/7530	2.41/NA	−4.0/1170
4-OH Tamoxifen	−7.11/6.09	−4.02/1130	−1.69/NA	−4.13/937
Tamoxifen	−7.40/3.79	−4.3/703.33	−3.08/5500	−6.07/35.69

NA: unavailable.

150.6, 161.4, HRMS (EI) calcd for $C_9H_9O_5F_3S_1$ (M^+) 286.0123, found 286.0113. The spectra of synthetic **4** are agreement with those reported by Subramanian et al.[19]

*4.1.2. 1,1-Dibromo-2-(2,4,5-trimethoxyphenyl)ethene (**9**)[20a].* A suspension of CBr_4 (6.76 g, 20.0 mmol) in CH_2Cl_2 (15.0 mL) was cooled to 0 °C, and a solution of PPh_3 (10.7 g, 40.0 mmol) in CH_2Cl_2 (15.0 mL) was added to the reaction mixture. After the mixture was stirred for 30 min at 0 °C, the color of the solution changed from pale yellow to orange. Into the reaction mixture was added a solution of 2,4,5-trimethoxybenzaldehyde (1.96 g, 10.0 mmol) in CH_2Cl_2 (5.0 mL) and Et_3N (11.4 mL, 80.0 mmol), and then the heterogeneous mixture was stirred for 8 h at room temperature. The resulting product was poured into ice water (50.0 mL), followed by extraction with CH_2Cl_2 (3×50.0 mL). After washing with saturated aqueous $NaHCO_3$ (5.0 mL), the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was reduced in vacuo, and the crude product was subjected to column chromatography (SiO_2, hexane/EtOAc 9/1) to provide the dibromide **9** (3.25 g, 93%) as a pale yellow solid. Characterization of **9**: mp 65–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3H), 3.82 (s, 3H), 3.87 (s, 3H), 6.44 (s, 1H), 7.36 (s, 1H), 7.56 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.9, 56.3, 56.4, 87.3, 96.5, 111.9, 115.4, 131.8, 142.3, 150.1, 151.6. The spectra of synthetic **9** are in agreement with those reported by Konno et al.[20a]

*4.1.3. 2,4,5-Trimethoxyphenylacetylene (**5a**)[20].* A solution of 1,1-dibromo-2-(2,4,5-trimethoxyphenyl)ethane (**9**) (0.98 g, 2.8 mmol) in THF (15.0 mL) was cooled at −78 °C for 30 min, and *n*-BuLi (3.6 mL, 1.6 M in hexane) was added with dropwise at −78 °C. The mixture was stirred at −78 °C for 30 min, and allowed to warm to room temperature. Then the resulting solution was quenched with saturated aqueous NH_4Cl (5.0 mL), followed by extraction with EtOAc (3×50.0 mL). The organic layers were dried and concentrated, and the residue was subjected to column chromatography (SiO_2, hexane/EtOAc 9/1) to obtain **5a** (0.52 g, 97%) as a white solid: mp 115–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22 (s, 1H), 3.78 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 6.44 (s, 1H), 6.90 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.9, 56.3, 56.6, 79.8, 80.2, 96.8, 101.8, 116.3, 142.6, 150.7, 156.0. The spectra of synthetic **5a** are in agreement with those reported by Konno and Brooks.[20]

*4.1.4. 3,5-Diacetoxy-1-[2-(2,4,5-trimethoxyphenyl)ethynyl]benzene (**10a**).* To a mixture of 3,5-diacetoxyphenyl triflate (1.04 g, 3.0 mmol), acetylene **5a** (0.58 g, 3.0 mmol), and $Pd(PPh_3)_4$ (0.18 g, 5 mol %) in anhydrous dioxane (30.0 mL) was added Et_3N (5.0 mL) at room temperature under nitrogen; the resulting mixture was heated to reflux at 110 °C for 6 h. After cooling, the reaction mixture was filtered through Celite. The filtrate was concentrated, and then purified by column chromatography (SiO_2, hexane/EtOAc 5/1) to afford **10a** (0.70 g, 67%) as light yellow solid: mp 118–120 °C; IR (KBr) ν_{max}: 3073, 3012, 2976, 2938, 2848, 2206, 1767, 1602, 1523, 1357, 1210, 1185, 1066, 895, 863 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (s, 6H), 3.77 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 6.42 (s, 1H), 6.81 (t, *J*=2.1 Hz, 1H), 6.89 (s, 1H), 7.10 (d, *J*=2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7, 55.7, 56.1, 56.4, 87.6, 90.2, 96.8, 102.2, 115.1, 115.5, 121.6, 125.4, 142.6, 150.5, 150.5, 155.4, 168.6; ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 20.0, 55.4, 55.9, 56.0, 88.2, 89.8, 97.7, 102.0, 115.8, 116.5, 121.6, 125.4, 143.2, 151.4, 151.7, 156.0, 168.5, HRMS (EI) calcd for $C_{21}H_{20}O_7$ (M^+) 384.1209, found 384.1213.

*4.1.5. 1-(3,5-Dimethoxyphenyl)-2-(2,4,5-trimethoxyphenyl) acetylene (**10b**).* To a mixture of 3,5-dimethoxyphenyl triflate (0.86 g, 3.0 mmol), acetylene **5a** (1.15 g, 6.0 mmol), $Pd(PPh_3)_2Cl_2$ (0.21 g, 10 mol %), and CuI (0.11 g, 20 mol %) in anhydrous dioxane (30.0 mL) was immediately added Et_3N (5.0 mL) at room temperature under nitrogen, and the mixture was heated to reflux at 100 °C for 8 h. After cooling, the reaction mixture was filtered through Celite. The filtrate was concentrated, and then purified by column chromatography (SiO_2, hexane/EtOAc 5/1) to give **10b** (0.84 g, 85%) as light yellow solid: mp 122–124 °C; IR (KBr) ν_{max}: 3003, 2966, 2940, 2842, 2200, 1586, 1362, 1210, 1066, 826 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 (s, 6H), 3.81 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 6.39 (t, *J*=2.4 Hz, 1H), 6.46 (s, 1H), 6.67 (d, *J*=2.4 Hz, 2H), 6.96 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.3, 55.9, 56.3, 56.7, 85.4, 92.1, 97.1, 101.4, 103.0, 109.1, 115.8, 124.9, 142.8, 150.4, 155.3, 160.4, HRMS (EI) calcd for $C_{19}H_{20}O_5$ (M^+) 328.1311, found 328.1312.

*4.1.6. cis-1-(3,5-Dimethoxy)-2-(2,4,5-trimethoxy)stilbene (**11b**)[21].* According to Hua's procedure, into a sealable tube were introduced **10b** (0.50 g, 1.5 mmol), KOH (0.13 g, 2.3 mmol), $Pd(OAc)_2$ (6.8 mg, 2 mol %), and DMF (5.0 mL) under a N_2 atmosphere. The tube was capped and the mixture was heated at 145 °C with stirring for 3 h. After cooling, the crude reaction mixture was diluted with CH_2Cl_2 (20.0 mL) and cyclohexane (10.0 mL), the mixture was filtered and the filtrate evaporated in vacuo. The residue was subjected to flash chromatography (SiO_2, hexane/EtOAc 5/1) to provide the *cis*-stilbene **11b** (0.46 g, 94%) as light yellow solid: mp 93–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45 (s, 3H), 3.64 (s, 6H), 3.80 (s, 3H), 3.86 (s, 3H), 6.27 (t, *J*=2.4 Hz, 1H), 6.44 (d, *J*=2.4 Hz, 2H), 6.47 (d, *J*=12.3 Hz, 1H), 6.48 (s, 1H), 6.65 (d, *J*=12.3 Hz, 1H), 6.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.2, 56.0, 56.1, 56.5, 97.2, 99.4, 106.7, 113.4, 117.2, 125.3, 128.7, 139.5, 142.4, 149.2, 151.7, 160.5, HRMS (EI) calcd for $C_{19}H_{22}O_5$ (M^+) 330.1467, found 330.1462. The spectra of synthetic **11b** are in agreement with those reported by Kim et al.[21]

*4.1.7. cis-1-(3,5-Diacetoxy)-2-(2,4,5-trimethoxy)stilbene (**11a**).* This compound was prepared from **10a** (0.59 g, 1.5 mmol), KOH (0.13 g, 2.3 mmol), and $Pd(OAc)_2$ (6.8 mg, 2 mol %) in DMF (5.0 mL) according to the preceding procedure. Compound **11a** was isolated in 90% yield as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 6H), 3.41 (s, 3H), 3.66 (s, 3H), 3.75 (s, 3H), 6.32 (d, *J*=12.3 Hz, 1H), 6.35 (s, 1H), 6.27 (t, *J*=1.5 Hz, 1H), 6.57 (s, 1H), 6.59 (d, *J*=12.3 Hz, 1H), 6.75 (d, *J*=1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0, 55.2, 55.3, 55.6, 97.7, 113.4, 114.4, 115.8, 119.1, 125.9, 126.8, 140.2, 142.8, 150.3, 151.3, 152.1, 168.5, ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 21.0, 55.8, 55.9, 56.3, 97.1, 112.6, 114.0, 116.3, 119.2, 126.5, 126.6, 140.1, 142.5, 149.3, 150.8, 151.7, 168.9, HRMS (EI) calcd for $C_{21}H_{22}O_7$ (M^+) 386.1366, found 386.1370.

*4.1.8. 3,5-Diacetoxy-1-[2-(2,4,5-trimethoxyphenyl)ethyl]benzene (**12a**).* The mixture of **10a** (0.13 g, 0.3 mmol) and palladium on carbon (10%, 0.074 g) in MeOH (3.0 mL) and EtOAc (3.0 mL) was placed with shaking in Parr-Shaker equipment under 50 psi of H_2. After shaking for 8 h at ambient temperature, the reaction mixture was filtered and evaporated to give the crude product, which was subjected to column chromatography (SiO_2, hexane/EtOAc 5/1) to obtain **12a** (0.12 g, 90%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.24 (s, 6H), 2.81 (br s, 4H), 3.74 (s, 3H), 3.75 (s, 3H), 3.83 (s, 3H), 6.49 (s, 1H), 6.56 (s, 1H), 6.71 (s, 1H), 6.77 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0, 31.3, 36.3, 56.1, 56.1, 56.5, 97.6, 112.7, 114.1, 119.0, 120.8, 142.6, 144.7, 147.8, 150.7, 151.4, 169.0, HRMS (EI) calcd for $C_{21}H_{24}O_7$ (M^+) 388.1522, found 388.1529.

*4.1.9. 1-(3,5-Dimethoxyphenyl)-2-(2,4,5-trimethoxyphenyl)ethane (**12b**).* Compound **11b** (1.00 g, 3.0 mmol) was dissolved in MeOH (10.0 mL) and EtOAc (10.0 mL). Palladium on carbon (10%, 0.35 g) was added and the reaction was placed with shaking in Parr-Shaker equipment under 50 psi of H_2. After shaking for 8 h at ambient temperature, the reaction mixture was filtered and evaporated to give the crude product, which was subjected to column chromatography (SiO_2, hexane/EtOAc 5/1) to provide **12b** (0.96 g, 96%) as white solid: mp 84.1–86.1 °C; IR (KBr) ν_{max}: 3013, 2972, 2938, 2836,

1603, 1523, 1462, 1155, 1046, 852 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 2.78–2.80 (m, 4H), 3.75 (s, 6H), 3.77 (s, 3H), 3.78 (s, 3H), 3.86 (s, 3H), 6.29 (t, J=2.4 Hz, 1H), 6.34 (d, J=2.4 Hz, 2H), 6.51 (s, 1H), 6.61 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.7, 36.9, 55.2, 56.2, 56.4, 56.6, 97.7, 97.9, 106.6, 114.3, 121.7, 142.7, 144.7, 147.8, 151.5, 160.6, HRMS (EI) calcd for $C_{19}H_{24}O_5$ (M^+) 332.1624, found 332.1626.

*4.1.10. 1-(2-Bromo-3,5-dimethoxyphenyl)-2-(2,4,5-trimethoxyphenyl) ethane (**13**).* NBS (29 mg, 1.6×10^{-1} mmol) was added in one portion to a well-stirred solution of **12b** (50 mg, 1.5×10^{-1} mmol) in CH_2Cl_2 (5.0 mL) at room temperature, and the mixture was stirred for 8 h. The resulting solution was quenched by adding saturated aqueous Na_2SO_3 (2.0 mL), and extracted with CH_2Cl_2 (3×5.0 mL). After removal of solvent, the residue was subjected to column chromatography (SiO_2, hexane/ether 5/1) to give **13** (58 mg, 95%) as a light yellow solid: mp 100–102 °C; IR (KBr) ν_{max}: 3002, 2967, 2939, 2849, 1592, 1524, 1468, 1363, 1335, 1210, 1205, 1083, 860, 816 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 2.83 (t, J=4.5 Hz, 2H), 2.94 (t, J=4.5 Hz, 2H), 3.73 (s, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 6.32 (d, J=2.4 Hz, 1H), 6.33 (d, J=2.4 Hz, 1H), 6.51 (s, 1H), 6.63 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.0, 37.3, 55.4, 56.1, 56.2, 56.4, 56.6, 97.4, 97.8, 104.7, 106.8, 114.3, 121.4, 142.7, 143.4, 147.8, 151.6, 156.6, 159.3, HRMS (EI) calcd for $C_{19}H_{23}BrO_5$ (M^+) 410.0729, found 410.0723.

*4.1.11. 1-(2-Iodo-3,5-dimethoxyphenyl)-2-(2,4,5-trimethoxyphenyl) ethane (**14**).* This compound was prepared as a white solid from **12b** (50 mg, 1.5×10^{-1} mmol), NIS (37 mg, 1.6×10^{-1} mmol), and one drop CF_3CO_2H in CH_3CN (5.0 mL) at 50 °C according to the preceding **13** procedure. Compound **14** was isolated in 90% yield as a solid: mp 94–96 °C; IR (KBr) ν_{max}: 3003, 2995, 2937, 2848, 1590, 1523, 1464, 1319, 1205, 1157, 1080, 1034, 938, 850, 816 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 2.81 (t, J=4.5 Hz, 2H), 2.96 (t, J=4.5 Hz, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.82 (s, 3H), 3.88 (s, 3H), 6.27 (d, J=2.7 Hz, 1H), 6.37 (d, J=2.7 Hz, 1H), 6.51 (s, 1H), 6.67 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.3, 41.8, 55.4, 56.2, 56.4, 56.4, 56.6, 81.7, 96.5, 97.8, 106.7, 114.3, 121.3, 142.7, 146.8, 147.8, 151.6, 158.7, 160.7, HRMS (EI) calcd for $C_{19}H_{23}IO_5$ (M^+) 458.0590, found 458.0590.

*4.1.12. 5-Methoxy-2-(3,5-dimethoxyphenethyl)-1,4-benzoquinone (**17**).* To a mixture of **12b** (0.41 g, 1.2 mmol) and AgO (0.59 g, 4.8 mmol) in dioxane (25 mL) was added 6 N HNO_3 (1.4 mL) dropwise at room temperature. The suspension was stirred for 20 min, and the reaction was quenched with saturated aqueous $NaHCO_3$ (10.0 mL). After extraction with EtOAc (3×20.0 mL), the organic layers were combined and concentrated and the residue was subjected to column chromatography (SiO_2, hexane/EtOAc 4/1) to give **17** (0.24 g, 64%) as orange solid: mp 149.5–150.4 °C; IR (KBr) ν_{max}: 3073, 2982, 2942, 2840, 1675, 1659, 1600, 1472, 1206, 1161, 981, 859 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 2.71–2.72 (m, 4H), 3.74 (s, 6H), 3.79 (s, 3H), 5.90 (s, 1H), 6.28 (t, J=2.4 Hz, 1H), 6.30 (d, J=2.4 Hz, 2H), 6.41 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.6, 34.3, 55.2, 56.2, 98.2, 106.4, 107.7, 130.9, 142.6, 149.2, 158.5, 160.8, 182.1, 187.2, HRMS (EI) calcd for $C_{17}H_{18}O_5$ (M^+) 302.1154, found 302.1146.

*4.1.13. 5-Methoxy-2-(2-bromo-3,5-dimethoxyphenethyl)-1,4-benzoquinone (**15**).* This compound was prepared from **13** (0.19 g, 4.6×10^{-1} mmol) and AgO (0.22 g, 1.8 mmol) in dioxane (10 mL) at room temperature according to the preceding procedure. Compound **15** was isolated in 62% yield as an orange solid: mp 95–96 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.60 (t, J=7.5 Hz, 2H), 2.80 (t, J=7.5 Hz, 2H), 3.63 (s, 3H), 3.68 (s, 3H), 3.71 (s, 3H), 5.79 (s, 1H), 6.22 (s, 2H), 6.29 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.4, 35.1, 55.5, 56.1, 56.2, 97.8, 104.7, 106.7, 107.7, 131.0, 141.6, 148.9, 156.8, 158.5, 159.6, 182.1, 187.2, HRMS (EI) calcd for $C_{17}H_{17}BrO_5$ (M^+) 380.0259, found 380.0264.

*4.1.14. 5-Methoxy-2-(2-iodo-3,5-dimethoxyphenethyl)-1,4-benzoquinone (**16**).* According to the previous procedure, compound **16** was isolated in 56% yield as an orange solid: mp 119–120 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.72 (t, J=7.5 Hz, 2H), 2.95 (t, J=7.5 Hz, 2H), 3.78 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 5.94 (s, 1H), 6.29 (d, J=2.7 Hz, 1H), 6.42 (d, J=2.7 Hz, 1H), 6.48 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.6, 39.6, 55.4, 56.2, 56.4, 81.6, 96.9, 106.6, 107.7, 131.0, 145.0, 148.9, 158.5, 158.9, 161.0, 182.1, 187.2, HRMS (EI) calcd for $C_{17}H_{17}IO_5$ (M^+) 428.0121, found 428.0126.

*4.1.15. 5′,7′-Dimethoxy-spiro(2,5-dimethoxy-cyclohexa-2,5-dien-4-one-1,1′-indan) (**18**). Method A*: To a solution of **12b** (0.10 g, 3.0×10^{-1} mmol) and $(CF_3CO)_2O$ (1.0 μL) in anhydrous CH_2Cl_2 (3.0 mL) was added VOF_3 (82 mg, 6.6×10^{-1} mmol) at 0 °C under N_2, and then a solution of CF_3CO_2H (0.2 mL) in CH_2Cl_2 (2.0 mL) was added dropwise slowly. After stirring at 0 °C for 4 h, the solution was diluted with CH_2Cl_2 (5.0 mL) and washed with cold 1 M aqueous citric acid (2×2.0 mL), 3 M NH_4OH (3×3.0 mL), and brine (2×4.0 mL). The resulting mixture was dried over Na_2SO_4 and filtered; the filtrate was concentrated in vacuo, and then the residue was subjected to column chromatography (SiO_2, hexane/EtOAc 4/1) to give **18** (78 mg, 82%) as an orange solid: mp 188–190 °C; IR (KBr) ν_{max}: 3073, 2976, 2940, 2842, 1675, 1636, 1597, 1457, 1369, 1195, 1081, 876, 842, 826 cm^{-1}, ^1H NMR (600 MHz, $CDCl_3$) δ 2.11–2.15 (m, 1H), 2.55–2.60 (m, 1H), 3.06–3.09 (m, 2H), 3.56 (s, 3H), 3.58 (s, 3H), 3.63 (s, 3H), 3.76 (s, 3H), 5.48 (s, 1H), 5.65 (s, 1H), 6.20 (d, J=1.2 Hz, 1H), 6.40 (d, J=1.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 31.9, 39.5, 53.6, 54.9, 55.1, 55.4, 56.0, 97.0, 100.8, 101.5, 115.5, 123.2, 146.8, 148.8, 156.6, 161.6, 178.7, 183.3, HRMS (EI) calcd for $C_{18}H_{20}O_5$ (M^+) 316.1311, found 316.1318.

Method B: To a solution of **12b** (0.10 g, 3.0×10^{-1} mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added a solution of $PhI(OCOCF_3)_2$ (0.13 g, 3.0×10^{-1} mmol) and $BF_3\cdot Et_2O$ (55 μL, 6.2×10^{-1} mmol) in CH_2Cl_2 (1.0 mL) dropwise at −40 °C under N_2. The reaction mixture was then stirred at −40 °C for 1 h and then evaporated in vacuo. After removal of solvent, the residue was subjected to column chromatography (SiO_2, hexane/EtOAc 4/1) to give **18** (65 mg, 68%) as an orange solid: mp 188–190 °C.

*4.1.16. 9,10-Dihydro-3,5,7-trimethoxy-1,4-phenanthrenedione (**19**)*[3]. To a solution of **17** (0.23 g, 7.6×10^{-1} mmol) in CH_2Cl_2 (10.0 mL) was added $TiCl_4$ (0.10 mL, 9.1×10^{-1} mmol) dropwise at 0 °C. The reaction was monitored by TLC and quenched after ca. 4 h by treatment with saturated aqueous $NaHCO_3$ (5.0 mL). The solvent was removed and the crude product was subjected to flash chromatography (SiO_2, hexane/EtOAc 3/1) to afford **19** (0.18 g, 78%) as a red solid and **20** (25 mg, 11%). Compound **19**: mp 194–195 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.56–2.66 (m, 4H), 3.76 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 5.84 (s, 1H), 6.34 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4, 28.8, 55.4, 55.8, 56.2, 97.6, 105.3, 105.8, 112.1, 139.0, 140.6, 142.5, 158.5, 159.9, 162.2, 180.0, 185.8, HRMS (EI) calcd for $C_{17}H_{16}O_5$ (M^+) 300.0998, found 300.1005.

*4.1.17. 5′,7′-Dimethoxy-spiro(4-methoxy-3-cyclohexen-2,5-dione-1,1′-indan) (**20**).* Into a solution of **17** (0.23 g, 7.6×10^{-1} mmol) in CH_2Cl_2 (10.0 mL) was introduced $BF_3\cdot Et_2O$ (70 μL, 7.6×10^{-1} mmol) dropwise at 0 °C, and the reaction was monitored by TLC until **17** was consumed (ca. 2 h). The reaction mixture was then evaporated in vacuo, and the residue subjected to column chromatography (SiO_2, hexane/EtOAc 3/1) to provide **19** (39 mg, 17%) and **20** (0.15 g, 67%) as an orange solid. Compound **20**: mp 145–146 °C; IR (KBr) ν_{max}: 3013, 2982, 2942, 2842, 1714, 1659, 1603, 1462, 1361, 1222, 1151, 1083, 839 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 1.95–2.02 (m,

1H), 2.40–2.49 (m, 1H), 2.81 (d, J=16.5 Hz, 1H), 2.87–2.95 (m, 1H), 3.04–3.36 (m, 1H), 3.33 (d, J=16.5 Hz, 1H), 3.65 (s, 3H), 3.75 (s, 3H), 3.79 (s, 3H), 5.96 (s, 1H), 6.24 (d, J=1.8 Hz, 1H), 6.37 (d, J=1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.7, 38.6, 47.3, 54.9, 55.4, 56.2, 59.0, 97.2, 101.1, 111.7, 123.4, 147.0, 156.2, 161.8, 162.5, 192.2, 199.7, HRMS (EI) calcd for $C_{17}H_{18}O_5$ (M^+) 302.1154, found 302.1147.

*4.1.18. 5-Hydroxy-3,7-dimethoxy-1,4-phenanthrenedione, denbinobin (**2**).* A solution of **19** (0.10 g, 3.3×10^{-1} mmol) in CH_2Cl_2 (12.0 mL) was stirred at room temperature, followed by addition of timethylsilyl iodide (TMSI, 95 μL, 6.6×10^{-1} mmol) dropwise. The resulting mixture was stirred at room temperature for 5 h, and quenched by treatment with MeOH (4.0 mL). After the reaction mixture was concentrated in vacuo, the residue was subjected to flash chromatography (SiO_2, hexane/EtOAc 3/1) to give 9,10-dihydro-5-hydroxy-3,7-dimethoxy-1,4-phenanthrenedione (95 mg, 92%) as a black solid: mp 162–164 °C; IR (KBr) ν_{max}: 3369, 3065, 3020, 2966, 2920, 2849, 1636, 1612, 1553, 1440, 1305, 1264, 1127, 1066, 843, 826 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66 (br s, 4H), 3.80 (s, 3H), 3.88 (s, 3H), 5.99 (s, 1H), 6.40 (d, J=2.7 Hz, 1H), 6.45 (d, J=2.7 Hz, 1H), 8.85 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 28.8, 55.3, 56.6, 102.8, 106.7, 108.4, 110.2, 137.0, 141.9, 144.6, 156.6, 158.8, 162.2, 185.3, 185.6, HRMS (EI) calcd for $C_{16}H_{12}O_5$ (M^+) 284.0685, found 284.0681, HRMS (EI) calcd for $C_{16}H_{14}O_5$ (M^+) 286.0841, found 286.0833. The spectra are in agreement with those reported for the synthetic product.[3]

A mixture of the preceding synthetic hydroxyphenanthrenedione (95 mg, 3.3×10^{-1} mmol) and DDQ (60 mg, 2.6×10^{-1} mmol) in anhydrous toluene (5.0 mL) was heated to reflux at 120 °C under N_2, and the reaction was monitored by TLC and quenched after ca. 12 h. After cooling, the resulting mixture was filtered and the solvent was removed, followed purification by column chromatography (SiO_2, hexane/EtOAc 5/1) to achieve **2** (75 mg, 80%) as gray solid: mp 215–217 °C; IR (KBr) ν_{max}: 3446, 3066, 2966, 2943, 2841, 1675, 1627, 1586, 1510, 1422, 1330, 1240, 1170, 1083, 852 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H), 3.96 (s, 3H), 6.14 (s, 1H), 6.80 (d, J=2.7 Hz, 1H), 6.91 (d, J=2.7 Hz, 1H), 8.04 (d, J=8.7 Hz, 1H), 8.10 (d, J=8.7 Hz, 1H), 11.01 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.5, 56.9, 101.7, 107.3, 108.6, 117.1, 122.6, 128.5, 132.3, 137.3, 139.9, 156.3, 160.7, 161.2, 184.3, 186.4, HRMS (EI) calcd for $C_{16}H_{12}O_5$ (M^+) 284.0685, found 284.0681. The spectra of synthetic **2** are in agreement with those reported for the natural product.[1a,5]

*4.1.19. 2,6-Dimethoxy-1,4,5,8-phenanthrenetetrone, moniliformediquinone (**1**).* To a solution of **2** (0.10 g, 3.5×10^{-1} mmol) in MeOH and DMF (10.0 mL; v/v 1/1) was added a solution of freshly prepared $(KSO_3)_2NO$ (0.33 g, 1.2 mmol) in water (6.0 mL) and 1 M aqueous NaOAc solution (0.18 mL) at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was extracted with ether (3×10.0 mL). The combined organic phase was washed with water (2.0 mL) and brine (2.0 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to flash chromatography (SiO_2, hexane/EtOAc 5/1) to provide **1** (95 mg, 91%) as yellow solid: mp 258–260 °C; IR (KBr) ν_{max}: 3082, 2986, 2946, 2843, 1688, 1652, 1610, 1458, 1354, 1232, 1139, 1085, 872, 822 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H), 3.95 (s, 3H), 6.17 (s, 1H), 6.32 (s, 1H), 8.36 (d, J=8.1 Hz, 1H), 8.41 (d, J=8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.6, 56.8, 108.2, 111.7, 129.6, 130.6, 133.1, 134.5, 135.0, 137.2, 158.9, 163.0, 178.5, 179.2, 182.4, 182.5, HRMS (EI) calcd for $C_{16}H_{10}O_6$ (M^+) 298.0477, found 298.0482. The spectra of synthetic **1** are in agreement with those reported for the natural product.[1a]

*4.1.20. 1-(4-Hydroxy-3-methoxyphenyl)-2-(4-hydroxy-2,5-dimethoxyphenyl)ethane, moscatilin (**21**).* The moscatilin (**21**) was prepared from 4-hydroxy-3-methoxybenzaldehyde, using the procedure used to make **12**. Compound **21** was isolated in 51% yield over five steps as a white solid: mp 84–86 °C (lit.[22] 84 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.80 (br s, 4H), 3.78 (s, 3H), 3.82 (s, 6H), 5.39 (br s, OH), 5.50 (br s, OH), 6.34 (s, 2H), 6.59 (d, J=1.8 Hz, 1H), 6.66 (dd, J=8.1,1.8 Hz, 1H), 6.82 (d, J=8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.9, 38.4, 55.8, 56.2, 105.1, 111.2, 114.1, 121.0, 132.7, 132.8, 133.6, 143.7, 146.2, 146.8, HRMS (EI) calcd for $C_{17}H_{20}O_5$ (M^+) 304.1311, found 304.1319. Melting point and the spectra of synthetic **21** are in agreement with those reported for the natural product.[22]

*4.1.21. 5-Hydroxy-3,6,7-trimethoxy-1,4-phenanthrenedione, calanquinone A (**22**).* According to the developed preparation of **1**, compound **22** was prepared from 2,4,5-trimethoxybenzaldehyde in 14% yield over eight steps as a yellow solid: mp 154–155 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96 (s, 3H), 4.02 (s, 3H), 4.03 (s, 3H), 6.15 (s, 1H), 6.85 (s, 1H), 8.04 (d, J=8.7 Hz, 1H), 8.10 (d, J=8.7 Hz, 1H), 10.73 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.9, 56.9, 60.8, 101.1, 107.2, 118.5, 121.7, 128.1, 132.8, 134.8, 136.9, 140.1, 148.1, 155.0, 161.5, 184.4, 186.0, HRMS (EI) calcd for $C_{17}H_{14}O_6$ (M^+) 314.0790, found 314.0788. The spectra of synthetic **22** are in agreement with those reported for the natural product.[6]

4.2. Computational methods

4.2.1. Preparation of ligand and protein complexes input structures. First, protein structures were extracted from the Protein Databank (pdb) file (containing only non-hydrogen atoms). The protein's pdb files were edited with AutoDockTools-1.5.1 (on a Linux system) to protonate polar hydrogen atoms, add Kollman charges, and saved as a pdbqt file. Furthermore, ligand structures were optimized by Chem 3D Ultra version 8.0 (Run MOPAC minimize energy to minimum rms Gradient 0.001) to get the structure (pdb file). Alternatively, the X-ray structure of morusin was extracted from Cambridge Chemical Database (pdb file). The ligand file was input into AutoDockTools-1.5.1 program, detected the root of molecule, and output as a pdbqt file as well.

4.2.2. Grid and docking studies. To predict the energetically favorable positions of ligand molecules in the interior structure of the protein target, a rectangular grid box of 21.75×21.75×21.75 Å^3 with grid points separated by 0.333 Å, it was centered on the midpoint of the ligand binding pocket. It was essential to modify the AutoGrid program to enable docking in the presence of metals and heteroatoms. Subsequently, automated docking studies were performed with Lamarckian Genetic Algorithm to provide the binding energies and conformations of protein-ligand complexes.

4.3. Sulforhodamine B (SRB) assays

Cells were seeded in 96-well plates in medium with 5% fetal bovine serum (FBS). After 24 h, cells were fixed with 10% trichloroacetic acid (TCA) to represent cell population at the time of compound addition (T_0). After additional incubation of dimethyl sulfoxide (DMSO) or test compound for 48 h, cells were fixed with 10% TCA and sulforhodamine (SRB) at 0.4% (w/v) in 1% acetic acid was added to stain cells. Unbound SRB was washed out by 1% acetic acid and SRB-bound cells were solubilized with 10 mM Trizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T_0), control growth (C), and cell growth in the presence of the compound (T_x), the percentage growth was calculated at each of the compound concentrations levels. Percentage growth inhibition was calculated as: $100-[(T_x-T_0)/(C-T_0)]\times100$. Growth inhibition of 50% (IC_{50}) is determined at the compound concentration which results in 50%

reduction of total protein increase in control cells during the compound incubation.

Acknowledgements

The research was supported by the National Science Council (Research Grants NSC98-2119-M-018-002-MY3 and NSC99-2811-M-018-011), National Tsing Hua University (NSC99-2323-B-007-001), and the Department of Health, Taiwan (DOH99-TD-C-111-004). We thank Professor T. Ross Kelly for valuable proofreading and the revised manuscript. We also thank Dr. Fang-Rong Chang for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.06.054.

References and notes

1. (a) Lin, T.-H.; Chang, S.-J.; Chen, C.-C.; Wang, J.-P.; Tsao, L.-T. *J. Nat. Prod.* **2001**, *64*, 1084–1086; (b) Talapatra, B.; Mukhopadhyay, P.; Chaudhury, P.; Talapatra, S. K. *Indian J. Chem.* **1982**, *21B*, 386–387; (c) Tezuka, Y.; Hirano, H.; Kikuchi, T.; Xu, G.-J. *Chem. Pharm. Bull.* **1991**, *39*, 593–598; (d) Tezuka, Y.; Yoshida, Y.; Kikuchi, T.; Xu, G.-J. *Chem. Pharm. Bull.* **1993**, *41*, 1346–1349; (e) Lee, Y. H.; Park, J. D.; Baek, N. I.; Kim, S. I.; Ahn, B. Z. *Planta Med.* **1995**, *61*, 178–180; (f) Chen, H.-Y.; Shiao, M.-S.; Huang, Y.-L.; Shen, C.-C.; Lin, Y.-L.; Kuo, Y.-H.; Chen, C.-C. *J. Nat. Prod.* **1999**, *62*, 1225–1227; (g) Lin, T. H.; Chang, S. J.; Chen, C. C. *Chin. Pharm. J.* **2000**, *52*, 251–259; (h) Huang, Y. L.; Lay, H. L.; Shen, C. C.; Chen, F. C.; Chen, C. C. *Chin. Pharm. J.* **2000**, *52*, 305–311; (i) Chen, C.-C.; Huang, Y.-L.; Teng, C.-M. *Planta Med.* **2000**, *66*, 372–374; (j) Huang, Y.-C.; Guh, J.-H.; Teng, C.-M. *J. Biomed. Sci.* **2005**, *12*, 113–121; (k) Yang, K.-C.; Uen, Y.-H.; Suk, F.-M.; Liang, Y.-C.; Wang, Y.-J.; Ho, Y.-S.; Li, I.-H.; Lin, S.-Y. *World J. Gastroenterol.* **2005**, *11*, 3040–3045.
2. (a) Kuo, C.-T.; Hsu, M.-J.; Chen, B.-C.; Chen, C.-C.; Teng, C.-M.; Pan, S.-L.; Lin, C.-H. *Toxicol. Lett.* **2008**, *177*, 48–58; (b) Sánchez-Duffhues, G.; Calzado, M. A.; de Vinuesa, A. G.; Caballero, F. J.; Ech-Chahad, A.; Appendino, G.; Krohn, K.; Fiebich, B. L.; Muñoz, E. *Biochem. Pharmacol.* **2008**, *76*, 1240–1250; (c) Sánchez-Duffhues, G.; Calzado, M. A.; de Vinuesa, A. G.; Appendino, G.; Fiebich, B. L.; Loock, U.; Lefarth-Risse, A.; Krohn, K.; Muñoz, E. *Biochem. Pharmacol.* **2009**, *77*, 1401–1409; (d) Yang, C. R.; Guh, J. H.; Teng, C. M.; Chen, C. C.; Chen, P. H. *Br. J. Pharmacol.* **2009**, *157*, 1175–1185; Commentary: Magwere, T. *Br. J. Pharmacol.* **2009**, *157*, 1172–1174; (e) Hu, J.-M.; Zhao, Y.-X.; Miao, Z.-H.; Zhou, J. *Bull. Korean Chem. Soc.* **2009**, *30*, 2098–2100; (f) Pettit, G. R.; Singh, S. B.; Schmidt, J. M.; Niven, M. L.; Hamel, E.; Lin, C. M. *J. Nat. Prod.* **1988**, *51*, 517–527; (g) Chen, C.-C.; Wu, L.-G.; Ko, F.-N.; Teng, C.-M. *J. Nat. Prod.* **1994**, *57*, 1271–1274; (h) Tanpure, R. P.; Strecker, T. E.; Chaplin, D. J.; Siim, B. G.; Trawick, M. L.; Pinney, K. G. *J. Nat. Prod.* **2010**, *73*, 1093–1101; (i) Schobert, R.; Biersack, B.; Dietrich, A.; Effenberger, K.; Knauer, S.; Mueller, T. *J. Med. Chem.* **2010**, *53*, 6595–6602.
3. Krohn, K.; Loock, U.; Paavilainen, K.; Hausen, B. M.; Schmalle, H. W.; Kiesele, H. *ARKIVOC* **2001**, *2*, 88–130.
4. (a) Kraus, G. A.; Melekhov, A. *J. Org. Chem.* **1999**, *64*, 1720–1722; (b) Kraus, G. A.; Hoover, K.; Zhang, N. *Tetrahedron Lett.* **2002**, *43*, 5319–5321; (c) Kraus, G. A.; Zhang, N. *Tetrahedron Lett.* **2002**, *43*, 9597–9599.
5. Wang, Y.-C.; Lin, C.-H.; Chen, C.-M.; Liou, J.-P. *Tetrahedron Lett.* **2005**, *46*, 8103–8104.
6. Lee, C.-L.; Nakagawa-Goto, K.; Yu, D.; Liu, Y.-N.; Bastow, K. F.; Morris-Natschke, S. L.; Chang, F.-R.; Wu, Y.-C.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4275–4277.
7. Tseng, T.-H.; Chuang, S.-K.; Hu, C.-C.; Chang, C.-F.; Huang, Y.-C.; Lin, C.-W.; Lee, Y.-J. *Tetrahedron* **2010**, *66*, 1335–1340.
8. Liu, L.; Yang, B.; Katz, T. J.; Poindexter, M. K. *J. Org. Chem.* **1991**, *56*, 3769–3775.
9. Campeau, L.-C.; Parisien, M.; Leblanc, M.; Fagnou, K. *J. Am. Chem. Soc.* **2004**, *126*, 9186–9187.
10. Baek, S. J.; Wilson, L. C.; Eling, T. E. *Carcinogenesis* **2002**, *23*, 425–434.
11. (a) Lee, S. K.; Nam, K. A.; Hoe, Y. H.; Min, H.-Y.; Kim, E.-Y.; Ko, H.; Song, S.; Lee, T.; Kim, S. *Arch. Pharmacal. Res.* **2003**, *26*, 253–257; (b) Lee, S. K.; Park, E.-J.; Lee, E.; Min, H.-Y.; Kim, E.-Y.; Lee, T.; Kim, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2105–2108.
12. McEwen, A. B.; Guttieri, M. J.; Maier, W. F.; Laine, R. M.; Shvo, Y. *J. Org. Chem.* **1983**, *48*, 4436–4438.
13. Li, J.; Hua, R.; Liu, T. *J. Org. Chem.* **2010**, *75*, 2966–2970.
14. Takada, T.; Arisawa, M.; Gyoten, M.; Hamada, R.; Tohma, H.; Kita, Y. *J. Org. Chem.* **1998**, *63*, 7698–7706.
15. (a) Wang, K.; Wang, Q.; Huang, R. *J. Org. Chem.* **2007**, *72*, 8416–8421; (b) Ong, C. W.; Hwang, J.-Y.; Tzeng, M.-C.; Liao, S.-C.; Hsu, H.-F.; Chang, T.-H. *J. Mater. Chem.* **2007**, *17*, 1785–1790.
16. The X-ray data for **20** is deposited at CCDC as number CCDC813052.
17. Hunter, D. H.; Luyt, L. G. *Bioconjugate Chem.* **2000**, *11*, 175–181.
18. Chao, E. Y. H.; Collins, J. L.; Gaillard, S.; Miller, A. B.; Wang, L.; Orband-Miller, L. A.; Nolte, R. T.; McDonnell, D. P.; Willson, T. M.; Zuercher, W. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 821–824.
19. Subramanian, L. R.; Hanack, M.; Chang, L. W. K.; Imhoff, M. A.; Schleyer, P. V. R.; Effenberger, F.; Kurtz, W.; Stang, P. J.; Dueber, T. E. *J. Org. Chem.* **1976**, *41*, 4099–4103.
20. (a) Konno, Y.; Nozaki, K.; Yamada, S.; Asao, T.; Suzuki, T.; Kimura, M. WO 9632384 A1, 1996. *Chem. Abstr.* **1997**, *126*, 906; (b) Tanaka, K.; Konno, Y.; Kuraishi, Y.; Kimura, I.; Suzuki, T.; Kiniwa, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 623–627; (c) Brooks, G. T.; Ottridge, A. P.; Jennings, R. C.; Mace, D. W.; Alexander, B. A. *Pestic. Sci.* **1985**, *16*, 571–588.
21. Kim, S.; Min, S. Y.; Lee, S. K.; Cho, W.-J. *Chem. Pharm. Bull.* **2003**, *51*, 516–521.
22. (a) Majumder, P. L.; Sen, R. C. *Phytochemistry* **1987**, *26*, 2121–2124; (b) Majumder, P. L.; Guha, S.; Sen, S. *Phytochemistry* **1999**, *52*, 1365–1369.