








Experimental and theoretical evaluation of two indol-steroid-cyclobuta-imidazole derivatives as antibacterial drugs

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ABSTRACT

There some for treatment of infectious diseases; however, some drugs can induce several adverse effects. The aim of this study was synthesizing and to determinate the antibacterial activity of two indol-steroid-cyclobuta-imidazole complex (compound **11** and **12**) against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* in a minimum inhibitory concentration (MIC) model, using gentamicin, ciprofloxacin, cefotaxime as controls. The following stage involved the theoretical evaluation of the interaction of both compounds **11** and **12** with the β -lactamase enzyme (5f1g) using a docking software. The data found indicate that compound **12** decrease the growth bacterial of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumoniae* in comparison with the compound **11** and this effect only was similar to cefotaxime. Other theoretical data indicated that compound **12** could interact with different type of amino acids residues such as Ser₆₁, Leu₁₁₆, Gln₁₁₇, Asp₁₂₀, Tyr₁₄₇, Asn₁₄₉, Ser₂₀₉, Tyr₂₁₈, Thr₃₁₈, Asn₃₄₂ involved in the surface of 5f1g compared with **11**. These data indicate that; i) the steroid derivative (**12**) show better affinity by the 5f1g protein in comparison with compound **11** which is translated as higher antibacterial activity; ii) this compound is particularly interesting because could constitute a novel therapy as antibacterial agent.

Keywords: Indol-steroid-cyclobuta-imidazolone; bacteria; β -lactamase enzyme; theoretical; docking.

1. INTRODUCTION

Some studies indicate that infectious diseases are a serious health problem in the worldwide [1, 2]; it is noteworthy that some studies suggest that several bacteria such as *Escherichia coli* *Staphylococcus aureus* [3], *Streptococcus pneumoniae* [4] are causal agents for the development of several infectious diseases. Here it is important to mention that there are some pharmacological tools for the treatment of bacterial diseases [5, 6]; unfortunately, since several years ago, bacterial resistance has increased, this phenomenon may be due to some factors, such as; 1) a prolonged antibiotic therapy; 2) uncontrolled antibacterial therapy; or 3) some bacteria have developed several molecular mechanisms as a defense against antibiotics [7]. In the search of new pharmacological tools for treatment of bacterial resistance, have been synthesized some antibacterial drugs to penicillinases inhibition; for example, the preparation of the CP-45,899-drug as an inhibitor of both penicillinases and cephalosporinases bacteria [8]. Additionally, a report showed the preparation of dihidroxifenil-propenona with antibacterial activity against β -lactamase of *Enterobacter cloacae* [9]. In addition, the CP-45,899 {3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0) heptane-2-carboxylic acid, 4,4-dioxide, [2S-(2 α ,5 α)]} drug was prepared as β -lactamase inhibitor against *Neisseria gonorrhoeae* [10]. Also, the BRL-42715 [C6-(N1-methyl-1,2,3-triazolylmethylene)penem]

drug showed biological activity against β -lactamase enzyme in *Escherichia coli* [11]. Other study showed that 6-Acetylmethylenepenicillanic acid (Ro 15-1903) exert β -lactamase inhibition against both *Enterobacter cloacae* and *Escherichia coli* [12]. Additionally, some sulfonamide derivatives were prepared and biological activity against β -lactamase enzymes was evaluated using a kinetic enzymatic model [13]. Another report indicated the synthesis of a tetrahydropyrimidine-2-thione as an inhibitor of *Klebsiella pneumoniae* [14].

To evaluate the antibacterial properties of some drugs some theoretical models have been used [15]. For example, to evaluate the theoretical antimicrobial activity of an imidazole-pyrazole analog against *Candida albicans*, *Cryptococcus neoformans* and *Staphylococcus aureus* some docking models were used [16]. All these experimental and theoretical data showed that some drugs can exert antibacterial activity against several bacterial strains. Therefore, in this investigation two steroid-cyclobuta-imidazolone analogs were prepared and its biological activity on *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumoniae* was evaluated. In addition, to evaluate the interaction between two indole-steroid-cyclobuta-imidazolone complexes with β -lactamase enzyme surface, some theoretical models were used.

2. MATERIALS AND METHODS

The compounds used in this investigation were acquired from Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were determinate using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^1H and ^{13}C NMR spectra were evaluated on a Varian VXR300/5 FT NMR spectrometer at 300 MHz in CDCl_3 using TMS as internal standard. EIMS spectra were determinate with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/O2400 elemental analyzer.

General procedure for preparation of two steroid-azeto-imidazol derivatives.

A solution of testosterone (**1**) or progesterone (**2**) [0.50 mmol], 2-methylimidazole [45 mg, 0.55 mmol], Copper(II) chloride [70 mg, 0.52 mmol] in 5 ml of methanol was stirred for 48 h to room temperature. Then, the solvent was evaporated to dryness under reduced pressure. Finally, the product was purified via crystallization using the methanol:water system (4:1) in which case two steroid-azeto-imidazol derivatives (compounds **2** or **3**) were obtained, which showed the following chemical properties.

(3aS,5bR)-3-hydroxy-3a,5b,12-trimethyl-1,2,3,3a,4,5,5a,5b,6,7, 12,12a,13,14,14a,14b-hexadecahydrocyclopenta [7',8']phenanthro[1':3,4]azeto[1,2-a]imidazol-8(8aH)-one (3) yielding 22 % of product, m.p. 124-126 °C; IR (V_{\max} , cm^{-1}) 3466, 3402, 1712 and 1620: ^1H NMR (300 MHz, Chloroform-*d*) δ_{H} : 0.84 (s, 3H) 0.94-1.06 (m, 2H), 1.08 (s, 3H), 1.12-1.90 (m, 14H), 1.96-3.64 (m, 6H), 4.14-4.26 (m, 2H), 8.26 (broad, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_{C} : 12.10, 15.00, 20.07, 21.10, 23.79, 25.18, 29.16, 30.71, 31.66, 37.27, 38.34, 38.88, 42.94, 44.05, 47.56, 51.17, 51.70, 52.66, 56.88, 67.96, 81.84, 156.86, 205.44 ppm. EI-MS *m/z*: 370.26 Anal. Calcd. for $\text{C}_{23}\text{H}_{34}\text{N}_2\text{O}_2$: C, 74.55; H, 9.25; N, 7.56; O, 8.64. Found: C, 74.48; H, 9.18.

(3aS,5bR)-3-acetyl-3a,5b,10-trimethyl-1,2,3,3a,4,5,5a,5b,6,7, 8a,8b,9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8'] phenanthro[1':3,4]cyclobuta[1,2-d]imidazol-8-one (4) yielding 38 % of product, m.p. 72-74 °C; IR (V_{\max} , cm^{-1}) 3466, 1712 and 1622: ^1H NMR (300 MHz, Chloroform-*d*) δ_{H} : 0.76 (s, 3H), 1.06 (s, 3H), 1.16-1.90 (m, 14H), 1.96 (s, 3H), 1.98-2.02 (m, 3H), 2.10 (s, 3H), 2.20-2.76 (m, 4H), 4.12- 4.26 (m, 2H), 10.12 (broad, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_{C} : 15.00, 15.80, 20.62, 22.00, 23.08, 24.40, 25.14, 29.14, 30.79, 31.65, 36.32, 38.60, 38.87, 42.96, 44.25, 47.60, 51.12, 54.08, 56.88, 57.42, 63.96, 67.93, 156.84, 205.42, 208.40 ppm. EI-MS *m/z*: 396.27 Anal. Calcd. for $\text{C}_{25}\text{H}_{36}\text{NO}_3$: C, 75.72; H, 9.15; N, 7.06; O, 8.07. Found: C, 75.68; H, 9.10.

Preparation of steroid-cyclobuta-imidazol-chloroacetate derivatives.

In a balloon flask was prepared a solution of **3** or **4** [0.50 mmol], triethylamine [70 μl , 0.50 mmol] and chloroacetyl chloride [50 μl , 0.62 mmol] in 5 ml of methanol which was stirred for 24 h to room temperature. Then, the solvent was evaporated to dryness and the reaction mixture was purified by crystallization using the methanol: hexane:water system (4:2:1), in which case two

chloroacetate derivatives (compounds **3** or **4**) were obtained showing the following chemical properties.

(3aS,5bR)-9-(2-chloroacetyl)-3a,5b,10-trimethyl-8-oxo-2,3,3a, 4,5,5a,5b,6,7,8,8a,8b,9,11a,12,13,13a,13b-octadecahydro-1H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta- [1,2-d]imidazol-3-yl 2-chloroacetate (5)

yielding 58 % of product, m.p. 140-142 °C; IR (V_{\max} , cm^{-1}) 3466, 1740, 1712 and 1622: ^1H NMR (300 MHz, Chloroform-*d*) δ_{H} : 0.80 (s, 3H), 1.06 (s, 3H), 1.08-1.50 (m, 10H), 1.72 (s, 3H), 1.76-2.78 (m, 10H), 4.08 (t, 2H, $J = 14.80$), 4.10-4.14 (m, 2H), 4.74 (m, 1H), 4.80 (m, 1H), 5.06 (m, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_{C} : 12.08, 15.80, 19.80, 20.90, 23.78, 25.16, 27.77, 29.16, 31.65, 37.12, 38.32, 38.84, 40.80, 41.76, 42.60, 42.96, 44.51, 48.70, 49.52, 51.50, 52.66, 66.42, 81.69, 154.56, 165.04, 168.00, 204.20 ppm. EI-MS *m/z*: 522.20 Anal. Calcd. for $\text{C}_{27}\text{H}_{36}\text{Cl}_2\text{N}_2\text{O}_4$: C, 61.95; H, 6.93; Cl, 13.54; N, 5.35; O, 12.23. Found: C, 61.84; H, 6.88.

(3aS,5bR)-3-acetyl-9-(2-chloroacetyl)-3a,5b,10-trimethyl-1,2,3, 3a,4,5,5a,5b,6,7,8a,8b, 9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta [1,2-d]imidazol-8-one (6)

yielding 44 % of product, m.p. 108-110 °C; IR (V_{\max} , cm^{-1}) 3464, 1742, 1712 and 1622: ^1H NMR (300 MHz, Chloroform-*d*) δ_{H} : 0.76 (s, 3H), 1.06 (s, 3H), 1.16-1.70 (m, 11H), 1.74 (s, 3H), 1.80-2.04 (m, 5H), 2.10 (s, 3H), 2.22-2.78 (m, 5H), 4.11- 4.14 (m, 2H), 4.76-5.10 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_{C} : 15.00, 15.80, 19.74, 22.00, 23.08, 24.44, 25.14, 29.16, 30.80, 31.62, 36.32, 38.61, 38.87, 41.74, 42.96, 44.25, 44.51, 48.70, 49.52, 54.04, 57.42, 63.96, 66.42, 154.56, 165.07, 204.22, 208.36 ppm. EI-MS *m/z*: 472.22 Anal. Calcd. for $\text{C}_{27}\text{H}_{37}\text{ClN}_2\text{O}_3$: C, 68.55; H, 7.88; Cl, 7.49; N, 5.92; O, 10.15. Found: C, 68.49; H, 7.80.

Preparation of two steroid-epoxide derivatives.

In a balloon flask was prepared a solution of **5** or **6** (0.40 mmol), 2-hydroxy- 1-naphthaldehyde (68 mg, 0.40 mmol), and sodium hydroxide (10 mg, 0.25 mmol) in 5 ml of ethanol was stirred for 72 h at room temperature. Then, the solvent was evaporated to dryness and the reaction mixture was purified by crystallization using the methanol:water:hexane (3:1:1) system in which case two epoxide derivatives (compounds **7** or **8**) were obtained showing the following chemical properties.

(3aS,5bR)-3-hydroxy-9-((2S)-3-(2-hydroxynaphthalen-1-yl)-oxirane-2-carbonyl)-3a,5b,10-trimethyl-1,2,3,3a,4,5,5a,5b,6,7, 8a,8b,9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8'] phenanthro[1':3,4]cyclobuta[1,2-d]imidazol-8-one (7)

yielding 54 % of product, m.p. 132-134 °C; IR (V_{\max} , cm^{-1}) 3462, 3400, 1712 and 1212: ^1H NMR (300 MHz, Chloroform-*d*) δ_{H} : 0.84 (s, 3H), 0.94 (m, 1H), 1.06 (s, 3H), 1.07-1.60 (m, 10H), 1.72 (s, 3H), 1.80-3.62 (m, 10H), 4.22-4.46, 4.76-4.84 (m, 2H), 7.22-7.70 (m, 5H), 7.78 (broad, 2H), 7.90 (m, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_{C} : 12.06, 15.80, 19.76, 21.04, 23.79, 25.16, 29.16, 30.701, 31.65, 37.24, 38.33, 38.86, 42.94, 44.05, 44.51, 48.70, 51.28, 51.72, 52.66, 53.46, 53.90, 66.42, 81.80, 118.85, 121.45, 122.58, 123.43, 126.81, 127.86, 129.2, 130.35, 134.66,

153.92, 156.28, 163.60, 204.20 ppm. EI-MS m/z : 582.30 Anal. Calcd. for $C_{36}H_{42}N_2O_5$: C, 74.20; H, 7.26; N, 4.81; O, 13.73. Found: C, 74.16; H, 7.20.

(3aS,5bR)-3-acetyl-9-((2S)-3-(2-hydroxynaphthalen-1-yl)oxirane-2-carbonyl)-3a,5b,10-trimethyl-1,2,3,3a,4,5,5a,5b,6,7,8a,8b,9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta[1,2-d]imidazol-8-one (8)

yielding 44 % of product, m.p. 156-158 °C; IR (V_{max} , cm^{-1}) 3464, 1710 and 1210: 1H NMR (300 MHz, Chloroform- d) δ_H : 0.76 (s, 3H), 1.06 (s, 3H), 1.16-1.72 (m, 11H), 1.74 (s, 3H), 1.80-2.02 (m, 5H), 2.10 (s, 3H), 2.22-2.76 (m, 5H), 4.26-4.48 (m, 2H), 4.74-4.84 (m, 2H), 7.22-7.90 (m, 6H), 9.08 (broad, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform- d) δ_C : 15.00, 15.80, 19.74, 22.00, 23.08, 24.44, 25.14, 29.12, 30.22, 31.64, 36.32, 38.61, 38.87, 42.96, 44.25, 44.51, 48.70, 51.24, 53.44, 53.89, 54.10, 57.42, 63.96, 66.42, 118.82, 121.42, 122.58, 123.43, 126.81, 127.86, 129.2, 130.35, 134.66, 153.91, 156.28, 163.64, 204.24, 208.34 ppm. EI-MS m/z : 608.32 Anal. Calcd. for $C_{38}H_{44}N_2O_5$: C, 74.97; H, 7.29; N, 4.60; O, 13.14. Found: C, 74.90; H, 7.20.

Preparation of steroid-ether derivatives.

In a balloon flask was prepared a solution of **7** or **8** (0.50 mmol), (4-nitro-phenyl)-acetonitrile (100 mg, 0.62 mmol), and potassium carbonate (40mg, 0.30 mmol) in 10 ml of dimethyl sulfoxide was stirred for 72 h at room temperature. The solvent was evaporated to dryness and the reaction mixture was purified by crystallization using the methanol:water:hexano (1:3:1) system, in which case two ether derivatives (compounds **9** or **10**) were obtained, which showed the following chemical properties.

2-(4-((1-((3S)-3-((3aS,5bR)-3-hydroxy-3a,5b,10-trimethyl-8-oxo-2,3,3a,4,5,5a,5b,6,7,8,8a,8b,9,11a,12,13,13a,13b-octadecahydro-1H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta[1,2-d]imidazole-9-carbonyl)oxiran-2-yl)naphthalen-2-yl)oxy)phenyl)aceto- nitrile (9)

yielding 56 % of product, m.p. 167-169 °C; IR (V_{max} , cm^{-1}) 3464, 3400, 2100, 1712 and 1210: 1H NMR (300 MHz, Chloroform- d) δ_H : 0.86 (s, 3H), 0.94 (m, 1H), 1.06 (s, 3H), 1.07-1.60 (m, 10H), 1.72 (s, 3H), 1.80-3.62 (m, 10H), 3.64 (m, 2H), 3.86-4.72 (m, 2H), 4.76-4.84 (m, 2H), 6.40 (broad 1H), 6.92-8.20 (m, 10H) ppm. ^{13}C NMR (300 MHz, Chloroform- d) δ_C : 12.10, 15.80, 19.74, 21.04, 23.45, 23.79, 25.16, 29.16, 30.71, 31.65, 37.27, 38.34, 38.87, 42.94, 44.02, 44.50, 48.70, 51.24, 51.72, 52.66, 53.44, 53.80, 66.42, 81.84, 117.38, 118.00, 118.10, 118.32, 122.48, 123.45, 123.65, 126.00, 126.66, 126.73, 128.27, 131.10, 133.70, 146.34, 156.28, 156.84, 163.62, 204.20 ppm. EI-MS m/z : 697.35 Anal. Calcd. for $C_{44}H_{47}N_3O_5$: C, 75.73; H, 6.79; N, 6.02; O, 11.46. Found: C, 75.68; H, 6.70.

2-(4-((1-((3S)-3-((3aS,5bR)-3-acetyl-3a,5b,10-trimethyl-8-oxo-2,3,3a,4,5,5a,5b,6,7,8,8a,8b,9,11a,12,13,13a,13b-octadecahydro-1H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta[1,2-d]imidazole-9-carbonyl)oxiran-2-yl)naphthalen-2-yl)oxy)phenyl)acetonitrile (10)

yielding 44 % of product, m.p. 122-125 °C; IR (V_{max} , cm^{-1}) 3462, 2100, 1712 and 1210: 1H NMR (300 MHz, Chloroform- d) δ_H : 0.76 (s, 3H), 1.06 (s, 3H), 1.16-1.72 (m, 11H), 1.74 (s, 3H), 1.80-2.02 (m, 5H), 2.10 (s, 3H), 2.22-2.76 (m, 5H), 3.64 (m, 2H), 3.86-4.72 (m, 2H), 4.76-4.84 (m, 2H), 6.92-8.20 (m, 10H) ppm. ^{13}C NMR (300 MHz, Chloroform- d) δ_C : 15.00, 15.80, 19.76, 22.00, 23.08, 23.44, 24.44, 25.14, 29.16, 30.21, 31.65, 36.32, 38.61, 38.87,

42.96, 44.25, 44.51, 48.70, 51.24, 53.44, 53.82, 54.08, 57.4, 63.96, 66.42, 117.40, 118.00, 118.10, 118.31, 122.48, 123.45, 123.65, 126.03, 126.66, 126.73, 128.27, 131.12, 133.72, 146.36, 156.24, 156.84, 163.64, 204.20, 208.30 ppm. EI-MS m/z : 723.36 Anal. Calcd. for $C_{46}H_{49}Cl_2N_3O_5$: C, 76.32; H, 6.82; N, 5.80; O, 11.05. Found: C, 76.28; H, 6.80.

Preparation of two indol-steroid derivatives.

In a balloon flask was prepared a solution of **9** or **10** (0.50 mmol), Copper(II) chloride anhydrous (100 mg, 0.74 mmol), in 5 ml of methanol was stirring for 72 h at room temperature. The solvent was evaporated to dryness and the reaction mixture was purified by crystallization using the methanol:water:benzene (2:1:2) system, in which case two ether derivatives (compounds **11** or **12**) were obtained, which showed the following chemical properties.

(3aS,5bR)-9-((2S)-3-(2-((3H-indol-6-yl)oxy)naphthalen-1-yl)oxirane-2-carbonyl)-3-hydroxy-3a,5b,10-trimethyl-1,2,3,3a,4,5,5a,5b,6,7,8a,8b,9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta[1,2-d]imidazol-8-one (11)

yielding 58 % of product, m.p. 189-192 °C; IR (V_{max} , cm^{-1}) 3464, 3400, 3320, 1644, 1712 and 1210: 1H NMR (300 MHz, Chloroform- d) δ_H : 0.84 (s, 3H), 0.94 (m, 1H), 1.06 (s, 3H), 1.07-1.60 (m, 10H), 1.72 (s, 3H), 1.80-2.76 (m, 9H), 3.40 (m, 2H), 3.64 (m, 1H), 3.86-4.72 (m, 2H), 4.76-4.84 (m, 2H), 6.40 (broad, 1H), 6.46-7.80 (m, 8H), 7.98 (d, 1H, $J = 1.20$ Hz), 8.20 (m, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform- d) δ_C : 12.10, 15.80, 19.78, 21.04, 23.79, 25.16, 29.16, 30.71, 31.65, 37.27, 38.34, 38.87, 42.96, 44.03, 44.50, 48.22, 48.70, 51.24, 51.72, 52.66, 53.44, 53.82, 66.42, 81.84, 108.84, 110.76, 116.35, 118.31, 120.05, 121.78, 123.65, 124.79, 126.03, 126.66, 126.73, 131.12, 133.72, 147.67, 154.57, 154.80, 156.28, 163.64, 164.86, 204.20, 208.34 ppm. EI-MS m/z : 697.35 Anal. Calcd. for $C_{44}H_{47}N_3O_5$: C, 75.73; H, 6.79; N, 6.02; O, 11.46. Found: C, 75.68; H, 6.70.

(3aS,5bR)-9-((2S)-3-(2-((3H-indol-6-yl)oxy)naphthalen-1-yl)oxirane-2-carbonyl)-3-acetyl-3a,5b,10-trimethyl-1,2,3,3a,4,5,5a,5b,6,7,8a,8b,9,11a,12,13,13a,13b-octadecahydro-8H-cyclopenta[7',8']phenanthro[1':3,4]cyclobuta[1,2-d]imidazol-8-one (12)

yielding 52 % of product, m.p. 178-180 °C; IR (V_{max} , cm^{-1}) 3464, 3320, 1644, 1712 and 1210: 1H NMR (300 MHz, Chloroform- d) δ_H : 0.76 (s, 3H), 1.06 (s, 3H), 1.17-1.72 (m, 11H), 1.74 (s, 3H), 1.80-2.02 (m, 5H), 2.10 (s, 3H), 2.22-2.78 (m, 5H), 3.38 (m, 2H), 3.86-4.72 (m, 2H), 4.76-4.84 (m, 2H), 6.46-7.78 (m, 8H), 7.98 (d, 1H, $J = 1.20$ Hz), 8.20 (m, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform- d) δ_C : 15.00, 15.80, 19.74, 22.00, 23.08, 24.44, 25.14, 29.12, 30.20, 31.65, 36.32, 38.61, 38.87, 42.96, 44.25, 44.50, 48.18, 48.70, 51.27, 53.44, 53.82, 54.08, 57.4, 64.00, 66.40, 108.87, 110.76, 116.35, 118.31, 120.05, 121.78, 123.65, 124.79, 126.03, 126.66, 126.73, 131.12, 133.72, 147.67, 154.57, 154.80, 156.28, 163.64, 164.82, 204.22, 208.34 ppm. EI-MS m/z : 723.36 Anal. Calcd. for $C_{46}H_{49}N_3O_5$: C, 76.32; H, 6.82; N, 5.80; O, 11.05. Found: C, 76.28; H, 6.78.

Antimicrobial activity.

A previously method reported [17] was used to evaluate the antimicrobial activity of compounds **11** or **12** against *Staphylococcus aureus* (ATCC 49775), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603). These

microorganisms were incubated using some growth means such as brain/heart infusion for *Escherichia coli* and *klebsiella pneumoniae* and *Staphylococcus* 110 for *Staphylococcus aureus* for 24 h at 37 °C in absence or presence of compounds **11** or **12** to determinate the growth bacterial. It is noteworthy that minimum inhibitory concentration (MIC) of all compounds involved in this study was evaluated.

Physicochemical parameters evaluation.

Some electronic parameters such as HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) energy, orbital coefficients distribution, molecular dipole moment and HBD (hydrogen bond donor groups) and HBA (hydrogen bond acceptor groups) and TPSA (topological polar

surface area) were evaluated using the SPARTAN'06 software [18]. In addition, to determinate both logP (LogKow) and π parameters, the KOWWIN program was used [19].

Statistical analysis.

The results are expressed as average \pm SE. In addition, the data were put under an analysis of variance (ANOVA) using the Bonferroni correction factor [20]. The degree of significance was $p \leq 0.05$.

Theoretical evaluation of interaction.

Theoretical analysis was using a Docking model [21, 22] The interaction of compounds **11** or **12** were determinate with a theoretical model for β -lactamase (5f1g) [23].

3. RESULTS

Two steroid-cyclobuta-imidazolone derivatives (compounds **11** and **12**) were prepared using some chemistry tools. In the first step, two steroid-azeto-imidazol analogs (compounds **3** or **4**) were synthesized; it is noteworthy that several catalytic reagents have been used to preparation of imidazole derivatives such as *N,N*-carbonyldiimidazole [24], $ZrCl_4$ [25], $Pd(OAc)_2$ [26], silica sulfuric acid [27], Iodine [28] and others. In this study, progesterone or testosterone reacted with 2-methylimidazol in presence of Copper(II) chloride to form the compounds **3** or **4** (Figure 1 and 2).

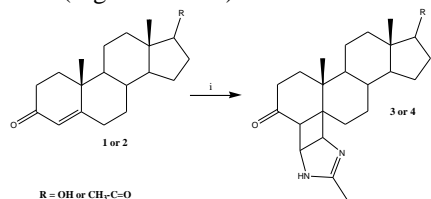


Figure 1. Preparation of two steroid-azeto-imidazol analogs (**3** or **4**). Reaction of testosterone (**1**) or progesterone (**2**) to form **3** or **4** in presence of Copper(II) chloride (i).

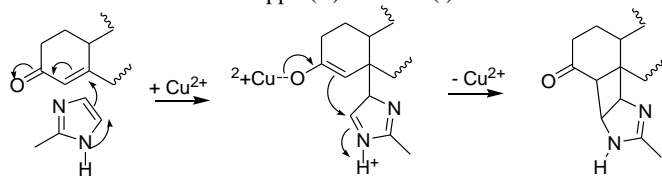


Figure 2. Mechanism of reaction involved in the synthesis of two steroid-azeto-imidazol derivatives.

The results of 1H NMR spectrum of **3** showed some bands at 0.84 and 1.10 ppm for methyl group linked to steroid nucleus; at 1.95 ppm for methyl bound to imidazole ring; at 0.94-1.06, 1.12-1.90 and 1.96-3.64 ppm for steroid moiety; at 4.14-4.26 ppm for imidazole ring; at 8.26 ppm for both amino and hydroxyl groups. The ^{13}C NMR spectra showed several signals at 12.10-15.00 ppm for methyl groups linked to steroid nucleus; at 20.07 ppm for methyl group bound to imidazole ring; at 21.10-52.66 and 81.84 ppm for steroid moiety; at 67.96 and 156.877 ppm for imidazole ring; at 205.44 ppm for ketone group. Additionally, the mass spectrum from **3** showed a molecular ion (m/z) 370.26.

Other results showed several signals of 1H NMR spectrum for **4** at 0.76-1.06 ppm for methyl groups bound to steroid nucleus; at 1.96 ppm for methyl group linked to imidazole ring; at 1.16, 1.90, 1.98-2.02, 2.20-2.76 ppm for steroid moiety; at 2.10 ppm for methyl group linked to ketone group; at 4.12-4.26 ppm for methylene groups of imidazole ring; at 10.12 ppm for amino group. The ^{13}C NMR spectra showed chemical shifts at 15.00-15.80 ppm for

methyl groups linked to steroid nucleus; at 20.62 ppm for methyl group linked to imidazole ring; at 22.00-54.08 and 57.42-63.96 ppm for steroid moiety; at 56.88, 67.93 and 156.84 ppm for imidazole ring; at 205.42-208.40 ppm for ketone groups. Finally, the mass spectrum from **4** showed a molecular ion (m/z) 396.23.

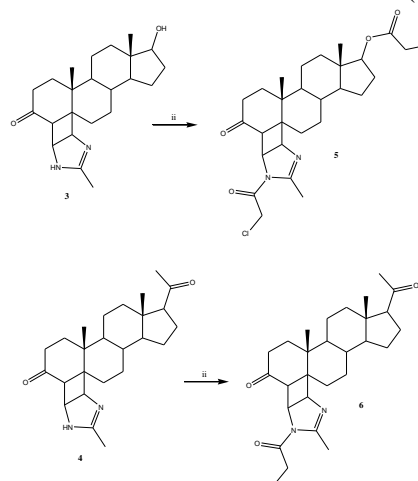


Figure 3. Synthesis of two chloroamide derivatives (**5** or **6**). Reaction of **3** or **4** with chloroacetyl chloride to form **5** or **6**. ii = triethylamine.

Synthesis of two chloroamide derivatives.

It is noteworthy that some chloroamide analogs were synthesized using trichloroisocyanuric Acid [29], *N*-chlorobenzotriazole [30], chloroacetyl chloride [31, 32] and others. In this investigation, two steroid-chloroamide analogs were synthesized via reaction of **5** or **6** with chloroacetyl chloride in the presence of triethylamine (Figure 3). The 1H NMR spectrum for **5** showed several bands at 0.80-1.06 ppm for methyl group linked to steroid nucleus; at 1.72 ppm for methyl group linked to imidazole ring; at 1.08-1.50, 1.76-2.78 and 4.80 ppm for steroid moiety, at 4.08 ppm for methylene group bound to both ester group and chloride atom; at 4.10-4.14 ppm for methylene group bound to both amide group and chloride atom; at 4.74 and 5.06 ppm for imidazole ring. The ^{13}C NMR spectra displayed several signals at 12.08-15.80 ppm for methyl group linked to steroid nucleus; at 19.80 ppm for methyl group linked to imidazole ring; at 20.90-38.84, 42.60-48.70, 51.50-56.66 and 81.69 ppm for steroid moiety; at 40.80 ppm for methylene group bound to both ester group and chloride atom; at 41.76 ppm for methylene group bound to both amide group and chloride atom; at 49.52, 66.42 and 154.56 ppm for imidazole ring; at 168.00 ppm for ester group; at 165.04 for amide group; at 204.20

ppm for ketone group. In addition, the mass spectrum from **5** showed a molecular ion (m/z) 522.20.

Additionally, the ^1H NMR spectrum for **6** displayed some bands at 0.76-1.06 ppm for methyl group bound to steroid nucleus; at 1.16-1.70, 1.80-2.04 and 2.22-2.78 ppm for steroid moiety; at 1.74 ppm for methyl group linked to imidazole ring; at 2.10 ppm for methyl group bound to ketone group; at 4.11-4.14 ppm for methylene group linked to both amide group and chloride atom; at 4.76-5.10 ppm for imidazole ring. The ^{13}C NMR spectra showed some signals at 15.00-15.80 ppm for methyl groups bound to steroid nucleus; at 19.74 ppm for methyl group bound to imidazole ring; at 22.00-29.16, 31.62-38.87, 42.96-46.70 and 54.04-63.96 ppm for steroid moiety; at 30.80 ppm for methyl group linked to ketone group; at 4.74 ppm for methylene group linked to both amide group and chloride atom; at 49.52, 66.42-154.56 ppm for imidazole ring; at 165.04 ppm for amide group; at 204.22-208.36 ppm for ketone group. In addition, the mass spectrum from **6** shown a molecular ion (m/z) 472.22.

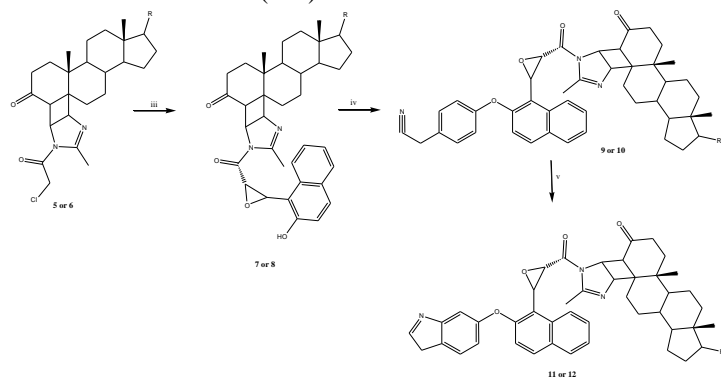


Figure 4. Preparation of two indol-steroid derivatives (**11** or **12**).

Reaction of **5** or **6** with 2-hydroxy-1-naphthaldehyde in basic medium (iii) to form two steroid-epoxides analogs (**7** or **8**). Then, **7** or **8** reacted with 1,3-(4-nitro-phenyl)-acetonitrile in the presence of dicyclohexylcarbodiimide/p-toluenesulfonic acid (iv) to synthesis of two steroid-ester derivatives (**9** or **10**). Finally, indol-steroid derivatives were prepared **11** or **12** were prepared by an internal reaction (2 + 2 addition) of **9** or **10** using Copper(II) chloride (v).

Preparation of two steroid-epoxy derivatives.

There are some studies which indicate the synthesis of several epoxide groups using several reagents such as Co(III) [33] and Cr(III) [34]; in addition, recently was prepared a steroid-epoxide using basic conditions. Analyzing this data, in this investigation, two new steroid-epoxide analogs (**7** or **8**) were prepared via reaction of **5** or **6** with 2-hydroxy-1-naphthaldehyde (Figure 4).

The ^1H NMR spectrum for **7** showed some signals at 0.84 and 1.06 ppm for methyl groups linked to steroid nucleus; at 1.72 ppm for methyl group bound to imidazole ring; at 0.94, 1.07-1.60 and 1.80-3.62 ppm for steroid moiety; at 4.22-4.46 ppm for oxirane ring; at 7.22-7.70 and 7.90 ppm for phenyl groups; at 7.78 ppm for hydroxyl groups. The ^{13}C NMR spectra showed several signals at 12.06-15.80 ppm for methyl groups linked to steroid nucleus; at 19.76 ppm for methyl group bound to imidazole ring; at 21.04-46.70, 51.72-56.66 and 81.80 ppm for steroid moiety; at 51.28, 66.42 and 156.28 ppm for imidazole ring; at 53.46-53.90 ppm for oxirane ring; at 118.85-153.92 for phenyl groups; at 163.60-204.20 ppm for ketone groups. In addition, the mass spectrum from **7** showed a molecular ion (m/z) 582.30.

Other results showed some signals of ^1H NMR spectrum for **8** at 0.76-1.06 ppm for methyl groups linked to steroid nucleus; at 1.16-1.72, 1.80-2.02 and 2.22-2.76 ppm for steroid moiety; at 1.74 ppm for methyl group bound to imidazole ring; at 2.10 ppm for methyl group linked to ketone group; at 4.26-4.84 ppm for oxirane ring; at 4.74-4.84 ppm for imidazole ring; 7.22-7.90 ppm for phenyl groups; at 9.08 ppm for hydroxyl group. The ^{13}C NMR spectra showed chemical shifts at 15.00-15.80 ppm for methyl groups; at 19.74 ppm for methyl group bound to imidazole ring; at 30.22 ppm for methyl group linked to ketone; at 22.00-29.12, 31.64-48.70 and 54.10-63.96 ppm; at 56.24 and 66.42 ppm for imidazole ring; at 53.44-53.84 and 156.28 ppm for oxirane ring; at 118.82-153.91 ppm for phenyl groups; at 163.64-208.34 ppm for ketone groups. Finally, the mass spectrum from **8** display a molecular ion (m/z) 608.32.

Preparation of steroid-ester derivatives.

Some reagents have used to preparation of ester analogs [35-38]; in this study, the compounds **9** or **10** were prepared by the reaction of **7** or **8** with (4-nitro-phenyl)-acetonitrile using 1,3-dicyclohexylcarbodiimide (DCC) (Figure 4). It is noteworthy that when DCC is used in some esterification reactions it can produce N-acylurea byproducts. Here it is important to mention that some reports suggest that the addition of a strong acid to the esterification reaction in the presence of DCC decreases the formation of N-acylurea [39]. In this study, *p*-toluenesulfonic acid was used to formation of **9** or **10** via esterification of **7** or **8** with (4-nitro-phenyl)-acetonitrile in the presence of DCC.

The ^1H NMR spectrum of **9** shows some signals at 0.86-1.06 ppm for methyl groups bound to steroid nucleus; at 0.94, 1.07-1.60 and 1.80-3.62 ppm for steroid moiety; at 1.72 ppm for methyl group bound to imidazole ring; at 3.64 ppm for methylene group bound to both cyanide and phenyl groups; at 3.86-4.72 ppm for oxirane ring; at 4.76-4.84 ppm for imidazole ring; at 6.40 ppm for hydroxyl group; at 6.92-8.20 ppm for phenyl groups. The ^{13}C NMR spectra display several signals at 12.10-15.80 ppm for methyl groups linked to steroid nucleus; at 19.74 ppm for methyl group bound to imidazole ring; at 21.04-48.70, 51.72-52.66 and 81.84 ppm for steroid moiety; at 51.24, 66.42 and 156.28 ppm for imidazole ring; at 53.44-53.80 ppm for oxirane ring; at 117.38 ppm for cyanide group; at 118.00-146.34 and 156.84 ppm for phenyl groups; at 163.62-204.20 for ketone groups. Additionally, the mass spectrum from **9** display a molecular ion (m/z) 697.35.

On the other hand, the ^1H NMR spectrum of **10** display several bands at 0.76-1.06 ppm for methyl groups bound to steroid nucleus; at 1.16-1.72, 1.80-2.02 and 2.22-2.76 ppm for steroid moiety; at 1.74 ppm for methyl group linked to imidazole ring; at 2.10 ppm for methyl group linked to ketone group; at 3.64 ppm for methylene group linked to both cyanide and phenyl group; at 3.86-4.72 ppm for oxirane ring; at 4.76-4.84 ppm for imidazole ring; at 6.92-8.20 ppm for phenyl groups. The ^{13}C NMR spectra display some signals at 15.00-15.80 ppm for methyl groups linked to steroid nucleus; at 19.76 ppm for methyl group linked to imidazole ring; at 22.00-23.08, 24.44-48.70 and 54.08-63.96 ppm for steroid moiety; at 23.44 for methylene group linked to both cyanide and phenyl group; at 51.24, 66.42 and 156.24 ppm for imidazole ring; at 53.44-53.82 ppm for oxirane ring; at 117.40 ppm for cyanide group; at 116.10-146.36 and 156.84 ppm for phenyl groups; at

163.64-208.30 ppm for ketone groups. Finally, the mass spectrum from **10** display a molecular ion (m/z) 723.36.

Preparation of two indol-steroid derivatives.

Several methods for synthesis de indoles, these methods use different reagents as such as o-Iodoanilines [40], benzonitrile derivatives [41], ruthenium [42], tetrabutylammonium fluoride [43] and others. In this investigation, either **9** or **10** reacted with Copper(II) chloride to form **11** or **12** (Figure 4); the mechanism involved an internal reaction (2 + 2 addition). The ^1H NMR spectrum of **11** display some signals at 0.86 and 1.06 ppm for methyl groups linked to steroid nucleus; at 0.94, 1.07-1.60, 1.80-2.70 and 3.04 ppm for steroid moiety; at 1.72 ppm for methyl group bound to imidazole ring; at 3.40 and 7.98 ppm for indole; at 3.86-4.72 ppm for oxirane ring; at 4.76-4.84 ppm for imidazole ring; at 6.40 ppm for hydroxyl group; at 6.46-7.80 and 8.20 ppm for phenyl groups. The ^{13}C NMR spectra showed several signals at 12.10-15.80 ppm for methyl groups bound to steroid nucleus; at 19.78 ppm for methyl group bound to imidazole ring; at 21.04-44.50, 48.70, 51.72-52.66 and 81.84 ppm for steroid moiety; at 48.22, 66.42 and 168.86 ppm for indole system; at 51.24, 156.28 and 156.08 ppm for imidazole ring; at 53.44-53.82 ppm for oxirane ring; at 108.84-154.80 ppm for phenyl groups; at 163.64-208.34 ppm for ketone groups. In addition, the mass spectrum from **11** display a molecular ion (m/z) 697.35.

On the other hand, the ^1H NMR spectrum for **12** showed some signals at 0.76-1.06 ppm for methyl groups linked to steroid nucleus; at 1.17-1.72, 1.80-2.02 and 2.22-2.78 ppm for steroid moiety; at 1.74 ppm for methyl group bound to imidazole ring; at 2.10 ppm for methyl group bound to ketone group; at 3.38 and 7.98 ppm for indole; at 3.86-4.72 ppm for oxirane ring; at 4.76-4.84 ppm for imidazole ring; at 6.46-7.78 and 8.20 ppm for phenyl groups.

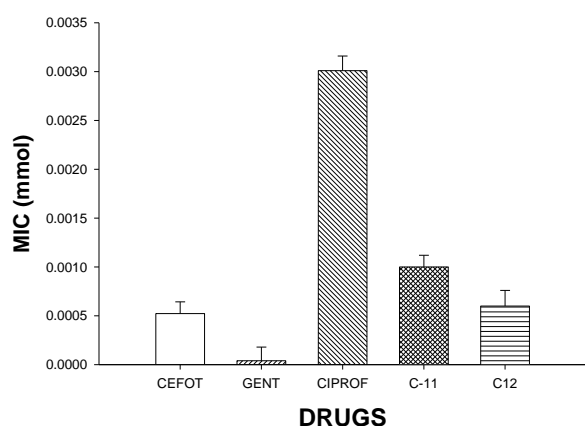


Figure 5. Antibacterial effects induced by two indol-steroid-cyclobuta-imidazolone (**11** or **12**) and the controls (cefotaxime, CEFOT; gentamicin, GENT; ciprofloxacin, CIPROF) against *Escherichia coli*. The experimental results showed that *Escherichia coli* was inhibited with cefotaxime (MIC = 5.2×10^{-4} mmol), gentamicin (MIC of 4.00×10^{-5} mmol) and ciprofloxacin (MIC of 3.01×10^{-3} mmol). In addition, bacterial growth of *Escherichia coli* was inhibited by **12** (MIC of 6.00×10^{-4} mmol) compared with **11** (MIC of 1.00×10^{-3} mmol). MIC = minimum inhibitory concentration.

The ^{13}C NMR spectra display several signals at 15.00-15.80 ppm for methyl groups bound to steroid nucleus; at 19.74 ppm for methyl group linked to imidazole ring; at 22.00-29.12, 44.50, 48.70 and 54.08-64.00 for steroid moiety; at 30.20 ppm for

methyl group bound to ketone; at 48.12, 108.87 and 164.82 ppm for indole system; at 51.27, 66.40 and 156.28 ppm for imidazole ring; at 53.44 and 53.82 ppm for oxirane ring; at 100.76-154.80 for phenyl groups; at 163.64-208.34 ppm for ketone groups. Additionally, the mass spectrum from **12** display a molecular ion (m/z) 723.36.

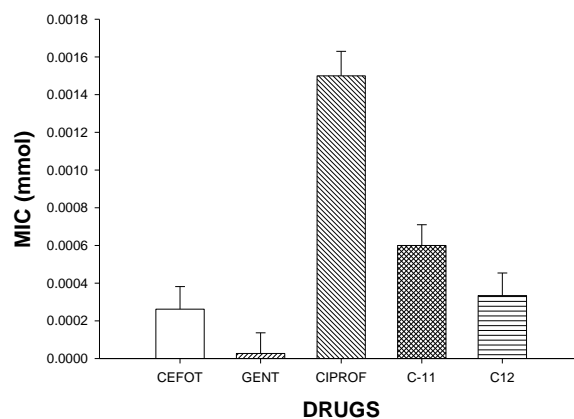


Figure 6. Antibacterial activity exerted by two indol-steroid-cyclobuta-imidazolone (**11** or **12**) and control (cefotaxime, CEFOT; gentamicin, GENT; ciprofloxacin, CIPROF) against *Klebsiella pneumoniae*. Bacterial growth of *Klebsiella pneumoniae* was blocked in presence of cefotaxime (MIC = 2.18×10^{-4} mmol), gentamicin (MIC of 2.68×10^{-5} mmol) and ciprofloxacin (MIC of 1.50×10^{-3} mmol). Finally, in the scheme is observed that *Klebsiella pneumoniae* was susceptible in presence of **11** (MIC of 6.00×10^{-4} mmol) compared with **12** (MIC of 3.34×10^{-4} mmol). MIC = minimum inhibitory concentration.

Evaluation of biological activity.

Antibacterial effect exerted by compounds **11** or **12** against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* was determinate using minimum inhibitory concentration [17].

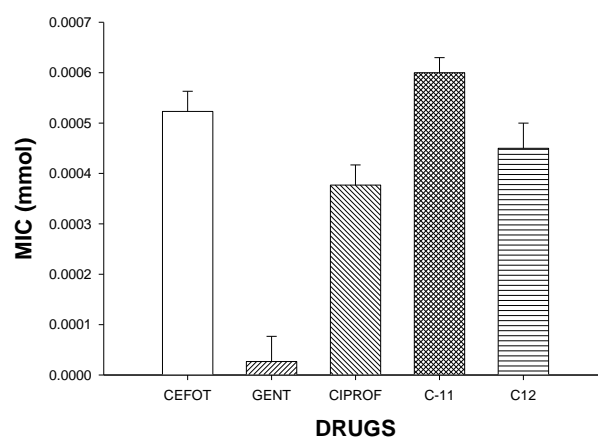


Figure 7. Antibacterial effect induced by two indol-steroid-cyclobuta-imidazolone (**11** or **12**) and the controls (cefotaxime, CEFOT; gentamicin, GENT; ciprofloxacin, CIPROF) against *Staphylococcus aureus*. Bacterial growth of *Staphylococcus aureus* was inhibited in presence of cefotaxime (MIC = 5.23×10^{-4} mmol), gentamicin (MIC of 2.68×10^{-5} mmol) and ciprofloxacin (MIC of 3.77×10^{-4} mmol). Other results indicated that *Staphylococcus aureus* was also inhibited with **11** (MIC of 6.00×10^{-4} mmol) and **12** (MIC of 3.34×10^{-4} mmol). MIC = minimum inhibitory concentration.

It is important to mention that some drugs such as gentamicin, ciprofloxacin and cefotaxime were used as controls. The results found (Figure 5) showed that bacterial growth of *Escherichia coli*

was inhibited with cefotaxime [$\text{MIC} = 5.23 \times 10^{-4}$ mmol], gentamicin [$\text{MIC} = 4.00 \times 10^{-5}$ mmol], ciprofloxacin [$\text{MIC} = 3.01 \times 10^{-3}$ mmol], compound **11** [$\text{MIC} = 1.00 \times 10^{-3}$ mmol], compound **12** [$\text{MIC} = 6.00 \times 10^{-4}$ mmol]. Other data (Figure 6) showed that bacterial growth of *Klebsiella pneumoniae* was blocked in presence of cefotaxime [$\text{MIC} = 2.61 \times 10^{-4}$ mmol], ciprofloxacin [$\text{MIC} = 1.50 \times 10^{-3}$ mmol], gentamicin [$\text{MIC} = 2.68 \times 10^{-5}$ mmol], compounds **11** [$\text{MIC} = 6.00 \times 10^{-4}$ mmol] and compound **12** [$\text{MIC} = 3.34 \times 10^{-4}$ mmol].

Finally, the bacterial growth of *Staphylococcus aureus* (Figure 7) was blocked with cefotaxime [$\text{MIC} = 5.23 \times 10^{-4}$ mmol], gentamicin [$\text{MIC} = 2.68 \times 10^{-5}$ mmol], ciprofloxacin [$\text{MIC} = 3.77 \times 10^{-4}$ mmol], compound **11** [$\text{MIC} = 6.00 \times 10^{-3}$ mmol], compound **12** [$\text{MIC} = 4.50 \times 10^{-4}$ mmol].

The experimental results showed that; i) compounds **11** and **12** has different biological activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* compared with gentamicin (protein synthesis inhibitor) [44], cefotaxime (β -lactamic) [45], ciprofloxacin (DNA gyrase inhibitor) [46]; ii) these phenomena could be product of different characteristic chemical involved of compounds studied; iii) the antibacterial activity exerts by compound **12** was higher compared with compound **11**; iv) the antibacterial effect exerted by **12** against the bacteria involved in this study was similar to cefotaxime which suggests that antibacterial activity of **12** could be the result of differences on interaction of compound **12** with β -lactamase enzyme compared with **11** or to differences in chemical structure of these compounds.

Theoretical analysis.

To evaluate the hypothesis above mentioned, it was necessary to carry out other type of studies, the first stage involves the interaction of compound **12** with β -lactamase enzyme using a theoretical model [22]; it is noteworthy, that the enzyme β -lactamase (5flg protein) [23] and compound **11** were used as controls.

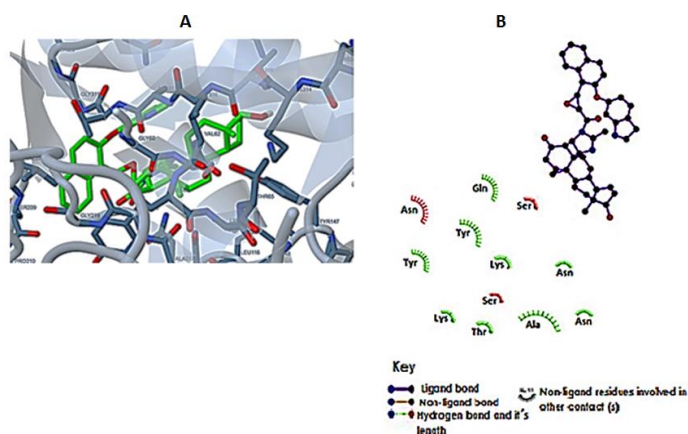


Figure 8. The schemes A and B showed the interaction of β -lactamase (5flg) surface with the compound **11**. Theoretical analysis was carried out using the Docking-server.

Theoretical data (Figure 8) shown the possible interaction of compound **11** with several amino acid residues of 5flg protein such as Ser₆₁, Lys₆₄, Gln₁₁₇, Tyr₁₄₇, Asn₁₄₉, Ser₂₀₉, Tyr₂₁₈, Lys₃₁₄, Thr₃₁₅, Ala₃₁₇, Asn₃₄₂, Asn₃₄₅. Analyzing these data, also the possible bind of compound **12** with 5flg protein was carried out to compare with the compound **11**; the theoretical data (Figure 9) found showed that compound **12** could interaction with Ser₆₁,

Leu₁₁₆, Gln₁₁₇, Asp₁₂₀, Tyr₁₄₇, Asn₁₄₉, Ser₂₀₉, Tyr₂₁₈, Thr₃₁₈, Asn₃₄₂ involved in 5flg protein surface. All these data indicate that compounds **11** or **12** could interact in a different way with the amino acid residues on the surface of the 5flg protein; however, it is also possible that Ser₆₁ is indispensable for the interaction of the free amino of compound **12** to form an amide bond. However, this phenomenon could involve some intramolecular interactions due to changes in the energy levels.

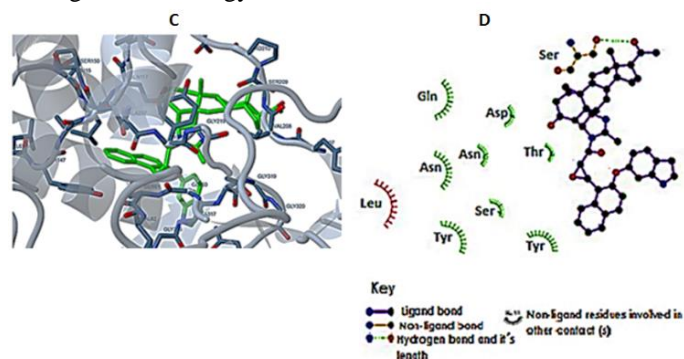


Figure 9. In the scheme is shown the interaction of β -lactamase (5flg) surface with the compound **12** (A and B). Theoretical analysis was carried out using the Docking-server.

Thermodynamic parameters.

There are some studies indicate that also some thermodynamic parameters are evidences for confirming the interaction drug-protein [47], in this study a theoretical evaluation was carried out on some thermodynamic parameters such as free energy of binding, electrostatic energy, total intermolecular energy, vdW + Hbond + desolv energy and inhibition constant. The results showed differences in the intramolecular energy involved in the interaction for compound **11** or **12** (Table 1 and 2) with the 5flg protein.

Table 1. Evaluation of energy levels involved in the interaction between compounds **11** or **12** with 5flg protein surface.

Compound	Est. Free Energy of Binding (kcal/mol)	Est. Inhib. Const. Ki (nM)	VdW + Hbond + desolv Energy
11	-5.54	87.28	-7.20
12	-5.88	48.81	-8.71

Table 2. Energy levels involved in the interaction between compounds **11** or **12** with 5flg protein surface.

Compound	Electrost. Energy (kcal/ mol)	Total Intermol Energy (kcal/ mol)	Inter. Surface
11	0.27	-0.693	1022.662
12	0.16	-8.56	1153.803

Finally, other data showed that inhibition constants were lower for **12** compared with the Ki for **11**. These data are interesting, which implies a higher interaction of the compound **12** with the 5flg protein which could be translated as a major theoretical antibacterial activity. However, it is noteworthy some reports indicate that some thermodynamic parameters may be relationship with other electronic factors of some compounds [48].

Electronic parameters (HOMO and LUMO).

There are several studies that suggest that HOMO and LUMO could be predictive factors of the biological activity exerted by some compounds on different microorganisms [49]. Analyzing these data, a theoretical study was carried out to evaluate the relationship between HOMO-LUMO and the experimental biological activity of compounds **11** and **12** using SPARTAN'06 software package. The results (Figures 10 and 11; Table 3) indicate that only there are differences in the LUMO value for compound **12** compared with **11**; this phenomenon may be

conditioned by differences of electron donating ability of functional groups involved in the chemical structure of **11** or **12**. However, was necessary to evaluate other physicochemical parameters such as HBD and HBA, TPSA that also may condition the biological activity of some compounds [50-52].

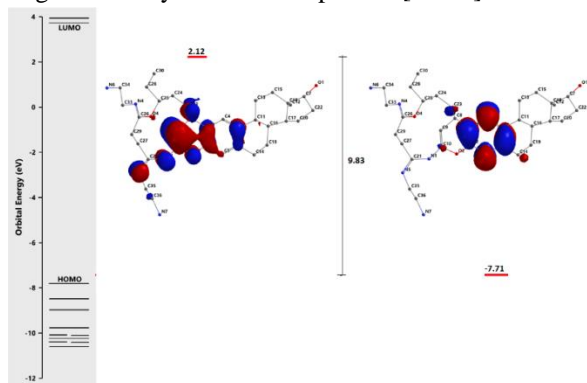


Figure 10. Molecular Orbitals (HOMO and LUMO) involved in the compound **11**. Visualized with SPARTAN'06 software.

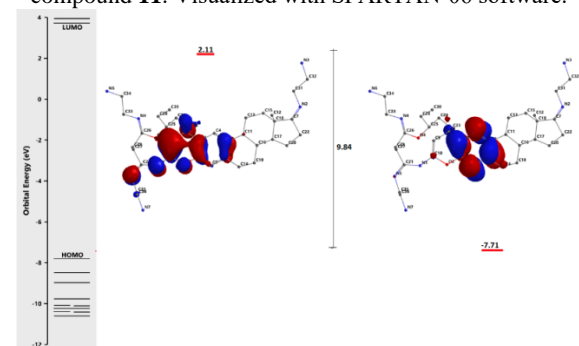


Figure 11. Molecular Orbitals (HOMO and LUMO) involved in the compound **12**. Visualized with SPARTAN'06 software. **12** and β -lactamase (5f1g protein) using dockingserver software.

Table 3. Theoretical electronic parameters involved in the compound **6** and **7** using Spartan software.

Parameter	Compound 6	Compound 7
Energy (kcal/mol)	-2218.55	-2295.03
Energy HOMO (ev)	-7.72	-7.72
Energy LUMO (ev)	2.12	2.11
Energy-gap = E. HOMO – E. LUMO (ev)	9.84	9.83

Physicochemical parameters.

To evaluate the premise above mentioned, in this study the physicochemical parameters HBD and HBA were evaluated using the Spartan 6.0 software. The data determinate (Table 4) indicated that the values of HBA were <10 and the values HBD <5 for compounds **11** and **12**; these data indicate that these compounds could be well absorbed according to Lipinski's rule for other types of compounds [53]. In addition, to confirm this hypothesis, the TPSA parameter involved in the chemical properties of compounds **11** and **12** was determinate; it is important to mention that the TPSA is a descriptor that is related to some properties of drugs [54]. The results (Table 4) indicated that TPSA was lower for **12** compared with **11**; These data suggest that TPSA could condition the ability of compounds **11** and **12** to penetrate some biological barrier such happening with other type of drugs [55].

Table 4. Evaluation of theoretical physicochemical parameters involved in the compound **11** and **12** using both ACDLabs and Spartan software.

Parameter	Compound 11	Compound 12
Molar refractivity (cm ³)	193.73 \pm 0.50	202.76 \pm 0.50
Molar volume (cm ³)	463.90 \pm 7.00	495.00 \pm 7.00

Parameter	Compound 11	Compound 12
Parachor (cm ³)	1301.5 \pm 8.00	1371.2 \pm 8.00
Index of refraction	1.77 \pm 0.05	1.75 \pm 0.05
Density (kg/cm ³)	1.50 \pm 0.10	1.46 \pm 0.10
Surface tension (dyne/cm)	61.9 \pm 7.00	58.80 \pm 7.00
Dipole momento (debye)	3.27	3.09
PSA (Å)	80.19	74.86
HBA	8	8
HBD	1	0
Polarizability	96.71	99.26

Table 5. Evaluation of physicochemical factors (logKow and π) involved in the compounds **11** and **12** using ECOSUITE 2.1 software.

Compound	Fragments	Values
11	-CH ₃ [aliphatic carbon]	1.6419
	-CH ₂ [aliphatic carbon]	4.4199
	-CH [aliphatic carbon]	3.6140
	-C [aliphatic carbon - No H, not tert]	0.9723
	-OH [hydroxy, aliphatic attach]	-1.4086
	-O [oxygen, aliphatic attach]	-1.2566
	-N< [aliphatic attach]	-1.8323
	Aromatic Carbon	4.7040
	-N [aliphatic N, one aromatic attach]	-0.9170
	-O [aliphatic O, two aromatic attach]	0.2923
	-C(=O)- [carbonyl, aliphatic attach]	-1.5586
	-C(=O)N [aliphatic attach]	-0.5236
	-tert Carbon [3 or more carbon attach]	0.8028
	-N=C [aliphatic attach]	-0.0010
	Ortho-subst on di-aromatic ether (non-cyl)	-0.8396
	Fused aliphatic ring unit correction	2.7368
	Aryloxy (or -C-O)-C-C(=O)NH- correction	0.4874
	-O-C-C(-O)-C(=O)- structure correction	1.0000
	Equation Constant	0.2290
	π	1.2200
	Log Kow	7.0895
12	-CH ₃ [aliphatic carbon]	2.1892
	-CH ₂ [aliphatic carbon]	4.4199
	-CH [aliphatic carbon]	3.6140
	-C [aliphatic carbon - No H, not tert]	0.9723
	-O [oxygen, aliphatic attach]	-1.2566
	-N< [aliphatic attach]	-1.8323
	Aromatic Carbon	4.7040
	-N [aliphatic N, one aromatic attach]	-0.9170
	-O [aliphatic O, two aromatic attach]	0.2923
	-C(=O)- [carbonyl, aliphatic attach]	-3.1172
	-C(=O)N [aliphatic attach]	-0.5236
	-tert Carbon [3 or more carbon attach]	0.8028
	-N=C [aliphatic attach]	-0.0010
	Ortho-subst on di-aromatic ether (non-cyl)	-0.8396
	Fused aliphatic ring unit correction	-2.7368
	Aryloxy (or -C-O)-C-C(=O)NH- correction	0.4874
	-O-C-C(-O)-C(=O)- structure correction	1.0000
	Equation Constant	0.2290
	π	0.3773
	Log Kow	7.4668

However, it is important to mention that there is another type of physicochemical parameters, such as logP and π , which can condition the degree of lipophilicity that can be translated as an increase or decrease in the passage through the lipid membrane of some drugs [56]. Analyzing this data, a theoretical analysis of logKow and π values was evaluated using a previously method reported [57]. The results shown in Table 5 indicate that the log Kow and π values were higher for compound **12** compared to **11**, which results in a greater degree of lipophilicity for **12**. However, it is noteworthy that there are some studies [56] which suggest that this phenomenon could be conditioned by other physicochemical parameters such as molar volume (V_m) and molar refractivity (R_m)

that are steric constant may induce changes in some biological model. Therefore, in this study, a theoretical analysis of both V_m and R_m descriptors was conducted (Table 4) using a previously method reported [56]. Theoretical results showed that R_m and V_m

were higher for **12** compared with **11**. All these data suggest that the steric hindrance and the different conformations of **12** compared to **11** could be some factors which exert a decrease the bacterial growth of the microorganisms involved in this study.

4. CONCLUSIONS

All these results suggested the following; 1) antibacterial activity of **12** depend on chemical structure; 2) possibly the biological activity involve the interaction with β -lactamase; and 3) physicochemical parameters may condition the antibacterial activity of compound **12** compared with **11**. All these data indicate

that compound **12** can be a good pharmacological alternative as antibacterial drug; however, it is important to carry out some toxicity studies of this compound to rule out any adverse effects involved in its administration.

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