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Design and Synthesis of New Steroid-Derivatives with Antibacterial Activity on *Salmonella typhi*

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In this study, the following estrogen derivatives were synthesized; oxazin-estradiol-3,17-diol (**6**), oxazine-estradiol-3,17-diyl*bis*-2-chloroacetate (**8**), chloro-acetic acid-estradiol ester (**9**), 3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-estradiol-1,2-diamine (**10**) and 3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-estradiol-chloro-acetamide (**11**) using several strategies. The structure of compounds obtained was confirmed by elemental analysis, spectroscopy and spectrometry data. On the other hand, antibacterial activity of compounds synthesized was evaluated on *Salmonella typhi* with broth dilution methods to determine the minimal inhibitory concentration using gentamycin, ciprofloxacin and cefotaxime as controls. The results indicate that only the compounds **6**, **9**, **10** and **11** decrease the growth of *Salmonella typhi*. The methods used for synthesis of estrogen derivatives offers some advantages such as simple procedure, low cost and ease of workup. In addition, the antibacterial activity showed the compounds **6**, **9**, **10** and **11** depend on chemical structure in comparison with the controls involved. These estrogenic derivatives could be used as a therapeutic alternative for treatment of infectious diseases induced by *Salmonella typhi*.

Keywords: Estrogen derivative, Estradiol, Chloroacetyl chloride, *Salmonella typhi*.

INTRODUCTION

There are reports which indicate that *Salmonella typhi* is a human pathogen that induces several deaths each year [1-3]. Diverse drugs have been used for their treatment. However, some strains of *Salmonella typhi* have induced resistance to chloramphenicol, ampicillin and trimethoprim, streptomycin, sulfonamides and tetracyclines in under developing countries [4,5]. In search of new alternative therapeutics for treatment of resistance exerted by *Salmonella typhi*, have developed several antibacterial drugs. For example, the synthesis of several hydrazones were prepared by reacting isatin and aromatic primary amines/hydrazines [6]. Other data showed that several polysaccharides-tetanus toxoid conjugates induce antibacterial activity on *Salmonella typhi* [7].

On the other hand, also some steroids as potential therapeutic agents have been developed for *Salmonella typhi*; for example, there is a study which showed the synthesis of steroidal thiocarbazone derivatives with antibacterial activity on *Salmonella typhimurium* [8]. In addition, a steroid derivative (cholest-5-en-3-oxazolo) was synthesized and their antibacterial effect was evaluated on *Salmonella typhimurium* [9]. Other data indicate the preparation of a steroid-thiourea derivative with antibacterial effect on *Salmonella typhimurium* [10]. Additionally, other steroid derivatives (3α-hydroxy-23,24-*bis*-norcholane polyamine carbamates) with antibacterial activity on *Salmonella typhimurium* were synthesized [11]. All these experimental results show several procedures which are available for synthesis of several antibacterial steroid-derivatives. Nevertheless, expensive reagents and special conditions are required. Therefore, in this study some steroid derivatives were synthesized using several strategies. It is noteworthy that antibacterial activity of these steroid derivatives on *Salmonella typhi* was evaluated *in vitro* in a bacteria model.

EXPERIMENTAL

The compound 4-[(2-amino-ethylamino)methyl]-13-methyl-7,8,9,11,12,13,14,15,17-decahydro-6*H*-cyclopenta-[a]phenanthrene-3,17-diol (**1**) was synthesized using reported method [12]. 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). Infrared spectra were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H NMR 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 3-(1*H*-naphtho[1,2-e][1,3]oxazin-2(3*H*)-yl)propan-1-amine (3): A solution of 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), ethylenediamine (50 µL, 0.75 mmol) and formaldehyde (3 mL) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to reduce the volume. After the mixture was diluted with water and extracted with chloroform (Fig. 1). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 54 % of product, m.p.: 100-102 °C; IR (ν_{max}, cm^{-1}): 3380 and 1196; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.68 (t, 2H, *J* = 7.00 Hz), 1.76 (broad, 2H), 2.50 (m, 2H), 2.56 (m, 2H), 4.12-4.86 (m, 4H), 7.04-7.80 (m, 6H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 30.90, 39.22, 50.20, 53.66, 82.00, 111.22, 117.90, 120.36, 123.30, 126.57, 127.90, 128.34, 133.08, 152.32 ppm. EI-MS *m/z:* 214.14 (M^+11). Anal. calcd. for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56, O, 6.60. Found: C, 74.30; H, 7.42.

Synthesis of 13-methyl-4{[2-(1*H*-naphto[1,2-e][1,3]oxazin-2-yl)-ethylamino]methyl}-7,8,9,11,12,13,14,15,16,17-decahydeo-6*H*-cyclopenta[a]phenanthrene-3,17-diol (6)

Method A: A solution of **1** (100 mg, 0.29 mmol), **4** (60 mg, 0.34 mmol) and formaldehyde (3 mL) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 66 % of product (Fig. 2), m.p.: 196-198 °C; IR (ν_{max}, cm^{-1}): 3412, 3380 and 1200; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.62 (s, 3H), 0.80-1.20 (m, 4H), 1.30-1.40 (m, 3H), 1.68- 1.84 (m, 4H), 2.06-2.50 (m, 4H), 2.54 (t, 2H, *J* = 6.90), 2.64 (t, 2H, *J* = 6.90), 3.60 (m 1H), 3.76 (t, 2H, *J* = 12.00), 4.30-5.00 (m, 3H), 5.08 (s, 3H), 5.10 (m, 1H), 6.52-6.80 (m, 2H), 7.00-7.72 (m, 6H) ppm.^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 15.68, 24.20, 25.30, 27.58, 27.68, 32.56, 33.70, 37.20, 44.28, 44.46, 44.52, 47.20, 50.22, 50.76, 55.00, 82.36, 82.40, 111.20, 112.48, 118.4, 120.78, 122.40, 123.3, 126.30, 127.86, 128.20, 128.40, 128.72, 131.7, 131.7, 137.25, 148.98, 151.64 ppm. EI-MS *m/z:* 512.30 (M^+12). Anal. calcd. for $C_{33}H_{40}N_2O_3$: C, 77.31; H, 7.86; N, 5.46, O, 9.36. Found: C, 77.27; H, 7.82.

Method B: A solution of estradiol (100 mg, 0.37 mmol), **3** (90 mg, 0.37 mmol) in formaldehyde (5 mL) was stirring for 72 h to reflux. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (3:1) yielding 44 % of product (Fig. 2). Similar ^{1}H NMR and ^{13}C NMR data were obtained compared with method A.

Synthesis of (13R)-4-((2-(1*H*-naphtho[1,2-e][1,3]-oxazin-2(3*H*)-yl)ethyl)amino)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta-[a]phenanthrene-3,17-diyl*bis*(2-chloroacetate) (8): A solution of **6** (250 mg, 0.48 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 3). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from hexane:methanol:water (1:3:1) yielding 55 % of product, m.p.: 178-180 °C; IR (ν_{max}, cm^{-1}): 1730, 1210 and 1192; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.82 (s, 3H), 1.12-1.28 (m, 3H), 1.40-1.50 (m, 3H), 1.68-1.74 (m, 4H), 2.12-2.22 (m, 2H), 2.40-2.52 (m, 3H), 2.58 (t, 2H, *J* = 6.90 Hz), 2.64 (t, 2H, *J* = 6.90 Hz), 3.60 (broad, 1H), 3.80 (m, 2H), 4.06-4.10 (m, 4H), 4.30-4.40 (m, 2H), 4.80 (m, 1H), 5.00-5.10 (m, 2H), 6.72-6.80 (m, 2H),, 7.00-7.80 ppm.^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 14.30, 24.58, 25.20, 27.60, 27.70, 30.00, 33.70, 37.20, 40.56, 40.80, 44.00, 44.50, 44.88, 47.30, 50.36, 50.94, 55.10, 82.34, 84.54, 111.20, 118.22, 118.40, 118.66, 120.72, 123.30, 126.30, 127.86, 128.38, 128.80, 130.64, 131.70, 135.52,

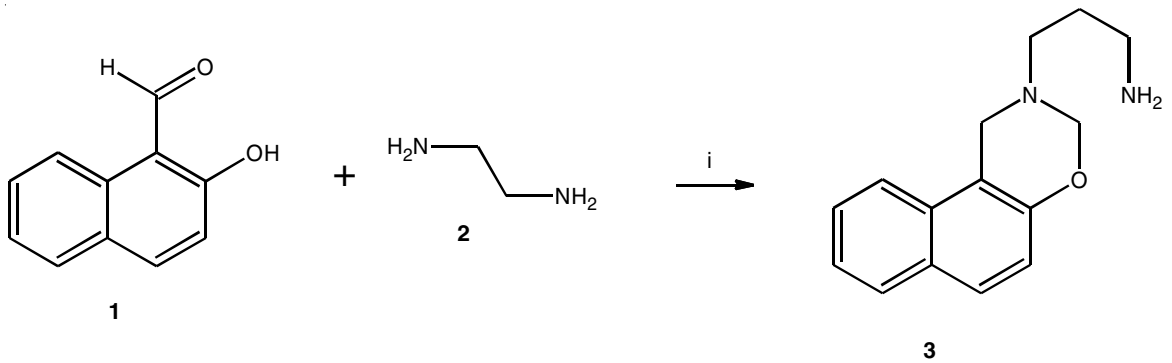


Fig. 1. Synthesis of 3-(1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)propan-1-amine (**3**). Reaction of 2-hydroxy-1-naphthaldehyde (**1**) with ethylenediamine (**2**) to form **3**.i = formaldehyde/room temperature

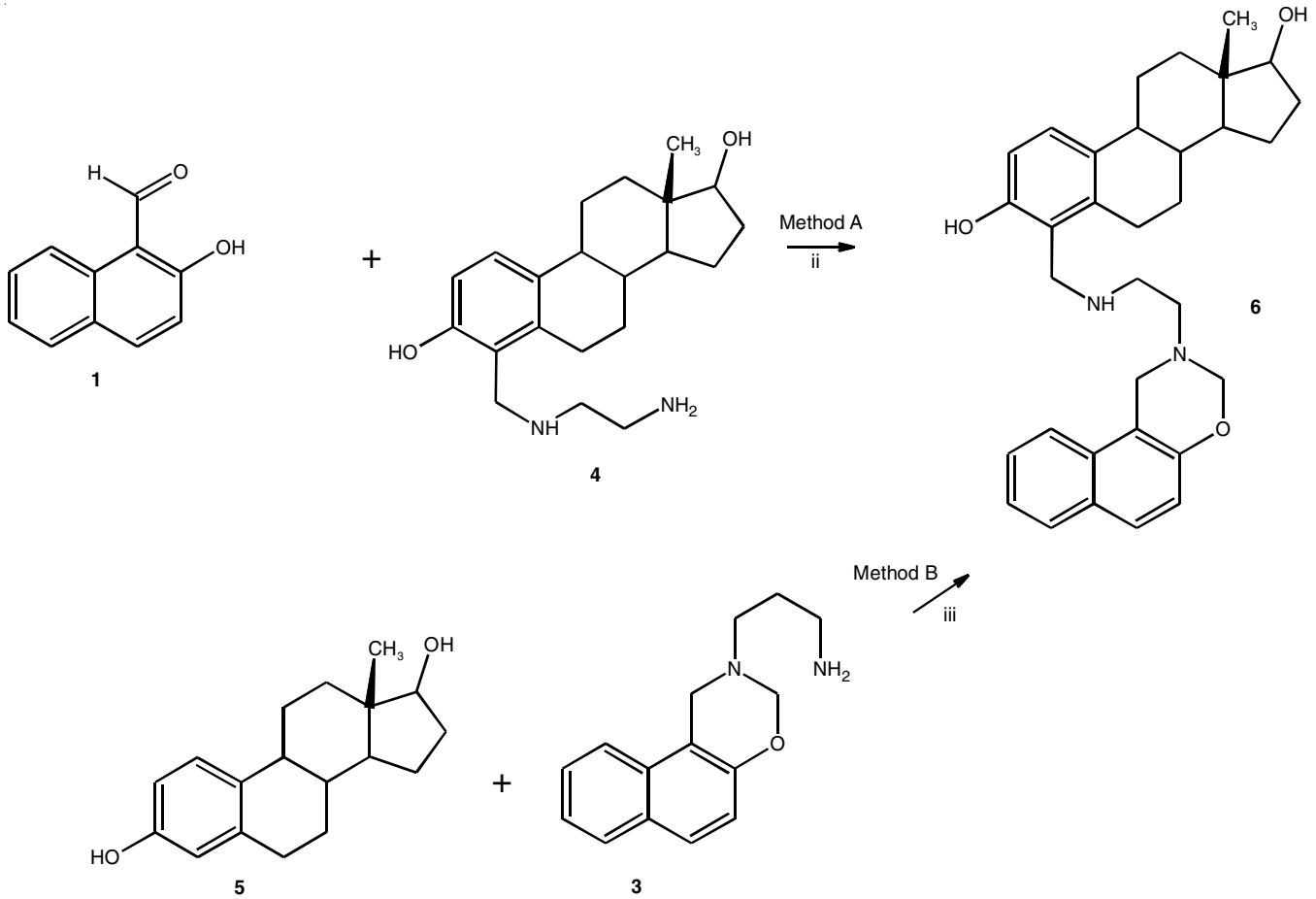
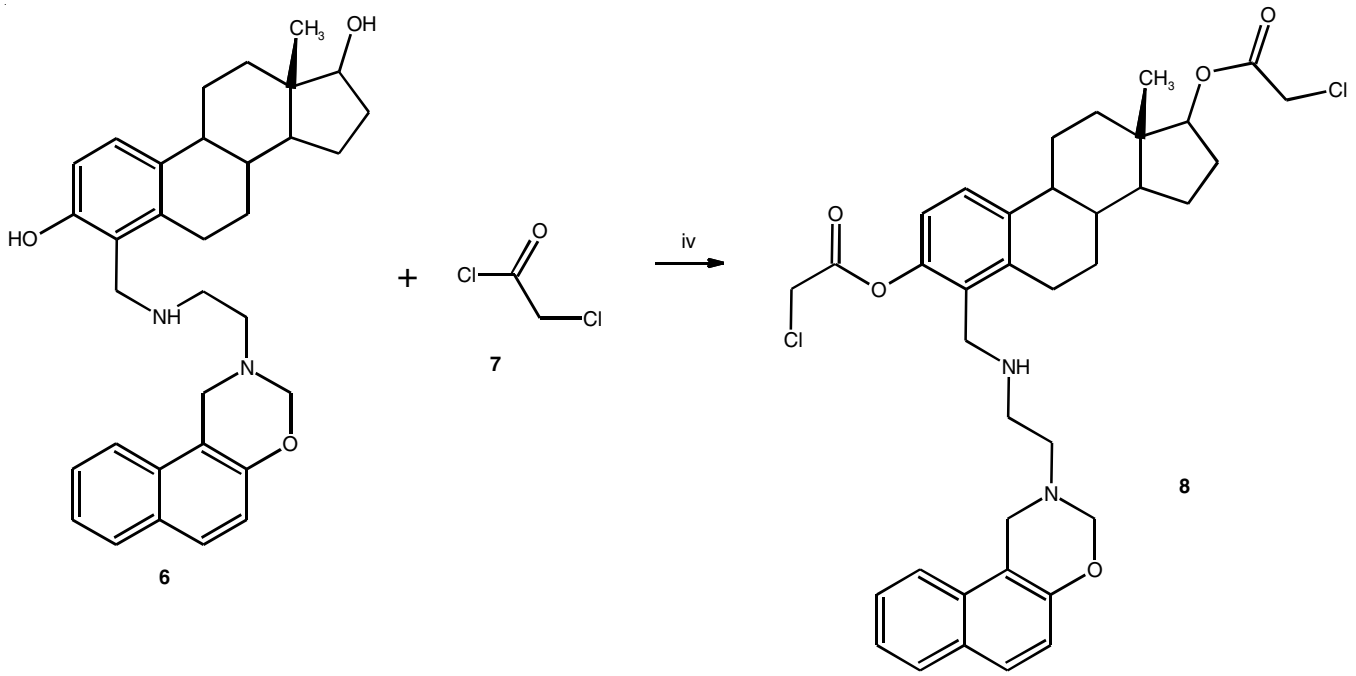


Fig. 2. Synthesis of an estrogen derivative (**6**). The first stage was achieved by reaction of 4-[(2-amino-ethylamino)-methyl]-13-methyl-7,8,9,11,12,13,14,15,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (**4**) with 2-hydroxy-1-naphthaldehyde (**1**) to form **6**. Also **6** was synthesized by the reaction ofestradiol (**5**) with 3-(1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)propan-1-amine (**3**). i = formaldehyde/MeOH/room temperature; ii = formaldehyde/MeOH/reflux



Fi. 3. Chloro-acetic acid 3-(2-chloro-acetoxy)-4-({(2-chloro-3-oxo-cyclobutyl)-[2-(1*H*-naphto[1,2-e][1,3]oxazin-2-yl)-ethyl]amino}methyl)-13-methyl-7,8,9,11,12,13, 14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl ester (**8**). Reaction of the compound **6** with chloroacetyl chloride to form **8**. iii = triethylamine/MeOH/room temperature

138.44,139.50, 144.52, 151.66, 166.74, 168.00 ppm. EI-MS *m/z:* 664.24 (M^+10). Anal. calcd. for $C_{37}H_{42}N_2O_5Cl_2$: C, 66.76; H, 6.36; Cl, 10.65; N, 4.21, O, 12.02. Found: C, 66.70; H, 6.30.

Synthesis of chloro-acetic acid 3-(2-chloro-acetoxy)-4-{[2-(2-chloro-acetylamino)ethylamino]methyl}-13-methyl-7,8,9,1,12,13,14,15,16,17-decahydro-6*H*-cyclopenta-[a]phenanthren-17-yl ester (9): A solution of **4** (200 mg, 0.58 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h at room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 4). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 56 % of product, m.p.: 268-270 °C; IR (ν_{max}, cm^{-1}): 3310, 1210 and 1192; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.82 (s, 3H), 1.12-1.30 (m, 3H), 1.40-1.50 (m, 3H), 1.68-1.76 (m, 4H), 2.12-2.52 (m, 5H), 2.66 (t, 2H, *J* = 6.44 Hz), 3.38 (t, 2H, *J* = 6.44 Hz), 3.80 (s, 2H), 4.00-4.12 (m, 6 H), 4.82 (s, 1H), 5.76 (broad, 2H), 6.72-6.84 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 14.22, 25.16.00, 27.50, 27.63, 30.00, 33.56, 37.20, 38.46, 40.50, 40.72, 42.36, 44.00, 44.50, 44.76, 50.82, 52.80, 84.56, 118.64, 130.68, 135.58, 138.48, 139.36, 144.36, 162.50, 166.76, 168.08 ppm. EI-MS *m/z:* 572.16 (M^+11). Anal. calcd. for $C_{27}H_{35}N_2O_5Cl_3$: C, 56.50; H, 6.15; Cl, 18.53; N, 4.88, O, 13.94. Found: C, 56.42; H, 6.10.

Synthesis of N-1-[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-4-ylmethyl]ethane-1,2-diamine (10): A solution of **4** (200 mg, 0.58 mmol) and *tert*-butyldimethylsilyl chloride (200 µL, 1.07 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 5). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 78 % of product, m.p.: 144-146 °C; IR (ν_{max}, cm^{-1}): 3380, 3310 and 1094. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.25 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.96 (s, 9H), 1.01-1.90 (m, 10H), 2.08 (m, 1H), 2.20 (broad, 3H), 2.42-2.50 (m, 3H), 2.62 (t, 2H, *J* =5.97 Hz), 2.76 (t, 2H, *J* = 5.97 Hz), 3.50 (m, 1H), 3.62 (m, 2H), 658-6.80 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: -4.50, -4.20, 15.18, 17.76, 18.44, 25.30, 25.54, 25.70,25.74, 27.65, 27.70, 32.97, 35.00, 37.28, 41.52, 43.70, 44.48, 45.88, 51.42, 53.30, 82.56, 115.12, 123.78, 127.20, 131.29, 136.19, 150.76 ppm. EI-MS *m/z:* 572.40 (M^+11). Anal. calcd. for $C_{33}H_{60}N_2O_2Si_2$: C, 69.17; H, 10.55; N, 4.89, O, 5.58; Si, 9.80. Found: C, 69.12; H, 10.48.

Synthesis of N-(2-{[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-4-ylmethyl]amino}-ethyl)-2-chloro-acetamide (11): A solution of **10** (400 mg, 0.70 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 5). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (2:1) yielding 52 % of product, m.p.: 238-240 °C; IR (ν_{max}, cm^{-1}): 3310, 1680 and 1096; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0..25 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 1.00 (s, 9H), 1.20-1.90 (m, 10H), 2.10-2.50 (m, 4H), 2.72 (t, 2H, *J* = 6.44), 3.40 (t, 2H, *J* = 6.44), 3.50 (m, 1H), 364 (t, 2H, *J* = 12.00), 4.00 (m, 2H), 5.00 (broad, 2H), 6.50-6.80 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: -4.50, -4.20, 15.18, 17.76, 18.42, 25.30, 25.54, 25.70, 25.68, 25.74, 27.60, 27.70, 32.97, 35.01, 37.28, 38.57, 42.40, 43.70, 44.45, 45.83, 51.49, 52.80, 82.60, 115.09, 124.13, 127.18, 131.30, 136.14, 150.78, 162.56ppm.EI-MS *m/z:* 648.36 (M^+10). Anal. calcd. for $C_{35}H_{61}N_2O_3Si_2Cl$: C, 64.72; H, 9.47; Cl, 5.46; N, 4.31; O, 7.39; Si, 8.65. Found: C, 64.68; H, 9.42.

Antimicrobial activity: The evaluation of antimicrobial effect of the different compounds on *Salmonella typhi* was made by described method [13]. In this method, *Salmonella typhi* was incubated on McConkey agar for 24 h at 37 °C. After some time, it was determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first contained 2 mL of culture medium (tripticasesoye) at double concentration and the remainder (11 tubes), contained

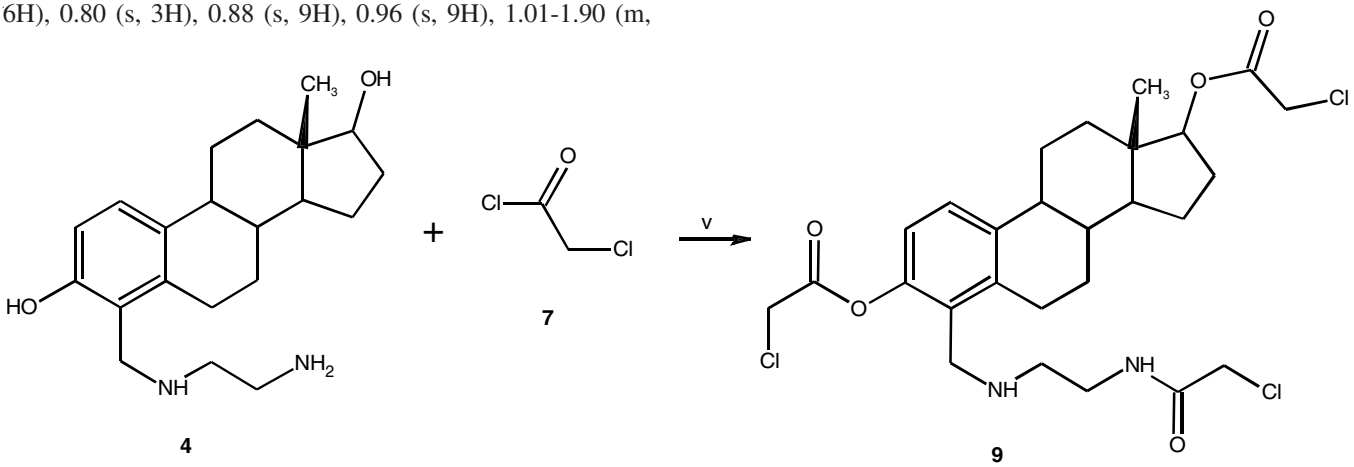


Fig. 4. Synthesis of chloro-acetic acid 3-(2-chloro-acetoxy)-4-{[2-(2-chloro-acetylamino)-ethylamino]-methyl}-13-methyl-7,8,9,1,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl ester (**9**). Reaction of compound **4** with chloroacetyl chloride (**7**) to form **9**. iv = triethylamine/MeOH/room temperature

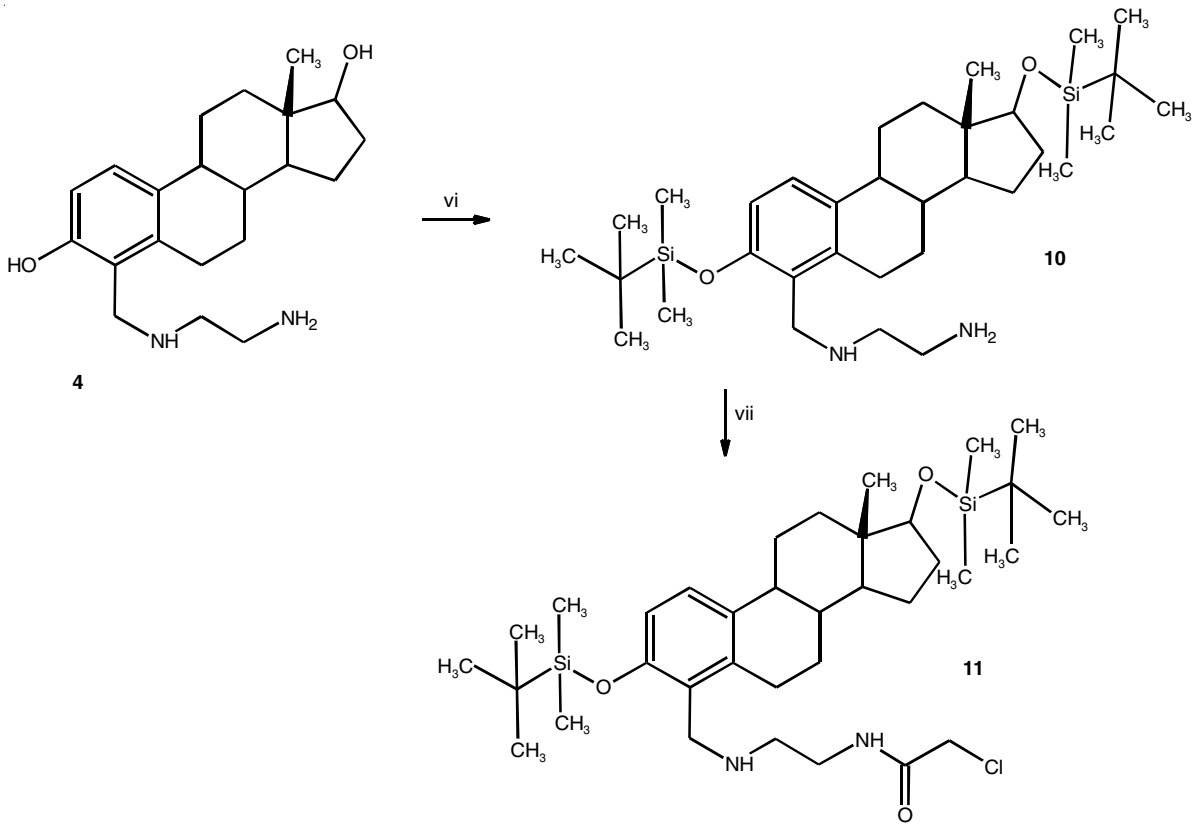


Fig. 5. Synthesis of an estrogen derivative (**11**). The first stage was achieved by reaction of **4** with *tert*-butyldimethylsilyl chloride (vi) to form the compound N-1-[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]-phenanthren-4-ylmethyl]ethane-1,2-diamine (**10**). After, **10** was made reacting with chloroacetyl chloride (vii) to form **11**. vii = triethylamine/MeOH/room temperature

the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 mL of the studied compound (1 mg/mL) was added and stirred, from this tube an aliquot of 2 mL was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 mL of dissolution had been used up. After this process, each tube was inoculated with 0.1 mL of the bacterial suspension, whose concentration corresponded to Mc-Farland scale (9×10^8 cells/mL) and all the tubes were incubated at 37 °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.

Statistical analysis: The obtained values are expressed as average ± SE [14]. The differences were considered significant when p was equal or smaller than 0.05.

RESULTS AND DISCUSSION

In this study, some antibacterial estrogen derivative was developed using several strategies. The first step was achieved by the synthesis of oxazine-steroid derivative (**3**). It is important to mention that many procedures for the synthesis of oxazine derivatives are available in the literature [15-18]. Nevertheless, expensive reagents and special conditions are required; therefore, in this study the compound **3** was synthesized using Mannich reaction [19]. ^{1}H NMR spectrum of **3** shows signals at 1.68 and 2.50-2.56 ppm for methylene groups bound to both amino groups; at 1.76 ppm for amino group; at 4.12-4.86 ppm for protons of oxazine ring; at 7.07-7.80 ppm for both phenyl groups. ^{13}C NMR spectrum of **3** contains peaks at 30.90-39.22 and 53.66 ppm for methylene groups bound to both amino groups; at 50.20 and 82.00 ppm for carbons of oxazine ring; at 11.22-152.32 ppm for both phenyl groups. Finally, the presence of compound **3** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 214.14.

The second stage was achieved using two methods; in the method A, the compound **6** was synthesized by condensation of an estrogen-derivative with2-hydroxy-1-naphthaldehyde using Mannich reaction [19]. ^{1}H NMR spectrum of **6** shows signals at 0.62 ppm for methyl group bound to steroid nucleus; at 0.80-2.50, 3.60 and 6.52-6.80 ppm for steroid moiety; at 2.54-2.64 ppm for methylene groups bound to both amine groups; at 3.76 for methylene group bound to both amino group and ring A of steroid nucleus; at 4.30-5.00 and 5.10 ppm for oxazine ring; at 5.08 ppm for both hydroxyl and amino groups. ^{13}C NMR spectrum of **3** contains peaks at 15.68 ppm for methyl group bound to steroid nucleus; at 24.20-44.46, 50.76, 82.40, 112.48, 122.40, 128.20 and 131.70-148.98 ppm for steroid

moiety; 47.20 and 55.00 ppm for methylene groups bound to both amino groups; at 50.22 and 82.36 ppm for oxazine ring; at 111.20, 118.40-120.78, 123.30, 128.40, 128.72, 131.66 and 151.64 ppm for naphthalene group. Finally, the presence of compound **6** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 512.30. Additionally, the compound **6** was prepared by the reaction of estradiol with the compound **3** in presence of formaldehyde. It is important to mention here that the yield was higher with the method A in comparison with method B. This phenomenon may possibly be due to reaction conditions.

The third stage was accomplished by the esterification of hydroxyl groups involved in the compound **6** for synthesis of **8**. Several methods have been used to prepare ester groups. Despite its wide scope, these protocols have several drawbacks such as low stability and use for hazardous reagents for their preparation [20,21]. In this study the compound **8** was synthesized by the reaction of **6** with chloroacetyl chloride using triethylamine as catalyst. It is important to mention that chlorobutanone was also formed in the chemical structure of **8** by the reaction of secondary amine with chloroacetyl chloride; this reaction is similar to other compounds with secondary amine [22].

^{1}H NMR spectrum of **8** shows signals at 0.82 ppm for methyl group bound to steroid nucleus; at 1.12-2.52, 4.80 and 6.72-6.80 ppm for steroid moiety; at 2.58 and 2.64 ppm for methylene groups bound to both amino groups; at 3.60 ppm for amino group; at 3.80 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 4.06-4.10 for methylene groups of both chloroacetic acid groups; at 4.30-4.40 and 5.00-5.10 ppm for oxazine ring; at 7.00-7.80 ppm for phenylgroups. ^{13}C NMR spectrum of **8** contains peaks at 14.30 ppm for methyl group bound to steroid nucleus; at 24.58-37.20, 44.00-44.50, 54.94, 84.54,118.66, 130.64 and 135.12-144.52 ppm for steroid moiety; at 40.56 and 40.80 ppm for methylene groups of both chloroacetic acid groups; at 44.88 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 47.30, 55.10 ppm for methylene groups bound to both amino groups; at 50.36 and 82.34 ppm for oxazine ring; at 111.20, 118.22, 120.72-128.80, 131.70 and 151.66 for phenyl groups; at 166.74 and 168.00 ppm for chloroacetic acid groups. Finally, the presence of compound **8** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 664.24.

On the other hand, the fourth stage was achieved by the reaction of **4** with chloroacetyl chloride to form the compound **9** using triethylamine as catalyst.In this reaction the hydroxyl group was esterified. However, a chloroamide group was also formed. It is important to mention that there are many procedures for the formation of chloroamides are known in the literature, for example the reaction of amine with trichloroisocyanuric acid [23] or secondary amide with *N*-chlorobenzotriazole to form a chloroamide derivative [24]. In addition, have been prepared some chloroamide groups using chloroacetyl chloride [25]. The results of ^{1}H NMR spectrum of **9** shows signals at 0.82 ppm for methyl group bound to steroid nucleus; at 1.12-2.52, 4.82 and 6.72-6.84 ppm for steroid moiety; at 2.66 and 3.38 for methylene groups bound to both amino groups; at 3.80 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 4.00 ppm for methylene group bound to amide group; at 4.08-4.12 ppm for both chloroacetic acid groups; at 5.76 ppm for both amino and amide groups. ^{13}C NMR spectrum of **9** contains peaks at 14.22 ppm for methyl group bound to steroid nucleus; at 24.58-37.20, 44.00-44.50, 50.82 and 84.56-144.36 ppm for steroid moiety; at 38.46 and 52.80 ppm for methylene groups bound to both amine groups; at 40.50 and 40.72 ppm for both chloroacetic acid groups; at 42.36 ppm for methylene bound to amide group; at 44.76 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 162.50 ppm for amide group; at 166.76-168.08 ppm for both ester groups. Finally, the presence of compound **9** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 572.16.

The fifth stage was accomplished by protecting the hydroxyl group of the compound **4**. It is important to mention that several triorganosilyl groups have been employed for protection of hydroxyl groups such as *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl [26]. In this study, the compound **4** was formed by reacting with *tert*-butyldimethylsilyl chloride to form the compound **10**. ^{1}H NMR spectrum of **10** shows signals at 0.08 and 0.88 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 0.25 and 0.96 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 0.80 ppm for methyl group bound to steroid nucleus; 1.01-2.08, 2.42-2.50, 3.50, 242-2.50 and 6.58-6.80 ppm for steroid moiety; at 2.20 ppm for both amino groups, at 2.62-2.76 ppm for methylene groups bound to both amine groups; at 3.62 ppm for methylene bound to both ring A of steroid nucleus and amine group. ^{13}C NMR spectrum of **9** contains peaks at 4.20, 18.44 and 25.70 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 4.50, 17.76 and 25.74 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 15.18 ppm for methyl group bound to steroid nucleus; at 25.30-25.54, 27.65-37.28, 43.70-44.48, 51.42 and 82.56-150.76 for steroid moiety; at 41.52 and 53.30 ppm for methylene groups bound to both amine groups; at 45.88 for methylene group bound to both ring A and amino groups. Finally, the presence of compound **10** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 572.40.

The last stage was achieved by reaction of **10** with chloroacetyl chloride to form the compound **11** using triethylamine as catalyst. ^{1}H NMR spectrum of **11** shows signals at 0.06 and 0.88 ppm for protons involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 0.22 and 1.00 ppm for protons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.02-2.50, 3.50 and 6.50-6.80 ppm for steroid moiety; at 2.72 and 3.40 ppm for methylene groups bound to amine groups; at 3.64 ppm for methylene group bound to both ring A of steroid nucleus and amine group; at 4.00 ppm for chloroamidegroup; at 5.00 ppm for amino and amide groups. ^{13}C NMR spectrum of **11** contains peaks at 4.50, 17.76 and 25.74 ppm for carbons involved in

the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 4.20, 18.42 and 25.70 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 15.18 ppm for methyl group bound to steroid nucleus; at 23.30-25.54, 27.60-37.28, 43.70-44.45, 51.49 and 82.60-150.78 ppm for steroid moiety; at 38.57 and 52.80 ppm for methylene groups bound to both amine groups: at 42.40 ppm for chloroamidegroup; at 45.83 ppm for methylene group bound to both ring A of steroid nucleus and amine group; at 162.56 for amide group. Finally, the presence of compound **11** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 648.36.

Biological activity: In order to evaluate the possibility of that compounds synthesized may have biological characteristics. In this study its antibacterial activity (minimal inhibitory concentration, MC) on Gram-negative (*Salmonella typhi*) bacteria was evaluated. The results showed (Figs. 6 and 7) that only the compounds **6** (MIC = 1.95×10^{-3} mmol), **9** (MIC = 1.74×10^{-3} mmol), **10** (MIC = 2.05×10^{-3} mmol) and **11** (MIC = 1.44×10^{-3} mmol) have antibacterial activity on *Salmonella typhi* in a dose manner dependent. Nevertheless, this effect was different in comparison with the controls (cefotaxime, MIC = 2.62×10^{-4} mmol; gentamycin, MIC = 1.29×10^{-4} mmol; and ciprofloxacin, MIC = 1.88×10^{-4} mmol). Analyzing these data, the antibacterial activity of a mixture of steroid derivatives was also evaluated using several systems (Fig. 7), The results showed that system G (mixture of all estrogen derivatives) exert higher antibacterial effect on *Salmonella typhi*. All these data indicate that antibacterial activity exerted by the estrogen derivatives on *Salmonella typhi* depend of their structure chemical in comparison with the controls and other steroid derivatives that are involved in this study. This phenomenon may involve the interaction of these compounds with some components of the bacterial cell, which may result in disturbance of bacterial growth and induce cell death, through perturbation of membrane bacterial. In this sense, the intramolecular interaction of compounds could be *via* divalent cations such as Mg^{2+} and Ca^{2+} involved in the membrane, consequently resulting a substantial increase the permeability of the outer membrane of *Salmonella typhi* as happening with other type of antibacterial agents [8-11,13].

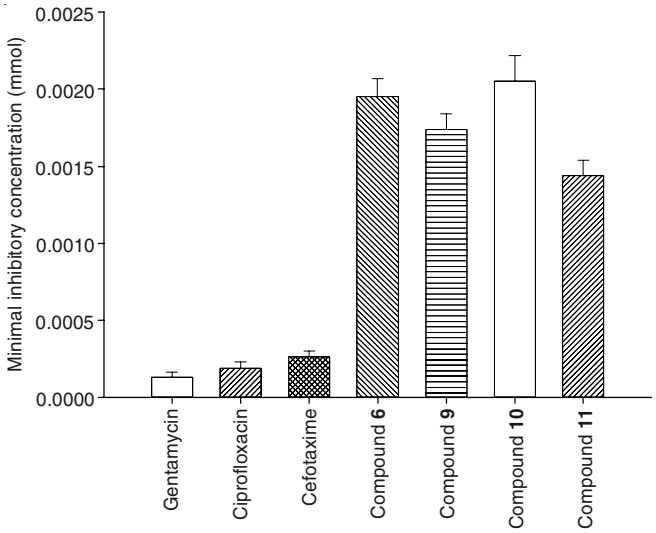


Fig. 6. Antibacterial activity induced by steroid derivatives (compound **6**, **9**, **10** and **11**) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on*Salmonella typhi*. Experimental data showed that *Salmonella typhi*was susceptibly to CEFOT (MIC = 2.62×10^{-4} mmol), GENT (MIC = 1.29×10^{-4} mmol) and CIPROF (MIC = 1.88×10^{-4} mmol). In addition, in presence of the compounds **6** (1.95×10^{-3} mmol), **9** (1.74×10^{-3} mmol), **10** (2.05×10^{-3} mmol) and **11**(1.44×10^{-3} mmol) the bacterial growth of this microorganism was inhibit. Each bar represents the mean ± S.E. of 9 experiments. MIC = Minimal inhibitory concentration

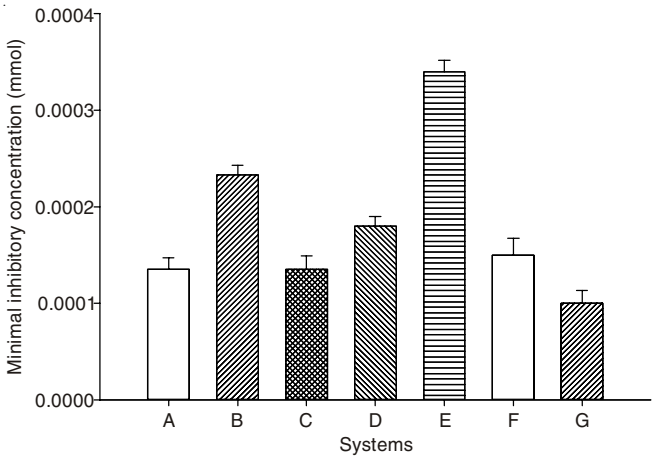


Fig. 7. Antibacterial activity induced by a steroid derivatives mixture on *Salmonella typhi*. Experimental data showed that *Salmonella typhi* was susceptibly to system A (compounds **6** and **9**; MIC = 1.35×10^{-4} mmol), system B (compounds **6** and **10**; MIC = 2.33×10^{-4} mmol), system C (compounds **6** and **11**; MIC = 1.35×10^{-4} mmol), system D (compounds **9** and **10**; MIC = 1.80×10^{-4} mmol), system E (compounds **9** and **11**; MIC = 3.40×10^{-4} mmol), system F (compounds **10** and **11**; MIC = 1.50×10^{-4} mmol), system G (compounds **6**, **9**, **10** and **11**; MIC = 1.20×10^{-4} mmol). Each bar represents the mean ± SE of 9 experiments. MIC = Minimal inhibitory concentration

Conclusion

In this study, new steroid derivatives with antibacterial activity on *Salmonella typhi* were synthesized using several strategies, which provide some advantages such as simple procedure and ease of workup in comparison with other techniques involved in the synthesis of other steroid derivatives.

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