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ORIGINAL PAPER

Synthesis of a dihydrotestosterone–ciprofloxacin conjugate: relationship between descriptors log*P*, π, R_m, and V_m and its antibacterial activity in *S. aureus* and *E. coli*

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Abstract In this work a dihydrotestosterone–ciprofloxacin conjugate was synthesized. The route involved preparation of a ciprofloxacin–ethylenediamine derivative by the reaction of ciprofloxacin with ethylenediamine using a carbodiimide or boric acid as catalysts. Additionally, the ciprofloxacin derivative was bound to dihydrotestosterone hemisuccinate to form the dihydrotestosterone–ciprofloxacin conjugate in the presence of a carbodiimide. The antibacterial activity of dihydrotestosterone–ciprofloxacin, as well as ciprofloxacin–ethylenediamine and ciprofloxacin, was evaluated in vitro on *S. aureus* and *E. coli* using the dilution method and the minimum inhibitory concentration. To delineate the structural chemical requirements of the compounds ciprofloxacin, ciprofloxacin–ethylenediamine and dihydrotestosterone–ciprofloxacin conjugate as antibacterial agents on *S. aureus* and *E. coli*, other parameters such as the descriptors log*P*, π, R_m, and V_m were calculated. The results showed that bacterial growth of the microorganisms studied was inhibited by ciprofloxacin, ciprofloxacin–ethylenediamine and dihydrotestosterone–ciprofloxacin in a dose-dependent manner. These data suggest that functional groups involved in the structure of the studied compounds are specific for their antibacterial activity.

Keywords Ciprofloxacin · Dihydrotestosterone · Ethylenediamine · Antibacterial

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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] and *E. coli* [5] among others [6, 7], have been shown to accelerate the 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]. Therefore, new drugs have been developed for control of bacterial resistance [15–17]. For example, there has been a resurgence of interest in fluoroquinolones as potential therapeutic agents for infectious diseases [18]. In this context, several ciprofloxacin derivatives have been synthesized and their antibacterial activity evaluated [19]. For example, 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-methylcarbamate and 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-3-oxopyrazolo[4,3-c]quinoline were synthesized and tested for their antibacterial effect [20]. Additionally, other studies by Foroumadi and coworkers [21] showed the synthesis of a series of *N*-[2-(5-bromo-2-thienyl)-2-oxoethyl]

and *N*-[2-(5-bromo-2-thienyl)-2-oximinoethyl] derivatives of piperazinylquinolones and the evaluation of their antimicrobial activity against Gram-positive and Gram-negative microorganisms. In addition, there are studies that show the synthesis of *N*-phenethylpiperazinylquinolones and the determination of their antibacterial activity on Gram-positive and Gram-negative bacteria [22]. Other studies showed a number of levofloxacin analogues with certain bulky residues on the piperazine ring that were prepared and evaluated as antibacterial agents on Gram-positive and Gram-negative bacteria [23]. Additionally, recently several enoxacin carboxamide derivatives were synthesized by the modification of a carboxyl group from enoxacin, and their biological activity was evaluated [24]. All these experimental data show several protocols for synthesis of fluoroquinolone derivatives with biological activity; nevertheless, the evaluation of antibacterial effects by modification of the carboxyl group of ciprofloxacin to produce new antibacterial drugs has not been studied extensively. Therefore, in this work, the objective is to synthesize a new ciprofloxacin derivative that can be used for treatment of infectious diseases. For this purpose, the dihydrotestosterone–ciprofloxacin conjugate was evaluated for its antibacterial activity on *S. aureus* and *E. coli* using the microbial minimal inhibitory concentration (MIC) method [25]. Additionally, to delineate the structural chemical requirements of the compounds ciprofloxacin (**1**), ciprofloxacin–ethylenediamine (**2**), and dihydrotestosterone–ciprofloxacin conjugate (**4**) as antibacterial agents on *S. aureus* and *E. coli*, several parameters such as the descriptors log*P*, π, R_m, and V_m were calculated.

Results and discussion

In this study, a straightforward route is reported for the synthesis of dihydrotestosterone–ciprofloxacin conjugate **4**, which has a spacer arm with both ester and amide groups (Fig. 2) between the fragments of dihydrotestosterone and ciprofloxacin (**1**). The first step was achieved by reacting ethylenediamine hydrochloride with **1** to form an amide bond to yield **2** (Fig. 1). Several procedures for the formation of amide groups are described in the literature

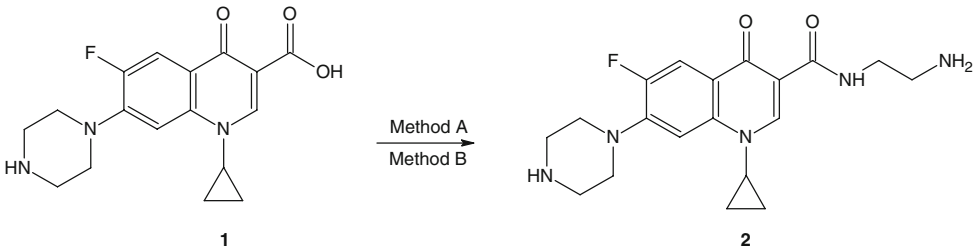

Fig. 1 Synthesis of *N*-(2-aminoethyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) quinoline-3-carboxamide (**2**) by reaction of ciprofloxacin (**1**) with ethylenediamine hydrochloride using 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (method A) or boric acid (method B) as catalysts

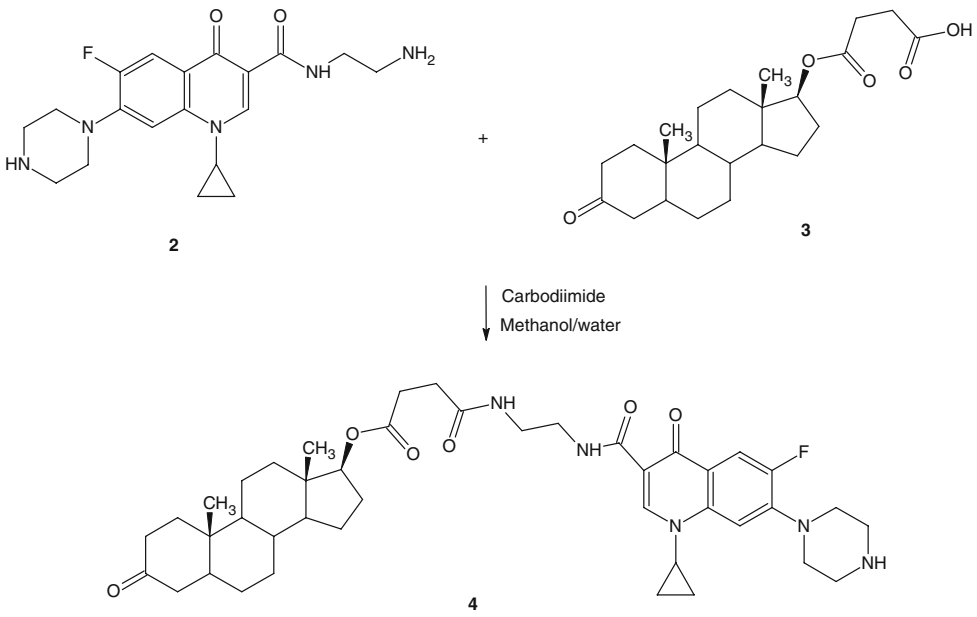

Fig. 2 Synthesis of dihydrotestosterone-ciprofloxacin (**4**). Reaction of **2** with dihydrotestosterone hemisuccinate (**3**) to form **4** using 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide as catalyst in acetonitrile/water

[26–28]; the most widely practiced method employs carboxylic acid chlorides as the electrophiles, which react with the amine in the presence of an acid scavenger [29]. Despite its wide scope, this protocol suffers from several drawbacks; most notable are the limited stability of many acid chlorides and the need for hazardous reagents (thionyl chloride) for their preparation [30]. In this work two different methods for amide formation were employed: in the first one the technique reported by Pingwah [31] for boric acid catalyzed amidation of carboxylic acids and amines (method B) was used; in the second one we used a carbodiimide derivate (method A) as catalyst [32] for the amide bond formation to obtain **2**. The use of carbodiimide was found to result in higher yields compared to the amide bond forming with method B.

In addition to the characteristic chemical shifts of compound **1**, the ^{1}H NMR spectra of **2** showed upfield chemical shifts at 0.96–1.17 ppm for methylene protons of the cyclopropane ring. Signals at 2.98 and 3.50 ppm were found for methylene protons involved in the arm bound to the quinone ring. Additionally, another signal at 4.95 ppm corresponding to both protons of amide and amino groups was found. The ^{1}H NMR spectra of secondary amides are usually more complex than those of primary amides because of the presence of a substituent bonded to the amide nitrogen atom. These substituents produce a much wider range of chemical shifts for the amide proton, which may, in addition, display coupling to aliphatic groups bonded to it. The chemical shifts of aliphatic groups bonded to the carbonyl group are similar to those observed for primary amides, while those groups bonded to the nitrogen resonate at slightly lower field than the corresponding amines [33]. Finally, other signals at 7.58–8.42 ppm for aromatic rings were found.

The ^{13}C NMR spectra displayed chemical shifts at 7.7 and 29.7 ppm for carbon atoms of the cyclopropane ring of **2**. The methylene groups at the free amine resonate at 42.6 and 43.7 ppm. In addition, there are two signals at 46.1 and 53.3 ppm corresponding to carbons in the piperazine ring and several signals at 102.4–153.2 for the aromatic rings. Additionally, a signal at 157.7 ppm for the amide group was found. Finally, a signal at 177.3 ppm corresponding to a ketone group was found. The presence of **2** was further confirmed from the mass spectrum, which showed a molecular ion at $m/z = 373.12$.

The second step was achieved by reacting **2** with dihydrotestosterone hemisuccinate **3** to form the dihydrotestosterone–ciprofloxacin conjugate **4**, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as catalyst. Compound **4** contains a spacer arm with both ester and amide functional groups which couples the D-ring of the steroid nucleus to the quinone ring of the ciprofloxacin fragment.

The ^{1}H NMR spectrum of **4** showed signals at 0.78 and 0.98 ppm corresponding to methyl groups present in the steroid nucleus. In addition, there are two signals at 0.96 and 1.16 ppm for methylene protons of the cyclopropane ring. Other signals at 2.46 ppm for the methylene group bound to the ester and at 3.33–3.50 ppm for the methylene group next to the amide group were found. Additionally, a signal at 7.16 ppm for the protons of the amide groups and the piperazine ring was found. Finally, several signals at 7.56–8.38 ppm corresponding to aromatic rings were found.

The ^{13}C NMR spectra display chemical shifts at 12.0 and 17.0 ppm for the methyl groups present in the steroid nucleus. The chemical shift of the methylene next to the ester group is found at 29.1 ppm. There are two signals at 38.5 and 39.7 ppm for methylene groups bound to the amide group. In addition, several signals at 42.6–45.5 ppm corresponding to carbons of the steroid nucleus and another signal at 46.0 ppm for carbons of the piperazine ring were found. Additionally, other signals at 103.0–148.9 ppm corresponding to aromatic rings were found. Finally, several signals at 157.0 and 171.6 ppm corresponding to amide groups, at 172.0 ppm for the ester group and at 177.2 and 212.4 ppm for ketones, were found. Additionally, the presence of **4** was further confirmed from the mass spectrum, which showed a molecular ion at $m/z = 745.42$.

Biological analyses

The antibacterial activity of **4** on *S. aureus* and *E. coli* was evaluated by means of the dilution method and the minimum inhibitory concentration (MIC), using gentamicin, ampicillin, and cefotaxime as control. The results obtained (Fig. 3) indicate that bacterial growth of *S. aureus* was inhibited with cefotaxime (MIC = 0.125 mg/cm^3, 2.74×10^{-4} mmol/cm^3), gentamicin (MIC = 0.125 mg/cm^3, 2.61×10^{-4} mmol/cm^3), and compound **1** (MIC = 0.125 mg/cm^3, 3.77×10^{-4} mmol/cm^3). In addition, the bacterial growth of *S. aureus* in the presence of compound **4** (MIC = 0.5 mg/cm^3, 6.70×10^{-4} mmol/cm^3) was blocked. Other results obtained (Fig. 4) showed that bacterial growth of *E. coli* in the presence of cefotaxime (MIC = 0.125 mg/cm^3, 2.74×10^{-4} mmol/cm^3), gentamicin (MIC = 0.062 mg/cm^3, 1.37×10^{-4} mmol/cm^3), compound **1** (MIC = 0.0625 mg/cm^3, 1.88×10^{-4} mmol/cm^3), and compound **4** (MIC = 0.5 mg/cm^3, 6.70×10^{-4} mmol/cm^3) was inhibited. All these data indicate that compound **4** has different antibacterial potency for *S. aureus* and *E. coli* in comparison with cefotaxime (a β-lactam antibiotic) [34], gentamicin (an inhibitor of protein synthesis) [35], and **1** (an inhibitor of DNA gyrase) [36]. This phenomenon may be attributed mainly to the different

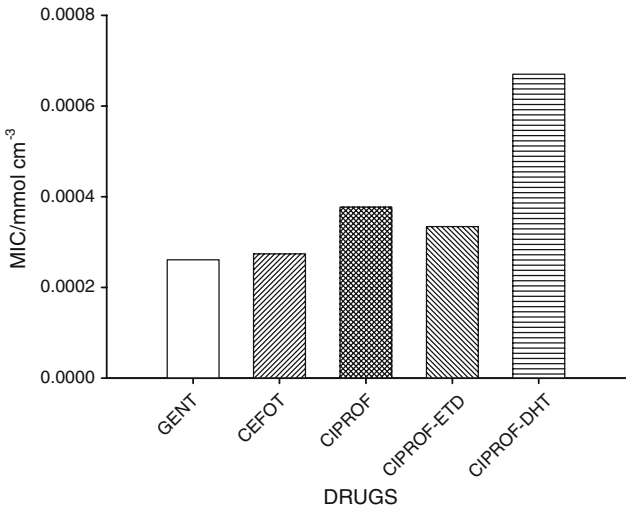

Fig. 3 Antibacterial effects induced by dihydrotestosterone–ciprofloxacin conjugate **4** and controls (cefotaxime, CEFOT; gentamycin, GENT; ciprofloxacin, CIPROF) on *S. aureus*. Experimental data showed that *S. aureus* was susceptible to cefotaxime (MIC = 2.74×10^{-4} mmol/cm^3), gentamycin (MIC = 2.61×10^{-4} mmol/cm^3), and compound **1** (MIC = 3.77×10^{-4} mmol/cm^3). In addition, the bacterial growth of this microorganism in the presence of the compounds **2** (MIC = 3.34×10^{-4} mmol/cm^3) and **4** (MIC = 6.70×10^{-4} mmol/cm^3) was inhibited

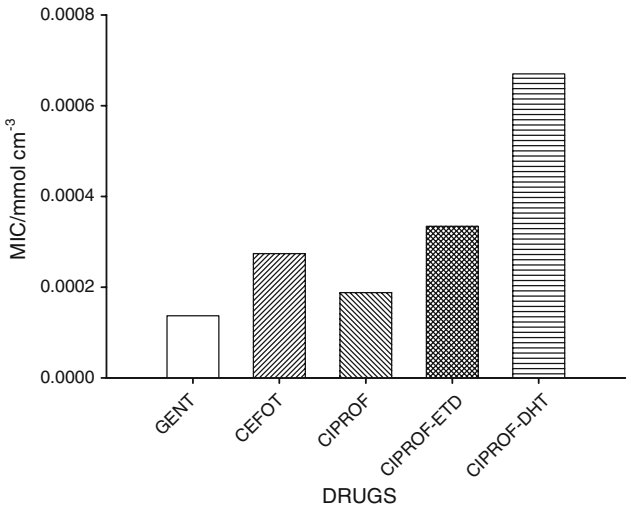

Fig. 4 Antibacterial effects induced by dihydrotestosterone–ciprofloxacin conjugate **4** and controls (cefotaxime, CEFOT; gentamicin, GENT; ciprofloxacin, CIPROF) on *E. coli*. There are differences in the antibacterial activity of CEFOT (MIC = 2.74×10^{-4} mmol/cm^3), GENT (MIC = 1.37×10^{-4} mmol/cm^3), and compound **1** (MIC = 1.88×10^{-4} mmol/cm^3) on *E. coli* in comparison with the compounds **2** (MIC = 3.34×10^{-4} mmol/cm^3) and **4** (MIC = 6.70×10^{-4} mmol/cm^3)

molecular mechanism involved and the characteristic chemical structure of the compounds studied in this work.

In this sense, it is interesting to consider the molecular mechanism involved in the effects induced by **4** on the growth of *S. aureus* and *E. coli*. Compound **3** was used as a pharmacological tool to evaluate the molecular mechanism involved in the antibacterial activity of **4**, because several reports have indicated that some steroid derivatives induce antibacterial effects on *S. aureus* and *E. coli* [37, 38]. The results showed that in the presence of **3** the bacterial growth of *E. coli* and *S. aureus* was not blocked (data not shown). These experimental data suggest that the dihydrotestosterone derivative by itself does not have antibacterial activity on the microorganisms studied and that the other fragment (**2**) involved in chemical structure of **4** could only be responsible for the antibacterial activity. In order to analyze this possibility, an alternative experiment with *S. aureus* and *E. coli* using **2** was made to compare its effects with those induced by **4**. The results obtained (Figs. 3, 4) indicate that bacterial growth of *S. aureus* and *E. coli* was inhibited by compound **2** (MIC = 0.125 mg/cm^3, 3.34×10^{-4} mmol cm^3). These results indicate that the antibacterial effect of **2** was high in comparison with **4**; these experimental data suggest that the antimicrobial effect induced by **2** can depend on the nature of the free amine group contained in its chemical structure, which could be a membrane-perturbing agent whose antibacterial activity is possibly induced by the interaction with teichoic acids, which are essential polymers that play a vital role in the growth and development of Gram-positive bacteria [39]. Another possibility is the intermolecular interaction of **2** with divalent cations (Mg^{2+} and Ca^{2+}) involved in the outer membrane of Gram-negative bacteria that consequently brings cell death.

Nevertheless, it is important to mention that when **2** is bound with **3** to form **4**, the antibacterial activity seems to be low, possibly because **2** does not require a hydrophobic region of the steroid derivative in order to interact with the cell surface and integrate into the cytoplasmic membrane and induce bacterial death. Nevertheless, in the case of **4**, such integration into the membrane could perturb bacterial growth in a dose-dependent manner; this phenomenon can induce, as consequence, an increase in the permeability of the outer membrane and induce bacterial growth inhibition on these pathogen microorganisms. This premise is supported by some mechanisms, based on experimental data indicating that several steroid derivatives can adopt several conformations to induce bacterial death [40, 41].

All data suggest that the structural chemistry of compounds studied in this work is specific for their antibacterial activity. Therefore, to delineate the structural chemical requirements of the compounds **1**, **2**, and **4** as antibacterial agents on *S. aureus* and *E. coli*, other parameters such as the descriptors log*P* and π [42] were calculated. Log*P* describes the logarithmic octanol-water partition coefficient; therefore, it represents the lipophilic

effects of a molecule that includes the sum of the lipophilic contributions of the parent molecule and its substituents [43]. The difference between the substituted and unsubstituted log*P* values is conditioned by the π value for a particular substituent. Hammett showed that π values measure the free energy change caused by a particular substituent to relate to biological activity [44]. Therefore, in this work, the log*P* and π parameters were calculated by the method reported by Leo and coworkers [45]. The results (Table 1) showed an increase in log*P* and π values in compounds **2** and **4** with respect to **1**. This phenomenon is conditioned mainly by the contribution of all substituent atoms involved in the chemical structure of the different compounds, as is showed in Table 1. These results showed that aliphatic carbons in compound **2** contribute to the high lipophilicity in comparison with **1**. Additionally, other results showed that the lipophilicity of **4** is high in comparison with **1** and **2**. This phenomenon is due to the presence of the methyl groups in the steroid nucleus and the aliphatic carbons.

All data indicate that an increase in the degree of lipophilicity could affect the antibacterial activity of compounds **1**, **2**, and **4** on the microorganisms studied. To prove the existence of a correlation between the calculated log*P* and antibacterial activities of all compounds studied, the MIC was calculated using the method proposed by Hansch [46] and compared with experimental MIC values (Tables 2, 3). The statistical analysis showed a correlation between the experimental and calculated MIC values of $r = 0.88$ on *S. aureus* (Fig. 5). The results on *E. coli* (Fig. 6) showed a relationship between the observed and the calculated MIC of $r = 0.96$. Nevertheless, it is important to mention that there is some variability in the comparison of experimental and calculated MIC that may be because of other chemical parameters involved in the antibacterial activity of the compounds studied. Therefore, in this work, some steric constants such as the molar volume (V_m) and molar refractivity (R_m) were calculated. These options are a useful tool for the correlation of different properties that depend on characteristics of substituents attached to a constant reaction center. The results showed an increase in both R_m and V_m values for **4** in comparison with **1** and **2**. These data indicate that steric impediment could affect the antibacterial activity of **4**. Additionally, different molecular mechanisms and conformational preferences and internal rotation of **4** could influence the antibacterial activity on *S. aureus* and *E. coli*. These data are supported by studies reported by Bryantsev and coworkers [47], who showed that conformational differences between several chemical functional groups have important consequences in the union to biological molecules by conformational changes.

Experimental

Dihydrotestosterone hemisuccinate (**3**) was prepared according to the method reported by Erlanger [48]. 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 was carried out in dry methanol on a Perkin-Elmer model 552 spectrophotometer, and infrared spectra were recorded on a Perkin-Elmer Lambda 40 spectrometer from KBr pellets. ^{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. The results were found to agree favorably with the calculated values.

N-(2-Aminoethyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxamide (**2**, $C_{19}H_{24}FN_5O_2$)

Method A: A solution of 200 mg **1** (0.60 mmol), 160 mg ethylenediamine hydrochloride (1.20 mmol), and 173 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.90 mmol) in 15 cm^3 of dioxane/water (3:1) was stirred at room temperature for 72 h. Then the solvent was removed under vacuum, and the crude product was purified by crystallization from methanol/*n*-hexane/water (3:2:1), yielding 60% of product **2**. M.p.: 175 °C; UV (MeOH): λ_{max} (log ε) = 223 (2.67) nm; IR: $\overline{\nu}$ = 3,380, 1,680, 1,118 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.96 (m, 2 H), 1.17 (m, 2H), 2.91 (m, 2H), 2.96–2.98 (m, 4H), 3.01–3.13 (m, 4 H), 3.50 (m, 2H), 3.85 (m, 1H), 4.95 (s, 4H), 7.58 (d, J = 5.1 Hz, 1H), 7.74 (s, 1H), 8.42 (d, J = 1.65 Hz, 1H) ppm; ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 7.7 (C-22, C-23), 29.7 (C-21), 42.6 (C-26), 43.7 (C-25), 46.1 (C-3, C-5), 53.3 (C-2), 53.4 (C-6), 102.4 (C-16), 109.3 (C-9), 117.2 (C-12), 119.3 (C-10), 136.8 (C-15), 142.9 (C-13), 146.7 (C-7), 153.2 (C-8), 157.7 (C-19), 177.3 (C-11) ppm; MS (70 eV): *m/z* = 373.12 ([M + 10]$^+$), 253.10, 205.17, 177.22.

Method B: 200 mg (0.60 mmol) of **1** was added to a solution of 160 mg ethylenediamine hydrochloride (1.20 mmol) and 64 mg boric acid (1.06 mmol) in 15 cm^3 acetonitrile/water (3:1), and the mixture was

Table 1 Physicochemical parameters (log*P*, [logK_{ow}], and π) of compounds **1**, **2**, and **4**

Compound	LogK_{ow} fragment	Contribution
1	–CH_2– [aliphatic carbon]	2.9466
	–CH [aliphatic carbon]	0.3614
	=CH– or =C < [olefinic carbon]	0.7672
	–NH– [aliphatic attach]	−1.4962
	Aromatic carbon	1.7640
	–N [aliphatic N, one aromatic attach]	−1.8340
	–CO_2H [acid, aliphatic attach]	−0.6895
	–F [fluorine, aromatic attach]	0.2004
	Ketone in a ring [olefin, aromatic attach]	−0.5497
	Amino acid [olefin; non-alpha carbon type]	−1.7000
	Equation constant	0.2290
	LogK_{ow}	−0.0008
	π	2.3895
2	–CH_2– [aliphatic carbon]	3.9288
	–CH [aliphatic carbon]	0.3614
	=CH– or =C < [olefinic carbon]	0.7672
	–NH_2 [aliphatic attach]	−1.4148
	–NH– [aliphatic attach]	−2.9924
	Aromatic carbon	1.7640
	–N [aliphatic N, one aromatic attach]	−1.8340
	–F [fluorine, aromatic attach]	0.2004
	–C(=O)N [aliphatic attach]	−0.5236
	Ketone in a ring [olefin, aromatic attach]	−0.5497
	C–(C(=O)–)–C(=)N structure correction	0.9755
	Equation constant	0.2290
	LogK_{ow}	0.9118
	π	2.3903
4	–CH_3 [aliphatic carbon]	1.0946
	–CH_2– [aliphatic carbon]	9.3309
	–CH [aliphatic carbon]	2.5298
	–C [aliphatic carbon: No H, not tert]	0.9723
	–NH– [aliphatic attach]	−4.4886
	Aromatic carbon	1.7640
	–N [aliphatic N, one aromatic attach]	−1.8340
	–C(=O)– [carbonyl, aliphatic attach]	−1.5586
	–C(=O)– [carbonyl, one aromatic attach]	−0.8667
	–C(=O)O [ester, aliphatic attach]	−0.9505
	–F [fluorine, aromatic attach]	0.2004
	–C(=O)N [aliphatic attach]	−1.0472
	–tert carbon [3 or more carbon attach]	0.5352
	C–(C(=O)–)–C(=)N [structure correction fused aliphatic ring unit correction]	0.9755
		−2.0526
	Equation constant	0.2290
	LogK_{ow}	4.8335
	π	2.4432

stirred at room temperature for 72 h. Then the solvent was removed under vacuum, and the crude product was purified by crystallization from methanol/*n*-hexane/water (3:2:1) yielding 40% of product **2**; ^{1}H NMR and ^{13}C NMR data obtained were similar to the product from method A.

Table 2 Experimental (log [mmol cm^{-3}/MIC_{EXP}]) and calculated (log [mmol cm^{-3}/MIC_{CALC}]) minimum inhibitory concentration

Compound	log [mmol cm^{-3}/ MIC_{EXP}]		log [mmol cm^{-3}/ MIC_{CALC}]	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
GENT	3.58	3.86	3.65	3.98
CEFOT	3.56	3.56	3.44	3.53
CIPROF	3.42	3.72	3.49	3.63
CIPROF-ETD	3.47	3.47	3.42	3.50
CIPROF-DT	3.17	3.17	3.19	3.17

Table 3 Physicochemical parameters of compounds **1**, **2**, and **4**

Compound	R_m (cm^3)	V_m (cm^3)
1	83.25	226.7
2	98.11	274.7
4	199.87	567.6

R_m molar refractivity, V_m molar volume

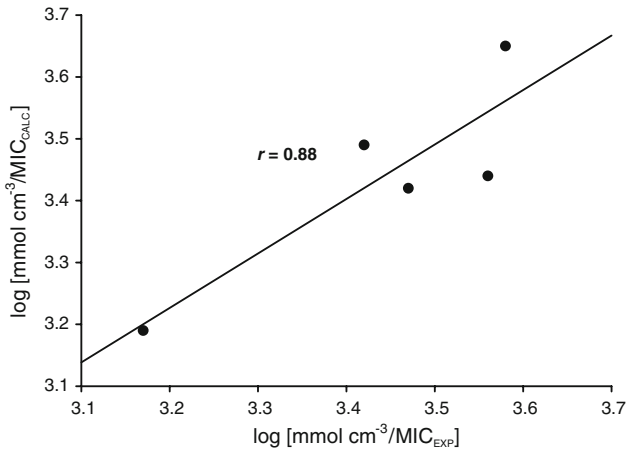

Fig. 5 Correlation between experimental (log [mmol cm^{-3}/ MIC_{EXP}]) and calculated MIC (log [mmol cm^{-3}/MIC_{CALC}]) on *S. aureus*

3-Oxoandrostan-17-yl 4-[2-[1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinolin-3-ylcarbonylamino]ethylamino]-4-oxobutanoate (**4**, $C_{42}H_{56}FN_5O_6$)

A solution of 200 mg **2** (0.53 mmol), 207 mg dihydrotestosterone hemisuccinate (0.53 mmol), and 200 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.04 mmol) in 15 cm^3 of methanol/water (3:1) was stirred at room temperature for 72 h. The reaction mixture was evaporated to a smaller volume, diluted with water, and extracted with chloroform. The organic phase was evaporated to dryness

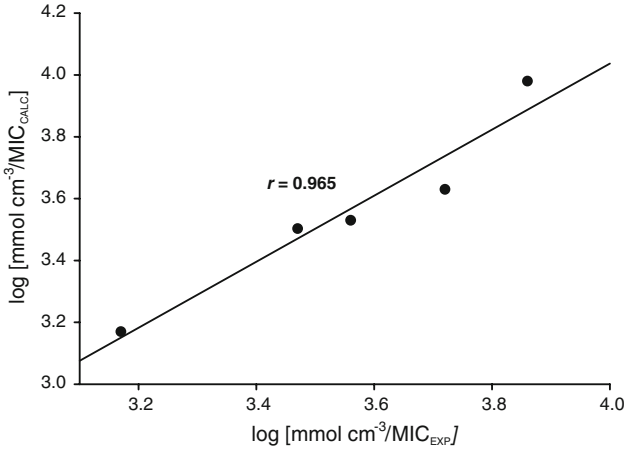

Fig. 6 Correlation between experimental (log [mmol cm^{-3}/ MIC_{EXP}]) and calculated MIC (log [mmol cm^{-3}/MIC_{CALC}]) on *E. coli*

under reduced pressure, and the residue was purified by crystallization from *n*-hexane/methanol/water (2:3:1), yielding 70% of product **4**. M.p.: 162–165 °C; UV (MeOH): λ_{max} (log ε) = 222 (3.14), 240 (3.89) nm; IR: $\bar{\nu}$ = 1,684, 1,670, 1,130 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (s, 3H), 0.90 (m, 1H), 0.96 (m, 2H), 0.98 (s, 3H), 1.02–1.08 (m, 2H), 1.12 (m, 1H), 1.16 (m, 3H), 1.18 (m, 2H), 1.21–1.35 (m, 3H), 1.50–1.58 (m, 3H), 1.70–1.86 (m, 4H), 2.02–2.22 (m, 5H), 2.46–2.52 (s, 4H), 2.88–2.96 (m, 4H), 3.02 (m, 2H), 3.33–3.50 (s, 4H), 3.80 (m, 1H), 4.62 (m, 1H), 7.16 (s, 3H), 7.56 (d, 1H), 7.70–8.38 (s, 2H) ppm; ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 7.7 (C-22, C-23), 12.0 (C-52), 17.0 (C-53), 20.4 (C-47), 23.5 (C-45), 27.1 (C-42), 27.5 (C-44), 28.8 (C-43), 29.1 (C-21), 30.3 (C-30), 35.2 (C-38), 35.3 (C-40), 36.8 (C-46), 37.4 (C-49), 38.0 (C-48), 38.5 (C-26), 39.7 (C-25), 42.6 (C-36), 43.4 (C-51), 45.5 (C-41), 46.0 (C-3, C-5), 47.3 (C-39), 50.5 (C-37), 53.2 (C-2, C-6), 81.6 (C-35), 103.0 (C-16), 110.0 (C-9), 117.0 (C-12), 120.0 (C-10), 136.0 (C-15), 140.6 (C-13), 142.0 (C-7), 148.9 (C-8), 157.0 (C-19), 171.6 (C-28), 172.0 (C-32), 177.2 (C-11), 212.4 (C-50) ppm; MS (70 eV): *m/z* = 745.42 ($[M + 10]^+$), 678.40, 456.87.

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) and *E. coli* (ATCC 25922). The strains were kept under refrigeration at 4 °C in special gel (BBL).

Antimicrobial agents

The 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 the antimicrobial effect of the different compounds on the bacterial species was made by a method described by Chiong et al. [25]. The bacterial species were incubated on McConkey (*E. coli*) and Staphylococcus 110 (*S. aureus*) agars for 24 h at 37 °C. 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 cm^3 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 cm^3 of the studied compound (1 mg/cm^3) was added and stirred; from this tube an aliquot of 2 cm^3 was taken and added to the following tube (simple concentration), and the process was successively repeated until the last 2 cm^3 of dissolution had been used up. After this process, each tube was inoculated with 0.1 cm^3 of the bacterial suspension, whose concentration corresponded to McFarland scale (9×10^8 $cells/cm^3$), 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 incubated for 24 h at 37 °C.

Statistical analysis

The relationship of MICs was calculated with the program SPSS 12.0.

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