JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Isolation and Identification of Cucurbitane-Type Triterpenoids with Partial Agonist/Antagonist Potential for Estrogen Receptors from *Momordica charantia*

Chin Hsu,[†] Chin-Lin Hsieh,[‡] Yueh-Hsiung Kuo,[*,§,∥,⊥] and Ching-jang Huang[*,†]

[†]Department of Biochemical Science and Technology, National Taiwan University, Tapei, Taiwan

[‡]Department of Disaster Management, Taiwan Police College, Taipei, Taiwan

[§]Tsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taichung, Taiwan

[∥]Department of Chemistry, National Taiwan University, Taipei, Taiwan

[⊥]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

ABSTRACT: This study aims at investigating the estrogenic activity and active cucurbitane-type triterpenoid compounds of bitter gourd (*Momordica charantia*, MC) using a transactivation assay for estrogen receptors (ER) α and β. The lyophilized fruits of MC were exhaustively extracted with ethyl acetate (EA) and 95% ethanol (EtOH), sequentially. The nonsaponifiable fraction (NS) of the EA extract as well as the acid hydrolyzed EtOH extract (AH) was fractionated and isolated by repeated column chromatography and further purified by preparative HPLC or RP-HPLC. One known compound, 5β,19-epoxycucurbita-6,24-diene-3β,23ξ-diol (**6**), was isolated from the NS, and five new compounds (**1**−**5**) were isolated from AH and identified as cucurbita-6,22(*E*),24-trien-3β-ol-19,5β-olide (**1**), 5β,19-epoxycucurbita-6,22(*E*),24-triene-3β,19-diol (**2**), 3β-hydroxycucurbita-5(10),6,22(*E*),24-tetraen-19-al (**3**), 19-dimethoxycucurbita-5(10),6,22(*E*),24-tetraen-3β-ol (**4**), and 19-*nor*-cucurbita-5(10),6,8,22(*E*),24-pentaen-3β-ol (**5**). In the noncytotoxic concentration range, compounds **1**, **2**, **5** and **6** showed weak agonistic activity via ER α and β. Compounds **1**, **2**, **3** and **6** significantly antagonized the transactvation of 17β-estradiol (E_2) via both ER α and β. In conclusion, this study demonstrates, for the first time as far as we know, the partial agonist/antagonist activity via ER of four new and one known cucurbitane-type triterpenoids from MC. Further studies are worthy to explore the selective estrogen receptor modulator (SERM) activity of MC.

KEYWORDS: cucurbitane-type triterpenoid, estrogen receptor, *Momordica charantia*, partial agonist/antagonist, transactivation assay

INTRODUCTION

Momordica charantia (MC), known as bitter gourd, bitter melon, balsam apple, ampalaya, karela, or cundeamor, is a common vegetable in tropical areas and has also been used in traditional medicine. Numerous studies have demonstrated various pharmacological activities of MC, such as antidiabetic (including its complications, nephropathy, cataract, insulin resistance), antibacterial, antiviral (including HIV infection) and anticancer.[1] The potential of MC for the treatment of cancer,[2] obesity,[3] diabetes and lipidemia [4,5] has also been reviewed.

Infertility was reported to be an adverse effect of MC,[5] as the fertility rate of mice fed with daily bitter melon juice dropped from 90% to 20%.[6] Solvent extract of MC was shown to inhibit spermatogenesis in male rats and speculated to result from a deficiency or unavailability of gonadotropin (FSH and/or LH).[7] Androgenic activity of these MC extracts was also demonstrated.[7] These reports imply that constituents of MC might modulate the reproductive hormone action at the molecular level, but such study has never been reported so far.

Estrogen receptors (ER), including ERα and ERβ, belong to the steroid nuclear receptor superfamily and are ligand-dependent, gene-specific transcription factors that mediate the physiological effects of estrogens. Selective estrogen receptor modulators (SERMs) are a class of ER ligands that display an agonist/antagonist activity profile on specific cell and tissue depending on specific conformational change of receptor which recruits coactivators or corepressors, or on ERα and ERβ expression ratio in specific tissues.[8] SERMs such as tamoxifen and raloxifene that are used to treat estrogen receptor-positive breast cancer have also been shown the efficacy of prevention for osteoporosis associated with hypoestrogenicity.[9−11] SERMs are thus of potential for the management of estrogen related health problems. Phytoestrogens are considered as SERMs and known to be beneficial to human health and may prevent certain diseases.[12]

We previously reported that the ethyl acetate (EA) extract of MC activated peroxisome proliferator activated receptors (PPARs) α and γ, another member of the nuclear receptor superfamily,[13] and identified 9-*cis*, 11-*trans*, 13-*trans*-conjugated linolenic acid (9*c*,11*t*,13*t*-CLN) as an active compound.[14] In a preliminary test, the nonsaponifiable (NS) fraction of the EA extract was found to activate ERs. This study thus aimed at isolation and identification of partial ER agonists/antagonists from MC. We hypothesized that some cucurbitane-type triterpenoids from MC might interact with ER and play some roles in the ER-regulated pathway.

As most of the triterpenoids in MC existed in the glycosylated form,[15,16] we have included an acid hydrolysis procedure in the

Received: January 28, 2011
Accepted: April 1, 2011
Revised: March 31, 2011
Published: April 01, 2011

search for more bioactive cucurbitane-type triterpenoid components from the ethanol (EtOH) extract of Taiwanese MC.

MATERIALS AND METHODS

Materials. Fresh wild bitter gourds (MC), provided by Hualien District Agricultural Research and Extension Station of Taiwan, were lyophilized and used for the following extraction.

Extraction and Isolation. The lyophilized powder of wild bitter gourds was extracted with 20-fold (w/v) ethyl acetate (EA) at room temperature for 2 days and repeated once. The residue was then extracted with 95% ethanol (EtOH) similarly. The respectively pooled extracts were evaporated to yield the EA and EtOH extracts.

The EA extract was saponified by dissolving in 10-fold (w/v) of a mixture of tetrahydrofuran (THF) and 10 N NaOH in equal volume and reacted at 60 °C overnight. The solvent was then evaporated and the residue partitioned in *n*-hexane and H_2O. The *n*-hexane extract (the nonsaponifiable fraction, NS) was loaded on a silica gel column and eluted by solvent mixtures of *n*-hexane and EA with increasing polarity. Four fractions, eluted by 50% EA/*n*-hexane, were collected. The first fraction (148 mg) was further separated by a preparative RP-HPLC eluted with 10% H_2O, 10% THF, and 80% acetonitrile (ACN) and yielded compound **6** (22 mg).

The EtOH extract (total 120 g) was separated by a Diaion HP-20 (SUPELCO) column eluted with H_2O, 50% MeOH/H_2O, MeOH and acetone sequentially. The evaporated MeOH fraction (20.4 g) was dissolved in 15% HCl and hydrolyzed at 90 °C for 1 h. The reaction mixture was then extracted with EA, washed with water and evaporated to give the acid hydrolyzed product (AH). The AH (10.4 g) was separated by a RP-18 column chromatography eluted with increasing proportion of MeOH in H_2O. The thirteen fractions obtained were checked by NMR and analyzed for estrogenic activity using the transactivation assay. The ninth (585 mg) fraction was further separated by a preparative RP-HPLC and eluted with 100% MeOH and yielded five subfractions. The second subfraction (89 mg) was continually separated by a preparative HPLC eluted with 30% EA/*n*-hexane and yielded compounds **1** (11 mg) and **5** (36 mg) (Figure 1). The third subfraction (154 mg) was separated by the same preparative HPLC and yielded compound **2** (24 mg). Moreover, the eighth (1.5 g) of the thirteen fractions was also separated by a preparative RP-HPLC using 0.1% acetic acid in MeOH as the mobile phase. The second (488 mg) of the nine subfractions was further separated by a subsequent normal phase preparative HPLC using 20% THF/*n*-hexane as mobile phase. Compounds **3** (77 mg) and **4** (45 mg) were obtained. The separation procedure is summarized in Figure 2.

General Experimental Procedures. Optical rotations, IR and UV spectra were respectively measured on a JASCO DIP-1000 digital polarimeter, a Perkin-Elmer 983G and a Thermo Helios Alpha&Beta spectrophotometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker Avance 400 MHz FT NMR spectrometer (^{1}H 400 MHz; ^{13}C 100 MHz). EIMS and HREIMS were recorded on a Finnigan TSQ-700 MS and a Finnigan MAT-95S MS spectrometer, respectively. Silica gel 60 F_{254} plates, 60 RP-18 F_{254S} plates (Merck), silica gel (230−400 mesh ASTM, Merck) and RP-18 silica gel (40−63 μm, Merck) were respectively used for TLC and column chromatography. HPLC was run with a semipreparative normal phase (250 × 10 mm, 5 μm, Thermo) or a reverse phase column (250 × 10 mm, 5 μm, Varian), respectively.

Transactivation Assay for ER Activity. The procedure of the transactivation assay has been described in our previous report.[17] This assay measured the ligand dependent transcription of a reporter gene in CHO-K1 cells (CCRC 60006) transiently cotransfected with vectors carrying estrogen receptor (GAL4-hERα or β ligand binding domain chimeric receptor) and (UAS)4-alkaline phosphatase (ALP) reporter

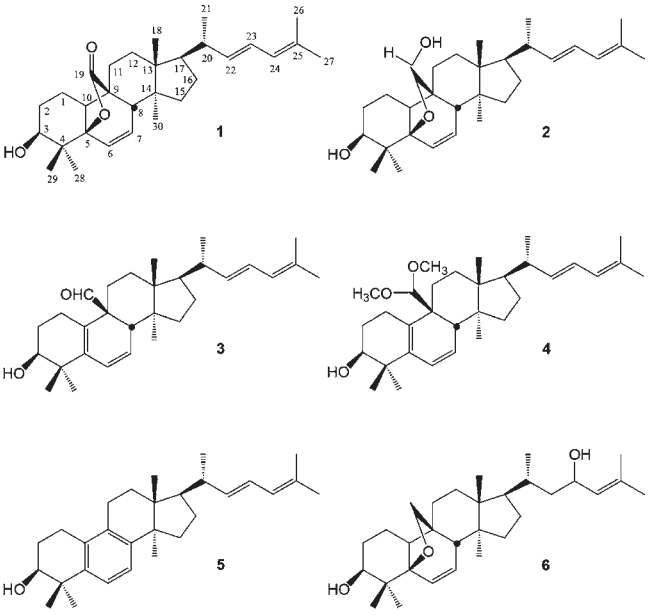

Figure 1. The chemical structures of compounds **1**−**6**.

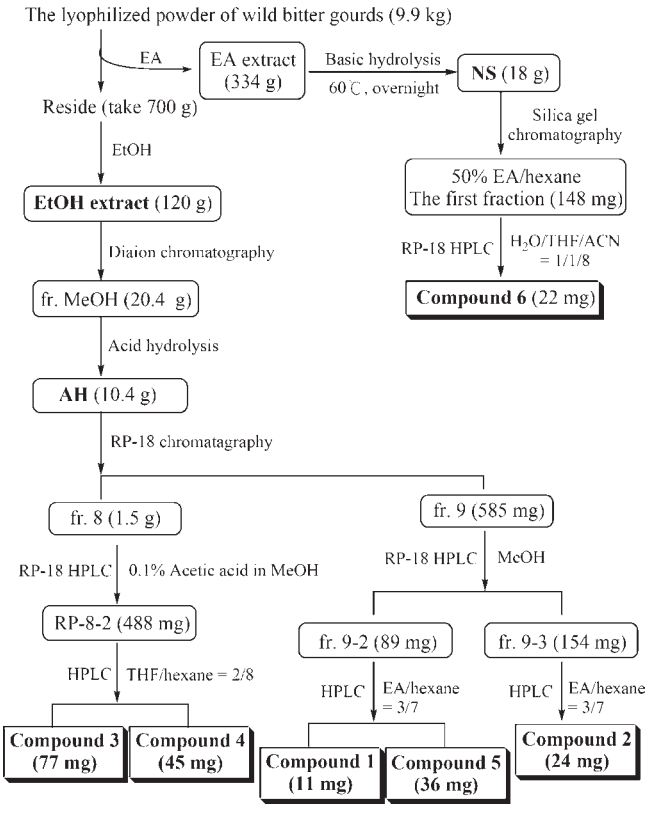

Figure 2. The scheme of separation and purification of *Momordica charantia* extracts.

gene. Cotransfected cells were treated with vehicles, tested samples at varying concentrations or positive control (1 nM E_2). The antagonistic activity was examined by measuring the transactivation of 1 nM E_2 together with samples at varying concentrations. For the transactivation experiments, the crude extracts, fractions, and isolated compounds from MC were dissolved in a minimal amount of absolute ethanol. The reporter gene (ALP) activity was assayed by using pNPP (*p*-nitrophenyl

dx.doi.org/10.1021/jf200418g |*J. Agric. Food Chem.* 2011, 59, 4553–4561

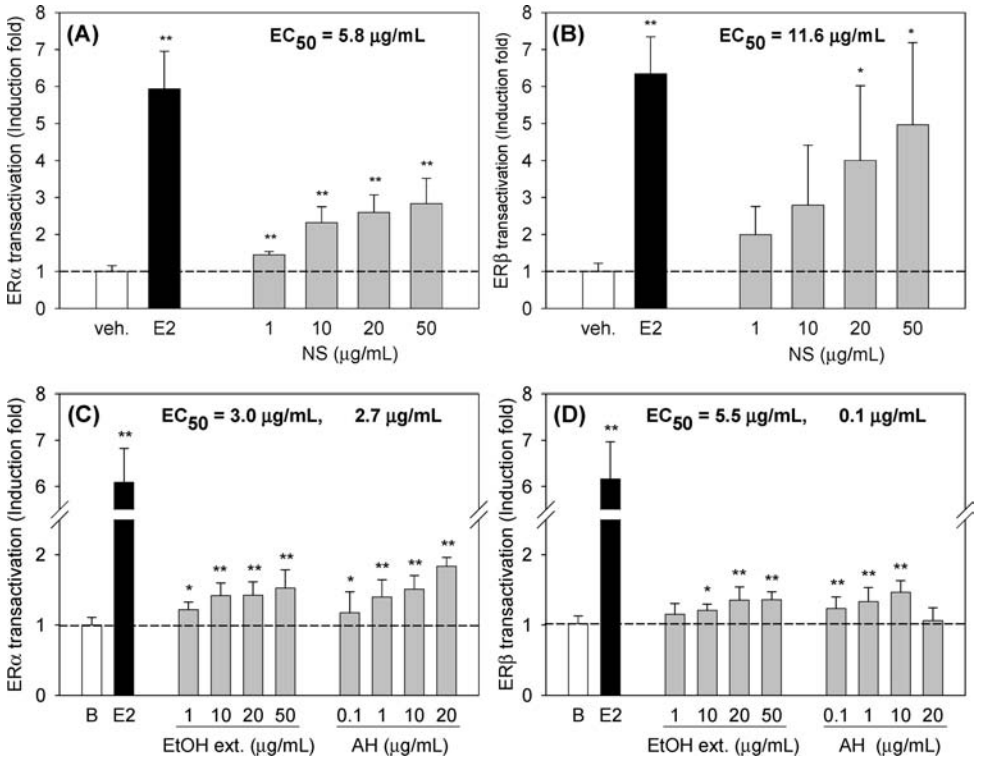

Figure 3. Transactivation of ERα and ERβ by nonsaponifiable (NS) fraction (A and B), EtOH extracts and their acid hydrolyzed extract (AH) (C and D) of *Momordica charantia*. Values are means ± SD of at least three separate experiments with triplicate wells in each. 1 nM 17β-estradiol (E_2) is used as the positive control. * and ** denote significant difference from vehicle at $p < 0.01$ (*) and $p < 0.001$ (**) analyzed by Student's *t*-test.

phosphate) as the substrate.[18] Folds of activation were calculated by taking the ALP activity of vehicle-treated cells as 1.0. The viability of treated cells was checked by the MTT assay. Data reported were confined to those that treatments did not significantly change the cell growth and viability.

Data Analysis. Data reported were expressed as means ± standard deviation of at least three separate experiments with triplicate wells in each. The significance of difference between each treatment and vehicle (or positive control) was analyzed by one-way ANOVA (analysis of variance) or Student's *t* test using SAS (SAS 9.0, Cary, NC) software. Data of heterogeneous variances were transformed to log or root square before the statistical analysis.

RESULTS

The Estrogenic Activity of MC Crude Extract. At a concentration of 50 μg/mL, the nonsaponifiable fraction (NS) transactivated via ERα and ERβ to a maximal activation that was respectively 37% and 75% that of 1 nM E_2 (Figures 3A and 3B). The EtOH extracts and their acid hydrolyzed extract (AH) also transactivated via ERα and ERβ, but to a lower extent (Figures 3C and 3D). The maximal activation via ERα and ERβ by EtOH extracts (at 50 μg/mL) was 10% and 7% that of 1 nM E_2, while AH achieved the maximal activation via ERα (at 10 μg/mL) and ERβ (at 20 μg/mL) that was 17% and 9% that of E_2, respectively.

Identification of New Cucurbitane-Triterpenoid Compounds Isolated from AH. Compound **6** is identified as a known triterpenoid (Figure 1), based on the spectroscopic (MS, ^{13}C and ^{1}H NMR) data identical to the reported data.[19] The spectroscopic data of compounds **1**−**5**, characterized for the first time in this study, are described below. They are all amorphous powders, and their ^{13}C and ^{1}H NMR data are listed in Table 1.

Compound **1** has an optical rotation $[\alpha]_D^{23}$ of −64.8 (*c* 0.01 in MeOH), IR (KBr) absorptions ν_{max} of 3510, 2954, 2876, 1759, 1744, 1696, 1469, 1449, 1404, 1377, 1163, 899, 738 cm^{-1}, UV absorption (MeOH) λ_{max} (log ε) of 238 nm (4.13) and EIMS (70 eV) fragments *m*/*z* (%) of 452 $[M]^+$ (8), 281 (31), 109 (100), 67 (41). The HREIMS of the ion peak at *m*/*z* 452.3279 and ^{13}C NMR data were consistent with the molecular formula $C_{30}H_{44}O_3$ (calculated for 452.3285). The IR spectrum of compound **1** showed absorption bands at 3501 and 1759 cm^{-1} suggestive of hydroxy and γ-lactone functionalities. The ^{1}H and ^{13}C NMR (Table 1) spectra of compound **1** showed the presence of six tertiary methyls [δ_H 0.84, 0.92, 0.93, 1.25, 1.71, 1.73 (3H each, s)], one secondary methyl [δ_H 1.02 (3H, d, J = 6.5 Hz)], and a carbinol proton [δ_H 3.45 (1H, br s)], in addition to the signals for an allyl ABX system of cyclohexene [δ_H 6.17 (1H, dd, J = 9.9, 2.2 Hz, H-6), 5.69 (1H, dd, J = 9.9, 3.3 Hz, H-7), 2.51 (1H, dd, J = 3.3, 2.2 Hz, H-8); δ_C 133.4 (d), 131.1 (d), 44.5 (d)]. These NMR data suggested a structure of cucurbitane-type triterpene, as signals due to the tetracyclic part were similar to those of karavilagenin D,[19] except for the signals of the side chain, 1,1-dimethy-4-alkyl-1,3-butadiene with *E*-form moiety, as revealed by NMR data [δ_H 5.37 (1H, dd, J = 15.0, 8.7 Hz, H-22), 6.14 (1H, dd, J = 15.0, 10.8 Hz, H-23), 5.72 (1H, d, J = 10.8 Hz, H-24), 1.71 and 1.73 (3H each, s); δ_C 138.1 (C-22), 124.4 (C-23), 125.1 (C-24), 133.1 (C-25), 18.2 (C-26), 25.9 (C-27)], the UV absorption band at 238 nm and the fragment ion in the EIMS at *m*/*z* 109 (100%). In a HMBC experiment (Figure 4A), correlations from H-27 (δ_H 1.73) to C-25 (δ_C 133.1), C-24 (δ_C 125.1), from H-24 (δ_H 5.72) to C-22 (δ_C 138.1), C-23 (δ_C 124.4), and from H-21 (δ_H 1.02) to C-17 (δ_C 50.4), C-20 (δ_C 40.3), C-22 (δ_C 138.1) were observed. The stereo structure of **1** was characterized by NOESY experiment (Figure 4B), which showed the correlations

Table 1. ^{1}H (J in Hz, 400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Data for Compounds 1−5

	1		2		3		4		5	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	18.4	1.27−1.32 m, 1.59−1.65 m	17.3	1.44−1.53 m	23.9	2.14−2.26 m	24.2	2.07−2.14 m, 2.55 dt (J = 18.5, 5.9)	24.1	2.52−2.68 m
2	26.5	1.77−1.82 m	27.0	1.71−1.80 m	26.4	1.71−1.88 m	26.6	1.66−1.69 m, 1.75−1.81 m	26.6	1.91−1.96 m, 2.00−2.06 m
3	75.3	3.45 br s	76.1	3.39 br s	75.1	3.50 dd (J = 8.8, 2.9)	75.3	3.44[a]	75.2	3.72 dd (J = 8.8, 2.9)
4	37.0		37.2		38.4		38.1		39.0	
5	85.4		86.5		137.7		133.1		140.6	
6	133.4	6.17 dd (J = 9.9, 2.2)	132.9	6.04 dd (J = 9.8, 2.0)	126.2	6.04 d (J = 10.0)	125.8	6.07 d (J = 9.7)	123.8	7.11 d (J = 8.1)
7	131.1	5.69 dd (J = 9.9, 3.3)	132.3	5.63 dd (J = 9.8, 3.5)	126.8	5.62 dd (J = 10.0, 6.0)	125.6	5.53 dd (J = 9.7, 6.2)	122.8	6.86 d (J = 8.1)
8	44.5	2.51 dd (J = 3.3, 2.2)	41.3	2.84 dd (J = 3.5, 2.0)	39.9	2.63 br d (J = 6.0)	39.8	2.40 d (J = 6.2)	144.5	
9	51.1		48.4		52.2		45.1		132.6	
10	39.9	2.63 dd (J = 12.3, 5.8)	40.5	2.44 dd (J = 10.6, 9.8)	126.8		133.3		132.0	
11	21.7	1.72−1.78 m, 2.19−2.28 m	23.1	1.68−1.77 m	20.9	1.79−1.86 m	18.4	1.79−1.87 m	24.1	2.52−2.68 m
12	29.8	1.22−1.25 m, 1.63−1.70 m	30.5	1.61 m	30.9	1.61−1.67 m	31.3	1.48−1.54 m	30.9	1.91−2.03 m
13	45.1		45.1		45.0		45.4		44.0	
14	47.8		48.0		48.2		48.0		50.2	
15	33.2	1.26−1.33 m	33.5	1.23−1.37 m	31.6	1.15−1.19 m, 1.20−1.28 m	32.1	1.08−1.12 m, 1.17−1.22 m	32.1	1.63−1.70 m, 1.75−1.85 m
16	27.8	1.29−1.36 m	28.3	1.29−1.38 m	27.9	1.22−1.30 m, 1.66−1.72 m	28.1	1.18−1.24 m, 1.63−1.67 m	28.6	1.42−1.46 m, 1.90−1.94 m
17	50.4	1.48−1.56 m	50.2	1.49 m	50.5	1.49−1.56 m	50.4	1.42−1.47 m	50.4	1.67−1.73 m
18	14.8	0.93 s	14.9	0.89 s	14.8	0.90 s	14.6	0.88 s	16.2	0.65 s
19	181.5		105.2	5.12 s	202.3	9.23 s	112.3	3.99 s		
20	40.3	2.12−2.20 m	40.3	2.14 m	40.4	2.10−2.14 m	40.4	2.07−2.14 m	40.9	2.17 m
21	20.5	1.02 d (J = 6.5)	20.4	1.00 d (J = 6.6)	20.5	0.98 d (J = 6.5)	20.5	0.96 d (J = 6.5)	20.7	1.07 d (J = 6.6)
22	138.1	5.37 dd (J = 15.0, 8.7)	138.4	5.38 dd (J = 14.9, 8.6)	138.3	5.35 dd (J = 15.0, 8.7)	138.8	5.34 dd (J = 15.0, 8.8)	138.6	5.44 dd (J = 15.0, 8.4)
23	124.4	6.14 dd (J = 15.0, 10.8)	124.2	6.13 dd (J = 14.9, 10.6)	124.3	6.12 dd (J = 15.0, 10.8)	123.9	6.09 dd (J = 15.0, 10.8)	124.3	6.16 dd (J = 15.0, 11.0)
24	125.1	5.72 d (J = 10.8)	125.1	5.73 d (J = 10.6)	125.1	5.72 d (J = 10.8)	125.2	5.70 d (J = 10.8)	125.2	5.76 d (J = 11.0)
25	133.1		132.5		132.9		132.5		132.8	
26	18.2	1.71 s	18.2	1.71 s	18.2	1.71 s	18.2	1.69 s	18.2	1.73 s
27	25.9	1.73 s	25.9	1.72 s	25.9	1.72 s	25.8	1.70 s	25.9	1.74 s
28	20.3	1.25 s	20.4	1.19 s	22.0	1.03 s	22.4	1.02 s	25.3	1.26 s
29	23.5	0.92 s	23.9	0.82 s	25.8	1.05 s	25.8	1.01 s	29.3	1.30 s
30	19.3	0.84 s	19.7	0.85 s	16.9	0.80 s	16.2	0.65 s	28.2	1.01 s
OCH_3							58.1	3.34 s		
OCH_3							58.8	3.44 s		

[a] Overlapping with other signals.

between the following proton pairs: H_3-18/H-8; H_3-28/H-3, H-10; H_3-30/H-10, H-17. Thus compound **1** was elucidated as cucurbita-6,22(*E*),24-trien-3β-ol-19,5β-olide, unambiguously.

Compound **2** has an optical rotation $[\alpha]_D^{23}$ of −55.0 (*c* 0.02 in MeOH), IR (KBr) absorptions ν_{max} of 3421, 2949, 2875, 1647, 1443, 1377, 1157, 1120, 1084, 961 cm^{-1}; UV absorption (MeOH) λ_{max} (log ε) of 238 nm (4.40) and EIMS (70 eV) fragments *m*/*z* (%) of 454 $[M]^+$ (2), 408 (55), 299 (35), 281 (100), 109 (39). Its HREIMS indicated a molecular ion at *m*/*z* 454.3471 corresponding to a molecular formula of $C_{30}H_{46}O_3$ (calculated for 454.3441). The IR spectrum showed bands attributable to hydroxy (3421 cm^{-1}) and double-bond (1647 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR (Table 1) indicated the presence of 6 tertiary methyls including 2 methyls attached at a double bond, a secondary methyl, a carbinol signal, and an allyl ABX system of cyclohexene. The fragment ion at EIMS *m*/*z* 109 and UV absorption at 238 nm (1,1,4-trialkylbuta-1,3-diene) confirmed the side chain formulas being C_8H_{13}. The NMR data of compounds **2** and **1** were similar except that a signal at δ_C 105.2 (resonance at δ_H 5.12 s) is observed in that of compound **2** instead of δ_C 181.5 suggestive of γ-lactone functionality of compound **1**. As no hydrogen is present at C-5 which occurred as a singlet at δ_C 86.5, an ether linkage between C-5 and C-19 was elucidated. The signal at δ_H 5.12 (s) (resonance at δ 105.2) was assigned to H-19 as the chemical shift coinciding with the hemiacetal functionality and the 5,19-hemiacetal ring is explicated. The stereochemistry of the hydroxyl group in the 5,19-hemiacetal ring should be endo form according to the NOESY correlation between H-19 and H-1 (Figure 4C).[20,21] Based on these and the 2-D NMR spectra, compound **2** was elucidated as 5β,19-epoxycucurbita-6,22(*E*),24-triene-3β,19-diol.

Compound **3** has an optical rotation $[\alpha]_D^{23}$ of −130.3 (*c* 0.01 in MeOH), IR (KBr) absorptions ν_{max} of 3438, 3037, 2956, 2875, 2723, 1712, 1452, 1379, 1035, 987, 960, 737 cm^{-1}, UV absorption (MeOH) λ_{max} (log ε) of 232 (4.42), 239 (4.44), 273 (3.61) and EIMS (70 eV) fragments *m*/*z* (%) of 436 $[M]^+$ (10), 406 (18), 390 (25), 389 (100), 297 (60), 109 (60). Based on the HREIMS *m*/*z* 436.2970, the molecular formula was assigned as $C_{30}H_{44}O_2$ (calculated for 436.3343). The IR spectrum showed bands attributable to hydroxy (3438 cm^{-1}) and aldehyde (2723, 1712 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR spectra (Table 1) indicated the presence of six methyl singlets [δ_H 0.80, 0.90, 1.03, 1.05, 1.71, 1.72 (3H each, s)], a methyl doublet [δ_H 0.98 (3H, d, J = 6.5 Hz)], an *E*-form 1,1,4-trialkyl-substituted buta-1,3-diene [δ_H 5.35 dd

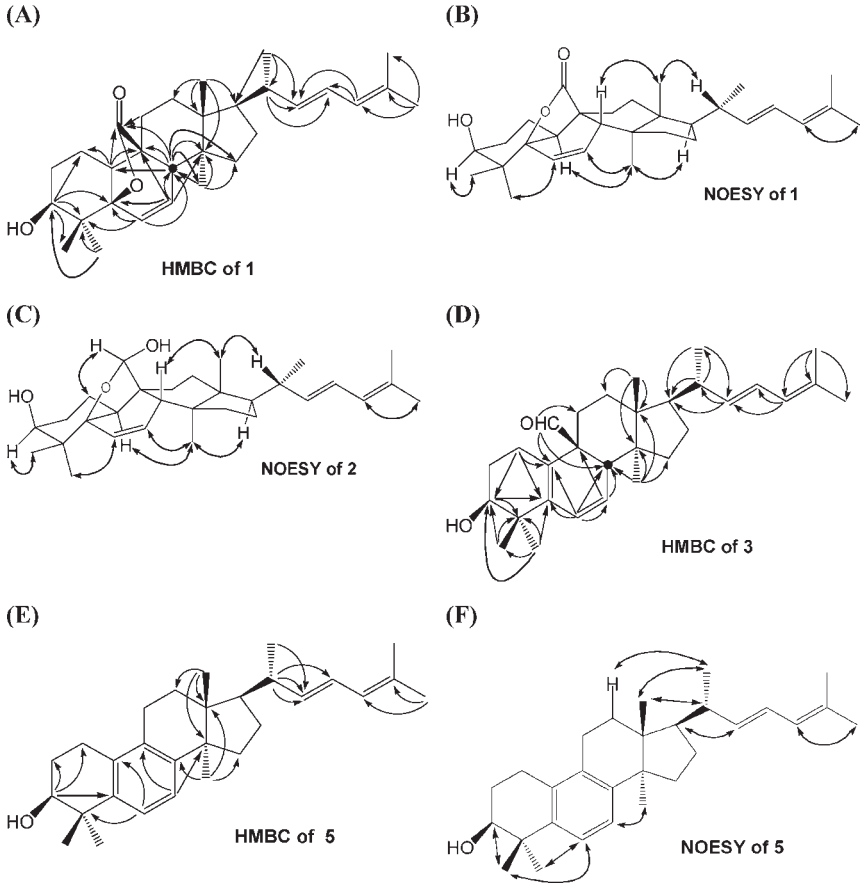

Figure 4. The main HMBC (H→C) correlation of compounds **1** (A), **3** (D), **5** (E) and NOESY (↔) correlations of compound **1** (B), **2** (C), **5** (F).

(1H, dd, J = 15.0, 8.7 Hz), 6.12 (1H, dd, J = 15.0, 10.8 Hz), 5.72 (1H, d, J = 10.8 Hz); δ_C 138.3 (d), 124.3 (d), 125.1(d), 132.9 (s)][UV λ_{max} 239 nm], a cyclohexene [δ_H 5.62 (1H, dd, J = 10.0, 6.0 Hz), 6.04 (1H, d, J = 10.0 Hz); δ_C 126.2 (d), 126.8 (d)], and an axial oxymethine proton [δ_H 3.50 (1H, dd, J = 8.8, 2.9 Hz, H-3)]. The NMR data showed that the signals of the side chain of compound **3** were similar to those of **1**. The tetracyclic skeleton of compound **3** exhibited a cyclohexa-1,3-diene moiety, which was proposed from the UV absorption band at 273 nm and the ^{13}C NMR signals [δ_C 126.8 (s) 137.7 (s), 126.2 (d), 126.8 (d)]. The HMBC correlations (Figure 4D), H-1 (δ_H 2.14−2.26)/C-5 (δ_C 137.7), C-10 (δ_C 126.8); H-6 (δ_H 6.04)/C-5 (δ_C 137.7), C-7(δ_C 126.8), C-8(δ_C 39.9), C-10(δ_C 126.8), suggested that the diene system was located at C-5, C-6, C-7, and C-10. The NMR data of the tetracyclic rings are in good agreement with those of cucurbita-5(10),6,23(*E*)-triene-3β,25-diol,[22] except for the signals of C-19. The NMR data [δ_H 9.23 (1H, s), δ_C 202.3 (s)] indicated the functionality is an aldehyde group. The HMBC correlation, δ_H 9.23/C-8 (δ_C 39.9), C-10 (δ_C 126.8), C-11 (δ_C 20.9), indicated the presence of aldehyde group at C-19. Therefore, compound **3** was determined as 3β-hydroxycucurbita-5(10),6,22(*E*),24-tetraen-19-al.

Compound **4** has an optical rotation $[\alpha]_D^{23}$ of −58.3 (*c* 0.04 in MeOH), IR (KBr) absorptions ν_{max} of 3421, 2964, 2927, 2875, 1715, 1694, 1464, 1452, 1397, 1156, 1099, 1069, 977, 737 cm^{-1}, UV absorption (MeOH) λ_{max} (log ε) of 232 (4.22), (log ε) 239 (4.25), 269 (3.53) and 482 $[M]^+$ (5), 406 (22), 390 (37), 389 (100), 297 (45), 109 (73), 75 (100). The HREIMS implied a molecular formula of $C_{32}H_{50}O_3$ based on the molecular ion at m/z 482.3767 (calculated for 482.3754) and indicated the presence of eight degrees of unsaturation. The IR absorption band at 3421 cm^{-1} designates a hydroxyl group. The ^{1}H and ^{13}C NMR (Table 1) showed the presence of six tertiary methyls, a secondary methyl, a cyclohexa-1,3-diene, a 1,1,4-trisubstituted 1,3-butadiene, an oxygened methine, two methoxys, and an acetal methine groups. The UV λ_{max} 239 and 269 nm confirmed the conjugated system of **4** resembling that of **3**. The ^{1}H and ^{13}C NMR data also resembled those of compound **3**, except that the aldehyde group was replaced by an acetal group at C-19. The NMR signals [δ_H 3.34, 3.44 (3H each, s), 3.99 (1H, s); δ_C 58.1, 58.8, 112.3] indicated the presence of acetal methine attached with two methoxy groups. Based on the above evidence and the 2-D NMR spectra, compound **4** was elucidated as 19-dimethoxycucurbita-5(10),6,22(*E*),24-tetraen-3β-ol.

Compound **5** has an optical rotation $[\alpha]_D^{23}$ of +6.4 (*c* 0.02 in MeOH), IR (KBr) absorptions ν_{max} of 3415, 3012, 2956, 2876, 1606, 1479, 1376, 1047, 987, 960, 821, 737 cm^{-1}, UV absorption (MeOH) λ_{max} (log ε) of 224 (4.14), 230 (4.14), 238 (4.13), 272 (3.01) and 406 $[M]^+$ (5), 309 (20), 297 (32), 279 (18), 109 (100), 67 (23). The molecular ion peak at m/z 406.3241 shown in HREIMS corresponded to the molecular formula $C_{29}H_{42}O$ (calculated for 406.3230) and indicated nine degrees of unsaturation. The IR spectrum suggested the presence of the aromatic (3012, 1606, 1479 cm^{-1}) and hydroxy (3415 cm^{-1}) functionalities. The UV λ_{max} absorption at 238 nm confirmed the presence of 1,1,4-trialkyl-substituted buta-1,3-diene. The ^{1}H NMR spectrum (Table 1) showed resonances for four tertiary

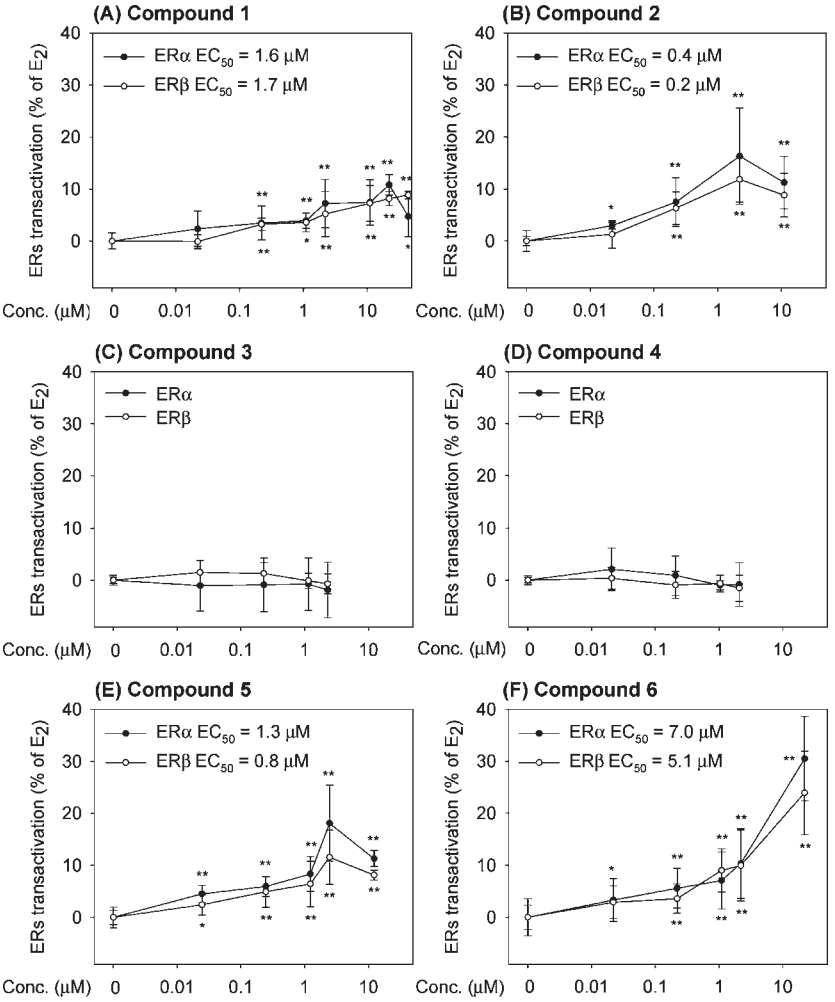

Figure 5. Transactivation of ERα (closed circle) and ERβ (open circle) by isolated compounds **1**−**6** (A−F). 1 nM 17β-estradiol (E_2) serves as the positive control, and its transactivation folds were taken as 100%. All the values are means ± SD of at least three separate experiments with triplicate wells in each. * and ** denote significant difference from vehicle at $p < 0.01$ (*) and $p < 0.001$ (**) analyzed by Student's *t*-test.

methyls [δ_H 0.65, 1.01, 1.26, 1.30 (3H each, s)], a secondary methyl [δ_H 1.07 (3H, d, J = 6.6 Hz)], two vinylic methyls [δ_H 1.73, 1.74 (3H each, s)], one oxygenated methine [δ_H 3.72 (1 H, dd, J = 8.8, 2.9 Hz, H-3)], and three olefinic protons [δ_H 5.44 (1H, dd, J = 15.0, 8.4 Hz), 6.16 (1H, dd, J = 15.0, 11.0 Hz), and 5.76 (1H, d, J = 11.0 Hz)]. Altogether, 29 carbon signals were observed from the ^{13}C NMR spectrum of compound **5** and sorted into seven methyls, six methylenes, two methines, three quaternary, six aromatic, four olefinic, and one oxygenated carbons. The ^{1}H and ^{13}C NMR data also showed that the signals of the side chain of compound **5** were almost the same as those of compound **3**. A benzene ring structure existed in the tetracyclic skeleton of compound **5** which was judged from the two *ortho*-phenyl protons at δ_H 7.11 (1H, d, J = 8.1 Hz) and 6.86 (1H, d, J = 8.1 Hz), and the six ^{13}C NMR signals at δ_C 122.8 (d), 123.8 (d), 140.6 (s), 132.0 (s), 132.6 (s) and 144.5(s). The HMBC correlations (Figure 4E) (H-6 (δ_H 7.11)/C-4 (δ_C 39.0); H-7 (δ_H 6.86)/C-14 (δ_C 50.2)) and NOESY correlation between H-6 and H-28 (Figure 4F) suggested that the benzene ring was located at the B-ring of the tetracyclic skeleton. The NOESY correlations between H-20 and H_3-18, H_3-21 and H-12, as well as H-22 and H-17, confirmed the configuration at C-20 as *R*.[23] Therefore, compound **5** was determined as 19-*nor*-cucurbita-5(10),6,8,22-(*E*),24-pentaen-3β-ol.

Estrogenic Activity of Isolated Compounds. Among the six isolated compounds, compounds **1**, **2**, **5** and **6** transactivated via both subtypes of ER in a dose-dependent manner. As shown in Figure 5, compound **6** showed the highest induction fold and achieved the maximal activation via ERα and ERβ that was 31% and 24% that of E_2 at the concentration of 22 μM. The EC_{50} values for ERα and ERβ were 7.0 and 5.1 μM, respectively. Compounds **1**, **2** and **5** activated via ERα and ERβ to a less extent. The maximal activation via ERα of compounds **1**, **2** and **5** were 12%, 16% and 18% that of 1 nM E_2, while those via ERβ were 9%, 12% and 12%, respectively. Based on the similar dose−response curves and the EC_{50} values, these compounds are not selective to a specific subtype of ER.

In contrast, neither ERα nor ERβ was activated by compounds **3** and **4** at all concentrations tested. When the tested concentration reached 10 μM, the cell survival rate dropped to below 70% that of the vehicle treated.

Antiestrogenic Activity of Isolated Compounds. Except for compound **4**, the remaining five isolated compounds showed significant antiestrogenic activity. As shown in Figure 6, compounds **1**, **2**, **3** and **6** antagonized the transcriptional activity of 1 nM E_2 via both ERα and β. At the concentration of 22 μM, compound **6** exhibited maximal inhibition on 1 nM E_2 via ERα

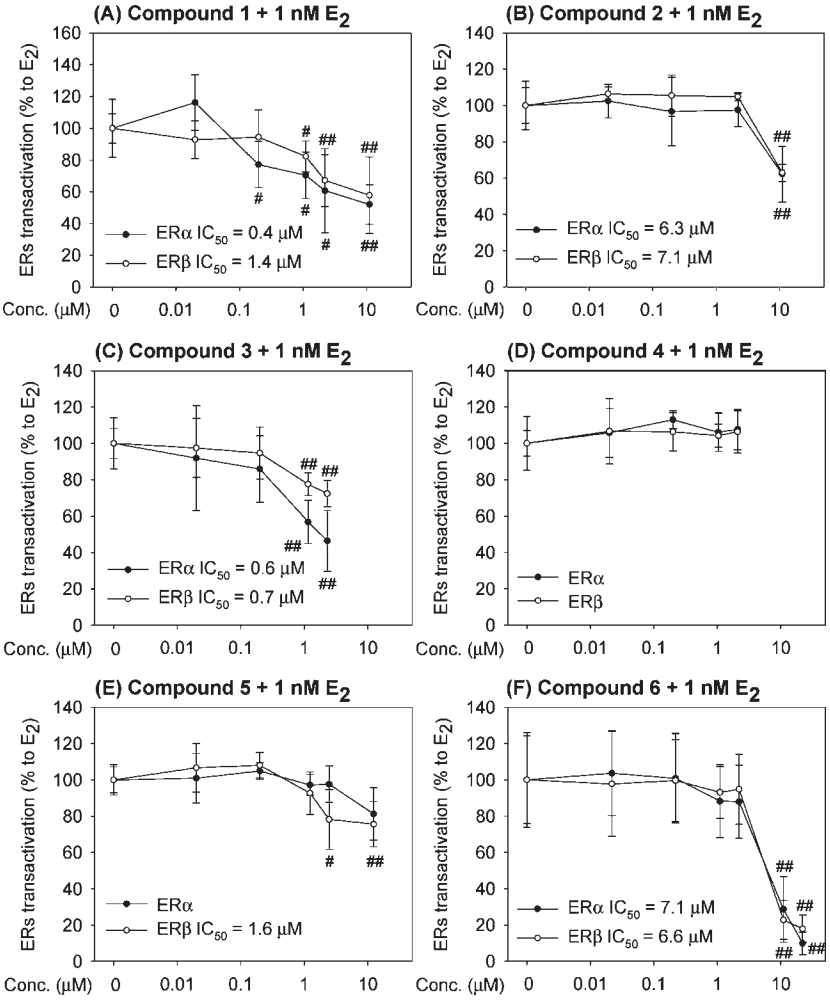

Figure 6. Transactivation of ERα (closed circles) and ERβ (open circles) by compounds **1**−**6** (A−F) in the presence of 17β-estradiol (E_2, 1nM). The transactivation folds of 1 nM E_2 only were taken as 100%. All the values are the means ± SD of at least three separate experiments with triplicate wells in each. # and ## denote significant difference from vehicle (1 nM E_2 only) at $p < 0.05$ (#) or $p < 0.01$ (##) analyzed by Student's *t*-test.

and β to 10% and 18% that of E_2. The IC_{50} values for ERα and ERβ were 7.1 and 6.6 μM, respectively. Compounds **1**−**3** also displayed significant inhibitions but to a less extent than compound **6**. The maximal inhibition on 1 nM E_2 via ERα of compounds **1**−**3** were 52%, 62% and 46% that of E_2, while those via ERβ were 58%, 63% and 72%, respectively. Likewise, the IC_{50} values of these three compounds for ERα and ERβ were also comparable. On the other hand, compound **5** only slightly suppressed the transactivaiton via ERβ of E_2 at a concentration of 12 μM (76% that of 1 nM of E_2), but not via ERα. Compound **4**, however, did not affect the estrogenic activity of 1 nM E_2 at concentrations below 2 μM.

DISCUSSION

In this study, we have isolated and identified five new and one known cucurbitane-type triterpenoid compounds from MC and tested their ER modulating potential. Except for compound **4**, all of the remaining showed partial agonistic and/or antagonistic activity on ERs. These results provide a rational basis, at least in part, to the effect of MC on reproductive function in animal studies.

Two types of triterpenoids, namely, cucurbitane-type (tetracyclic skeleton) and oleanene-type (pentacyclic skeleton), have been isolated from the fruits,[20,24] stems,[22,25] leaves and vines [26] of MC [15] and exist mostly in glycosylated form. It has been suggested that the cucurbitane aglycons may be more important as the pharmacophore.[27,28] Some cucurbitane-type triterpenoids have been demonstrated for their cytotoxic,[29,30] hepatoprotective,[25] chemopreventive,[24] antitumor promoter and antidiabetic activities.[27,31]

Among the compounds isolated in this study, compound **6**, namely, karavilagenine E, has been identified from the dried fruits of MC [19] and reported for its in vitro cytotoxicity against human breast cancer cells (MCF-7).[32] Its ER antagonistic activity observed in this study provides a possible mechanism to its cytotoxicity [32] as the growth of MCF-7 cells is estrogen dependent. The remaining five new compounds identified are characterized by a Δ22,24-conjugated diene in their side chain. A possibility that this conjugated diene resulted from the dehydration of hydroxyl group through the acid hydrolysis cannot be excluded. The tetracyclic cucurbitane nucleus skeleton with oxygenated functionalities of compounds **1** and **2** has been reported [21,24] while that of compounds **3**, **4**, and **5** was further characterized by additional double bonds in the B ring, especially the aromatic B ring of compound **5**, which would contribute to a relatively plane conformation. These identified compounds might (1) exist as aglycons in MC; (2) exist as glycosides in MC; or (3) be formed as a result of the acid hydrolysis. In any

case, the partial agonist/antagonist activity of cucurbitane-type triterpenoids toward ERs is, to our knowledge, first reported in this study.

Among the six cucurbitane-type triterpenoids identified in this study, compounds **1**, **2**, **5** and **6** showed weak ER agonistic activity, while compounds **1**, **2**, **3**, **5** and **6** showed significant antagonist activity. Compounds **3** and **4** showed no ER transactivation mainly because they exhibited substantial cytotoxicity at the concentration level (10 μM) at which the remaining compounds transactivated ERs. However, compound **3**, but not compound **4**, inhibited the transactivation of 1 nM E_2 via both ERs. The steric hindrance from the two methoxy groups attached to C19 of compound **4** might contribute to the little interaction between this compound and ERs.

27-Hydroxycholesterol (27HC) has been shown to be an endogenous SERM as it showed weak agonistic and high antagonistic ERα transcriptional activities, recruited coactivator peptides, provoked ligand-induced conformation change and modulated ERα-target gene expression in MCF-7 cells, in a manner similar to, but to a much lower extent than, E_2.[33,34] Moreover, it showed an antiestrogenic effect on the vasculature in vivo.[34] In addition, β-sitosterol and oxidation products of stigmasterol were reported to induce a weak estrogenic response in cultured human breast cells (MCF-7), to reduce the E_2-induced proliferation of MCF-7 cells, and to decrease the E_2-induced ALP activity in the endometrium Ishikawa cell line.[35,36] It is conceivable that our cucurbitane-type triterpenoid aglycons of MC, also with a 3-hydroxyl group and the tetracyclic steroid-like nucleus, could also interact with ER, as they are structurally similar to 27HC and phytosterol mentioned above.

The relatively weak agonist activity but more marked antagonist activities toward ERs of our compounds are similar to those of many antiestrogens, like 27HC, tamoxifen and other newly developed antiestrogen.[37] The partial agonist/antagonist activity toward ER seems to be an important characteristic of SERMs which are considered potential candidates for managing ER related pathological conditions. It is thus worthy to further examine the SERM potential of our compounds in appropriate models.

Whether an exogenous ER modulator acts as an agonist or an antagonist depends on tissues and endogenous estrogen status.[33] Tamoxifen is well-known to be antiestrogenic in breast tissue but estrogenic in the uterus tissue. Tamoxifen and raloxifene, typical SERMs, display a tissue-selective activity profile, thereby showing estrogenic actions on bone and antiestogenic effects on the breast.[9−11] They are clinically used to treat ER positive breast cancer in pre- and postmenopausal women and considered to function as competitive antagonists that compete with estrogen for ER binding sites in breast and other tissues.

Structurally, many ER antagonists are characteristic of a steroid or steroid-like skeleton with a side chain attached.[38−40] The bulky side chains of antagonists hinder the LBD of ERs from folding into a transcriptionally active conformation, which recruits other proteins known as corepressors to stop genes being switched on by estrogen.[39,41] Noticeably, the cucurbitane-type triterpenoids identified in this study are also characterized by harboring an alkyl side chain and showed significant antagonist activity toward ER.

Compared to the isolated cucurbitane-type triterpenoids, the NS fraction transactivated ERs to a higher extent (Figure 3 and 5). This implies that estrogenic compounds other than these identified cucurbitane-type triterpenoids exist in MC. Indeed, we have also isolated and identified several other types of compounds from MC that showed partial ER agonistic activity (data not shown), including phytol, lutein, phytosterol and loliolide.[42] It would also be of interest to further explore their potential as SERMs.

In conclusion, five new together with one known cucurbitane-type triterpenoids were isolated and identified from MC. Except for compound **4**, the remaining 5 isolates showed partial agonist/antagonist activity on ERs in the cell-based transactivation assay. To our knowledge, this study is thus the first to report the ER modulating activity of cucurbitane type triterpenoids. The significance of these compounds in managing ER related pathological condition is worthy of further investigation.

AUTHOR INFORMATION

Corresponding Author

*Phone: +886-2-33662276. Fax: +886-2-23621301. E-mail: cjjhuang@ntu.edu.tw.

Funding Sources

The study is supported by a grant from National Science Council of Taiwan (NSC 96-2317-B-002-016). The authors also thank China Medical University (CMU98-CT-05), Taiwan Department of Health Clinical Research Center of Excellence (DOH 100-TD-B-111-004) and of Health Cancer Research Center of Excellence (DOH 100-TD-C-111-005) for financial support.

ABBREVIATIONS USED

AH, acid hydrolysis; ALP, alkaline phosphatase; E_2, 17β-estradiol; ER, estrogen receptor; EA, ethyl acetate; EtOH, ethanol; MC, *Momordica charantia*; NS, nonsaponifiable; SERM, selective estrogen receptor modulator

REFERENCES

(1) Grover, J. K.; Yadav, S. P. Pharmacological actions and potential uses of Momordica charantia: a review. *J. Ethnopharmacol.* **2004**, *93*, 123–32.

(2) Nerurkar, P.; Ray, R. B. Bitter melon: antagonist to cancer. *Pharm. Res.* **2010**, *27*, 1049–53.

(3) Hasani-Ranjbar, S.; Nayebi, N.; Larijani, B.; Abdollahi, M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World J. Gastroenterol.* **2009**, *15*, 3073–85.

(4) Yin, J.; Zhang, H.; Ye, J. Traditional chinese medicine in treatment of metabolic syndrome. *Endocr. Metab. Immune Disord. Drug Targets* **2008**, *8*, 99–111.

(5) Basch, E.; Gabardi, S.; Ulbricht, C. Bitter melon (Momordica charantia): a review of efficacy and safety. *Am. J. Health. Syst. Pharm.* **2003**, *60*, 356–9.

(6) Stepka, W.; Wilson, K. E.; Madge, G. E. Antifertility investigation on Momordica. *Lloydia* **1974**, *37*, 645.

(7) Naseem, M. Z.; Patil, S. R.; Patil, S. R.; Ravindra; Patil, S. B. Antispermatogenic and androgenic activities of Momordica charantia (Karela) in albino rats. *J. Ethnopharmacol.* **1998**, *61*, 9–16.

(8) McDonnell, D. P.; Connor, C. E.; Wijayaratne, A.; Chang, C.-y.; Norris, J. D. Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. *Recent Prog. Horm. Res.* **2002**, *57*, 295–316.

(9) Oseni, T.; Patel, R.; Pyle, J.; Jordan, V. C. Selective estrogen receptor modulators and phytoestrogens. *Planta Med.* **2008**, *74*, 1656–65.

(10) Love, R. R.; Mazess, R. B.; Barden, H. S.; Epstein, S.; Newcomb, P. A.; Jordan, V. C.; Carbone, P. P.; DeMets, D. L. Effects of Tamoxifen on Bone Mineral Density in Postmenopausal Women with Breast Cancer. *N. Engl. J. Med.* **1992**, *326*, 852–6.

(11) Cummings, S. R.; Eckert, S.; Krueger, K. A.; Grady, D.; Powles, T. J.; Cauley, J. A.; Norton, L.; Nickelsen, T.; Bjarnason, N. H.; Morrow, M.; Lippman, M. E.; Black, D.; Glusman, J. E.; Costa, A.; Jordan, V. C. The Effect of Raloxifene on Risk of Breast Cancer in Postmenopausal Women. *JAMA, J. Am. Med. Assoc.* **1999**, *281*, 2189–97.

(12) Ososki, A. L.; Kennelly, E. J. Phytoestrogens: a review of the present state of research. *Phytother. Res.* **2003**, *17*, 845–69.

(13) Chao, C. Y.; Huang, C. J. Bitter gourd (Momordica charantia) extract activates peroxisome proliferator-activated receptors and upregulates the expression of the acyl CoA oxidase gene in H4IIEC3 hepatoma cells. *J. Biomed. Sci.* **2003**, *10*, 782–91.

(14) Chuang, C. Y.; Hsu, C.; Chao, C. Y.; Wein, Y. S.; Kuo, Y. H.; Huang, C. J. Fractionation and identification of 9c, 11t, 13t-conjugated linolenic acid as an activator of PPARalpha in bitter gourd (Momordica charantia L.). *J. Biomed. Sci.* **2006**, *13*, 763–72.

(15) Murakami, T.; Emoto, A.; Matsuda, H.; Yoshikawa, M. Medicinal foodstuffs. XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a, -b, -c, -d, -e, -f, -g, and -h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese Momordica charantia L. *Chem. Pharm. Bull. (Tokyo)* **2001**, *49*, 54–63.

(16) Chen, J. C.; Chiu, M. H.; Nie, R. L.; Cordell, G. A.; Qiu, S. X. Cucurbitacins and cucurbitane glycosides: structures and biological activities. *Nat. Prod. Rep.* **2005**, *22*, 386–99.

(17) Cheng, W. Y.; Kuo, Y. H.; Huang, C. J. Isolation and identification of novel estrogenic compounds in yam tuber (Dioscorea alata Cv. Tainung No. 2). *J. Agric. Food Chem.* **2007**, *55*, 7350–8.

(18) Durocher, Y.; Perret, S.; Thibaudeau, E.; Gaumond, M.-H.; Kamen, A.; Stocco, R.; Abramovitz, M. A Reporter Gene Assay for High-Throughput Screening of G-Protein-Coupled Receptors Stably or Transiently Expressed in HEK293 EBNA Cells Grown in Suspension Culture. *Anal. Biochem.* **2000**, *284*, 316–26.

(19) Matsuda, H.; Nakamura, S.; Murakami, T.; Yoshikawa, M. Structure of New Cucurbitane-Type Triterpenes and Glycosides, Karavilagenins D and E, and Karavilosides VI, VII, VIII, IX, X, and IX, from the Fruit of Momordica charantia. *Heterocycles* **2007**, *71*, 331–41.

(20) Li, Q. Y.; Chen, H. B.; Liu, Z. M.; Wang, B.; Zhao, Y. Y. Cucurbitane triterpenoids from Momordica charantia. *Magn. Reson. Chem.* **2007**, *45*, 451–6.

(21) Mulholland, D. A.; Sewram, V.; Osborne, R.; Pegel, K. H.; Connolly, J. D. Cucurbitane triterpenoids from the leaves of Momordica foetida. *Phytochemistry* **1997**, *45*, 391–5.

(22) Chang, C. I.; Chen, C. R.; Liao, Y. W.; Cheng, H. L.; Chen, Y. C.; Chou, C. H. Cucurbitane-type triterpenoids from the stems of Momordica charantia. *J. Nat. Prod.* **2008**, *71*, 1327–30.

(23) Chen, C. R.; Liao, Y. W.; Wang, L.; Kuo, Y. H.; Liu, H. J.; Shih, W. L.; Cheng, H. L.; Chang, C. I. Cucurbitane triterpenoids from Momordica charantia and their cytoprotective activity in tert-butyl hydroperoxide-induced hepatotoxicity of HepG2 cells. *Chem. Pharm. Bull. (Tokyo)* **2010**, *58*, 1639–42.

(24) Akihisa, T.; Higo, N.; Tokuda, H.; Ukiya, M.; Akazawa, H.; Tochigi, Y.; Kimura, Y.; Suzuki, T.; Nishino, H. Cucurbitane-type triterpenoids from the fruits of Momordica charantia and their cancer chemopreventive effects. *J. Nat. Prod.* **2007**, *70*, 1233–9.

(25) Chang, C. I.; Chen, C. R.; Liao, Y. W.; Shih, W. L.; Cheng, H. L.; Tzeng, C. Y.; Li, J. W.; Kung, M. T. Octanorcucurbitane triterpenoids protect against tert-butyl hydroperoxide-induced hepatotoxicity from the stems of Momordica charantia. *Chem. Pharm. Bull. (Tokyo)* **2010**, *58*, 225–9.

(26) Chen, J. C.; Liu, W. Q.; Lu, L.; Qiu, M. H.; Zheng, Y. T.; Yang, L. M.; Zhang, X. M.; Zhou, L.; Li, Z. R.; Kuguacins, F-S cucurbitane triterpenoids from Momordica charantia. *Phytochemistry* **2009**, *70*, 133–40.

(27) Tan, M. J.; Ye, J. M.; Turner, N.; Hohnen-Behrens, C.; Ke, C. Q.; Tang, C. P.; Chen, T.; Weiss, H. C.; Gesing, E. R.; Rowland, A.; James, D. E.; Ye, Y. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. *Chem. Biol.* **2008**, *15*, 263–73.

(28) Ukiya, M.; Akihisa, T.; Tokuda, H.; Toriumi, M.; Mukainaka, T.; Banno, N.; Kimura, Y.; Hasegawa, J.; Nishino, H. Inhibitory effects of cucurbitane glycosides and other triterpenoids from the fruit of Momordica grosvenori on epstein-barr virus early antigen induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate. *J. Agric. Food Chem.* **2002**, *50*, 6710–5.

(29) Clericuzio, M.; Mella, M.; Vita-Finzi, P.; Zema, M.; Vidari, G. Cucurbitane triterpenoids from Leucopaxillus gentianeus. *J. Nat. Prod.* **2004**, *67*, 1823–8.

(30) Zhang, J.; Guo, H.; Tian, Y.; Liu, P.; Li, N.; Zhou, J.; Guo, D. Biotransformation of 20(S)-protopanaxatriol by Mucor spinosus and the cytotoxic structure activity relationships of the transformed products. *Phytochemistry* **2007**, *68*, 2523–30.

(31) Harinantenaina, L.; Tanaka, M.; Takaoka, S.; Oda, M.; Mogami, O.; Uchida, M.; Asakawa, Y. Momordica charantia constituents and antidiabetic screening of the isolated major compounds. *Chem. Pharm. Bull. (Tokyo)* **2006**, *54*, 1017–21.

(32) Ramalhete, C.; Mansoor, T. A.; Mulhovo, S.; Molnar, J.; Ferreira, M. J. Cucurbitane-type triterpenoids from the African plant Momordica balsamina. *J. Nat. Prod.* **2009**, *72*, 2009–13.

(33) DuSell, C. D.; Umetani, M.; Shaul, P. W.; Mangelsdorf, D. J.; McDonnell, D. P. 27-Hydroxycholesterol Is an Endogenous Selective Estrogen Receptor Modulator. *Mol. Endocrinol.* **2008**, *22*, 65–77.

(34) Umetani, M.; Domoto, H.; Gormley, A. K.; Yuhanna, I. S.; Cummins, C. L.; Javitt, N. B.; Korach, K. S.; Shaul, P. W.; Mangelsdorf, D. J. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat. Med.* **2007**, *13*, 1185–92.

(35) Ju, Y. H.; Clausen, L. M.; Allred, K. F.; Almada, A. L.; Helferich, W. G. beta-Sitosterol, beta-Sitosterol Glucoside, and a Mixture of beta-Sitosterol and beta-Sitosterol Glucoside Modulate the Growth of Estrogen-Responsive Breast Cancer Cells In Vitro and in Ovariectomized Athymic Mice. *J. Nutr.* **2004**, *134*, 1145–51.

(36) Newill, H.; Loske, R.; Wagner, J.; Johannes, C.; Lorenz, R. L.; Lehmann, L. Oxidation products of stigmasterol interfere with the action of the female sex hormone 17beta-estradiol in cultured human breast and endometrium cell lines. *Mol. Nutr. Food Res.* **2007**, *51*, 888–98.

(37) Yamamoto, Y.; Shibata, J.; Yonekura, K.; Sato, K.; Hashimoto, A.; Aoyagi, Y.; Wierzba, K.; Yano, S.; Asao, T.; Buzdar, A. U.; Terada, T. TAS-108, a Novel Oral Steroidal Antiestrogenic Agent, Is a Pure Antagonist on Estrogen Receptor α and a Partial Agonist on Estrogen Receptor β with Low Uterotrophic Effect. *Clin. Cancer Res.* **2005**, *11*, 315–22.

(38) von Angerer, E.; Biberger, C.; Holler, E.; Koop, R.; Leichti, S. 1-carbamoylalkyl-2-phenylindoles: Relationship between side chain structure and estrrogen antagonism. *J. Steroid Biochem. Mol. Biol.* **1994**, *49*, 51–62.

(39) Dayan, G.; Lupien, M.; Auger, A.; Anghel, S. I.; Rocha, W.; Croisetière, S.; Katzenellenbogen, J. A.; Mader, S. Tamoxifen and Raloxifene Differ in Their Functional Interactions with Aspartate 351 of Estrogen Receptor α. *Mol. Pharmacol.* **2006**, *70*, 579–88.

(40) Roelens, F.; Heldring, N.; Dhooge, W.; Bengtsson, M.; Comhaire, F.; Gustafsson, J.-Å.; Treuter, E.; De Keukeleire, D. Subtle Side-Chain Modifications of the Hop Phytoestrogen 8-Prenylnaringenin Result in Distinct Agonist/Antagonist Activity Profiles for Estrogen Receptors α and β. *J. Med. Chem.* **2006**, *49*, 7357–65.

(41) Nettles, K. W.; Sun, J.; Radek, J. T.; Sheng, S.; Rodriguez, A. L.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S.; Greene, G. L. Allosteric control of ligand selectivity between estrogen receptors alpha and beta: implications for other nuclear receptors. *Mol. Cell* **2004**, *13*, 317–27.

(42) Hong, Y. H.; Wang, S. C.; Hsu, C.; Lin, B. F.; Kuo, Y. H.; Huang, C. J. Phytoestrogenic Compounds in Alfalfa Sprout (Medicago sativa) beyond Coumestrol. *J. Agric. Food Chem.* **2011**, *59*, 131–7.