ANTIMICROBIAL ACTIVITY OF PREGNENOLONE-CARBAMAZEPINE COMPLEX ON S. AUREUS, K. PNEUMONIAE AND E. COLI.

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ABSTRACT

In this work the antibacterial activity of *pregnenolone-carbamazepine* complex was evaluated on *S. Aureus*, *K. pneumoniae* and *E. coli.* The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by the method of microbial minimal inhibitory. The results indicate that bacterial growth of *S. Aureus* was inhibited with *cefotaxime* (MIC=5.23 x 10^{-4} mmol), *gentamicin* (MIC=2.68 x 10^{-5} mmol), ciprofloxacin (MIC=3.77 x 10^{-4} mmol), *hemisuccinate-pregnenolone* (MIC=2.40 x 10^{-3} mmol), carbamazepine-*aminocapric acid* (MIC=6.80 x 10^{-4} mmol) and the *pregnenolone-carbamazepine* complex (MIC=1.39 x 10^{-4} mmol). Other results showed that bacterial growth of *E. coli* was inhibited with *cefotaxime* (MIC=5.23 x 10^{-4} mmol), *gentamicin* (MIC=1.34 x 10^{-5} mmol), *ciprofloxacin* (MIC=3.01 x 10^{-3} mmol), *hemisuccinate-pregnenolone* (MIC=2.40 x 10^{-3} mmol), carbamazepine-*aminocapric acid* (MIC=1.36 x 10^{-3} mmol) and the *pregnenolone-carbamazepine* complex (MIC=3.18 x 10^{-4} mmol). Alternative experimental, indicate that *bacterial* growth of *K. pneumoniae* was inhibited with *cefotaxime* (MIC=2.61 x 10^{-4} mmol), *gentamicin* (MIC=2.68 x 10^{-5} mmol), *ciprofloxacin* (MIC=1.50 x 10^{-3} mmol), *hemisuccinate-pregnenolone* (MIC=2.40 x 10^{-3} mmol), carbamazepine-*aminocapric acid* (MIC=1.36 x 10^{-3} mmol) and the *pregnenolone-carbamazepine* complex (MIC=3.18 x 10^{-4} mmol). In conclusion, *S. Aureus*, *K. pneumoniae* and *E. Coli* were more susceptible to both *gentamicin* and *pregnenenolone-carbamazepine* complex and were more resistant to that *hemisuccinate-pregnenenolone* conjugate. Possibly the molecular mechanism of *pregnenolone-carbamazepine* could be by mimic the actions of several steroid-derivates with antibacterial characteristics.

Keywords: *Pregnenolone-carbamazepine,* complex, antibacterial activity.

INTRODUCTION

Infectious diseases are one of the main causes of morbidity-mortality in the world[1,2,3]. Several causal agents, such as *S. Aureus*[4], *K. pneumoniae*[5] and *E. Coli*[6] among others[7] have been shown to accelerate the progression of these pathologies. Although there are many therapeutic agents for treatment of these bacterial microorganisms[8,9,10] unfortunately, prolonged antibiotic therapy 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*) predispose to patients for acquisition of resistance to *S. Aureus*[15,16]. Other reports showed that antibiotic-resistant strains have emerged among Gram-negative bacilli such as *K. pneumoniae*[17] and *E. Colli*[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[19,20,21] 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 behavior 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]. Therefore, in this work the antibacterial effect of *pregnenolone-carbamazepine complex* on *S. proteus*, *K. pneumoniae* and *E. coli* was evaluated using the method of microbial minimal inhibitory[27]. It is important to mention that the steroid-derivative has a spacer arm in the A-ring of steroid bound to quaternary amine in the azepine ring involved in the *pregnenolone-carbamazepine* complex. In addition were used such biological tools the *carbamazepine-aminocaproic acid* and *hemisuccinate of pregnenolone* to evaluate the molecular mechanism involved in the antibacterial activity of *pregnenolone-carbamazepine* complex. In addition, in this study our aim was to have new drugs that can be used for treatment of infectious disease.

MATERIALS AND METHODS

General methods:

Strains: The microorganisms in this study belonged to the strain bank at the Departament of Pharmaco-Chemistry at the Faculty of Chemical Biological Sciences of the Universidad Autonóma de Campeche. The strains are certified by 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°C in special gel (BBL).

Antimicrobial agents: *Pregnenolone-hemisuccinate* (*5-Pregnen-20-one, 3-(3-carboxy-1-oxopropoxy*), *carbamazepine-amnocaproic acid* conjugate (*Dibenzo [b,f]azepine-5-carboxyl acid (6-amino-hexanoyl)-amide* and *pregnenolone-carbamazepine* complex (*N-{6-[(Dibenzo[b,f]azepine-5-carbonyl)-amino]-6-oxo-hexyl}-succin amic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradeca-hydro-1H-cyclo- penta-phenanthren-3-yl ester)* (Figure 1) were synthesized by the method reported by Figueroa[28,29]. The compounds were dissolved in methanol and diluted with distilled water. *Cefotaxime, gentamicin, methicillin* and *ciprofloxacin* were used as the standard drugs.

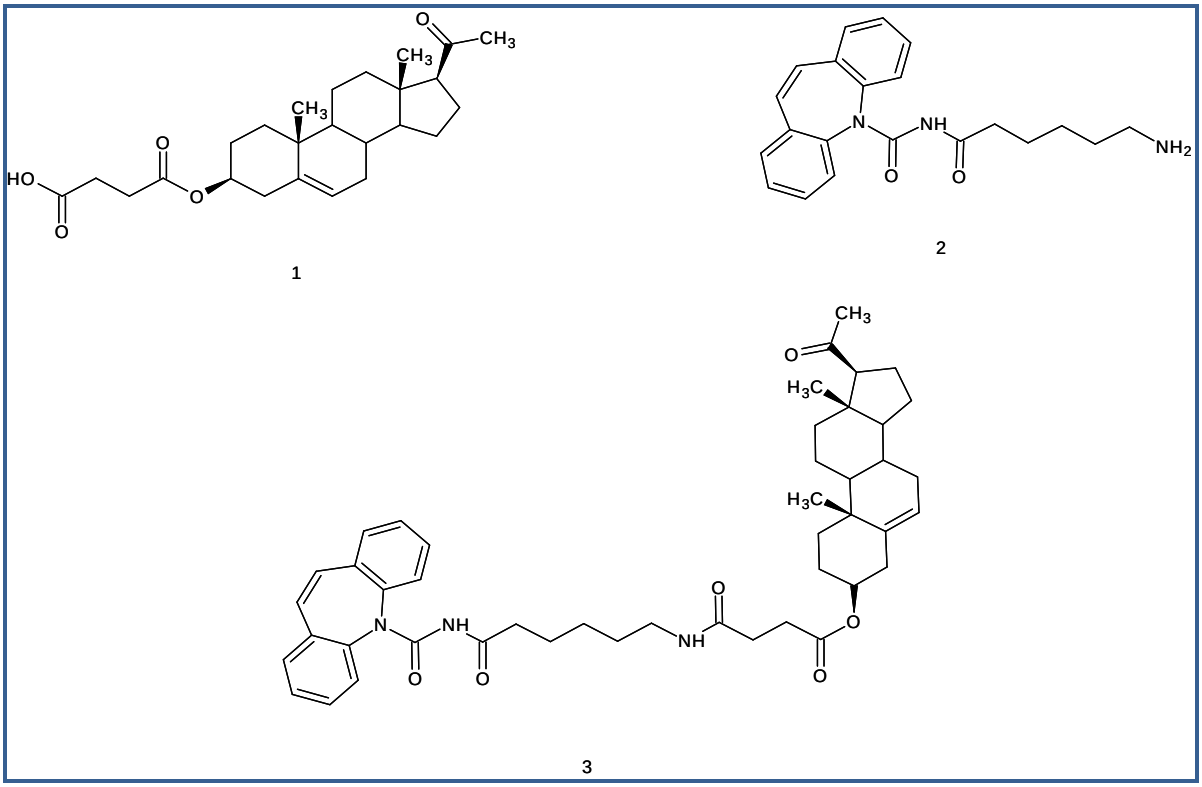

Figure 1: Chemical structure of *hemisuccinate-pregnenolone* (1), *carbamazepine-aminocaproic acid* (2) and *pregnenolone-carbamazepine* complex *(3).*

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 Mc Conkey (*E.coli* and *K. pneumoniae*) and *Staphylococcus* 110 (*S. aureus*) agars for 24 hours at 37°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 (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 Mc-Farland scale (9 x 10^8 cells/ml) and all the tubes were incubated at 37°C for 24 hours. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms, and were incubated for 24 hours 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.

RESULTS

The bacterial activity of *pregnenolone-carbamazepine* complex was compared with the antibacterial effect of *cefotoxime*, *gentamicin, methicillin*, and *ciprofloxacin* (controls) in such bacterial microorganism studied. The results obtained (Figure 2) indicate that bacterial growth of *S. aureus* was inhibited by *cefotaxime* (MIC= 5.23 x 10^{-4} mmol), *gentamicin* (MIC= 2.68 x 10^{-5} mmol), and ciprofloxacin (MIC = 3.77 x 10^{-4} mmol). It is important to mention that ampicillin non inhibit the bacterial growth of same microorganism. Nevertheless in presence of *hemisuccinate-pregnenolone* (MIC= 2.40 x 10^{-3} mmol) and *carbamazepine-aminocaproic acid* (MIC= 6.8 x 10^{-4} mmol) the bacterial growth was blocked in a manner dose-dependent. In addition, the results obtained of the *pregnenolone-carbamazepine* complex showed a MIC of 1.39 x 10^{-4} mmol on bacterial growth of this microorganism.

On the other hand, alternative experimental were made in Gram-negative bacterial (*K. pneumoniae* and *E. coli*) using the same controls to evaluate the antibacterial effect of *pregnenolone-carbamazepine* complex. The results indicate that bacterial growth of *E. coli* was inhibited (Figure 3) in presence of the *hemisuccinate-pregnenolone* compound (MIC= 2.40 x 10^{-3} mmol) and *carbamazepine-aminocaproic acid* conjugate (MIC= 1.36 x 10^{-3} mmol). In addition the *pregnenolone-carbamazepine* complex showed a MIC of 3.18 x 10^{-4} mmol) on *E. coli.* This experimental data showed different antibacterial activity on this same bacterial microorganism in comparison with *cefotaxime* (MIC = 5.23 x 10^{-4} mmol), gentamicin (MIC= 1.34 x 10^{-5} mmol) and ciprofloxacin (MIC = 3.01 x 10^{-3} mmol). It is important to mention that in presence of ampicillin, the bacterial growth of *E. colli* non was blocked.

Other results obtained (Figura 4) showed that *hemisuccinate-pregnenolone* compound (MIC = 2.4 x 10^{-3} mmol) and *carbamazepine-aminocaproic acid* conjugate (MIC= 1.36 x 10^{-3} mmol) blocked the growth bacterial of *K. pneumoniae* in a manner dose-dependent. In addition, this same bacterial organism in presence of the *pregnenolone-carbamazepine* complex the bacterial growth was inhibited (MIC= 3.18 x 10^{-4} mmol). This results were compared with the antibacterial effect of controls, in this case, the cefotaxime showed a MIC of 2.61 x 10^{-4} mmol, the MIC for gentamicin was of 2.68 x 10^{-5} mmol and the MIC for ciprofloxacin was MIC = 1.50 x 10^{-3} mmol.

DISCUSSION

The experimental data obtained indicate that *pregnenolone-carbamazepine complex* had different antibacterial activity in comparison with the controls (*cefotaxime, gentamicin and ciprofloxacin*), this phenomenon could be mainly because different molecular mechanisms exist involved in the antibacterial effect induced by the different compounds. To evaluate the mechanism involved in the antibacterial activity induced by *pregnenolone-carbamazepine complex* on *S. aureus, K. pneumoniae* and *E. coli*, in this study the *hemisuccinte of pregnenolone and carbamazepine-aminocaproic acid* compounds were used as a biological tool. The result showed that bacterial growth of *S. aureus* in presence of hemisuccinte *of pregnenolone* was blocked. The antibacterial activity of this compound may depend on the nature of the free *carboxyl* group contained in their chemical structure (Figure 1), which is a membrane-perturbing agent whose antibacterial activity can be possibly by the interaction with the positively charged *amino* groups contained in the *D-alanyl* incorporated in the teichoic acids that are essential polymers that plays a vital role in the growth and development of the gram-positive bacteria (*S. aureus*)[30]. Here is important to mention that experimental data exist which indicate that *D-alanyl* can modulate cell envelope properties and function of teichoic acids[31] and consequently *D-alanyl ester* group contribute to resistance bacterial on several antimicrobial peptides[32]. In this case, the interaction of *hemisuccinate of pregnenolone* compound with this molecule can blocked the bacterial growth of *S. aureus* in a manner dose-dependent.

Nevertheless, the coupling of *hemisuccinate of pregnenolone* compound to *carbamazepine-aminocaproic acid* conjugate to generate *pregnenolone-carbamazepine* complex, induced a greater antibacterial effect on *S. aureus*. Therefore, this phenomenon indicate that dibenzo-cycloheptano group and functional groups bound to quaternary amine in the azepine ring contained in the *carbamazepine-aminocaproic acid* conjugate appears to be the key requirement for increase the antibacterial activity of *pregnenolone-carbamazepine* complex. To evaluate this possibility the antibacterial activity of *carbamazepine-aminocaproic acid* compound was evaluated. The results (Figure 2) showed that the antibacterial effect on *S. aureus* of this substance was greater in comparison with the antibacterial activity induced by *hemisuccinate of pregnenolone* compound. Nevertheless this phenomenon was smaller in comparison with the antibacterial activity induced by *carbamazepine-pregnenolone* complex. Therefore, these data suggest that dibenzo-cycloheptano group and functional groups bound to quaternary amine in the azepine ring involved in the *pregnenolone-carbamazepina* complex are important to antibacterial activity. This premise is availed by the studies done by Heller and coworkers[33] which indicate that dibenzo-cycloheptano group and functional groups bound to quaternary amine in the azepine ring of both tofranil and perofrane compounds can be specific to their antibacterial activity on *S. aureus* and *E. coli*. Nevertheless, it is important to mention that antibacterial effect induced by

carbamazepine-pregnenolone complex requires also of the hydrophobic region of the hemisuccinate-pregnenolone in order to interact with some components of bacterial cell, disturbing the bacterial growth and to cause cell death.

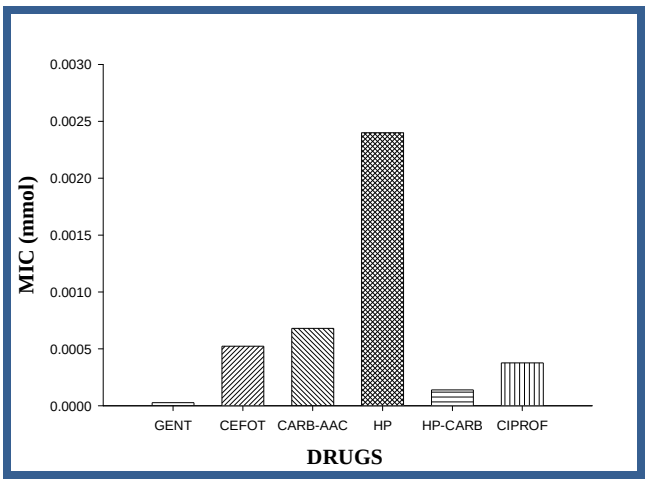

Figure 2: Antibacterial effects induced by *pregnenolone-carbamazepine* complex (HP-CARB), *hemisuccinate of pregnenolone* (HP), *carbamazepine-aminocaproic acid* and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *S. aureus*. The results showed that *S. aureus* was susceptibly to cefotaxime (MIC = 5.23 x 10^{-4} mmol), gentamicin (MIC = 2.68 x 10^{-5} mmol) and ciprofloxacin (MIC = 3.77 x 10^{-4} mmol). In addition, the bacterial growth in presence of the hemisuccinate-pregnenolone (MIC = 2.40 x 10^{-3} mmol) and the *carbamazepine-aminocaproic acid* (MIC = 6.80 x 10^{-4} mmol) was inhibited. This pathogen microorganism was inhibited with the *pregnenolone-carbamazepine* complex (MIC = 1.39 x 10^{-4} mmol).

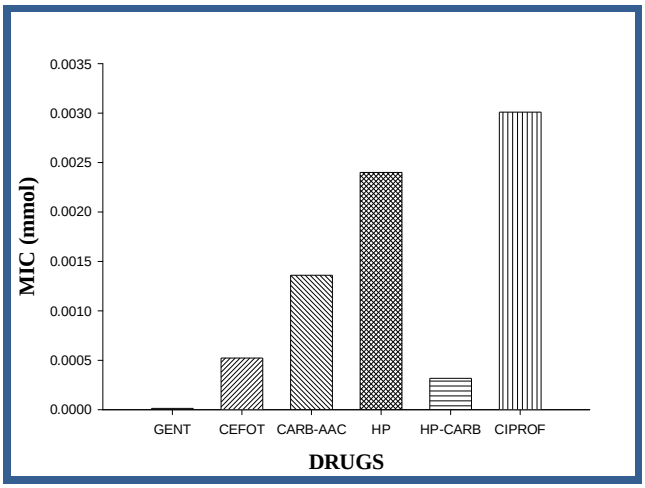

Figure 3: Antibacterial activity induced by *pregnenolone-carbamazepine* complex (HP-CARB), *hemisuccinate of pregnenolone* (HP), *carbamazepine-aminocaproic acid* (CARB-AAC) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *E. coli*. Experimental data showed that *E. coli* was susceptibly to CEFOT (MIC = 5.23 x 10^{-4} mmol), GENT (MIC = 1.34 x 10^{-5} mmol) and CIPROF (MIC = 3.01 x 10^{-3} mmol). Nevertheless, in presence of HP the MIC was of 2.40 x 10^{-3} mmol and for CARB-AAC the MIC was of 1.36 x 10^{-3} mmol. In addition, the bacterial growth of *E. coli* with *pregnenolone-carbamazepine* complex was inhibit (MIC = 3.18 x 10^{-4} mmol).

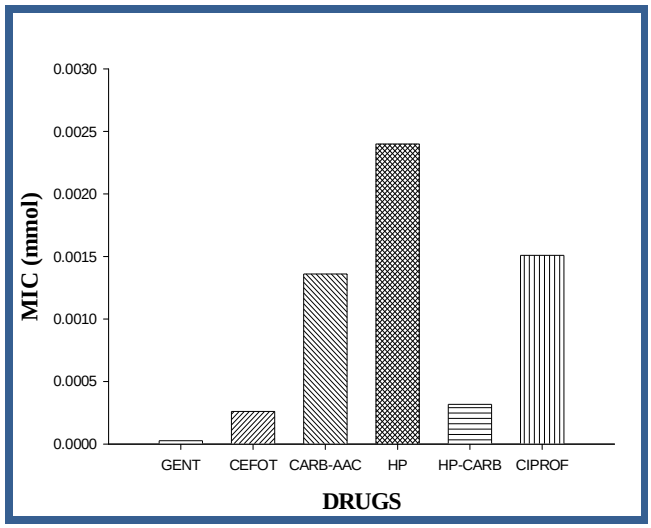

Figure 4: Effects induced by *pregnenolone-carbamazepine* complex (HP-CARB), *hemisuccinate of pregnenolone* (HP), *carbamazepine-aminocaproic acid* (CARB-AAC) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *K. pneumoniae*. The results showed that bacterial growth of *K. pneumoniae* in presence of CEFOT (MIC = 2.61 x 10^{-4} mmol), GENT (MIC = 2.68 x 10^{-5} mmol), CIPROF (1.50 x 10^{-3} mmol), HP (MIC = 2.40 x 10^{-3} mmol) and CARB-AAC (1.36 X 10^{-3} mmol) was inhibited. In addition, the *pregnenolone-carbamazepine* complex showed a MIC = 3.18 x 10^{-4} mmol.

On the other hand, the results obtained to evaluate the antibacterial effects of the pregnenolone-carbamazepine complex on Gram-negative bacteria (E. coli, Figure 3; and K. pneumoniae, Figure 4) indicate that differences between the pregnenolone-derivatives and the controls exist. In addition the results showed that bacterial activity of *pregnenolone-carbamazepine complex* was greater in comparison with the *hemisuccinate-pregnenolone* compound and *carbamazepine-aminocaproic acid* conjugate. Nevertheless, the molecular mechanism could be different in comparison with the antibacterial activity exert on *S. aureus*. Possibly the antibacterial activity involve the intramolecular interaction of hemisuccinate of pregnenenolone via divalent cations (Mg2+ and Ca2+), involved in the membrane cell providing a substantial increase the permeability of the outer membrane of Gram-negative bacteria include bactericidal/ permeability increasing protein. In addition, the antibacterial effect of *carbamazepine-aminocaproic acid* possibly could be mainly by the interaction of free amine group with the lipid A of Gram-negative bacteria, this premise is availed by Li[34] and Ding[35], who developed a class of cationic compounds-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.

In conclusion, in this work the bacterial microorganism such as *S. Aureus*, *K. pneumoniae* and *E. Coli* were more susceptible to both *gentamicin* and *pregnenenolone-carbamazepine* complex and were more resistant to that *hemisuccinate-pregnenenolone* conjugate. Possibly the molecular mechanism could be by mimic the actions of several steroid-derivates with antibacterial characteristics. The molecular mechanism suggest that *pregnenolone-carbamazepine* complex can adopt cationic, facially amphiphilic conformations and involved the nature of functional groups contained in the chemical structure, which appears to be key requirement for antibacterial activity. This chemistry structure allows them to disrupt bacterial membranes at relatively different concentrations by the interaction with some factors in the bacterial membrane as effective targets of the *pregnenolone-carbamazepine* complex.

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