Tetrahedron 65 (2009) 10535–10543

Contents lists available at ScienceDirect

Tetrahedron

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

Structure elucidation by synthesis of four metabolites of the antitumor drug ENMD-1198 detected in human plasma samples

Zhenglai Fang [a], Gregory E. Agoston [b], Gaetan Ladouceur [b], Anthony M. Treston [b], LiQuan Wang [c], Mark Cushman [a,d,*]

[a] Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, IN 47907, USA
[b] EntreMed, Inc., Rockville, MD 20850, USA
[c] XenoBiotic Laboratories, Inc.,107 Morgan Lane, Plainsboro, NJ 08536, USA
[d] The Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Received 11 August 2009
Received in revised form 12 October 2009
Accepted 13 October 2009
Available online 15 October 2009

ABSTRACT

ENMD-1198 is a biologically active analogue of the antitumor drug 2-methoxyestradiol. Four human metabolites of ENMD-1198 were identified through synthesis and liquid chromatography/mass spectrometry comparisons of the metabolites with the synthetic standards. Two metabolites (**3** and **4**) are epimers resulting from benzylic hydroxylation at C-6. Two additional metabolites (**5** and **6**) are formed by epimeric hydroxylation at C-6 and α-epoxidation of the 16,17-alkene. The syntheses provided sufficient quantities of the metabolites for cytotoxicity studies to proceed. The 6-β-ol **4** was moderately less cytotoxic than the parent drug, while the remaining three metabolites (**3**, **5**, and **6**) were significantly less cytotoxic.

1. Introduction

Microtubules are essential components of cellular structure. They are made of heterodimeric α- and β-tubulin subunits and display highly dynamic behavior.[1] Microtubules have been a popular anticancer drug target for decades due to their participation in a wide variety of critical cellular functions, such as motility, division, shape maintenance, and intracellular transport.[2] Antitubulin drugs can be categorized by their mechanism of action as either tubulin polymerization inhibitors or microtubule stabilizers.[3,4] Some common tubulin polymerization-inhibiting anticancer drugs have been derived from natural sources, including colchicine, 2-methoxyestradiol (**1**), vinblastine, vincristine, and combretastatin A4. Well-known microtubule-stabilizing anticancer drugs include paclitaxel (Taxol®), docetaxel (Taxotere®), the epothilones, the eleutherobins, and discodermolide.[5] These small molecules bind to different pockets on tubulin and thereby exert diverse effects on microtubule dynamics. Limitations of these compounds often include poor oral bioavailability, high toxicity, difficulty of isolation or synthesis from natural sources, and drug resistance. Therefore, there is continued interest in modifications of these natural products to improve their pharmacological properties. Recently, researchers at EntreMed, Inc. developed a promising analogue of 2-methoxyestradiol (**1**), ENMD-1198 (**2**), for the treatment of various cancers (Fig. 1).[6–8]

Both in vitro liver microsomal studies and investigation of the metabolism of **2** in rats and dogs suggested a number of possible metabolites. Analysis by HPLC and mass spectrometry indicated that one predominant metabolite has a molecular weight that is consistent with a benzylic hydroxylation product (6-hydroxy metabolite of **2**), while another metabolite has a molecular weight indicative of further epoxidation of the 16(17)-alkene in the D-ring. Both alkene epoxidation and benzylic hydroxylation are common metabolic reactions catalyzed by cytochrome P450 enzymes. Four possible structures **3**, **4**, **5**, and **6** of these metabolites were

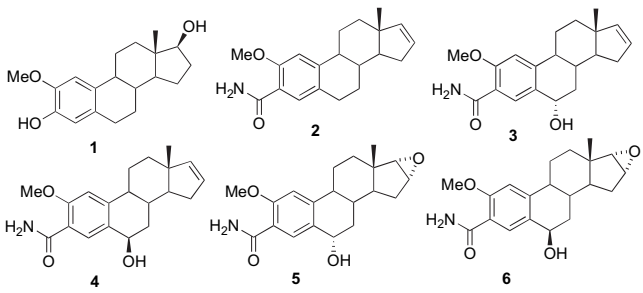


Figure 1. 2-Methoxyestradiol, ENMD-1198, and possible metabolites of ENMD-1198.

* Corresponding author.
E-mail address: cushman@purdue.edu (M. Cushman).

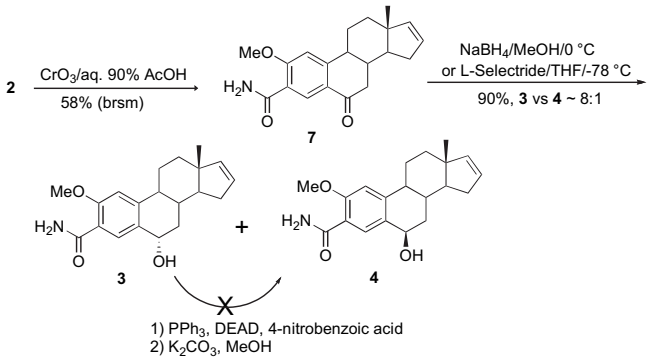
0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.10.046

considered. The present study had two objectives. The first goal was to determine the structures of the metabolites of ENMD-1198 through stereoselective syntheses of all four of them and comparison of their properties with those of the trace quantities of the metabolites that were available in human blood plasma. The second aim was to carry out practical syntheses of the metabolites in sufficient quantities so that their biological properties could be determined. This paper describes the synthesis of all four compounds **3**–**6**, as well as their in vitro characterization and correlation with human metabolites of **2**.

2. Results and discussion

2.1. Synthesis

The initial approach toward these four possible metabolites was to use readily available **2** (Scheme 1) as starting material. When **2** was oxidized with CrO_3 in 90% AcOH under optimized conditions,[9] benzylic ketone **7** was obtained in 58% yield (based on recovered starting material) with 68% conversion by weight. The moderate yield is understandable since oxidation could easily occur at the competing allylic carbon C-15. When **7** was reduced with either $NaBH_4$ or L-Selectride at 0 °C, a mixture of **3** and **4** (8:1 ratio by ^1H NMR) was obtained that could not be separated on a preparative scale. Attempts to make separable derivatives of the 6-OH and 3-

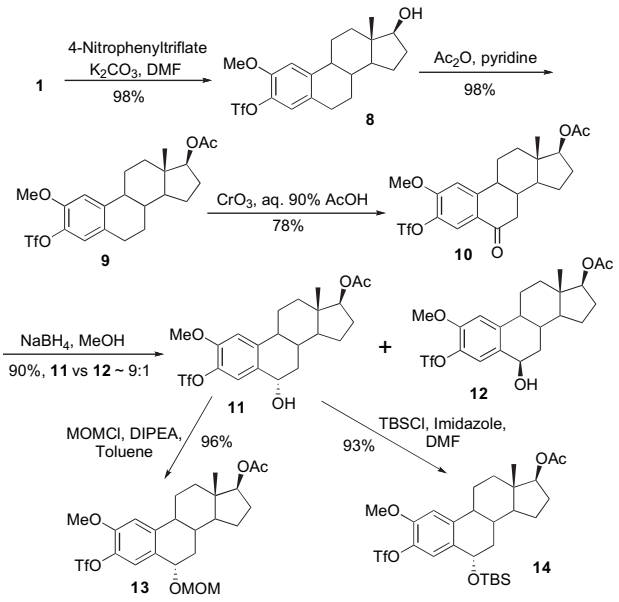


Scheme 1. Attempted synthesis of metabolites **3** and **4** from **2**.

$CONH_2$ of **3** and **4** also resulted in mixtures that could not be separated by flash column chromatography or recrystallization.

Attempted use of the Mitsunobu reaction/basic hydrolysis to invert the C-6 stereocenters of **3** and **4** led to mixtures of **3** and **4** with the same or with increased ratio of **3** versus **4**. A potential explanation for these results is that the Mitsunobu reaction takes place through an S_N1 mechanism instead of the more common S_N2 mechanism with ordinary secondary alcohol substrates. In this system, the 2-methoxy group in the A ring is *para* to the C-6 benzylic alcohol and could stabilize a benzylic cation intermediate.[10]

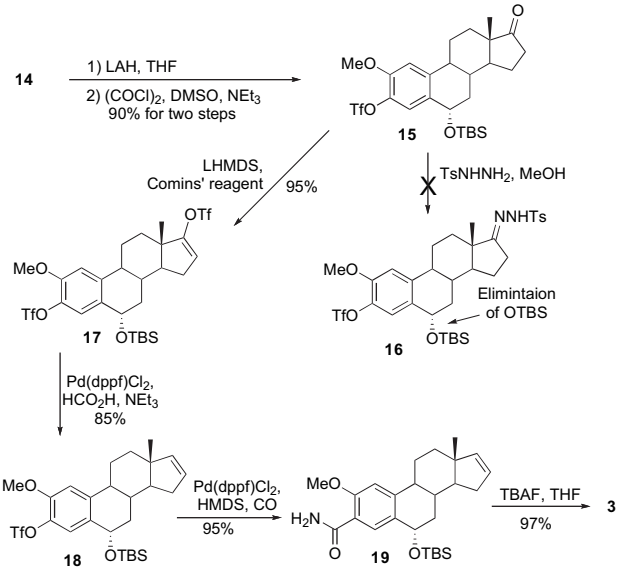
Since the attempts to prepare pure **3** and **4** from **2** were unsuccessful, attention was directed to use of **1** as a starting material, which is commercially available in enantiomerically pure form. The resulting synthetic approach toward pure **3** is shown in Scheme 2. Initially, the 3-phenol was selectively converted to triflate ester **8**. The 17-hydroxy group of compound **8** was protected as its acetate to afford intermediate **9**. Benzylic oxidation of **9** under optimized conditions with CrO_3 in 90% aqueous acetic acid at 15 °C for 2 h afforded ketone **10** in 78% yield. Reduction of compound **10** with sodium borohydride gave a mixture of 6α-ol **11** and 6β-ol **12** in a 9:1 ratio, respectively. The stereochemistry of **11** and **12** was assigned according to the appearance of the C6 methine proton. For 6α-ol **11**, the axial C6 methine appears as a doublet of doublets with a characteristic axial–axial coupling constant of 17.7 Hz and an axial–



Scheme 2. Approach to synthesis of possible metabolites from **1**.

equatorial coupling constant of 7.7 Hz. In contrast, the equatorial C6 methine of **12** overlapped with the C17 methine to produce a two-proton apparent triplet with an average coupling constant of 8.7 Hz. Compounds **11** and **12** were easily separated by column chromatography.

With the pure alcohol **11** in hand, an appropriate protecting group must be selected before the transformations in Scheme 3. When the MOM-protected compound **13** was treated with various acids for deprotection of the alcohol, only elimination product having a 6(7)-alkene was obtained. Based on previous experience with the Mitsunobu reaction in this series, a stabilized benzylic cation is very easily formed even under mild acidic conditions even though the 6α-OMOM of **13** is pseudo-equatorial. A successful protecting group must therefore be removed under non-acidic conditions so that elimination of 6-OMOM can be avoided. Additionally, the protecting group for the 6α-hydroxy group should ideally be compatible with the acetate-protecting group for the 17β-hydroxy group. TBS was therefore chosen as the protecting



Scheme 3. Synthesis of metabolite **3**.

group for the 6α-hydroxy group and alcohol **11** was transformed to its TBS ether **14**.

Compound **14** was then converted to the 17-ketone **15** in a two-step transformation (Scheme 3). Basic hydrolysis of the C-17 acetate protecting group, followed by oxidation under Swern conditions, resulted in a 75% yield of **15** over two steps. Alternatively, reductive deprotection of the C-17 acetate group, followed by Swern oxidation, gave a better yield of 90% for the conversion of **14** to **15**.

When **15** was reacted with $TsNHNH_2$ in refluxing MeOH in order to produce a hydrazone for a Shapiro sequence to introduce the 16(17)-alkene,[11] the 6α-OTBS ether eliminated to afford 6(7)-alkene. Consequently, the strategy for introduction of the 16(17)-alkene was changed to the palladium-catalyzed reduction of enol triflates.[12,13] To this end, compound **15** was treated with LHMDS in THF followed by trapping the enolate with Comins reagent to give enol triflate **17**.[14] Several reaction conditions were tested to selectively reduce the 17-vinyl triflate in the presence of the 3-aryl triflate. The best reaction conditions used $Pd(dppf)Cl_2$–dichloromethane adduct as the catalyst at room temperature for 12 h (entry 4 in Table 1). Under

Table 1
Optimization of selective reduction of enol triflate **17**[a]

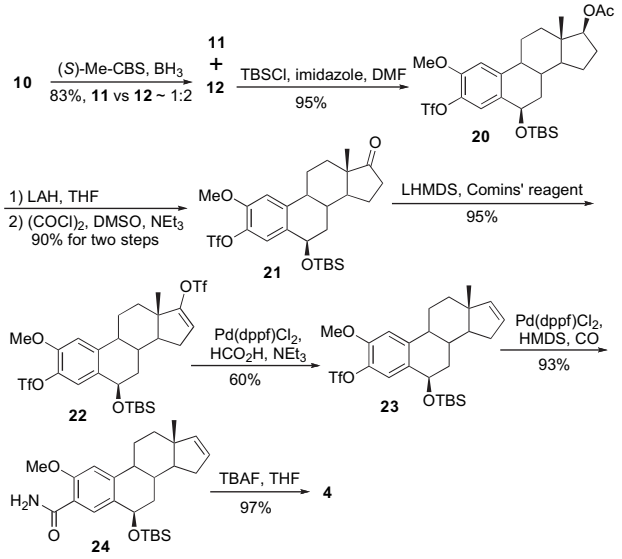
Entry	Catalyst	Temp (°C)	Time (min)	Conversion (%)	Yield (%)
1	$Pd(OAc)_2/PPh_3$	60	10	100	74
2	$Pd(OAc)_2/PPh_3$	rt	720	0	0
3	$Pd(dppf)Cl_2$	60	5	100	62
4	$Pd(dppf)Cl_2$	rt	720	100	85

[a] All reactions were carried out with 0.2 mmol of **17**, 0.21 mmol of formic acid, and 0.30 mmol of triethylamine in 2.0 mL of DMF.

these optimized conditions, the desired alkene **18** was obtained in 85% yield.

The completion of the synthesis of possible metabolite **3** was accomplished successfully in two steps from intermediate **18**. First, carbonylation of aryl triflate **18** followed by trapping with HMDS and acidic workup gave the primary amide **19** in 95% yield.[15] Deprotection of the TBS group furnished the metabolite candidate **3** in almost quantitative yield.

The synthesis of the isomeric potential metabolite **4** utilized a similar reaction sequence except CBS reduction of ketone **10** was utilized to afford the 6β-ol **12** as the major product. Under optimized conditions, ketone **10** was reduced with 1.2 equiv of $BH_3\cdot THF$ catalyzed by (*S*)-Me-CBS at 0 °C for 2 h to provide a mixture of **11**



Scheme 4. Synthesis of metabolite **4**.

and **12** in about 83% yield in a 1:2 ratio (Scheme 4).[16,17] The 6β-ol **12** was then protected as its TBS ether **20**. Reductive deprotection of the 17-acetate followed by oxidation under Swern conditions gave 17-ketone **21** in about 90% yield. Unfortunately, conversion of **20** to **21** using basic hydrolysis and Swern oxidation did not work due to the concomitant hydrolysis of the 3-aryl triflate even under very mild basic conditions. Ketone **21** was then transformed to enol triflate **22** in 95% yield.

The optimized conditions listed as entry 4 in Table 1 were used to selectively reduce the 17-vinyl triflate in compound **22** to give **23** in a moderate yield (40%). The reduction conditions were further refined by using less coordinating solvents (Table 2). THF affords the best balance between reactivity and selectivity of the solvents

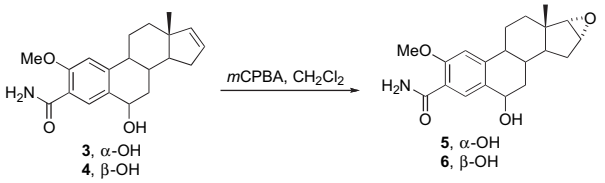
Table 2
Optimization of selective reduction of enol triflate **22**[a]

Entry	Solvent	Time (min)	Conversion (%)	Yield (%)
1	DMF	240	100	40
2	Toluene	720	60	50
3	THF	720	100	60

[a] All reactions were carried out with 0.2 mmol of **22**, 0.21 mmol of formic acid, and 0.30 mmol of triethylamine in 2.0 mL specified solvent at room temperature with $Pd(dppf)Cl_2$ dichloromethane adduct as catalyst.

tested (entry 3 in Table 2). Under such conditions, the alkene **23** was obtained in 60% yield. Advanced intermediate **23** was then converted to potential metabolite **4** in two steps under identical conditions used for the synthesis of metabolite **3**.

With compounds **3** and **4** in hand, the other two potential metabolites **5** and **6** were prepared (Scheme 5). Treatment of a solution of **3** in dichloromethane with *m*-CPBA at 0 °C for 30 min afforded the desired compound **5** in 95% yield. Similarly, **6** was obtained from **4** in 95% yield. The stereochemistries of the 16,17-epoxides in **5** and **6** are expected to be 16α,17α due to the more shielded β face by the C18 angular methyl group. Furthermore, the chemical shifts of 16-H and 17-H and their coupling constants in both **5** (3.41 ppm, 3.18 ppm, 2.8 Hz) and **6** (3.20 ppm, 3.43 ppm, 2.8 Hz) were con-



Scheme 5. Synthesis of metabolites **5** and **6**.

sistent with those reported by Che and Zhang[18] in a similar product (3.20 ppm, 3.42 ppm, 2.8 Hz). In comparison, the chemical shifts of 16-H and 17-H in a similar 16β,17β-epoxide show a set of significantly different values (3.23 ppm, 3.50 ppm, 3.0 Hz).[19]

2.2. Metabolism

An LC/MS study was initiated in order to investigate the possible metabolism of **2** to compounds **3**–**6**. The metabolites were obtained from human plasma samples of two patients who had been treated with **2** for either 31 days (Patient 122) or for 4 h (Patient 121). Acetonitrile extracts of the plasma samples were evaporated and the residues were dissolved in methanol–water (1:1) for analysis. HPLC elution was carried out using a gradient from 20 mM ammonium formate to methanol. The HPLC retention times, full scan mass spectra, and product ion scan mass spectra of the metabolites were compared with those of the synthetic materials by LC/MS and LC/MS/MS.

Table 3
HPLC retention times and mass spectral data of synthetic standards and metabolites

Metabolite	Standard HPLC RT (min)[a]	Metabolite HPLC RT (min)[b]	Mass (MH^+)[c]	Standard ESIMS MH^+, m/z[d]	Standard MS/MS MH^+, m/z[e]	Metabolite MS/MS MH^+, m/z[f]
3	69.02	69.00	328.18	328.24	328.20	328.22
4	69.73	69.71	328.18	328.26	328.18	328.20
5	51.96	51.89	344.18	344.23	344.19	344.22
6	53.99	54.00	344.18	344.24	344.18	344.23

[a] HPLC retention times of synthetic standards.
[b] HPLC retention times of human metabolites.
[c] Calculated molecular weights.
[d] ESIMS m/z values of the protonated molecular ions determined for synthetic standards.
[e] MS/MS m/z values of the protonated molecular ions determined for synthetic standards.
[f] MS/MS m/z values of the protonated molecular ions determined for human metabolites.

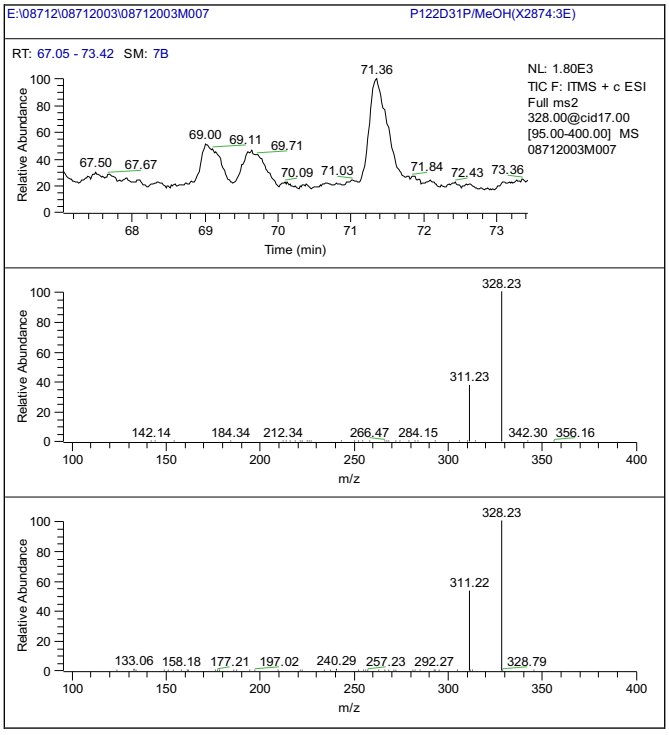


Figure 2. LC/(+)CID-MS/MS spectral data of **3** (retention time 69.00 min) and **4** (retention time 69.71 min) from plasma sample of Patient 122 day 31. Top: TIC of MS^2 at m/z 328. Middle: product ion scan of m/z 328 at 69.07 min. Bottom: product ion scan of m/z 328 at 69.68 min.

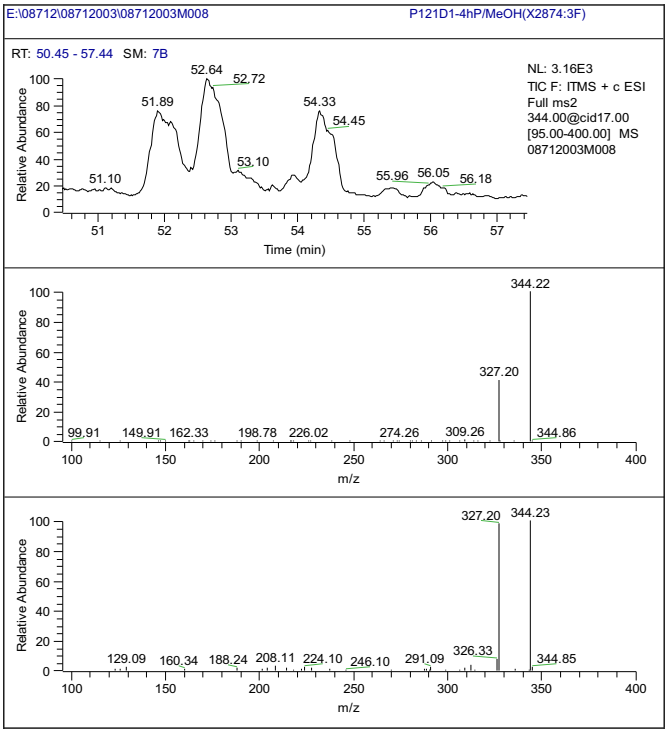


Figure 3. LC/(+)CID-MS/MS spectral data of **5** (retention time 51.89 min) and **6** (retention time 54 min) from plasma sample of Patient 121 day 1, 4 h. Top: TIC of MS^2 at m/z 344. Middle: product ion scan of m/z 344 at 51.89 min. Bottom: product ion scan of m/z 344 at 54.00 min.

The HPLC retention times and mass spectrometry m/z values of the synthetic compounds and the metabolites are listed in Table 3. Close correspondence was observed between the mass spectrometry data and the retention times of the synthetic compounds and the metabolites. As shown in Figures 2 and 3, metabolites **3** and **4** appeared with retention times of 69.00 and 69.71 min, respectively, while **5** and **6** eluted earlier, with retention times of 51.89 and 54.00 min. The presence of all four metabolites in both patients was confirmed by both full scan and product ion scan experiments in this study. More detailed results are included in Supplementary data.

2.3. Cytotoxicity studies

The four possible metabolites and **2** were tested for their inhibitory properties against five cell lines: HCT-116 human colon carcinoma cells, MDA-MB-453 breast carcinoma cells, SkBr3 breast carcinoma cells, SW626 ovarian carcinoma cells, and HUVEC human umbilical vein endothelial cells. The biological results are shown in Table 4. The relative cytotoxicities were in the order **2**>**4**≫**3**>**5**>**6**, with the metabolite **4** being only moderately less active than the parent compound **2** (i.e., 1.3- to 5-fold less active in 4/5 cell types). Overall, the biological consequence of ENMD-1198 (**2**) metabolism is to convert it to less cytotoxic metabolites that are more polar and are expected to be more readily eliminated by urinary excretion.

Table 4
Cytotoxicities of possible metabolites

Compound	Cytotoxicity (GI_{50} in μM)[a]				
	HCT-116	MDA-MB-453	SkBr3	SW626	HUVEC
2	0.7	0.02	0.4	1.5	0.05
3	40	10	25	50	7
4	1	0.4	2	2	0.5
5	80	15	50	80	10
6	>100	100	>100	>100	15

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. The values are representative of replicate independent experiments.

3. Conclusion

Stereoselective syntheses of four human metabolites of the anticancer drug ENMD-1198 have been executed in stereochemically defined fashion, and the products have been used to establish the structures of the metabolites. The metabolic pathway involves benzylic hydroxylation at C-6 with formation of both epimers, as well as stereoselective α-epoxidation of the C16–C17 double bond. The synthetic compounds were obtained in sufficient quantities to allow cytotoxicity studies to be performed. The 6-β alcohol **4** was moderately less cytotoxic than the parent compound, while the remaining three metabolites were significantly less cytotoxic. The synthetic routes to the four metabolites will provide sufficient material to allow future biological studies to proceed.

4. Experimental section

4.1. General information

All solvents were purified by standard procedures. THF and Et_2O were dried over 4 Å molecular sieves and distilled prior to use from sodium–benzophenone under argon. Toluene was distilled from sodium and stored over 4 Å molecular sieves under argon. CH_2Cl_2 and CH_3CN were distilled from CaH_2 under argon prior to use. Unless noted otherwise, NMR spectra were obtained at 300 MHz (^1H) and 75 MHz (^{13}C) in $CDCl_3$ using $CHCl_3$ as the internal standard. Multiplicities were recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), and m (multiplet). IR spectra were recorded using FT-IR. Flash chromatography was performed with 230–400 mesh silica gel. TLC was carried out using silica gel IB2-F plates of 2.5 mm thickness. Melting points are uncorrected. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. All yields given refer to isolated yields.

*4.1.1. (6S,13R)-6-Hydroxy-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3-carboxamide (**3**).* Compound **19** (33.5 mg, 0.076 mmol) was dissolved in anhydrous THF (1.0 mL). TBAF solution (1.0 M in THF, 83.5 μL, 0.0835 mmol) was added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H_2O (2.0 mL). The reaction mixture was extracted with ethyl acetate (5 mL×3). The combined organic layer was washed with H_2O and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with hexanes–ethyl acetate–methanol (46:46:8, v/v/v), to afford the desired product **3** (24.0 mg, 97%) as a white solid: mp 140–142 °C. ESIMS *m*/*z* (rel intensity) 328 (MH^+, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.72 (br s, 1H), 6.85 (s, 1H), 6.13 (br s, 1H), 5.90 (dd, *J*=1.5, 5.7 Hz, 1H), 5.74 (m, 1H), 4.92 (dd, *J*=6.8, 10.0 Hz, 1H), 3.94 (s, 3H), 2.82 (br s, 1H), 2.55–1.40 (m, 11H), 0.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.4, 156.9, 146.9, 143.6, 133.3, 132.1, 129.5, 118.6, 107.8, 68.7, 56.0, 55.0, 45.6 (2C), 38.2, 36.7, 35.8, 31.8, 26.4, 17.1; HRESIMS *m*/*z* calcd for $C_{20}H_{26}NO_3$ (MH^+) 328.1913, found 328.1917. The purity was determined to be 100% by HPLC analysis performed on a Waters Symmetry® C18 column (5 μm, 4.8×150 mm), eluting with 60% MeOH–40% H_2O at a flow rate of 1 mL/min (UV detection at 254 nm). The compound had a retention time of t_R=28.030 min.

*4.1.2. (6R,13R)-6-Hydroxy-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3-carboxamide (**4**).* Compound **24** (82.0 mg, 0.186 mmol) was dissolved in anhydrous THF (2.0 mL). TBAF solution (1.0 M in THF, 204 μL, 0.204 mmol) was added. The reaction mixture was stirred at room temperature overnight. The reaction was then quenched with H_2O (5 mL) and the mixture was extracted with ethyl acetate (10 mL×3). The combined organic solution was washed with H_2O (5 mL) and brine (5 mL). The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography on SiO_2, eluting with pure ethyl acetate to ethyl acetate–methanol (96:4), afforded the desired product **4** (59 mg, 97%) as colorless oil. ESIMS *m*/*z* (rel intensity) 328 (MH^+, 99), 310 ($MH-H_2O^+$, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.71 (br s, 1H), 6.93 (s, 1H), 5.91 (m, 1H), 5.77 (m, 1H), 4.89 (br s, 1H), 3.97 (s, 3H), 2.40–1.50 (m, 12H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 157.3, 146.9, 143.6, 134.3, 131.8, 129.6, 118.8, 108.0, 66.4, 56.0, 55.2, 45.9, 36.6, 35.9, 31.8, 31.2, 26.1, 17.3; HRESIMS *m*/*z* calcd for $C_{20}H_{26}NO_3$ (MH^+) 328.1913, found 328.1915. The purity was determined to be 92% by HPLC analysis carried out on a Waters Symmetry® C18 column (5 μm, 4.8×150 mm), eluting with 60% MeOH–40% H_2O at a flow rate of 1 mL/min (UV detection at 254 nm). The compound had a retention time of t_R=33.246 min.

*4.1.3. (6S,13S,16R,17S)-6-Hydroxy-2-methoxy-13-methyl-6,8,9,11,12,13,14,15,16,17-decahydro-7H-20-oxa-cyclopropa[16,17]cyclopenta[a]phenanthrene-3-carboxylic acid amide (**5**).* A solution of **3** (34 mg, 0.104 mmol) in anhydrous dichloromethane (1.0 mL) at 0 °C was treated with *m*-CPBA (77%, 24.5 mg, 0.109 mmol). The reaction mixture was stirred at this temperature for 30 min. The reaction mixture was filtered to remove the white 3-chlorobenzoic acid. The organic solution was diluted with dichloromethane (5 mL) and washed with aqueous $NaHSO_3$, aqueous $NaHCO_3$, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography on SiO_2, eluting with 100% ethyl acetate to ethyl acetate–methanol (20:1, v/v), afforded the desired product **5** (34 mg, 95%) as white solid: mp 218–220 °C. ESIMS *m*/*z* (rel intensity) 344 (MH^+, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.71 (br s, 1H), 6.83 (s, 1H), 6.05 (br s, 1H), 4.85 (t, *J*=6.3 Hz, 1H), 3.95 (s, 3H), 3.41 (d, *J*=2.8 Hz, 1H), 3.18 (d, *J*=2.8 Hz, 1H), 2.87 (br s, 1H), 2.45–1.20 (m, 11H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 156.0, 144.5, 133.2, 130.6, 119.8, 108.0, 67.3, 61.2, 55.8, 52.9, 45.3, 42.8, 37.9, 35.6, 32.3, 26.6, 25.5, 15.3; HRESIMS *m*/*z* calcd for $C_{20}H_{26}NO_4$ (MH^+) 344.1862, found 344.1857. The purity was determined to be 100% by HPLC analysis performed on a Waters Symmetry® C18 column (5 μm, 4.8×150 mm), eluting with 50% MeOH–50% H_2O at a flow rate of 1 mL/min (UV detection at 254 nm). The compound had a retention time of t_R=11.137 min.

*4.1.4. (6R,13S,16R,17S)-6-Hydroxy-2-methoxy-13-methyl-6,8,9,11,12,13,-14,15,16,17-decahydro-7H-20-oxa-cyclopropa[16,17]cyclopenta[a]phenanthrene-3-carboxylic acid amide (**6**).* A solution of **4** (35 mg, 0.107 mmol) in anhydrous dichloromethane (1.0 mL) at 0 °C was treated with *m*-CPBA (77%, 25.2 mg, 0.109 mmol). The reaction mixture was stirred at this temperature for 30 min. The reaction mixture was filtered to remove the white 3-chlorobenzoic acid. The organic solution was diluted with dichloromethane (5 mL) and washed with aqueous $NaHSO_3$, aqueous $NaHCO_3$, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography on SiO_2, eluting with 100% ethyl acetate to ethyl acetate–methanol (20:1, v/v), afforded the desired product **6** (35 mg, 95%) as white solid: mp 222–224 °C. ESIMS *m*/*z* (rel intensity) 344 (MH^+, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.70 (br s, 1H), 6.92 (s, 1H), 5.76 (br s, 1H), 4.86 (br s, 1H), 3.97 (s, 3H), 3.43 (d, *J*=2.8 Hz, 1H), 3.20 (d, *J*=2.8 Hz, 1H), 2.45–1.20 (m, 11H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.0, 156.4, 144.9, 133.2, 131.4, 119.8, 107.9, 64.7, 61.2, 55.8, 53.0, 36.7, 32.3, 30.0, 26.6, 25.3, 15.4; HRESIMS *m*/*z* calcd for $C_{20}H_{26}NO_4$ (MH^+) 344.1862, found 344.1859. The purity was determined to be 100% by HPLC analysis performed on a Waters Symmetry® C18 column (5 μm, 4.8×150 mm), eluting with 50%

MeOH–50% H_2O at a flow rate of 1 mL/min (UV detection at 254 nm). The compound had a retention time of t_R=10.501 min.

*4.1.5. (13R)-2-Methoxy-13-methyl-6-oxo-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3-carboxamide (**7**).* A solution of CrO_3 (435 mg, 4.35 mmol) in 90% aqueous AcOH (3.0 mL) was added dropwise at 5 °C to a solution of **2** (311 mg, 1.0 mmol) in 90% AcOH–CH_2Cl_2 (6.0 mL, 1:1, v/v). The reaction mixture was stirred at 15 °C for 1 h. The mixture was poured into ice-water (30 g) and extracted with EtOAc (20 mL×3). The combined organic solution was washed with aqueous $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on SiO_2, eluting with 100% EtOAc, to afford the desired product **7** (130 mg, 58.4%) as white solid, while **2** (98 mg) was recovered. ESIMS *m*/*z* (rel intensity) 651 (2M, 100), 206 (24), 326 (MH^+, 57); ^1H NMR (300 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.47 (br s, 1H), 6.96 (s, 1H), 5.91 (m, 2H), 5.76 (m, 1H), 4.05 (s, 3H), 2.79 (dd, *J*=3.3, 16.8 Hz, 1H), 2.64 (m, 1H), 2.45–1.95 (m, 6H), 1.90–1.65 (m, 3H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.11, 166.23, 161.31, 153.13, 143.30, 133.00, 129.50, 126.55, 119.77, 107.63, 56.35, 55.32, 45.56, 44.48, 44.41, 38.53, 35.54, 31.63, 25.76, 16.96; HRESIMS *m*/*z* calcd for $C_{20}H_{24}NO_3$ (MH^+) 326.1756, found 326.1752.

*4.1.6. (13S,17S)-17-Hydroxy-2-methoxy-13-methyl-7,8,9,11,12,13,14,15,-16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl trifluoromethanesulfonate (**8**).* 2-Methoxyestradiol (**1**, 4.54 g, 15.0 mmol) was dissolved in anhydrous DMF (50.0 mL). K_2CO_3 (4.14 g, 30.0 mmol) was added, followed by 4-nitrophenyl trifluoromethanesulfonate (4.50 g, 16.5 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water (100 mL) and ether (100 mL). The mixture was extracted with ether (100 mL×3). The combined organic solution was washed with cold 1 N HCl solution, water, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with hexanes–EtOAc (1:1), to afford the desired product **8** (6.43 g, 98%) as a white solid: mp 146–148 °C. ESIMS *m*/*z* (rel intensity) 434 (MH^+, 20), 416 (100); ^1H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.90 (s, 1H), 3.87 (s, 3H), 3.74 (dd, *J*=8.2, 14.2 Hz, 1H), 2.78 (dd, *J*=3.8, 8.3 Hz, 2H), 2.28–1.83 (m, 5H), 1.78–1.68 (m, 1H), 1.59–1.16 (m, 8H), 0.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.9, 141.7, 136.6, 129.8, 122.2, 118.8 (q, *J*=320.5 Hz, CF_3), 110.5, 81.8, 56.2, 50.1, 44.6, 43.2, 38.3, 36.7, 30.6, 28.7, 27.0, 26.4, 23.2, 11.1. Anal. Calcd for $C_{20}H_{25}F_3O_5S$: C, 55.29; H, 5.80. Found: C, 55.68; H, 5.46.

*4.1.7. (13S,17S)-2-Methoxy-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**9**).* Acetic anhydride (11.0 mL) was added dropwise under argon at 0 °C to a solution of compound **8** (6.43 g 14.8 mmol) in anhydrous pyridine (22.0 mL). The resulting mixture was stirred at 110 °C for 1 h. The reaction mixture was cooled to room temperature and poured into an ice-cold 1 N aqueous HCl solution (100 mL). The reaction mixture was extracted with ether (100 mL×3). The combined organic solution was washed with saturated aqueous $NaHCO_3$, water, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with hexanes–EtOAc (4:1), to afford the desired product **9** (6.90 g, 98%) as white solid: mp 110–111 °C. CIMS *m*/*z* (rel intensity) 477 (MH^+, 100), 417 (55), 344 (18); ^1H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.90 (s, 1H), 4.69 (dd, *J*=7.8, 9.0 Hz, 1H), 3.87 (s, 3H), 2.79 (dd, *J*=4.0, 8.6 Hz, 2H), 2.28–2.16 (m, 3H), 2.06 (s, 3H), 1.97–1.70 (m, 3H), 1.56–1.22 (m, 7H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 148.9, 141.4, 136.6, 129.7, 122.2, 118.8 (q, *J*=320.5 Hz, CF_3), 110.5, 82.6, 56.2, 49.9, 44.4, 42.8, 38.0, 36.8, 28.6, 27.6, 27.0, 26.2, 23.2, 21.2, 12.1. Anal. Calcd for $C_{22}H_{27}F_3O_6S$: C, 55.45; H, 5.71. Found: C, 55.40; H, 5.67.

*4.1.8. (13S,17S)-2-Methoxy-13-methyl-6-oxo-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**10**).* A solution of CrO_3 (132 mg, 1.32 mmol) in 90% aqueous AcOH (1.0 mL) was added dropwise at 5 °C to a solution compound **9** (143 mg, 0.30 mmol) in 90% AcOH (1.0 mL). The reaction mixture was stirred at 15 °C for 2 h. The mixture was then poured into ice-water (8 g) and extracted with EtOAc (10 mL×3). The combined organic solution was washed with aqueous $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on SiO_2, eluting with 100% EtOAc, to afford the desired product **10** (114 mg, 78%) as white solid: mp 149–151 °C. ESIMS *m*/*z* (rel intensity) 491 (MH^+, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.00 (s, 1H), 4.72 (dd, *J*=8.0, 8.9 Hz, 1H), 4.00 (s, 3H), 2.74 (dd, *J*=3.3, 16.8 Hz, 1H), 2.55 (m, 1H), 2.40–2.15 (m, 3H), 2.07 (s, 3H), 2.06–1.94 (m, 2H), 1.80–1.58 (m 3H), 1.55–1.21 (m, 3H), 0.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.2, 171.2, 155.4, 149.2, 137.7, 126.4, 121.5, 118.8 (q, *J*=320.4 Hz, CF_3), 109.5, 82.1, 56.6, 49.9, 43.5, 43.4, 42.7, 39.5, 36.4, 27.5, 25.5, 23.0, 21.3, 12.0. Anal. Calcd for $C_{22}H_{25}F_3O_7S$: C, 53.87; H, 5.14. Found: C, 53.77; H, 5.02.

*4.1.9. (6S,13S,17S)-6-Hydroxy-2-methoxy-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**11**).* Sodium borohydride (396 mg, 10.4 mmol) was added under argon at room temperature to a solution of compound **10** (981 mg, 2.0 mmol) in anhydrous methanol (10 mL). The reaction mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure. The residue was then dissolved in water (100 mL) and ice-cold HCl (1 N, 50 mL), and extracted with dichloromethane (100 mL×3). The combined organic solution was washed with saturated $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with hexanes–EtOAc (2:1), to afford the desired product **11** (790 mg, 81%) as white solid: mp 155–156 °C. ESIMS *m*/*z* (rel intensity) 515 (MNa^+, 100), 493 (MH^+, 10); ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 6.90 (s, 1H), 4.79 (dd, *J*=7.7, 17.5 Hz, 1H), 4.70 (dd, *J*=7.8, 8.8 Hz, 1H), 3.89 (s, 3H), 2.40–2.18 (m, 4H), 2.07 (s, 3H), 1.93–1.70 (m, 3H), 1.64–1.26 (m, 7H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 150.1, 141.3, 137.2, 133.0, 121.2, 118.8 (q, *J*=324.8 Hz, CF_3), 109.9, 82.5, 68.9, 56.2, 49.2, 44.8, 42.8, 37.7, 37.6, 36.8, 27.5, 26.1, 23.2, 21.2, 12.0. Anal. Calcd for $C_{22}H_{27}F_3O_7S$: C, 53.65; H, 5.53. Found: C, 53.47; H, 5.52.

*4.1.10. (6R,13S,17S)-6-Hydroxy-2-methoxy-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**12**).* A flame-dried round-bottomed flask was charged with a solution of (*S*)-Me-CBS (1.37 mL, 1.0 M in toluene, 1.37 mmol) followed by a solution of BH_3·THF (5.48 mL, 1.0 M in THF, 5.48 mmol) at 0 °C. Compound **10** (6.72 g, 13.7 mmol) in anhydrous THF (50.0 mL) was dropped in for 1 h using a syringe pump. The reaction mixture was stirred for an additional 1 h at this temperature. The reaction mixture was quenched with H_2O (20 mL) and extracted with ethyl acetate (50 mL×3). The combined organic solution was washed with H_2O (30 mL) and brine (20 mL). The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was then purified by column chromatography on SiO_2, eluting with dichloromethane–ethyl acetate (30:1 to 20:1 to 10:1, v/v), to afford **6** (3.67 g, 54.5%) and **5** (1.93 g, 28.6%). Compound **6** was obtained as a white solid: mp 170–172 °C. ESIMS *m*/*z* (rel intensity) 514 (MNa^+, 100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1H), 6.94 (s, 1H), 4.78–4.62 (app t, *J*=8.7 Hz, 2H), 3.88 (s, 3H), 2.32–1.20 (m, 14H), 2.14 (s, 3H), 0.85 (s,

3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 150.7, 141.9, 137.0, 131.0, 123.6, 118.8 (q, J=320.5 Hz, CF_3), 110.0, 82.5, 66.5, 56.2, 49.5, 44.5, 43.0, 36.8, 36.2, 32.3, 27.6, 25.8, 23.2, 21.2, 12.1. Anal. Calcd for $C_{22}H_{27}F_3O_7S$: C, 53.65; H, 5.53. Found: C, 53.49; H, 5.44.

*4.1.11. (6S,13S,17S)-2-Methoxy-6-(methoxymethoxy)-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**13**).* Compound **11** (440 mg, 0.894 mmol) was dissolved in anhydrous toluene (10 mL). Diisopropylethylamine (234 μL, 1.34 mmol) was added to the solution under argon. Then MOMCl (114 μL, 1.34 mmol) was slowly dropped in at room temperature. The resulting mixture was stirred at 60 °C for 24 h. The reaction mixture was cooled to room temperature and quenched with saturated aqueous NH_4Cl solution (5 mL). The crude product was extracted with ethyl acetate (10 mL×3). The combined organic solution was washed with water and brine. The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with pure dichloromethane, to afford compound **13** (460 mg, 96%) as colorless oil. ESIMS m/z (rel intensity) 559 (MNa^+, 100), 537 (MH^+, 40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1H), 6.90 (s, 1H), 4.88 (d, J=7.0 Hz, 1H), 4.74 (d, J=7.0 Hz, 1H), 4.75–4.66 (m, 2H), 3.89 (s, 3H), 3.46 (s, 3H), 2.42–2.18 (m, 4H), 2.06 (s, 3H), 1.95–1.72 (m, 2H), 1.60–1.20 (m, 7H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 150.2, 142.1, 137.0, 130.5, 121.6, 118.8 (q, J=320.4 Hz, CF_3), 109.8, 95.4, 82.3, 74.3, 56.1, 55.7, 49.4, 44.1, 42.7, 37.3, 36.6, 34.1, 27.5, 26.0, 23.2, 21.1, 11.9; HRESIMS m/z calcd for $C_{24}H_{32}F_3O_8S$ (MH^+) 537.1770, found 537.1773.

*4.1.12. (6S,13S,17S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**14**).* Compound **11** (1.84 g, 3.74 mmol) and imidazole (763 mg, 11.22 mmol) were dissolved in anhydrous DMF (10.0 mL). *tert*-Butyldimethylsilyl chloride (846 mg, 5.61 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with dichloromethane (50 mL×3). The combined organic layer was washed with diluted HCl, H_2O, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by flash column chromatography on SiO_2, eluting with hexanes–ethyl acetate (3:1, v/v), afforded the desired product **14** (2.09 g, 93%) as a colorless oil. ESIMS m/z (rel intensity) 629 (MNa^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 1H), 6.87 (s, 1H), 4.79 (dd, J=10.3, 6.1 Hz, 1H), 4.69 (dd, J=8.8, 8.0 Hz, 1H), 3.88 (s, 3H), 2.07 (s, 3H), 2.42–1.12 (m, 13H), 0.95 (s, 3H), 0.84 (s, 3H), 0.17 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 150.1, 141.1, 137.3, 133.6, 121.1, 118.9 (q, J=324.8 Hz, CF_3), 110.0, 82.5, 70.0, 56.4, 49.2, 44.9, 42.9, 37.7, 37.5, 36.9, 27.6, 26.3, 25.9, 23.2, 21.3, 18.2, 12.2, −3.9, −4.8. Anal. Calcd for $C_{28}H_{41}F_3O_7SSi$: C, 55.42; H, 6.81. Found: C, 55.34; H, 6.70.

*4.1.13. (6S,13S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl trifluoromethanesulfonate (**15**).* Compound **14** (2.7 g, 4.45 mmol) was dissolved in anhydrous THF (30 mL) and cooled to 0 °C. Lithium aluminum hydride solution (2.67 mmol, 2.67 mL, 1.0 M in THF) was added dropwise in 10 min. The reaction mixture was stirred at 0 °C for 30 min. The reaction was then quenched with H_2O (10 mL) and the mixture was extracted with ethyl acetate (30 mL×3). The combined organic solution was washed with cold 0.1 N HCl solution (15 mL), H_2O (15 mL), and brine (15 mL). The solvent was removed and the residue was purified by column chromatography on SiO_2, eluting with hexanes–ethyl acetate (3:2, v/v), to afford the intermediate C-17 alcohol. The C-17 alcohol was dissolved in dry dichloromethane (10 mL). Oxalyl chloride (415 μL, 603 mg, 4.75 mmol) was dissolved in dry dichloromethane (10 mL) and cooled to −78 °C. DMSO (739 μL, 813 mg, 10.4 mmol) was added dropwise in 5 min. The reaction mixture was stirred for 10 min. The solution of C-17 alcohol in dry dichloromethane was dropped in the solution at −78 °C in 5 min. The reaction mixture was then stirred at −60 °C for 30 min. Triethylamine (3.46 mL) was added and the cooling bath was removed. After 5 min, H_2O (20 mL) was added. The reaction mixture was extracted with dichloromethane (40 mL×3). The combined organic solution was washed with H_2O and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by flash column chromatography on SiO_2, eluting with 100% CH_2Cl_2, afforded the desired product **15** (2.25 g, 90% for two steps) as a colorless oil. ESIMS m/z (rel intensity) 569 (MLi^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 1H), 6.88 (s, 1H), 4.84 (dd, J=10.4, 6.2 Hz, 1H), 3.88 (s, 3H), 2.60–1.45 (m, 13H), 0.95 (s, 9H), 0.93 (s, 3H), 0.19 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 220.2, 150.2, 140.7, 137.4, 133.5, 121.1, 118.9 (q, J=321.0 Hz, CF_3), 109.9, 69.8, 56.3, 49.8, 47.9, 45.0, 37.4, 36.8, 35.8, 31.6, 25.9, 21.6, 18.1, 13.9, −3.9, −4.8. Anal. Calcd for $C_{26}H_{37}F_3O_6SSi\cdot 0.2H_2O$: C, 55.14; H, 6.66. Found: C, 55.02; H, 6.62.

*4.1.14. (6S,13S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diyl bis(trifluoromethanesulfonate) (**17**).* Anhydrous THF (6.0 mL) was added to a flame-dried 100 mL round-bottom flask and cooled to −78 °C. A THF solution of LHMDS (1.0 M in THF, 1.32 mL, 1.32 mmol, 2.0 equiv) was added to the flask. Then a solution of **15** (370 mg, 0.656 mmol) in anhydrous THF (7.0 mL) was added in 2 min. The resulting solution was stirred at −78 °C for 20 min. Then a solution of Comins' reagent (386 mg, 0.984 mmol) in anhydrous THF (2.5 mL) was added in 2 min. The reaction mixture was then stirred at −78 °C for 30 min and at room temperature for 30 min. The reaction mixture was then quenched with aqueous NH_4Cl solution (8 mL) and extracted with ether (20 mL×3). The combined organic solution was washed with H_2O and brine. The organic solution was then dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography on SiO_2, eluting with 3:1 hexanes–dichloromethane to 2:1 hexane–dichloromethane (v/v), afforded compound **17** (433 mg, 95%) as colorless oil. ESIMS m/z (rel intensity) 701 (MLi^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 1H), 6.85 (s, 1H), 5.63 (dd, J=1.5 and 2.9 Hz, 1H), 4.84 (dd, J=3.8 and 6.2 Hz, 1H), 3.89 (s, 3H), 2.50–1.45 (m, 11H), 1.02 (s, 3H), 0.95 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.0, 150.2, 140.8, 137.4, 133.4, 121.2, 118.9 (CF_3, q, J=319.6 Hz), 118.7 (CF_3, q, J=321.1 Hz), 114.5, 109.5, 69.7, 56.2, 52.9, 45.2, 45.0, 37.1, 36.0, 32.7, 28.3, 25.8, 25.7, 18.1, 15.4, −3.9, −4.8. Anal. Calcd for $C_{27}H_{36}F_6O_8S_2Si$: C, 46.68; H, 5.22. Found: C, 46.58; H, 5.09.

*4.1.15. (6S,13R)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl trifluoromethanesulfonate (**18**).* Compound **17** (65 mg, 0.094 mmol), triethylamine (19.7 μL, 14.3 mg, 0.141 mmol), and Pd(dppf)Cl_2 dichloromethane adduct (3.9 mg, 0.0047 mmol) in anhydrous DMF (0.95 mL) in a flame-dried flask were degassed with argon for 5 min. Formic acid (4.74 mg, 0.103 mmol) was injected through a syringe. The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was diluted with H_2O (10 mL) and extracted with dichloromethane (8 mL×3). The combined organic layer was washed with dilute HCl, H_2O, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography on SiO_2, eluting with hexanes–dichloromethane (2:1, v/v), to afford the desired product **18** (43.5 mg, 85%) as a colorless oil. ESIMS m/z (rel intensity) 569 (MNa^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 1H), 6.88 (s, 1H), 5.92 (dd, J=5.7, 1.5 Hz, 1H), 5.76 (m, 1H), 4.85 (dd, J=10.5, 6.3 Hz, 1H), 3.88 (s, 3H),

2.50–1.47 (m, 11H), 0.95 (s, 9H), 0.81 (s, 3H), 0.19 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.8, 143.6, 141.5, 137.0, 133.4, 129.1, 120.9, 118.7 (q, J=320.4 Hz, CF_3), 109.4, 69.8, 56.1, 54.5, 45.5, 45.4, 38.2, 36.5, 35.6, 31.5, 26.3, 25.7, 18.0, 16.9, −4.0, −5.0. Anal. Calcd for $C_{26}H_{37}F_3O_5SSi$: C, 57.12; H, 6.82. Found: C, 56.75; H, 6.49.

*4.1.16. (6S,13R)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3-carboxamide (**19**).* A mixture of **18** (46.0 mg, 0.084 mmol), HMDS (140.4 μL, 108.6 mg, 0.673 mmol), and Pd(dppf)Cl_2–dichloromethane adduct (3.44 mg, 0.0042 mmol) in anhydrous DMF (0.8 mL) was purged with CO for 5 min. Then the reaction mixture was stirred under a CO balloon at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with H_2O (3 mL). The reaction mixture was extracted with ethyl acetate (5 mL×3). The combined organic layer was washed with dilute HCl, H_2O, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on SiO_2, eluting with hexanes–ethyl acetate (2:5), to afford the desired product **19** (35.5 mg, 95%) as colorless oil. ESIMS m/z (rel intensity) 442 (MH^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.67 (br s, 1H), 6.83 (s, 1H), 5.91 (dd, J=1.3, 5.7 Hz, 1H), 5.85 (br s, 1H), 5.75 (m, 1H), 4.92 (dd, J=6.5, 9.9 Hz, 1H), 3.94 (s, 3H), 2.55–1.48 (m, 11H), 0.96 (s, 9H), 0.80 (s, 3H), 0.24 (s, 3H), 0.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 156.8, 146.5, 143.7, 133.3, 132.3, 129.4, 118.8, 107.7, 70.1, 56.0, 55.0, 45.7, 38.7, 36.7, 35.9, 31.8, 26.4, 26.1, 18.3, 17.1, −3.9, −4.4; HRESIMS m/z calcd for $C_{26}H_{40}NO_3Si$ (MH^+) 442.2777, found 442.2773.

*4.1.17. (6R,13S,17S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-3-(trifluoromethylsulfonyloxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl acetate (**20**).* Compound **12** (3.21 g, 6.52 mmol) and imidazole (1.33 g, 19.56 mmol) were dissolved in anhydrous DMF (20 mL). *tert*-Butyldimethylsilyl chloride (1.48 g, 9.78 mmol) was added to the solution. The reaction mixture was stirred at room temperature under argon for 24 h. The reaction mixture was then diluted with H_2O (100 mL) and extracted with dichloromethane (100 mL×3). The combined organic solution was washed with diluted HCl solution (50 mL), H_2O (100 mL×2), and brine (100 mL). The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was then purified by column chromatography on SiO_2, eluting with hexanes–ethyl acetate (3:1, v/v), to afford compound **20** (3.76 g, 95%) as white solid: mp 146–148 °C. ESIMS m/z (rel intensity) 629 (MNa^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.92 (s, 1H), 4.75–4.63 (m, 2H), 3.89 (s, 3H), 2.30–1.25 (m, 13H), 2.07 (s, 3H), 0.89 (s, 9H), 0.85 (s, 3H), 0.13 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 150.2, 141.7, 137.0, 132.3, 122.8, 118.9 (q, J=320.6 Hz, CF_3), 109.8, 82.6, 67.1, 56.2, 49.6, 44.2, 43.1, 36.8, 36.7, 32.6, 27.6, 25.8, 23.3, 21.3, 18.1, 12.1, −4.3, −4.4. Anal. Calcd for $C_{28}H_{41}F_3O_7SSi$: C, 55.42; H, 6.81. Found: C, 55.32; H, 6.74.

*4.1.18. (6R,13S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl trifluoromethanesulfonate (**21**).* Compound **20** (1.68 g, 2.79 mmol) was dissolved in anhydrous THF (20 mL) and the solution was cooled to 0 °C. Lithium aluminum hydride solution (1.67 mmol, 1.67 mL, 1.0 M in THF) was added dropwise in 10 min. The reaction mixture was stirred at 0 °C for 30 min. The reaction was then quenched with H_2O (7 mL) and the mixture was extracted with ethyl acetate (20 mL×3). The combined organic solution was washed with cold 0.1 N HCl solution (10 mL), H_2O (10 mL), and brine (10 mL). The solvent was removed and the residue was purified by column chromatography on SiO_2, eluting with hexanes–ethyl acetate (3:2, v/v), to afford the intermediate C17-ol. The C17-ol was dissolved in dry dichloromethane 7 mL. Oxalyl chloride (262 μL, 382 mg, 3.00 mmol) was dissolved in dry dichloromethane (7 mL) and cooled to −78 °C. DMSO (466 μL, 512 mg, 6.56 mmol) was added dropwise in 5 min. The reaction mixture was stirred for 10 min. The solution of C-17 alcohol in dry dichloromethane was dropped into the solution at −78 °C in 5 min. The reaction mixture was then stirred at −60 °C for 30 min. Triethylamine (2.18 mL) was added and the cooling bath was removed. After 5 min, H_2O (15 mL) was added. The reaction mixture was extracted with dichloromethane (25 mL×3). The combined organic solution was washed with H_2O and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by flash column chromatography on SiO_2, eluting with 100% CH_2Cl_2, afforded the desired product **21** (1.42 g, 90% for two steps) as a colorless oil. ESIMS m/z (rel intensity) 569 (MLi^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (s, 1H), 6.94 (s, 1H), 4.71 (dd, J=3.5, 3.5 Hz, 1H), 3.90 (s, 3H), 2.60–1.40 (m, 13H), 0.93 (s, 3H), 0.90 (s, 3H), 0.15 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 220.3, 150.3, 141.2, 137.0, 132.1, 122.8, 118.8 (q, J=320.6 Hz, CF_3), 109.8, 67.0, 56.1, 50.0, 48.1, 44.3, 35.9, 35.8, 32.4, 31.5, 25.8, 25.5, 21.5, 18.0, 13.7, −4.4, −4.5. Anal. Calcd for $C_{26}H_{37}F_3O_6SSi\cdot 0.2H_2O$: C, 55.14; H, 6.66. Found: C, 54.93; H, 6.75.

*4.1.19. (6R,13S)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diyl bis(trifluoromethanesulfonate) (**22**).* Anhydrous THF (5.0 mL) was added to a flame-dried, 100 mL round-bottomed flask and the mixture was cooled to −78 °C. A THF solution of LHMDS (1.0 M in THF, 1.03 mL, 1.03 mmol, 2.0 equiv) was added to the flask. A solution of compound **21** (289 mg, 0.514 mmol) in anhydrous THF (6.0 mL) was added in 2 min. The resulting solution was stirred at −78 °C for 20 min. A solution of Comins' reagent (303 mg, 0.771 mmol) in anhydrous THF (2.0 mL) was added in 2 min. The reaction mixture was then stirred at −78 °C for 30 min and at room temperature for 30 min. The reaction mixture was then quenched with aqueous NH_4Cl solution (7 mL) and extracted with ether (16 mL×3). The combined organic solution was washed with H_2O and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography on SiO_2, eluting with 3:1 hexanes–dichloromethane to 2:1 hexane–dichloromethane (v/v), afforded the title compound **22** (340 mg, 95%) as colorless oil. ESIMS m/z (rel intensity) 701 (MLi^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (s, 1H), 6.91 (s, 1H), 5.63 (dd, J=1.5, 1.6 Hz, 1H), 4.73 (dd, J=3.7, 3.7 Hz, 1H), 3.90 (s, 3H), 2.40–1.55 (m, 11H), 1.03 (s, 3H), 0.89 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 150.4, 141.3, 137.1, 132.2, 123.0, 118.9 (q, J=320.6 Hz, CF_3), 118.8 (q, J=320.6 Hz, CF_3), 114.7, 109.5, 67.0, 56.3, 53.2, 45.3, 44.6, 36.2, 32.8, 31.0, 28.4, 25.9, 25.5, 18.1, 15.3, −4.26, −4.31. Anal. Calcd for $C_{27}H_{36}F_6O_8S_2Si$: C, 46.68; H, 5.22. Found: C, 46.74; H, 5.14.

*4.1.20. (6R,13R)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl trifluoromethanesulfonate (**23**).* A mixture of **22** (217 mg, 0.312 mmol), triethylamine (65.4 μL, 47.5 mg, 0.468 mmol), and Pd(dppf)Cl_2 dichloromethane adduct (12.8 mg, 0.0156 mmol) in anhydrous THF (3.1 mL) in a flame-dried flask was degassed with argon for 5 min. Formic acid (15.8 mg, 0.344 mmol) was injected through a syringe. The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was diluted with H_2O (30 mL) and extracted with dichloromethane (30 mL×3). The combined organic layers were washed with diluted HCl, H_2O, and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography on SiO_2, eluting with hexanes–dichloromethane (2:1, v/v), to afford the desired product **23** (102 mg, 60%) as a colorless oil. ESIMS m/z (rel intensity) 553 (MLi^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (s, 1H), 6.93 (s, 1H), 5.92 (dd, J=4.2, 5.5 Hz, 1H), 5.76 (m, 1H), 4.72 (dd, J=3.6, 3.6 Hz, 1H), 3.90 (s, 3H),

2.33–1.47 (m, 11H), 0.89 (s, 9H), 0.82 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.3, 143.9, 142.3, 137.0, 132.6, 129.4, 122.7, 118.9 (q, J=320.4 Hz, CF_3), 109.6, 67.3, 56.3, 55.2, 46.0, 45.0, 37.4, 35.9, 31.9, 31.6, 26.3, 25.9, 18.1, 17.1, −4.2, −4.3. Anal. Calcd for $C_{27}H_{37}F_3O_5SSi$: C, 57.12; H, 6.82. Found: C, 57.01; H, 6.76.

*4.1.21. (6R,13R)-6-(tert-Butyldimethylsilyloxy)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthrene-3-carboxamide (**24**).* A mixture of compound **23** (73 mg, 0.134 mmol), HMDS (223 μL, 173 mg, 1.07 mmol), and Pd(dppf)Cl_2 dichloromethane adduct (5.46 mg, 0.0067 mmol) in anhydrous DMF (1.4 mL) was degassed five times with argon. The reaction flask was then degassed five times with CO. The reaction mixture was heated at 100 °C under a CO balloon for 1 h. After cooling to room temperature, the reaction mixture was diluted with H_2O (15 mL) and extracted with dichloromethane (10 mL×3). The combined organic solution was washed with diluted HCl (5 mL), H_2O (10 mL), and brine (10 mL). Purification by column chromatography on SiO_2, eluting with hexane–ethyl acetate (1:1, v/v), afforded the desired product **24** (55 mg, 93%) as a colorless oil. ESIMS m/z (rel intensity) 464 (MNa^+, 100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.68 (br s, 1H), 6.89 (s, 1H), 5.93 (m, 1H), 5.85 (br s, 1H), 5.76 (m, 1H), 4.83 (dd, J=3.0, 3.0 Hz, 1H), 3.95 (s, 3H), 2.40–1.50 (m, 11H), 0.87 (s, 9H), 0.82 (s, 3H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 157.0, 146.7, 143.9, 134.5, 132.2, 129.5, 118.7, 107.9, 67.5, 56.0, 55.1, 46.0, 45.9, 37.7, 36.0, 31.8, 31.1, 26.3, 26.0, 18.2, 17.1, −3.8, −4.3.

4.1.22. Human plasma extraction. Acetonitrile (3×, v/v) was added to each human plasma sample (200 μL, completely thawed). The mixture was vortexed for 2 min, followed by centrifugation at ca. 10,000 *g* and 4 °C for 10 min. The pellets were rinsed twice with acetonitrile (200 μL). The acetonitrile extracts were combined and evaporated to dryness by N-Evap. The residues were then reconstituted with methanol–water (200 μL, 1:1) for LC/MS analysis.

4.1.23. Preparation of reference standard solutions. Each of the reference standards **3**–**6** was dissolved in methanol (ca. 1 mg/mL), and the solution was further diluted in 50% aqueous methanol (500×, v/v) for LC/MS analysis.

4.1.24. Cell culture. Human MDA-MB-231 breast carcinoma, MDR1/231 multidrug-resistant cells derived from MDA-MB-231 cells, U87-MG glioblastoma, and PC3 prostate cancer cells were maintained in DMEM/F-12 (Life Technologies) supplemented with 10% (v/v) fetal bovine serum (Hyclone Laboratories). Mouse Lewis lung carcinoma (LLC) cells were maintained in DMEM (Life Technologies) supplemented with 5% fetal bovine serum, glutamine, non-essential amino acids, vitamins, and sodium pyruvate. HT-29 human colon cancer cells were grown in DMEM (Bio-Whittaker) supplemented with 10% fetal bovine serum and L-glutamine. Human umbilical vein endothelial cells were grown in EGM medium (Clonetics). Cells were grown in a humidified chamber at 37 °C with 5% CO_2.

4.1.25. Proliferation assays. IC_{50} values for 2ME2 and analogues were obtained by assessment of DNA synthesis using a bromodeoxyuridine incorporation ELISA kit assay (Roche) or by cleavage of the tetrazolium salt (WST-1) to soluble formazan dye by cellular mitochondrial dehydrogenases using the WST-1 Cell Proliferation Reagent (Roche). For bromodeoxyuridine and WST-1 proliferation assays, cells were seeded on 96-well plates at 1000–5000 per well depending on growth rate, allowed to attach overnight, and were then exposed to compounds for 48 h. For bromodeoxyuridine incorporation, cells were incubated with the bromodeoxyuridine solution for 2 h followed by fixation, labeling, and absorbance measurement (370/492 nm) according to the manufacturer's instructions. WST-1 reagent was added to cell medium (1:10) followed by incubation for 0.5–4 h (depending on rate of cell metabolism) before reading absorbance at 450/650 nm. IC_{50} values were calculated using the mean from usually two independent experiments, each conducted in triplicate.

4.1.26. Liquid chromatography/mass spectrometry (LC/MS). The HPLC system used in the metabolism studies consisted of Shimadzu LC-10ADVP pumps, a Shimadzu SIL-HTA autosampler, and a Shimadzu SCL-10A System Controller. The mass spectrometer used in the metabolism studies was a ThermoFinnigan LTQ™. Liquid chromatography was performed on an Ace 3 C18 3 μm, 4.6×150 mm column at 40 °C, and an autosampler temperature of 4 °C with a gradient of 20 mM ammonium formate to methanol during 102 min. Mass spectrometry was performed with heated electrospray ionization (HESI) in both positive and negative modes with an ion source temperature of 380 °C, a capillary temperature of 320 °C, a spray voltage of 5 kV, and a capillary voltage of −9 V. Both MS and MS/MS scans were obtained with N_2 sheath and auxiliary gas flows and He as the collision gas.

Supplementary data

Results from LC/MS analyses of synthetic standards **3**–**6** and metabolites, as well as ^{1}H NMR and ^{13}C NMR spectra of all compounds. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.10.046.

References and notes

1. Jordan, M. A. *Curr. Med. Chem.—Anti-Cancer Agents* **2002**, *2*, 1–17.
2. Downing, K. H.; Nogales, E. *Curr. Opin. Cell Biol.* **1998**, *10*, 16–22.
3. Li, Q.; Sham, H. L. *Expert Opin. Ther. Pat.* **2002**, *12*, 1663–1702.
4. Beckers, T.; Mahboobi, S. *Drugs Future* **2003**, *28*, 767–785.
5. Kavallaris, M.; Verrills, N. M.; Hill, B. T. *Drug Resist. Updat.* **2001**, *4*, 392–401.
6. Moser, C.; Lang, S. A.; Mori, A.; Hellerbrand, C.; Schlitt, H. J.; Geissler, E. K.; Fogler, W. E.; Stoeltzing, O. *BMC Cancer* **2008**, *8*, 206–216.
7. LaVallee, T.; Swartz, G. M.; Kifle, G.; Fogler, W. E.; Treston, A. M.; Gustafson, D. L.; Sidor, C.; Camidge, D. R. *Mol. Cancer Ther.* **2007**, *6*, 3464S–3465S.
8. LaVallee, T. M.; Burke, P. A.; Swartz, G. M.; Hamel, E.; Agoston, G. E.; Shah, J.; Suwandi, L.; Hanson, A. D.; Fogler, W. E.; Sidor, C. F.; Treston, A. M. *Mol. Cancer Ther.* **2008**, *7*, 1472–1482.
9. Cushman, M.; He, H.-M.; Katzenellenbogen, J.; Varma, R. K.; Hamel, E.; Lin, C. M.; Ram, S.; Sachdeva, Y. P. *J. Med. Chem.* **1997**, *40*, 2323–2334.
10. Brown, R. F. C.; Jackson, W. R.; McCarthy, T. D. *Tetrahedron Lett.* **1993**, *34*, 1195–1196.
11. Shapiro, R. H.; Heath, M. J. *J. Am. Chem. Soc.* **1967**, *89*, 5734–5735.
12. Cacchi, S.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1984**, *25*, 4821–4824.
13. Liu, P.; Hong, S.; Weinreb, S. M. *J. Am. Chem. Soc.* **2008**, *130*, 7562–7563.
14. Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299–6302.
15. Morera, E.; Ortar, G. *Tetrahedron Lett.* **1998**, *39*, 2835–2838.
16. Corey, E. J.; Shibata, S.; Bakshi, R. K. *J. Org. Chem.* **1988**, *53*, 2861–2863.
17. Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1987–2012.
18. Zhang, J. L.; Che, C. M. *Chem.—Eur. J.* **2005**, *11*, 3899–3914.
19. Arunachalam, T.; Caspi, E. *J. Org. Chem.* **1981**, *46*, 3415–3420.