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Synthesis and antibacterial activity evaluation of two androgen derivatives

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ABSTRACT

In this study two androgen derivatives were synthesized using several strategies; the first stage an aza-steroid derivative (**3**) was developed by the reaction of a testosterone derivative (**1**) with thiourea (**2**) in presence of hydrogen chloride. The second step, involves the synthesis of an amino-steroid derivative (**4**) by the reaction of **1** with **2** using boric acid as catalyst. The third stage was achieved by the preparation of an aminoaza-androgen derivative (**6**) by the reaction of **3** with ethylenediamine using boric acid as catalyst. In addition, the compound **6** was made reacting with dihydrotestosterone to form a new androgen derivative (**7**) in presence of boric acid. The following step was achieved by the reaction of **7** with chloroacetyl chloride to synthesize an azetidinone-androgen derivative (**8**) using triethylamine as catalyst. Additionally, a thiourea-androgen derivative (**9**) was synthetized by the reaction of **4** with dihydrotestosterone using boric acid as catalyst. Finally, the compound **9** was made reacting with chloroacetyl chloride in presence of triethylamine to synthesize a new azetidinone-androgen derivative (**10**).

On the other hand, antibacterial activity of compounds synthesized was evaluated on Gram negative (*Escherichia coli* and *Vibrio cholerae*) and Gram positive (*Staphylococos aureus*) bacteria. The results indicate that only the compound **3** and **8** decrease the growth bacterial of *E. coli* and *V. cholerae*. Nevertheless, growth bacterial of *S. aureus* was not inhibited by these compounds. These data indicate that antibacterial activity exerted by the compounds **3** and **8** depend of their structure chemical in comparison with the controls and other androgen derivatives that are involved in this study

1. Introduction

Since several years ago, some steroid derivatives have been developed to evaluate its biological activity; for example, a series of antibacterial steroid-derivatives were synthesized by the reaction of steroidal thiosemicarbazones with 2,3-dichloroquinoxalines [1]. Other data showed the reaction of 16-(bis-methylsulfanyl-methylene)-3-hydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-17-one with 2-aminophenylamine to form the compound 3β-hydroxy-1′-methyl-16-(benzimidazol-2′-ylidino)androstan-17-one which exert antibacterial activity on bacteria negative [2]. In addition, other reports show the synthesis of steroid-thiosemicarbazone conjugates and their antibacterial activity exerted on Gram positive and Gram negative bacteria [3]. Other study showed the synthesis of an antibacterial steroid-derivative (cholest-5-en-3-one semicarbazone) by the reaction of cholest-5-en-3-one with semicarbazone hydrochloride [4]. Additionally, other report indicates the preparation of antibacterial cationic-steroids by conjugating tripeptides with derivatives of cholic acid [5]. Recently, was synthesized an antibacterial-steroid derivative (pregnenolone–vitamin B1 conjugate) by the reaction of hemisuccinate of pregnenolone and vitamin-B_1 [6]. Also, some ω-pyridinium alkylethers-steroid derivatives were synthesized as antimicrobial agents by the reaction of 3-hydroxy-estra-1,3,5(10)-triene-17-one and 1-hydroxy-4-methyl-estra-1,3,5(10)-triene-17-one with ω,ω′-dibromoalkanes/pyridine [7]. Other data indicate the synthesis of an antibacterial steroid (dihydrotestosterone–ciprofloxacin conjugate) via the reaction of a ciprofloxacin derivative with dihydrotestosterone [8]. All these experimental results show several

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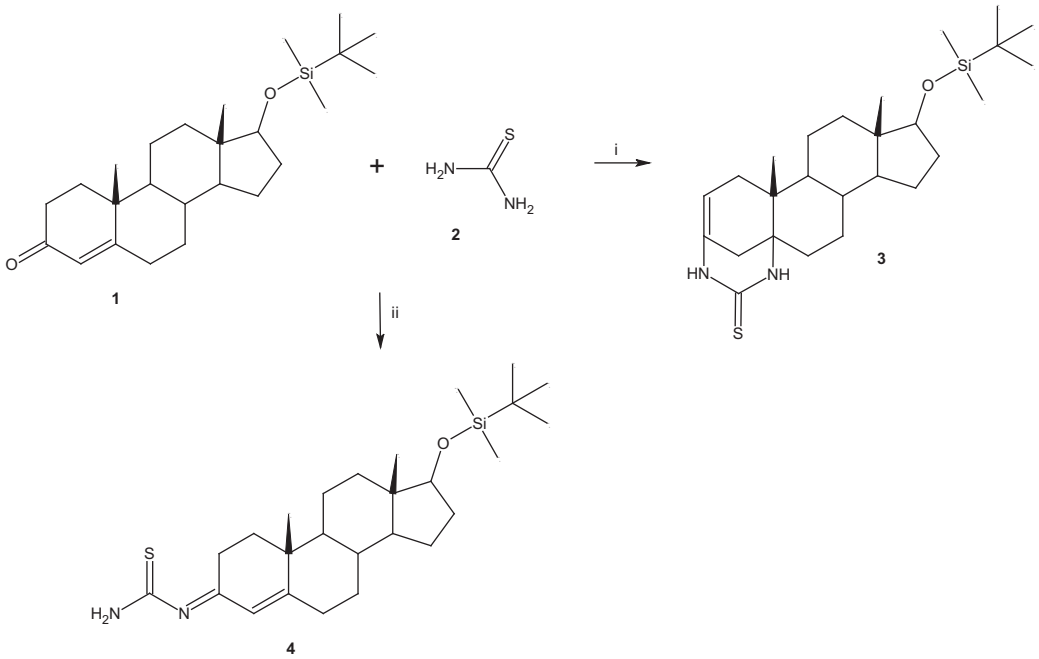
procedures which are available for synthesis of several antibacterial steroid-derivatives; nevertheless, expensive reagents and special conditions are required. Therefore, in this study two androgen derivatives were synthesized using several strategies. It is noteworthy that antibacterial activity of these androgen derivatives was evaluated *in vitro* on a bacteria model.

2. Results and discussion

In this study several straightforward routes are reported for synthesis of two androgen derivatives using strategies different; the first stage was achieved by the synthesis of 3-(terbuthyl-dimethyl-silanyloxyl)-5b,3a-dimethyl-octahydro-indeno[4,5-d]10, 12-diazatri-cycle[7.3.1.01,611]tridecan-9-one (**3**). It is important to mention that there are many procedures for preparation of several aza-steroid derivatives; nevertheless, despite its wide scope, have some drawbacks; for example, several agents used have limited stability and their preparation requires special conditions [9–13]. Therefore **3** was synthesized by the reaction of 17β-[(tert-butyldimethylsilyl)oxy]androst-4-en-3-one with thiourea in presence of hydrogen chloride (Scheme 1). The ^{1}H NMR spectrum of **3** shows signals at 0.06–0.84 ppm for methyl groups involved the tert-butyldimethylsilane fragment; at 0.67 and 0.82 ppm for methyl groups bound to steroid nucleus; at 0.90–5.20 ppm for steroid moiety; at 9.80 ppm for both amino groups. The ^{13}C NMR spectra displays chemical shifts at −4.50, 17.98 and 25.44 ppm for methyl groups involved in the tert-butyldimethylsilane fragment; at 11.18 and 13.60 ppm for methyl groups bound to steroid nucleus; at 21.44–23.50 and 27.90–134.98 ppm for steroid moiety; at 156.74 ppm for carbon bound to both amino groups. Finally, the presence of **3** was further confirmed from mass spectrum which showed a molecular ion at 460.20 *m*/*z*.

The second step was achieved by the synthesis of an imine group involved in the compound **4** (Scheme 1). It is important to mention, that there are several procedures for the synthesis of imines which are described in the literature [14,15]. For example, the synthesis of imine derivatives by the reaction of the compound 1-[(2-amino-ethylamino)phenyl-methyl]-naphta-len-2-ol with androsterone using as catalyst boric acid [16]. Therefore, in this study the synthesis of the compound **4** was developed by the reaction of 17β-[(tert-butyldimethylsilyl)oxy]androst-4-en-3-one with thiourea to form the compound **4** using boric acid as catalyst. The results indicate that ^{1}H NMR spectrum of **4** showed signals at 0.06–0.82 ppm for methyl groups involved in the tert-butyldimethylsilane fragment; at 0.90–1.02, 1.06–1.10 and 1.30–6.00 ppm for steroid moiety; at 1.04 and 1.22 ppm for methyl groups bound to steroid nucleus; at 6.90 ppm for both amino groups. The ^{13}C NMR spectra displays chemical shifts at −4.50, 17.98 and 25.44 ppm for methyl groups involved in the tert-butyldimethylsilane fragment; at 11.22 and 17.69 ppm for methyl groups bound to steroid nucleus; at 20.90–23.50 and 30.40–162.26 ppm for steroid moiety; at 185.26 ppm for thiourea group. Finally, the presence of **4** was further confirmed from mass spectrum which showed a molecular ion at 460.26 *m*/*z*.

The third step was achieved by the reaction of **3** with ethylenediamine to form the compound **6** (*N*-17-[3-(tert-butyl-dimethyl-silanyloxyl)-5b,3a-dimethyl-octahydro-indeno [4,5-d]10,12-diaza-tricyclo[7.3.1.01,68]tridec-7-en-9-yli-dene]-ethane-1,2-diamine) using boric acid (Scheme 2). It is noteworthy that the fragment bound to both amino groups of compound **6**, has a free amino group which can react with other type of compounds with specific functional groups. Also, this fragment can serve as a spacer arm with other molecules to decrease some steric hindrance when their pharmacological activity will be assessed in any biological model. The results indicate that ^{1}H NMR spectrum of **6** showed signals at 0.06 and 0.87 ppm for methyl groups bound to steroid nucleus; at 0.68 and 0.82 ppm for methyl groups bound to steroid nucleus; at 0.94–2.36, 3.50 and 4.65 ppm for steroid moiety; at 3.18 and 3.70 ppm for both methylene groups bound to both amine groups; at 3.90 ppm for both amino groups. The ^{13}C NMR spectra displays chemical shifts at −4.50, 17.96 and 25.46 ppm for methyl groups involved in the tert-butyldimethylsilane fragment; at 11.30 and 13.58; at 21.50, 27.90–40.10, 43.32–51.90 and 61.16–130.16 ppm for steroid moiety; at 41.30 and 52.48 ppm for methylene groups bound to both amino groups; at 156.60 ppm for imino group.



Scheme 1. Synthesis of **3** by the reaction of 17β-[(tert-butyldimethylsilyl)oxy]androst-4-en-3-one (**1**) with thiourea (**2**) in 6 presence of hydrogen chloride (i). In addition, the compound **4** was synthetized by the reaction of **1** with **2** using boric acid as catalyst (ii).