



Antibacterial activity induced by several steroid derivatives against *E. coli*, *S. Typhi*, *K. pneumoniae* and *S. aureus*

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ARTICLE INFO

Article history:

Received: 26 August 2011;

Received in revised form:

26 October 2011;

Accepted: 5 November 2011;

Keywords

Antimicrobial activity,

Antimicrobial agents,

Biological activity.

ABSTRACT

In this work the antibacterial activity of several steroid derivatives (compounds 1, 2 and 3) against *E. coli*, *S. Typhi*, *K. pneumoniae* and *S. aureus* was performed according to NCCLS (now CLSI)²² with some modifications. The results indicate that compounds 1, 2 and 3 induce antibacterial activity against both *K. pneumoniae* and *S. Typhi* in a dose-dependent way. In addition, the growth bacterial of *E. coli* was inhibited in presence of the compound 2 and 3. These experimental data obtained in this study, suggest that antibacterial activity of steroid derivatives against to *E. coli*, *K. pneumoniae*, *V. cholerae* and *S. typhi* may depend of chemical structure.

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Introduction

Infectious diseases are one of the main causes of morbidity-mortality in the world¹⁻³. Several causal agents, such as *V. cholerae* and *E. coli*⁴⁻⁶ among others⁷ have been shown to accelerate the progression of infectious diseases. Although, there are many therapeutic agents for treatment of these bacterial microorganisms^{7,8}, unfortunately, prolonged antibiotic therapy can induce bacterial resistance, because some bacteria have developed ways to circumvent the effects of antibiotics^{9,10}. For example, several studies indicate that *V. cholerae* is not sensitive to trimetoprim and sulphametoxazol¹¹. In addition, clinical data suggest that isolates of *E. coli* can show resistance to quinolones¹². Other studies¹³ also indicate that *E. coli* induce bacterial resistance to fluoroquinones and this phenomenon is linked to decreased cell permeability. Other reports showed that chloramphenicol used in animals exert bacterial resistance to gram-negative bacilli such as *E. coli*¹⁴.

All data suggest that bacterial 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^{15,16} for example, several steroid derivatives have been developed as potential therapeutic agents for infectious diseases¹⁷ which mimic the antibacterial behavior of some endogenous peptide antibiotics¹⁸. This task includes selective association of the steroid-antibiotic with disruption of bacterial membranes¹⁹. 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 membranes²⁰. In addition, several studies suggest that functional groups of steroid-derivative are involved in the bacterial activity²¹. Therefore, in this work the antibacterial activity of several steroid

derivatives against *S. Typhi*, *S. aureus*, *K. pneumoniae* and *E. coli* was performed according to NCCLS (now CLSI)²² with some modifications.

It is important to mention that were used such pharmacological tools at cefotaxime (β -lactam antibiotic)²³, gentamicin (inhibitor of protein synthesis)²⁴ and ciprofloxacin (inhibitor of DNA-gyrase)²⁵.

Material and Methods

Biological activity

The microorganisms in this study belonged to the strain bank at the Department of Pharmaco-Chemistry at the Faculty of Chemical Biological Sciences of the Universidad Autónoma de Campeche.

These strains were certified by the Centers for Disease Control and Prevention in Atlanta (USA) and were *S. Typhi* (ATCC 23564) and *S. aureus* (ATCC 25923), *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922).

The strains were kept under refrigeration at 4 °C for its conservation in a mixture of culture mediums (caseine peptone [2.5 g/L], extract of meat [1.5g/L] and columbia agar base [42/L]).

Antimicrobial agents

The Compounds 1 (Succinic acid mono-[(2-aminoethylamino)-methyl]-1-ethynyl-10a,12a-dimethyl 2, 3, 3a, 3b, 4, 5, 10, 10a, 10b, 11, 12, 12a -dodecahydro-1H-7-oxa-8-aza-dicyclo-penta-[a,h]-phenanthren-1-yl)ester, 2(30,60-Dihydro-17-hydroxy-60-phenyl-androst-2-eno[3,2-d]pyrimidine-20(10H)-thione) and 3 (11a,13a-dimethyl-phenyl-1-[1-(6-phenyl-2-thioxo-1,2,3,6-tetrahydro-pyrimidin-4-yl)-ethyl] 1, 2, 3, 3a, 3b, 4, 5, 5a, 7,9,10,10a,11,11a,11b,12,13,13a-octadecahydro-1H-7, 9-diaza-indeno[5,4a]anthracene-8-thione) were synthesized by methods reported previously²⁶⁻²⁸. Steroid derivatives were dissolved in methanol and diluted with distilled water. Cefotaxime, gentamicin, methicillin and ciprofloxacin were used as standard drugs.

Antimicrobial activity

Evaluation of the antimicrobial effect of different compounds on the bacterial species was performed according to NCCLS with some modifications ²²*E. coli*, *S. tiphy*, *S. aureus* and *K. pneumoniae* isolate were cultured on McConkey agar for 24 h at 37 °C. In addition, a series of tubes were prepared, the first of which contained 2 mL of culture medium (trypticase soy) 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 McFarland scale (9×10^8 cells/mL) and all the tubes were incubated at 37 °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 °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.

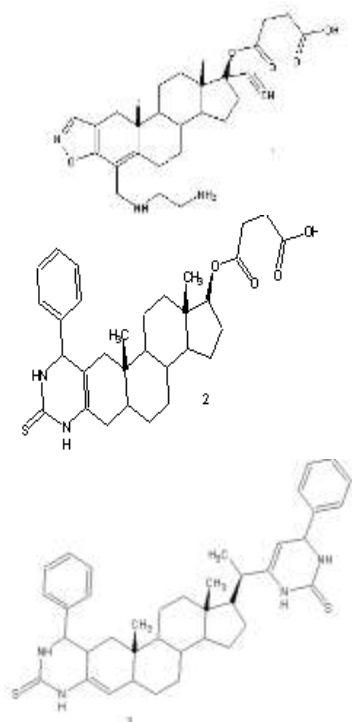


Figure 1. Chemical structure of steroid derivatives Results

The results obtained (Figure 2, see) indicate that bacterial growth of *E. coli* was inhibited by cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamicin (MIC = 1.34×10^{-5} mmol/mL), and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). In addition, in presence of compounds 2 (MIC = 2.32×10^{-4} mmol/mL) and 3 (MIC = 1.96×10^{-4} mmol/mL) the bacterial growth was blocked in a dose-dependent way.

On the other hand, alternative experimental were made in *K. pneumoniae* (Figure 3, see) using the same controls to evaluate

the antibacterial effect of compounds studied. The results indicate that bacterial growth of *K. pneumoniae* was inhibited by cefotaxime (MIC = 2.61×10^{-4} mmol/mL), gentamicin (MIC = 2.68×10^{-5} mmol/mL), and ciprofloxacin (MIC = 1.50×10^{-3} mmol/mL). In addition, the bacterial growth of *K. pneumoniae* was inhibited by administration of the compounds 1 (MIC = 1.96×10^{-3} mmol/mL), 2 (MIC = 1.86×10^{-3} mmol/mL) and 3 (MIC = 1.96×10^{-4} mmol/mL).

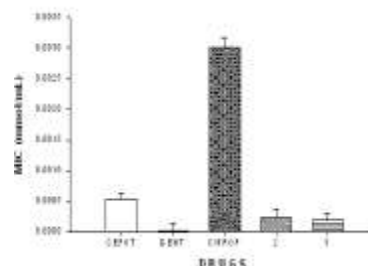


Figure 2. Antibacterial activity induced by the steroid derivatives (compounds 2 and 3) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *E. coli*. The results showed differences in the antibacterial effect exerted by GENT in comparison with the antibacterial activity induced by steroid derivatives against *E. coli*. Nevertheless, the compounds 2 and 3 induce higher antibacterial effect with respect to CEFOT and CIPROF.

Each bar represents the mean \pm S.E. of 9 experiments

Other results indicate that bacterial growth of *S. tiphy* (Figure 4, see) was inhibited by cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamicin (MIC = 1.34×10^{-5} mmol/mL), and ciprofloxacin (MIC = 3.01×10^{-3} mmol/mL). In addition, the bacterial growth of *S. tiphy* was inhibited by administration of the compounds 1 (MIC = 1.96×10^{-3} mmol/mL), 2 (MIC = 1.86×10^{-3} mmol/mL) and 3 (MIC = 1.96×10^{-4} mmol/mL) was blocked.

Finally, the bacterial growth of *S. aureus* (Figure 5, see) in presence of cefotaxime (MIC = 5.23×10^{-4} mmol/mL), gentamicin (MIC = 2.68×10^{-5} mmol/mL), and ciprofloxacin (MIC = 3.77×10^{-3} mmol/mL) was inhibited. In addition, the bacterial growth of *S. aureus* was blocked by the administration of the compound 1 (MIC = 2.45×10^{-4} mmol/mL) and 2 (MIC = 1.86×10^{-3} mmol/mL).

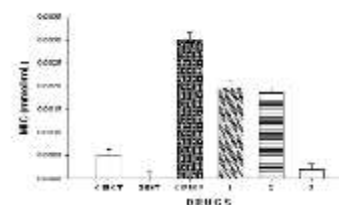


Figure 3. Antibacterial activity exerted by the steroid derivatives (compounds 1, 2, and 3) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *K. pneumoniae*. There are differences in the antibacterial effect exerted by CEFOT, GENT and CIPROF in comparison with the antibacterial activity induced by steroid derivatives against *K. pneumoniae*. The compound 3 had higher antibacterial potency in comparison with the compounds 1 and 2. Each bar represents the mean \pm S.E. of 9 experiments.

Discussion

The bacterial activity of several steroid derivatives was compared with the antibacterial effect of cefotaxime, gentamicin

and ciprofloxacin (controls) in such bacterial microorganism studied. The results showed that steroid derivatives (2 and 3) have different antibacterial effects against *E. coli* in comparison with cefotaxime and gentamicin. This phenomenon may be attributed mainly to different molecular mechanisms involved in the antibacterial activity of the steroid derivatives and controls. In addition, it is important to mention that compounds 2 and 3 had higher antibacterial activity in comparison with ciprofloxacin. Nevertheless, the bacterial growth of *E. coli* in presence of 1 was not inhibited (data not shown). These experimental data suggest that 1) the antibacterial activity depend of different chemical structures of each steroid derivative which may consequently bring the interaction with some cell molecules involved in the cell membrane of *E. coli* such happened with other type of dihydropyrimidine derivatives²⁹; 2) the antibacterial activity of compounds 2 and 3 against *E. coli* may depend of the dihydropyrimidine ring involved in its chemical structure.

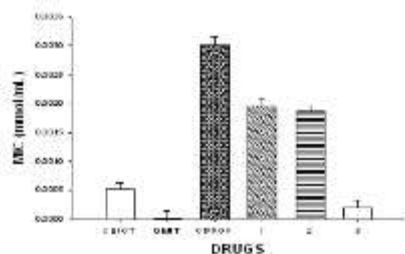


Figure 4. Antibacterial activity exerted by the steroid derivatives (compounds 1, 2 and 3) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) against *S. tify*. The results showed differences in the antibacterial effect induced by CEFOT and GENT in comparison with the antibacterial activity exerted by steroid derivatives against *S. tify*. Nevertheless, the compounds 1, 2, and 3 induce higher antibacterial effect with respect to ciprofloxacin. In addition, the compound 3 had higher antibacterial potency in comparison with the compounds 1, and 2. Each bar represents the mean \pm S.E. of 9 experiments.

On the other hand, analyzing these results and evaluating the possibility of that the compounds studied could exert antibacterial effect on another type of bacteria; in this study the antibacterial activity induced by the compounds 1, 2 and 3 against both *K. pneumoniae* and *S. tify* was evaluated. The results showed that the compounds 1, 2 and 3 had antibacterial effect against both *K. pneumoniae* and *S. tify*; in addition, this effect was different in comparison with cefotaxime, gentamicin and ciprofloxacin. It is important to mention that 3 have higher antibacterial potency in comparison with 1 and 2. These experimental data indicate that dihydropyrimidine rings involved in the compound 3 may be the responsible of increase the antibacterial activity against *S. tify*.

All these experimental data obtained suggest that; 1) activity antibacterial of the steroid derivatives (1, 2 and 3) against *K. pneumoniae* and *S. tify* may depend; 1) of both carboxyl and amino groups involved in the structure of danazol derivative (compound 1 and 2) Possibly the antibacterial activity of compound 1 involve the intramolecular interaction of via divalent 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 include bactericidal/permeability increasing protein such happened with other type of steroid derivatives. In addition, the antibacterial effect of the compounds 1 possibly could be mainly by the interaction of free amine group with the lipid A of Gram-negative bacteria; this

premise is availed by Li³⁰ and Ding³¹, who developed a class of cationic steroids-antibiotics with the intent of mimicking the antibacterial activities of polymyxin B on Gram-negative bacteria. These authors proposed a compelling model of complex formation involving ionic interactions between the phosphates on lipid A and the amine groups on polymyxin B. This phenomenon may increase the permeability of the outer membrane and induce bacterial growth inhibition on this gram-negative microorganism; 3) the dihydropyrimidine rings of compound 2 and 3 may be the responsible of its antibacterial activity against *K. pneumoniae* and *S. tify*. This phenomenon may involve interaction of dihydropyrimidine ring with some substance at cellular level and induce inhibition of bacterial growth of these microorganisms.

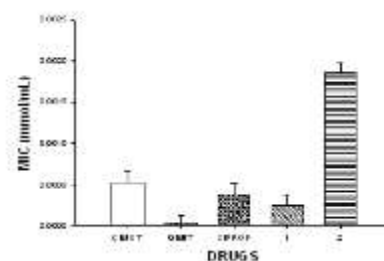


Figure 5. Antibacterial activity induced by the steroid derivative (compounds 1 and 2) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) against *S. aureus*. There are differences in the antibacterial effect exerted by CEFOT and GENT in comparison with the antibacterial activity induced by steroid derivatives against *S. aureus*. Nevertheless, the compound 1 had higher antibacterial potency in comparison with the compounds 2. Each bar represents the mean \pm S.E. of 9 experiments

Analyzing these data and other reports which show that some steroid derivatives exert antibacterial activity against Gram positive bacteria³², in this study the antibacterial activity induced by the compound 1, 2 and 3 against *S. aureus* was evaluated. The results showed that only the compounds 1 and 2 exert antibacterial effect on this microorganism; in addition, the antibacterial activity of the compound 2 was less in comparison with 1. These experimental data suggest that; 1) *S. aureus* induce bacterial resistance to compound 3 possibly because exist some steric impediment involved in its chemical structure.

Conclusions

The experimental data obtained in this study, suggest that antibacterial activity of steroid derivatives against to *E. coli*, *K. pneumoniae*, *V. cholerae* and *S. tify* may depend of chemical structure.

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