Design and synthesis of a new steroid-macrocyclic derivative with biological activity

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Abstract The aims of this study were to evaluate the positive inotropic effect of a new macrocyclic derivative (compound **11**) and characterize the molecular mechanism involved in its biological activity. The first step was achieved by synthesis of a macrocyclic derivative involving a series of reactions for the preparation of several steroid derivatives such as (a) steroid-pyrimidinone (**3** and **4**), (b) steroid-amino (**5**), (c) steroid-imino (**6**), (d) ester-steroid (**7** and **8**), and (e) amido-steroid (**9** and **10**). Finally, **11** was prepared by removing the *tert*-butyldimethylsilane fragment of **10**. The biological activity of compounds on perfusion pressure and vascular resistance was evaluated on isolated rat heart using the Langendorff model. The inotropic activity of **11** was evaluated in presence of prazosin, metoprolol, indomethacin, nifedipine, and flutamide to characterize its molecular mechanism. Theoretical experiments were carried out with a Docking model, to assess potential interactions of androgen receptor with **11**. The results showed that only this macrocyclic

derivative exerts changes on perfusion pressure and vascular resistance translated as the positive inotropic effect, and this effect was blocked with flutamide; these data indicate that the positive inotropic activity induced by this macrocyclic derivative was via androgen receptor activation. The theoretical results indicated that the interaction of the macrocyclic derivative with the androgen receptor involves several amino acid residues such as Leu₇₀₄, Asn₇₀₅, Met₇₈₀, Cys₇₈₄, Met₇₄₉, Leu₇₆₂, Phe₇₆₄, Ser₇₇₈, and Met₇₈₇. In conclusion, all these data suggest that the positive inotropic activity of the macrocyclic derivative may depend on its chemical structure.

Keywords Testosterone · Cycloheptadecaphane · Inotropic activity · Steroid · Macrocyclic

Introduction

For several years, some macrocyclic derivatives have been developed in various fields of medicinal chemistry for the preparation of new drugs or for evaluation of its activity in other areas of science. For example, there is a report which showed the preparation of a series of macrocyclic polyethers with inotropic activity [1]. Other data indicate that macrocyclic callipeltin exerts a positive inotropic effect in the left atria from the hearts of guinea pigs [2]. In addition, a study showed that a series of macrocyclic polyamines can induce a positive inotropic effect in atrial cells of dog [3]. Also, there is a study which shown the positive inotropic activity of the macrocyclic d-HP-DO3A in male Sprague-Dawley rats [4]. Additionally, other studies indicate that the macrocyclic polyester 15-crown-5 exerts a positive inotropic effect via calcium channels in isolated guinea pig atrium [5]. Contrary these experimental results, a study indicates that the macrocyclic xestospongina

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attenuates the positive inotropic effect by α -adrenergic stimulation in guinea pig papillary muscle [6]. In addition, other studies made in the tracheal smooth muscle of guinea pig and the heart muscle showed a variety of positive and negative inotropic responses in the presence of some crown ethers-macrocyclic derivatives in a dose-dependent manner [7]. Also, a study showed that macrocyclic derivatives *cis*-[Ru(bpy)₂Cl(NO)]⁺, *trans*-[RuCl([15]aneN₄)NO]²⁺, and [Ru(terpy)(bdq-COO)NO]³⁺ can induce smooth muscle relaxation via activation of cGMP production and K⁺ channel in normotensive rat aorta [8]. All these data show that some macrocyclic derivatives can induce inotropic effects in the cardiovascular system; nevertheless, the cellular site and molecular mechanism involved in their inotropic activity are very confusing; perhaps, this phenomenon is due to differences in the chemical structure of macrocyclic derivatives or to the different pharmacological approaches used. Therefore, more pharmacological data are needed to characterize the activity induced by the macrocyclic derivatives at cardiovascular level. To provide this information, the present study was designed to investigate the effects of the steroid-cycloheptadecaphane-tetraone macrocyclic derivative on perfusion pressure and coronary resistance in isolated rat hearts using the Langendorff technique. In addition, to evaluate the molecular mechanism involved in the inotropic activity induced by this compound on left ventricular pressure, some pharmacological tools were used.

Material and methods

General methods

The testosterone derivative (OTBS-testosterone) was prepared mainly previously reported [9]. The other reagents used in this study were purchased from Sigma-Aldrich Co. Ltd. The melting point was determined on an Electrothermal (900 model). ¹H and ¹³C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in CDCl₃ using TMS as an internal standard. Electron impact (EI)-MS spectra were obtained with a Finnigan Trace GC Polaris Q. spectrometer. Elementary analysis data were acquired from a PerkinElmer Ser. II CHNS/O 2400 elemental analyzer.

Chemical synthesis

4-(2-Hydroxy-naphthalen-1-yl)-5-phenyl-5H-pyrimidine-2-thione (3)

A solution of 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), acetophenone (97 μ l, 0.83 mmol), thiourea (70 mg, 0.91 mmol), and potassium hydroxide (30 mg,

0.53 mmol) in 5 ml of methanol was stirred for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 55% of product, m.p. 136–138 °C; IR ($V_{\max}$, cm⁻¹) 3330 and 1182; ¹H NMR (300 MHz, CDCl₃) δ_{H} 4.60 (m, 1H), 6.50–7.26 (m, 6H), 7.28 (m, 1H), 7.60–8.56 (m, 5H), and 12.66 ppm (broad, 1H). ¹³C NMR (75.4 Hz, CDCl₃) δ_{C} 42.00 (C-5), 119.32 (C-14), 122.72 (C-20), 122.72 (C-22), 125.40 (C-16), 127.06 (C-11), 127.08 (C-21), 127.41 (C-10, C-12), 128.90 (C-23), 131.62 (C-18), 131.70 (C-19), 131.92 (C-9, C-13), 136.24 (C-17), 138.12 (C-8), 148.18 (C-4), 157.90 (C-15), 164.95 (C-6), and 185.66 ppm (C-2). EI-MS m/z 330.08 Anal. Calcd. for C₂₀H₁₄N₂OS: C, 72.70; H, 4.27; N, 8.48; O, 4.84; S, 9.71. Found: C, 72.62; H, 8.36.

4-(2-Hydroxy-naphthalen-1-yl)-5-phenyl-5H-pyrimidine-2-one (4)

A solution of 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), acetophenone (97 μ l, 0.83 mmol), urea (50 mg, 0.83 mmol), and potassium hydroxide (30 mg, 0.53 mmol) in 5 ml of methanol was stirred for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 55% of product, m.p. 142–144 °C; IR ($V_{\max}$, cm⁻¹) 3400, 1725, and 1248; ¹H NMR (300 MHz, CDCl₃) δ_{H} 4.90 (m, 1H), 6.50–7.10 (m, 3H), 7.22 (m, 1H), 7.28–8.76 (m, 8H), and 12.68 ppm (broad, 1H). ¹³C NMR (75.4 Hz, CDCl₃) δ_{C} 47.10 (C-5), 117.90 (C-14), 121.92 (C-20), 122.72 (C-22), 124.60 (C-16), 126.76 (C-21), 126.98 (C-10, C-12), 127.04 (C-11), 128.50 (C-23), 129.50 (C-18), 130.12 (C-9, C-13), 132.32 (C-19), 135.94 (C-17), 136.62 (C-8), 1151.12 (C-4), 157.70 (C-15), 163.45 (C-2), and 167.60 ppm (C-6). EI-MS m/z 330.08 Anal. Calcd. for C₂₀H₁₄N₂O₂: C, 76.42; H, 4.49; N, 8.91; O, 10.18. Found: C, 76.34; H, 4.40.

N¹-[17-(tert-butyl-dimethyl-silanyloxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-cyclopenta[a]phenanthren-3-ylidene]-ethane-1,2-diamine (5)

A solution of OTBS-testosterone (200 mg, 0.49 mmol), ethylenediamine (60 μ l, 0.90 mmol), and boric acid (50 mg, 0.80 mmol) in 5 ml of methanol was stirred for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 66% of product, m.p. 144–146 °C; IR ($V_{\max}$, cm⁻¹) 3380, 3330, and 1248; ¹H NMR (300 MHz, CDCl₃) δ_{H} 0.06 (s, 6H), 0.86 (s, 9H), 0.94–1.02 (m, 2H), 1.04 (s, 3H), 1.08–1.10 (m, 2H), 1.22 (s, 3H), 1.38–1.60 (m, 6H), 1.62–1.90 (m, 5H), 2.22–2.40 (m, 3H), 3.12–

3.50 (m, 4H), 3.58 (m, 1H), 4.33 (broad, 1H), and 5.96 ppm (d, 1H, $J = 1.84$ Hz). ^{13}C NMR (75.4 Hz, CDCl_3) δ_{C} –4.60 (C-21, C-27), 11.40 (C-18), 17.80 (C-26), 18.00 (C-28), 21.00 (C-5), 23.52 (C-9, C-29, C-30, C-31), 30.44 (C-15), 31.08 (C-8), 31.16 (C-16), 31.32 (C-11), 31.70 (C-10), 35.30 (C-17), 35.38 (C-3), 37.10 (C-6), 40.82 (C-24), 43.36 (C-1), 50.60 (C-4), 52.33 (C-2), 53.18 (C-23), 81.70 (C-7), 115.54 (C-13), 158.12 (C-12), and 166.00 ppm (C-14). EI-MS m/z 444.35 Anal. Calcd. for $\text{C}_{27}\text{H}_{48}\text{N}_2\text{OSi}$: C, 72.91; H, 10.88; N, 6.30; O, 3.60; Si, 6.31. Found: C, 72.84; H, 10.72.

1-(2-{[17-(Tert-butyl-dimethyl-silanyloxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-cyclopenta[a]phenanthren-3-ylideneamino]ethylimino}-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-ol (6)

A solution of **5** (200 mg, 0.45 mmol) and **3** or **4** (200 μl , 1.07 mmol) in 5 ml of chloroform/dimethyl sulfoxide (3:2) was stirred for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 44% of product, m.p. 142–144 °C; IR (V_{max} , cm^{-1}) 3400, 3380, and 1248; ^1H NMR (300 MHz, CDCl_3) δ_{H} 0.08 (s, 6H), 0.88 (s, 9H), 0.94–1.02 (m, 2H), 1.05 (s, 3H), 1.08–1.10 (m, 2H), 1.22 (s, 3H), 1.38–1.60 (m, 6H), 1.62–1.90 (m, 5H), 2.22–3.56 (m, 5H), 3.84–3.90 (m, 4H), 4.34 (m, 1H), 5.96 (d, 1H, $J = 1.84$ Hz), 6.70–6.92 (m, 3H), 7.24 (m, 1H), 7.36–8.50 (m, 8H), and 12.70 ppm (broad, 1H). ^{13}C NMR (75.4 Hz, CDCl_3) δ_{C} –4.60 (C-48, C-50), 11.40 (C-45), 17.78 (C-44), 18.02 (C-51), 21.00 (C-26), 23.50 (C-21), 25.50 (C-52, C-53, C-54), 30.40 (C-22), 31.02 (C-20), 31.16 (C-23), 31.30 (C-24), 31.76 (C-25), 35.30 (C-14), 35.36 (C-16), 37.10 (C-27), 42.90 (C-5), 43.30 (C-18), 50.60 (C-15), 51.44 (C-9), 52.26 (C-8), 52.30 (C-17), 81.70 (C-19), 115.52 (C-12), 118.14 (C-34), 122.32 (C-40), 122.72 (C-38), 124.96 (C-42), 127.06 (C-31), 127.88 (C-39), 128.20 (C-30, C-32), 129.52 (C-36), 131.50 (C-29, C-33), 132.04 (C-35), 137.06 (C-43), 137.23 (C-28), 146.54 (C-4), 157.00 (C-41), 158.10 (C-13), 163.30 (C-6), 165.54 (C-2), and 166.00 ppm (C-11). EI-MS m/z 740.44 Anal. Calcd. for $\text{C}_{47}\text{H}_{60}\text{N}_4\text{O}_2\text{Si}$: C, 76.17; H, 8.16; N, 7.56; O, 4.32; Si, 3.79. Found: C, 76.04; H, 8.08.

3-((1-((Z)-2-((2-(((10R,13S,17S,E)-17-(tert-butyl dimethylsilyl)oxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-3-ylidene)amino)ethyl)imino)-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-yl)oxy)-5-nitrobenzoic acid (7)

A solution of **6** (200 mg, 0.27 mmol), 3,5-dinitrobenzoic acid (70 μl , 0.33 mmol), and potassium carbonate (30 mg, 0.21 mmol) in 5 ml of methanol/dimethyl sulfoxide (2:1)

was stirred for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol/hexane (3:1) yielding 38% of product, m.p. 78–80 °C; IR (V_{max} , cm^{-1}) 3398, 3332, and 1248; ^1H NMR (300 MHz, CDCl_3) δ_{H} 0.08 (s, 6H), 0.88 (s, 9H), 0.94–1.02 (m, 2H), 1.05 (s, 3H), 1.08–1.10 (m, 2H), 1.22 (s, 3H), 1.38–1.60 (m, 6H), 1.62–1.90 (m, 5H), 2.22–3.56 (m, 5H), 3.84–3.90 (m, 4H), 4.34 (m, 1H), 5.96 (d, 1H, $J = 1.84$ Hz), 6.70 (m, 2H), 7.24 (m, 1H), 7.36–8.80 (m, 12H), and 11.62 ppm (broad, 1H). ^{13}C NMR (75.4 Hz, CDCl_3) δ_{C} –4.60 (C-48, C-50), 11.40 (C-45), 17.78 (C-44), 18.02 (C-57), 21.00 (C-26), 23.50 (C-21), 25.50 (C-52, C-53, C-54), 30.40 (C-22), 31.02 (C-20), 31.16 (C-23), 31.30 (C-24), 31.76 (C-25), 35.30 (C-14), 35.36 (C-16), 37.10 (C-27), 42.90 (C-5), 43.30 (C-18), 50.60 (C-15), 51.44 (C-9), 52.26 (C-8), 52.30 (C-17), 81.70 (C-19), 115.52 (C-12), 115.70 (C-55), 118.44 (C-38), 119.00 (C-51), 119.12 (C-53), 122.32 (C-42), 123.64 (C-34), 124.72 (C-39), 125.30 (C-40), 126.50 (C-37), 127.08 (C-31), 127.20 (C-35), 127.24 (C-29, C-33), 128.12 (C-36), 128.20 (C-30, C-32), 134.02 (C-28), 134.24 (C-43), 136.62 (C-54), 146.54 (C-4), 146.90 (C-52), 158.10 (C-13), 159.18 (C-41), 163.29 (C-6), 164.60 (C-61), 165.54 (C-2), and 166.00 ppm (C-11). EI-MS m/z 905.45 Anal. Calcd. for $\text{C}_{54}\text{H}_{63}\text{N}_5\text{O}_6\text{Si}$: C, 71.57; H, 7.01; N, 7.73; O, 10.59; Si, 3.10. Found: C, 71.46; H, 7.00.

3-((1-((Z)-2-((2-(((10R,13S,17S,E)-17-(tert-butyl dimethylsilyl)oxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-3-ylidene)amino)ethyl)imino)-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-yl)oxy)-5-(4-carboxybenzyl)benzoic acid (8)

A solution of **7** (200 mg, 0.22 mmol) and 4-hydroxybenzoic acid (50 mg, 0.36 mmol) in 6 ml of ethanol/dimethyl sulfoxide (1:1) and potassium carbonate (30 mg, 0.21 mmol) was stirred for 80 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 56% of product, m.p. 72–74 °C; IR (V_{max} , cm^{-1}) 3332, 1712, and 1248; ^1H NMR (300 MHz, CDCl_3) δ_{H} 0.08 (s, 6H), 0.88 (s, 9H), 0.94–1.02 (m, 2H), 1.05 (s, 3H), 1.08–1.10 (m, 2H), 1.22 (s, 3H), 1.38–1.60 (m, 6H), 1.62–1.90 (m, 5H), 2.22–3.56 (m, 5H), 3.84–3.90 (m, 4H), 4.30 (m, 1H), 5.96 (d, 1H, $J = 1.84$ Hz), 6.70–7.08 (m, 2H), 7.24 (m, 1H), 7.32–7.90 (m, 10H), 8.04–8.10 (m, 4H), 8.24 (broad, 2H), and 8.78 ppm (m, 1H). ^{13}C NMR (75.4 Hz, CDCl_3) δ_{C} –4.60 (C-48, C-56), 11.40 (C-45), 17.78 (C-44), 18.02 (C-57), 21.00 (C-26), 23.50 (C-21), 25.50 (C-58, C-68, C-69), 30.40 (C-22), 31.02 (C-20), 31.16 (C-23), 31.30 (C-24), 31.76 (C-25), 35.30 (C-14), 35.36 (C-16), 37.10 (C-27), 42.90 (C-5), 43.30

(C-18), 50.60 (C-15), 51.44 (C-9), 52.26 (C-8), 52.30 (C-17), 81.70 (C-19), 104.24 (C-53), 105.86 (C-55), 111.98 (C-12), 113.70 (C-61, C-65), 115.52 (C-12), 118.44 (C-38), 120.64 (C-42), 122.00 (C-34), 124.22 (C-63), 124.74 (C-39), 124.90 (C-42), 125.30 (C-40), 125.80 (C-61, C-65), 126.30 (C-34), 126.50 (C-37), 127.08 (C-31), 125.30 (C-40), 126.50 (C-37), 127.06 (C-31), 127.24 (C-35), 128.12 (C-36), 128.20 (C-30, C-32), 133.88 (C-62, C-64), 133.60 (C-54), 134.02 (C-28), 134.24 (C-43), 140.00 (C-54), 146.56 (C-4), 157.06 (C-41), 158.10 (C-13), 158.82 (C-60), 163.69 (C-6), 165.54 (C-2), 166.00 (C-11), 168.44 (C-70), 168.78 (C-66), 171.24 (C-52), and 171.59 ppm (C-50). EI-MS m/z 996.48 Anal. Calcd. for $C_{61}H_{68}N_4O_7Si$: C, 73.46; H, 6.87; N, 5.62; O, 11.23; Si, 2.82. Found: C, 73.38; H, 6.76.

N-(2-aminoethyl)-3-(4-((2-aminoethyl)carbamoyl)phenoxy)-5-((1-(*E*)-2-((2-(((10*R*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[*a*]phenanthren-3-ylidene)amino)ethyl)imino)-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-yl)oxy)-benzamide (**9**)

A solution of **8** (100 mg, 0.50 mmol), ethylenediamine (80 μ l, 0.74 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (150 mg, 0.78 mmol), and *p*-toluenesulfonic acid anhydrous (100 mg, 0.86 mmol) in 5 ml of methanol was stirred for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 54% of product, m.p. 94–96 °C; IR (V_{max} , cm^{-1}) 3330, 1678, and 1244; 1H NMR (300 MHz, $CDCl_3$) δ_H 0.08 (s, 6H), 0.88 (s, 9H), 0.94–1.02 (m, 2H), 1.05 (s, 3H), 1.08–1.10 (m, 2H), 1.22 (s, 3H), 1.38–1.60 (m, 6H), 1.62–1.90 (m, 5H), 2.22–2.40 (m, 4H), 3.12 (m, 4H), 3.50 (m, 4H), 3.56 (m, 1H), 3.84–3.90 (m, 4H), 4.30 (m, 1H), 4.62 (broad, 6H), 5.96 (d, 1H, J = 1.84 Hz), 6.70–7.22 (m, 8H), 7.24 (m, 1H), and 7.36–8.78 ppm (m, 10H). ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C -4.60 (C-48, C-56), 11.40 (C-45), 17.78 (C-44), 18.02 (C-57), 21.00 (C-26), 23.50 (C-21), 25.52 (C-58, C-68, C-69), 30.40 (C-22), 31.02 (C-20), 31.16 (C-23), 31.30 (C-24), 31.76 (C-25), 35.30 (C-14), 35.36 (C-16), 37.10 (C-27), 42.90 (C-5), 43.30 (C-73, C-77), 43.32 (C-18), 43.70 (C-74, C-78), 50.56 (C-15), 51.44 (C-9), 52.26 (C-8), 52.30 (C-17), 81.70 (C-19), 112.00 (C-61, C-65), 114.10 (C-51), 115.52 (C-12), 116.16 (C-53), 117.78 (C-55), 118.44 (C-38), 118.82 (C-36), 120.12 (C-34), 124.74 (C-39), 125.30 (C-40), 126.50 (C-37), 127.00 (C-31), 127.24 (C-35), 127.28 (C-29, C-33), 128.12 (C-38), 128.20 (C-30, C-32), 128.73 (C-63), 130.90 (C-62, C-64), 134.02 (C-28), 134.24 (C-37), 146.14 (C-54), 146.56 (C-4), 155.40 (C-

41), 158.10 (C-13), 160.78 (C-52), 162.30 (C-66), 162.60 (C-60), 162.94 (C-50), 163.72 (C-6), 165.52 (C-2), 166.00 (C-11), and 169.72 ppm (C-70). EI-MS m/z 1080.60 Anal. Calcd. for $C_{65}H_{80}N_8O_5Si$: C, 72.19; H, 7.46; N, 10.36; O, 7.40; Si, 2.60. Found: C, 72.08; H, 7.38.

*1*⁵-((1-(*E*)-2-((2-(((10*R*,13*S*,17*S*)-17-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[*a*]phenanthren-3-ylidene)amino)ethyl)imino)-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-yl)oxy)-2-oxa-5,8,13,16-tetraza-1(1,3),3(1,4)-dibenzenacycloheptade-caphane-4,9,12,17-tetraone (**10**)

A solution of **9** (200 mg, 0.18 mmol), succinic acid (200 μ l, 1.07 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (150 mg, 0.78 mmol), and *p*-toluenesulfonic acid anhydrous (100 mg, 0.86 mmol) in 5 ml of methanol was stirred for 78 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 52% of product, m.p. 82–84 °C; IR (V_{max} , cm^{-1}) 3334, 1678, and 1248; 1H NMR (300 MHz, $CDCl_3$) δ_H 0.08 (s, 6H), 0.86 (s, 9H), 0.94–1.02 (s, 2H), 1.05 (s, 3H), 1.06–1.08 (m, 2H), 1.22 (s, 3H), 1.34–1.90 (m, 11H), 2.22–2.40 (m, 4H), 3.12 (m, 4H), 3.55 (t, 2H, J = 6.44 Hz), 3.56 (m, 1H), 3.62 (t, 2H, J = 6.44 Hz), 3.64 (m, 4H), 3.72 (m, 4H), 4.30 (m, 1H), 5.96 (d, 1H, J = 1.80 Hz), 6.20–7.20 (m, 8H), 7.22 (m, 1H), 7.36 (m, 1H), 7.38 (broad, 2H), 7.42–7.66 (m, 5H), 7.72 (broad, 2H), and 7.90–8.80 ppm (m, 4H). ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C -4.40 (C-80, C-81), 11.40 (C-77), 17.78 (C-76), 18.02 (C-82), 20.76 (C-68), 23.50 (C-63), 25.52 (C-83, C-84, C-85), 28.70 (C-54), 30.14 (C-65), 30.42 (C-64), 31.02 (C-62), 33.00 (C-66), 33.24 (C-67), 35.60 (C-56), 36.20 (C-13, C-14), 37.88 (C-56), 38.12 (C-69), 39.50 (C-9, C-18), 40.12 (C-10, C-17), 42.90 (C-48), 43.30 (C-60), 51.00 (C-57), 51.58 (C-51), 52.26 (C-59), 52.40 (C-50), 81.70 (C-61), 113.37 (C-27), 114.94 (C-5), 115.50 (C-3), 115.52 (C-54), 116.70 (C-23, C-25), 118.44 (C-37), 118.82 (C-34), 120.16 (C-30), 124.74 (C-36), 125.30 (C-35), 126.50 (C-38), 126.96 (C-21), 127.10 (C-73), 127.24 (C-31), 127.28 (C-71, C-75), 128.12 (C-32), 128.20 (C-72, C-74), 131.10 (C-22, C-26), 134.02 (C-70), 134.24 (C-33), 138.00 (C-6), 146.56 (C-47), 155.40 (C-29), 157.80 (C-55), 163.70 (C-43), 163.94 (C-7), 165.52 (C-45), 165.72 (C-20), 166.00 (C-53), 166.66 (C-2), 167.50 (C-12, C-15), 168.80 (C-4), and 169.00 ppm (C-24). EI-MS m/z 1162.60 Anal. Calcd. for $C_{69}H_{82}N_8O_7Si$: C, 71.23; H, 7.10; N, 9.63; O, 9.63; Si, 2.41. Found: C, 71.14; H, 7.04.

1⁵-(1-(E)-2-((2-(((10R,13S,17S,E)-17-hydroxy-10,13-dimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetra-decahydro-3H-cyclopenta[a]phenanthren-3-ylidene)amino)ethyl)imino)-5-phenyl-2,5-dihydropyrimidine-4-yl)naphthalen-2-yl)oxy)-2-oxa-5,8,13,16-tetraza-1(1,3),3(1,4)-dibenzenacycloheptadecaphane-4,9,12,17-tetraone (11)

A solution of **10** (200 mg, 0.17 mmol) in 5 ml of hydrofluoric acid was stirred for 8 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After that, the residue was purified by crystallization from methanol yielding 67% of product, m.p. 110–112 °C; IR ($V_{\max}$, cm^{-1}) 3400, 3322, and 1678; ^1H NMR (300 MHz, CDCl_3) δ_{H} 0.80 (s, 3H), 0.96–1.02 (m, 2H), 1.05 (s, 3H), 1.08–1.60 (m, 8H), 1.62–1.90 (m, 4H), 2.10–2.40 (m, 5H), 3.10 (m, 4H), 3.53–3.62 (m, 4H), 3.64 (m, 4H), 3.66 (m, 1H), 3.72 (m, 4H), 4.30 (m, 1H), 5.96 (d, 1H, $J = 1.80$ Hz), 6.20 (m, 1H), 6.26 (broad, 1H), 6.68–7.20 (m, 7H), 7.24 (m, 1H), 7.36 (m, 1H), 7.38 (broad, 2H), 7.42–7.66 (m, 5H), 7.72 (broad, 2H), and 7.90–8.78 ppm (m, 4H). ^{13}C NMR (75.4 Hz, CDCl_3) δ_{C} 11.12 (C-77), 17.80 (C-76), 20.78 (C-68), 23.40 (C-63), 30.14 (C-66), 30.30 (C-62), 30.42 (C-64), 32.96 (C-66), 33.20 (C-67), 35.60 (C-58), 36.20 (C-13, C-14), 36.64 (C-69), 37.88 (C-56), 39.50 (C-9, C-18), 40.12 (C-10, C-17), 42.80 (C-60), 42.88 (C-48), 50.56 (C-59), 51.00 (C-57), 51.60 (C-51), 52.40 (C-50), 80.72 (C-61), 113.37 (C-27), 114.94 (C-5), 115.50 (C-3), 115.52 (C-54), 116.70 (C-23, C-25), 118.44 (C-37), 118.82 (C-34), 120.16 (C-30), 124.74 (C-36), 125.30 (C-35), 126.50 (C-38), 126.96 (C-21), 127.10 (C-73), 127.24 (C-31), 127.28 (C-71, C-75), 128.12 (C-32), 128.20 (C-72, C-74), 131.10 (C-22, C-26), 134.02 (C-70), 134.24 (C-33), 138.00 (C-6), 146.56 (C-47), 155.40 (C-29), 157.80 (C-55), 163.70 (C-43), 163.96 (C-7), 165.54 (C-45), 165.72 (C-20), 166.00 (C-53), 166.62 (C-2), 167.48 (C-12, C-15), 168.86 (C-4), and 169.00 ppm (C-24). EI-MS m/z 1048.52 Anal. Calcd. for $\text{C}_{63}\text{H}_{68}\text{N}_8\text{O}_7$: C, 72.11; H, 6.53; N, 10.68; O, 10.67. Found: C, 72.04; H, 7.46.

Biological activity

The experimental methods used in this investigation were reviewed and approved by the Animal Care and Use Committee of University Autonomous of Campeche (no. PI-420/12) and were in accordance with the guide for the care and use of laboratory animals [10]. Male Wistar rats, weighing 200–250 g, were obtained from University Autonomous of Campeche.

Reagents

The compounds involved in this study were dissolved in methanol, and different dilutions were obtained using the Krebs-Henseleit solution ($\leq 0.01\%$, v/v).

Experimental design

Male rats (200–250 g) were anesthetized by injecting them with pentobarbital at a dose rate of 50 mg/kg body weight [11]. After that, the chest was opened, and a loose ligature was passed through the ascending aorta. Then, the heart was removed and immersed in ice-cold physiologic saline solution¹. The heart was trimmed of non-cardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate. Following, an initial perfusion rate of 15 ml/min for 5 min was followed by a 15-min equilibration period at a perfusion rate of 10 ml/min. All experimental measurements were done after this equilibration period.

Evaluation of left ventricular pressure

The contractile activity was evaluated by measuring the left ventricular developed pressure (LV/dP), using a saline-filled latex balloon (0.01-mm diameter) inserted into the left ventricle via the left atrium [12]. It is important to mention that the latex balloon was linked to a cannula which was bound to a pressure transducer that was connected with the MP100 data acquisition system.

First stage

Effect induced by testosterone and the compounds **1 and **5–11** on perfusion pressure** Changes in perfusion pressure as a consequence of increase in time (3 to 18 min) in the absence (control) and presence of the compounds **1** and **5–11** and testosterone at a concentration of 0.001 nM were determined. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min.

Evaluation of effects exerted by the compounds **1 and **5–11** and testosterone on coronary resistance** Coronary resistance in the absence (control) and presence of the compounds **1** and **5–11** and testosterone at a concentration of 0.001 nM was evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. Since a constant flow was used, changes in coronary pressure reflected the changes in coronary resistance.

¹ Physiologic saline solution (Krebs-Henseleit solution; it was actively bubbled with a mixture of O_2/CO_2 (95:5/5%) and regulated at a pH of 7.4 and 37 °C) was composed of NaCl, 117.8 mmol; KCl, 6 mmol; CaCl_2 , 1.75 mmol; NaH_2PO_4 , 1.2 mmol; MgSO_4 , 1.2 mmol; NaHCO_3 , 24.2 mmol; glucose, 5 mmol; and sodium pyruvate 5 mmol, and the coronary flow was adjusted with a variable speed peristaltic pump.

Krebs-Henseleit solution was actively bubbled with a mixture of O_2/CO_2 (95:5/5 %) and regulated at a pH of 7.4 and 37°C.

Second stage

Effect of the compound 11 on left ventricular pressure through the calcium channel activation Intracoronary boluses (50 μ l) of the compound **11** (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 1 nM (duration of the preincubation with nifedipine was 10 min).

Effect exerted by the compound 11 on left ventricular pressure through synthesis of prostaglandins The boluses (50 μ l) of the compound **11** (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was evaluated. The bolus was injected at the point of cannulation. The dose-response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (duration of the preincubation with indomethacin was 10 min).

Effect induced by the compound 11 on left ventricular pressure through adrenergic receptors Intracoronary boluses (50 μ l) of the compound **11** (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of prazosin or metoprolol at a concentration of 1 nM (duration of preincubation with prazosin or metoprolol was 10-min equilibration period).

Effect induced by the compound 11 on left ventricular pressure via androgen receptor Intracoronary boluses (50 μ l) of the compound **11** (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of flutamide at a concentration of 1 nM (duration of preincubation with flutamide was 10-min equilibration period).

Statistical analysis

The obtained values are expressed as average $\pm$ SE, using each heart ($n = 9$) as its own control. The data obtained were put under analysis of variance (ANOVA) with the Bonferroni correction factor [13] using the SPSS 12.0 program. The differences were considered significant when p was equal or smaller than 0.05.

Docking Server

Docking calculations were carried out using Docking Server [14]. The MMFF94 force field [15] was used for energy minimization of ligand molecule (compound **11**) using the

Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on the 2axa-transcription protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [16]. Affinity (grid) maps of $20 \times 20 \times 20$ -Å grid points and 0.375-Å spacing were generated using the Autogrid program [17]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [18]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

Results and discussions

Evaluation chemistry

There are several reports on the preparation of pyrimidine-thiones and pyrimidine-ones derivatives using several reagents such as 3-isothiocyanato-propene [19], oxalyl chloride [20], cyanothioacetamide [21], formaldehyde [22], furoyl chloride [23], and diazotized anilines [24]; however, some of these reagents require condition specials. In this study, the first stage involved the preparation of a pyrimidine-thione using the three-component system (α -naphthol, benzaldehyde, and thiourea) in mild conditions (Fig. 1). The ^1H NMR spectrum of **3** showed several signals at 4.60 and 7.28 ppm for dihydropyrimidine ring, at 6.50–7.26 and 7.60–8.56 ppm for phenyl groups, and at 12.66 ppm for hydroxyl group. The ^{13}C NMR spectra display chemical shifts at 42.00, 148.18, and 164.95–185.66 ppm for dihydropyrimidine ring and at 119.32–138.12 and 157.90 ppm for phenyl groups.

The second stage was achieved by the reaction of α -naphthol, benzaldehyde, and urea with the same conditions mentioned earlier to form the compound **4** (Fig. 1). The results showed several signals involved in the ^1H NMR spectrum of **4** at 4.90 and 7.22 ppm for dihydropyrimidine ring, at 6.50 and 7.28–8.76 ppm for phenyl groups, and at 12.68 ppm for hydroxyl group. The ^{13}C NMR spectra display chemical shifts at 47.10, 151.12, and 163.45–167.65 ppm for dihydropyrimidine ring and at 117.90–136.62 and 157.70 ppm for phenyl groups (Fig. 2).

The following stage was achieved by preparation of imino groups involved in the compound **5**. It is important to mention

Fig. 1 Synthesis of pyrimidin-2-thione (**3**) or pyrimidin-2-one derivatives (**4**) using the three-component system (2-hydroxy-1-naphthaldehyde (**2**), acetophenone, and urea or thiourea) to form **3** or **4**. *i* potassium hydroxide

that there are several procedures for the synthesis of imino groups which are described in the literature [25]. For example, the synthesis of imino derivatives by the reaction of the compound 1-[(2-amino-ethylamino)phenyl-methyl]-naphthalen-2-ol with androsterone used boric acid as the catalyst [26]. In this study, the compound **5** was synthesized (Fig. 3) by the reaction of **3** or **4** using boric acid as the catalyst, because it is not an expensive reagent and no special conditions are required for use [26]. The ^1H NMR spectrum of **5** showed several signals at 0.06 and 0.86 ppm for the *tert*-butylsilane fragment; at 1.04–1.22 ppm for methyl groups bound to nucleus steroid; at 0.94–1.02, 1.08–2.40, 3.58, and 5.96 ppm for steroid moiety; and at 4.33 ppm for amino group. The ^{13}C NMR spectra display chemical shifts at –4.60, 18.00, and

23.52 ppm for *tert*-butylsilane fragment; at 11.40 and 17.80 ppm for methyl groups bound to steroid nucleus; at 21.00, 30.40–37.10, 43.36–52.33, and 81.70–158.12 ppm for steroid moiety; at 40.82 and 53.18 ppm for methylene groups bound to amino and imino groups; and at 166.00 ppm for imino group. EI-MS m/z 444.76 Anal. Calcd. for $\text{C}_{27}\text{H}_{48}\text{N}_2\text{OSi}$: C, 72.91; H, 10.88; N, 6.30; O, 3.60; Si, 6.31. Found: C, 72.84; H, 10.74.

It is important to mention that the compound **5** was reacted with **3** or **4** for synthesis of **6** using boric acid as the catalyst (Fig. 3). The ^1H NMR spectrum of **6** showed several signals at 0.08–0.88 ppm for the *tert*-butylsilane fragment; at 1.05 and 1.22 ppm for methyl groups bound to steroid nucleus; at 0.94–1.02, 1.08–1.10, 1.38–3.56, and 5.96 ppm for steroid moiety;

Fig. 2 Synthesis of OTBS-steroid-amino derivative (**5**). Reaction of OTBS-testosterone with ethylenediamine to form **5**. *ii* boric acid

Fig. 3 Synthesis of the OTBS-steroid-dihydropyrimidine-naphthalen-2-ol derivative (**6**). Reaction of OTBS-steroid-amino (**5**) with the compounds **3** or **4** to form **6**. *iii* chloroform/dimethyl sulfoxide

at 4.34 and 7.24 ppm for dihydropyrimidine ring; at 3.84–3.90 ppm for methylene bound to imino groups; at 6.70 and 7.36–8.50 ppm for phenyl groups; and at 12.70 ppm for hydroxyl group. The ^{13}C NMR spectra display chemical shifts at –4.60, 18.00, and 25.50 ppm for the *tert*-butylsilane fragment; at 11.40 and 17.78 ppm for methyl groups bound to steroid nucleus; at 21.00–23.50, 30.40–37.10, 43.30–50.60, 52.30–115.53, and 158.10 ppm for steroid moiety; at 42.90, 146.54, and 163.30 ppm for dihydropyrimidine ring; at 51.44–52.26 ppm for methylene groups bound to imino groups; at 118.14–137.26 and 157.00 ppm for phenyl groups; and at 165.54 and 166.00 for imino groups.

On the other hand, the following stage was achieved by the preparation of an ether group involved in the chemical structure of **7** (Fig. 4). It is important to mention that several ether derivatives have been synthesized using some reagents such as aluminum oxide [27], zinc chloride [28], 2,2'-dibromo-4,4'-dinitrobenzophenone [29], and dimethyl sulfoxide [30]. In this study, the compound **7** was prepared via displacement of nitro group from 3,5-dinitrobenzoic acid with **6** in presence of dimethyl sulfoxide at mild conditions. The ^1H NMR spectrum of **7** showed several signals at 0.08–0.88 ppm for the *tert*-butylsilane fragment; at 1.05 and 1.22 ppm for methyl groups bound to steroid nucleus; at 0.94–1.02, 1.08–1.10, 1.38–3.56, and 5.96 ppm for steroid moiety; at 4.34 and 7.24 ppm for dihydropyrimidine ring; at 3.84–3.90 ppm for methylene bound to imino groups; at 6.70 and 7.36–8.80 ppm for phenyl groups; and at 11.62 ppm for carboxyl group. The ^{13}C NMR spectra display chemical shifts at –4.60, 18.02, and 25.50 ppm for the *tert*-butylsilane fragment; at 11.40 and 17.78 ppm for methyl groups bound to steroid nucleus; at 21.00–23.50, 30.40–37.10, 43.30–50.60, 52.30–81.70, 115.52, and 158.10 ppm for steroid moiety; at 42.90, 146.56, and 163.69 ppm for dihydropyrimidine ring; at 51.44–52.26 ppm for methylene groups bound to imino groups; at 104.24–113.70, 118.44–140.00, 155.06–158.82, and 171.24–171.59 ppm for phenyl groups; at 168.42–168.78 ppm for carboxyl group; and at 165.54 and 166.00 for imino groups. EI-MS m/z 996.48 Anal.

17.78 ppm for methyl groups bound to steroid nucleus; at 21.00–23.50, 30.40–37.10, 43.30–50.60, 52.30–115.53, and 158.10 ppm for steroid moiety; at 42.90, 146.54, and 163.30 ppm for dihydropyrimidine ring; at 51.44–52.26 ppm for methylene groups bound to imino groups; at 115.70–136.62, 146.90, and 159.18–167.70 ppm for phenyl groups; at 164.54 ppm for carboxyl group; and at 165.54 and 166.00 for imino groups.

A second esterification was carried out via displacement of nitro group of the compound **7** with 4-hydroxybenzoic acid using the same conditions mentioned earlier to form the compound **8** (Fig. 4). The ^1H NMR spectrum of **8** showed several signals at 0.08–0.88 ppm for the *tert*-butylsilane fragment; at 1.05 and 1.22 ppm for methyl groups bound to steroid nucleus; at 0.94–1.02, 1.08–1.10, 1.38–3.56, and 5.96 ppm for steroid moiety; at 4.30 and 7.24 ppm for dihydropyrimidine ring; at 3.84–3.90 ppm for methylene bound to imino groups; at 6.70–7.08 and 7.32–7.90, 8.04–8.10, and 8.78 ppm for phenyl groups; and at 8.24 ppm for carboxyl group. The ^{13}C NMR spectra display chemical shifts at –4.60, 18.02, and 25.50 ppm for the *tert*-butylsilane fragment; at 11.40 and 17.78 ppm for methyl groups bound to steroid nucleus; at 21.00–23.50, 30.40–37.10, 43.30–50.60, 52.30–81.70, 115.52, and 158.10 ppm for steroid moiety; at 42.90, 146.56, and 163.69 ppm for dihydropyrimidine ring; at 51.44–52.26 ppm for methylene groups bound to imino groups; at 104.24–113.70, 118.44–140.00, 155.06–158.82, and 171.24–171.59 ppm for phenyl groups; at 168.42–168.78 ppm for carboxyl group; and at 165.54 and 166.00 for imino groups. EI-MS m/z 996.48 Anal.

Fig. 4 Synthesis of the OTBS-steroid-benzamide derivative (**9**). The first stage was achieved by the reaction of **6** with 3,5-dinitrobenzoic acid (iv) to form the OTBS-steroid-nitrobenzoic acid derivative (**7**). The second

stage involves the preparation of a OTBS-steroid-benzoic acid derivative (**8**) by the reaction of **7** with 4-hydroxybenzoic acid (v). Finally, **8** was reacted with ethylenediamine to form **9** in presence of EDC (vi)

On the other hand, the following stage was achieved by the preparation of an amide group involved in the compound **9** (Fig. 4). Many procedures for the formation of amide groups are known in the literature; the most widely practiced method employs carboxylic acid chlorides as the electrophiles which react with the amine in the presence of an acid scavenger [31]. Despite its wide scope, this protocol suffers from several drawbacks; most notable are the limited stability of many acid chlorides and the need for hazardous reagents for their preparation (thionyl chloride) [32]. In this work, the technique reported by Pingwah [33] for boric acid catalyzed amidation of carboxylic acids and amine was used. Therefore, boric acid was used as the catalyst in the reaction of **8** with ethylenediamine to form the compound **9** (Fig. 6). The ^1H NMR spectrum of **9** showed several signals at 0.08–

0.88 ppm for the *tert*-butylsilane fragment; at 1.05 and 1.22 ppm for methyl groups bound to steroid nucleus; at 0.94–1.02, 1.08–1.10, 1.38–2.40, 3.56, and 5.96 ppm for steroid moiety; at 4.30 and 7.24 ppm for dihydropyrimidine ring; at 3.84–3.90 for methylene bound to imino groups; at 3.12 and 3.50 ppm for both amino and amide groups; at 4.62 ppm for amino groups; at 6.70–7.00, 7.12–7.18, and 7.36–8.78 ppm for phenyl groups. The ^{13}C NMR spectra display chemical shifts at –4.60, 18.02, and 25.50 ppm for the *tert*-butylsilane fragment; at 11.40 and 17.78 ppm for methyl groups bound to steroid nucleus; at 21.00–23.50, 30.40–37.10, 43.32–50.56, 52.30–81.70, 115.52, 146.14, 146.56, and 158.10 ppm for steroid moiety; at 42.90, 146.56, and 163.69 ppm for dihydropyrimidine ring; at 43.30 and 43.70 ppm for methylene groups bound to both amino

Fig. 5 Steroid-dihydropyrimidine-dibenzenacycloheptadecaphane-tetraone (**11**). Reaction of **9** with succinic acid in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (*vii*) to form the compound

OTBS-steroid-dibenzenacycloheptadecaphane (**10**). Finally, the *tert*-butylsilyl fragment involved in the compound **10** was removed with hydrofluoric acid (*viii*) to form **11**

and amide groups; at 51.54–52.26 ppm for methylene groups bound to imino groups; at 112.00, 114.10, 116.16, 146.14, 155.40, 160.75, and 162.60–162.94 ppm for phenyl groups; at 162.30 and 169.72 ppm for both amino and amide groups; and at 165.56 and 166.00 for imino groups. EI-MS m/z 1080.60.

The following stage was achieved by the reaction of **9** with succinic acid to form a new amide involved in the compound **10** using the same conditions for preparation of **9** (Fig. 5). The ^1H NMR spectrum of **10** showed several signals at 0.08–0.86 ppm for the *tert*-butylsilyl fragment; at 1.04 and

1.20 ppm for methyl groups bound to steroid nucleus; at 1.06–1.08, 1.38–2.40, 3.36, and 5.96 ppm for steroid moiety; at 4.30 and 7.22 ppm for dihydropyrimidine ring; at 3.02–3.10 for methylene bound to imino groups; at 7.38 and 7.74 ppm for amide groups; at 3.63–3.70 ppm for methylene groups bound to amide groups; at 3.55–3.62 for methylene groups bound to both imino groups; and at 6.20–7.20, 7.36, 7.42–7.66, and 7.90–8.80 ppm for phenyl groups. The ^{13}C NMR spectra display chemical shifts at –4.40, 18.02, and 25.52 ppm for the *tert*-butylsilyl fragment; at 11.40 and 17.78 ppm for methyl groups bound to steroid nucleus; at 20.76–23.50,

Fig. 6 Effect induced by the compounds **1** and **5–11** on perfusion pressure. The results showed in the graphic **a** indicate that perfusion pressure was not inhibited in presence of the compounds **1** and **5–7**. The scheme **b** shows that testosterone ($p = 0.06$) and the compound **11**

significantly increase ($p = 0.05$) the perfusion pressure in comparison with the control conditions and the compounds **8–10**. Each bar represents the mean $\pm$ SE of nine experiments

30.11–35.60, 37.88–38.12, 43.30–51.00, 52.26, 81.70, 115.50, and 157.80 ppm for steroid moiety; at 42.90, 146.56, and 163.70 ppm for dihydropyrimidine ring; at 36.20 and 39.50–40.12 ppm for methylene groups bound to amide groups; at 51.58 and 52.40 ppm for methylene groups bound to imino groups; at 113.37–114.94, 115.52–138.00, 155.40, 166.66, and 168.80–169.00 ppm for phenyl groups; at 163.94, 165.72, and 167.50 ppm for amide groups; and at 165.52 and 166.00 for imino groups. EI-MS m/z 1162.60.

Finally, the fifth stage was performed by the removal of the *tert*-butyldimethylsilyl of **10** to form the compound **11** (Fig. 5); it is important to mention that several reagents have been used to remove the protector groups such as dimethylaluminum chloride [34], palladium(II) [35], and hydrofluoric acid [36]. In this study, aqueous hydrofluoric acid was used to remove the *tert*-butyldimethylsilyl group. The ^1H NMR spectrum of **11** showed several signals at 0.80 and 1.05 ppm for methyl groups bound to steroid nucleus; at 0.96–1.02, 1.08–2.40, 3.36, and 5.96 ppm for steroid moiety; at 4.30 and 7.24 ppm for dihydropyrimidine ring; at 3.53–3.62 and 3.72 for methylene bound to imino groups; at 7.38 and 7.74 ppm for amide groups; at 3.10 and 3.64 ppm for methylene groups bound to amide groups; at 6.26 ppm for hydroxyl group; and at 6.20, 6.68–7.20, 7.36, 7.42–7.66, and 7.90–8.78 ppm for phenyl groups. The ^{13}C NMR spectra display chemical shifts at 11.12 and 17.78 ppm for methyl groups bound to steroid nucleus; at 20.78–35.60, 36.64–37.88, 42.80, 50.56–51.00, 80.72, 115.50, and 157.80 ppm for steroid moiety; at 42.88, 146.56, and 163.70 ppm for dihydropyrimidine ring; at 36.20 and 39.50–40.12 ppm for methylene groups bound to amide

Fig. 7 Activity exerted by the compound **11** on coronary resistance. The results show that coronary resistance was higher ($p = 0.05$) in the presence of the compound **11** in comparison with the control conditions and testosterone. Each bar represents the mean $\pm$ SE of nine experiments

Fig. 8 Effects induced by the compound **11** on LVP through calcium channel or prostaglandin activation. Intracoronary boluses (50 μl) of the compound **11** (0.001 to 100 nM) were administered, and the corresponding effect on the LVP was determined. The results showed that the compound **11** increases the LVP in a dose-dependent manner and this effect was not inhibited in the presence of nifedipine or indomethacin. Each bar represents the mean $\pm$ SE of nine experiments. LVP left ventricular pressure

groups; at 51.60–52.40 ppm for methylene groups bound to imino groups; at 113.37–114.94, 115.52–138.00, 155.40, 166.62, and 168.80–169.00 ppm for phenyl groups; at 163.94, 165.72, and 167.48 ppm for amide groups; and at 165.54 and 166.00 for imino groups. EI-MS m/z 1048.52.

Fig. 9 Activity exerted by the compound **11** on LVP through adrenergic receptors. The compound **11** (0.001 to 100 nM) was administered (intracoronary boluses, 50 μl), and the corresponding effect on the LVP was evaluated in the absence and presence of prazosin or metoprolol at a dose of 1 nM. The results showed that the activity induced by the compound **11** on LVP was not inhibited in the presence of prazosin or metoprolol. Each bar represents the mean $\pm$ SE of nine experiments. LVP left ventricular pressure

Fig. 10 Effect induced by the compound **11** on LVP via androgen receptor. The compound **11** (0.001 to 100 nM) was administered (intracoronary boluses, 50 μ l), and the corresponding effect on the LVP was evaluated in the absence and presence of flutamide at a dose of 1 nM. The results showed that the activity induced by the compound **11** on LVP was blocked by flutamide. Each *bar* represents the mean $\pm$ SE of nine experiments. LVP left ventricular pressure

Biological evaluation

There are several studies which indicate that some macrocyclic derivatives exert a positive inotropic activity on the heart [3–6]. Nevertheless, the cellular site and molecular mechanism involved in their inotropic activity are very confusing; perhaps, this phenomenon is due to differences in the chemical structure of macrocyclic derivatives or to the different pharmacological approaches used. Therefore, in this study, the inotropic activity by a new macrocyclic derivative was evaluated using several strategies. The first stage involves the evaluation of effect exerted by the compounds **1** and **5**–

11 on blood vessel capacity (which is translated as changes in perfusion pressure) and coronary resistance using an isolated rat heart model. The results (Fig. 6) show that only the compound **11** ($p = 0.05$) significantly increases the perfusion pressure over time (3–18 min) compared to the control conditions and the compounds **1** and **5**–**10**. To test whether the effect exerted by the macrocyclic derivative on perfusion pressure could be the result from the presence of the steroid nucleus involved in its chemical structure, in this study, testosterone was used as a control. The results showed that perfusion pressure was higher in presence of compound **11** compared with testosterone. These data suggest that compound **11** exerts

Fig. 11 Site of binding for testosterone (*I*) and the compound **11** (*II*) with human androgen receptor (PDB ID: 2axa) visualized with GL mol Viewer after docking analysis with one-click docking

Fig. 12 The scheme shown the contact site of amino acid residues involved in the androgen receptor with testosterone (a) and the compound **11** (b). Visualized with GL mol Viewer after docking analysis with one-click docking

different effects on blood pressure, which could subsequently modify the vascular tone and coronary resistance of the heart.

In order to evaluate the activity exerted by testosterone, the compound **11** on coronary resistance was evaluated. The

Table 1 Decomposed interaction energies (kcal/mol) for the compound **11**

Hydrogen bonds	Polar	Cation-pi	Hydrophobic	Other
Met ₇₈₀ (−0.0024)	Ser ₇₇₈ (−0.4191)	Phe ₇₆₄ (−2.14)	Phe ₈₉₁ (−4.537)	Trp ₇₄₁ (−3.0227)
Cys ₇₈₄ (5.6332)	Ser ₇₅₃ (−0.3602)		Leu ₈₈₀ (3.3153)	Gln ₇₈₃ (−0.8379)
Asn ₇₀₅ (6.7385)			Phe ₈₇₆ (−3.2921)	Pro ₆₈₂ (−0.1276)
Leu ₇₀₄ (9.1575)			Met ₈₉₅ (−1.92679)	Thr ₈₇₇ (259142)
			Met ₇₄₂ (−1.6645)	
			His ₈₇₄ (−1.3527)	
			Ile ₈₉₉ (−0.7194)	
			Val ₈₀₃ (−0.1966)	
			Phe ₇₇₀ (0.4181)	
			Leu ₇₆₂ (0.9449)	
			Val ₇₁₅ (1.529)	
			Val ₇₄₆ (2.2911)	
			Leu ₈₇₃ (4.2247)	
			Met ₇₈₇ (6.6042)	
			Met ₇₄₉ (8.9214)	
			Met ₇₄₅ (9.5214)	

results indicated that coronary resistance was higher in presence of **11** compared with testosterone and control conditions (Fig. 7). All these data suggest that the effects exerted by testosterone and the compound **11** on perfusion pressure and vascular tone are different, which could involve the generation or activation of vasoactive substances which is translated as changes in blood pressure. This premise may be supported by some studies which indicate that the macrocyclic derivative (gadoteridol) [37] can induce changes on blood pressure via increasing the intracellular calcium. Analyzing these data, in this study, the activity induced by the compound **11** on left ventricular pressure was evaluated in the absence or presence of nifedipine. The results showed that the effect exerted by the compound **11** was not inhibited in the presence of nifedipine (Fig. 8). Furthermore, these data indicate that the activity exerted by the compound **11** was not via activation calcium channel.

In the search of molecular mechanism involved in the positive inotropic activity of compound **11**, the possibility of this macrocyclic derivative to exert its effect through prostaglandin activation (which is involved in the regulation of blood pressure) was also analyzed such as the effect exerted by the bryostatin (macrocyclic derivative) on the prostaglandins [38]. The results showed that the effect induced by the compound **11** was not inhibited in the presence of this compound (Fig. 8). All these data suggest that the molecular mechanism involved in the activity of the compound **11** was not via prostaglandins.

Table 2 Interaction of amino acid fragments with the compound **11**

Hydrogen bonds	Polar	Hydrophobic	Pi-pi	Other
Leu ₇₀₄	Ser ₇₅₃	Leu ₇₀₄	Phe ₇₆₄	Pro ₆₈₂
Asn ₇₀₅		Val ₇₁₅	Phe ₇₆₄	Leu ₇₀₄
Met ₇₈₀		Met ₇₄₂	His ₈₇₄	Asn ₇₀₅
Cys ₇₈₄		Met ₇₄₅	Phe ₈₇₆	Gln ₇₁₁
Met ₇₄₉		Val ₇₄₆		Val ₇₁₅
Leu ₇₆₂		Met ₇₄₉		Trp ₇₄₁
Phe ₇₆₄		Leu ₇₆₂		Met ₇₄₂
Ser ₇₇₈		Phe ₇₆₄		Met ₇₄₅
Met ₇₈₇		Phe ₇₇₀		Val ₇₄₆
		Cys ₇₈₄		Met ₇₄₉
		Met ₇₈₇		Leu ₇₆₂
		Leu ₈₇₃		Phe ₇₆₄
		Leu ₈₈₀		Phe ₇₇₀
		Met ₈₉₅		Met ₇₈₀
		Ile ₈₉₉		Gln ₇₈₃
		Val ₉₀₃		Cys ₇₈₄
				Met ₇₈₇
				Leu ₈₇₃
				Thr ₈₇₇

Table 3 Decomposed interaction energies (kcal/mol) for testosterone

Hydrogen bonds	Polar	Cation-pi	Hydrophobic	Other
Gln ₇₁₁	Asn ₇₀₅	Phe ₈₉₁	Phe ₇₆₄	Met ₈₉₅
	Thr ₈₇₇		Met ₇₄₅	Met ₇₄₉
	Arg ₇₅₂		Leu ₇₀₁	
			Met ₇₄₂	
			Leu ₇₀₄	

Analyzing the obtained experimental data, we also considered validating the inotropic activity exerted by some macrocyclic derivatives via adrenergic stimulation [39] and evaluating the possibility that the activities exerted by the compound **11** involve stimulation and secretion of catecholamines; in this experimental study, the activity exerted by the compound **11** on left ventricular pressure in the absence or presence of prazosin and metoprolol was evaluated. The results showed that the effect induced by the compound **11** on left ventricular pressure was not blocked by prazosin or metoprolol (Fig. 9). Therefore, these results indicate that the molecular mechanism involved in the effect exerted by the compound **11** was not via the adrenergic system.

In the search of the molecular mechanism involved in the activity induced by the compound **11** on left ventricular pressure and analyzing previous reports, it is found that the macrocyclic bisbibenzyls exert activities on androgen receptor [40]. In this study, the positive inotropic activity of the compound **11** was evaluated in presence of flutamide. The results showed that the effect induced by the compound **11** was significantly inhibited in the presence of flutamide (Fig. 10). All these data suggest that the molecular mechanism involved in the activity of the compound **11** is via androgen receptor. This phenomenon is similar to the activity exerted by other drugs on left ventricular pressure [41], however via a different molecular mechanism, which may contribute to decreased cell death caused by heart failure. There are some reports which indicate that several macrocyclic derivatives have biological activity and can modulate the activity exerted by the androgen receptor [41, 42]. In order to evaluate this hypothesis, several studies have been conducted to predict the interaction of

Table 4 Interaction of amino acid fragments with testosterone

Hydrogen bonds	Polar	Hydrophobic	Cation-pi	Other
Gln ₇₁₁	Asn ₇₀₅	Leu ₇₀₁	Phe ₈₉₁	Asn ₇₀₅
	Arg ₇₅₂	Leu ₇₀₄		Gln ₇₁₁
	Thr ₈₇₇	Met ₇₄₂		Met ₇₄₅
		Met ₇₄₅		Met ₇₄₉
		Phe ₇₆₄		Thr ₈₇₇
				Met ₈₉₅

macrocyclic with the androgen receptor using theoretical models [13–16].

Docking evaluation

In order to evaluate the possibility that the compound **11** could interact with the androgen receptor in this study, a molecular docking model (Docking Server) [43, 44] was used to evaluate the theoretical interactions of the compound **11** with human androgen receptor (PDB ID: 2axa) [45, 46]. Theoretical results indicate that the hydrogen interaction between compound **11** and the androgen receptor (Figs. 11 and 12 and Table 1) involves several amino acid residues such as Leu₇₀₄, Asn₇₀₅, Met₇₈₀, Cys₇₈₄, Met₇₄₉, Leu₇₆₂, Phe₇₆₄, Ser₇₇₈, and Met₇₈₇. In addition, other theoretical results showed the decomposed interaction energies (kcal/mol) between the compound **11** with other types of amino acid residues from androgen receptor (Table 2). These interaction energies involved amino acid residues such as Cys₇₈₄, Asn₅₇, Leu₇₀₄, and Met₇₈₀. Analyzing these data and the different functional groups involved on chemical structure of compound **11**, an additional study was conducted using testosterone as a chemical tool to know whether the interaction of compound **11** with androgen receptor could depend on steroid nucleus or on its functional groups. The results showed that the hydrogen interaction between testosterone and androgen receptor (Figs. 11 and 12 and Table 3) involves several amino acid residues such as Leu₇₀₁, Leu₇₀₄, Asn₇₀₅, Gln₇₁₁, Met₇₄₂, Met₇₄₅, Met₇₄₉, Arg₇₅₂, Phe₇₆₄, Thr₈₇₇, Phe₈₉₁, and Met₈₉₅. Other results (Table 4) showed different decomposed interaction energies for the interaction of testosterone-androgen receptor compared with compound **11**. All these data suggest that (1) the interaction of compound **11** with androgen receptor is conditioned by its physicochemical properties and (2) the interaction of compound **11** involves different amino acid residues compared with testosterone.

Conclusions

The macrocyclic derivative (compound **11**) is a particularly interesting drug, because the positive inotropic activity induced in the heart involves a different molecular mechanism in comparison with other drugs. This phenomenon may constitute a novel therapy for heart failure.

Compliance with ethical standards The experimental methods used in this investigation were reviewed and approved by the Animal Care and Use Committee of University Autonomous of Campeche (no. PI-420/12) and were in accordance with the guide for the care and use of laboratory animals [10]. Male Wistar rats, weighing 200–250 g, were obtained from University Autonomous of Campeche.

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