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Synthesis, Characterization and Antibacterial Activity of Danazol-Pregnenolone Conjugate

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This work reports the synthesis of the danazol-pregnenolone conjugate (**5**). The route involved preparation of danazol succinate (**3**) by esterification of danazol (**1**) with succinic anhydride (**2**) followed by the reaction of **3** with pregnenolone (**4**) for synthesis of **5**. Additionally, another route used in this work for synthesis of **5**, was made by means of the reaction between pregnenolone-succcinate (**6**) and danazol to form **5**. The antibacterial activity of compound **3**, as well as **5** and **6**, was evaluated *in vitro* on *S. aureus, K. pneumoniae* and *E. coli* using dilution method and the minimum inhibitory concentration (MIC). The structure of **5** was confirmed by spectroscopy and spectrometry data. The ^{1}H NMR spectrum showed, up field shifts at 0.64, 0.85, 1.02 and 1.03 ppm for methyls present in the heterocyles rings at 1.87 ppm for proton present in alkyne (C≡CH). In addition, a signal at 2.62 ppm was found for methylene involved in arm spacer between the fragments of danazol and pregnenolone. Another chemical shifts at 5.39 and 7.89 ppm were exhibited for the protons involved in azole-ring. Finally, the presence of the danazol-pregnenolone conjugate was further confirmed from mass spectrum which showed a molecular ion at m/z 797.94. The results of the biological activity indicate that the bacterial growth of the microorganisms studied was inhibited by **3**, **5** and **6** in a manner dose-dependent. In conclusion, experimental data suggest that quaternary amine group involved in the danazol-pregnenolone conjugate require only the hydrophobic region of pregnenolone, in order to interact with the cell surface and perturb bacterial growth of *S. aureus*, *E. coli* and *K. pneumoniae*.

Key Words: Danazol, Pregnenolone, Antibacterial activity, Quaternary amine.

INTRODUCTION

Epidemiological and clinical studies suggest that infectious diseases are one of the main causes of mortality in the world[1-3]. Several causal agents, such as *S. aureus*[4], *K. pneumoniae*[5] and *E. coli*[6] among others[7], have been shown to accelerate the

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progression of these pathologies. Although there are many therapeutic agents for the treatment of these bacterial microorganisms[8-10], unfortunately, prolonged antibiotic therapy may induce bacterial-resistance[11,12], because some bacteria have developed ways to circumvent the effects of antibiotics[13,14]. For example, several studies indicate that β-lactam antibiotics (methicilin/oxacillin) induced resistance in *S. aureus*[15,16]. Other studies showed that antibiotic-resistant strains have emerged among Gram-negative bacilli such as *K. pneumoniae*[17] and *E. coli*[18]. Therefore, antibiotic resistance can be considered a serious threat for 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[19-21]. For example, there has been a resurgence of interest in steroids as potential therapeutic agents for infectious diseases[22]. In this context, several steroid-antibiotics have been developed to mimic the antibacterial behaviour of endogenous peptide antibiotics[23]. This task includes selective association of the steroid-antibiotic with disruption of bacterial membranes[24]. The association relates to the chemical structural characteristics of the steroid-antibiotic agents such as, cationic forms and facially amphiphilic conformations, which seems to be the key required for antibacterial activity. It has also been suggested that membrane selectivity is primarily derived from ionic recognition of negatively charged bacterial membranes[25]. In addition, several studies suggest that functional groups of steroid-derivative are involved in the bacterial activity[26]. In present work, the objective is to synthesize a new drug that can be used for treatment of infectious diseases. Therefore, our initial design included the synthesis of danazol-pregnenolone conjugate. It is important to mention that this steroid-conjugate has a spacer arm with ester group between the fragments of danazol and pregnenolone involved in their chemical structure.

On the other hand, the Danazol-pregnenolone conjugate was used to evaluate their antibacterial activity on *S. proteus, K. pneumoniae* and *E. coli* using the microbial minimal inhibitory (MIC 90) method[27].

EXPERIMENTAL

Pregnenolone succinate (5-pregnen-20-one,3-(3-carboxy-1-oxopropoxy) was prepared according to reported method by Figueroa and coworkers[28]. The other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Ultraviolet spectroscopy (UV) was carried out in dry methanol on a Perkin-Elmer model 552 spectrophotometer and infrared spectra (IR) was recorded using KBr pellets on a Perkin-Elmer Lambda 40 spectrometer. ^{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.

Synthesis of danazol succinate (5-(1-ethynil-10a,12a-dimethyl-2,3,3a,3b,4,5,10,10a,10b,11,12,12a-dodecahydro-1*H*-7-oxa-8-aza-dicyclopenta-[a,h]phenanthren-1-yl)-4-oxo-pentanoic acid: A solution of danazol (17-pregna-2,4-dien-20-yno[2,3-d]-isoxazol-17-ol) 200 mg (0.59 mmol), succinic anhydride 118 mg (1.18 mmol), 3 mL of pyridine in 10 mL of toluene was gently refluxed for 24 h and then cooled to room temperature. The reaction mixture was evaporated to a smaller volume, diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from hexane:methanol:water (1:2:1), yielding 60 % of product; m.p. 156 ºC; UV (MeOH) λ_{max} (log) 210 (1.80) 284 (2.82) nm; IR ν_{max} 3302, 2941, 2876, 1736 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.90 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.25-1.78 (1H, m), 1.88 (1H, s, C≡CH), 2.02 (1H, m), 213-231 (1H, m), 2.52 (2H, s, CH_2-CO_2), 2.58 (2H, m, CH_2-CO_2H), 3.06 (1H, m, HC-C≡C), 3.28 (1H, m), 4.86 (1H, m, OCH, cyclopentane), 5.67 (1 H, m, OCH, oxazole-ring), 6.17 (1H, s, *J* = 3.63, C=CH), 8.01, (1H, s, N=CH), 10.02 (1H, s, CO_2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 12.61 (C-18), 18.66 (C-19), 21.12 (C-11), 30.52 (CO_2-CH_2-CH_2-CO_2H), 32.13 (C-15), 32.48 (C-6), 33.21 (C-7), 36.02 (C-12), 36.81 (C-8), 37.48 (C-16), 38.75 (C-10) 40.91 (C-1), 46.59 (C-13), 49.70 (C-2), 51.20 (C-9), 53.71 (C-14), 67.55 (C≡CH), 83.28 (OCH, oxazole-ring) 87.51 (C-17), 87.68 (C≡CH), 118.62 (HC=C), 148.54 (HC=C), 154.41 (N=CH), 164.80 (CH_2-CO_2), 171.02 (CO_2H). EIMS [M+] m/z 439.54 (12), 421.49 (100), 412.22 (8), 339.12 (15). Anal. found to $C_{26}H_{33}NO_5$: C, 71.18; H, 7.58; N, 3.20. Calcd. C,71.05; H, 7.57; N, 3.19.

Succinic acid (17.acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetra- decahydro-1*H*-cyclopentan[a]phenanthren-3-yl)ester(1-ethynyl-10a,12a,-dimethyl-2,3,3a,3b,4,5,6a,9a,10,10a,10b,11,12,12a-tetradecahydro-1*H*-7-oxa-8-aza-dicyclopental[a,h]phenanthren-1-yl)ester.

Method A: Danazol succinate (200 mg, 0.26 mmol) was added to a solution of pregnenolone (84 mg, 0.26 mmol) and 1,3-dicyclohexylcarbodiimide (107 mg, 0.52 mmol) in acetonitrile. The mixture was stirred at room temperature for 72 h, the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) yielding 48 % of product; m.p. 148 ºC; UV (MeOH) λ_{max} (log e) 207 (1.59) 288 (0.45) nm; IR ν_{max} 2931, 2852, 2117, 1702 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.64 (3H, s, methyl-pregn), 0.85 (3H, s, methyl-danazol), 1.02 (3H, s, methyl-pregn), 1.03 (3H, s, methyl-danazol), 116-1.39 (1H, m), 1.45-1.48 (1H, m), 1.61-1.81 (1 H, m), 1.87 (C≡CH), 1.88-2.10 (1 H, m), 2.13 (3H, s, CH_3-C=O), 2.22-2.52 (1H, m), 2.62 (CCH_2-CO_2), 312 (CH-C≡C), 3.46 (1H, m), 4.49 (1H, m, O-CH, pregnen), 4.84 (1H, m, HC-O, danazol), 5.36 (1H, m, O-CH, oxazole-ring), 5.39 (1H, m, *J* = 1.70, C=CH, pregnen), 6.26 (1H, d, *J* = 3.60, CH=C, danazol), 7.89 (1H, s, N=CH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 12.92 (CH_3, pregn), 13.45 (CH_3, danazol), 18.98 (CH_3, pregn), 19.62 (CH_3, danazol),

21.23, 21.45, 23.04, 23.61 (O=C-CH_3), 27.87, 29.84 (2HC-CO_2), 31.76, 31.91, 31.97, 32.05, 32.45, 33.54, 35.15, 35.22, 36.73, 37.13, 37.47 (HC-C≡CH), 38.20, 39.03, 39.07, 39.97, 42.41, 44.25, 48.91 (C-CH, oxazole-ring), 49.22, 51.16, 54.03, 57.11, 63.88, 67.92 (C≡CH), 74.23 (O-CH-pregn), 84.69 (N-O-CH, oxazole-ring), 87.58 (C≡CH), 89.84 (HC-O-danazol), 118.93 (HC=C, danazol), 122,62 (C=CH, pregn), 139.80 (C=CH, pregn), 141.05 (HC=C, danazol), 149.04 (N=CH), 164.23 (CCO_2), 173.23 (CCO_2), 210.02 (CC=O). EIMS [M+] M/Z 737.58 (12), 710.91(8), 439.12 (15), 416.51 (100), 398.51 (35). Anal. found to $C_{47}H_{63}NO_6$: C, 76.52; H, 8.62, N, 1.88. Calcd. C, 76.49; H, 8.60; N, 1.90.

Method B: A solution of pregnenolone succinate (5-pregnen-20-one, 3-(3-carboxy-1-oxopropoxy) (200 mg, 0.48 mmol), danazol (162 mg, 0.48 mmol) and 1,3-dicyclohexylcarbodiimide (198 mg, 0.96 mmol) in acetonitrile. The mixture was stirred at room temperature for 72 h. The solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) yielding 39 % of product. The ^{1}H NMR and ^{13}C NMR spectrums for danazol-pregnenolone (**5**) were similar to method A reported.

Biological evaluation

Strains: The microorganisms in this study belonged to the strain bank at the Department of Pharmaco-Chemistry at the Facultad of Ciencias Quimico-biologicas of the Universidad Autonoma de Campeche. The strains are certified by the Center for Disease Control in Atlanta and were as follows. *S. aureus* (ATCC 25923), *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922). The strains are kept under refrigeration at 4 ºC in special gel (BBL).

Antimicrobial agents: The steroids derivatives and the other compounds studied were dissolved in methanol and diluted with distilled water. Cefotaxime, gentamycin, methicillin and ciprofloxacin were used as control drugs.

Antimicrobial activity: The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by method described by Chiong *et al.*[27]. The bacterial species were incubated on McConkey (*E. coli* and *K. pneumoniae*) and *Staphylococcus* 110 (*S. aureus*) agars for 24 h at 37 ºC. After 24 h, it was 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 (11 tubes), 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 McArland scale (9×10^8 cells/mL) and all the tubes were incubated at 37 ºC 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 ºC.

After such time, the minimum inhibitory concentration (MIC) was evaluated to consider the antimicrobial effect of all 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 at pH 7.0.

RESULTS AND DISCUSSION

In this study, a straightforward route is reported for the synthesis of danazol-pregnenolone conjugate (Figs. 1-3). The first step involves the esterification of the hydroxyl group of danazol to form danazol succinate by the method reported by Figueroa[28], using toluene to avoid hydrolysis in the new arm formed in cyclopentane-ring of danazol-succinate, which has characteristic of a free carboxyl group. The results indicate that ^{1}H NMR spectrum of danazol succinate showed signals at 0.90 and 1.02 ppm corresponding to methyls presents in the heterocycles rings. In addition, another signals at 1.88 ppm for proton of alkyne (C≡CH) and 2.58 ppm for methylene bound to carboxyl group were found. Another signals at 5.67 (OCH) and 8.01 ppm (N=CH) involved in oxazole-ring were shown. Finally, a signal at 10.02 ppm corresponding to the acidic hydrogen of C(=O)-OH was found.

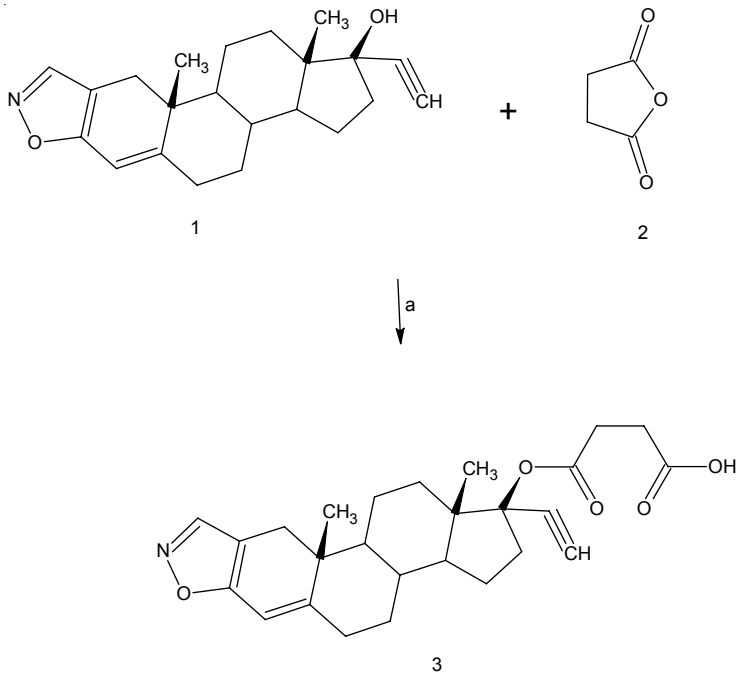

Fig. 1. Synthesis of danazol succinate, Esterification of danazol (**1**) using succinic acid (**2**) to form danazol succinate (**3**), a = conditions; pyridine/toluene

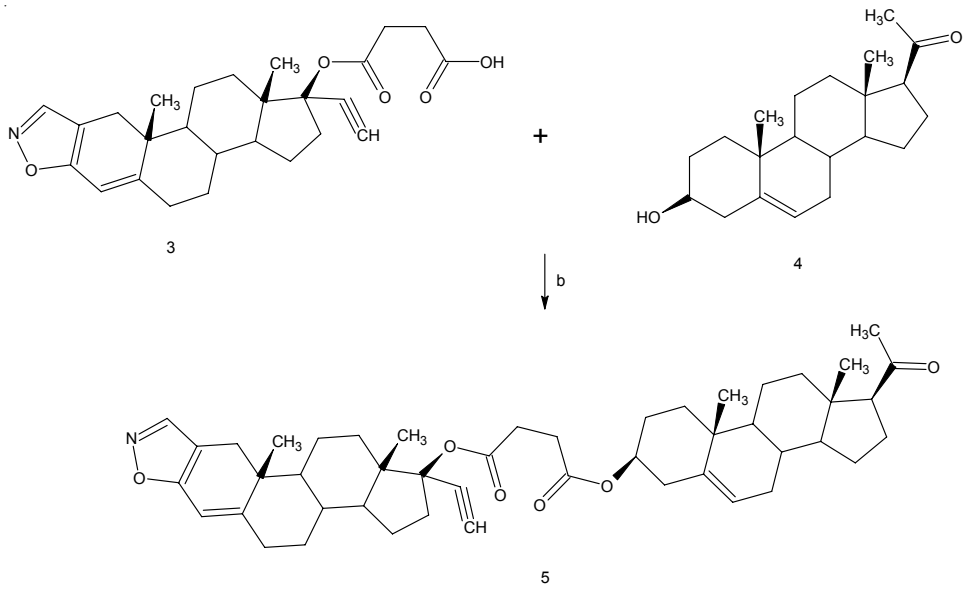

Fig. 2. Synthesis of danazol-pregnenolone conjugate, Reaction of danazol succinate (**3**) with pregnenolone (**4**) to form danazol-pregnenolone conjugate (**5**). b = conditions; 1,3-dicyclohexylcarbodiimide/acetonitrile

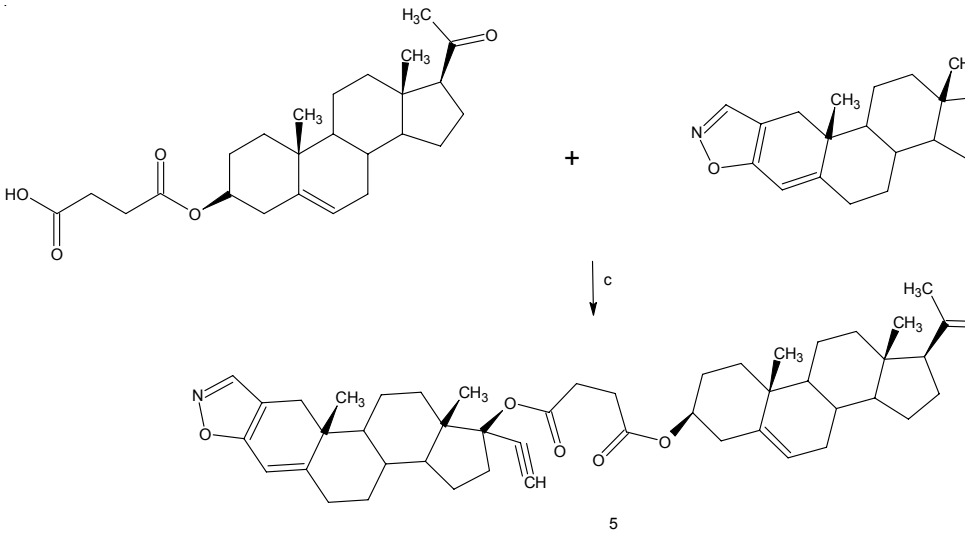

Fig. 3. Preparation of danazol-pregnenolone conjugate. Reaction of pregnenolone succinate (**6**) with danazol (**1**) to form danazol-pregnenolone conjugate (**5**), c = conditions; 1,3-dicyclohexylcarbodiimide/acetonitrile

The ^{13}C NMR spectra displays chemical shifts at 12.61 and 18.66 ppm for the carbons of methyls groups presents in the heterocyles. The chemical shift of the methylene bound to carboxyl group is found out at 30.52 ppm. In addition, there are several signals (32.13-53.71 ppm) corresponding to carbons involved in the heterocycle rings and at 67.55 ppm (C≡CH) and 87.68 ppm (C≡CH) for alkyne. Additionally, two signals characteristics at 83.28 ppm (OCH) and 154.41 ppm (N=CH) for carbons corresponding to oxazole-ring. Finally, a signal at 171.02 ppm for the carbon of CO_2H was found. Additionally, the presence of the danazol succinate was further confirmed from mass spectrum which showed a molecular ion at m/z 439.54.

The second step was achieved with the synthesis of danazol-pregnenolone conjugate using two methods. In method A, in this work was synthesized the danazol-pregnenolone conjugate (**5**) by the reaction of danazol-succinate (**3**) with pregnenolone (**4**) in presence of a carbodiimide-derivative, for ester bond formation involved in steroid-conjugate. In method B, the danazol (**1**) and pregnenolone succinate (**6**) were made react for the formation of danazol-pregnenolone conjugate (**5**) with the same conditions. It is important to mention that many procedures for the formation of ester groups are known in the literature[29,30]. In the case of steroid alcohols esterified with carboxylic acids, can preferably be synthesized with dicyclohexylcarbodiimide[31]. Therefore, in this work, 1,3-dicyclohexylcarbodiimide was used for ester bond formation in the danazol-pregnenolone conjugate. The ^{1}H NMR spectra of the danazol-pregnenolone conjugate shows in addition of the characteristic chemical shifts of the danazol succinate, upfield chemical shifts at 0.64, 1.02 and 2.13 ppm for methyls present in the pregnenolone fragment. In addition, a signal at 2.62 ppm was found for methylenes involved in arm spacer between the fragments of danazol and pregnenolone. Additionally, other signals at 4.49 (O-CH) and 5.36 ppm (C=CH) were found. Finally, several chemical shifts (116-181, 188-210, 22-252 and 346 ppm) corresponding to protons in the heterocyles were found.

On the other hand, ^{13}C NMR spectra displays chemical shifts at 12.92 and 18.98 ppm for the carbons of methyls groups presents in the pregnenolone fragment. In addition, another chemical shifts at 13.45 and 19.62 ppm for methyls groups involved in danazol fragment were found. Another chemical shifts at 29.84 ppm for carbons of methylenes involved in arm spacer between the fragments of danazol and pregnenolone were exhibited. In addition, two signals at 67.92 and 89.84 ppm for carbons of alkyne were found. Additionally, several signals at 48.91 (CCH, oxazole-ring), 74.23 (O-CH-pregn), 84.69 (N-O-CH, oxazole-ring), 87.58 (HC-O-danazol), 149.04 (N=CH), 164.23 (CCO_2), 173.23 (CCO_2), 210.02 (CCO, ketone) were exhibited. Finally other chemical shift at 21.23-23.04, 27.87-29.84, 31.76-37.47, 38.20-44.25, 49.22-63.88 and 118.93 for carbons corresponding to heterocycles were found. In addition, the presence of the danazol-pregnenolone conjugate was further confirmed from mass spectrum which showed a molecular ion at m/z 737.46.

On the other hand, the antibacterial activity of danazol-pregnenolone conjugate on *S. aureus*, *K. pneumoniae* and *E. coli* was evaluated by means of dilution method and the minimum inhibitory concentration (MIC)[27], using gentamycin, ampicillin, cefotaxime and cifroflaxin as control in this study. The results obtained (Fig. 4) indicate that bacterial growth of *S. aureus* was inhibited with cefotaxime (MIC 5.23×10^{-4} mmol), gentamycin (MIC = 2.68×10^{-5} mmol) and ciprofloxacin (MIC = 5.23×10^{-4} mmol). It is important to mention, that in presence of ampicillin, the bacterial growth of *S. aureus* was not blocked (data not shown). In addition, the bacterial growth of *S. aureus* in presence of danazol-pregnenolone conjugate (MIC = 6.63×10^{-4} mmol) was blocked. All this data indicate that antibacterial activity induced by danazol-pregnenolone conjugate was lower in comparison with cefotaxime (β-lactam antibiotic) and gentamycin (inhibitor of synthesis of protein). This phenomenon can be due mainly to the different molecular mechanism involved and the characteristic chemical structure of the compounds studied in this work.

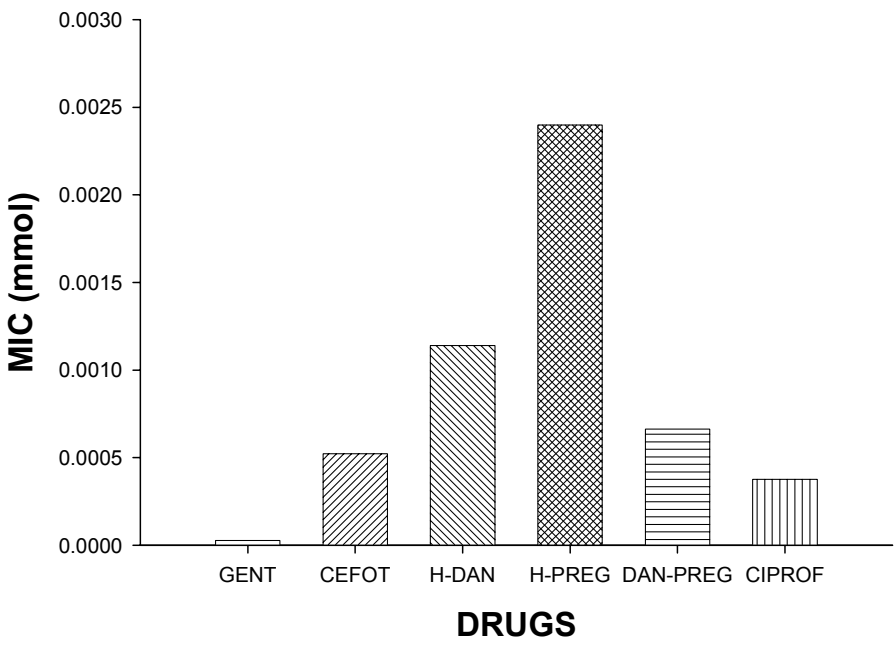

Fig. 4. Antibacterial effects induced by danazol-pregnenolone and controls (cefotaxime, CEFOT; gentamycin, GENT and ciprofloxacin CIPROF) on *S. aureus*. Data showed that *S. aureus* was susceptibly to cefotaxime (MIC of 5.23×10^{-4} mmol), gentamycin (MIC = 2.68×10^{-5} mmol) and CIPROF (MIC = 3.77×10^{-4} mmol). In addition, the bacterial growth of this microorganism in presence of the pregnenolone succinate compound (MIC = 2.40×10^{-3} mmol), danazol succinate (MIC = 1.14×10^{-3} mmol) and danazol-pregnenolone conjugate (MIC = 6.63×10^{-4} mmol) was inhibited. MIC = minimum inhibitory concentration

It is interesting to consider the different molecular mechanisms involved in the antibacterial activity induced by danazol-pregnenolone conjugate. This compound contains a spacer arm with ester group connected between the fragments of danazol and pregnenolone, in addition involve a quaternary amine in the oxazole-ring. Several reports have shown that drugs with quaternary amine exert antibacterial activity against both Gram-positive and Gram-negative bacteria through perturbation of lipid bi-layer membranes that constitute the bacterial cytoplasmic membrane and the outer-membrane of bacteria[32]. To evaluate this premise, we used the danazol, since the nature of functional groups contained in their chemical structure have a quaternary amine in the oxazole-ring. The results showed that in presence of danazol the bacterial growth of *S. aureus* was not blocked (data not showed). The experimental data suggest that quaternary amine of danazol by itself, does not have antibacterial activity on the pathogen microorganism studied. Additionally, those experimental data indicate that fragments of danazol or pregnenolone involved in the chemical structure of danazol-pregnenolone conjugate, could be the responsible of the antibacterial activity. In order to analyze this possibility, the antibacterial effects of both danazol succinate and pregnenolone succinate on *S. aureus*, were evaluated to compare with the antibacterial activity induced by the danazol-pregnenolone conjugate on *S. aureus*. The obtained results showed that the bacterial growth of *S. aureus* was blocked in presence of danazol succinate (MIC = 1.14×10^{-3} mmol) and pregnenolone hemisuccinate (MIC = 2.40×10^{-3} mmol) in a dose-dependent manner. These experimental data suggest that antimicrobial effect induced by danazol succinate and pregnenolone hemisuccinate can depend on the nature of the free carboxyl group present in its chemical structure, which could be a membrane-perturbing agent whose antibacterial activity is induced, possibly, by the interaction with the positively charged amino groups present in the D-alanyl incorporated in the teichoic acids. They are essential polymers that plays a vital role in the growth and development of the gram-positive bacteria[33].

Nevertheless, it is important to mention that when steroid-succinates are bound with free steroid (danazol or pregnenolone) to form the danazol-pregnenolone conjugate, the antibacterial activity seems to be greater, possibly because the quaternary amine group involved in oxazole-ring require only the hydrophobic region of pregnenolone in order to interact with the cell surface and integrate into the cytoplasmic membrane. Such integration into the membrane is sufficient to perturb bacterial growth to cause the membrane to lose fluidity and for the cell to die. This phenomenon can be associated by interaction of danazol-pregnenolone conjugate with teichoic acid that is an element of Gram-positive bacteria[33].

On the other hand, in alternative experiments on the antibacterial activity of danazol succinate, pregnenolone succinate and danazol-pregnenolone conjugate was evaluated on *E. coli* and *K. pneumoniae* using the same controls. The results showed that bacterial growth of *E. coli* and *K. pneumoniae* (Figs. 5 and 6) in presence

of controls was inhibited, in a manner dose dependent. In addition, another results showed that bacterial growth of this pathogen microorganisms in presence of danazol succinate (MIC = 2.28 × 10^{-3} mmol), pregnenolone succinate (MIC = 2.4 × 10^{-3} mmol) and danazol-pregnenolone conjugate (1.32 × 10^{-3} mmol) was blocked. This data indicate that steroid-conjugate have different antibacterial activity on *E. coli* and *K. pneumoniae* in comparison with steroid-succinates. This fact can be due mainly to the different molecular mechanism involved. The molecular mechanism implied in the antibacterial activity induced by steroid-succinates can be shown by the intermolecular interaction with the cations (Mg^{2+} and Ca^{2+}), involved in the membrane cell providing a substantial increase the permeability of the outer membrane of Gram-negative bacteria and induce cell death.

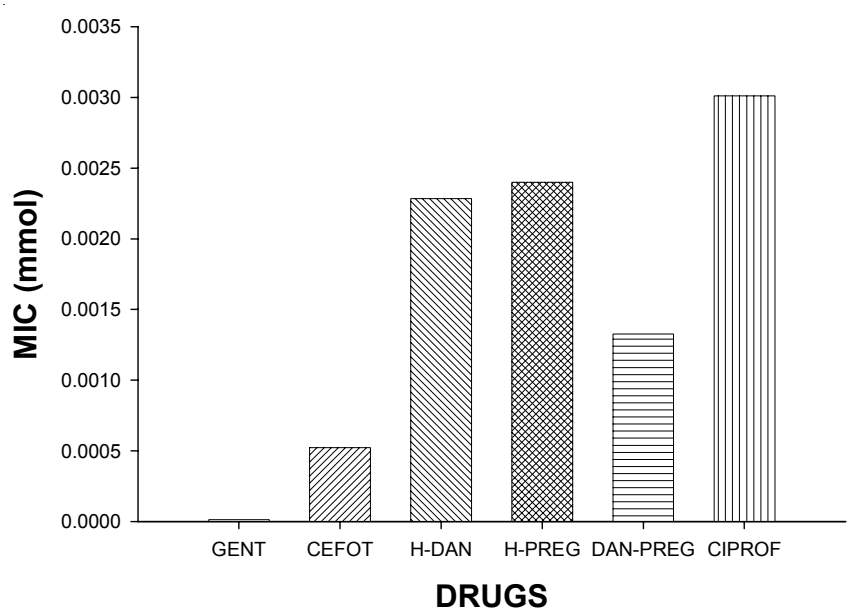

Fig. 5. Antibacterial effects induced by danazol-pregnenolone and controls (cefotaxime, CEFOT; gentamicin, GENT and ciprofloxacin CIPROF) on *E. coli*. It is showed that exist differences of antibacterial activity of CEFOT (MIC = 5.23 × 10^{-4} mmol), GENT (MIC = 1.34 × 10^{-5} mmol) and CIPROF (MIC = 3.01 × 10^{-3} mmol) on *E. coli* in comparison with the pregnenolone succinate compound (MIC = 2.40 × 10^{-3} mmol), danazol succinate (MIC = 2.28 × 10^{-3} mmol) and danazol-pregnenolone conjugate (MIC = 1.32 × 10^{-3} mmol). MIC = minimum inhibitory concentration

Nevertheles, the antibacterial activity of danazol-pregnenolone conjugate can depend on the intermolecular interaction with the lipopolysaccharide of Gram-negative bacteria. This premise is based on the works by several investigators whose developed a class of steroid antibiotics with the intent of mimicking the antibacterial activities of polymyxin B on Gram-negative bacteria[34]. In addition, this phenomenon

can induce, as consequence, an increase in the permeability of the outer membrane and induce growth bacterial inhibition on these pathogen microorganisms. This premise is supported by some mechanisms, based in experimental data, which proposed that steroid-antibiotics can adopt cationic conformations to induce bacterial death[35].

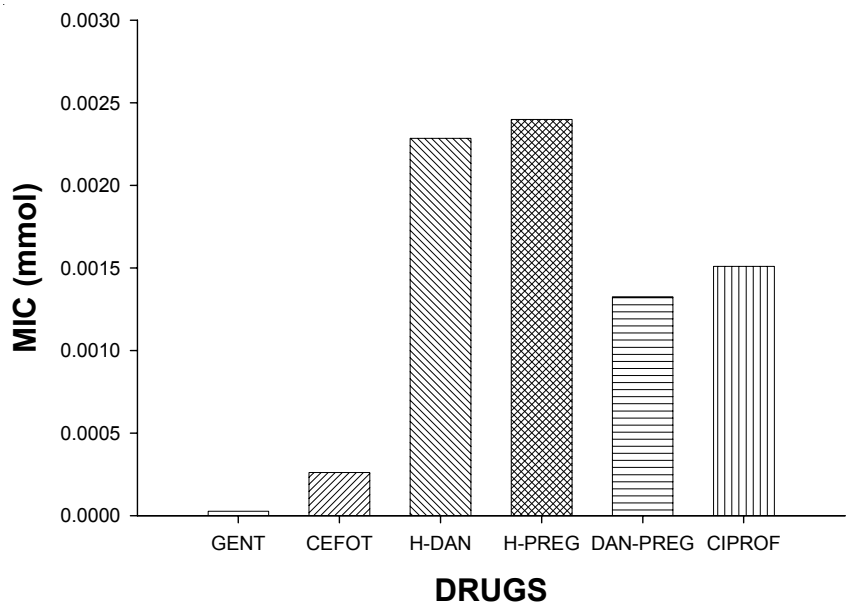

Fig. 6. Antibacterial activity induced by danazol-pregnenolone and control (cefotaxime, CEFOT; gentamicin, GENT and ciprofloxacin CIPROF) on *K. pneumoniae*. It is showed that exist differences of antibacterial activity of CEFOT (MIC = 2.61×10^{-4} mmol), GENT (MIC = 2.68×10^{-5} mmol) and CIPROF (MIC = 1.50×10^{-3} mmol) on *K. pneumoniae* in comparison with the pregnenolone succinate compound (MIC = 2.40×10^{-3} mmol), danazol succinate (MIC = 2.28×10^{-3} mmol) and danazol-pregnenolone conjugate (MIC = 1.32×10^{-3} mmol). MIC = minimum inhibitory concentration

Conclusion

Experimental data suggest that the antibacterial activity of danazol-pregnenolone conjugate depend on quaternary amine group and the hydrophobic region of pregnenolone, in order to interact with the cell surface and perturb bacterial growth of *S. aureus*, *E. coli* and *K. pneumoniae*.

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