



## Preparation of two Cyclobutadiene-steroid derivatives Theoretical Analysis of its Interaction with the $\mu$ , $\delta$ , and $\kappa$ Opioid-receptors

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### ABSTRACT

The objective of this investigation was to develop two cyclobutadiene-steroid derivatives (compounds 6 or 7) to evaluate its theoretical interaction on  $\mu$ ,  $\delta$ , and  $\kappa$  opioid-receptors. The synthesis of 6 or 7 was carried out using a series of reactions which involves. 1) addition/cyclization: 2) imination, 3) etherification and 4) oxy-functionalization. Chemical structure of all compounds was confirmed using elemental analysis and NMR spectra. In addition, a theoretical analysis on the interaction of compounds 6 or 7 with  $\mu$ ,  $\delta$ , and  $\kappa$  opioid-receptors was evaluated using a docking model. The results showed that 6 or 7 may interact with different type of amino acids residues on surface of the  $\mu$ ,  $\delta$ , and  $\kappa$  opioid-receptors. Other data, indicated that inhibition constant ( $K_i$ ) involved in the interaction of compounds 6 or 7 with  $\kappa$ -receptor was less compared with the  $K_i$  present in the interaction with  $\mu$ ,  $\delta$ , receptors. These data indicated that 1) compounds 6 or 7 show a high affinity by  $\kappa$ -receptor; 2) the cyclobutadiene analogs are particularly interesting, because these drugs may constitute a novel therapy for pain

**Keywords:** Cyclobutadiene, Steroid derivatives, Opioid, Receptors.

### INTRODUCTION

There are several drugs for treatment of pain such as codeine, fentanyl<sup>1</sup>, buprenorphine, butorphanol and others<sup>1,2</sup>, however some these drugs

can cause some adverse effects such addiction<sup>3</sup>, respiratory depressant<sup>4</sup>, sedation, dizziness, nausea, vomiting, constipation<sup>5</sup>. In the search new therapeutic alternatives for treatment of pain, several drugs were developed. In this sense, there



is reports that a diethyl-benzamide analog have high affinity by  $\kappa$ -receptor<sup>6</sup>. Additionally, a report indicates that an acetamide derivative exert an analgesic effect via  $\kappa$ -receptor activation using a rat inodel<sup>7</sup>. Another study showed that a  $\kappa$ -receptor agonist (U-50,4H8) was prepared and their biological activity was evaluated in a guinea pig model<sup>8</sup>. Other data describes the synthesis of 2-[(Acylamino)ethyl]-1,4-benzodiazepines and their interaction with  $\kappa$ -receptor using a theoretical docking model<sup>9</sup>. Also, a report showed that arylacetamidic and benzomorphan derivatives can act as agonists of  $\kappa$ -receptor using a model based on pharmacophores and coupling<sup>10</sup>. Finally, a report indicated the preparation and interaction of a piperidine analog on  $\kappa$ -receptor using a docking model<sup>11</sup>. All these data indicate that some drugs may interact with different types of opioid-receptors; this phenomenon could be due to different functional groups involved in the chemical structure or to diverse protocols used. The objective of this study was synthesizing two cyclobutadiene-steroid analogs to evaluate their theoretical interaction with  $\mu$ ,  $\delta$ , and  $\kappa$  opioid-receptors.

## EXPERIMENTAL

### Chemical synthesis

The nitro-progesterone was prepared using previously method reported<sup>13</sup>. In addition, all the reagents used in this study were purchased from Sigma-Aldrich Co., Ltd. Infrared spectra (IR) were determined using KBr pellets on a PerkinElmer Lambda 40 spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR300/5 FT NMR spectrometer at 300 and 75.4 MHz (megahertz) in CDCl<sub>3</sub> (deuterated chloroform) using TMS (tetramethylsilane) as an internal standard. EIMS (electron impact mass spectroscopy) spectra were determined using a Finnegan trace gas chromatography Polaris Q-spectrometer. Elementary analysis data were determined from a PerkinElmer Ser. II CHNS/02400 elemental analyser.

### NI-{1-[3-(2-Amino-ethylimino)-10,13-dimethyl-4-nitro-hexadecahydro-cyclopenta[a] phenanthren-17-yl]-ethylidene)-ethane-1,2-diamine (2)

A mixture of 1 (200 mg, 0.55 mmol), ethylenediamine (60  $\mu$ l, 0.90 mmol) and boric acid (50 mg, (1.80 mmol) and 5 ml of methanol was stirred for 72 h to room temperature. The solvent of

reaction product was evaporated to dryness under reduced pressure. Then, the residue was purified via crystallization with methanol:water (4:1) system; yielding 52 % of product, M. P. 215-220 °C: IR ( $V_{max}$ ,  $cm^{-1}$ ): 3380 and 15St). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta_H$ : 0.88 (s, 2H), 0.92 (s, 3H), 1.06-1.68 (m, 11H), 1.80 (s, 3H), 1.84-2.50 (m, 10H), 3.10 (m, 4H), 3.52 (m, 2H), 3.82 (m, 2H), 4.34 (broad, 4H), 5.14 (m, 1 H) ppm. <sup>13</sup>C NMR (75.4 Hz, CDCl<sub>3</sub>)  $\delta_C$ : 13.22, 14.52, 16.70, 21 24, 22 36, 26,42, 26.62, 29.53, 30.94, 35.76, 36, 82, 38.14, 41.00, 42.82, 43.54, 50.99, 52.13, 52.44, 52.84, 53.12, 63.16, 79.22, 156.70, 161.60 ppm. EI-MS: m/z 445.34. Anal. Calcd. for C<sub>25</sub>H<sub>43</sub>N<sub>5</sub>O<sub>2</sub>: C, 67.38; H, 9.73; N, 15.72; O, 7.18. Found. C, 67.29; H, 9.68.

### 6-[2-(17-(1-[2-(4-Hydroxy-hex-1-ynylamino)-ethylimino]-ethyl)-10,15-dimethyl-4-nitro-hexadecahydro-cyclopenta[a]phenanthren-3-ylideneamino)-ethylamino]-hex-5-yn-3-ol (3)

A mixture of 2 (200 mg, 0.45 mmol), 1-hexyn-3-ol (80  $\mu$ l, 72 mmol), Copper(II) chloride (68 mg, 0.50 mmol), 5 ml of methanol was stirred for 72 h to room temperature. Following, the residue was purified via crystallization using methanol:hexane:water (3:1:2) system; yielding 44 % of product. m.p. 58-60°C; IR ( $V_{max}$ ,  $cm^{-1}$ ). 3400, 3322, 3310 and 1552: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta_H$ : 0.88 (s, 3H), 0.90 (s, 3H), 1.00 (s, 6H), 1.06-1.35 (m, 6H), 1.40-1.46 (m, 3H), 1.52-1.54 (m, 2H), 1.60 (m, 2H), 1.64-1.67 (m, 2H), 1.80 (s, 3H), 1.82-2.50 (m, 10H), 2.60-2.66 (m, 4H), 3.20 (m, 4H), 3.32 (broad, 4H), 3.54 (m, 2H), 3.70 (m, 2H), 3.88 (m, 2H), 5.14 (m, 1H) ppm. <sup>13</sup>C NMR (75.4 Hz, CDCl<sub>3</sub>)  $\delta_C$ : 10.70, 13.22, 14.50, 16.70, 21.20, 22.36, 24.10, 26.62, 29.41, 29.53, 30.91, 35.76, 36.82, 35.17, 42.82, 43.54, 50.26, 51.00, 51.25, 52.44, 52.87, 54.14, 63.11, 75.83, 79.14, 79.22, 92.40, 156.70, 161.60 ppm. EI-MS: m/m 637.45. Anal. Calcd. for C<sub>37</sub>H<sub>59</sub>N<sub>5</sub>O<sub>4</sub>: C, 69.67; H, 9.32; N, 10.98; O, 10.03. Found. C, 69.54; H, 9.26.

### 6-((2-(E)-1-((4Z,9E,11aR,13aS)-2-ethyl-11a,13a-dimethyl-3,6,7,8,10,11,11a,11b,12,13, 13a,14,15, 16, 16a,16b,17,18, 18a,18b-icosahydro-2H-cyclopenta[7,8]phenanthro(1,2-b)[1]oxa[4,7]diazacycloundec-3-en-8-yn-14-yl)ethylidene)amino)ethylamino)hex-5-yn-3-ol (4)

A mixture of 3 (200 mg, 0.31 mmol), potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirred for 72 h to room temperature. Following, the residue

was purified via crystallization from methanol:water (3:1) yielding 56 % of product, m.p. 240-242°C: IR ( $V_{\max}$ ,  $\text{cm}^{-1}$ ) 3400, 3322, 3310 and 1170.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 0.80 (s, 3H), 0.86 (s, 3H), 0.88 (s, 3H), 0.92 (m, 1H), 1.00 (s, 3H), 1.06-1.50 (m, 9H), 1.51 (m, 2H), 1.52 (m, 2H), 1.60 (m, 1H), 1.64 (m, 1H), 1.70 (m, 1H), 1.76 (m, 1H), 1.80 (s, 3H), 1.82-1.84 (m, 2H), 1.88 (m, 1H), 1.94-2.50 (m, 6H), 2.60-2.64 (m, 2H), 3.10 (m, 2H), 3.18-3.56 (m, 4H), 3.66 (m, 1H), 3.70 (m, 1H), 4.20 (m, 2H), 4.56 (m, 1H), 5.60 (broad, 3H) ppm.  $^{13}\text{C}$  NMR (75.4 Hz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 10.74, 11.06, 13.24, 16.25, 16.70, 21.22, 23.26, 23.42, 24.09, 24.12, 26.44, 26.62, 28.92, 29.41, 31.37, 35.37, 36.69, 38.17, 42.29, 42.82, 51.25, 51.27, 52.39, 52.44, 53.65, 54.14, 56.15, 63.11, 75.83, 79.12, 79.32, 81.26, 86.38, 88.94, 92.39, 156.70, 164.60 ppm. EI-MS.  $m/z$  590.45. Anal. Calcd. for  $\text{C}_{37}\text{H}_{58}\text{N}_4\text{O}_2$ : C, 75.21; H, 9.89; N, 9.48; O, 3.42. Found: C, 75.14; H, 9.80.

**5-ethyl-N-(2-(((E)-1-((4Z,9E,11aR,13aS)-2-ethyl-11a,13a-dimethyl-3,6,7,8, 10,11,11a,11b,12,13,13a,14,15,16,16a,16b,17,18,18a,18b-icosahydro-2H-cyclopenta[7,8]phenanthro-[1,2-b][1]oxa[4,7]diazacycloundec-3-en-8-yn-14-yl)ethylidene)amino)ethyl)-4,3-dihydrofuran-2-amine (5)**

A mixture of 4 (200 mg, 0.34 mmol). Copper(II) chloride (46 mg, 0.34 mmol), 5 ml of methanol was stirred for 72 h at room temperature. Then, the mixture was purified via crystallization using methanol:hexane:water (4:2:1) system, yielding 66 % of product. m.p. 90-92°C. IR ( $V_{\max}$ ,  $\text{cm}^{-1}$ ) 3330, 3310 and 1172.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 0.80 (s, 3H), 0.86 (s, 3H), 0.88 (s, 3H), 0.90 (s, 3H), 0.96-1.50 (m, 9H), 1.51 (m, 2H), 1.52-1.64 (m, 1H), 1.66 (m, 1H), 1.70 (m, 1H), 1.72 (m, 1H), 1.76 (m, 1H), 1.80 (s, 3H), 1.82-1.84 (m, 2H), 1.90 (m, 1H), 1.94 (m, 1H), 2.00 (m, 1H), 2.12-2.36 (m, 3H), 2.39 (m, 1H), 2.40-2.50 (m, 2H), 3.10 (m, 2H), 3.44-3.56 (m, 4H), 3.64 (m, 1H), 4.22 (m, 2H), 4.56 (m, 1H), 4.60 (m, 1H), 4.74 (d, 1H,  $J = 1.05$  Hz), 7.32 (broad, 2H) ppm.  $^{13}\text{C}$  NMR (75.4 Hz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 9.84, 1.10, 13.24, 16.22, 16.73, 21.21, 23.26, 23.30, 23.34, 23.42, 24.09, 26.42, 26.62, 28.92, 31.37, 35.76, 35.78, 36.69, 38.17, 42.29, 42.82, 42.95, 51.27, 52.35, 52.39, 52.44, 53.65, 56.15, 63.11, 78.66, 79.32, 81.26, 86.38, 88.94, 92.94, 155.22, 156.70, 164.60 ppm. EI-MS  $m/z$  590.45. Anal. Calcd. for  $\text{C}_{37}\text{H}_{58}\text{N}_4\text{O}_2$ : C, 75.21; H, 9.89; N, 9.48; O, 3.42. Found: C, 75.16; H, 9.78.

**(3-ethyl-1-((2-(((E)-1-((5aS,7bR,E)-17-ethyl-14-(hydroxy(phenyl)methyl)-5a,7b-dimethyl-2, 2a, 2b,3,4,5,5a,6,7,7a,7b,8,9,11,12,13,16,17, 18a,18b-icosahydro-1H-cyclobuta[h]cyclopenta[7,8]phenanthro[1,2-b][1]oxa[4,7]diazacycloundec-5-yl)ethylidene)amino)ethyl)amino)-2-oxabicyclo[3.2.0]hept-6-en-6-yl)butan-2-ol (7)**

A mixture of 5 (200 mg, 0.34 mmol), 1-phenyl-2-propyn-1-ol (40  $\mu\text{l}$ , 0.33 mmol), Copper(II) chloride (46 mg, 0.34 mmol), 5 ml of methanol was stirring for 72 h at room temperature. Then, the mixture was purified via crystallization using the methanol:hexane (4:1) system, yielding 55% of product. m.p. 98-100°C: IR ( $V_{\max}$ ,  $\text{cm}^{-1}$ ): 3400, 3332, 3322, 1624 and 1170:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 0.84 (s, 3H), 0.88 (s, 3H), 0.92 (s, 3H), 0.94-1.28 (m, 4H), 1.34 (m, 1H), 1.38-1.46 (m, 4H), 1.47 (m, 1H), 1.50-1.52 (m, 3H), 1.53 (m, 1H), 1.62 (m, 1H), 1.64-1.76 (m, 2H), 1.80 (s, 3H), 1.81 (m, 1H), 1.83-1.94 (m, 2H), 1.96 (m, 1H), 2.12-2.50 (m, 5H), 2.52 (m, 1H), 3.00-3.06 (m, 2H), 3.26 (broad, 4H), 3.34 (m, 2H), 3.36 (m, 1H), 3.44 (m, 1H), 3.54 (m, 2H), 3.90 (m, 2H), 3.96 (m, 1H), 4.66 (m, 1H), 5.18 (m, 1H), 5.60 (m, 2H), 6.06 (d, 1H,  $J = 1.82$  Hz), 6.12 (d, 1H,  $J = 1.33$  Hz), 7.26-7.60 (m, 10H) ppm.  $^{13}\text{C}$  NMR (75.4 Hz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 9.60, 10.30, 13.24, 16.25, 16.73, 21.21, 21.51, 23.42, 24.09, 26.42, 26.62, 27.92, 31.33, 31.37, 35.76, 36.69, 38.17, 39.75, 42.29, 42.82, 43.79, 44.52, 50.34, 51.27, 52.44, 52.83, 53.65, 56.98, 63.11, 75.04, 75.86, 79.62, 81.53, 82.73, 89.50, 105.98, 126.42, 126.73, 127.82, 128.45, 128.65, 128.71, 129.26, 129.34, 137.16, 138.04, 140.59, 149.29, 156.70, 164.60 ppm. EI-MS.  $m/z$  654.57. Anal. Calcd. for  $\text{C}_{55}\text{H}_{74}\text{N}_4\text{O}_4$ : C, 77.24; H, 8.72; N, 6.55; O, 7.48. Found: C, 77.18; H, 8.66.

**1-(3-ethyl-1-((2-(((E)-1-((5aS,7bR,E)-17-ethyl-14-(2-hydroxybutyl)-5a,7b-dimethyl-2, 2a,2b, 3,4,3,5a,6,7,7a,7b,8,9,11,12,13,16,17,18a,18 b-icosahydro-1H-cyclobuta[h]cyclopenta- [7,8]phenanthro[1,2-b][1]oxa[4,7]diazacycloundec-5-yl)ethylidene)amino)ethyl)-amino)-2-oxabicyclo[3.2.0]hept-6-en-6-yl)butan-2-ol (7)**

A mixture of 6 (200 mg, 0.34 mmol). 1-hexyn-3-ol (80  $\mu\text{l}$ , 0.72 mmol), Copper(II) chloride (68 mg, 0.50 mmol), 5 ml of methanol was stirred for 72 h at room temperature. Following, the residue was purified via crystallization using the

methanol;hexane:water (4:2:1) system; yielding 38% of product, m.p. 130-132°C: IR ( $V_{\max}$ ,  $\text{cm}^{-1}$ ): 3400. 3332. 3320 and 1172:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta\text{H}$ : 0.84 (s, 3H), 0.88 (s, 3H), 0.92 (s, 3H), 0.93 (s, 3H), 0.94 (m, 1H), 0.96 (s, 6H), 1.06 (m, 1H), 1.20 (m, 2H), 1.26-1.28 (m, 2H), 1.34 (m, 2H), 1.38 (m, 2H), 1.40-1.46 (m, 4H), 1.47 (m, 1H), 1.50-1.52 (m, 3H), 1.53 (m, 1H), 1.62 (m, 1H), 1.64-1.76 (m, 2H), 1.80 (s, 3H), 1.81 (m, 1H), 1.82-1.83 (m, 2H), 1.93 (m, 1H), 1.94-2.12 (m, 2H), 2.22 (m, 1H), 2.28 (m, 1H), 2.30 (m, 1H), 2.36-2.38 (m, 2H), 2.42 (m, 1H), 2.48 (m, 1H), 2.50 (m, 1H), 2.52 (m, 1H), 2.96 (broad, 4H), 3.00-3.06 (m, 2H), 3.22 (m, 1H), 3.26 (m, 1H), 3.34 (m, 2H), 3.44 (m, 1H), 3.53 (m, 1H), 3.54 (m, 2H), 3.90 (m, 2H), 3.96 (m, 1H), 4.66 (m, 1H), 5.86 (d, 1H,  $J = 1.82$  Hz), 6.46 (d, 1H,  $J = 1.33$  Hz) ppm.  $^{13}\text{C}$  NMR (75.4 Hz,  $\text{CDCl}_3$ )  $\delta\text{C}$ : 9.56, 9.95, 10.30, 13.24, 16.25, 16.73, 21.21, 23.42, 24.09, 26.42, 26.62, 27.92, 29.22, 31.23, 31.37, 33.91, 35.76, 36.69, 38.17, 39.75, 42.29, 42.82, 43.79, 44.32, 44.52, 46.32, 51.27, 51.36, 52.44, 52.83, 53.65, 56.98, 63.11, 71.02, 74.04, 75.08, 81.53, 82.73, 87.86, 104.05, 126.34, 126.42, 131.11, 133.82, 146.34, 156.70, 164.60 ppm, EI-MS.  $m/z$  756.60. Anal. Calcd. for  $\text{C}_{49}\text{H}_{78}\text{N}_4\text{O}_4$ : C, 74.76; H, 9.99; N, 7.12; O, 8.13. Found. C, 74.70; H, 9.90

#### Evaluation of physicochemical parameters from compounds 2-7.

Physicochemical factors of compounds 2 to 7 such as lipophilicity degree (LogKow), HBD (hydrogen bond donor groups) and HBA (hydrogen bond acceptor groups), TPSA (topological polar surface area) and number of rotatable bonds (RB) were evaluated using a previously methods reported<sup>13,14</sup>.

#### Theoretical evaluation

The interaction between compounds 6 or 7 with opioid-receptors was determinate using a Dockingserver<sup>15,16</sup>. In addition, the interaction of compounds 6 or 7 were carried out using a theoretical model for  $\mu$  (4DKL)<sup>17</sup>,  $\mu$  (4RWA)<sup>18</sup> and  $\kappa$  (4DJH)<sup>19</sup> opioid-receptors.

#### Toxicity analysis.

Theoretical toxicity of compounds 6 or 7 was determined using PASS online software<sup>20</sup>.

## RESULTS AND DISCUSSION

### Chemical synthesis

The cyclobutadiene-steroid derivatives (compound 6 and 7) were synthesized using some chemical strategies:

### Preparation of an imino-steroid derivative

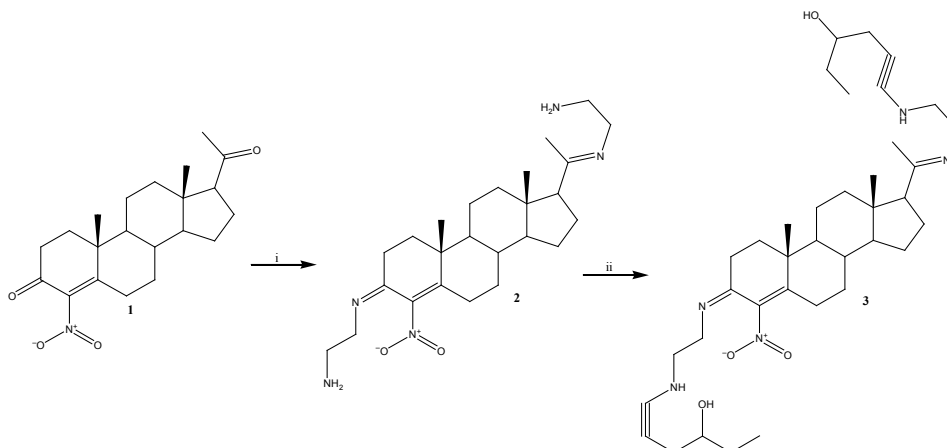
There are some reports that shown the synthesis of several imino analogs; however, the protocols require special conditions<sup>21,22</sup>. The first stage was achieved by reaction of a nitro-progesterone (1) with ethylenediamine Fig. 1 using boric as catalyst to synthesis of an imino-steroid analog (2); it is noteworthy that boric acid did not require special conditions<sup>23</sup>. The  $^1\text{H}$  NMR spectra of 2 shows bands at 0.88-0.92 ppm for methyl groups bound to steroid nucleus: at 1.80 ppm for methyl bound to imino group: at 1.06-1.68, 1.84-2.50 and 5.14 ppm for steroid moiety; at 3.10-3.82 ppm for methylene groups bound to both amino and imino groups: at 4.34 ppm for amino groups. The  $^{13}\text{C}$  NMR spectra showed chemical shifts at 13.22-14.52 ppm for methyl groups bound to steroid nucleus; at 16.70 ppm for methyl group bound to imino group: at 21.24-38.53.12 and 63.16-79.22 ppm for steroid moiety: at 41.00-52.84 ppm for methylene groups bound to both amino and imino groups; at 156.70-161.60 ppm for imino groups. Finally, the mass spectrum shown a molecular ion at 445.34.

### Preparation of hydroxy-hexynylamino-4-nitrosteroid derivative

Several studies showed the synthesis of some hexynyl-amino analogs by addition of amine groups to alkyne-derivatives using different reagents such as Cu/DMSO<sup>24</sup> and Ru-Cp<sup>25</sup>. In this investigation, the compound 2 reacted with 1-hexyn-3-ol Fig.1 in presence of Copper(II) to form a hydroxy-hexynylamino-4-nitrosteroid derivative (3). The  $^1\text{H}$  NMR spectra of 3 display several signals at 0.88-0.92 ppm for methyl groups bound to steroid nucleus: at 1.00 ppm for methyl group of the arm which was bound to hydroxyl group; at 1.80 ppm for methyl bound to imino group: at 1.06-1.38, 1.52-1.54, 1.64-1.67, 1.82-2.50 and 5.14 ppm for steroid moiety; at 1.40-1.46, 1.60, 3.70 and 2.60-2.66 ppm for methylene groups bound to hydroxyl

and alkyne groups: at 3.32 ppm for both amino and hydroxyl groups: at 3.20. 3.54 end 3.56 ppm for methylene groups binding to imino and amino groups. The  $^{13}\text{C}$  NMR spectrum showed several signals at 10.70 ppm for methyl group of the arm linked to hydroxyl group; at 13.22-14.50 ppm for methyl groups binding to steroid nucleus: at 16.70 ppm for methyl group linked to imino group; at 21.20-

22.36, 26.62, 29.53, 43.54, 51.00, 52.44-52.57, 63.11 and 79.22 ppm for steroid moiety, at 24.10, 29.41 and 75.83-79.14 ppm for methylene groups binding to hydroxyl and alkyne groups: at 50.26, 51.25 and 54.14 ppm for methylene groups linked to both imino and amino groups: at 92.40 ppm for alkyne group: at 156.70-161.60 ppm for imino groups. The mass spectrum showed a molecular ion at 637.45.



**Fig. 1. Preparation of hydroxy-hexynylamino-4-nitrosteroid derivative (3). Reaction of 4-nitroprogesterone (1) with ethylenediamine (i) to form an imino-steroid analog (2). Then, 2 reacted with 5-hexyn-3-ol (ii) to synthesis of 3**

#### Synthesis of an ether-steroid derivative (4)

There are several protocols to preparation of ether which use some reagents such as methoxy analogs<sup>26</sup>, fluoride ion<sup>27</sup>, D-glucose<sup>28</sup>, sodium phenoxide<sup>29</sup>, palladium(II)<sup>30</sup>, dimethyl sulfoxide/potassium carbonate<sup>31</sup> and others. In this study, an ether derivative (compound 4) was prepared by the reaction of 3 with 2-hydroxy-1-naphthaldehyde Fig. 2 in presence of dimethyl sulfoxide. The  $^1\text{H}$  NMR spectrum of 4 display bands at 0.80-0.86 ppm for methyl groups binding to steroid nucleus: at 1.00 ppm for methyl group of arm linked to hydroxyl group: at 1.80 ppm for methyl linked to imino group. at 0.88 ppm for methyl group of arm binding to 1-Oxa-4,7-diaza-cycloundec-3-en-8-yne ring: at 0.92, 1.06-1.50, 1.52, 1.64-1.76, 1.82-1.84, 1.94-2.50 and 3.66 ppm for steroid moiety at 1.51. 3.10 and 4.20-4.56 ppm for 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 1.60, 2.60-2.64 and 3.70 ppm for methylene groups linked to hydroxyl and alkyne groups; at 1.70 and 1.60 ppm for methylene group binding to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 3.18-3.56 ppm for methylene groups binding to both imino and amino groups; at 5.00 ppm for hydroxyl and amino groups. The  $^{13}\text{C}$  NMR spectrum showed several signals at 10.70 ppm for methyl group of

arm binding to hydroxyl group: at 13.22-16.25 ppm for methyl group linked to steroid nucleus. at 11.06 ppm for methyl group of arm binding to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 16.70 ppm for methyl group binding to imino group: at 21.22-23.42, 24.09, 26.44-26.62, 31.37-42.82, 52.44-53.65 and 63.11 ppm for steroid moiety; at 23.26, 52.39. 56.15 and 1.26-88.94 ppm for 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 24.12, 29.41 and 75.83 ppm for methylene groups linked to hydroxyl and alkyne groups: at 28.92 ppm for methyl group of arm bound 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 51.25 and 54.14 ppm for methylene groups bound to imino and amino groups; at 79.12. 79.32 and 92.39 ppm for alkyne groups, at 156.70-161.60 ppm for imino groups. Finally, the mass spectrum of 5 showed a molecular ion at 590.45.

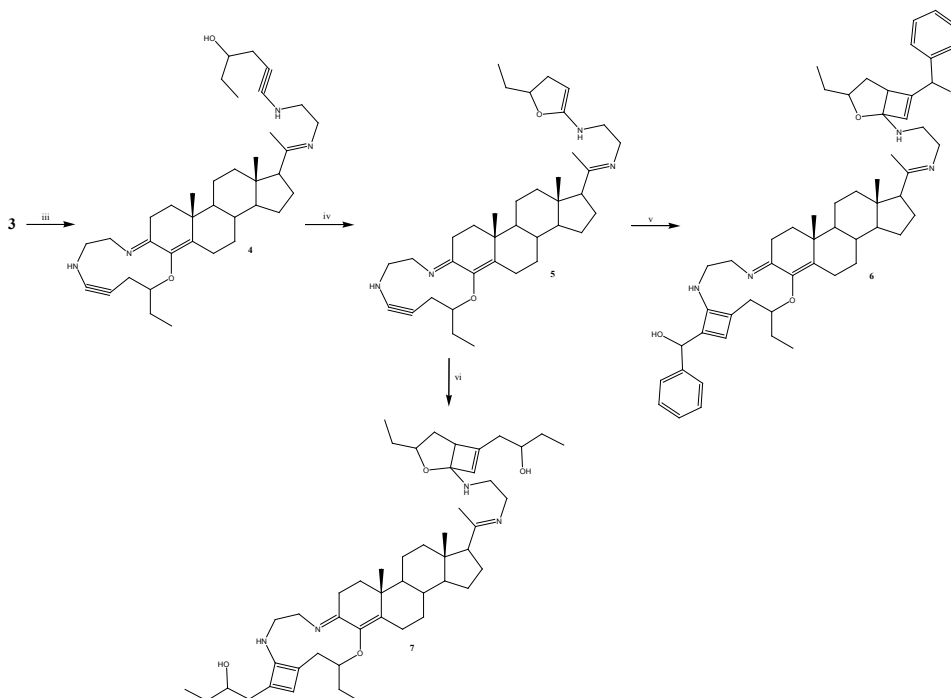
#### Preparation of Furanyl-steroid tlcristii»alive (5)

Analyzing the chemical structure of 4 and some reports which indicate that several metals can catalyze oxy-functionalization of alkynes<sup>32</sup>. Therefore, in this investigation 4 was reacted with Cooper(II) to form the compound 5 Fig. 2. The  $^1\text{H}$  NMR spectra of 5 display several signals at 0.80-0.86 ppm for methyl groups binding to steroid



nucleus: at 0.90 ppm for methyl group of arm linked to dihydrofuran ring; at 1.80 ppm for methyl binding to imino group; at 0.88 ppm for methyl group of arm bound to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 0.96-1.50, 1.54-1.64, 1.76, 1.82-1.84, 1.94, 2.12-2.36, 2.40-2.50 and 3.64 ppm for steroid moiety; at 1.51, 3.10 and 4.22-4.56 ppm for 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 1.66 and 1.72 ppm for dihydrofuran ring; at 1.70 and 1.90 ppm for methylene group binding to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 2.00, 2.39 and 4.60-4.74 ppm for dihydrofuran ring: at 3.44-3.56 ppm for methylene groups linked to both imino and amino groups; at 7.32 ppm for amino groups, The  $^{13}\text{C}$  NMR spectrum showed several signals at 9.84 ppm for methyl group of arm binding to dihydrofuran

ring; at 11.10 ppm for methyl group of arm linked to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 13.22-16.22 ppm for methyl groups bound to steroid nucleus; at 16.73 ppm for methyl group bound to imino group; at 21.21, 26.62, 31.37-35.76, 36.69-42.82, 51.27, 52.44-53.65 and 63.11 ppm for steroid moiety : at 23.26, 52.39, 56.15, 81.26 and 88.94 ppm for 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 23.34 ppm for methylene binding to dihydrofuran ring; at 28.92 ppm for methylene bound to 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring: at 35.78, 78.66 and 92.94-155.22 ppm for dihydrofuran ring; at 42.95 and 52.35 ppm for methylene groups bound to both imino and amino groups, at 156.70-164.60 ppm for imino groups. Finally, the compound 5 display a molecular ion at 590.45.



**Fig. 2.** Preparation of cyclobutene-steroid derivatives (6 or 7). Etherification of 3 using DMSO/ $\text{K}_2\text{CO}_3$  (iii) to synthesis of an ether-steroid analog (4). Then 4 reacted with Cooper(II) to formation of a Furanyl-steroid derivative (5). Finally, 6 or 7 were synthesized via reaction of 5 with 1-phenyl-2-propyn-1-ol (v) or 1-hexyn-3-ol (vi)

#### Preparation of cyclobutene-steroid derivatives (compounds 6 or 7)

There are some reports which show the [2 + 2] cycloaddition of alkene with alkyne groups and cycloaddition of alkyne groups to alkyne derivatives using metals as catalyst<sup>33,34</sup>. Therefore in this study 5 was reacted with 1-phenyl-2-propyn-1-ol or 1-hexyn-3-ol to form 6 or 7 in presence of copper(II) as catalyst Fig. 2. The  $^1\text{H}$  NMR spectra of 6 show several bands at 0.84-0.88 ppm for methyl groups binding to steroid

nucleus: at 0.93 ppm for methyl group of arm linked to 2-oxa-bicyclo[3.2.0]hept-6-ene ring: at 1.80 ppm for methyl bound to imino group: at 0.92 ppm for methyl group of arm binding to 1-Oxa-4,7-diaza-cycloundec-8-yne ring: at 0.94-1.28, 1.38-1.46, 1.50-1.52, 1.64-1.76, 1.83, 2.12-2.50 and 3.44 ppm for steroid moiety, at 1.34, 3.34, 3.90 and 4.66 ppm for 1-Oxa-4, 7-diaza-cycloundec-3-en-8-yne ring, at 1.47 and 1.53 ppm for methylene group binding to 1-Oxa-4,7-diaza-cycloundec-3-en-8-yne ring: 1.96,

2.52, 3.36, 3.96 and 6.12 ppm for 2-oxa-bicyclo(3.2.0)hept-6-ene ring: at 3.00-3.06 and 3.54 for methylene groups linked to both imino and amino groups; at 7.32 ppm for amino groups; at 3.26 for both amino and a hydroxyl groups: at 5.18 ppm for methylene group bound to both phenyl and hydroxyl groups, at 5.60 ppm for methylene binding to both cyclobutadiene ring and phenyl group: at 6.06 ppm for cyclobutadiene ring. at 7.26-7.60 ppm for phenyl groups. The  $^{13}\text{C}$  NMR spectrum display several signals at 9.60 ppm for methyl group of arm bound to 1-Oxa-4.7-diaza-cycloundec-3-en-8-yne ring; at 27.92 ppm for methylene group linked to 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 10.30 ppm for methyl group of arm bound to 2-oxa-bicyclo[3.2.0]hept-6-ene ring, at 13.24-16.25 ppm for methyl groups binding to steroid nucleus; at 16.73 ppm for methyl group bound to imino group: at 21.21-26.62, 31.37-38.17, 42.29-42.82, 51.27-52.44, 53.65 and 63.11 ppm for steroid moiety; at 29.92 ppm for methylene bound to 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 31.33, 50.34, 75.86, 89.50, 129.26 and 149.59 ppm for 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 39.75, 43.79, 56.98 and 81.53-82.73 ppm for 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 44.52 and 52.53 ppm for methylene groups bound to both amino and imino groups: at 79.62 ppm for methylene group binding to both phenyl group and 2-oxa-bicyclo[3.2.0]hept-6-ene; at 105.92, 126.73 and 129.34-137.16 ppm for cyclobutadiene ring; at 126.43, 127.82-128.71 and 138.04-140.59 ppm for phenyl groups: at 156.70-164.60 ppm for imino groups. Additionally, the compound 6 showed a molecular ion at 854.57.

Finally, the signals found of the  $^1\text{H}$  NMR spectrum for 7 showed several bands at 0.84-0.88 ppm for methyl groups binding to steroid nucleus: at 0.92 ppm for methyl group of arm bound to 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 1.80 ppm for methyl linked to imino group; at 0.92 ppm for methyl group of arm bound to 1-oxa-4.7-diaza-cycloundec-3-en-8-yne ring; at 0.94, 1.06, 1.26-1.28, 1.40-1.46, 1.50-1.52, 1.64-1.76, 1.82-1.83, 1.94-2.12, 2.28, 2.36, 2.50 and 3.44 ppm for steroid moiety; at 1.34, 3.34, 3.90 and 4.66 ppm for 1-Oxa-4.7-diaza-cycloundec-3-en-8-yne ring; at 1.20, 1.38, 2.22, 2.30, 2.42, 2.52, 3.22 and 3.53 ppm for methylene groups of arm binding to both cyclobutadiene ring and hydroxyl group; at 1.47 and 1.53 ppm for methylene group

bound to 1-Oxa-4.7-diaza-cycloundec-3-en-8-yne ring; at 1.62 and 1.81 ppm for methylene bound to 2-oxa-bicyclo[3.2.0]hept-6-ene ring: at 1.93, 2.48, 3.26, 3.96 and 6.46 ppm for 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 2.96 ppm for both amino and hydroxyl groups; at 3.00-3.06 and 3.54 ppm for methylene groups linked to both amino and imino groups; at 58.6 ppm for cyclobutadiene ring. The  $^{13}\text{C}$  NMR spectrum display several signals at 9.56 ppm for methyl group of arm bound to 1-Oxa-4.7-diaza-cycloundec-3-en-8-yne ring; at 10.30 ppm for methyl group of arm bound to 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 13.24-16.25 ppm for methyl groups binding to steroid nucleus: at 16.73 ppm for methyl group bound to imino group; at 9.95 ppm for methyl group of arm bound to both cyclobutadiene ring and hydroxyl group; at 21.21-26.62, 31.37, 35.76-35.17, 42.29-42.82, 51.27, 52.44, 53.65 and 63.11 ppm for steroid moiety; at 27.92 ppm for methylene group binding to 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 31.33, 51.36, 75.08, 87.86, 126.34, and 146.34 ppm for 2-oxa-bicyclo[3.2.0]hept-6-ene ring; at 33.91, 44.30, 46.32 and 71.02-74.04 ppm for methylene groups of arm bound to both cyclobutadiene ring and hydroxyl group; at 44.52 and 52.83 ppm for methylene groups bound to both amino and imino groups; at 156.70-164.60 ppm for imino groups. Finally, the compound 7 display a molecular ion at 786.60.

### Physicochemical parameters

Analyzing some reports, which indicate that some physicochemical factors of several drugs such as hydrogen bond donor groups (HBD) and hydrogen bond acceptor groups (HBA), topological polar surface area (TPSA) and number of rotatable bonds (RB) are used to predict the biological activity of some compounds in different theoretical models<sup>35-37</sup>; therefore, in this study these physicochemical parameters Table 1 were evaluated using the Schrodinger Software<sup>38</sup>. The results indicate that both HBA, HBD values were  $< 5$ , these data indicate that 7 could be well absorbed, such happening with other type of compounds<sup>39</sup>. Another result showed that polarity for 7 was higher compared the compounds 2 to 6; here it is noteworthy some studies indicate that this physicochemical parameter could condition the ability of several drugs to penetrate the blood-brain barrier affinity and exhibit biological activity on nervous central system<sup>40</sup>.

**Table 1: Physicochemical parameters of compounds 2 to 7**

Compound	MW	HBA	HBD	RB	TPSA	Polar
2	797.24	4	2	14	107.04	92.44
3	450.68	1	1	8	122.63	50.83
4	650.99	3	3	20	135.11	74.12
5	602.97	2	1	11	77.58	71.47
6	600.96	2	0	8	66.58	70.77
7	877.37	4	4	12	107.04	109.91

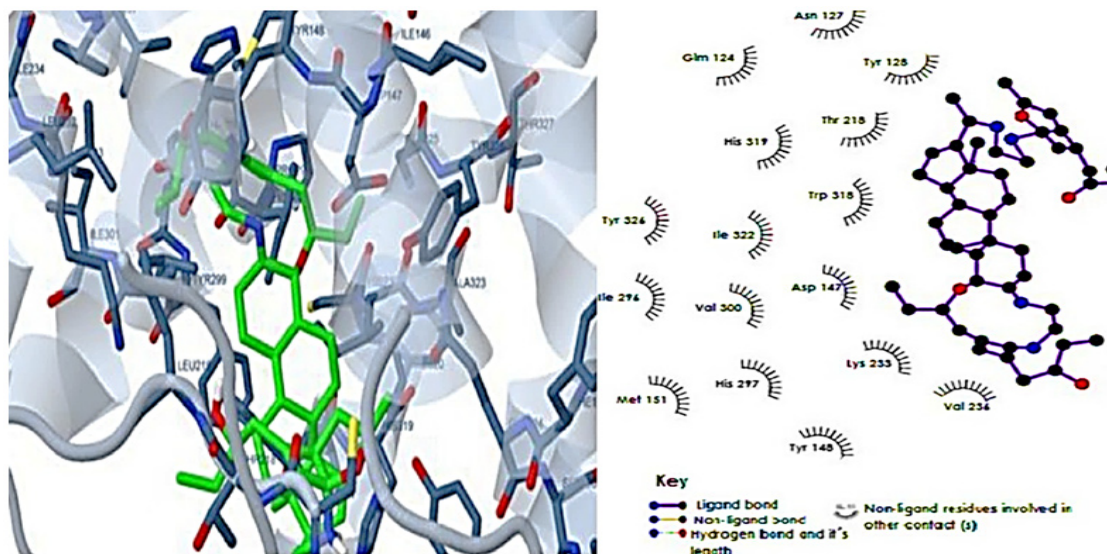
Bond donor groups (HBD) and hydrogen bond acceptor groups (HBA), topological polar surface area (TPSA) and number of rotatable bonds (RB).

On the other hand, also another parameters such as logP and  $\pi^{41}$  were used to delineate the structural chemical requirements of compounds 1 to 7; it is noteworthy that logP can be used to determinate lipophilicity degree of either molecule, therefore, in this study these parameters were calculated. The results shown in the Tables 3 and 4 indicate that aliphatic carbons (-CH<sub>3</sub>, -CH<sub>2</sub> and aromatic carbon) involved in the compound 6 contribute to increase the lipophilicity compared with the compounds 2-5 and 7. All this data suggest that changes in the degree of lipophilicity depend of structural chemical characteristic of compounds studied.

### Docking evaluation

Some studies suggest that some

compounds exert their biological activity via opioid receptors; therefore, in this study was evaluated that two cyclobutadiene-steroid derivatives (compound 6 or 7) could interact with opioid receptors such as  $\mu$  (4DKL)<sup>16</sup>,  $\delta$  (4RWA)<sup>17</sup> and  $\kappa$  (4DJH)<sup>18</sup> using a docking model<sup>13</sup>. The results Fig. 3 show the possible interaction of compound 6 with several aminoacid residues involved in the structure of  $\mu$  opioid receptor such as Gln<sub>124</sub>, Asn<sub>127</sub>, Tyr<sub>128</sub>, Asp<sub>147</sub>, Tyr<sub>148</sub>, Met<sub>151</sub>, Thr<sub>218</sub>, Lys<sub>233</sub>, Cys<sub>210</sub>, Val<sub>236</sub>, Ile<sub>296</sub>, His<sub>297</sub>, Val<sub>300</sub>, Trp<sub>318</sub>, His<sub>319</sub>, Ile<sub>322</sub>, Tyr<sub>326</sub>. In addition, theoretical interaction of compound 7 with this same opioid-receptor Fig. 6 shown that could bind with several aminoacid residues such as Asp<sub>147</sub>, Tyr<sub>148</sub>, Met<sub>151</sub>, Thr<sub>218</sub>, Leu<sub>219</sub>, Lys<sub>233</sub>, Val<sub>236</sub>, Phe<sub>237</sub>, His<sub>297</sub>, Val<sub>300</sub>, Ile<sub>301</sub>, Lys<sub>303</sub>, Glu<sub>310</sub>, Gln<sub>314</sub>, Thr<sub>315</sub>, Trp<sub>318</sub>, His<sub>319</sub>, Ile<sub>322</sub>.



**Fig. 3.** Contact site of aminoacid residues involved in the surface of  $\mu$ -opioid receptor (4DKL) and the compound 6 using Dockingserver.

Also, was evaluated the possibility of that cyclobutadiene-steroid derivative may interact with  $\delta$  opioid-receptor. the results Fig. 4 showed the

interaction of 6 with several amino acid residues such as Tyr<sub>109</sub>, Asp<sub>128</sub>, Tyr<sub>129</sub>, Met<sub>132</sub>, Leu<sub>200</sub>, Asp<sub>210</sub>, Lys<sub>214</sub>, Trp<sub>274</sub>, Ile<sub>277</sub>, Val<sub>281</sub>, Trp<sub>284</sub>, His<sub>301</sub>, Ile<sub>304</sub>, and



Tyr<sub>308</sub> involved in the structure of  $\delta$  opioid receptor. In addition, theoretical asses indicated the interaction of several aminoacid residues of this receptor with the compound 7 such as Gln<sub>105</sub>, Lys<sub>108</sub>, Tyr<sub>109</sub>, Asp<sub>128</sub>, Tyr<sub>129</sub>, Val<sub>197</sub>, Leu<sub>200</sub>, Asp<sub>210</sub>, Lys<sub>214</sub>, Val<sub>281</sub>, Trp<sub>284</sub>, Thr<sub>285</sub>, Val<sub>297</sub>, Leu<sub>300</sub> and Tyr<sub>308</sub> Figure 7.

Finally, the possible interaction of compounds 6 or 7 with  $\kappa$ -opioid receptor was evaluated. The interaction of 6 with  $\kappa$ -receptor involved several aminoacid residues Fig. 5 such as Val<sub>108</sub>, Thr<sub>111</sub>, Gln<sub>115</sub>, Trp<sub>124</sub>, Asp<sub>138</sub>, Glu<sub>209</sub>, Cys<sub>210</sub>,

Ser<sub>211</sub>, Tyr<sub>219</sub>, Lys<sub>227</sub>, Trp<sub>287</sub>, Ile<sub>290</sub>, Ile<sub>294</sub>, Tyr<sub>312</sub>, Ile<sub>316</sub> and Tyr<sub>320</sub>. Additionally, the interaction of 7 with  $\kappa$ -receptor involves the following aminoacid residues Fig. 8 such as Thr<sub>111</sub>, Phe<sub>114</sub>, Gln<sub>115</sub>, Val<sub>118</sub>, Trp<sub>124</sub>, Val<sub>134</sub>, Leu<sub>135</sub>, Asp<sub>138</sub>, Tyr<sub>139</sub>, Met<sub>142</sub>, Glu<sub>209</sub>, Cys<sub>210</sub>, Ser<sub>211</sub>, Tyr<sub>219</sub>, Asp<sub>223</sub>, Met<sub>226</sub>, Lys<sub>227</sub>, Trp<sub>287</sub>, Ile<sub>290</sub>, Ile<sub>294</sub>, Tyr<sub>312</sub>, and Ile<sub>316</sub>. All this data indicates that compound 6 or 7 interact in a manner different with aminoacid residues on surface of  $\mu$ ,  $\delta$  and  $\kappa$  opioid-receptors. This phenomenon could involve other type of intramolecular interactions due to changes in the energy levels.

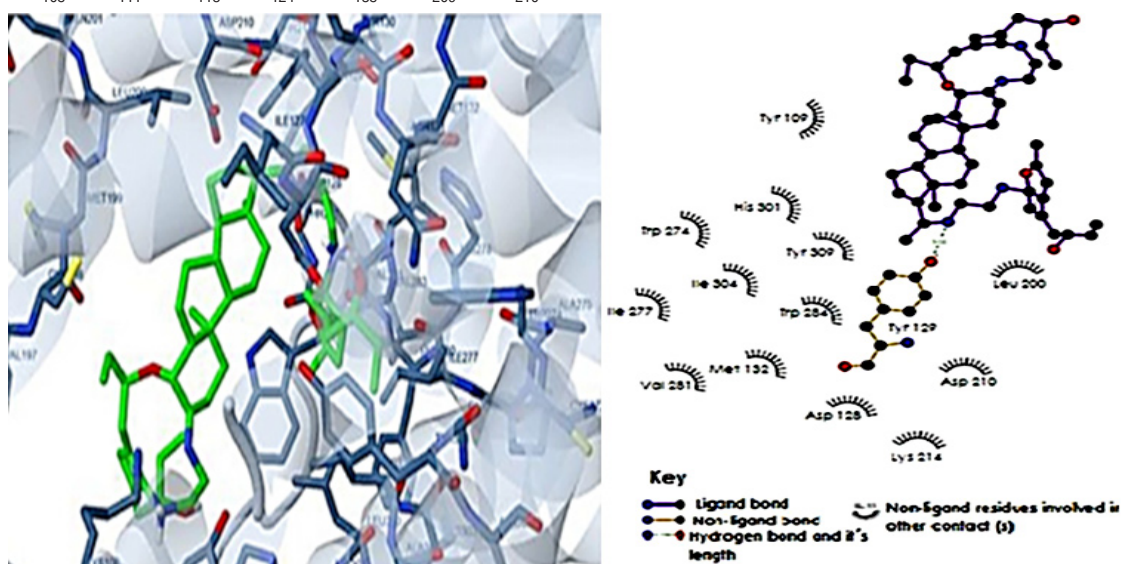


Fig. 4. Contact site of aminoacid residues involved in the surface of  $\delta$ -opioid receptor (4RWA) and the compound 6 using Dockingserver

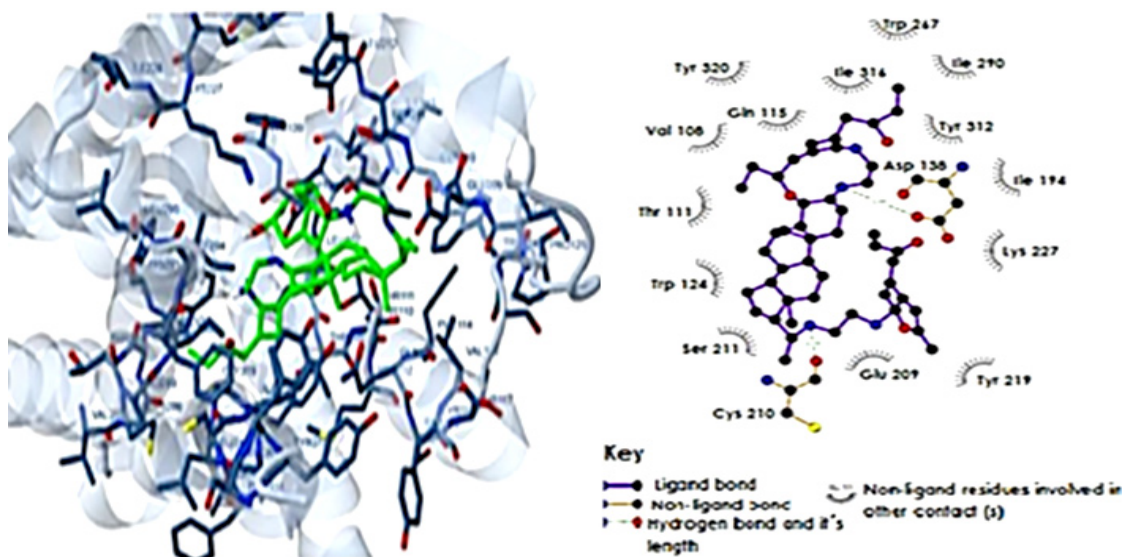


Fig. 5. Contact site of aminoacid residues involved in the surface of  $\kappa$ -opioid receptor (4DJH) and the compound 6 using Dockingserver

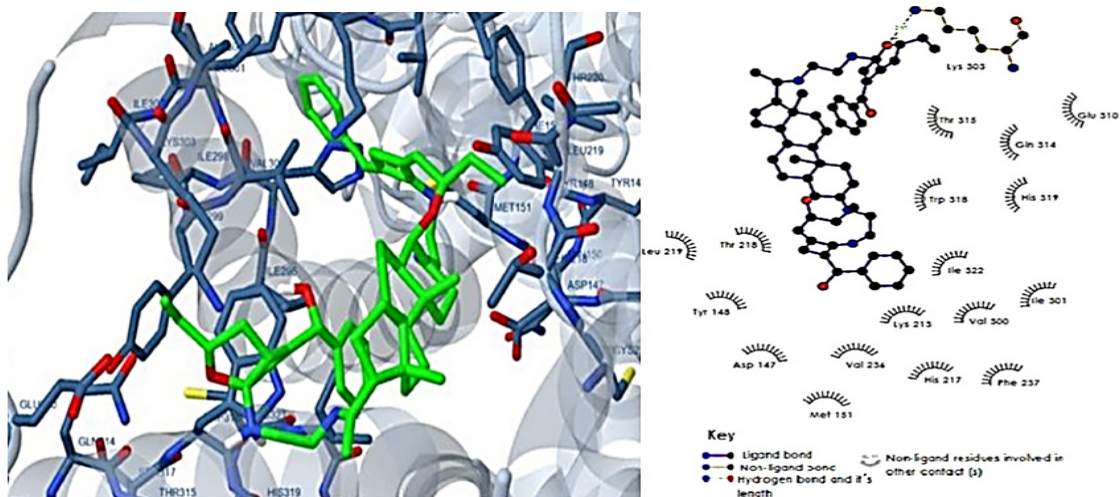


Fig. 6. Contact site of amino acid residues involved in the surface of  $\mu$ -opioid receptor (4DKL) and the compound 7 using Dockingserver

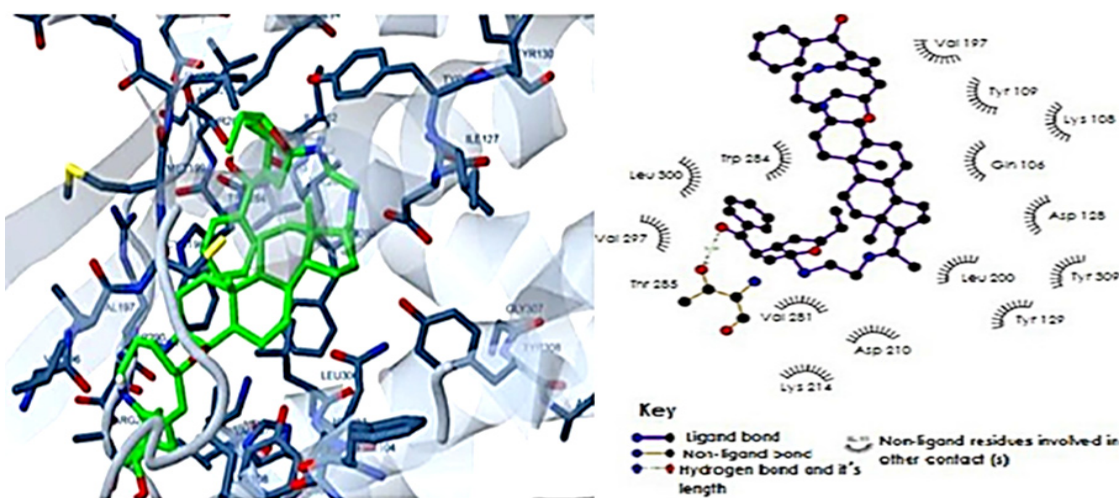


Fig. 7. Contact site of amino acid residues involved in the surface of  $\delta$ -opioid receptor (4RWA) and the compound 7 using Dockingserver

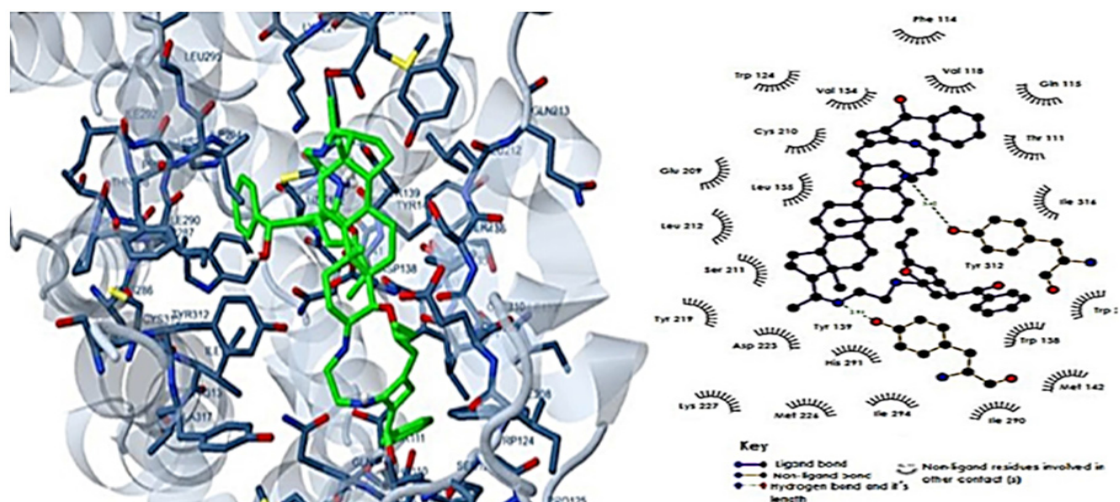


Fig. 8. Contact site of amino acid residues involved in the surface of  $\kappa$ -opioid receptor (4DJH) and the compound 7 using Dockingserver

**Table 2: Physicochemical parameters LogP and  $\pi$  of compounds 1-3**

Compound	Fragment	Value
1	-CH <sub>3</sub> [aliphatic carbon]	1.6419
	-CH <sub>2</sub> [aliphatic carbon]	3.9288
	-CH [aliphatic carbon]	2.1684
	-C(=O)- [aliphatic carbon attach]	-3.1172
	-NO <sub>2</sub> [nitro aliphatic attach]	-0.8132
	-tert Carbon [3 or more carbon attach]	0.5352
	Fused aliphatic ring unit correction	-2.0526
	C-(NO <sub>2</sub> )-CO- structure correction	1
	Equation constant	0.229
	$\pi$	-0.52
	LogKow	3.5203
	-CH <sub>3</sub> [aliphatic carbon]	1.64.19
	-CH <sub>2</sub> [aliphatic carbon]	5.8932
	-CH [aliphatic carbon]	2.1684
2	-C [aliphatic carbon – No H, no tert]	1.9446
	-NH <sub>2</sub> [aliphatic attach]	-2.8296
	-NO <sub>2</sub> [nitro aliphatic attach]	-0.8132
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	-0.002
	Fused aliphatic ring unit correction	-2.0526
	>C=N-C [cyclic-type imine, ali carbor att]	-1.55
	Equation constant	0.229
	$\pi$	1.6446
	LogKow	5.1649
	-CH <sub>3</sub> [aliphatic carbon]	2.7365
	-CH <sub>2</sub> [aliphatic carbon]	7.8576
	-CH [aliphatic carbon]	2.8912
	-C [aliphatic carbon – No, H no tert]	1.9446
3	#C [acetylenic carbon]	0.5336
	-OH [hydroxyl, aliphatic attach]	-2.8172
	-NH <sub>2</sub> [aliphatic attach]	-2.9924
	-NO <sub>2</sub> [nitro aliphatic attach]	-0.8132
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	-0.002
	multi-alcohol correction	0.4064
	Fused aliphatic ring unit correction	-2.0526
	>C=N-C [cyclic-type imine ali carbor att]	-1.55
	Equation constant	0.229
	$\pi$	1.7418
	LogKow	6.9067

**Table 3: Physicochemical parameters LogP and  $\pi$  of compounds 4 and 5**

Compound	Fragment	Value
4	-CH <sub>3</sub> [aliphatic carbon]	2.7365
	-CH <sub>2</sub> [aliphatic carbon]	7.9576
	-CH [aliphatic carbon]	2.8912
	-C [aliphatic carbon – No H, no tert]	1.9446
	#C [acetylenic carbon]	0.5336
	-OH [hydroxyl, aliphatic attach]	-1.4086
	-O- [oxygen, aliphatic attach]	-1.2566
	-NH- [aliphatic attach]	-2.9924
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	-0.002
	Fused aliphatic ring unit correction	-2.7368
	Equation constant	0.229
	$\pi$	1.4246
	LogKow	8.3313
5	-CH <sub>3</sub> [aliphatic carbon]	2.7365
	-CH <sub>2</sub> [aliphatic carbon]	7.8576
	-CH [aliphatic carbon]	2.8912
	-C [aliphatic carbon – No H, no tert]	1.9446
	=CH- or =C< [olefin carbon]	1.5344
	-O- [oxygen, aliphatic attach]	-2.5132
	-NH- [aliphatic attach]	-2.9924
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	-0.002
	Fused aliphatic ring unit correction	-2.7368
	Equation constant	0.229
	$\pi$	2.5774
	LogKow	9.4841

**Thermodynamic parameters**

Some studies indicate that thermodynamic parameters are evidences for confirming the interaction drug-protein<sup>42</sup>. In this study a theoretical evaluation ii as carried out on some thermodynamics parameters such as free energy of binding, electrostatic energy, total intermolecular energy, vdW + Hbond + desol energy and inhibition constant. The results showed differences in the intramolecular



**Table 4: Physicochemical parameters LogP and  $\pi$  of compounds 6 and 7**

Compound	Fragment	Value
6	-CH <sub>3</sub> [aliphatic carbon]	2.7365
	-CH <sub>2</sub> [aliphatic carbon]	7.8576
	-CH [aliphatic carbon]	3.9754
	-C [aliphatic carbon – No H, no tert]	2.9169
	=CH- or =C< [olefin carbon]	2.3016
	-OH [hydroxyl, aliphatic attach]	-2.8172
	-O- [oxygen, aliphatic attach]	-2.5132
	-NH- [aliphatic attach]	-2.9924
	Aromatic carbon	3.528
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	0.0020
	multi-alcohol correction	0.4064
	-C-O structure correction	0.5494
	Fused aliphatic ring unit correction	-2.3947
	Equation constant	0.229
	$\pi$	4.8355
	LogKow	14.3165
	-CH <sub>3</sub> [aliphatic carbon]	3.8311
	-CH <sub>2</sub> [aliphatic carbon]	9.822
	-CH [aliphatic carbon]	3.9754
	-C [aliphatic carbon – No H, no tert]	2.9169
	=CH- or =C< [olefin carbon]	2.3016
	-OH [hydroxyl, aliphatic attach]	-2.8172
7	-O- [oxygen, aliphatic attach]	-2.5132
	-NH- [aliphatic attach]	-2.9924
	-tert Carbon [3 or more carbon attach]	0.5352
	-N=C [aliphatic attach]	-0.002
	multi-alcohol correction	0.4064
	-C-O structure correction	0.5494
	Fused aliphatic ring unit correction	-2.3947
	Equation constant	0.229
	$\pi$	4.3634
	LogKow	13.8475

energy involved in the interaction for compound 6 (Table 5) or 7 (Table 6) with  $\mu$ ,  $\delta$  and  $\kappa$  opioid-receptors. In addition, other data shown different inhibition constants involved in the interaction or compounds 6 or 7 ( $K_i = 11.23$ ;  $K_i = 0.20$  with  $\kappa$ -opioid receptor). However, the values of these inhibition constants were lower compared with the  $K_i$  involved in the interaction of compounds 6 or 7 with  $\mu$  and  $\delta$ -opioid receptors; these data are interesting, which implies a higher interaction of 6 or 7 with the  $\kappa$ -opioid receptor (which is related to analgesia effect<sup>43</sup>). This phenomenon opens the possibility that these compounds could be evaluated experimentally in some biological system. In addition, it is important to perform some toxicity studies to evaluate if there are any side effects in the biological activity of the compounds involved in this study.

**Table 5: Intramolecular parameters involved between of interaction of the compound 6 and  $\mu$ ,  $\delta$  and  $\kappa$ -opioid receptors**

Opioid receptor	Est. Free Energy of Binding (Kcal/mol)	Inhibition Constant (K <sub>i</sub> , mM)	vdW + Hbond desolv Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Total Intermol Energy (Kcal/mol)	Surface
$\mu$ (4DKL)	-4.15	901.28	-8.39	-0.04	-8.43	1373.291
$\delta$ (4RWA)	-4.33	673.31	0.8	-0.55	-0.86	1398.405
$\kappa$ (4DJH)	-2.66	11.23	-4.1	-0.84	-4.94	1534.901

**Table 6: Intramolecular parameters involved between of interaction of the compound 7 and  $\mu$ ,  $\delta$  and  $\kappa$ -opioid receptors**

Opioid receptor	Est. Free Energy of Binding (Kcal/mol)	Inhibition Constant (K <sub>i</sub> , mM)	vdW + Hbond desolv Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Total Intermol. Energy (Kcal/mol)	Surface
$\mu$ (4DKL)	-3.73	1.84	-7.07	0.52	-6.55	1331.641
$\delta$ (4RWA)	-9	254.19	9.89	-0.39	-10.28	1520.856
$\kappa$ (4DJH)	119.06	0.2	91.8	-0.47	91.34	1600.575

**Table 7: Theoretical analyses of LD50 of compounds 6 or 7 using GUSAR software**

Compounds	Rat IP, LD <sub>50</sub> (Log <sub>10</sub> ; mmo/Kg)	Rat IV, LD <sub>50</sub> (Log <sub>10</sub> ; mmo/Kg)	Rat Oral LD <sub>50</sub> (Log <sub>10</sub> ; mmo/Kg)	Rat SC, LD <sub>50</sub> (Log <sub>10</sub> ; mmo/Kg)
6	-0.465 out of AD	-1.640 in AD	0.132 in AD	-1.309 in AD
7	-0.109 in AD	-1.678 in AD	0.184 in AD	-1.190 in AD

**Theoretical analysis of toxicity**

Analyzing the premise above mentioned, in this study also was evaluated the possible toxicity induced by the compounds 6 or 7 using the GUSAR software<sup>44</sup>. The results Table 7 showed that toxicity could be higher via intraperitoneal administration of compounds 6 or 7 compared to the other type of administration routes such as intravenous, oral and subcutaneous.

**CONCLUSIONS**

In conclusion, all these data indicate that 1) the compounds 6 or 7 show a high affinity by  $\kappa$ -receptor. 2) the cyclobutadiene-steroid derivatives are particularly interesting, because these drugs may constitute a novel therapy for treatment of pain.

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