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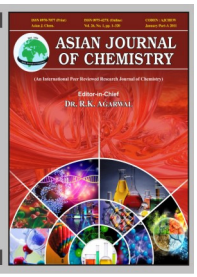

Design and Synthesis of Estrogen Derivative

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In this study, an estrogen derivative was synthesized. The first stage involves the synthesis of 17-(2-amino-ethylimino)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-3-ol (**3**) by the reaction of estrone with ethylenediamine using boric acid as catalyst. The second stage was achieved by reaction of compound **3** with chloroacetyl chloride in presence of triethylamine to form *N*-{2-[(3-chloro-2-oxo-cyclobutyl)-(3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl)-amino]ethylcarbamic chloride (**5**). The third stage was achieved by reaction of compound **5** with thiourea to form 2-[(2-amino-ethyl)-(3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16-17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl)amino]-4-chloro-cyclobutanone (**7**) in methanol. The structures of compounds obtained were confirmed by spectroscopically.

Keywords: Estrogen, Estrone, Thiourea, Ethylenediamine.

INTRODUCTION

Some estrogen derivatives have been developed for use in different biological and analytical methods[1-3]. For example, there are studies which show the synthesis of 2-alkylsulfanyl estrogens which are achieved by a directed ortho-lithiation reaction of suitably protected estrone followed by disulfide quench and final deprotection[4]. Other data showed the synthesis of nitrile derivatives of estrogens by reaction of 7α-cyano-19-nortestosterone with copper(II) bromide in acetonitrile at room temperature, resulting in aromatization of the A-ring of steroid[5]. In addition, other estradiol derivative (17α-(ruthenocenylmethyl)estra-1,3,5(10)-trien-3,17β-diol) was synthetized by the reaction between 17β-spiro-oxiranyl estradiol and lithium ruthenocenyl in THF[6]. Also, several 17β-O-alkyl ethers (methyl, ethyl, propyl, butyl, hexyl and octyl) of estradiol were obtained from 3-O-benzyl-17β-estradiol with sodium hydride/alkyl halide, followed by the removal of O-benzyl protecting group *via* catalytic transfer hydrogenation[7]. Additionally, there are some reports which shown the synthesis of an estradiol derivative by the Reformatsky reaction of 3,17β-*bis*[(2-trimethylsilyl)- ethoxymethyl]-1,3,5(10)-estratrien-6-one with bromoethyl acetate and zinc[8]. In addition, other aromatic derivative (14β-dihydroxyestrone) has been synthesized from 6-methoxy-tetralone in five steps involving a new acid catalyzed 1,3-shift of an allylic nitro group[9]. Recently, an estradiol analog is developed by the reaction of the estradiol-ethylenediamine derivative with HCl/$AlCl_3$ and water[10]. All these experimental data revealed that several procedures for synthesis of estrogen derivatives are available, however, expensive reagents and special conditions are required. Therefore, in this study a new estrogen was synthesized using several chemical methods.

EXPERIMENTAL

The 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 (IR) were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{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 GC Polaris Q. spectrometer. Elementary analysis data were

acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

17-(2-Amino-ethylimino)-13-methyl-7, 8,9,11,12,13, 14,15,16,17-decahydro-6*H*-cyclopenta[a] phenanthren-3-ol (3): A solution of estrone (100 mg, 0.40 mmol), ethylenediamine (50 µL, 0.75 mmol) and boric acid (50 mg, 0.83 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. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 83 % of product, m.p. 188-190 °C; IR (KBr, cm^{-1}, ν_{max}) 3460, 3380. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.94 (s, 3H), 1.22-1.54 (m, 2H), 1.72-1.89 (m, 2H), 2.06-2.17 (m, 3H), 2.23-2.27 (m, 2H), 2.39-2.82 (m, 4H), 2.97 (t, 1H, *J* = 6.4, Hz), 3.31 (t, 2H, *J* = 6.4, Hz), 4.40 (broad), 6.56-7.27 (d, 3H, *J* = 2.55, Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 15.70 (C-18), 21.92 (C-7), 25.70 (C-10), 26.12 (C-3) 27.48 (C-8), 29.22 (C-11), 32.40 (C-4), 37.56 (C-1), 41.02 (C-21), 41.08 (C-5), 43.30 (C-2), 54.22 (C-20), 54.30 (C-6), 112.39 (C-15), 114.90 (C-17), 125.37 (C-14), 130.70 (C-13), 135.15 (C-12), 152.84 (C-16), 176.80 (C-9), ppm. EI-MS *m/z*: 312.20 (M+, 17). Anal. Calcd. (%) for $C_{20}H_{28}N_2O$: C, 76.88; H, 9.03; N, 8.97. Found (%): C, 76.80; H, 9.00.

***N*-{2-[(3-Chloro-2-oxo-cyclobutyl)-(3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta-[a]phenanthren-17-yl)amino]ethylcarbamic chloride (5):** A solution of compound **3** (100 mg, 0.32 mmol), triethylamine (70 µL, 0.50 mmol) and chloroacetylchloride (70 µL, 0.88 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 the precipitate was separated, yielding 78 % of product, m.p. 198-200 °C; IR (KBr, cm^{-1}, ν_{max}) 3460, 1712, 1160. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.87 (s, 3H), 0.95-1.66 (m, 7H),1.67 (m, 1H), 1.91-1.95 (m, 4H), 1.98 (m, 1H), 2.02-2.30 (m, 2H), 2.31-2.37 (m, 2H), 2.40-2.86 (m, 3H), 3.67(d, 2H, *J* = 8.5, Hz), 3.72-4.26(d, 2H, *J* = 8.5, Hz), 6.59 (broad, 3H), 6.66-7.17(m, 3H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C:14.80 (C-24), 25.12 (C-9), 25.90 (C-6), 27.31 (C-10), 27.40 (C-8), 31.30(C-11), 32.70 (C-27), 36.48(C-7), 37.52 (C-4), 46.18 (C-20), 46.30 (C-2), 46.37 (C-5), 51.12 (C-19), 52.50 (C-3), 62.84 (C-28), 67.70 (C-1), 72.20 (C-26), 113.34 (C-15), 115.50 (C-17), 127.39 (C-14), 132.22 (C-13), 138.15 (C-12), 144.30 (C-22), 155.04 (C-16), 202.12 (C-29) ppm. EI-MS *m/z*: 478.10 (M+, 17). Anal. Calcd. (%) for $C_{25}H_{32}N_2O_3Cl_2$: C, 62.63; H, 6.73; Cl, 14.79; N, 5.04. Found (%): C, 62.60; H, 6.68.

2-[(2-Amino-ethyl)-(3-hydroxy-13-methyl-7,8,9,11,12, 13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl)-amino]-4-chloro-cyclobutanone (7): A solution of compound **5** (100 mg, 0.32 mmol), thiourea (70 mg, 0.91 mmol) and 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 the precipitate was separated, yielding 78 % of product, m.p. 218-220 °C; IR (KBr, cm^{-1}, ν_{max}) 3458, 3380, 1710. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.76 (s, 3H), 1.02-1.67 (m, 7H),1.68 (m, 1H), 1.91-1.97 (m, 4H), 1.98 (m, 1H), 2.02-2.40 (m, 3H), 2.52 (broad, 3H), 2.58 (t, 2H, *J* = 6.2, Hz), 2.70 (t, 2H, *J* = 6.2, Hz), 2.76-2.80 (m, 2H), 3.66-4.70 (d, 2H, *J* = 8.5, Hz), 6.56-7.10 (d, 3H, *J* = 8.5, Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 14.70 (C-22), 25.30 (C-9), 25.90 (C-6) 27. 32 (C-10), 27.40 (C-8), 31.32 (C-11), 32.40 (C-25), 36.50 (C-7), 37.56 (C-4), 46.30 (C-2), 46.42 (C-5), 47.12 (C-20), 50.98 (C-19),52.22 (C-3), 62.90 (C-26), 67.56 (C-1), 72.16 (C-24),113.39 (C-15), 115.50 (C-17), 125.37 (C-14), 130.78 (C-13), 138.15 (C-12), 155.14 (C-16), 202.10 (C-27), ppm. EI-MS *m/z*: 416.18 (M+, 17). Anal. Calcd. for $C_{24}H_{33}N_2O_2Cl$: C, 69.13; H, 7.98; Cl, 8.50; N, 6.72; O, 7.67. Found: C, 69.10; H, 7.92.

RESULTS AND DISCUSSION

There are many procedures for development of new estrogen derivatives. Nevertheless, despite its wide scope, have some drawbacks *e.g.*, several agents used have a limited stability and their preparation require condition specials[11-14]. Analyzing these data, in this study we report a straightforward route for synthesis of an estrogen derivative using some strategies. In the first stage, the compound **3** was formed by reaction of estrone with ethylenediamine to form an imino group involved in the compound **3** (Fig. 1) using boric acid as catalyst. It is important to mention that there are several procedures for the synthesis of imino groups which are described in the literature[15-17]. Nevertheless, in this study

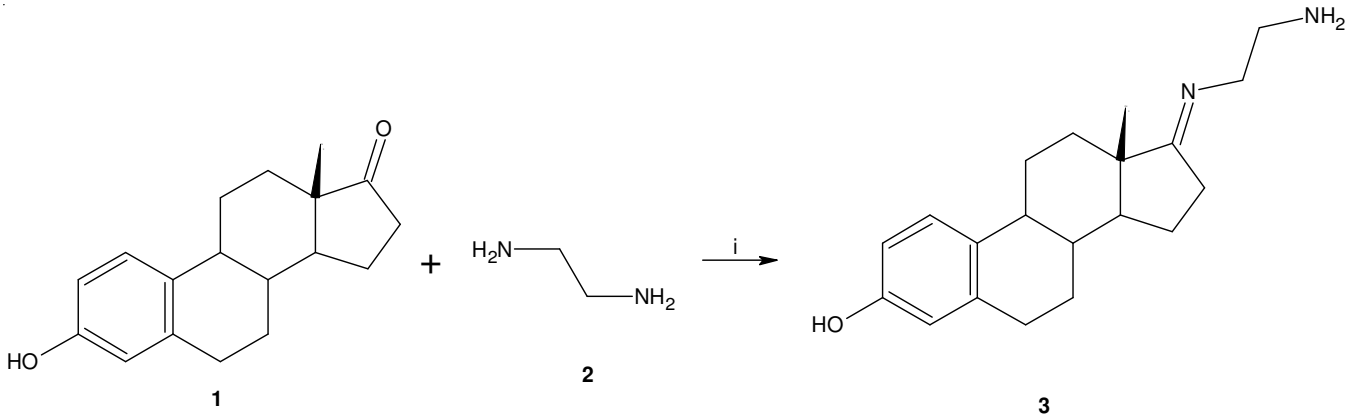

Fig. 1. Synthesis of 17-(2-amino-ethylimino)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-3-ol (**3**). Reaction of estrone (**1**) with ethylenediamine (**2**) to form 3. i = boric acid/rt

boric acid was used as catalyst, because it is not an expensive reagent and special conditions for its use are not required[18]. ^{1}H NMR spectrum of **3** shows signals at 0.94 ppm for methyl group involved in steroid moiety; at 1.22-2.82 ppm for steroid nucleus; at 2.97 and 3.31 ppm for methylene groups involved in the arm bound to imino group. Finally, other signals at 4.40 ppm for both amino and hydroxyl groups; at 6.56-7.27 ppm for protons of aromatic ring of steroid were found. The ^{13}C NMR spectrum contains peaks at 15.70 ppm for methyl group; at 21.92-37.56, 41.08-45.30 and 54.30 ppm for carbons presents in the steroid nucleus; at 41.02 and 52.22-176.80 ppm for methylene groups involved in the arm bound to imino group. Finally, the presence of **3** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 312.20.

The second step was achieved by the reaction of **3** with chloroacetyl chloride to form a chloroamide derivative involved in compound **5** (Fig. 2). It is important to mention that although many procedures for the formation of chloroamides are known in the literature, for example the reaction of amine with trichloroisocyanuric acid[19] or secondary amide with N-chlorobenzotriazoleto form a chloroamide derivative[20]. In addition, since several years ago, have been prepared some chloroamides using chloroacetyl chloride[21,22]. Therefore, in this study chloroacetyl chloride was used to form a chloroamide involved in the compound **5**. ^{1}H NMR spectrum of compound **5** shows signals at 0.87 ppm for methyl group of steroid nucleus; at 0.95-1.66, 1.91-1.95, 2.02-2.30, 2.40-2.86 and 6.66-7.17 ppm for steroid nucleus; at 1.67, 1.98 and 3.72-4.26 ppm for the protons involved in the cyclobutane group. Finally, the spectrum contains other signals at 2.31 to 2.37 and 3.67 ppm for arm bound to both amino and chloro-amide groups; at 6.59 ppm for protons involved in both hydroxyl and chloroamide groups. ^{13}C NMR spectrum contains peaks at 14.80 ppm for methyl group; at 25.12-31.30, 36.08-37.52, 46.30-46.37, 52.50, 67.71, 113.34-138.13 and 155.04 ppm for steroid nucleus; at 32.70, 62.84 and 72.20 ppm for cyclobutane group; at 46.18 and 51.12 ppm for arm bound to chloroamide group; at 144 for carbon of chloroamide group; and at 202.12 for ketone group. In addition, the presence of **5** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 478.10.

On the other hand, the third stage was achieved by the removal of chloroacetyl group involved in the compound **5**. There are some methods that show the removal of chloroacetyl

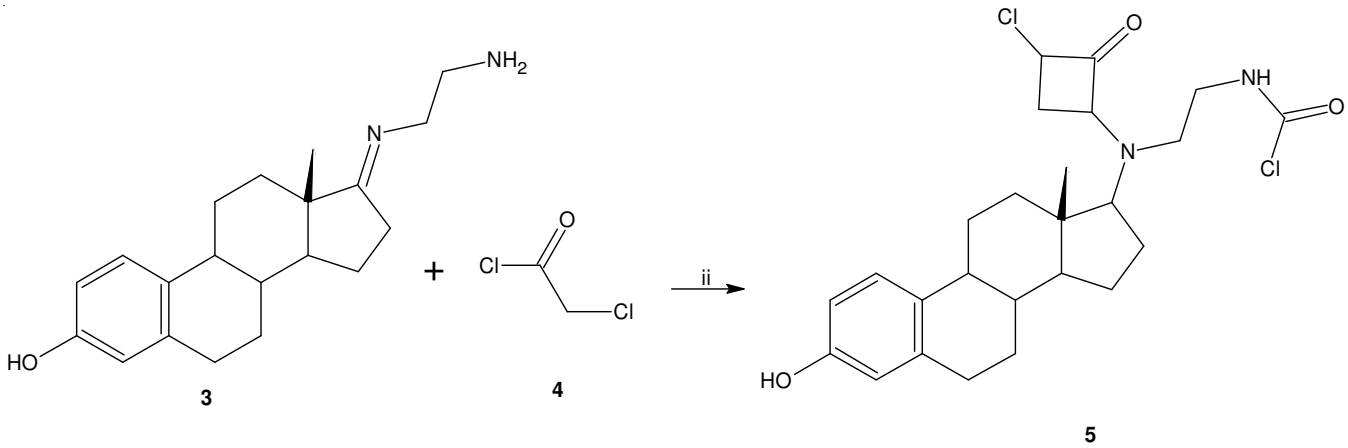

Fig. 2. Synthesis of N-{2-[(3-chloro-2-oxo-cyclobutyl)-(3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl)-amino]ethyl-carbamicchloride (**5**). Reaction of 17-(2-amino-ethylimino)-13-methyl-7,8,9,11,12,13-14,15,16,17-decahydro-6*H*-cyclo-penta[a]phenanthren-3-ol (**3**) with chloroacetyl chloride (**4**) to form 5.ii = triethylamine/rt

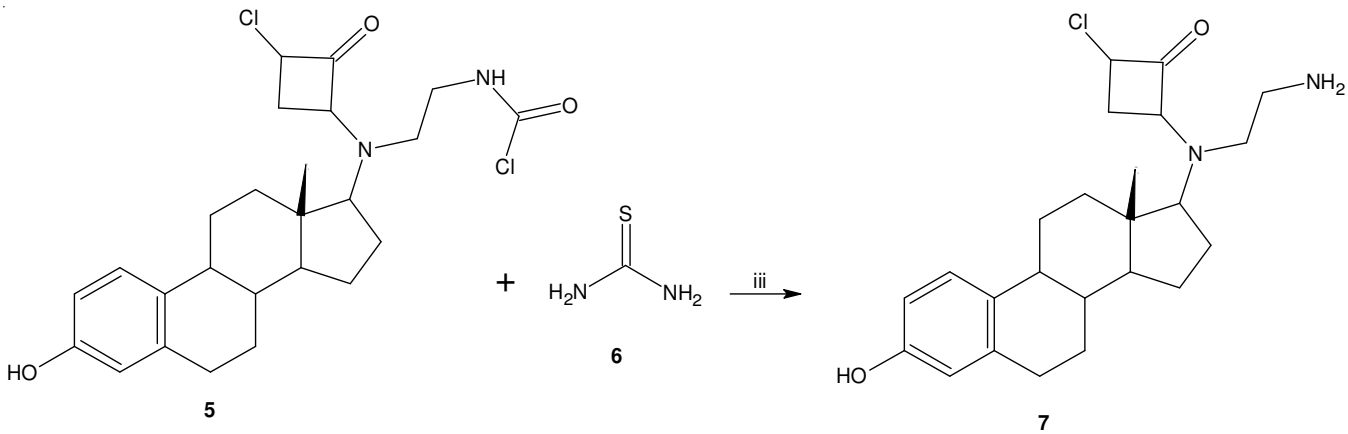

Fig. 3. Synthesis of 2-[(2-amino-ethyl)-(3-hydroxy-13-methyl,7,8,9,11,12,13,14,15,16- 17-decahy-dro-6*H*-cyclopenta[a]phenanthren-17-yl)-amino]4-chloro-cyclobutanone (**7**). Reaction of N-{2-[(3-chloro-2-oxo-cyclobutyl)-(3-hydroxy-13-methyl-7,8,9,11,12,13,14- 15,16,17-decahydro-6*H*-cyclopenta[a]-phenanthren-17-yl)-amino]ethyl-carbamic chloride (**5**) with thiourea (**6**) to form 7. iii = methanol/rt

groups of some compounds[23,24]; for example, the reaction of chloroacetylamine with thiourea to form an amine derivative[25,26]. In this study, for the removal of chloroacetyl group was used thiourea to form a primary amine involved in the compound **7**. This reaction is carried out an intramolecular amidinolysis to release the corresponding amine,as it happens with other compounds[26]. ^{1}H NMR spectrum of compound **7** shows signals at 0.76 ppm for methyl group; at 1.02-1.67, 1.91-1.97, 2.02-2.40, 2.76-2.80 and 6.56-7.10 ppm for steroid nucleus; at 1.68, 1.98 and 3.66-4.70 ppm for cyclobutane group. Finally, other signals at 2.58 and 2.70 ppm for protons involved in the arm bound to amino group; at 2.52 ppm for both hydroxyl and amino groups were found.^{13}C NMR spectrum contains peaks at 14.70 ppm for methyl group; at 26.30-31.32, 36.50-46.42, 52.22, 67.56, 113.39-155.14 ppm for carbons presents in the steroid nucleus; at 32.40, 62.90 and 72.16 ppm for methylene groups involved in the cyclobutane group; at 47.12 and 50.98 ppm for carbons presents in the arm bound to amino group; at 202.10 ppm for ketone group. Finally, the presence of **7** (Fig. 3) was further confirmed from mass spectrum which showed a molecular ion at *m/z* 416.18.

In conclusion, a facile procedure for the formation of estrogen derivative was developed in this study.

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