Published in final edited form as:

Bioorg Med Chem. 2015 September 1; 23(17): 5985–5998. doi:10.1016/j.bmc.2015.06.055.

Chemical Synthesis of Tetracyclic Terpenes and Evaluation of Antagonistic Activity on Endothelin-A Receptors and Voltage-gated Calcium Channels

Jianyu Lu¹, Angelo Aguilar¹, Bende Zou², Weier Bao¹, Serkan Koldas¹, Shi Aibin¹, John Desper¹, Philine Wangemann³, Xinmin Simon Xie², and Duy H. Hua^{1,*}

¹Department of Chemistry, Kansas State University, Manhattan, KS 66506, U.S.A

²AfaSci Research Laboratories, 522 Second Avenue, Redwood City, CA 94063, U.S.A

³Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, U.S.A

Abstract

A class of tetracyclic terpenes was synthesized and evaluated for antagonistic activity of endothelin-1 (ET-1) induced vasoconstriction and inhibitory activity of voltage-activated Ca^{2+} channels. Three repeated Robinson annulation reactions were utilized to construct the tetracyclic molecules. A stereoselective reductive Robinson annulation was discovered for the formation of optically pure tricyclic terpenes. Stereoselective addition of cyanide to the hindered α -face of tetracyclic enone (-)-**18** was found and subsequent transformation into the aldehyde function was affected by the formation of bicyclic hemiiminal (-)-**4**. Six selected synthetic tetracyclic terpenes show inhibitory activities in ET-1 induced vasoconstriction in the gerbil spiral modiolar artery with putative affinity constants ranging between 93 and 319 nM. Moreover, one compound, (-)-**3**, was evaluated further and found to inhibit voltage-activated Ca^{2+} currents but not to affect Na^{+} or K^{+} currents in dorsal root ganglion cells under similar concentrations. These observations imply a dual mechanism of action. In conclusion, tetracyclic terpenes represent a new class of hit molecules for the discovery of new drugs for the treatment of pulmonary hypertension and vascular related diseases.

Keywords

Antagonistic activity; endothelin-1 (ET-1); pulmonary hypertension; tetracyclic terpenes; vascular related diseases; vasoconstriction; voltage-activated Ca^{2+} channels

*Corresponding author. Tel: 785-532-6699; fax: 785-532-6666; duy@ksu.edu.

Supplementary data: Supplementary data associated with this article can be found, in the online version, at ... These data include ^1H and ^{13}C NMR spectra of all new compounds and single-crystal X-ray (CIF) data of compound (-)-**20**.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Endothelin-1 (ET-1), a potent 21-residue peptide produced mainly by vascular endothelial cells, binds to endothelin A receptors (ET_A) expressed in vascular smooth muscle cells and causes blood vessels to constrict.¹ Overproduction of this peptide is believed to be a key factor in pulmonary arterial hypertension² and pathological vascular spasms (vasospasms) that compromise the function of various organs³ and cause failure of vein grafts in coronary artery bypass surgery.⁴ Hence, ET-1 receptor antagonists may serve as hit compounds for the development of drugs that are useful for the treatment of various diseases including pulmonary arterial hypertension, vasospasms, renal failure, sudden hearing loss, and asthma⁵ as well as being useful in support for surgical procedures such as coronary bypass surgery. In search of compounds that possess ET-1 antagonistic activity, we investigated a number of tetracyclic terpenes, whose structures are derived from the A-B-C-D ring of myriceric acid A (Figure 1), a pentacyclic triterpene isolated from the crude extract of southern bayberry, *Myrica cerifera* twigs.⁶ Myriceric acid A binds selectively to ET_A receptor, antagonizes specific binding of [¹²⁵I]endothelin-1 (ET-1) to rat cardiac membranes, and inhibits ET-1 induced increase in cytosolic free Ca²⁺ concentration (IC₅₀ = 11 ± 2 nM) and ET-1 induced contraction of rat aortic strips (K_i = 66 ± 15 nM).⁶⁻⁹ A partial synthesis of (+)-myriceric acid A starting from a pentacyclic triterpene, oleanolic acid, involving 14 steps, has been reported.¹⁰ Its structure and absolute configuration was determined from a single-crystal X-ray analysis of its chemically modified 27-*O*-acetylmyricerone methyl ester.⁶ Despite these encouraging inhibitory results, only limited follow up studies were reported on myriceric acid A and its analogs.⁶⁻¹⁰ We envision that a reduction of the size and complexity of the structure would facilitate the synthesis of a library of molecules for the discovery of hit compounds. Hence, we report herein an asymmetric synthesis of optically pure tetracyclic terpenes (+)-**1** – (-)-**6** (Figure 1), evaluation of antagonistic activity on ET_A receptor mediated vasoconstriction of the spiral modiolar artery,^{11,12} and evaluation of inhibitory activity of voltage-activated Ca²⁺ currents.

2. Results and Discussion

2.1. Organic Synthesis

Compounds (+)-**2** – (-)-**4** were synthesized from (+)-**1**, and (-)-**5** and (-)-**6** were derived from (-)-**7**, which also leads to (+)-**1**. The retrosynthetic analysis of (+)-**1** is depicted in Figure 1, where the tetracyclic terpene is synthesized from tricyclic (-)-**7**, which is made in optically pure form from chiral Wieland-Miescher ketone (-)-**8**.¹³⁻¹⁵ In the study, a one-pot reductive/Robinson annulation was developed for the synthesis of optically pure tricycle (-)-**7**, and a stereoselective introduction of a cyano function to the tetracyclic enone from the concave face generating the stereochemistry at quaternary C10a carbon was achieved. Due to the bowl-shaped tetracyclic structure, the reduction of the shielded cyano function, which oriented in the concave face, was accomplished by manipulation of the D-ring.

Our synthesis of tetracyclic terpenes started from optically pure Wieland-Miescher ketone (-)-**8**, which was made by following the reported synthesis¹³ from 2-methyl-1,3-cyclohexanedione (**9**) (Scheme 1). Michael addition reaction of **9** with ethyl vinyl ketone (EVK) and KOH in methanol gave 2-methyl-2-(3-oxopentyl)cyclohexane-1,3-dione, which

was treated¹³ with an equivalent of D-phenylalanine as an organo-catalyst, and 0.5 equivalents of D-camphorsulfonic acid in DMF to produce (-)-**8**, having $[\alpha]_D^{25} = -142$ ($c = 0.21$, MeOH) after crystallization; ~100% enantiomeric excess (ee) [lit.¹³ -140 ($c = 0.20$, MeOH)].

A number of total syntheses¹⁶⁻¹⁹ of natural products have been reported by reducing (-)-**8** to alcohol (-)-**10** followed by the treatment with sodium methoxide in methanol and EVK to give (+)-**11**. However, the ee of (+)-**11** has decreased significantly to 23.5% ee.^{16,17} The loss of optical activity was explained by the formation of aldehyde **10B** via a retro-aldol reaction which competes with the formation of the dienolate **10A** (Scheme 1).¹⁶ Attempts to protect the hydroxyl function of (-)-**10** with various protecting groups led to either racemization of (-)-**10** or decomposition of the protected compound under the Robinson annulation conditions.¹⁶

To circumvent this racemization process, we investigated a one-pot reductive-Michael reaction^{20,21} of the silyloxy protected compound (-)-**12**, which was readily available from the silylation of (-)-**10** with *t*-butyldimethylsilyl chloride (TBSCl) (Scheme 1). Attempts to reduce enone (-)-**12** with K-Selectride²⁰ in THF followed by EVK gave only the corresponding reduced ketone (the double bond was reduced) but no Michael addition product was found. Similarly, reduction of (-)-**12** with lithium in liquid ammonia²¹ followed by EVK resulted in the reduced ketone and decomposition by-products along with the polymers of EVK. Apparently, EVK decomposes or oligomerizes under basic conditions, hence a more robust enone, the Stork-Ganem's ethyl α -(trimethylsilyl)vinyl ketone,^{22,23} was used. To our delight, lithium-liquid ammonia reduction of the enone function of (-)-**12** followed by ethyl α -(trimethylsilyl)vinyl ketone and cyclization with KOH in methanol afforded (+)-**13** as a single stereoisomer [presumably in optically pure form since C4a and C5 of (-)-**12** should not be affected under the reaction conditions]. Reductive methylation of the enone function of (+)-**13** with lithium in liquid ammonia/methyl iodide followed by protection of the resulting ketone function with ethylene glycol under acidic medium gave (-)-**14**, which was readily converted into (-)-**7**¹⁹ by removal of the TBS protecting group and subsequent IBX-DMSO oxidation (Scheme 1). Compound (-)-**7** possesses specific rotation $[\alpha]_D^{25} = -28$ ($c = 0.14$, CHCl₃), which is slightly larger than that reported ($[\alpha]_D^{25} = -26$; 98% ee)¹⁹ using the same solvent and concentration.

The assembly of tetracyclic compounds (+)-**1** – (-)-**6** from (-)-**7** requires an activation of the keto function by a phenylsulfide moiety to the α -position, since direct coupling of (-)-**7** with various bases and methyl vinyl ketone failed, and decomposition by-products were found. The C8a angular methyl of (-)-**7** likely hinders the 1,4-addition with methyl vinyl ketone. Hence, treatment of (-)-**7** with LDA in THF at -78 °C followed by diphenyl disulfide gave α -sulfide ketone (+)-**15** (Scheme 2) as a single isomer in a 92% yield. The stereochemistry at C7 of (+)-**15** was shown by its 2D NOESY NMR spectrum in which correlation between C8a-methyl (δ 1.19 ppm, s) and C7-hydrogen (δ 4.21 ppm, dd, $J = 13.1, 6.4$ Hz indicating J_{axial} and $J_{equatorial}$ coupling constant, respectively) was found. Presumably, C8a-methyl blocks the β -face of the enolate ion of (-)-**7** resulting in the sulfenylation at the α -face exclusively. Michael addition reaction of (+)-**15** with sodium methoxide and methyl vinyl

ketone led to a 2.6:1 ratio of (-)-**16a** and (-)-**16b**, the β - and α -adduct, respectively. Interestingly, only the α -adduct (-)-**16b** cyclized to the corresponding tetracyclic compound, while the β -adduct (-)-**16a** does not suggesting the C8a-methyl of (-)-**16a** shields the intramolecular aldol-type addition reaction from the β -face. Hence, to control the stereochemistry at C7 [such as that in (+)-**1**] and simplify the synthesis, the phenylsulfonyl function of (-)-**16** (using a mixture of 2.6:1 of **16a** and **16b**) was reduced with *n*-Bu₃SnH in the presence of a catalytic amount of azobisisobutyronitrile (AIBN), and a 1:1 ratio of (-)-**17a** and (-)-**17b** was resulted. Notably, (-)-**16a**, (-)-**16b**, (-)-**17a**, and (-)-**17b** are all separable by silica gel column chromatography and the stereochemistry of (-)-**16a** and (-)-**16b** were determined using 2D NOESY NMR. Presumably, the equatorial attached bulkier phenylsulfonyl moiety in (-)-**16a** is thermodynamically more stable than that in (-)-**16b**, hence (-)-**16a** formed predominantly. Again, it was found that (-)-**17b** converted smoothly to (-)-**18** by the treatment with NaOMe in methanol and THF at 58 °C in an 80% yield. A higher temperature such as 58 °C is needed for the cyclization. Under similar reaction conditions, treatment of (-)-**17a** with NaOMe in refluxing MeOH gave only a poor yield of (-)-**18**. Due to the shielding of C8a-methyl, the axial oriented C7 enolate ion of 3-ketobutyl group apparently does not attack at the C8-keto moiety readily (*vide supra*). Unidentifiable by-products were formed as major products. To increase the yield, (-)-**17a** was first transformed into (-)-**17b** in an 81% yield by the treatment with NaOMe in methanol at 25 °C (epimerization of C7-H) followed by the treatment with NaOMe in methanol at 58 °C to give (-)-**18**. Removal of the cyclic ketal protecting group of (-)-**18** with HCl in THF-water furnished (+)-**1**.

The introduction of a substituent at C10a of (-)-**18** to produce a tetra-substituted carbon for the synthesis of tetracyclic terpenes (+)-**2** – (-)-**4** can be accomplished by the 1,4-addition reaction with KCN and NH₄Cl in H₂O and DMF at 100 °C. The α -cyano adduct (-)-**19** was isolated in a 91% yield (Scheme 3) as the only stereoisomer. Likely, the cyanide ion approaches C10a from the opposite face of the C10b-methyl and the stereochemistry at C10a was unequivocally confirmed by a single-crystal X-ray analysis of its derivative (-)-**20** (*vide infra*). Aqueous hydrolysis of the ketal function of (-)-**19** with HCl in THF-H₂O gave (+)-**2** in a 95% yield. To our surprise, the cyano function of (-)-**19** can't be reduced by excess of diisobutylaluminum hydride (Dibal-H) in THF or toluene, and only C9 keto function was reduced. The resulting tetracyclic C9-alcohol was repeatedly treated with Dibal-H, however, the cyano function was not reduced and only starting material was recovered. The cyano function of (-)-**19** appears to be extremely robust and failed to undergo hydrolysis under mild acidic or basic conditions and only unidentifiable decomposition products were found from the hydrolysis reactions. The lack of reactivity of the cyano function likely attributes from (-)-**19**'s bowl-shape structure in which the concaved oriented cyano function is shielded by the tetracyclic skeleton. Fortuitously, a change of the C9-keto function of (-)-**19** to an enol silyl ether (a change of the D ring from cyclohexane chair conformation to a half-chair cyclohexene conformation) provided a solution to the reduction issue. Treatment of (-)-**19** with *t*-butyldimethylsilyl triflate and triethylamine gave a quantitative yield of (-)-**20**, which was reduced by Dibal-H in toluene to provide imine (-)-**3** in a 58% yield. The structure of (-)-**20** was firmly established by a single-crystal X-ray analysis (Figure 2), which shows the α -oriented cyano moiety and regiochemistry of the enol silylether function. The crystal is

orthorhombic and has space group of P2(1)2(1)2(1).²⁴ Interestingly, desilylation of (-)-**3** with *n*-Bu₄NF provided the corresponding imino ketone, in which the imine function does not undergo hydrolysis under acidic medium (only the C2 ketal protecting group was hydrolyzed) supporting the shielding effect of the bowl-shape structure (*vide supra*). It was discovered that by a change of the half-chair cyclohexene conformation to a bicyclo[2.3.1]azaoctene system would enhance the reactivity of the imine function. Hence, protonation of the alkene function of (-)-**3** with acetic acid in 1,4-dioxane resulted in a ring closure by the imine nitrogen, which underwent desilylation with *n*-Bu₄NF to give (-)-**4** in 81% overall yield. Hydrolysis of (-)-**4** with acetic acid in THF and water gave keto aldehyde (-)-**21**. The sequence of reactions demonstrates a regio- and stereo-selective introduction of a formyl moiety to the hindered C10a of (-)-**18** creating a tertiary stereocenter.

The synthesis of (-)-**5** can be achieved by the intramolecular aldol condensation of (-)-**16b** followed by *in situ* dehydrosulfenylation with NaOMe in methanol and removal of the cyclic ketal protecting group with 1 N HCl in THF (Scheme 4). Alkylation of (-)-**22** with sodium carbonate and ethyl chloroacetate followed by reduction with sodium borohydride and deprotection of the ketal function with HCl afforded (-)-**6** in 55% overall yield.

A stereo- and regioselective introduction of a formyl group at the sterically hindered C10a of the tetracyclic terpene was accomplished via the addition of cyanide ion followed by the transformation to bicyclo[2.3.1]azaoctene (-)-**4** allowing the reduction of the C10a C=N function. The conversion of tricyclic intermediate (-)-**16b** to the aromatic D-ring of tetracyclic (-)-**5** and (-)-**6** was also readily carried out affording the syntheses of various novel D-ring benzeno intermediates.

2.2. Bioactivities

2.2.1. Inhibition of ET-1 induced vasoconstriction—The gerbil spiral modiolary artery expresses ET_A receptors and responds to the peptide agonist ET-1 with a vasoconstriction (EC₅₀ = 0.74 nM).²⁵ ET-1 induced vasoconstrictions are inhibited in the presence of competitive ET_A receptor antagonists.²⁵ Here we used the spiral modiolary artery as a bioassay to evaluate antagonistic activity (or putative affinity value, A₂) of compounds (+)-**1** – (-)-**6** and (+)-myriceric acid. Based on a number of assumptions (*see below*), putative affinity constants (pA₂ values) were computed from the effects of different concentrations of tetracyclic terpenes on vasoconstrictions induced by 1 nM ET-1. Table 1 summarizes these putative pA₂ values for the synthetic tetracyclic terpenes and for myriceric acid A, which served as a positive control. The putative affinity values of the six synthetic compounds are between 93 and 318 nM. The affinity constant for the known antagonist myriceric acid A was 85 nM.

In brief, segments of the gerbil spiral modiolary artery were superfused with physiological saline and the vascular diameter was recorded by videomicroscopy.^{11,12} Representative recordings are shown in Figure 3. Experiments began with two standard vasoconstrictions that were induced by raising the Ca²⁺ concentration in the superfusate from 1 to 10 mM for the duration of 1 min.^{11,12} This maneuver elicited a transient vasoconstrictions that was quantified. Note, the magenta circles in Figure 3 that mark the decrease in the vascular

diameter from 68 μm to 55 μm (Left panel). Vasoconstrictions in response to 10 mM Ca^{2+} were used to normalize vasoconstrictions induced by the ET_A receptor agonist 1 nM ET-1. In the next step of the experiment, the antagonist myriceric acid A or a tetracyclic terpene of unknown antagonist-activity was added to the superfusate for the duration of 2 min. After the first minute, the agonist 1 nM ET-1 was added for the duration of 1 min. The magnitude of the ET-1 induced vasoconstriction in the presence of myriceric acid A or in the presence of a tetracyclic terpene was normalized and normalized values were used to calculate the affinity constants. Affinity constants were computed under the assumption of competitive inhibition according to the equation $\text{pA}_2 = -\log [(A/A')-1] - \log (B)$, where A and A' are molar EC_{50} values for the agonist ET-1 in the presence and absence of antagonist, respectively, and B is the molar concentration of the antagonist. A' (EC_{50} : 74 nM) was taken from a previous study²⁵ and A was estimated from individual experiments according to the equation:

$$\text{EC}_{50} = 10^{\log\left\{\frac{E_{\max} \times [\text{Agonist}]^h}{E - [\text{Agonist}]^h}\right\}},$$

where E is the normalized vasoconstriction measured, $E_{\max}$ is the normalized maximal vasoconstriction obtained from a previous study ($E_{\max}$: 140%),²⁵ $[\text{Agonist}]$ is the molar concentration of the agonist ($[\text{Agonist}]$: 1 nM), and h is the Hill-coefficient obtained from this previous study ($h = 1.2$).²⁵

Following the determination of ET-1 induced vasoconstriction in the presence of myriceric acid A or tetracyclic terpenes, the reversibility of the putative or established antagonists were evaluated in the experiments, see Figure 3. Agonist and antagonist were washed out by superfusing the spiral modiolar artery with physiological saline for the duration of 2 min after which the agonist 1 nM ET-1 was again added to the superfusate. A vasoconstriction that is larger in magnitude than the vasoconstriction observed in the presence of an established or putative antagonist would indicate at least partial reversibility. A vasoconstriction of 83% (normalized to the vasoconstriction induced by 10 mM Ca^{2+}) would indicate complete reversibility. Note, a nearly complete reversibility for myriceric acid A (Figure 3) and compounds **1** – **6** were found.

Interestingly, the selected six tetracyclic terpenes (+)-**1** – (-)-**6** possess strong antagonistic activity. The inhibitory activities of vasoconstriction induced by ET-1 decrease in the order: (+)-myriceric acid > (-)-**3** > (+)-**2** > (+)-**1** > (-)-**5** > (-)-**4** > (-)-**6** (Table 1). Compound (-)-**3** contained a cyclohexenyl D ring and an imino function at C10a possesses a similar antagonistic activity as that of myriceric acid A, where putative affinity value of (-)-**3** is 93 nM and that of myriceric acid A is 85 nM. Cyano diketo (-)-**2**, $\text{A}_2 = 134$ nM, shows slightly less activity as that of (-)-**3**, which may due to a difference in the cyano moiety at C10a. Compounds (+)-**1**, (-)-**5**, and (-)-**6** possessed no substitution at C10a show weaker antagonistic activities. Similarly, the rigid skeletal structure in ring D of (-)-**4** also diminishes the activity.

2.2.2. Inhibition of voltage-gated Ca^{2+} currents—Reduction of extracellular Ca^{2+} entry into cells of blood vessel will widen the blood vessel and allows the heart to pump easier resulting in lowering blood pressure. Since the vasoconstriction of artery smooth muscle caused by ET-1 can be partially antagonized by the dihydropyridine Ca^{2+} channel blocker, nicardipine,²⁶ inhibition of Ca^{2+} channels by tetracyclic terpenes was studied. Noteworthy, myriceric acid A has been shown to inhibit ET-1 induced increase in cytosolic free Ca^{2+} concentration with an IC_{50} value of 11 nM,⁶ studies of the blockage of voltage-gated Ca^{2+} current provide a different method in an evaluation of inhibition of Ca^{2+} channel. Compound (-)-**3**, possessing the strongest antagonistic activity, was selected for the evaluation of potential direct interaction with Ca^{2+} channels due to its potent inhibition of ET-1 induced vasoconstriction (see Figure 3). Compound (-)-**3** was subjected to patch clamp studies in varying concentrations to assess the Ca^{2+} channel inhibition. Low- (LVA, principally Cav3.2 T-type) and high-voltage-activated (HVA, mainly L-type) Ca^{2+} currents were activated following step depolarization to various test potentials from a holding potential (V_h) of -90 mV (Figure 4). Bath application of compound (-)-**3** (0.1 - 3 μM) caused concentration-dependent inhibition of both LVA- and HVA- Ca^{2+} currents (Figure 4A). The current-voltage relationship (I-V plot) shows that the peak Ca^{2+} currents occurred at -10 mV in control and the inhibition of (-)-**3** is evident across all voltage activation and in a concentration-dependent manner (Figure 4B). Single depolarizing steps to -10 mV from a V_h of -90 mV for a short duration (20 ms) were delivered every 10 s to minimize current rundown. Compound (-)-**3** at 0.1 and 0.3 μM substantially inhibited the Ca^{2+} currents in a concentration-dependent manner, and at 3 μM completely block Ca^{2+} currents (Figure 4C). The interaction of (-)-**3** with voltage-gated Na^+ and K^+ currents in mouse dorsal root ganglion (DRG) neurons were investigated to evaluate ion channel modulation selectivity. The tetrodotoxin (TTX)-sensitive inward Na^+ currents or outward K^+ currents were elicited by depolarizing to 0 mV for 20 ms or to 70 mV for 300 ms, respectively (delivered at 0.1 Hz) and shown stable over 30 min under control conditions. As shown in Figure 5, (-)-**3** at 0.1 - 0.3 μM produced little inhibition of the Na^+ and K^+ currents, indicating high selectivity against the Ca^{2+} over Na^+ , and K^+ channels. At 3 μM , (-)-**3** caused non-specific inhibition on Na^+ , K^+ and Ca^{2+} currents in DRG cells. Hence, a new class of compounds with a dual mechanism of action, namely antagonizing ET receptors and inhibition of Ca^{2+} channels was discovered.

3. Conclusions

A new class of tetracyclic terpenes was synthesized in optically pure form via three sequential Robinson annulation reactions. A reductive-Michael addition process was found for the synthesis of optically pure tricyclic intermediates such as compound (+)-**13**. Regio- and stereo-selective 1,4-addition of a cyano moiety at the sterically hindered C10a of the tetracyclic enone (-)-**18** and subsequent transformation into (-)-**21** by exposing the imino function through a azabicyclic structure, i.e., (-)-**4** were found. The inhibitions of ET-1 induced vasoconstriction of six tetracyclic terpenes were examined along with myriceric acid A. Compound (-)-**3** showed the strongest inhibitory activity among the six tested terpenes and has a similar antagonistic activity as that of myriceric acid A, with a putative affinity value of 93 nM. The new tetracyclic terpene molecules possess a less complex

structure and lower molecular weight than those of myriceric acid A, which may enhance their penetration ability into cells and allow an easy synthesis of a library of analogs for the discovery of hit molecules. Furthermore, compound (-)-**3** (0.1 - 3 μ M) caused a concentration-dependent blockage of Ca^{2+} channel, and little inhibitions of Na^{+} or K^{+} currents were found at 0.3 and 1 μ M of (-)-**3**, respectively, indicating a degree of selectivity for Ca^{2+} channel over Na^{+} or K^{+} channels. The dual mechanism of (-)-**3** on the inhibition of ET receptors and calcium ion channel may provide a new tool for the mechanistic investigation on the vasoconstriction and may lead to a new class of compounds for the treatment of pulmonary hypertension.

4. Experimental Section

4.1. General Methods

NMR spectra were recorded on a 400-MHz spectrometer (Varian Inc.), in CDCl_3 , unless otherwise indicated, and reported in ppm. Low-resolution mass spectra were taken from an API 2000-triple quadrupole ESI-MS/MS mass spectrometer (from Applied Biosystems). High-resolution Mass spectra were obtained using a LCT Premier time of flight mass spectrometer. Chemicals were purchased from Fisher Scientific, Chem-Impex International, and VWR. Methanol was distilled over magnesium and dichloromethane and *N,N*-dimethylformamide (DMF) were distilled over calcium hydride under argon. THF was freshly distilled over sodium and benzophenone under argon and *t*-butanol was distilled over sodium under argon. Myriceric acid A was obtained from Shionogi & Co., Osaka, Japan. The purities of compounds submitted for biological evaluation were >95% as determined by HPLC/MS/MS.

4.2.1. (-)-(R)-5,8a-Dimethyl-3,4,8,8a-tetrahydronaphthalene-1,6(2H,7H)-dione (8).¹³—To a refluxing solution of 5.0 g (39.6 mmol) of 2-methyl-1,3-cyclohexanedione and 89 mg (1.6 mmol) of potassium hydroxide in 30 mL of dry methanol under argon, was added 5.0 g (59.4 mmol) of ethyl vinyl ketone over five min. After refluxing for 12 h, the solution was diluted with saturated aqueous ammonium chloride and 1 M hydrochloric acid to pH $\sim$ 7.0, and extracted with ethyl acetate three times. The combined extracts were washed with brine, dried (MgSO_4), and concentrated to give 8.32 g (100% yield) of 2-methyl-2-(3-oxopentyl)cyclohexane-1,3-dione as a yellow oil, which was used in the next reaction without purification. ^1H NMR δ 2.78 - 2.59 (m, 4 H), 2.39 (q, J = 7.3 Hz, 2 H), 2.32 (t, J = 7.3 Hz, 2 H), 2.07 (t, J = 7.3 Hz, 2 H), 2.10 - 1.84 (m, 2 H), 1.24 (s, 3 H), 1.02 (t, J = 7.3 Hz, 3 H); ^{13}C NMR δ 210.5, 210.3 (2 C), 64.6, 38.0 (2 C), 37.2, 36.2, 30.0, 20.0, 17.8, 7.9. To a solution of 8.32 g (39.6 mmol) of 2-methyl-2-(3-oxopentyl)cyclohexane-1,3-dione in 200 mL of dry DMF under argon were added 6.54 g (39.6 mmol) of D-phenylalanine and 4.6 g (19.8 mmol) of *S*-(+)-camphor-10-sulfonic acid. After stirring for 2 h at 25 $^\circ\text{C}$, the reaction temperature was increased by 15 $^\circ\text{C}$ every 2 hours until it reached 85 $^\circ\text{C}$ and stirred for additional 12 h. After cooling to 25 $^\circ\text{C}$, the reaction solution was diluted with saturated aqueous NaHCO_3 , extracted three times with diethyl ether, and the combined extract was washed with brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 4.6 g (61% yield) of (-)-**8**: $[\alpha]_{\text{D}}^{25}$ = -114.5 (c = 0.21, MeOH). Crystallization from hexane and diethyl ether gave

white needles, mp. 51.5 – 52.0 °C; $[\alpha]_D^{25} = -142$ ($c = 0.21$, MeOH); 100% ee. (lit.¹³ -125, $c = 0.54$, MeOH, after column; and -140 ($c = 0.20$, MeOH, after crystallization). ¹H NMR δ 2.88 (dt, $J = 15.6, 5.8$ Hz, 1 H), 2.73 - 2.64 (m, 1 H), 2.66 – 2.40 (m, 4 H), 2.20 - 2.04 (m, 3 H), 1.81 (s, 3 H), 1.80 – 1.71 (m, 1 H), 1.42 (s, 3 H); ¹³C NMR δ 211.7, 197.2, 158.1, 130.4, 50.4, 37.1, 33.1, 29.4, 27.0, 23.1, 21.3, 11.0. MS (electrospray ionization), m/z 215.1 (M + Na)⁺.

4.2.2. (-)-(4a*R*,5*R*)-5-Hydroxy-1,4a-dimethyl-4,4a,5,6,7,8-

hexahydronaphthalen-2(3*H*)-one (10)—To a solution of 4.62 g (24.1 mmol) of optically pure ketone (-)-**8** in 60 mL of dry ethanol at 0 °C under argon was added 0.23 g (6.0 mmol) of sodium borohydride. After stirring for 1 h, the reaction was diluted with saturated aqueous NH₄Cl and 1 M HCl to pH ~ 7.0. The solution was extracted three times with diethyl ether, and the combined extract was washed with brine, dried (MgSO₄), concentrated and chromatographed on silica gel using a gradient mixture of hexane and diethyl ether to give 4.10 g (87% yield) of compound **10**¹⁶ as a yellow oil: $[\alpha]_D^{25} = -170$ ($c = 1.20$, CHCl₃) [lit.¹⁶ $[\alpha]_D^{25} = -170$ ($c = 1.20$, CHCl₃)]; ¹H NMR δ 3.42 (dt, $J = 11.4, 4.7$ Hz, 1 H), 2.69 (dm, $J = 14.9$ Hz, 1 H), 2.46 - 2.43 (m, 2 H), 2.14 (dt, $J = 13.2, 4.4$ Hz, 1 H), 2.10 - 2.02 (m, 1 H), 1.95 - 1.81 (m, 3 H), 1.78 (d, $J = 1.5$ Hz, 3 H), 1.70 (ddd, $J = 24.6, 12.9, 4.1$ Hz, 1 H), 1.52 (bd, $J = 5.6$, 1 H), 1.37 (qt, $J = 13.5, 4.4$ Hz, 1 H), 1.18 (s, 3 H); ¹³C NMR δ 200.1, 162.5, 130.2, 78.6, 42.6, 34.0 (2 C), 30.5, 27.7, 23.5, 16.4, 11.9. MS (electrospray ionization), m/z 217.7 (M + Na)⁺.

4.2.3. (+)-(4a*S*,8*R*,8a*R*)-8-Hydroxy-1,4a,8a-trimethyl-4,4a,6,7,8,8a,9,10-octahydrophenanthren-2(3*H*)-one (11)

—To a stirred solution of 4.18 g (21.5 mmol) of (-)-**10** in 60 mL of dry methanol was added 3.5 g (65 mmol) of sodium methoxide, and the solution was stirred for 3 h at 25 °C. To it, 6.47 mL (65 mmol) of ethyl vinyl ketone was added drop wise over 5 min, and the solution was stirred for 12 h and then heated to reflux for 6 h, cooled to 25 °C, and diluted with saturated aqueous NH₄Cl and 1 N HCl to pH ~ 7.0. The mixture was extracted three times with diethyl ether, and the combined extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 3.83 g (69% yield) of (+)-**11** as an off white solid: mp. 120 - 125 °C (after recrystallization from a mixture of 2:1 of diethyl ether and hexane) (lit.¹⁶ 107 - 110 °C); $[\alpha]_D^{25} = +21$ ($c = 0.14$, CHCl₃); 23.5% ee (calculated), [lit.¹⁶ after recrystallization, $[\alpha]_D^{27} = +50$ ($c = 2.0$, CHCl₃); 56% ee]; ¹H NMR δ 5.50 (t, $J = 3.8$ Hz, 1 H) 3.48 (dd, $J = 8.77, 7.71$ Hz, 1 H), 2.67 - 2.42 (m, 4 H), 2.28 - 2.20 (m, 2 H), 2.10 – 1.96 (m, 3 H), 1.81 - 1.73 (m, 2H), 1.78 (s, 3 H), 1.53 - 1.44 (m, 2 H), 1.42 (s, 3 H), 1.27 (s, 3 H); ¹³C NMR δ 198.7, 163.4, 148.3, 128.3, 120.2, 76.1, 41.4, 39.1, 35.1, 34.5, 34.1, 28.1, 26.1, 24.8, 24.6, 20.6, 11.4.

4.2.4. (-)-(4a*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3*H*)-one (12)

—To a solution of (-)-**10** (0.40 g, 2.1 mmol) and imidazole (0.27 g, 4.0 mmol) in 15 mL dry DMF under argon at 25 °C, was added TBSCl (0.47 g, 3.1 mmol), and the mixture was stirred for 12 h, diluted with 50 mL aqueous NH₄Cl, and extracted three times with ethyl acetate (50 mL each). The combined extract was washed with water, and brine, dried (MgSO₄), concentrated, and column

chromatographed on silica gel using a mixture of hexane and diethyl ether (5:1) as an eluent to give (-)-**12** [0.43 g, 90% yield based on recovered (-)-**10**] as a colorless oil and 0.11 g (28% recovery) of alcohol (-)-**10**. Compound (-)-**12**: mp 53.5 - 54.0 °C; $[\alpha]_D^{25} = -114$ ($c = 0.20$, CHCl_3); $^1\text{H NMR}$: δ 3.35 (dd, $J = 10.8, 5.6$ Hz, 1 H), 2.65 (d, $J = 14.8$ Hz, 1 H), 2.42 - 2.38 (m, 2 H), 2.09 - 2.00 (m, 2 H), 1.88 - 1.81 (m, 1 H), 1.77 (s, 3 H), 1.71 - 1.62 (m, 3 H), 1.36 - 1.24 (m, 1 H), 1.14 (s, 3 H), 0.90 (s, 9 H), 0.04 (s, 3 H), 0.03 (s, 3 H); $^{13}\text{C NMR}$ δ 199.4, 161.5, 129.9, 79.2, 42.7, 34.2, 33.9, 30.7, 27.3, 26.1 (3 carbons, *t*-Bu), 22.9, 18.3, 16.2, 11.5, -3.7, -4.7. MS (electrospray ionization), m/z 331.3 ($\text{M}+\text{Na}^+$). HRMS calcd. for $\text{C}_{18}\text{H}_{32}\text{O}_2\text{SiNa}$ ($\text{M}+\text{Na}^+$) 331.2069, found 331.2044.

4.2.5. (+)-(4a*R*,8*R*,8a*R*)-8-(*tert*-Butyldimethylsilyloxy)-1,4a,8a-trimethyl-4,4a,4b,5,6,7,8,8a,9,10-decahydrophenanthren-2(3*H*)-one (13)—To a solution of 40 mL of liquid NH_3 at -78 °C under argon was added lithium (56 mg, 8.1 mmol), and the solution was stirred for 30 min. To it, a solution of (-)-**12** (1.0 g, 3.2 mmol) and *t*-butanol (0.18 g, 2.6 mmol) in 10 mL of diethyl ether was added via syringe, and the mixture was stirred at -35 °C for 2 h, added a few drops of isoprene (freshly distilled over sodium; to quench the excess of lithium), warmed to 25 °C (to remove liquid NH_3), connected to a vacuum (0.1 mm Hg pressure), and heated at 40 °C for 10 min. The resulting white solids were dissolved in 40 mL of diethyl ether, cooled to -78 °C, and protected from light by covering with a black cloth. To it, a solution of ethyl α -trimethylsilylvinyl ketone (0.75 g, 4.8 mmol) in 10 mL of diethyl ether was added over 30 min. via a syringe pump under argon. The reaction mixture was gradually warmed to -40 °C and then to -10 °C over 2 h, diluted with aqueous NH_4Cl , and extracted three times with ethyl acetate (60 mL each). The combined extract was washed with water and brine, and concentrated under reduced pressure to dryness. The residue was dissolved in 100 mL of methanol and 20 mL 6% (by weight) of aqueous KOH, stirred, heated to reflux for 2 h, cooled to 25 °C, neutralized with acetic acid to pH ~ 7.0 , and concentrated on a rotary evaporator. The residue was diluted with aqueous NH_4Cl , extracted three times with ethyl acetate (60 mL each), and the combined extract was washed with water and brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and diethyl ether (3:1) as eluent to give 0.68 g (58% yield) of (+)-**13** as a colorless oil: $[\alpha]_D^{25} = +45.2$ ($c = 0.25$, CHCl_3); $^1\text{H NMR}$ δ 3.06 (dd, $J = 11.2, 4.8$ Hz, 1 H), 2.67 (dt, $J = 11.6, 3.2$ Hz, 1 H), 2.49 - 2.31 (m, 2 H), 2.23 (td, $J = 12.8, 2.8$ Hz, 1 H), 2.04 - 1.97 (m, 2 H), 1.79 (s, 3 H), 1.78 - 1.75 (m, 1 H), 1.59 - 1.11 (m, 6 H), 1.15 (s, 3 H), 1.11 - 1.02 (m, 2 H), 1.05 (s, 3 H), 0.89 (s, 9 H), 0.020 (s, 3 H), 0.019 (s, 3 H); $^{13}\text{C NMR}$ δ 199.2, 164.6, 128.4, 81.1, 55.6, 40.0, 39.4, 38.2, 36.5, 33.5, 31.1, 26.1 (3 carbons, *t*-Bu), 24.6, 24.5, 20.6, 18.6, 18.3, 12.5, 11.3, -3.7, -4.6. MS (electrospray ionization), m/z 377.5 ($\text{M}+\text{H}^+$). HRMS calcd. for $\text{C}_{23}\text{H}_{40}\text{O}_2\text{SiNa}$ ($\text{M}+\text{Na}^+$) 399.2695, found 399.2676.

4.2.6. (-)-(4a'*R*,8'*R*,8a'*R*)-1',1',4a',8a'-8-*tert*-Butyldimethylsilyloxy-tetramethyldodecahydro-1'*H*-spiro[1,3]dioxolane-2,2'-phenanthrene (14)—To a solution of liquid NH_3 (20 mL) at -78 °C under argon was added lithium (30 mg, 4.3 mmol) and the solution was stirred for 30 min. To it, a solution of (+)-**13** (0.40 g, 1.1 mmol) in 2 mL of dry THF was added slowly, stirred at -35 °C for 2 h, cooled to -78 °C, added dry THF (35 mL), stirred at -78 °C for 10 min, and added methyl iodide (1.4 mL, 22 mmol) into the bottom part of the reaction solution via syringe. After stirring at -78 °C for 30 min,

additional methyl iodide (1.4 mL, 22 mmol) was added to the reaction mixture, warmed to 25 °C, stirred for 14 h, diluted with aqueous NH₄Cl, and extracted three times with ethyl acetate (40 mL each). The combined extract was washed with water and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane and diethyl ether (10:1) as an eluent to give 0.27 mg (65% yield) of (+)-(4a*R*,8*R*,8a*R*)-8-(*tert*-butyldimethylsilyloxy)-1,1,4a,8a-tetramethyl-decahydrophenanthren-2(1*H*,3*H*,4*bH*)-one as a colorless solid: mp 93.5 - 94.5 °C; [α]_D²⁵ = +9.3 (c = 0.44, CHCl₃); ¹H NMR δ 3.06 (dd, *J* = 11.2, 4.8 Hz, 1 H), 2.56 - 2.38 (m, 2 H), 1.96 - 1.90 (m, 2 H), 1.78 - 1.69 (m, 1 H), 1.55 - 1.17 (m, 11 H), 1.08 (s, 3 H), 1.04 (s, 3 H), 0.94 (s, 3 H), 0.90 (s, 3 H), 0.89 (s, 9 H), 0.03 (6 H); ¹³C NMR δ 218.2, 81.5, 56.7, 55.4, 47.7, 40.4, 39.6, 39.5, 37.0, 34.4, 31.3, 26.8, 26.1 (3 carbons, *t*-Bu), 24.8, 21.3, 20.7, 19.6, 18.3, 16.4, 13.2, -3.7, -4.5. MS (electrospray ionization), *m/z* 415.1 (M+Na)⁺. To a flask equipped with a Dean-Stark apparatus under argon, were added 2.0 g (5.25 mmol) of the above tricyclic ketone, 50 mg (0.30 mmol) of *p*-toluenesulfonic acid, 3.25 g (52 mmol) of ethylene glycol, and 30 mL of toluene. The reaction solution was stirred under reflux for 2 h and water was removed through the Dean-Stark apparatus. The solution was cooled to 25 °C, diluted with aqueous NaHCO₃, and extracted three times with diethyl ether (50 mL each). The combined extract was washed with water and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane and ethyl acetate (5:1) as an eluant to give 2.10 g (95% yield) of (-)-**14** as a white solid: mp 97.0 - 101.0 °C; [α]_D²⁵ = -49 (c = 0.23, CHCl₃); ¹H NMR δ 3.97 - 3.87 (m, 4 H), 3.05 (dd, *J* = 11.2, 4.8 Hz, 1 H), 1.88 - 1.76 (m, 2 H), 1.73 - 1.65 (m, 1 H), 1.60 (ddd, *J* = 13.2, 7.2, 3.6 Hz, 1 H), 1.52 - 1.32 (m, 6 H), 1.29 - 1.10 (m, 5 H), 1.04 - 0.95 (m, 1 H), 0.93 (s, 3 H), 0.87 (s, 12 H), 0.86 (s, 3 H), 0.84 (s, 3 H), 0.00 (s, 6 H); ¹³C NMR δ 113.6, 81.7, 65.1, 65.07, 57.2, 54.0, 42.4, 40.4, 40.3, 37.3, 37.1, 31.4, 27.1, 26.2 (3 carbons, *t*-Bu), 24.8, 23.1, 20.3, 20.1, 18.4, 18.3, 16.4, 13.6, -3.7, -4.6; MS (electrospray ionization), *m/z* 459.1 (M+Na)⁺. HRMS calcd. for C₂₆H₄₉O₃Si (M+H⁺) 437.3451, found 437.3457.

4.2.7. (-)-(4a'*S*,8a'*R*)-1',1',4a',8a'-Tetramethyldecahydro-1'*H*-

spiro[[1,3]dioxolane-2,2'-phenanthren-8'(3'*H*)-one (7)—To a solution of 2.0 g (4.6 mmol) of (-)-**14** in 30 mL of THF under argon was added a dried solution (dried over 3 Å molecular sieves) of 27 mL of 1.0 M tetra-*n*-butylammonium fluoride in THF and 20 mL of THF. The resulting solution was stirred at 60 °C for 14 h, cooled to 25 °C, diluted with aqueous NH₄Cl, extracted three times with ethyl acetate (50 mL each), and the combined extract was washed with water and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of diethyl ether and hexane (1:1) as an eluent to give 1.4 g (95% yield) of (-)-(4a'*R*,8'*R*,8a'*R*)-1',1',4a',8a'-tetramethyldodecahydro-1'*R*-spiro[[1,3]dioxolane-2,2'-phenanthren-8'-ol as a white solid: mp 193.5 - 194 °C; [α]_D²⁵ = -46 (c = 0.1, CHCl₃; lit.^{16,17} [α]_D²⁵ = -44 (c = 0.1, CHCl₃; 98% ee); ¹H NMR δ 3.99 - 3.86 (m, 4 H), 3.13 (ddd, *J* = 11.4, 8.4, 4.1 Hz, 1 H), 1.90 - 1.74 (m, 2 H), 1.73 - 1.08 (m, 15 H), 0.93 (s, 3 H), 0.88 (s, 6 H), 0.85 (s, 3 H); ¹³C NMR δ 113.5, 81.4, 65.1, 56.8, 53.8, 42.4, 39.8, 39.4, 37.2, 37.0, 30.4, 27.1, 26.1, 24.8, 23.2, 20.2, 20.1, 18.3, 16.5, 13.3; MS (electrospray ionization), *m/z* 377.4 (M+Na)⁺. To a solution of 0.24 g (0.75 mmol) of the above tricyclic alcohol in 5 mL of DMSO under argon at 25 °C, was added 0.21 g (0.89 mmol) of 2-iodoxybenzoic acid (IBX). The reaction solution was stirred for 14 h, and the

resulting white participate was filtered and rinsed with diethyl ether. The filtrate was diluted with aqueous NaHCO₃, extracted three times with diethyl ether (60 mL each), and the combined extract was washed with water and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane, dichloromethane, and diethyl ether (5:3:1) as an eluent to give 0.22 g of pure (-)-**7** (95% yield) as a white solid: mp. 189.0 – 191.0 °C; [α]_D²⁵ = -28 (c = 0.14, CHCl₃); lit.¹⁹ [α]_D²⁵ = -26 (c = 0.14, CHCl₃; 98% ee); ¹H NMR δ 3.98 – 3.86 (m, 4 H), 2.54 (td, *J* = 14.0, 6.9 Hz, 1 H), 2.18 (dm, 1 H), 2.08 – 2.01 (m, 1 H), 1.82 (td, *J* = 13.9, 4.0 Hz, 1 H), 1.75 – 1.35 (m, 10 H), 1.26 (dd, *J* = 12.3, 2.1 Hz, 1 H), 1.18 (dd, *J* = 11.8, 3.0 Hz, 1 H), 1.14 (s, 3 H), 0.98 (s, 3 H), 0.94 (s, 3 H), 0.84 (s, 3 H); ¹³C NMR δ 215.9, 113.2, 65.1, 65.1, 57.6, 53.2, 49.4, 42.4, 38.3, 37.9, 37.1, 34.7, 27.0, 26.4, 23.1, 20.3, 20.07, 20.04, 18.0, 16.8; MS (electrospray ionization), *m/z* 343.6 (M+Na)⁺.

4.2.8. (+)-(4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one (15)—

To a solution of 0.30 g (0.94 mmol) of ketone (-)-**7** in 10 mL of THF at -20 °C under argon was added 28 mL (2.8 mmol) of freshly prepared 0.1 M lithium diisopropylamine (LDA) in THF. Immediately after the addition, the reaction solution was warmed to 25 °C, stirred for 1 h, and added a solution of 0.31 g (1.41 mmol) of phenyl disulfide in 10 mL of THF via cannula. The resulting solution was stirred for 12 h, diluted with saturated aqueous NH₄Cl, and extracted three times with diethyl ether. The combined organic layer was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane, methylene chloride, and diethyl ether to give 0.37 g (92% yield) of (+)-**15** as a white solid: mp. 218.0 – 219.5 °C; [α]_D²⁵ = +21.2 (c = 0.37, CHCl₃); ¹H NMR δ 7.41 – 7.37 (m, 2 H), 7.30 – 7.21 (m, 3 H), 4.21 (dd, *J* = 13.1, 6.4 Hz, 1 H), 3.97 – 3.86 (m, 4 H), 2.35 – 2.28 (m, 1 H), 1.84 – 1.36 (m, 10 H), 1.29 – 1.21 (m, 3 H), 1.19 (s, 3 H), 0.96 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H); ¹³C NMR δ 209.9, 134.4, 132.6 (2C), 129.1 (2C), 127.3, 113.0, 65.1 (2C), 57.9, 54.9, 53.0, 50.0, 42.4, 38.3, 37.0, 34.9, 34.7, 27.0, 23.1, 21.0, 20.0, 19.9, 17.9, 16.7; MS (electrospray ionization), *m/z* 451.4 (M+Na)⁺; HRMS calcd. for C₂₆H₃₆O₃S (M+H⁺) 429.2463, found 429.2444.

4.2.9. (4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(3-oxobutyl)-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one (16a) and (4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-tetramethyl-7'-(3-oxobutyl)-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one (16b)—

A solution of 0.50 g (1.17 mmol) of (+)-**15** and 0.13 g (2.33 mmol) of sodium methoxide in 8 mL of methanol and 16 mL of THF was stirred for 1 h at 25 °C. To it, 0.29 mL (3.51 mmol) of methyl vinyl ketone was added, stirred at 25 °C for 15 min and then 55 °C for 1 h. After cooling to 25 °C, the solution was diluted with saturated aqueous NH₄Cl, extracted three times with diethyl ether, and the combined organic layer was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 0.27 g (70% yield) of compound **16a** and 0.12 g (27% yield) of compound **16b**. Compound **16a** (less polar): white solids, mp. 134.5 – 135 °C; [α]_D²⁵ = -41.5 (c = 0.39, CHCl₃); ¹H NMR δ

7.30 - 7.40 (m, 5 H), 3.99 – 3.90 (m, 4 H), 2.55 – 2.35 (m, 2 H), 2.27 (dd, $J = 6.9$, 11.8 Hz, 1 H), 2.90 (s, 3H), 2.06 -1.78 (m, 7 H), 1.68 – 1.24 (m, 8 H), 1.06 (s, 3 H), 0.98 (s, 3 H), 0.95 (s, 3 H), 0.87 (s, 3 H); ^{13}C NMR δ 211.5, 208.2, 137.6 (2C), 130.4, 129.7, 128.9 (2C), 113.2, 65.1 (2C), 58.0, 53.3, 49.1, 47.9, 42.4, 39.2, 38.1, 36.9, 36.2, 30.9, 30.6, 30.2, 26.9, 23.1, 20.7, 20.2, 18.3, 16.5, 16.1; MS (electrospray ionization), m/z 521.4 ($\text{M}+\text{Na}$) $^{+}$; HRMS calcd. for $\text{C}_{30}\text{H}_{42}\text{O}_4\text{S}$ ($\text{M}+\text{H}^{+}$) 499.2882, found 499.2884. Compound **16b** (more polar): white solids, mp. 173.5 – 175.0 °C; $[\alpha]_{\text{D}}^{25} = -78.4$ ($c = 0.1$, CHCl_3); ^1H NMR δ 7.23 - 7.28 (m, 5 H), 3.99 – 3.87 (m, 4 H), 2.50 – 2.30 (m, 2 H), 2.09 (s, 3 H), 2.02 – 1.66 (m, 9 H), 1.50 (s, 3 H), 1.47 -1.12 (m, 7 H), 1.01 (s, 3 H), 0.94 (s, 3 H), 0.84 (s, 3 H); ^{13}C NMR δ 211.2, 208.2, 136.6 (2C), 130.7, 129.6, 129.0 (2C), 113.3, 65.1 (2C), 58.2, 56.7, 53.2, 49.4, 42.4, 39.1, 38.0, 36.9, 36.8, 36.4, 31.1, 30.2, 27.0, 24.0, 23.0, 19.9, 18.1, 17.4, 17.1; MS (electrospray ionization), m/z 521.4 ($\text{M}+\text{Na}$) $^{+}$; HRMS calcd. for $\text{C}_{30}\text{H}_{42}\text{O}_4\text{S}$ ($\text{M}+\text{H}^{+}$) 499.2882, found 499.2886. In the 2D NOESY spectrum of (-)-**16a**, no correlation was found between the aromatic hydrogens and C8a-methyl, while in that of (-)-**16b**, correlation was found between the aromatic signals and C8a-methyl (δ 1.50 ppm).

4.2.10. (-)-(4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(3-oxobutyl)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one (17a) and (-)-(4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-tetramethyl-7'-(3-oxobutyl)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one (17b)—To a solution of 0.43 g (0.85 mmol) of a mixture of (-)-**16a** and (-)-**16b** (a ratio of 2.6:1) and 0.46 mL of tri-*n*-butyltin hydride in 17 mL of dry toluene under argon, was added 14 mg (0.09 mmol) of AIBN. The solution was stirred under reflux and stirred for 12 h. The reaction solution was cooled to 25 °C, concentrated under reduced pressure, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 0.15 g (45% yield) of compound **17a** (more polar) and 0.15 g (45% yield) of compound **17b** (less polar). Compound **17a**: a white solid, mp. 118 - 120 °C; $[\alpha]_{\text{D}}^{25} = -33^{\circ}$ ($c = 0.22$, CHCl_3); ^1H NMR δ 3.97 – 3.85 (m, 4 H), 2.58 – 2.51 (m, 2 H), 2.41 – 2.33 (m, 1 H), 2.12 (s, 3 H), 2.10 – 2.04 (m, 1 H), 1.92 – 1.55 (m, 7 H), 1.51 – 1.12 (m, 8 H), 1.11 (s, 3 H), 0.97 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H); ^{13}C NMR δ 215.9, 209.4, 113.2, 65.1, 65.1, 58.5, 53.2, 49.3, 44.7, 42.4, 41.7, 38.3, 37.0, 34.6, 34.0, 30.1, 27.0, 24.2, 23.1, 20.6, 20.1, 20.07, 18.1, 16.7; MS (electrospray ionization), m/z 413.3 ($\text{M}+\text{Na}$) $^{+}$; HRMS calcd. for $\text{C}_{24}\text{H}_{38}\text{O}_4$ ($\text{M}+\text{H}^{+}$) 391.2848, found 391.2838. Compound **17b**: a white solid, mp. 169.5 - 171.0 °C; $[\alpha]_{\text{D}}^{25} = -120$ ($c = 0.13$, CHCl_3); ^1H NMR δ 3.99 – 3.86 (m, 4 H), 2.47 (t, $J = 7.3$ Hz, 2 H), 2.63 – 2.30 (m, 1 H), 2.13 (s, 3 H), 1.96 – 1.74 (m, 5 H), 1.70 – 1.40 (m, 7 H), 1.38 - 1.15 (m, 4 H), 1.07 (s, 3 H), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H); ^{13}C NMR δ 219.8, 209.1, 113.2, 65.1 (2C), 53.6, 51.6, 48.0, 44.4, 42.4, 41.7, 37.7, 36.6, 36.3, 30.2, 26.9, 25.4, 25.0, 23.1, 20.3, 20.1, 16.2, 17.2, 16.1; MS (electrospray ionization), m/z 413.2 ($\text{M}+\text{Na}$) $^{+}$; HRMS calcd. for $\text{C}_{24}\text{H}_{38}\text{O}_4$ ($\text{M}+\text{H}^{+}$) 391.2848, found 391.2856.

4.2.11. (-)-(4aR,4bR,6aR,10bR)-1,1,4a,10b-Tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolan]-9(3H)-one (18)—A solution of (-)-**17b** (8.0 mg, 20 μmol) and NaOMe (3.5 mg, 60 μmol) in 1.0 mL of dry MeOH and 0.5 mL of THF was stirred at 25 °C for 20 h and 58 °C for 4 h. It was diluted with saturated aqueous NH_4Cl , extracted three times with diethyl ether, and the combined

extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 6.0 mg (80% yield) of (-)-**18** as a white solid: mp. 213.5 – 215 °C; [α]_D²⁵ = -24.1° (c = 0.11, CHCl₃); ¹H NMR δ 8.82 (d, *J* = 1.8 Hz, 1 H), 3.98–3.86 (m, 4 H), 2.58–2.47 (m, 1 H), 2.37 (dt, *J* = 16.4, 5.0 Hz, 1 H), 2.28–2.19 (m, 1 H), 2.11–1.97 (m, 2 H), 1.88–1.75 (m, 2 H), 1.72–1.45 (m, 8 H), 1.30–1.16 (m, 4 H), 1.12 (s, 3 H), 0.94 (s, 6 H), 0.86 (s, 3 H); ¹³C NMR δ 201.7, 176.9, 120.0, 113.2, 65.1, 65.1, 57.3, 53.4, 42.4, 41.4, 38.5, 38.1, 37.2, 36.2, 35.4, 34.7, 29.6, 27.1, 23.1, 22.8, 20.6, 20.1, 18.6, 16.7; MS (electrospray ionization), *m/z* 395.1 (M+Na)⁺; HRMS calcd. for C₂₄H₃₆O₃ (M+H⁺) 373.2743, found 373.2729.

4.2.12. Conversion of (-)-17b to (-)-17a—To a solution of 20 mg of (50 μ mol) of (-)-**17b** in 2 mL of dry methanol was added 10 mg (200 μ mol) of NaOMe at 25 °C under argon. The reaction solution was stirred for 48 h, diluted with 10 mL of saturated aqueous NH₄Cl, and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 16.2 mg (81% yield) of (-)-**17a**.

4.2.13. (+)-(4a*R*,10b*R*)-1,1,4a,10b-Tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydro- chrysene-2,9(1H,3H)-dione (1)—To a solution of 5.0 mg (13.4 μ mol) of (-)-**18** in 1 mL THF was added 0.2 mL of 2 N HCl, and the solution was allowed to stir at 25 °C for 4 h. It was diluted with aqueous NH₄OH and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 4.0 mg (90% yield) of (+)-**1** as a viscous oil. [α]_D²⁵ = +47.5° (c = 0.12, CHCl₃); ¹H NMR δ 5.84–5.83 (m, 1H), 2.61–2.34 (m, 4 H), 2.29–2.20 (m, 1H), 2.15–1.93 (m, 3H), 1.86–1.83 (m, 1H), 1.70–1.57 (m, 3H), 1.48–1.40 (m, 3H), 1.25–1.18 (m, 2H), 1.16 (s, 3H), 1.11 (s, 3H), 1.06 (s, 3H), 0.99 (s, 3H), 0.93–0.78 (m, 2H); ¹³C NMR δ 214.6, 201.4, 175.9, 120.2, 56.8, 54.7, 47.5, 41.2, 39.3, 37.8, 36.2, 35.3, 34.6, 34.1, 29.9, 29.6, 27.0, 22.2, 21.2, 21.0, 19.7, 16.6; MS (electrospray ionization), *m/z* 351.3 (M+Na)⁺. HRMS calcd. for C₂₂H₃₃O₂ (M+H⁺) 329.2481, found 329.2470.

4.2.14. (-)-(4a*R*,4b*R*,6a*R*,10a*R*,10b*R*,12a*R*)-1,1,4a,10b-Tetramethyl-9-oxohexadecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbonitrile (19)—To a solution of 0.21 g (0.57 mmol) of (-)-**18** in 3 mL of THF and 22.5 mL of DMF and H₂O (2:1) under argon, were added 0.18 mg (3.4 mmol) of NH₄Cl and 0.56 g (8.6 mmol) of KCN. The reaction solution was heated to 100–110 °C for 12 h, cooled to 25 °C, diluted with water, and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 0.21 g (91% yield) of (-)-**19** as a white solid: mp. 103–104 °C; [α]_D²⁵ = -27° (c = 0.045, CHCl₃); ¹H NMR δ 3.97–3.86 (m, 4 H), 2.54 (d, *J* = 14 Hz, 1 H), 2.46 (dm, *J* = 14 Hz, 1 H), 2.40 (d, *J* = 14 Hz, 1 H), 2.29 (td, *J* = 14, 6 Hz, 1 H), 2.00–1.94 (m, 2 H), 1.87–1.74 (m, 3 H), 1.68–1.57 (m, 6 H), 1.52–1.26 (m, 9 H), 1.05 (s, 3 H), 0.91 (s, 3 H), 0.83 (s, 3 H); ¹³C NMR δ 207.6, 120.5, 112.9, 65.2, 65.0, 55.4, 53.8, 52.4, 44.0, 42.4, 40.6, 40.5, 37.3, 37.2, 36.9 (2C), 30.7 (2C),

27.1, 23.0, 20.12, 20.10, 18.3, 16.5, 15.4; HRMS calcd. for $C_{25}H_{37}NO_3$ ($M+H^+$) 400.2852, found 400.2851.

4.2.15. (+)-(4a*S*,4b*R*,6a*R*,10a*R*,10b*R*,12a*R*)-4b,7,7,10a-Tetramethyl-3,8-dioxo-octadecahydrochrysene-4a-carbonitrile (2)—A solution of 4.0 mg (10 μ mol) of (-)-**19** in 1 mL of THF and 0.2 mL of 2 N HCl was stirred at 25 °C for 3 h, diluted with 10 mL of diethyl ether, and neutralized with aqueous $NaHCO_3$. The mixture was extracted with diethyl ether three times and the combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 3.2 mg (95% yield) of (+)-**2**. $[\alpha]_D^{25} = +31.25$ ($c = 0.08$, $CHCl_3$); 1H NMR δ 2.58 – 2.25 (m, 6H), 2.04 – 1.75 (m, 6H), 1.68 – 1.58 (m, 4H), 1.55 – 1.44 (m, 4H), 1.256 – 1.252 (m, 1H), 1.10 (s, 3H), 1.08 (s, 3H), 1.04 (s, 3H), 0.95 (s, 3H); ^{13}C NMR δ 207.1, 199.4, 120.4, 55.2, 53.7, 53.2, 47.2, 43.8, 40.55, 40.48, 39.1, 37.04, 37.01, 36.3, 33.9, 30.67, 30.61, 27.2, 21.1, 20.6, 19.5, 16.6, 15.1; MS (electrospray ionization), m/z 378.5 ($M+Na$) $^+$. HRMS calcd. for $C_{23}H_{33}NO_2Na$ ($M+Na$) $^+$ 378.2409, found 378.2392.

4.2.16. (-)-(4a*R*,4b*R*,6a*R*,10a*S*,10b*R*,12a*R*)-9-(*tert*-Butyldimethylsilyloxy)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1 *H*-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbonitrile (20)—To a solution of 25 mg (63 μ mol) of (-)-**19** and 70 μ L of triethylamine in 2.5 mL of CH_2Cl_2 at 0 °C under argon, was added 50 μ L (0.22 mmol) of *tert*-butyldimethylsilyl triflate. After stirring for 2 h, the reaction solution was diluted with aqueous $NaHCO_3$, extracted three times with diethyl ether, and the combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel (the silica was deactivated by washing with 1% triethylamine in hexane prior to the subjection of the crude product) using a gradient mixture of hexane and diethyl ether as eluent to give 32 mg (100% yield) of (-)-**20** as a white solid: mp. 173.0 – 174.5 °C; $[\alpha]_D^{25} = -16$ ($c = 0.055$, $CHCl_3$); 1H NMR δ 4.87 – 4.84 (m, 1 H), 3.97 – 3.85 (m, 4 H), 2.82 (dm, $J = 17.3$ Hz, 1 H), 2.12 – 1.93 (m, 3 H), 1.83 – 1.22 (m, 15 H), 0.99 (s, 3 H), 0.91 (s, 12 H), 0.90 (s, 3 H), 0.83 (s, 3 H), 0.15 (s, 3 H), 0.14 (s, 3 H); ^{13}C NMR δ 147.1, 122.1, 113.0, 102.8, 65.1, 65.0, 54.1, 52.4, 51.3, 42.4, 40.0, 37.5, 37.3, 36.8, 33.1, 33.0, 31.1, 30.0, 27.1, 25.9 (3C), 23.1, 20.2 (2C), 18.2, 18.17, 16.6, 15.6, -4.10, -4.18; MS (electrospray ionization), m/z 536.4 ($M+Na$) $^+$. HRMS calcd. for $C_{31}H_{51}NO_3Si$ ($M+H^+$) 514.3716, found 514.3718. Crystallization of (-)-**20** from diethyl ether gave white crystal suitable for single-crystal x-ray analysis.

4.2.17. (-)-((4a*R*,4b*R*,6a*R*,10a*S*,10b*R*,12a*R*)-9-(*tert*-Butyldimethylsilyloxy)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1 *H*-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-yl)methanimin (3)—To a solution of 10 mg (19.5 μ mol) of (-)-**20** in 1 mL of dry toluene at 25 °C under argon, was added 0.10 mL (0.10 mmol) of 1.0 M diisobutylaluminum hydride in toluene. After stirring for 30 min, the reaction solution was diluted with aqueous $NaHCO_3$ and extracted three times with diethyl ether. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel (deactivated by washing with 1% triethylamine in hexane) using a gradient mixture of hexane and diethyl

ether as eluent to give 5.8 mg (58% yield) of (-)-**3**: $[\alpha]_D^{25} = -35$ ($c = 0.106$, CHCl_3); ^1H NMR δ 8.38 (bs, 1 H), 4.69 – 4.68 (m, 1 H), 3.98 – 3.87 (m, 4 H), 2.19 – 2.03 (m, 2 H), 1.96 – 1.88 (m, 1 H), 1.85 – 1.77 (m, 2 H), 1.72 – 1.64 (m, 4 H), 1.51 – 1.46 (m, 5 H), 1.41 – 1.30 (m, 4 H), 1.23 – 1.14 (m, 2 H), 1.07 (s, 3 H), 0.93 (s, 3 H), 0.92 (s, 9 H), 0.90 (s, 3 H), 0.80 (s, 3 H), 0.14 (s, 3 H), 0.12 (s, 3 H); ^{13}C NMR δ 168.2, 125.7, 113.3, 102.1, 65.1, 65.0, 54.5, 53.6, 53.4, 52.4, 42.4, 37.6, 37.5, 34.1, 30.5, 29.9, 29.5, 28.9, 27.1, 26.1, 26.0, 23.0, 21.1, 20.2, 20.1, 18.2, 18.2, 17.5, 16.8, -4.07, -4.17; MS (electrospray ionization), m/z 538.4 ($\text{M} + \text{Na}^+$). HRMS calcd. for $\text{C}_{31}\text{H}_{53}\text{NO}_3\text{Si}$ ($\text{M} + \text{H}^+$) 516.3828, found 516.3865.

4.2.18. (-)-Spiro-(10a, 9)-{(4a*R*,4b*R*,6a*R*,10a*S*,10b*R*,12a*R*)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1*H*-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-yl}methyl-hemiaminal (4**)**—A solution of 40 mg (78 μmol) of (-)-**3** in a solution of 9:1:0.05 ratio of 1,4-dioxane, water, and acetic acid was stirred at 25 °C for 24 h, diluted with diethyl ether and saturated aqueous NaHCO_3 carefully, and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 30 mg of the crude cyclic silyloxy iminal, which was used in the following step without purification. ^1H NMR δ 7.70 (s, 1 H), 4.05 – 3.85 (m, 4 H), 2.03 – 1.64 (m, 6 H), 1.38 – 1.19 (m, 9 H), 0.93 (s, 3 H), 0.89 (bs, 15 H), 0.85 (s, 3 H), 0.14 (s, 3 H), 0.09 (s, 3 H). To a solution of 46 mg (89 μmol) of the above cyclic silyloxy iminal in 3 mL of THF at 25 °C under argon, was added 0.18 mL of 1.0 M tetra-*n*-butylammonium flouride in THF. After 1 h, the reaction solution was diluted with water and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as *eluent* to give 25 mg [81% yield after 2 steps from (-)-**3**] of (-)-**4**: $[\alpha]_D^{25} = -52$ ($c = 0.06$, CHCl_3); ^1H NMR δ 7.76 (bs, 1 H), 4.00 – 3.84 (m, 4 H), 1.97 (bd, $J = 10.5$ Hz, 1 H), 1.83 (td, $J = 14.1$, 3.5 Hz, 1 H), 1.73 – 1.47 (m, 11 H), 1.41 – 1.15 (m, 9 H), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.85 (s, 3 H); ^{13}C NMR δ 169.8, 113.3, 98.8, 65.1, 65.07, 54.6, 53.7, 47.5, 47.4, 42.4, 37.3, 37.2, 36.3, 33.9, 33.6, 32.1, 30.5, 28.9, 27.1, 23.1, 20.7, 20.1, 18.3, 18.2, 16.7; MS (electrospray ionization), m/z 424.3 ($\text{M} + \text{Na}^+$). HRMS calcd. for $\text{C}_{25}\text{H}_{40}\text{NO}_3$ ($\text{M} + \text{H}^+$) 402.3008, found 402.3021.

4.2.19. (-)-(4a*R*,4b*R*,6a*R*,10a*S*,10b*R*,12a*R*)-1,1,4a,10b-Tetramethyl-9-oxohexadecahydro-1*H*-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbaldehyde (21**)**—A solution of 10 mg (0.025 mmol) of (-)-**4** in 1.3 mL (0.13 mmol) of 0.1 M acetic acid in THF and 1.3 mL of water was stirred under reflux for 5 h, cooled to 25 °C, and diluted with diethyl ether and saturated aqueous sodium bicarbonate. The aqueous layer was extracted three times with diethyl ether, and the combined ether layers were washed with brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 8 mg (80% yield) of (-)-**21**: $[\alpha]_D^{25} = -101.3$ ($c = 0.04$, CHCl_3); ^1H NMR δ ppm 10.31 (s, 1 H), 4.01–3.84 (m, 4 H), 2.49 (d, $J = 14.1$ Hz, 1 H), 2.39 (dd, $J = 16.0$, 5.1 Hz, 1 H), 2.27 – 2.18 (m, 1 H), 2.15 – 2.00 (m, 3 H), 1.92 – 1.16 (m, 15 H), 1.11 (s, 3 H), 0.98 (s, 3 H), 0.92 (s, 3 H), 0.81 (s, 3 H); ^{13}C NMR δ 209.8, 209.1, 113.1, 65.2, 65.1, 60.0, 53.5, 53.1, 42.4, 42.4, 40.7, 39.9, 37.7, 37.6, 36.3,

31.2, 30.6, 29.0, 27.1, 23.0, 21.3, 20.1, 18.3, 17.3, 17.1; MS (electrospray ionization), m/z 425.3 ($M+Na$)⁺. HRMS calcd. for $C_{25}H_{39}O_4$ ($M+H$)⁺ 403.2848, found 403.2856.

4.2.20. (4b*R*,6a*S*,10a*S*,10b*R*)-4b,7,7,10a-Tetramethyl-4b,5,6,6a,7,8,9,10,10a,10b,11,12-dodecahydro-7*H*-spiro[chrysene-8,8'-[1,3]dioxolane]-3-ol (-)-(22)—

A solution of (-)-**16b** (20 mg, 40 μ mol) and NaOMe (3.5 mg, 60 μ mol) in 2 mL of methanol was stirred under reflux for 10 h, cooled to 25 °C, diluted with aqueous NH_4Cl , and extracted three times with diethyl ether. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 6.4 mg (42% yield) of (-)-**22** as a white solid: mp. 256 °C (dec); $[\alpha]_D^{25} = -77.0$ ($c = 0.1$, $CHCl_3$); 1H NMR δ 6.86 (d, $J = 8.0$ Hz, 1H), 6.70 (d, $J = 2.0$ Hz, 1H), 6.54 (dd, $J = 8.0, 2.4$ Hz, 1H), 4.55 (s, 1H), 3.96 – 3.85 (m, 4H), 2.86 – 2.66 (m, 2H), 2.31 – 2.25 (m, 1H), 1.91 – 1.58 (m, 5H), 1.53 – 1.46 (m, 4H), 1.37 – 1.21 (m, 2H), 1.18 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.85 (s, 3H); ^{13}C NMR δ 153.6, 151.9, 129.9, 127.5, 113.4, 112.8, 111.4, 65.12, 65.06, 55.0, 53.2, 42.4, 40.7, 38.4, 37.4, 37.0, 30.3, 21.7, 26.3, 23.1, 20.0, 19.1, 18.5, 16.5; MS (electrospray ionization), m/z 371.3 ($M+H$)⁺. HRMS calcd. for $C_{24}H_{35}O_3$ ($M+H$)⁺ 371.2581, found 371.2571.

4.2.21. (-)-(4a*R*,4b*R*,10b*R*)-9-Hydroxy-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,10b,11,12,12a-decahydrochrysen-2(1*H*)-one (5)—

A solution of 6.4 mg (17.0 μ mol) of (-)-**22** in 1 mL of THF and 0.2 mL of 2 N HCl was stirred at 25 °C for 3 h, diluted with water, and extracted three times with diethyl ether. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 5.5 mg (94% yield) of (-)-**5** as a white solid: mp. 265 °C (dec); $[\alpha]_D^{25} = -28.0$ ($c = 0.21$, $CHCl_3$); 1H NMR δ 6.90 (d, $J = 8.2$ Hz, 1 H), 6.73 (d, $J = 2.3$ Hz, 1 H), 6.58 (dd, $J = 8.2, 2.7$ Hz, 1 H), 4.55 (s, 1 H), 2.92 – 2.84 (m, 1 H), 2.80 – 2.71 (m, 1 H), 2.60 – 2.44 (m, 2 H), 2.86 (dt, $J = 12.5, 3.1$ Hz, 1 H), 2.09 – 2.03 (m, 1 H), 1.83 – 1.66 (m, 6 H), 1.52 – 1.46 (m, 4 H), 1.35 – 1.32 (m, 2 H), 1.22 (s, 3 H), 1.11 (s, 3 H), 1.09 (s, 3 H), 1.02 (s, 3 H); ^{13}C NMR δ 218.3, 153.9, 151.2, 130.1, 127.2, 113.1, 111.3, 54.8, 54.5, 47.5, 39.9, 39.3, 38.2, 37.2, 34.2, 30.1, 27.0, 25.7, 21.2, 20.3, 18.9, 16.3; MS (electrospray ionization), m/z 349.2 ($M+Na$)⁺. HRMS calcd. for $C_{22}H_{31}O_2$ ($M+H$)⁺ 327.2319, found 327.2311.

4.2.22. (-)-(4a*R*,10b*R*)-9-(2-Hydroxyethoxy)-1,1,4a,10b-tetramethyl-4,4a,5,6,10b,11,12,12a-octahydro-(1*H*,3*H*,4b*H*)-chrysen-2-one (6)—

A solution of 5.0 mg (13.4 μ mol) of (-)-**22**, 3.0 mg (27 μ mol) of ethyl chloroacetate, and 1.5 mg (14 μ mol) of sodium carbonate in 0.2 mL of DMF under argon was heated at 120 °C for 14 h. The mixture was cooled to 25 °C, diluted with saturated aqueous NH_4Cl , and extracted three times with diethyl ether. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 5.2 mg (88% yield) of the corresponding aryl ether as a colorless oil: $[\alpha]_D^{25} = -9.2$ ($c = 0.055$, $CHCl_3$); 1H NMR δ 6.91 (d, $J = 8.0$ Hz, 1 H), 6.82 (d, $J = 2.4$ Hz, 1 H), 6.61 (dd, $J = 8.0, 2.4$ Hz, 1 H), 4.57 (s, 2 H, Ar-O- CH_2 CO), 4.27 (q, $J = 7.2$ Hz, 2 H), 3.98 – 3.84 (m, 4 H), 2.87 – 2.68 (m, 2 H), 2.32 – 2.27 (m, 1 H), 1.90 – 1.58 (m, 7 H), 1.53 – 1.47 (m, 2 H), 1.36 – 1.31 (m, 3 H), 1.30 (t, $J = 6.8$ Hz, 3 H), 1.17 (s, 3 H), 0.96 (s, 3 H),

0.95 (s, 3 H), 0.85 (s, 3 H); MS (electrospray ionization), m/z 479.2 ($M+Na$)⁺. To a solution of the aryl ether (5.0 mg, 11 μ mol) in 0.5 mL of a mixture of dichloromethane, methanol, and ethanol (5:2:3) at 0 °C was added sodium borohydride (14.0 mg, 37 μ mol) in portion over 3 h. This mixture was stirred at 0 °C for 2 h and acidified with 2 N HCl to pH $\sim$ 2. The resulting solution was stirred at 25 °C for 6 h, diluted with saturated aqueous NH₄Cl, and extracted three times with diethyl ether. The combined extract was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 2.8 mg (63% yield) of (-)-**6** as a colorless oil: $[\alpha]_D^{25} = -27$ ($c = 0.12$, CHCl₃); ¹H NMR δ 6.96 (d, $J = 8.4$ Hz, 1 H), 6.83 (d $J = 2.8$ Hz, 1 H), 6.68 (dd, $J = 8.4, 2.4$ Hz, 1 H), 4.06 – 4.04 (m, 2 H, ArO-CH₂CH₂-), 3.96 – 3.93 (m, 2 H, ArO-CH₂CH₂-); 2.94 – 2.72 (m, 2 H), 2.60 – 2.42 (m, 2 H), 2.38 (dt, $J = 16, 6.4$ Hz, 1 H), 2.10 – 2.00 (m, 2 H), 1.85 – 1.64 (m, 4 H), 1.52 – 1.46 (m, 3 H), 1.33 (dd, $J = 12, 2.0$ Hz, 1 H), 1.23 (s, 3 H), 1.10 (s, 3 H), 1.08 (s, 3 H), 1.02 (s, 3 H); ¹³C NMR δ 218.1, 157.1, 151.2, 130.0, 127.8, 111.8, 111.3, 69.4, 61.8, 54.7, 54.5, 47.5, 39.9, 39.2, 37.2, 34.2, 30.6, 30.1, 26.8, 25.7, 21.2, 20.4, 18.9, 16.4; MS (electrospray ionization), m/z 393.3 ($M+Na$)⁺. HRMS calcd. for C₂₄H₃₅O₃ ($M+H$)⁺ 371.2581, found 371.2570.

4.3. Bioevaluation

4.3.1. Inhibition of ET-1 induced vasoconstriction in the gerbil spiral modiolar artery

—All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Kansas State University (IACUC#: 2613). Young adult female gerbils (Mongolian Gerbils, *Meriones unguiculatus*, Charles River, Wilmington, MA) were deeply anesthetized with tribromoethanol (560 mg/kg i.p) and sacrificed by decapitation. Temporal bones were removed and spiral modiolar arteries were isolated by microdissection at 4 °C.

Segments of the spiral modiolar artery with a length of $\sim$ 200 μ m were transferred into a warmed (37 °C) custom-built bath chamber (volume: 75 μ L) that was mounted on the stage of an inverted microscope (Nikon).^{11,12} Vessel segments were held in place by two very fine blunted glass needles mounted on micromanipulators (NM-151, Narishige) and superfused with physiological saline at a rate of 9 mL/min. This flow rate corresponds to an exchange rate of 2 bath chamber volumes per second. All experiments were performed at 37 °C.

Physiological saline contained (in mM) 150 NaCl, 3.6 KCl, 1.0 MgCl₂, 1.0 CaCl₂, 5.0 HEPES, and 5.0 glucose, pH 7.4. A solution containing 10 mM Ca²⁺ was prepared by addition of 9 mM CaCl₂ to the physiological saline. Synthetic tetracyclic terpenes, myriceric acid A and endothelin-1 were pre-dissolved in anhydrous DMSO. The final concentration of DMSO did not exceed 0.1 vol%.

The vascular diameter was continuously monitored via the microscope as described previously.^{11,12} Briefly, the magnified image of the spiral modiolar was monitored by a video camera (WV-1410, Panasonic), displayed on a monitor (PVM-122, Sony), and recorded on video tape (AG-1960 Panasonic). The vascular outer diameter was detected by two video detectors (Crescent Electronics, East Sandy, UT) and the calibrated analog signal

was charted (Kipp & Zonen), digitized at a rate of 12 Hz, and recorded by a data acquisition system (Axoscope 8.0, Axon Instruments, Union City, CA).

4.3.2. Preparation of DRG neurons—Mouse *dorsal root ganglion* (DRG) neurons were prepared from 1-2 months old C57/BC6 mice. All the procedures related with animal handling are strictly in accordance with the NIH guidelines and institute IACUC committee approved protocols. Briefly, the spine was taken out and split into two halves from the middle line after sacrificing the mice by decapitation. Lumber DRGs were collected into modified Krebs's solution (in mM: 130 NaCl, 10 HEPES-Na, 5 KCl, 1 CaCl₂, 10 glucose, 2 MgCl₂, pH adjusted to 7.35 with 1 N HCl) in a 1.5 mL tube. For digestion, the DRGs were removed into 0.5 mL of Hank's balanced salt solution (HBSS) with 1 mg/mL Collagenase and 0.5 mg/mL Trypsin added. The DRGs were minced with a fine scissors and incubated at 35 °C for 50 min. After removing the HBSS solution, the DRGs were dispersed into modified Krebs's solution and triturated with fire polished glass pipettes until no clump is visible. Finally the cells was dispersed onto poly-l-ornithine (Sigma) coated cover slips and maintained in a modified Krebs's solution with streptomycin sulfate (0.2 mM), Penicillin G Sodium (0.3 mM), Gentamycin (0.1 mM) at 21 °C.

4.3.3. Patch clamp recordings—Whole-cell voltage clamp recordings were performed on large size (30 – 50 µM in diameter) mouse DRG neurons within 2 days after acutely dissociation. All experiments were performed at room temperature (around 21 °C). Whole-cell currents were recorded using a MultiClamp 700B amplifier and analyzed offline with pCLAMP10.4 software (Axon CNS, Molecular Devices, formerly Axon Instruments). To record calcium currents, the external solution was composed of (in mM) 115 choline-Cl, 30 TEA-Cl, 2 CaCl₂, 10 Glucose and 10 HEPES (pH 7.3 -7.4 adjusted with TEA-OH and osmolality verified as 295 mOsm/kg). The internal solution was composed of (in mM) 125 CsCl, 10 HEPES(acid), 10 EGTA, 2 CaCl₂, 1 MgCl₂, 4 MgATP and 0.3 MgGTP (pH 7.3 - 7.4 adjusted with CsOH, and the osmolality verified as 295 mOsm/kg). Since sodium component was removed in the external solution, TTX was not needed. To record sodium currents, the external solution was remained as modified Krebs's solution specified above. The internal solution was composed of (in mM) 120 CsF, 10 HEPES, 11 EGTA, 1 CaCl₂, 1 MgCl₂, 10 TEA-Cl, 4 MgATP and 0.3 MgGTP (pH 7.3 - 7.4 adjusted with CsOH). In this recording condition, no obvious calcium components were observed in most cells. Occasionally, some L-type calcium components were observed but completely decayed quickly, perhaps due to high concentration of sodium component in the external solution. To record potassium currents, the external solution was remained as a modified Krebs's solution specified above. The internal solution was composed of (in mM) 65 KCl, 80 KF, 5 KOH, 10 EGTA, 2 MgATP (pH 7.35 - 7.4 adjusted with KOH). Recording electrodes were pulled by P-87 puller (Sutter Instrument Co.). The tip of resistance was 3-4 MΩ in bath and the series resistance was less than 10 MΩ after whole cell configuration. All compounds were purchased from Sigma unless specifically remarked. Drugs were bath applied to 1.5 mL external solution in the recording chamber and the final concentration was calculated as an even diffusion system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge financial support by the American Heart Association (0750115Z) and the National Institute of Neurological Disorders and Stroke grant (NS086343). SK thanks the Council of Higher Education of Turkey for a research fellowship.

References and Notes

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. *Nature*. 1988; 332:411. [PubMed: 2451132]
2. Bishop BM, Mauro VF, Khouri SJ. *Pharmacotherapy*. 2012; 32:838. [PubMed: 22806762]
3. Rubanyi GM, Polokoff MA. *Pharmacol Rev*. 1994; 46:325. [PubMed: 7831383]
4. Jeremy JY, Shukla N, Angelini GD, Wan S. *Pharmacol Res*. 2011; 63:483. [PubMed: 21056670]
5. Webb ML, Meek TD. *Med Res Rev*. 1997; 17:17. [PubMed: 8979248]
6. Sakurawi K, Yasuda F, Tozoy T, Nakamura M, Sato T, Kikuchi J, Terui Y, Ikenishi Y, Iwata T, Takahashi K, Konoike T, Mihara S, Fujimoto M. *Chem Pharm Bull*. 1996; 44:343. [PubMed: 8998841]
7. Fujimoto M, Mihara S, Nakajima S, Ueda M, Nakamura M, Sakurai K. *FEBS*. 1992; 305:41.
8. Mihara S, Sakurai K, Nakamura M, Konoike T, Fujimoto M. *Europ J Pharmacol*. 1993; 247:219.
9. Mihara S, Tozawa F, Itazaki K, Fujimoto M. *Europ J Pharmacol*. 1998; 342:319.
10. Konoike T, Takahashi K, Araki Y, Horibe I. *J Org Chem*. 1997; 62:960.
11. Wangemann P, Cohn ES, Gruber DD, Gratton MA. *Hear Res*. 1998; 118:90. [PubMed: 9606064]
12. Wangemann P, Gruber DD. *Hear Res*. 1998; 115:113. [PubMed: 9472740]
13. Hagiwara H, Uda H. *J Org Chem*. 1988; 53:2308.
14. Harada N, Sugioka T, Uda H, Kuriki T. *Synthesis*. 1990:53.
15. Takahashi S, Oritani T, Yamashita K. *Tetrahedron*. 1988; 44:7081.
16. Honda T, Favaloro FG Jr, Janosik T, Honda Y, Suh N, Sporn MB, Gribble GW. *Org Biomol Chem*. 2003; 1:4384. [PubMed: 14685310]
17. Takikawa H, Imamura Y, Sasaki M. *Tetrahedron*. 2006; 62:39.
18. Favaloro FG Jr, Honda T, Honda Y, Gribble GW, Suh N, Risingsong R, Sporn MB. *J Med Chem*. 2002; 45:4801. [PubMed: 12383005]
19. Yokoe H, Mitsunashi C, Matsuoka Y, Yoshimura T, Yoshida M, Shishido K. *J Am Chem Soc*. 2011; 133:8854. [PubMed: 21557626]
20. Fortunato JM, Ganem B. *J Org Chem*. 1976; 41:2194.
21. Cheung A, Snapper ML. *J Amer Chem Soc*. 2002; 124:11584. [PubMed: 12296709]
22. Stork G, Ganem B. *J Amer Chem Soc*. 1973; 95:6152.
23. Monti SA, Yang YL. *J Org Chem*. 1979; 44:897.
24. The authors have deposited atomic coordinates for structure (-)-20 with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1048742. The coordinates can be obtained on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EK, U.K.
25. Scherer EQ, Wonneberger K, Wangemann P. *J Membr Biol*. 2001; 182:183. [PubMed: 11547341]
26. Trezise DJ, John VH, Xie XM. *Br J Pharmacol*. 1998; 124:953. [PubMed: 9692781]

Abbreviations

2D NOESY	two-dimensional nuclear Overhauser effect spectroscopy
Dibal-H	diisobutylaluminum hydride
DRG	dorsal root ganglion
ee	enantiomeric excess
ET-1	endothelin-1
ET_A	endothelin A receptors
EGTA	ethylene glycol tetraacetic acid
EC₅₀	median effective concentration
EVK	ethyl vinyl ketone
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HVA	high-voltage-activated
K_i	inhibitory constant
K-Selectride	potassium tri- <i>sec</i> -butylborohydride
LDA	lithium diisopropylamide
LVA	low-voltage-activated
MA	myricic acid A
MgATP	magnesium adenosine 5'-triphosphate
ORTEP	Oak Ridge thermal ellipsoid plot
SEM	standard error of the mean
TBSCl	<i>t</i> -butyldimethylsilyl chloride
TTX	tetrodotoxin

Retrosynthetic analysis:

Figure 1. Synthesized tetracyclic terpenes and their retrosynthetic analysis and myriceric acid A

Figure 2.
An ORTEP drawing of a single-crystal X-ray structure of compound (-)-20.

Figure 3.

Representative inhibition graphs of ET-1 induced vasoconstrictions of the spiral modiolar artery by compounds (+)-1, (+)-2, (-)-3, (-)-4, (-)-5, and (-)-6, and myriceric acid A (MA). Isolated segments of the spiral modiolar artery were superfused by physiologic saline and vascular diameter was recorded by video microscopy. The duration of changes in the composition of the superfusate was marked by black bars and the nature of the changes in the composition of the superfusate was annotated. MA, myriceric acid; ET-1, endothelin-1. 10 mM Ca²⁺ indicated an increase in the Ca²⁺ concentration from 1 to 10 mM.

Figure 4.

Compound (-)-3 inhibits Ca^{2+} currents in mouse DRG neurons. A. The representative traces of voltage-gated Ca^{2+} currents in control conditions and during application of (-)-3 at different concentrations (0.1 – 1 μM). The DRG neuron was held at -90 mV and depolarized to 70 mV for 100 ms in a 10 mV increment (depolarizing pulses were delivered at a 2 s interval, which did not show obvious frequency dependent inhibition). As shown in panel A, both low (T-type) and high (mainly L-type) voltage-activated Ca^{2+} currents were elicited. Application of (-)-3 inhibited both low- and high-voltage-activated Ca^{2+} currents in dose-dependent manner. The control traces were recorded in the presence of 0.1% DMSO (the highest one in vehicles). The panel B shows an I/V plot of high voltage-activated Ca^{2+} currents in the absence and presence of different concentrations of (-)-3. Similar results were recorded in 3 DRG neurons. C. The dose-response curve was plotted and the curve was fitted with a formula of $y = a + b/(1 + (x/\text{IC}_{50})^c)$. IC_{50} was calculated as 0.29 μM . Data were presented as Mean $\pm$ SEM (n = 4 per data point).

Figure 5.

Compound (-)-**3** effects on voltage-activated Na^+ and K^+ currents in mouse DRG neurons. Left panels show representative traces of Na^+ (top) and K^+ (bottom) currents in the presence of different concentrations and absence of (-)-**3**. At 0.1 - 0.3 μM , (-)-**3** produced little inhibitory effect on both Na^+ and K^+ currents. The neurons were held at -90 mV. Na^+ and K^+ currents were elicited by depolarizing to 0 mV for 20 ms, and 70 mV for 300 ms, respectively. Na^+ currents were measured at the peak and K^+ currents were measured at the end (298 ms) of depolarization steps. Traces were original individuals and not averaged. Note the tail currents were truncated for clarity. Right panels show the time course of Na^+ (top) or K^+ (bottom) currents upon application of (-)-**3** at different concentrations. Application of 0.1% DMSO (the highest concentration contained in highest drug solution tested) only caused transient slight inhibition and fully recovered after removal. It desensitized the repeating application of vehicles (data not shown). Each point was the average of three consecutive traces (in 10 sec interval).

Scheme 1. Synthesis of tricyclic intermediate (-)-7

Scheme 2. Synthesis of tetracyclic terpene (+)-1

Scheme 3. Synthesis of tetracyclic terpenes (+)-2 - (-)-4 and compound (-)-21

Scheme 4. Synthesis of tetracyclic terpenes 5 and 6

Affinity constants of compounds (+)-**1** – (-)-**6** and myricic acid A. Data represent averages $\pm$ standard deviations of putative pA₂ values. Calculations were based on a number of assumptions (*see text*). Each number is based on 11 to 17 individual experiments [N = 14 for (+)-**1**, (+)-**2**, and (-)-**5**; N = 16 for (-)-**3**; N = 17 for (-)-**4**; N = 11 for (-)-**6**; and N = 15 for myricic acid A]. in which the agonist 1 nM ET-1 was applied in the presence of a putative antagonist presented at one of three different concentrations that ranged between 0.1 μ M and 1 μ M. ET-1 induced vasoconstrictions in the presence or absence of antagonist were normalized to a standard vasoconstriction that was induced by 10 mM of Ca²⁺.²⁵

Table 1

Compound	(+)- 1	(+)- 2	(-)- 3	(-)- 4	(-)- 5	(-)- 6	(+)-myricic acid A
pA ₂ (M)	6.7 $\pm$ 0.3	6.9 $\pm$ 0.2	7.0 $\pm$ 0.2	6.5 $\pm$ 0.2	6.7 $\pm$ 0.3	6.5 $\pm$ 0.3	7.1 $\pm$ 0.3
A ₂	193 nM	134 nM	93 nM	301 nM	205 nM	318 nM	85 nM