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Antibacterial activity exerted by some diazabicyclo-steroid derivatives against *Staphylococcus aureus* and *Streptococcus pneumoniae*. Theoretical analysis of its interaction with the DNA-gyrase

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ABSTRACT

The aim of this study was to synthesize three diazabicyclo-steroid derivatives to evaluate its antibacterial activity. This process involved a series of reactions such as; *i*) cycloaddition [2 + 2] of 5-hexyn-1-ol to OTBS-testosterone (**1**) or progesterone (**2**) or OTBS-pregnenolone (**3**) to form cyclobutene-ol-steroid derivatives (**4** or **5** or **6**); *ii*) the compounds **4** or **5** or **6** were reacted with ethylenediamine to form steroid-amino conjugates (**7** or **8** or **9**); *iii*) alkynylation of **7** or **8** or **9** with 5-hexyn-1-ol to form the steroid-amino-hexynol conjugates (**10** or **11** or **12**); *iv*) preparation of the cyclobuta-ynal-steroid derivatives (**13** or **14** or **15**) by the reaction of **10** or **11** or **12** with DMSO; *v*) amination of **13** or **14** or **15** with ethylenediamine to form new amino-steroid derivatives **16** or **19** or **21;** *vi*) removal of the *tert*-butyldimethylsilyl from **16** or **21** with hydrofluoric acid to form hydroxyl-steroids (**17** and **22**); *vii*) preparation of 1,4-diazacycloundeca-5,11-dien-steroid derivatives by the reaction **17** or **19** or **22** with Copper(II) chloride. In order to evaluate the possibility of that compounds synthesized may have biological activity; in this study its antibacterial effect on *Streptococcus pneumoniae* and *Staphylococcus aureus* bacteria was evaluated. The results indicate that compound **20** exert higher antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* compared with **18** and **23** via interaction DNA-gyrase. In conclusion, these data indicate that antibacterial activity exerted by the compounds **20** depend of their structure chemical in comparison with the other steroid derivatives involved in this study.

Keywords: diazabicyclo, steroid, testosterone, antibacterial, DNA-girase.

1. INTRODUCTION

Infectious diseases are one of the main causes of morbidity-mortality in the world [1, 2]. There are several reports which indicate that some causal agents, such as *Staphylococcus aureus* [3], *Streptococcus pneumoniae* [4] and others are involved in the development of infectious diseases. Although there are many therapeutic agents for treatment of these bacterial microorganisms [5, 6], unfortunately prolonged antibiotic therapy induces bacterial-resistance, because some bacteria have developed ways to circumvent the effects of antibiotics [7, 8]. Therefore, antibiotic resistance to bacteria can be considered a serious threat to the human health; this fact requires an international approach to its management. In this sense, new drugs have been developed for control of bacterial resistance; for example, the preparation of diazabicycle derivatives as antibacterial agents against *Staphylococcus aureus* strains [9] Other data indicate the synthesis and antibacterial activity of some 1,3-diazabicyclo-carbapen derivatives 2,4-dichloro-5-fluorophenyl derivatives against *Staphylococcus aureus* strain [10]. In addition, some 3,7-diazabicyclo[3.3.1]nonane azines were prepared as antibacterial agents against *Staphylococcus aureus* [11]. In addition, a study showed the synthesis of 9-alkyl-l,5-diazabicyclo[4.3.0]non-5-enes which decreased the bacterial growth of *Staphylococcus aureus* [12]. Also, other report showed the preparation of 1,4-diazabicyclo[2.2.2]octane with antibacterial activity against a *Staphylococcus aureus* strain [13]. Additionaly, other report shown that the diazabycycle derivative (1,3-diazaadamante) exerted antibacterial activity against *Staphylococcus aureus* [14].

On the other hand, a 3,7-diazabicyclo[3.3.1]nonane derivative was synthesized as antibacterial agent against *Streptococcus pneumoniae* [15, 16]. Additionally, other data showed the synthesis of the compound 3,8-Diazahicyclo[3.2.l]octane [17] which inhibits the bacterial growth of *Streptococcus pneumoniae* [18, 19]. All these experimental results show that several diazabicyclo derivatives can induce antibacterial effects against *Staphylococcus aureus* and *Streptococcus pneumoniae*. Analyzing these data, in this study three diazabicyclo-steroid derivatives were

synthesized and their antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* was evaluated *in vitro*.

2. EXPERIMENTAL SECTION

2.1. General methods. The testosterone derivative and pregnenolone (OTBS-Testosterone and OTBDS-pregnenolone) were prepared using methods previously reported [20]. The other reagents used in this study were purchased from Sigma-Aldrich Co. Ltd. The melting point was determined on an Electrothermal (900 model). ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

2.2. Preparation of cyclobuta-3-one-steroid derivatives. A solution of **1** or **2 or 3** (0.50 mmol), 5-hexyn-1-ol (60 µl, 0.54 mmol), Iron(III) chloride anhydrous (80 mg, 0.49 mmol) in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (4:1).

8-(tert-Butyl-dimethyl-silanyloxy)-2-(4-Hydroxy-butyl)-5a,7a-dimethyl-4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-tetradecahydro-2aH-cyclobuta[*j*]cyclopenta[a]phenanthren-3-one (4)

yielding 67 % of product, m.p. 74-76 ^{o}C; IR (V_{max}, cm^{-1}): 3400, 1720 and 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.68 (s, 3H), 0.86 (s, 9H), 0.94-1.04 (m, 2H), 1.08 (s, 3H), 1.20-1.48 (6H), 152-1.60 (m, 4H), 1.62-2.10 (m, 8H), 2.20 (m, 2H), 2.22-3.54 (m, 5H), 3.60 (broad, 1H), 3.66 (m, 2H), 5.70 (d, 1H, J = 1.82 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-24, C-31), 11.32 (C-21), 14.80 (C-20), 18.02 (C-32), 21.34 (C-18), 23.52 (C-8), 24.30 (C-27), 25.24 (C-11), 25.50 (C-33, C-34, C-35), 31.06 (C-7), 31.82 (C-10), 32.44 (C-12), 32.54 (C-28), 36.54 (C-3), 36.70 (C-19), 37.02 (C-26), 38.50 (C-13), 40.40 (C-9), 42.24 (C-1), 43.30 (C-5), 49.78 (C-2), 52.00 (C-4), 60.16 (C-15), 62.55 (C-29), 81.62 (C-6), 134.52 (C-17), 147.68 (C-16), 210.62 (C-14) ppm. EI-MS *m/z:* 500.36 Anal. Calcd. for $C_{31}H_{52}O_3Si$: C, 74.34; H, 10.47; O, 9.58; Si, 5.61. Found: C, 74.26; H, 10.36.

8-Acetyl-2-(4-hydroxy-butyl)-5a,7a-dimethyl-4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-tetradecahydro-2aH-cyclobuta[*j*]cyclopenta[a]phenanthren-3-one (5)

yielding 44 % of product, m.p. 80-82 ^{o}C; IR (V_{max}, cm^{-1}): 3404 and 1722; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.70 (s, 3H), 1.06 (s, 3H), 1.20-1.46 (8H), 1.50-1.58 (m, 4H), 1.66-2.10 (m, 8H), 2.12 (s, 3H), 2.20 (t, 2H, J = 13.20 Hz), 2.22-3.00 (m, 5H), 3.60 (broad, 1H), 3.66 (t, 2H, J = 11.00 Hz), 5.70 (d, 1H, J = 1.32 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.40 (C-21), 14.80 (C-20), 21.52 (C-7), 23.02 (C-18), 24.30 (C-24), 24.44 (C-17), 25.24 (C-10), 30.74 (C-30), 31.82 (C-9), 32.44 (C-11), 32.54 (C-25), 35.64 (C-3), 37.02 (C-23), 38.48 (C-12), 39.02 (C-6), 40.40 (C-8), 42.24 (C-1), 44.28 (C-5), 51.19 (C-2), 57.72 (C-4), 60.16 (C-14), 62.55 (C-26), 63.98 (C-19), 134.52 (C-16), 147.68 (C-15), 208.28 (C-28), 210.62 (C-13) ppm. EI-MS *m/z:* 412.29 Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.60; H, 9.77; O, 11.63. Found: C, 78.52; H, 9.68.

1-[8-(*tert*-Butyl-dimethyl-silanyloxy)-10-(4-hydroxy-butyl)-3a,3b-dimethyl-1,2,3,3a,4,5,5a,5b,6,7,11a,12,12a,12b-hexadecahydro-cyclobuta[*k*]cyclopenta[*a*]phenanthren-3-yl]ethanonone (6)

yielding 56 % of product, m.p. 148-150 ^{o}C; IR (V_{max}, cm^{-1}): 3402, 1720 and 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.70 (s, 3H), 0.90 (s, 9H), 0.98 (s, 3H), 1.14-1.46 (m, 9H), 1.50 (m, 2H), 1.52 (m, 1H), 1.56-2.00 (m, 7H), 2.02 (m, 2H), 2.12 (s, 3H), 2.16-2.20 (m, 3H), 2.58-3.50 (m 5H), 3.60 (broad, 1H), 3.66 (t, 2H, J = 11.00 Hz), 5.40 (d, 1H, J = 1.32 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-22, C-32), 13.70 (C-24), 16.90 (C-24), 18.50 (C-33), 21.22 (C-10), 24.12 (C-15), 24.20 (C-16), 24.90 (C-28), 26.10 (C-34, C-36, C-37), 28.34 (C-35), 28.52 (C-2), 32.54 (C-29), 32.88 (C-6), 33.34 (C-14), 35.00 (C-3), 35.14 (C-11), 35.62 (C-27), 38.68 (C-4), 39.02 (C-9), 43.20 (C-13), 44.14 (C-8), 44.80 (C-5), 51.12 (C-12), 56.42 (C-7), 62.55 (C-30), 63.78 (C-17), 71.56 (C-1), 133.92 (C-19), 153.90 (C-18), 208.58 (C-25) ppm. EI-MS *m/z:* 528.39 Anal. Calcd. for $C_{33}H_{56}O_3Si$: C, 74.94; H, 10.67; O, 9.08; Si, 5.31. Found: C, 74.83; H, 10.54.

2.3. Preparation of cyclobutene-1-ol-steroid-amino conjugates. A solution of **4** or **5 or 6** (0.50 mmol), ethylenediamine (60 µl, 0.90 mmol) and boric acid (50 mg, 0.80 mmol), in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (3:1).

4[3-(2-Amino-ethylimino)-8-(*tert*-butyl-dimethyl-silanyloxy)-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[*j*]cyclopenta[a]phenan- thren-2yl]-butan-1-ol (7)

yielding 55 % of product, m.p. 120-122 ^{o}C; IR (V_{max}, cm^{-1}): 3402, 3370 and 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.68 (s, 3H), 0.88 (s, 9H), 0.94 (m, 1H), 0.98 (s, 3H), 1.06-1.48 (8H), 1.50 (m, 2H), 1.56-1.60 (m, 2H), 1.62 (m, 2H), 1.64-1.88 (m, 5H), 2.15 (t, 2H, J = 13.20 Hz), 2.22-2.42 (m, 3H), 3.10-3.50 (m, 4H), 3.54 (m, 1H), 3.66 (t, 2H, J = 11.00 Hz), 4.10 (broad, 3H), 5.20 (m, 1H), 5.40 (d, 1H, J = 1.32 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-24, C-34), 11.34 (C-21), 15.60 (C-20), 18.02 (C-35), 21.32 (C-18), 23.52 (C-8), 24.38 (C-30), 25.14 (C-11), 25.50 (C-36, C-37, C-38), 26.64 (C-12), 27.22 (C-13), 31.04 (C-7), 32.02 (C-10), 32.54 (C-31), 36.50 (C-29), 36.54 (C-3), 36.70 (C-19), 41.00 (C-27), 42.04 (C-9), 43.30 (C-5), 45.16 (C-1), 47.40 (C-2), 49.08 (C-15), 52.00 (C-4), 53.60 (C-26), 62.55 (C-32), 81.70 (C-6), 132.34 (C-17), 150.38 (C-16), 169.34 (C-14) ppm. EI-MS *m/z:* 542.42 Anal. Calcd. for $C_{33}H_{58}N_2O_2Si$: C, 73.01; H, 10.77; N, 5.16; O, 5.89; Si, 5.17. Found: C, 73.00; H, 10.68.

4-{3-(2-Amino-ethylimino)-8-[1-(2-amino-ethylimino)-ethyl]-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[*j*]cyclopenta[*a*]phenanthren-2-yl}-butan-1-ol (8)

yielding 64 % of product, m.p. 70-72 °C; IR (V_{max}, cm^{-1}): 3402 and 3332; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.20-1.44 (m, 7H), 1.50 (m, 2H), 1.52-1.58 (m, 3H), 1.62 (m, 2H), 1.76 (m, 1H), 1.80 (s, 3H), 1.82-2.12 (m, 4H), 2.18 (t, 2H, J = 13.20 Hz), 2.22-2.40 (m, 5H), 3.09 (m, 2H), 3.10-3.50 (m, 4H), 3.52 (m, 2H), 3.66 (t, 2H, J = 11.00 Hz), 4.20 (broad, 5H), 5.20 (m, 1H), 5.40 (d, 1H, J = 1.82 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-21), 15.60 (C-20), 16.70 (C-36), 21.92 (C-7), 24.40 (C-27), 25.18 (C-10), 25.28 (C-17), 26.40 (C-18), 26.62 (C-11), 27.28 (C-12), 32.04 (C-9), 32.54 (C-28), 36.52 (C-26), 36.56 (C-3), 38.28 (C-6), 41.00 (C-24), 41.02 (C-34), 42.04 (C-8), 42.82 (C-5), 45.17 (C-1), 48.80 (C-2), 49.06 (C-14), 53.10 (C-33), 53.60 (C-23), 56.22 (C-4), 62.55 (C-29), 63.08 (C-19), 132.32 (C-16), 150.38 (C-15), 156.78 (C-31), 169.28 (C-13) ppm. EI-MS *m/z* 496.41 Anal. Calcd. for $C_{31}H_{52}N_4O$: C, 74.95; H, 10.55; N, 11.28; O, 3.22. Found: C, 74.84; H, 10.46.

4-[3-[1-(2-Amino-ethylimino)-ethyl]-8-(isopropyl-dimethyl-silanyloxy)-3a,5b-dime- thyl-1,2,3,3a,4,5,5a,5b,6,7,8,9,11a,12,12a,12b-hexadecahydro-cyclobuta[*k*]cyclopenta [*a*]phenanthren- 10-yl]-butan-1-ol (9)

yielding 45 % of product, m.p. 258-260 °C; IR (V_{max}, cm^{-1}): 3370, 3330 and 1060; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.07 (s, 6H), 0.88 (s, 3H), 0.90 (s, 9H), 0.98 (s, 3H), 1.14-1.40 (m, 7H), 1.50 (m, 2H), 1.51 (m, 1H), 1.52 (m, 2H), 1.53-1.78 (m, 6H), 1.80 (s, 3H), 1.82-1.94 (m, 2H), 2.00 (m, 2H), 2.12-2.40 (m, 5H), 3.08-3.52 (m, 4H), 3.53 (m, 1H), 3.66 (t, 2H, J = 11.00 Hz), 4.10 (broad, 3H), 5.40 (d, 1H, J = 1.82 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-22, C-35), 13.20 (C-24), 16.70 (C-38), 16.90 (C-23), 18.44 (C-36), 21.68 (C-10), 24.90 (C-31), 25.22 (C-15), 26.04 (C-37, C-39, C-40), 26.40 (C-16), 28.52 (C-2), 32.54 (C-32), 33.30 (C-14), 33.80 (C-6), 35.00 (C-3), 35.14 (C-11), 35.57 (C-30), 38.28 (C-9), 38.60 (C-4), 41.00 (C-28), 42.82 (C-8), 43.15 (C-13), 44.80 (C-5), 51.14 (C-12), 53.10 (C-27), 57.36 (C-7), 62.55 (C-33), 63.08 (C-17), 71.56 (C-1), 133.94 (C-19), 153.88 (C-18), 156.70 (C-25) ppm. EI-MS *m/z* 570.45 Anal. Calcd. for $C_{35}H_{62}N_2O_2Si$: C, 73.63; H, 10.95; N, 4.91; O, 5.60; Si, 4.92. Found: C, 73.52; H, 10.83.

2.4. Alkynylation of amino groups. A solution of 7 or 8 or 9 (0.50 mmol), 5-hexyn-1-ol (130 µl, 1.18 mmol) and cupric chloride (120 mg, 0.89 mmol), in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from hexane:methanol:water (1:4:2).

6-{2-[8-*tert*-Butyl-dimethyl-silanyloxy)-2-(3-hydroxy-propyl)-5a,7a-dimethyl-4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-tetrade-cahydro-2a*H*-cyclobuta[*j*]cyclopenta[*a*]phenanthren-3-ylidene amino]-ethylamino}-hex-5-yn-1-ol (10)

yielding 38 % of product, m.p. 130-132 °C; IR (V_{max}, cm^{-1}): 3400 and 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.68 (s, 3H), 0.88 (s, 9H), 0.90 (m, 1H), 0.98 (s, 3H), 1.04-1.56 (9H), 1.58 (m, 2H), 1.60-1.62 (m, 2H), 1.64 (m, 2H), 1.68 (m, 2H), 1.78-2.22 (m, 6H), 2.26 (m, 2H), 2.30 (m, 2H), 2.40 (m, 1H), 3.20 (t, 2H, J = 13.60 Hz), 3.52 (m, 2H), 3.53 (m, 1H), 3.56 (m, 2H), 3.66 (t, 2H, J = 11.00 Hz), 3.80 (broad, 3H), 5.40 (d, 1H, J = 1.32 Hz), 5.60 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-22, C-40), 11.40 (C-23), 15.60 (C-24), 16.18 (C-31), 17.80 (C-41), 21.32 (C-13), 23.14 (C-37), 23.54 (C-11), 25.14 (C-9), 25.72 (C-42, C-43, C-44), 25.80 (C-32), 26.64 (C-14), 27.22 (C-15), 30.06 (C-33), 31.04 (C-10), 32.02 (C-8), 33.40 (C-36), 36.50 (C-4), 36.72 (C-12), 42.04 (C-7), 43.30 (C-2), 45.16 (C-6), 47.40 (C-5), 47.68 (C-17), 51.72 (C-26), 51.98 (C-3), 54.14 (C-27), 61.80 (C-38), 62.05 (C-34), 78.44 (C-30), 81.66 (C-1), 87.30 (C-29), 133.52 (C-18), 150.58 (C-16), 169.34 (C-16) ppm. EI-MS *m/z:* 624.46 Anal. Calcd. for $C_{38}H_{64}N_2O_3Si$: C, 73.02; H, 10.32; N, 4.48; O, 7.68; Si, 4.49. Found: C, 73.00; H, 10.26.

6-{2-[8-{1-[2-(6-Hydroxy-hex-1-ynylamino)-ethylimino]-ethyl}-2-(3-hydroxy-propyl)-5a,7a-dimethyl-4,5,5a,5b,6,7,7a,8,9,10, 10a,10b,11,12-tetradecahydro-2aH-cyclobuta[*j*]cyclopenta[*a*] phenanthren-3-ylideneamino]-ethylamino}-hex-5-yn-1-ol (11)

yielding 64 % of product, m.p. 133-134 °C; IR (V_{max}, cm^{-1}): 3402 and 3330; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.20-1.56 (m, 10H), 1.58 (m, 4H), 1.66 (m, 4H), 1.68 (s, 3H), 1.69 (m, 2H), 1.78-2.22 (m, 7H), 2.26 (t, 2H, J = 13.20 Hz), 2.28 (m, 1H), 2.30 (m, 4H), 2.38-2.40 (m, 2H) 3.16 (m, 4H), 3.52 (t, 2H, J = 11.00 Hz), 3.56 (m, 4H), 3.64 (m, 4H), 3.70 (broad, 5H), 5.40 (d, 1H, J = 1.82 Hz), 5.60 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-20), 15.60 (C-21), 16.20 (C-29, C-40), 21.92 (C-10), 22.60 (C-49), 23.20 (C-46), 25.18 (C-8), 25.80 (C-30, C-41), 26.40 (C-12), 26.62 (C-13), 26.64 (C-14), 27.28 (C-15), 30.02 (C-31, C-42), 32.04 (C-7), 33.42 (C-45), 36.52 (C-3), 38.18 (C-9), 42.06 (C-8), 42.82 (C-1), 45.17 (C-5), 47.68 (C-17), 48.80 (C-4), 51.22 (C-24), 51.76 (C-35), 52.44 (C-2), 54.10 (C-25, C-36), 56.70 (C-11), 61.80 (C-47), 62.05 (C-32, C-43), 78.48 (C-28, C-39), 87.28 (C-27, C-38), 133.52 (C-19), 150.58 (C-18), 162.68 (C-22), 169.28 (C-16) ppm. EI-MS *m/z* 674.51 Anal. Calcd. for $C_{42}H_{66}N_4O_3$: C, 74.73; H, 9.86; N, 8.30; O, 7.11. Found: C, 74.64; H, 9.78.

6-(2-{1-[8-(*tert*-Butyl-dimethyl-silanyloxy)-10-(4-hydroxy-butyl)-3a,5b-dimethyl-1,2,3, 3a,4,5,5a,5b, 6,7,8,9,11a,12, 12a, 12b-hexadecahydro-cyclobuta[*k*]cyclo penta[*a*]phenan thren-3-yl]-ethylideneamino}-ethylamino)-hex-5-yn-1-ol (12)

yielding 64 % of product, m.p. 82-84 °C; IR (V_{max}, cm^{-1}): 3400, 3332 and 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.88 (s, 3H), 0.90 (s, 9H), 0.98 (s, 3H), 1.16-1.42 (m, 7H), 1.50 (m, 2H), 1.51 (m, 1H), 1.52 (m, 2H), 1.53 (m, 1H), 1.58 (m, 2H), 1.59 (m, 1H), 1.64 (m, 2H), 1.59 (m, 1H), 1.64 (m, 2H), 1.66 (m, 1H), 1.68 (s, 3H), 1.70-1.96 (m, 5H), 2.00 (m, 2H), 2.14-2.28 (m, 4H), 2.30 (m, 2H), 2.40 (m, 1H), 3.18 (m, 2H), 3.50 (m, 1H), 3.54 (m, 2H), 3.58 (broad, 3H), 3.66 (m, 2H), 3.67 (m, 2H), 5.40 (d, 1H, J = 1.32 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.56 (C-22, C-42), 13.20 (C-24), 16.20 (C-32), 16.90 (C-23), 18.44 (C-43), 21.68 (C-10), 22.62 (C-45), 24.90 (C-38), 25.82 (C-33), 26.10 (C-44, C-46, C-47), 26.40 (C-16), 26.60 (C-15), 28.50 (C-2), 30.10 (C-34), 32.54 (C-39), 33.30 (C-14), 33.80 (C-6), 35.00 (C-3), 35.14 (C-11), 35.57 (C-37), 38.18 (C-9), 38.64 (C-4), 42.82 (C-8), 43.15 (C-13), 44.80 (C-5), 51.12 (C-12), 51.24 (C-27), 53.60 (C-7), 54.16 (C-28), 56.70 (C-17), 62.08 (C-35), 62.50 (C-40), 71.52 (C-1), 78.44 (C-31), 87.32 (C-30), 133.84 (C-19), 153.88

(C-18), 162.70 (C-25) ppm. EI-MS *m/z* 666.51 Anal. Calcd. for $C_{41}H_{70}N_2O_3Si$: C, 73.82; H, 10.58; N, 4.20; O, 7.20; Si, 4.21. Found: C, 73.74; H, 10.44.

2.5. Preparation of cyclobuta-ynal-steroid derivatives. A solution of **10** or **11 or 12** (0.50 mmol), in 5 ml of dimethylsulfoxyde was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, 5 ml of water was added and stirring for 72 h to room temperature. The residue was purified by crystallization from hexane:methanol:water (1:4:2).

7-{2-[8-(*tert*-Butyl-dimethyl-silanyloxy)-5a,7a-dimethyl-2-(4-oxo-butyl)-4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-tetradecahydro-2aH-cyclobuta[*j*]cyclopenta[*a*]phenanthren-3-ylidene amino]-ethylamino}-hept-6-ynal (13)

yielding 44 % of product, m.p. 50-52 °C; IR (V_{max}, cm^{-1}): 3332 and 1058; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.69 (s, 3H), 0.84 (s, 9H), 0.92 (m, 1H), 0.98 (s, 3H), 1.02-1.62 (11H), 1.70 (m, 2H), 1.78-1.82 (m, 3H), 1.84 (m, 2H), 1.86 (m, 1H), 1.94 (m, 2H), 2.20-2.22 (m, 2H), 2.24 (m, 2H), 2.26-2.30 (m, 4H), 2.40 (m, 1H), 2.56 (m, 2H), 3.20 (t, 2H, J = 13.34 Hz), 3.52 (m, 1H), 3.56 (t, 2H, J = 8.79 Hz), 5.20 (broad, 1H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.32 Hz), 9.70 (CHO), 9.82 (CO_2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-22, C-42), 11.40 (C-23), 15.60 (C-24), 16.18 (C-31), 17.80 (C-43), 21.00 (C-38), 21.30 (C-13), 22.14 (C-33), 23.54 (C-11), 25.14 (C-9), 25.70 (C-44, C-45, C-46), 26.64 (C-14), 26.94 (C-32), 27.22 (C-15), 31.02 (C-10), 32.06 (C-8), 35.50 (C-37), 36.56 (C-4), 36.70 (C-12), 42.04 (C-7), 43.30 (C-2), 43.52 (C-39), 44.60 (C-34), 45.16 (C-6), 47.40 (C-5), 49.08 (C-17), 51.72 (C-26), 51.98 (C-3), 54.14 (C-27), 79.64 (C-30), 81.66 (C-1), 87.30 (C-29), 132.38 (C-19), 147.80 (C-18), 169.34 (C-16), 202.18 (C-40), 202.44 (C-35) ppm. EI-MS *m/z:* 648.46 Anal. Calcd. for $C_{40}H_{64}N_2O_3Si$: C, 74.02; H, 9.94; N, 4.32; O, 7.40, Si, 4.33. Found: C, 74.00; H, 9.88.

7-[2-(5a,7a-Dimethyl-2-(4-oxo-butyl)-8-{1-[2-(7-oxo-hept-1-ynylamino)-ethylimino]ethyl}-4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-tetradecahydro-2aH-cyclobuta[*j*]cyclopenta[*a*]phenanthren-3-ylideneamino]-ethylamino]-hept-6-ynal (14)

yielding 64 % of product, m.p. 48-50 °C; IR (V_{max}, cm^{-1}): 3330 and 1742; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.20-1.56 (m, 10H), 1.68 (s, 3H), 1.70 (m, 4H), 1.78-1.84 (m, 7H), 1.86 (m, 4H), 1.94 (m, 1H), 1.96 (m, 2H), 2.12-2.22 (m, 3H), 2.24 (m, 4H), 2.26 (m, 2H), 2.28 (m, 1H), 2.30 (m, 2H), 2.38-2.40 (m, 2H), 2.56 (m, 4H), 3.16 (m, 4H), 3.56 (m, 4H), 5.20 (broad, 5H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.82 Hz), 9.70 (CHO), 9.82 (CO_2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-20), 15.60 (C-21), 16.12 (C-29, C-41), 21.00 (C-48), 21.92 (C-10), 22.10 (C-31, C-43), 22.60 (C-52), 25.18 (C-8), 26.40 (C-12), 26.62 (C-13), 26.64 (C-14), 26.90 (C-30, C-42), 27.28 (C-15), 32.04 (C-7), 35.54 (C-47), 36.52 (C-3), 38.18 (C-9), 42.06 (C-6), 42.82 (C-1), 43.50 (C-49), 44.62 (C-32, C-44), 45.17 (C-5), 48.80 (C-4), 49.02 (C-17), 51.22 (C-24), 51.76 (C-36), 52.44 (C-2), 54.10 (C-25, C-37), 56.70 (C-11), 79.60 (C-28, C-40), 87.28 (C-27, C-39), 132.40 (C-19), 147.80 (C-18), 162.68 (C-22), 169.28 (C-16), 202.20 (C-50), 202.42 (C-33, C-45), ppm. EI-MS *m/z* 710.51 Anal. Calcd. for $C_{45}H_{66}N_4O_3$: C, 76.01; H, 9.36; N, 7.88; O, 6.75. Found: C, 76.00; H, 9.28.

6-(2-{1-[8-(*tert*-Butyl-dimethyl-silanyloxy)-3a,5b-dimethyl-10-(5-oxo-pentyl) -1,2,3,3a, 4, 5,5a,5b,6,7,8,9, 11a,12,12a,12b-hexadecahydro-cyclobuta[*k*]cyclopenta[*a*] phenan- thren-3-yl]ethyl ideneamino}-ethylamino)hex-5-ynal (15)

yielding 64 % of product, m.p. 148-150 °C; IR (V_{max}, cm^{-1}): 3332, 1749 and 1182; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.88 (s, 3H), 0.90 (s, 9H), 0.98 (s, 3H), 1.14-1.40 (m, 7H), 1.46 (m, 2H), 1.50-1.52 (m, 2H), 1.56 (m, 2H), 1.58-1.66 (m, 2H), 1.68 (s, 3H), 1.70-1.82 (m, 4H), 1.84 (m, 2H), 1.94 (m, 1H), 1.96 (m, 2H), 2.12-2.28 (m, 4H), 2.34 (m, 2H), 2.40 (m, 1H), 2.44-2.45 (m, 4H), 3.18 (m, 2H), 3.50 (m, 1H), 3.56 (m, 2H), 5.20 (broad, 1H), 5.40 (d, 1H, J = 1.32 Hz), 9.66 (d, 1H, J = 1.90 Hz), 9.72 (d, 1H, J = 1.90 Hz)ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-22, C-43), 13.20 (C-24), 15.20 (C-32), 16.90 (C-23), 18.44 (C-44), 21.68 (C-10), 21.72 (C-33), 22.62 (C-46), 23.00 (C-39), 25.30 (C-38), 26.06 (C-45, C-47, C-48), 26.40 (C-16), 26.60 (C-15), 28.52 (C-2), 33.30 (C-14), 33.80 (C-6), 35.00 (C-3), 35.17 (C-11), 35.52 (C-37), 38.18 (C-9), 38.66 (C-4), 42.80 (C-8), 43.12 (C-34), 43.17 (C-13), 43.69 (C-40), 44.80 (C-5), 51.14 (C-12), 51.26 (C-27), 53.60 (C-7), 54.16 (C-28), 56.70 (C-17), 71.56 (C-1), 75.90 (C-31), 87.32 (C-30), 133.94 (C-19), 154.08 (C-18), 162.70 (C-25), 198.62 (C-35), 202.48 (C-41) ppm. EI-MS *m/z* 676.49 Anal. Calcd. for $C_{42}H_{68}N_2O_3Si$: C, 74.50; H, 10.12; N, 4.14; O, 7.09; Si, 4.15. Found: C, 74.42; H, 10.02.

2.6. Amination of cyclobuta-ynal-steroid derivatives. A solution of **13** or **14 or 15** (0.50 mmol), ethylenediamine (60 µl, 0.90 mmol) and boric acid (50 mg, 0.80 mmol), in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (3:1).

2-[4-(2-Amino-ethylimino)-butyl]-3-{2-[7-(2-amino-ethylimino)-hept-1-ynylamino]-ethylimino}-8-(tert-butyl-dimethyl-silanyloxy)-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[j]cyclopenta[a]phenanthrene (16).

yielding 63 % of product, m.p. 102-104 °C; IR (V_{max}, cm^{-1}): 3378, 3330 and 1058; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.69 (s, 3H), 0.84 (s, 9H), 0.92 (m, 1H), 0.98 (s, 3H), 1.04-1.56 (9H), 1.58 (m, 2H), 1.60-1.62 (m, 2H), 1.64 (m, 2H), 1.74 (m, 2H), 1.78-1.88 (m, 4H), 2.04 (m, 2H), 2.20 (m, 4H), 2.21-2.24 (m, 2H), 2.28 (m, 2H), 2.36 (m, 2H), 2.40 (m, 1H), 3.10 (m, 4H), 3.20 (t, 2H, J = 13.34 Hz), 3.52 (m, 4H), 3.53 (m, 1H). 3.56 (t, 2H, J = 8.79 Hz), 4.52 (broad, 5H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.32 Hz), 7.70 (m, 1H), 8.20 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-24, C-48), 11.40 (C-21), 15.60 (C-20), 16.38 (C-31), 17.98 (C-49), 21.30 (C-18), 23.54 (C-8), 24.92 (C-34), 25.14 (C-11), 25.22 (C-41), 25.50 (C-50, C-51, C-52), 26.64 (C-12), 26.94 (C-33), 27.22 (C-13), 27.40 (C-32), 31.02 (C-7), 31.10 (C-42), 32.06 (C-10), 36.10 (C-40), 36.56 (C-3), 36.70 (C-19), 40.54 (C-38, C-46), 42.02 (C-9), 43.30 (C-5), 45.16 (C-1), 47.40 (C-2), 49.08 (C-15), 51.62 (C-37), 51.70 (C-26), 51.98 (C-4), 54.14 (C-27), 58.00 (C-45), 79.64 (C-30), 81.66 (C-6), 87.30 (C-29), 132.38

(C-17), 152.24 (C-43), 156.18 (C-35), 156.22 (C-16), 169.34 (C-14) ppm. EI-MS *m/z:* 732.58 Anal. Calcd. for $C_{44}H_{76}N_6OSi$: C, 72.08; H, 10.45; N, 11.46; O, 2.18, Si, 3.83. Found: C, 72.00; H, 10.34.

(Z)-7-((2-Aminoethyl)imino)-N-(2-(((Z)-1-((5aR,7aS,(S,E)-2-(E)-4-((2-aminoethyl)-imino)butyl)-3-[2-(((Z)-7-((2-amino-ethyl)imino)hept-1-yn-1-yl)amino)ethyl)imino)-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[j]cyclopenta[a]phenanthren-8-yl)ethylidene)amino) ethyl)hept-1-yn-1-amine (19).

yielding 44 % of product, m.p. 84-86 ^oC; IR (V_{max}, cm^{-1}): 3378 and 3330; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.20-1.56 (m, 10H), 1.58 (m, 4H), 1.66 (m, 4H), 1.68 (s, 3H), 1.74 (m, 2H), 1.78-1.94 (m, 4H), 2.06 (m, 2H), 2.12 (m, 1H), 2.22 (m, 4H), 2.21-2.28 (m, 3H), 2.30 (m, 2H), 2.36 (m, 4H), 2.39-2.40 (m, 2H), 3.10 (m, 6H), 3.16 (m, 4H), 3.52 (m, 4H), 3.56 (m, 4H), 4.60 (broad, 8H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.82 Hz), 7.70 (m, 2H), 8.20 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-21), 15.60 (C-20), 16.42 (C-28, C-52), 21.92 (C-7), 22.60 (C-61), 24.90 (C-31, C-55), 25.18 (C-10), 25.22 (C-17, C-38), 26.40 (C-18), 26.62 (C-11), 27.00 (C-30, C-54), 27.28 (C-12), 27.44 (C-29, C-53), 31.10 (C-39), 32.04 (C-9), 36.14 (C-37), 36.52 (C-3), 38.28 (C-6), 40.56 (C-35, C-43, C-59), 42.02 (C-8), 42.82 (C-5), 45.20 (C-1), 48.80 (C-2), 49.02 (C-14), 51.22 (C-47), 51.60 (C-34, C-58), 51.76 (C-23), 54.10 (C-24, C-48), 56.20 (C-4), 56.70 (C-19), 58.00 (C-42), 79.60 (C-27, C-51), 87.28 (C-26, C-50), 132.40 (C-16), 152.26 (C-40), 156.20 (C-32, C-56), 156.28 (C-15), 162.68 (C-45), 169.28 (C-13) ppm. EI-MS *m/z* 836.68 Anal. Calcd. for $C_{51}H_{84}N_{10}$: C, 73.16; H, 10.11; N, 16.73. Found: C, 73.07; H, 10.04.

(E)-6-((2-Aminoethyl)imino)-N-(2-(((Z)-1-((3R,3aS,5bR,8S)-10-((E)-5-((2-aminoethyl)-imino)pentyl)-8-(((tert-butyldimethylsilyl)oxy)-3a,5b-dimethyl-1,2,3,3a4,5,5a,5b,6,7,8,9,11a,12,12a,12b-hexadecahydrocyclobuta[k]cyclopenta[a]phenanthren-3-yl) ethylidene)amino)ethyl)hex-1-yn-1-amine (21).

yielding 45 % of product, m.p. 142-144 ^oC; IR (V_{max}, cm^{-1}): 3378, 3330 and 1058; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.88 (s, 3H), 0.90 (s, 9H), 0.98 (s, 3H), 1.14-1.38 (m, 6H), 1.40 (m, 2H), 1.41-1.66 (m, 5H), 1.68 (s, 3H), 1.70 (m, 1H), 1.76 (m, 2H), 1.78-1.84 (m, 3H), 1.90 (m, 2H), 1.94 (m, 1H), 2.06-2.10 (m, 4H), 2.12 (m, 2H), 2.14-2.40 (m, 5H), 2.42 (m, 2H), 3.10 (m, 4H), 3.18 (m, 2H), 3.52 (m, 4H), 3.54 (m, 1H), 3.56 (m, 2H), 4.52 (broad, 5H), 5.40 (d, 1H, J = 1.32 Hz), 8.10 ((m, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.60 (C-24, C-49), 13.20 (C-21), 16.80 (C-32), 16.90 (C-20), 18.44 (C-50), 21.68 (C-7), 22.62 (C-52), 25.30 (C-15), 26.06 (C-51, C-53, C-54), 26.40 (C-16), 26.80 (C-41), 27.46 (C-33), 27.82 (C-42), 28.50 (C-9), 30.30 (C-34), 30.40 (C-43), 33.30 (C-14), 33.80 (C-3), 35.00 (C-8), 35.17 (C-11), 35.82 (C-40), 38.28 (C-6), 38.66 (C-1), 40.50 (C-38, C-47), 42.80 (C-5), 43.17 (C-13), 44.80 (C-2), 51.14 (C-12), 51.26 (C-27), 54.16 (C-28), 56.70 (C-17), 57.34 (C-4), 58.00 (C-37, C-46), 71.56 (C-10), 86.46 (C-31), 87.32 (C-30), 133.94 (C-19), 154.08 (C-18), 154.50 (C-35, C-44), 162.70 (C-25) ppm. EI-MS *m/z* 760.61 Anal. Calcd. for $C_{46}H_{80}N_6OSi$: C, 72.58; H, 10.59; N, 11.04; O, 2.10; Si, 3.69. Found: C, 72.42; H, 10.42.

2.7. Removal of the tert-butyldimethylsilylane fragment of 16 or 21. A solution of **16** or **21** (0.50 mmol) in 5 ml of hydrofluoric acid was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (3:1).

2-[4-(2-Amino-ethylimino)butyl]-3-{2-[7-(2-amino-ethylimino)-hept-1-ynylamino]-ethylimino}-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[j]cyclo-penta[a]phenanthren-8-ol (17).

yielding 55 % of product, m.p. 100-102 ^oC; IR (V_{max}, cm^{-1}): 3400, 3380 and 3330; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.84 (s, 3H), 0.94 (m, 1H), 0.98 (s, 3H), 1.10-1.56 (10H), 1.58 (m, 2H), 1.62 (m, 1H), 1.66 (m, 2H), 1.74 (m, 2H), 1.78-1.98 (m, 4H), 2.04 (m, 2H), 2.20 (m, 2H), 2.21-2.24 (m, 2H), 2.30 (m, 2H), 2.36 (m, 2H), 2.40 (m, 1H), 3.10 (m, 4H), 3.20 (t, 2H, J = 13.34 Hz), 3.52 (m, 4H), 3.56 (t, 2H, J = 8.79 Hz), 3.64 (m, 1H), 4.80 (broad, 6H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.32 Hz), 7.70 (m, 1H), 8.20 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 11.80 (C-21), 15.60 (C-20), 16.38 (C-29), 21.30 (C-18), 23.74 (C-8), 24.92 (C-32), 25.14 (C-11), 25.22 (C-39), 26.64 (C-12), 26.94 (C-31), 27.22 (C-13), 27.40 (C-30), 30.72 (C-7), 31.10 (C-40), 32.06 (C-10), 36.10 (C-38), 36.56 (C-3), 37.30 (C-19), 40.54 (C-36, C-44), 42.02 (C-9), 44.00 (C-5), 45.16 (C-1), 47.40 (C-2), 49.08 (C-15), 51.12 (C-4), 51.62 (C-35), 51.78 (C-24), 54.14 (C-25), 58.00 (C-43), 79.64 (C-28), 81.80 (C-6), 87.30 (C-27), 132.38 (C-17), 152.24 (C-41), 156.18 (C-33), 156.22 (C-16), 169.34 (C-14) ppm. EI-MS *m/z:* 618.49 Anal. Calcd. for $C_{38}H_{62}N_6O$: C, 73.74; H, 10.10; N, 13.58; O, 2.58. Found: C, 73.66; H, 10.04.

3-(1-{2-[6-(2-Aminoethylimino)-hex-1-ynylamino]ethylimino}-ethyl)-10-[5-(2-amino-ethylimino)-penthyl]-3a,5b-dimethyl-1,2,3,3a4,5,5a,5b,6,7,8,9,11a,12,12a,12b-hexadecahydrocyclo-buta[k]cyclopenta[*a*]phenanthren-8-ol (22).

yielding 38 % of product, m.p. 122-124 ^oC; IR (V_{max}, cm^{-1}): 3402 and 33323; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.14-1.38 (m, 6H), 1.40 (m, 2H), 1.42-1.66 (m, 5H), 1.68 (s, 3H), 1.70-1.74 (m, 2H), 1.76 (m, 2H), 1.78-1.84 (m, 2H), 1.90 (m, 2H), 1.94 (m, 1H), 2.06-2.08 (m, 4H), 2.12 (m, 2H), 2.14-2.40 (m, 5H), 2.42 (m, 2H), 3.10 (m, 4H), 3.18 (m, 2H), 3.52 (m, 4H), 3.56 (m, 2H), 3.80 (m, 1H), 4.24 (broad, 6H), 6.00 (d, 1H, J = 1.00 Hz), 7.70 (m, 1H), 8.10 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-21), 16.80 (C-30), 16.90 (C-20), 21.68 (C-47), 22.62 (C-47), 24.00 (C-41), 25.30 (C-15), 26.40 (C-16), 26.80 (C-39), 27.46 (C-31), 27.82 (C-40), 30.30 (C-32), 30.90 (C-9), 33.33 (C-14), 33.66 (C-8), 33.80 (C-3), 34.80 (C-11), 35.82 (C-38), 38.28 (C-6), 38.66 (C-1), 40.50 (C-36, C-45), 42.80 (C-5), 43.17 (C-13), 44.80 (C-2), 51.24 (C-25), 51.26 (C-12), 51.62 (C-44), 54.16 (C-26), 56.70 (C-17), 57.34 (C-4), 58.00 (C-35), 69.70 (C-10), 86.46 (C-29), 87.32 (C-28), 133.94 (C-19), 154.08 (C-18), 154.50 (C-33), 156.20 (C-42), 162.70 (C-23) ppm. EI-MS *m/z* 646.52 Anal. Calcd. for $C_{40}H_{66}N_6O$: C, 74.26; H, 10.28; N, 12.99; O, 2.47. Found: C, 74.13; H, 10.14.

2.8 Preparation of 1,4-diazacycloundeca-5,11-dien-steroid derivatives

A solution of **17** or **19 or 22** (0.50 mmol), Copper(II) chloride anhydrous (70 mg, 0.52 mmol), in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (3:1).

2-[4-(2-Amino-ethylimino)butyl]-3-[2-(1,4-diaza-cycloundeca-5,11-dien-5-ylamino)-ethylimino]-5a,7a-dimethyl-2a,3,4,5,5a, 5b,6,7,7a,8,9,10,10a,10b,11,12-hexadecahydro-cyclobuta[j]cyclopenta[a]phenanthren-8-ol (18).

yielding 55 % of product, m.p. 116-118 ^{o}C; IR (V_{max}, cm^{-1}):3402, 3380 and 3330; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.84 (s, 3H), 0.94 (m, 1H), 0.98 (s, 3H), 1.10-1.64 (11H), 1.74 (m, 2H), 1.78-1.82 (m, 2H), 1.84 (m, 2H), 1.86 (m, 1H), 1.88 (m, 2H), 1.98 (m, 1H), 2.04 (m, 2H), 2.06 (m, 2H), 2.21-2.24 (m, 2H), 2.30 (m, 2H), 2.32 (m, 2H), 2.40 (m, 1H), 3.10 (m, 2H), 3.12 (m, 2H), 3.48 (m, 2H), 3.52-3.58 (m, 4H), 3.64 (m, 1H), 3.90 (m, 2H), 4.44 (d, 1H, J = 0.78 Hz), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.32 Hz), 5.50 (broad, 5H), 6.70 (m, 1H), 8.20 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 11.80 (C-36), 15.60 (C-35), 21.30 (C-33), 23.74 (C-26), 25.14 (C-32), 25.22 (C-39), 26.64 (C-28), 27.22 (C-7), 27.25 (C-27), 28.88 (C-8), 30.72 (C-25), 31.10 (C-40), 32.06 (C-31), 32.70 (C-10), 34.12 (C-9), 36.10 (C-38), 36.56 (C-21), 37.30 (C-34), 40.54 (C-44), 42.02 (C-18), 43.60 (C-3), 44.00 (C-23), 45.16 (C-19), 45.87 (C-13), 47.40 (C-20), 49.08 (C-17), 51.12 (C-22), 52.72 (C-14), 58.00 (C-43), 61.27 (C-2), 73.34 (C-6), 81.80 (C-24), 132.38 (C-30), 149.60 (C-5), 152.24 (C-41), 156.28 (C-29), 159.88 (C-11), 169.34 (C-16) ppm. EI-MS *m/z:* 618.49 Anal. Calcd. for $C_{38}H_{62}N_6O$: C, 73.74; H, 10.10; N, 13.58; O, 2.58. Found: C, 73.62; H, 10.02.

(1Z,5E)-N-(2-(((Z)-1-((5aR,7aS,8S,E)-3-((2-(((1E,5Z)-1,4-diaza cycloundeca-5,11-dien-5-yl)amino)ethyl)imino)-2-(E)-4-((2-aminoethyl)imino)butyl)-5a,7a-dimethyl-2a,3,4,5,5a,5b,6,7,7a,8,9, 10,10a,10b,11,12-hexadecahydro-cyclobuta[j]cyclopenta[a] phenanthren-8-yl)ethylidene)amino)ethyl)-1,4-diazacycloundeca 5,11-dien-5-amine (20).

yielding 66 % of product, m.p. 62-64 ^{o}C; IR (V_{max}, cm^{-1}): 2402, 3376 and 3330; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.20-1.58 (m, 10H), 1.68 (s, 3H), 1.74 (m, 2H), 1.78-1.82 (m, 3H), 1.86 (m, 4H), 1.88 (m, 4H), 1.94 (m, 1H) 2.04 (m, 2H), 2.06 (m, 4H), 2.12-2.28 (m, 4H), 2.29 (m, 2H), 2.30 (m, 4H), 2.39-2.40 (m, 2H), 2.80 (m, 2H), 3.10 (m, 2H), 3.14 (m, 4H), 3.46 (m, 4H), 3.52 (m, 2H), 3.56-3.58 (m, 4H), 3.90 (m, 2H), 3.96-4.42 (m, 2H), 5.22 (m, 1H), 5.40 (d, 1H, J = 1.82 Hz), 5.60 (broad, 6H), 6.70 (m, 2H), 8.20 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-36), 15.60 (C-35), 21.92 (C-25), 22.60 (C-61), 25.18 (C-31), 25.22 (C-32), 25.24 (C-38), 26.30 (C-56), 26.40 (C-33), 26.62 (C-27), 26.64 (C-27), 27.20 (C-7, C-59), 27.28 (C-26), 28.84 (C-8, C-8, 31.10 (C-39), 32.04 (C-30), 32.70 (C-10), 34.16 (C-9, C-57), 36.14 (C-37), 36.52 (C-21), 38.28 (C-28), 40.56 (C-43), 42.02 (C-18), 42.82 (C-23), 43.60 (C-3, C-52), 45.20 (C-19), 45.86 (C-13, C-48), 48.80 (C-20), 49.02 (C-17), 52.22 (C-47), 52.76 (C-14), 54.90 (C-53), 56.20 (C-22), 56.70 (C-34), 58.00 (C-42), 61.26 (C-2), 73.40 (C-6, C-60), (C-18), 132.40 (C-29), 149.60 (C-5, C-50), 152.26 (C-40), 156.20 (C-28), 159.90 (C-11, C-55), 162.68 (C-45), 169.28 (C-16) ppm. EI-MS *m/z* 836.68 Anal. Calcd. for $C_{51}H_{84}N_{10}$: C, 73.16; H, 10.11; N, 16.73. Found: C, 73.08; H, 10.02.

10-[5-(2-Aminoethylimino)-penthyl]-3-{1-[2-(1,4-diaza-cycloundeca-5,11-dien-5-ylamino)-ethylimino]-ethyl}-3a,5b-dimethyl-1,2,3,3a4,5,5a,5b,6,7,8,9,11a,12,12a,12b-hexadecahydrocyclobuta[k]cyclopenta[*a*]phenanthren-8-ol (23).

yielding 64 % of product, m.p. 150-152 ^{o}C; IR (V_{max}, cm^{-1}): 3380 and 3332; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (s, 3H), 0.98 (s, 3H), 1.14-1.38 (m, 6H), 1.40 (m, 2H), 1.42-1.66 (m, 5H), 1.68 (s, 3H), 1.70-1.84 (m, 4H), 1.86-1.88 (m, 4H), 1.90 (m, 2H), 1.94 (m, 1H), 2.04 (m, 2H), 2.06-2.10 (m, 4H), 2.12-2.28 (m, 4H), 2.30 (m, 2H), 2.40 (m, 1H), 2.78 (m, 2H), 3.10 (m, 2H), 3.12 (m, 2H), 3.48 (m, 2H), 3.52 (m, 2H), 3.56 (m, 2H), 3.80 (m, 1H), 3.96 (m, 1H), 4.80 (broad, 5H), 5.40 (d, 1H, J = 0.70 Hz), 6.70 (m, 1H), 8.10 (m, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.20 (C-37), 16.90 (C-38), 21.68 (C-33), 22.62 (C-36), 25.30 (C-31), 26.30 (C-10), 26.40 (C-30), 26.80 (C-41), 27.20 (C-7), 27.82 (C-42), 28.88 (C-8), 30.40 (C-43), 30.90 (C-24), 33.33 (C-29), 33.66 (C-23), 33.80 (C-9), 34.12 (C-9), 34.80 (C-26), 35.82 (C-40), 38.28 (C-32), 38.66 (C-22), 40.50 (C-47), 42.80 (C-47), 42.80 (C-18), 43.17 (C-28), 43.60 (C-3), 44.80 (C-21), 45.82 (C-13), 51.24 (C-27), 52.26 (C-14), 54.88 (C-2), 56.70 (C-17), 57.34 (C-19), 58.00 (C-46), 69.70 (C-25), 73.40 (C-6), 133.94 (C-35), 149.60 (C-5), 154.08 (C-34), 154.50 (C-44), 159.90 (C-11), 162.70 (C-16) ppm. EI-MS *m/z* 646.52 Anal. Calcd. for $C_{41}H_{68}N_6O$: C, 74.26; H, 10.37; N, 12.71; O, 2.42. Found: C, 74.14; H, 10.24.

2.9. Antimicrobial activity. The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by a previously method described [21]. The bacterial species were incubated on brain/heart Infusion (*Streptococcus pneumoniae*) and *Staphylococcus* 110 (*Staphylococcus aureus*) agars for 24 h at 37 ^{O}C. After such time, it was be determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first of which contained 2 ml of culture medium (tripticase soye) at double concentration and the remainder (11tubes), contained the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 ml of the studied compound (1 mg/ml) was added and stirred, from this tube an aliquot of 2 ml was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 ml of dissolution had been used up. After this process, each tube was inoculated with 0.1 ml of the bacterial suspension, whose concentration corresponded to Mc-Farland scale (9×10^8 cells/ml) and all the tubes were incubated at 37 ^{o}C for 24 h. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms, and were incubated for 24 h at 37 ^{o}C. After such time, the minimum inhibitory concentration (MIC) was evaluated to consider the antimicrobial effect of the different compounds. In order to discard the effect of methanol (solvent) on the bacterial species studied, a series of the same number of tubes

was prepared in parallel, to which 2 ml of methanol at 60% was added to the first and corresponding successive dilutions were added in the same way as before. In addition a control series was also performed using distilled water to pH 7.0.

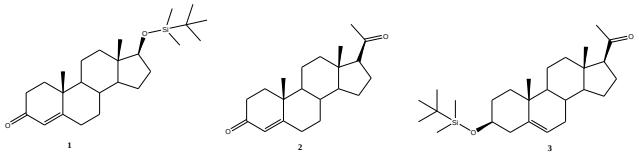
2.10. Docking Server. Docking calculations were carried out using Docking Server [22]. The MMFF94 force field [23] was used for energy minimization of ligand molecule using the Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on the HERD2 [24] and PARP [25] protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [26]. Affinity (grid) maps of 20 × 20 × 20-Å grid points and 0.375-Å spacing were generated using the Autogrid program [26]. AutoDock parameter set and distance dependent dielectric functions were used in the calculation of the Van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [27]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2Å and quaternion and torsion steps of 5 were applied.

2.11. Statistical analysis. The obtained values are expressed as average ± SE, using each heart (n = 9) as its own control. The data obtained were put under Analysis of Variance (ANOVA) with the Bonferroni correction factor [28] using the SPSS 12.0 program. The differences were considered significant when *p* was equal or smaller than 0.05.

3. RESULTS SECTION

There are reports which indicate the preparation of diazahicyclo derivatives as antibacterial agents; nevertheless, expensive reagents and special conditions are required [9-17]. Therefore, in this study three diazahicyclo-steroid derivatives were synthetized using several strategies to evaluate the biological activity against *Staphylococcus aureus and Streptococcus pneumoniae*

3.1. Preparation of three diazecin-steroid-hexahydroazocin derivatives. In this study several straightforward routes are reported for synthesis of three diazahicyclo-steroid derivatives using OTBS-testosterone (**1**), progesterone (**2**) and OTBS-pregnenolone (**3**) as chemical tools (Scheme 1). The first stage was achieved by the synthesis of three cyclobutane-steroid derivatives (**4** or **5** or **6,** Scheme 2); it is important to mention that there are several reports to preparation of cyclobutene rings using some reagents such as $Co(PPh_3)_2I_2/PPh_3/Zn$ [29], rodhium [30], nikel [31], ruthenium [32] and others.



Scheme 1. Chemical structure of OTBS-Testosterone (**1**), Progesterone (**2**) and OTBS-pregnenolone (**3**).

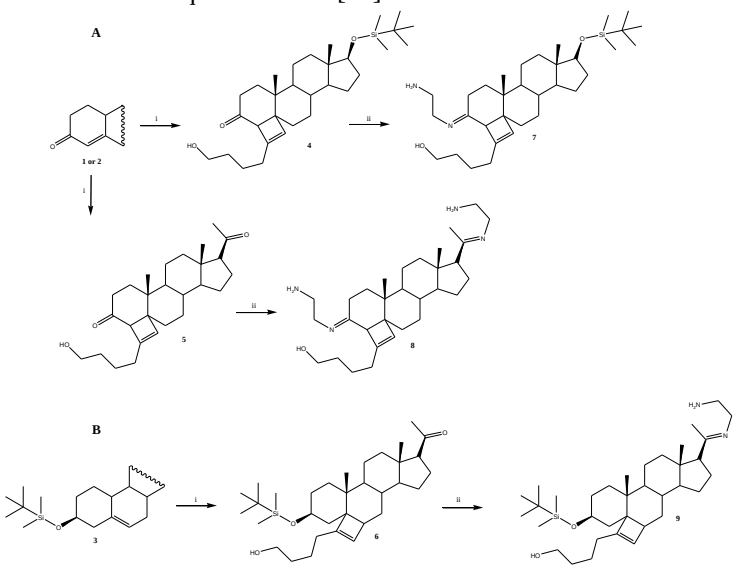
In this study, the compounds **1**, **2** and **3** were reacted with 5-hexyn-1-ol using Cooper II chloride as catalyst to form **4** or **5** or **6**. The ^{1}H NMR spectrum of **4** shows signals at 0.08 and 0.86 ppm for *ter*-butyldimethylsylane fragment; at 0.68 and 1.08 for methyl groups bound to steroid nucleus; at 0.94-1.04, 1.20-1.48, 1.62-2.10 and 2.22-3.54 ppm for steroid moiety; at 1.52-1.60, 2.20 and 3.66 ppm for methylene groups involved in the arm bound to A-ring; at 5.70 ppm for cyclobutane. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.02 and 25.50 ppm for *ter*-butyldimethylsylane fragment; at 11.32-14.80 ppm for methyl groups bound to steroid nucleus; at 21.34-23.52, 25.24, 31.06-32.44, 36.54-36.70, 38.50-60.16 and 81.62 ppm for steroid moiety; at 24.30, 32.54, 37.02 and 62.5 ppm for methylene groups involved in the arm bound to A-ring; at 134.52-147.68 ppm for cyclobutane ring; at 210.62 ppm for ketone group. Finally, the presence of **4** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 500.36.

On the other hand, the ^{1}H NMR spectrum of **5** showed signals at 0.70-1.06 for methyl groups bound to steroid nucleus; at 2.12 for methyl group bound to ketone; at 1.20-1.46, 1.66-2.10 and 2.22-3.00 ppm for steroid moiety; at 1.50-1.58, 2.20 and 3.66 ppm for methylene groups involved in the arm bound to A-ring; at 3.60 ppm for hydroxyl group; at 5.70 ppm for cyclobutane ring. The ^{13}C NMR spectra showed chemical shifts at 13.40-14.80 ppm for methyl groups bound to steroid nucleus; at 30.74 ppm for methyl group bound to ketone; at 21.52-23.02, 24.44-25.24, 31.82-32.44, 35.64, 38.88-60.16 and 63.98 ppm for steroid moiety; at 24.30, 32.54, 37.02 and 62.55 ppm for methylene groups involved in the arm bound to A-ring; at 134.52-147.68 ppm for cyclobutane ring; at 208.28-210.62 ppm for ketone groups. In addition, the presence of **5** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 412.29.

Finally, the ^{1}H NMR spectrum of **6** shows signals at 0.06 and 0.90 ppm for *ter*-butyldimethylsylane fragment; at 0.70 and 0.98 ppm for methyl groups bound to steroid nucleus; at 2.12 ppm for methyl group bound to ketone; at 1.14-1.46, 1.56-2.00 and 2.58-3.50 ppm for steroid moiety; at 1.501.52, 2.02 and 3.66 ppm for methylene groups involved in the arm bound to A-ring; at 3.60 ppm for hydroxyl group at 5.40 for cyclobutane ring. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.50 and 26.10 ppm for *ter*-butyldimethylsylane fragment; at 13.70-16.90 ppm for methyl groups bound to steroid nucleus; at 28.34 ppm for methyl group bound to ketone; at 21.22-24.20, 28.52, 32.88-35.14, 36.68-56.42 and 63.78 ppm for steroid moiety; at 24.90 and 32.54, 35.62, 62.55 and 71.56 ppm for methylene groups involved in the arm bound to A-ring; at 133.92-153.90 ppm for cyclobutane ring; at 208.58 ppm for ketone group. Finally, the presence of **6** was

further confirmed from mass spectrum which showed a molecular ion at m/z*:* 528.39.

3.2. Preparation of Cyclobutene-ol-steroid-amino conjugates. The following stage was achieved by preparation of imino groups involved in the compounds **7** or **8** or **9 (**Scheme 2). It is important to mention, that there are several procedures for the synthesis of imino groups which are described in the literature [33, 34]. In this study the compounds **7** or **8** or **9** were synthesized (Figure 3) by the reaction of **3** or **5** or **6** with ethylenediamine using boric acid as catalyst, because it is not an expensive reagent and no special conditions are required for use [35].



Scheme 2. Preparation of cyclobutene-1-ol-steroid-amino conjugates (**7** or **8** or **9**). Reaction of **1** or **2** or **3** with 5-hexyn-1-ol/Cooper(II) chloride (**i**) the cyclobuta-3-one-steroid derivatives (**4** or **5** or **6**). After, **4** or **5** or **6** were reacted with nediamine/boric acid (**ii**) to form **7** or **8** or **9**.

The ^{1}H NMR spectrum of **7** showed signals at 0.08 and 0.88 ppm for *ter*-butyldimethylsylane fragment; at 0.68 and 0.98 for methyl groups bound to steroid nucleus; at 0.94, 1.06-1.48, 1.56-1.60, 1.64-1.88, 2.22-2.42, 3.54 and 5.20 ppm for steroid moiety; at 1.50, 1.62, 2.15 and 3.66 ppm for methylene groups of arm bound to cylobutene ring; at 3.10-3.50 ppm for methylene groups involved in the arm bound to A-ring of steroid; at 4.10 ppm for both hydroxyl and amino groups; at 5.40 for cyclobutane ring. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.50 and 25.50 ppm for *ter*-butyldimethylsylane fragment; at 11.34-15.60 ppm for methyl groups bound to steroid nucleus; at 21.32-23.52, 25.14, 26.64.32.02,36.54-36.70, 42.04-52.00 and 81.70 ppm for steroid nucleus; at 24.38, 32.54-36.50 and 62.55 ppm for methylene groups of arm bound to cyclobutane ring; at 41.00 and 53.60 ppm for methylene groups involved in the arm bound to A-ring of steroid; at 132.34-150.38 for cyclobutane ring; at 169.34 ppm for imino group. In addition, the presence of **7** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 542.42.

On the other hand, the ^{1}H NMR spectrum of **8** showed signals at 0.88-0.98 ppm for methyl groups bound to steroid nucleus; at 1.80 ppm for methyl group bound to imino; at 1.20-1.44, 1.52-1.58, 1.76, 1.82-2.12, 2.22-2.40 and 5.20 ppm for steroid moiety; at 1.50, 1.62, 2.18 and 3.66 ppm for methylene groups of arm bound to cyclobutane ring; at 3.09 and 3.52 ppm for methylene groups bound to both amino and imino groups; at 3.10-3.50 ppm for methylene groups involved in the arm bound to A-ring of steroid; at 4.20 ppm for both hydroxyl and amino groups; at 5.40 ppm for cyclobutane ring. The ^{13}C NMR spectra showed chemical shifts at 13.20-15.60 ppm for methyl groups; at 16.70 ppm for methyl group bound to imino; at 21.92-25.18-32.04, 36.56-38.28, 42.04-49.06, 56.22 and 63.08 ppm; at 24.40, 32.54-36.52 and 62.55 ppm for methylene groups of arm bound to cyclobutane ring; at 41.00 and 53.60 ppm for methylene groups involved in the arm bound to A-ring of steroid; at 41.02 and 53.10 ppm for methylene groups bound to both amino and imino groups; at 132.32-150.38 ppm for cyclobutane ring; at 156.78-169.28 ppm for imino group. In addition, the presence of **8** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 496.41.

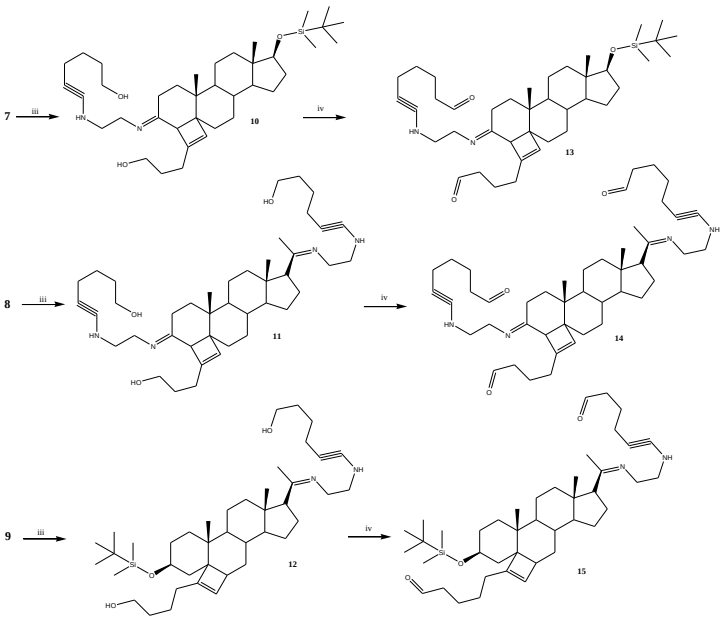
Finally, other results showed several signals involved in the ^{1}H NMR spectrum for **9** at; 0.07 and 0.90 ppm for *ter*-butyldimethylsylane fragment; at 0.88 and 0.98 ppm for methyl groups bound to steroid nucleus; at 1.80 ppm for methyl group bound to imino; at 1.14-1.40, 1.51, 1.53-1.78, 1.82-1.94, 2.12-2.40 and 3.53 ppm for steroid moiety; at 1.50, 1.52 and 2.00 ppm for methylene groups of arm bound to cyclobutane ring; at 3.08-3.52 and 3.66 ppm for methylene groups involved in the arm bound to A-ring of steroid; at 3.08-3.52 and 3.66 ppm for methylene groups bound to both imino and amino groups; at 4.10 ppm for both hydroxyl and amino groups; at 5.40 ppm for cyclobutane ring. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.44 and 26.04 ppm for *ter*-butyldimethylsylane fragment; at 13.20 and 16.90 ppm for methyl groups bound to steroid nucleus; at 16.70 ppm for methyl group bound to imino; at 21.68, 25.22, 26.40-28.52, 33.30-35.14, 38.28-38.60, 42.82-51-14, 57.36 and 63.08-71.56 ppm for steroid moiety; at 29.90, 32.54, 35.57 and 62.55 ppm for methylene groups of arm bound to cyclobutane ring; at 41.00 and 53.10 ppm for methylene groups bound to both imino and amino groups; at 133.94-153.88 ppm for cyclobutene ring; at 156.70 ppm for imino group. In addition, the presence of **9** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 570.45.

3.3. Alkynylation of amino groups. There are some studies which shown the reaction of chloro-hexyne derivatives with secondary amines; for example, the preparation of β-Alkynyl-β-amino Esters via the Mannich reaction with silyl ketene acetals and alkynyl imines using silver as catalyst [36]. Other data indicate the preparation of an indole-alkyne derivative by the reaction of 5-chloro-1-pentyne or 6-chloro-1-hexyne with indole-3-acetamide in basic medium [37]. In this investigation the compounds 7, 8 or 9 were reacted with 5-hexyn-2-ol in presence of CopperII chloride to form 10 or 11 or 12 (Scheme 3). The mechanism involves the compounds 7 or 8 via SN_2 mechanism (Fig.4 and 5). The ^{1}H NMR spectrum of 10 showed signals at 0.06 and 0.88 ppm for *ter*-butyldimethylsylane fragment; at 0.68 and 0.98 ppm for methyl groups bound to steroid nucleus; at 0.90, 1.04-1.56, 1.60-1.62, 1.78-2.22, 2.40, 3.53, and 5.60 ppm for steroid nucleus; at 1.58, 1.64, 2.30 and 3.66 ppm for methylene groups bound to both alkyne and hydroxyl groups; at 1.68, 2.26 and 3.52 ppm for methylene groups of arm bound to cyclobutene ring; at 3.20 and 3.56 ppm for methylene groups bound to both amino and imino groups; at 3.80 ppm for both hydroxyl and amino

groups; at 5.40 for cyclobutene ring. The ^{13}C NMR spectra showed chemical shifts at -4.60, 17.80 and 25.72 ppm for *ter*-butyldimethylsylane fragment; at 11.40-15.60 ppm for methyl groups bound to steroid nucleus; at 16.18, 25.80 and 30.06 ppm for methylene groups bound to both alkyne and hydroxyl groups; at 21.32, 23.54-25.14, 26.64-27.22, 31.04-32.02, 36.50-47.68, 51.98 and 81.60 ppm for steroid moiety; at 23.14, 33.40 and 61.86 for methylene groups of arm bound to cyclobutene ring; at 51.72 and 54.14 ppm for methylene groups bound to both amino and imino groups; at 78.44 and 87.30 ppm for alkyne group; at 133.52-150.58 ppm for cyclobutene ring; at 169.34 ppm for imino group. Finally, the presence of 10 was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 624.46.

On the other hand, the ^{1}H NMR spectrum of **11** showed signals at 0.88-0.98 ppm for methyl groups bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino; at 1.20-1.56, 1.78-2.22, 2.28, 2.38-2.40 and 5.60 ppm for steroid nucleus; at 1.58-1.66, 2.30 and 3.64 ppm for methylene groups bound to both hydroxyl and alkyne groups; at 1.69, 2.26 and 3.52 ppm for methylene groups involved in the arm bound to cyclobutene ring; at 3.16 and 3.56 ppm for methylene groups bound to both amino and imino groups; at 3.70 ppm for both hydroxyl and amino groups; at 5.40 for cyclobutene ring. The ^{13}C NMR spectra showed chemical shifts at 13.20-15.60 ppm for methyl groups bound to steroid nucleus; at 22.60 ppm for methyl group bound to imino; at 16.20, 25.80, 30.02 and 62.05 ppm for methylene groups bound to both hydroxyl and alkyne groups; at 21.92, 25.18, 26.40-27.28, 32.04, 36.52-48.80, 52.44 and 56.70 ppm for steroid moiety; at 23.20, 33.42 and 61.80 ppm for methylene groups involved in the arm bound to cyclobutene ring; at 51.22-51.76 and 54.10 ppm for methylene groups bound to both amino and imino groups; at 78.48-87.28 ppm for alkyne group at 133.52-150.58 ppm for cyclobutene ring; at 162.68- 169.28 ppm for imino groups. In addition, the presence of **11** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 674.51.

Finally, The ^{1}H NMR spectrum of **12** showed signals at 0.06 and 0.90 ppm for *ter*-butyldimethylsylane fragment; at 0.88 and 0.98 ppm for methyl group bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino; at 1.16, 1.70-1.96, 21.4-2.28, 2.40 and 3.50 ppm for steroid moiety; at 1.52, 2.00 and 3.66 ppm for methylene groups involved in the arm bound to cyclobutene ring; at 1.58, 1.64, 2.30 and 3.67 ppm for methylene groups bound to both hydroxyl and alkyne groups; at 3.18 and 3.54 ppm for methylene groups bound to both amino and imino groups; at 3.58 ppm for both hydroxyl and amino groups; at 5.40 ppm for cyclobutene ring. The ^{13}C NMR spectra showed chemical shifts at -4.56, 18.44 and 26.10 ppm for *ter*-butyldimethylsylane fragment; at 22.62 for methyl bound to imino group; at 13.20-16.94 ppm for methyl group bound to steroid nucleus; at 21.68, 26.40-28.50, 33.30-35.14, 38.17-51.12, 53.60,56.70 and 71.52 ppm for steroid moiety. At 16.20, 25.82, 30.10 and 62.08 ppm for methylene groups bound to both hydroxyl and alkyne groups; at 24.90, 32.54, 35.52 and 62.50 ppm for methylene groups involved in the arm bound to cyclobutene ring; at 51.24 and 56.16 ppm for methylene groups bound to both amino and imino groups; at 78.44-87.32 ppm for alkyne group; at 133.84-153.88 ppm for cyclobutene ring; at 162.70 ppm for imino group. Finally, the presence of **12** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 666.51.



Scheme 3. *Preparation of cyclobuta-ynal-steroid derivatives* (**13** or **14** or **15**). *Alkynylation 0f* **7** or **8** or **9** with 5-hexyn-1-ol/Cooper(II) chloride **(**iii) to form the alkyne-steroid derivatives **(10** or **11** or **12). After, 10** or **11** or **12** were reacted with DMSO (iv) to form **13** or **14** or **15**.

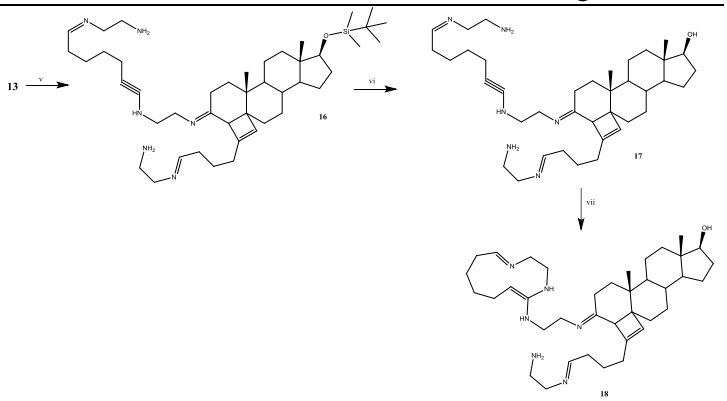
3.4. Preparation of aldehyde-steroid derivative. The sixth stage was achieved by the synthesis of a aldehyde-steroid derivatives (**13** or **14** or **15,** Scheme 3); it is noteworthy that there are several reports on the oxidation of primary alcohols to form the corresponding aldehydes. In addition, some reports indicate the preparation of aldehyde derivatives using several reagents such as morpholinium bisulfate [38], calcium hydride [39], 2-(hydroxyalky1)dithianes [40], KN(TMS)$_2$ [41], chromium(VI) [42], ruthenium [43] and others. However, some these reagents may induce risks of toxicity by generation of several substances involved on the reaction mixtures. Therefore, in this study a method previously reported[37] for oxidation of hydroxyl groups was used for synthesis of **13** or **14** or **15** by the reaction of **10** or **11** or **12** with dimethyl sulfoxide. The ^{1}H NMR spectrum of **13** shows signals at 0.08 and 0.84 ppm for *ter*-butyldimethylsylane fragment; at 0.69 and 0.98 ppm for methyl groups bound to steroid nucleus; at 0.92, 1.02-1.62, 1.78-1.82, 1.86, 2.20-2.22, 2.40, 3.54, 5.22 ppm for steroid moiety; at 1.70, 1.84, 2.24 and 2.56 ppm for methylene groups bound to both aldehyde and alkyne groups; at 1.94 and 2.26-2.30 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 3.20 and 3.56 for methylene groups bound to both amino and imino groups; at 5.20 ppm amino group; at 5.40 ppm for cyclobutene ring; at 9.70-9.82 ppm for aldehyde groups. The ^{13}C NMR spectra showed chemical shifts at -4.60, 17.80 and 25.70 ppm for *ter*-butyldimethylsylane fragment; at 11.40 and 15.60 ppm for methyl groups bound to steroid nucleus; at 16.18, 22.14, 26.94 and 44.52 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 21.30, 23.54-25.14, 26.64, 27.22-32.06, 36.56-43.30, 45.16-59.08, 51.98 and 81.66 ppm for steroid moiety; at 51.72 and 54.14 for methylene groups bound to both amino and imino groups; at 79.64 and 87.30 ppm for alkyne

group; at 132.38-147.80 for cyclobutene ring; at 169.34 ppm for imino group; at 202.18-202.44 for aldehyde groups. In addition, the presence of **13** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 666.51.

Other signals of ^{1}H NMR spectrum for **14** were found at 0.88-0.98 ppm for methyl groups bound to steroid nucleus; at 1.20-1.50, 1.78-1.84, 1.94, 2.12-2.22, 2.28, 2.38-2.40 and 5.22 ppm for steroid moiety; at 1.68 ppm for methyl group bound to imino group; at 1.70, 1.86, 2.24 and 2.56 ppm for methylene groups bound to both aldehyde and alkyne groups; at 1.96, 2.26 and 2.30 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 3.16 and 3.56 ppm for methylene groups bound to both amino and imino groups; at 5.20 ppm for amino group; at 5.40 ppm for cyclobutene group; at 9.70-9.82 ppm for aldehyde groups. The ^{13}C NMR spectra showed chemical shifts at 13.20 and 15.60 ppm for methyl groups bound to steroid nucleus; at 22.60 ppm for methyl group bound to imino; at 16.12, 22.10 and 26.90 ppm for methylene groups bound to both alkyne and aldehyde groups; at 21.00, 35.54, 43.50 and 44.62 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 21.92, 25.18-26.64, 27.28-32.04, 36.52-42.82, 45.17-49.02, 52.44 and 56.70 ppm for steroid moiety; at 51.22-51.76 and 54.10 ppm for methylene groups bound to amino and imino groups; at 79.60-87.28 ppm for alkyne group; at 132.40-147.80 ppm for cyclobutene ring; at 162.68-169.28 ppm for imino groups; at 202.20-202.42 ppm for aldehyde groups. Finally, the presence of **14** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 710.51.

Other results indicated that ^{1}H NMR spectrum of **15** shows signals at 0.06 and 0.90 ppm for *ter*-butyldimethylsylane fragment; at 0.88 and 0.98 ppm for methyl groups bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino group; at 1.14-1.40, 1.50-1.52, 1.58-1.66, 1.70-1.82, 1.94, 2.12-2.28, 2.40 and 3.50 ppm for steroid nucleus; at 1.84 and 2.44-2.45 ppm for methylene groups bound to both alkyne and aldehyde groups; at 1.46 and 1.56, 1.96 and 2.34 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 3.18 and 3.56 for methylene groups bound to both amino and imino groups; at 5.20 ppm for amino group; at 5.40 for cyclobutene ring; at 9.66-9.72 ppm for aldehyde groups. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.44 and 26.06 ppm for *ter*-butyldimethylsylane fragment; at 13.20 and 16.90 ppm for methyl groups; at 22.62 for methyl group bound to imino; at 15.20, 21.72 and 43.12 ppm for methylene groups bound to both alkyne and aldehyde groups; at 21.68, 26.40-35.17, 38.18-42.80, 43.17, 44.80-51.14, 53.60 and 56.70-71.56 ppm for steroid moiety; at 23.00-25.30, 35.52 and 43.69 ppm for methylene groups involved in the arm bound to both aldehyde and cyclobutene ring; at 51.26 and 54.16 ppm for methylene groups bound to both amino and imino groups; at 133.90-154.08 ppm for cyclobutene ring; at 162.70 ppm for imino group; at 198.62-202.48 ppm for aldehyde groups. Finally, the presence of **15** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 666.51.

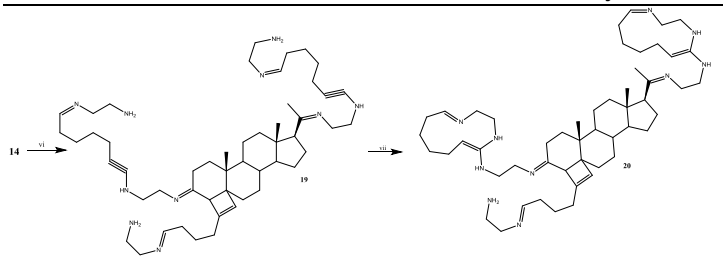
3.5. Preparation of imino-steroid derivatives (Scheme 4, 5 and 6). The compounds **13** or **14** or **15** were reacted with ethylenediamine to form the compounds **16** or **19** or **21** using boric acid as catalyst.



Scheme 4. Synthesis of a *diazacycloundeca-5,11-dien-steroid-8-ol derivative* (**18**). Reaction of **13** with ethylenediamine (v) to form an amino-steroid derivative (**16**). After, **17** was prepared by removal of the ter-buthylsylane fragment of **16** with hydrofluoric acid (v). Finally, **17** was reacted with Copper(II) chloride (vi) to form **18**.

The ^{1}H NMR spectrum of **16** showed several signals at 0.08 and 0.84 ppm for *ter*-butyldimethylsylane fragment; at 0.69 and 1.00 ppm for methyl groups bound to steroid nucleus; at 0.92, 1.04-1.56, 1.60-1.62, 1.78-1.88, 2.20-2.24, 2.40, 3.53 and 5.22 ppm for steroid moiety; at 1.58, 1.64, 2.20 and 2.36 ppm for methylene bound to both amino and alkyne groups; at 1.74, 2.04 and 2.28 ppm for methylene groups bound to both imino and cyclobutene ring; at 3.10, 3.18, 3.52 and 3.56 ppm for methylene groups bound to both imino and amino groups; at 4.52 ppm for amino groups; at 5.40 for proton of cyclobutene ring; at 7.70 and 8.20 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at -4.60, 17.98 and 25.50 for *ter*-butyldimethylsylane fragment; at 11.40-15.60 ppm for methyl groups bound to steroid nucleus; at 21.30-23.54, 25.14, 26.64, 27.22-28.30, 31.02, 32.06, 36.56-36.70, 42.02-49.08, 51.98 and 81.66 ppm for steroid moiety; at 16.38, 24.92, 29.94 and 27.40 ppm for methylene groups bound to both imino and alkyne; at 25.22, 31.10 and 36.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.54, 51.62-51.70 and 54.14-58.00 ppm for methylene bound to both imino and amino groups; 79.64 and 87.30 ppm for alkyne group; at 132.38 and 156.22 ppm for cyclobutene ring; at 152.24-156.18 and 169.34 ppm for imino groups. Finally, the presence of **16** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 732.58.

Other data showed several signals of the ^{1}H NMR spectrum for **19** at 0.87 and 0.98 ppm for methyl groups bound to steroid nucleus; at 1.74 ppm for methyl group bound to imino; at 1.20-1.56, 1.78-1.94, 2.12-2.28, 2.39-2.40 and 5.22 ppm for steroid moiety; at 1.58, 1.66, 2.20 and 2.36 ppm for methylene groups bound to both imino and alkyne; at 2.06 and 2.30 ppm for methylene groups bound to both imino and cyclobutene ring; at 3.10, 3.18, 3.52 and 3.56 ppm for methylene groups bound to both amino and imino groups; at 4.60 ppm for amino groups; at 5.40 ppm for cyclobutene ring; at 7.70-8.20 ppm for imino groups.



Scheme 5. Preparation of a *di-diazacycloundeca-5,11-dien-steroid-5-amine derivative* (**20**). Reaction of **14** with ethylenediamine (vi) to form an amino-steroid derivative (**19**). After, **19** was reacted with Copper(II) chloride (vi) to form **20**.

The ^{13}C NMR spectra showed chemical shifts at 13.20-25.60 ppm for methyl groups bound to steroid nucleus; at 22.60 for methyl group bound to imino; at 21.92, 25.18, 26.40-26.62, 27.28, 32.04, 36.52-38.28, 42.02-49.02 and 56.20-56.70 ppm for steroid moiety; at 16.42, 24.90, 27.00 and 27.44 ppm for methylene groups bound to both alkyne and imino groups; at 25.22, 31.10 and 36.14 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.56, 51.22-54.10 and 58.00 ppm for methylene groups bound to both imino and amino groups; at 79.60-87.28 for alkyne groups; at 132.40 and 156.28 for cyclobutene ring; at 152.26, 156.20, 162.68 and 169.28 ppm for imino groups. In addition, the presence of **19** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 836.68.

Finally, the ^{1}H NMR spectrum of **21** shows signals at 0.06 and 0.90 ppm for methyl groups of terbuthyldimethylsylane fragment; at 0.88 and 0.98 ppm for methyl bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino group; at 1.14-1.38, 1.41-1.66, 1.78-1.84, 1.94, 2.14-2.40 and 3.54 ppm for steroid moiety; at 1.40, 1.90 and 2.06-2.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 1.76, 2.12 and 2.42 ppm for methylene bound to both alkyne and imino groups; at 3.10-3.52 ppm for methylene groups bound to both imino and amino groups; at 4.52 ppm for cyclobutene ring; at 8.10 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at -4.60, 18.44 and 26.06 ppm for methyl groups of the terbuthyldimethylsylane fragment; at 13.20 and 16.90 ppm for methyl groups bound to steroid nucleus; at 22.62 for methyl group bound to imino; at, 26.40, 28.50, 33.30-35.17, 38.28-38.66, 42.80-51.44, 56.70-57.34 and 71.56 ppm for steroid moiety; at 16.80, 27.46 and 30.30 ppm for methylene groups bound to both alkyne and imino groups; at 26.80, 27.82, 30.40 and 35.82 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.50, 51.26-54.16 and 58.00 ppm for methylene groups bound to both amino and imino groups; at 86.46-87.32 ppm for alkyne groups; at 133.94-154.08 ppm for cyclobutene ring; at 154.50-167.70 ppm for imino groups. Finally, the presence of **21** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 760.61.

3.6. Removal of silyl fragment of 16 or 22 via hydrofluoric acid to form 17 or 21 (Scheme 4 and 5). There are several reagent for removal of silyl protecting groups from hydroxyl such as ammonium fluoride [44], tris(dimethylamino) sulfonium/difluoro-trimethylsilicate [45], hydrofluoric acid [46] and others. In this study, hydrofluoric acid was used to removal of silyl-protecting group from hydroxyl of the compound 8 to form 9 (Scheme 5). The ^{1}H NMR spectrum of 17 showed several signals at 0.84 and 0.98 ppm for methyl groups bound to steroid nucleus; at 0.94, 1.10-1.56, 1.62, 1.78-1.98, 2.21-2.24, 2.40, 3.64 and 5.22 ppm for steroid moiety; at 1.58, 1.66, 2.20 and 2.36 ppm for methylene bound to both amino and alkyne groups; at 1.74, 2.04 and 2.28 ppm for methylene groups bound to both imino and cyclobutene ring; at 3.10, 3.18 and 3.52-3.56 ppm for methylene groups bound to both imino and amino groups; at 4.80 ppm for both hydroxyl and amino groups; at 5.40 for proton of cyclobutene ring; at 7.70 and 8.20 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at 11.80-15.60 ppm for methyl groups bound to steroid nucleus; at 21.30-23.74, 25.14, 26.64, 27.22, 30.72, 32.06, 36.56-37.30, 42.02-51.12 and 81.80 ppm for steroid moiety; at 16.38, 24.92, 26.94 and 27.40 ppm for methylene groups bound to both imino and alkyne; at 25.22, 31.10 and 36.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.54, 51.62-58.00 ppm for methylene bound to both imino and amino groups; at 79.64-87.30 ppm for alkyne groups; at 132.38 and 156.22 ppm for cyclobutene ring; at 152.24-156.18 and 169.34 ppm for imino groups. Finally, the presence of 17 was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 618.49.

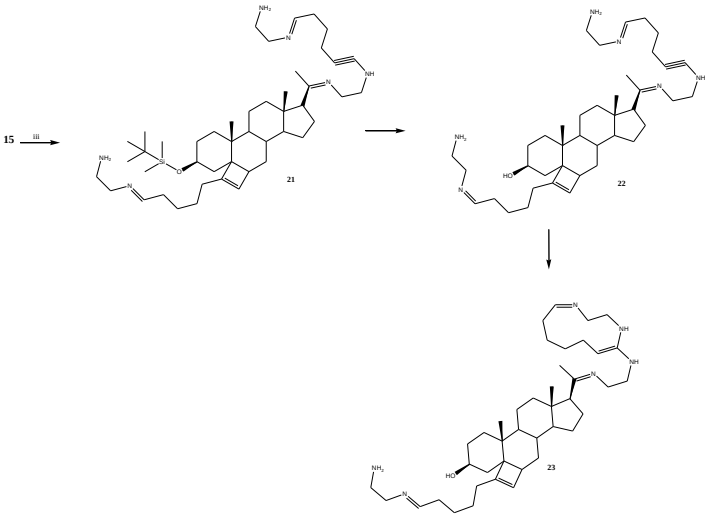
Other results showed several signals of the ^{1}H NMR spectrum for **22** at 0.88 and 0.98 ppm for methyl bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino group; at 1.14-1.38, 1.42-1.66, 1.70-1.74, 1.78-1.84, 1.94, 2.14-2.40 and 3.80 ppm for steroid moiety; at 1.40, 1.90 and 2.06-2.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 1.76, 2.12 and 2.42 ppm for methylene bound to both alkyne and imino groups; at 3.10-3.56 ppm for methylene groups bound to both imino and amino groups; at 4.24 ppm for both hydroxyl and amino groups; at 6.00 ppm for cyclobutene ring; at 7.70-8.10 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at 13.20 and 16.90 ppm for methyl groups bound to steroid nucleus; at 22.62 for methyl group bound to imino; at 21.68, 25.30-26.40, 30.90-34.80, 38.28-38.66, 42.80-44.80, 51.26, 56.70-57.34 and 69.70 ppm for steroid moiety; at 16.80, 26.80, 27.46 and 35.82 ppm for methylene groups bound to both alkyne and imino groups; at 40.50, 51.24, 51.62-54.16 and 58.00 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.50, 51.26-54.16 and 58.00 ppm for methylene groups bound to both amino and imino groups; at 86.46-87.32 ppm for alkyne groups; at 133.94-154.08 ppm for cyclobutene ring; at 154.50-162.70 ppm for imino groups. Finally, the presence of **22** was further confirmed from mass spectrum which showed a molecular ion at m/z: 646.52.

3.7. Synthesis of 1,4-diazacycloundeca-5,11-dien-steroid derivatives (Scheme 4, 5 and 6). Thre are several reports shown the preparation of diazabicycle derivatives using different reagents such as piperidinone [47], lithium hexamethyldisilazane [48], sodium hydride [49], azodicarboxylate [50], di-tertbutyl dicarbonate [51], palladium on carbon [52]. In this study, the compounds **17**, **19** or **22** were reacted with Copper (II) chloride to form the bicycle derivatives **18** or **20** or **23**. The ^{1}H NMR spectrum of **18** showed several signals at 0.84 and 0.98 ppm for methyl groups bound to steroid nucleus; at 0.94, 1.10-1.64, 1.78-

1.82, 1.86, 1.98, 2.20-2.24, 2.40, 3.64 and 5.22 ppm for steroid moiety; at 1.74, 2.04 and 2.30 ppm for methylene groups bound to both imino and cyclobutene ring; at 3.10 and 3.52-3.58 ppm for methylene groups bound to both imino and amino groups; at 1.84, 1.88, 2.06, 2.32, 3.12 and 3.90-4.40 ppm for bicycle ring (1,4-diaza-cycloundeca-diene); at 5.40 ppm for cyclobutene ring; at 5.50 ppm for amino groups; at 6.70-8.20 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at 11.80-15.60 ppm for methyl groups bound to steroid nucleus; at 21.30-25.14, 26.64, 27.25, 30.72, 32.06, 36.56-37.30, 42.02, 44.00-45.16, 47.40-51.12 and 81.80 ppm for steroid moiety; at 25.22, 31.10 and 36.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.54, 45.87 and 52.72-58.00 ppm for methylene bound to both imino and amino groups; at 27.22, 28.88, 32.70-34.12, 43.60, 61.27-73.34 and 149.60 for bicycle ring (1,4-diaza-cycloundeca-diene); 132.38 and 156.28 ppm for cyclobutene ring; at 152.24, 159.88-169.34 ppm for imino groups. In addition, the presence of **18** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 618.49.

Other data showed several signals of the ^{1}H NMR spectrum for **20** at 0.88 and 0.98 ppm for methyl groups bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino; at 1.20-1.58, 1.78-1.82, 1.94, 2.12-2.28, 2.39-2.40 and 5.22 ppm for steroid moiety; at 1.74, 2.04 and 2.29 ppm for methylene groups bound to both imino and cyclobutene ring; at 1.86-1.88, 2.06, 2.30, 2.80, 3.14 and 3.90-4.42 ppm for bicycle ring (1,4-diaza-cycloundeca-diene); at 3.10 and 3.46-3.58 ppm for methylene groups bound to both amino and imino groups; at 5.60 ppm for amino groups; at 5.40 ppm for cyclobutene ring; at 6.70-8.20 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at 13.20-15.60 ppm for methyl groups bound to steroid nucleus; at 22.60 for methyl group bound to imino; at 21.92, 25.18, 25.22, 26.40-26.62, 27.28, 32.04, 36.52-38.28, 42.02-42.82, 45.20, 48.80-49.02 and 56.20-56.70 ppm for steroid moiety; at 25.24, 31.14 and 36.14 ppm for methylene groups bound to both imino and cyclobutene ring; at 26.30, 27.20, 28.84, 32.70-34.16, 43.60, 54.90, 61.26-73.40 and 149.60 ppm for bicycle ring (1,4-diaza-cycloundeca-diene); at 36.14, 40.56, 45.86, 52.22-52.76 and 58.00 ppm for methylene groups bound to both imino and amino groups; at 132.40 and 156.20 for cyclobutene ring; at 152.26, 159.90-169.28 ppm for imino groups. In addition, the presence of **20** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 836.68.

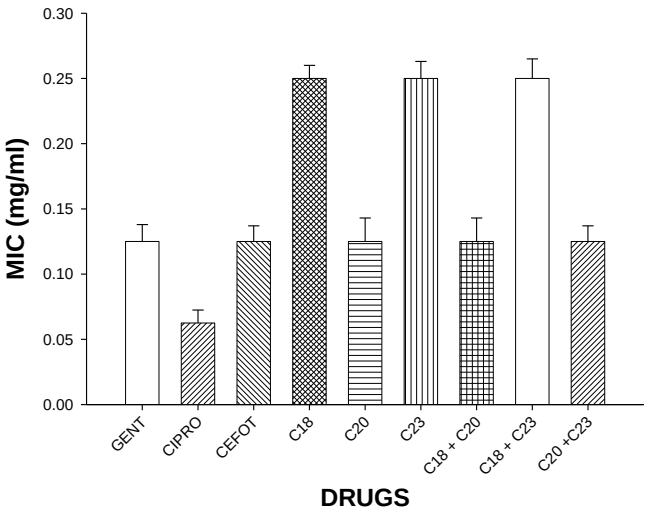
Finally, other data showed several signals of the ^{1}H NMR spectrum for **23** at 0.88 and 0.98 ppm for methyl bound to steroid nucleus; at 1.68 ppm for methyl group bound to imino group; at 1.14-1.38, 1.42-1.66, 1.70-1.84, 1.94, 2.12-2.28, 2.40 and 3.80 ppm for steroid moiety; at 1.40, 1.90 and 2.06-2.10 ppm for methylene groups bound to both imino and cyclobutene ring; at 1.86-1.88, 2.04, 2.30, 2.78, 3.12 and 3.96 ppm for bicycle ring (1,4-diaza-cycloundeca-diene); at 3.10-3.56 ppm for methylene groups bound to both imino and amino groups; at 4.80 ppm for both hydroxyl and amino groups; at 5.40 ppm for cyclobutene ring; at 6.70-8.10 ppm for imino groups. The ^{13}C NMR spectra showed chemical shifts at 13.20 and 16.90 ppm for methyl groups bound to steroid nucleus; at 22.62 for methyl group bound to imino; at 21.68, 25.30-26.40, 30.90-33.80, 34.80, 38.28-38.66, 42.80-43.17, 44.80, 51.24, 56.70-57.34 and 69.70 ppm for steroid moiety; 26.30, 27.20, 28.88, 34.12, 43.60, 54.88, 73.40 and 149.57 ppm for bicycle ring (1,4-diaza-cycloundeca-diene); at 26.80, 27.82, 30.40, 35.82, 54.88 and 154.50 ppm for methylene groups bound to both imino and cyclobutene ring; at 40.50, 45.82, 52.24 and 58.00 ppm for methylene groups bound to both amino and imino groups; at 133.94-154.08 ppm for cyclobutene ring; at 159.90-162.70 ppm for imino groups. Finally, the presence of **23** was further confirmed from mass spectrum which showed a molecular ion at m/z*:* 646.52.



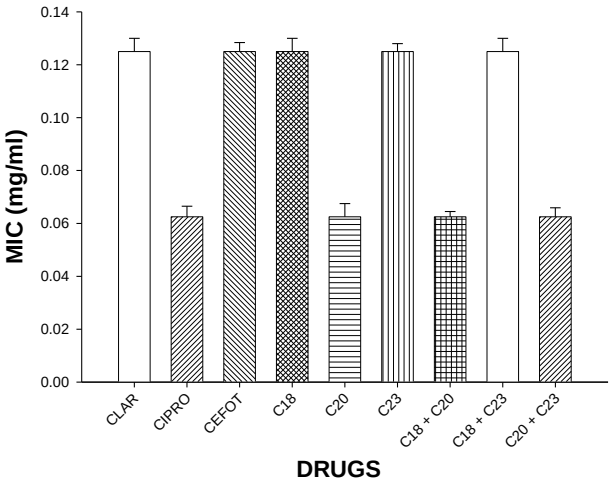
Scheme 6. Synthesis of a *diazacycloundeca-5,11-dien-steroid-5-amine derivative* (**23**). Reaction of **15** with ethylenediamine (v) to form an amino-steroid derivative (**21**). After, **22** was prepared by removal of the ter-buthylsylane fragment of **21** with hydrofluoric acid (v). Finally, **22** was reacted with Copper(II) chloride (vi) to form **23**.

3.8. Evaluation of biological activity for steroid derivatives. In this study the antibacterial activity of some steroid derivatives (compounds 1-23) on *Staphylococcus aureus* and *Streptococcus pneumoniae* was evaluated by means of the dilution method and the minimum inhibitory concentration [MIC], using gentamicin, clarithromycin, ciprofloxacin and cefotaxime as control. The results showed that the bacterial growth of *Staphylococcus aureus* was inhibited with cefotaxime [MIC = 0.12 mg/ml], gentamicin [MIC = 0.12 mg/ml], ciprofloxacin [MIC = 0.06 mg], compound **18** [MIC = 0.25 mg/ml], compound **20** [MIC = 0.12 mg/ml], compound **23** [MIC = 0.25 mg/ml]. In addition, the growth of this microorganism was inhibited with the following mixtures; **18** + **20** [MIC = 0.12 mg/ml], **18** + **23** [MIC = 0.25 mg/ml] and **20** + **23** [MIC = 0.12 mg/ml].

Other results showed that bacterial growth of *Streptococcus pneumoniae* was inhibited in the presence of cefotaxime [MIC = 0.12 mg/ml, 2.74), ciprofloxacin [MIC = 0.06 mg/ml], clarithromycin [MIC = 0.12 mg/ml] and compounds **18** [MIC = 0.12 mg/ml], compound **17** [MIC = 0.06 mg/ml], **20** [MIC = 0.12 mg/ml] and **23** [MIC = 0.12 mg/ml]. Finally, the bacterial growth of *Streptococcus pneumoniae* was inhibited with the following mixtures; **18** + **20** [MIC = 0.06 mg/ml], **18** + **23** [MIC = 0.12 mg/ml], **20** + **23** [MIC = 0.06 mg/ml].



Scheme 7. Antibacterial activity induced by the diazabicyclo-steroid derivatives (compounds C18, C20 and C23) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPRO) on *Staphylococcus aureus*. The results showed that the bacterial growth of *Staphylococcus aureus* was inhibited with cefotaxime [MIC = 0.12 mg/ml], gentamicin [MIC = 0.12 mg/ml], ciprofloxacin [MIC = 0.06 mg], compound **18** [MIC = 0.25 mg/ml], compound **20** [MIC = 0.12 mg/ml], compound **23** [MIC = 0.25 mg/ml]. In addition, the growth of this microorganism was inhibited with the following mixtures; **18** + **20** [MIC = 0.12 mg/ml], **18** + **23** [MIC = 0.25 mg/ml] and **20** + **23** [MIC = 0.12 mg/ml]. MIC = Minimal inhibitory concentration.



Scheme 8. Effect exerted by the diazabicyclo-steroid derivatives (compounds C18, C20 and C23) and controls (cefotaxime, CEFOT; clarithromicin, CLAR; and ciprofloxacin, CIPRO) on *Streptococcus pneumoniae*. The results showed that bacterial growth of *Streptococcus pneumoniae* was inhibited in the presence of cefotaxime [MIC = 0.12 mg/ml, 2.74), ciprofloxacin [MIC = 0.06 mg/ml], clarithromycin [MIC = 0.12 mg/ml] and compounds **18** [MIC = 0.12 mg/ml], **20** [MIC = 0.0.06 mg/ml] and **23** [MIC = 0.12 mg/ml]. Additionally, the bacterial growth of *Streptococcus pneumoniae* was inhibited with the following mixtures; **18** + **20** [MIC = 0.06 mg/ml], **18** + **23** [MIC = 0.12 mg/ml], **20** + **23** [MIC = 0.06 mg/ml]. MIC = Minimal inhibitory concentration.

All these data indicate that; *i*) compounds **18**, **20** and **22** has different antibacterial potency for *Staphylococcus aureus* and *Streptococcus pneumoniae* in comparison with gentamicin (an inhibitor of protein synthesis) [53], and clarithromycin (protein synthesis inhibitor) [54], this phenomenon may be attributed mainly to the different molecular mechanism involved and the characteristic chemical structure of the compounds studied; *ii*) the compound **20** exerts greater antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* compared with the compounds **18** and **23**; *iv*) the antibacterial effect of **20** was similar to cycprofloxacin. This phenomenon could depend on the interaction of two diazabicyclo rings involved in the chemical structure of **20** with some cellular structure involved in the microorganisms studied. This hypothesis can be availed by some reports which indicate that antibacterial activity of cycprofloxacin is via interaction with DNA gyrase [54]; *v*) finally, the different mixtures evaluated in this study do not increase the antibacterial activity compared with the compound **20** against *Staphylococcus aureus* and *Streptococcus pneumoniae*.

3.9. Docking evaluation. In order, to evaluate the possibility that the compound **20** could interact with DNA gyrase (PDB ID:2xcr) [55] in this study a molecular docking model (serverdoking) [56] was used [57]. Theoretical results indicate that hydrogen-interaction between compound **20** and DNA gyrase (Figure 9 and Table 1) involves several amino acid residues such as Leu_{704}, Asn_{705}, Met_{780}, Cys_{784}, Met_{749}, Leu_{762}, Phe_{764}, Ser_{778} and Met_{787}.

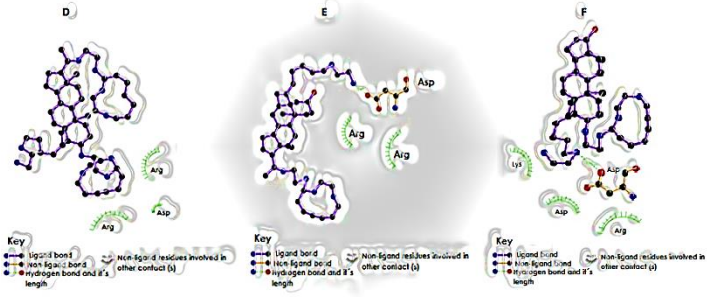


Figure 9. The scheme shown the contact site of amino acid residues involved in the interaction of DNA-gyrase with the compound **18** (D), **20** (E) and **23** (F). Visualized with GL mol Viewer after docking analysis with one-click docking

Table 1. Aminoacid residues involved between the interaction of diazabicyclo-steroid derivatives (18, 20 and 23) with the DNA-gyrase surface.

	Compounds		
Interactions*	**18**	**20**	**23**
Hidrogen bonds	Asp_{470}		Arg_{470}
Polar	Asp_{470}; Arg_{471}	Arg_{471}	Arg_{468}; Arg_{470}
Others	Lys_{466}; Asp_{470}; Arg_{471}	Arg_{468}; Asp_{470}; Arg_{471}	Arg_{468}; Arg_{477}

*aminoacids residues

In addition, other theoretical results showed the decomposed interaction energies (Kcal/mol) between the compound **20** and the amino acid residues from DNA gyrase (table 2). All these data suggest that the interaction of compound **20** with DNA gyrase is conditioned by their physicochemical properties.

4. CONCLUSIONS

The diazabicyclo-steroid derivative (compound **20**) is a particularly interesting drug, because its antibacterial activity exerted against *Staphylococcus aureus* and *Streptococcus pneumoniae* involves a molecular mechanism different in comparison with other drugs; this phenomenon may constitute a novel therapy for infectious diseases.

Table 2. Descompesed interaction energies (Kcal/mol) involved between the diazabicyclo-steroid derivatives (**18**, **20** and **23**) and DNA-gyrase surface.

	Compounds		
Interactions*	**18**	**20**	**23**
Hidrogen bonds	Asp_{470} (-0.3491)		Arg_{470} (0.0708)
Polar	Arg_{471} (-1.4456)	Arg_{471} (-1.6256)	Arg_{468} (-1.2042)
Others	Arg_{468} (-1.0132) Lys_{466} (-0.9578)	Arg_{468} (-2.2648) Asp_{470} (-0.1188)	Arg_{471} (-0.8197)

*aminoacids residues

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