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Synthesis and biological activity of steroidal imides

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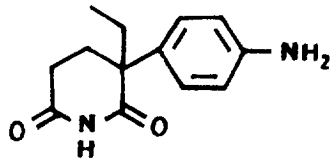
aromatase / aromatase inhibitor / breast cancer / antineoplastic agent

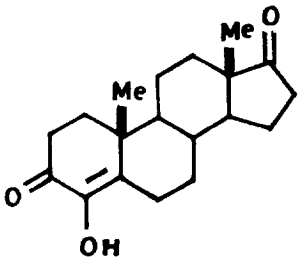
Introduction

Female sex hormones have been implicated [1–3] in a number of human diseases including breast cancer. The blockade of aromatization of androgens by inhibiting the aromatase activity to reduce circulating oestrogens has been intensely pursued with the goal of the treatment of several oestrogen-dependent pathologies like hormone-dependent mammary carcinomas [4].



1

Non-steroidal aromatase inhibitor, aminoglutethimide **1**, introduced as an anticonvulsant, is now being used in the clinical treatment of oestrogen-dependent breast cancer [5]. Several modified androgen analogues have also been reported to possess potent aromatase inhibitory activity [6, 7]. One of them, 4-hydroxy-4-androstene-3,17-dione **2** has shown a response rate of 21% in patients with advanced breast cancer [1, 5].



2

Among the modified D-ring steroidal analogues, testolactone has been used for the therapy of breast-cancer for many years [8].

In the present investigation, our approach was to design compounds with the 4-hydroxy-4-en-3-one moiety of 4-hydroxyandrostenedione **2** and the imide functionality of aminoglutethimide **1** in the same steroid nucleus.

Chemistry

Alkaline hydrogen peroxide treatment of the unsaturated ketone **5** [9] yielded the product **6**. Alkaline epoxidation of 3-ketones proceeds with variable stereochemistry [10]. From the NMR spectrum, **6** was found to be a 40:60 mixture of 4α, 5α-oxido **6B** and 4β, 5β-oxido **6A** isomers, respectively. In the NMR spectrum, a singlet for 4α-H appeared at δ 3.00 (19-methyl singlet at δ 1.17) while the singlet for 4β-H was at 3.06 ppm (30:20 area ratio); together they inte-

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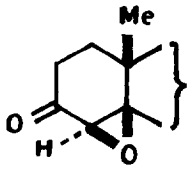
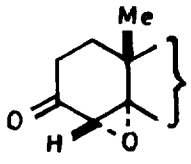


6A



6B

grated for one proton (19-methyl singlet at δ 1.03). The mixture of oxiranes **6** was treated with sulfuric acid in glacial acetic acid to afford **7**. The enolic OH was free since the product gave a positive ferric chloride test and had a bathochromic shift in alkaline methanol from 276 to 315 nm. The NMR spectrum showed the singlets at δ 1.18 (3H; 19-CH_3), 1.22 (3H; 18-CH_3), 6.20 (1H; exch D_2O; 4-OH) and 8.43 ppm (br, 1H; exch D_2O; N-H) (scheme 1).

17-Aza-D-homo-4-androstene-3,16,17a-trione **5** was treated with methyliodide and anhydrous potassium carbonate, in ethyl methyl ketone, to yield the *N*-methyl derivative **8**. Its NMR spectrum exhibited singlet at δ 3.16 (s, 3H; N-CH_3). The imide **3** on treatment with hydrazine hydrate followed by refluxing with xylene afforded the amino derivative **9**. Its NMR spectrum showed a broad signal around 4.96 (2H; disappeared an deuterium exchange; NH_2) (scheme 1).

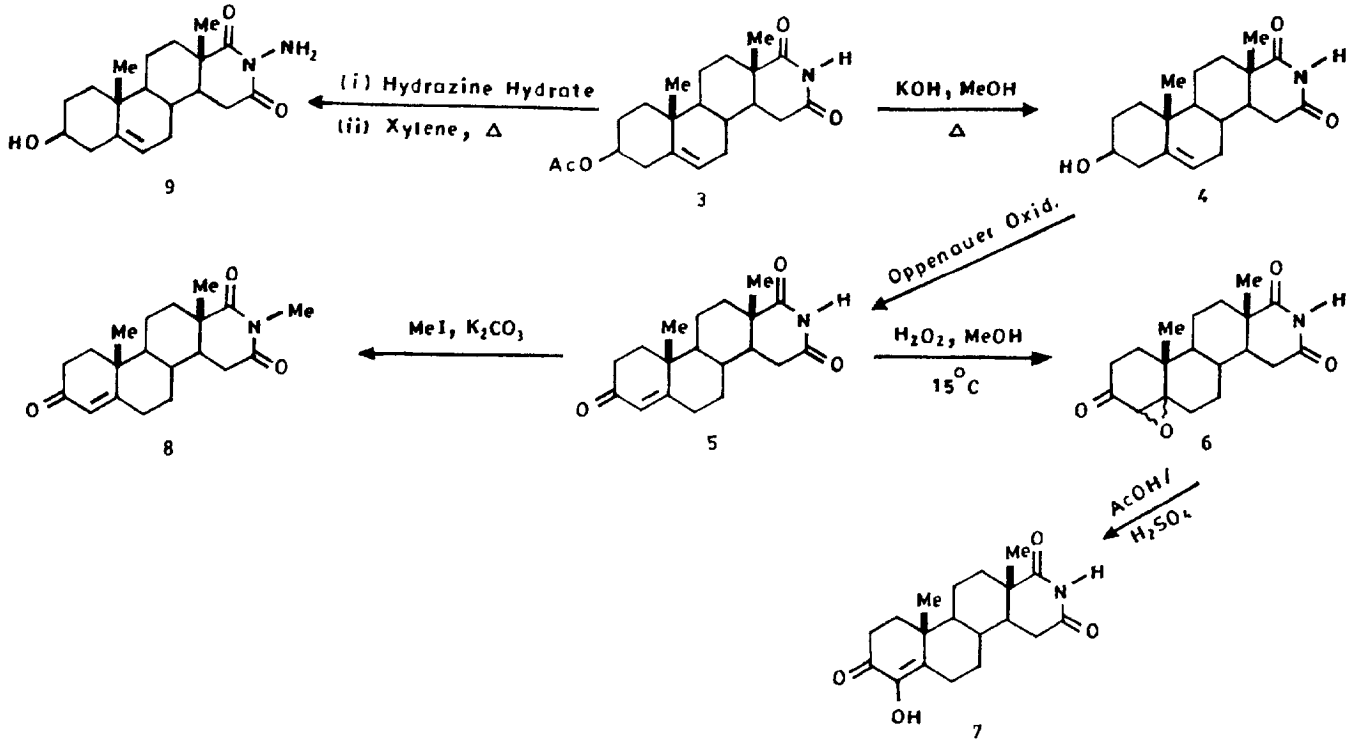
Pharmacology

Aromatase assay in placental microsomes

The aromatase enzyme inhibitory activity of compounds **3–5** and **7–9** was monitored by quantifying the tritiated water released from radiolabelled androstenedione during aromatisation to oestrone [11]. None of the compounds displayed any significant inhibition of aromatase activity at 1, 5 and 20 μm concentrations. The modification of the D-ring to six members and increased polarity due to the presence of imide function resulted in total loss of aromatase inhibitory activity.

Antineoplastic activity

Compounds **3–5** and **7–9** were tested at the National Cancer Institute, Bethesda, MD, *in vitro* against the cell panel consisting of 60 lines. A 48 h continuous drug exposure protocol was used to estimate cell



Scheme 1.

viability or growth, a sulforhodamine B (SRB) protein assay was used. The selectivity analyses of the compounds for differential cellular sensitivity based on the response parameters GI 50, TGI and LC 50 were found to be statistically insignificant.

Experimental protocols

Chemistry

The melting points reported are uncorrected. NMR spectra were recorded on EM-390, 90 MHz model for solutions in deuteriochloroform containing tetramethylsilane (TMS) as an internal reference. IR and UV spectra were obtained with Perkin-Elmer-882 and Lambda-15 models, respectively. Mass spectra were recorded on Vg-11-250J 70S model. Elemental analysis was carried out on Perkin-Elmer-2400 model.

TLC plates were prepared with silica gel G using ethyl acetate and activated at 110°C for 30 min and developed by exposure to iodine vapours. Anhydrous sodium sulfate was used as a drying agent for the organic extracts unless otherwise indicated. Ultraviolet spectra were recorded in methanol (λ_{max} in nm), figures within parentheses refer to log (values) and IR spectra were obtained in a potassium bromide pellet (ν_{max} in cm^{-1}).

17-Aza-D-homo-4ξ, 5-oxido-5ξ-androstane-3,16,17a-trione **6**
Chilled hydrogen peroxide solution (30% v/v, 4 ml) and aqueous sodium hydroxide solution (4 N, 2 ml) were simultaneously added to a stirred solution of 17-aza-D-homo-4-androstene-3,16,17a-trione **5** [9] (1.0 g) in methanol (100 ml) at 15°C. The reaction mixture was kept at 0°C for 12 h, poured into ice-cold water (1000 ml), and the aqueous suspension was extracted with chloroform (6 x 100 ml). The combined chloroform extracts were washed with water, dried and the solvent was removed under vacuum. The fluffy material so obtained was triturated with *n*-hexane, filtered, washed, and dried to afford **6** (0.8 g, 74%); mp 186–188°C; IR (KBr) ν 3200, 1722, 1702, 1380, 1272, 1180 and 860 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.03 (s, 3H, 19-CH_3, α-isomer), 1.17 (s, 3H, 19-CH_3, β-isomer), 1.23 (s, 3H, 18-CH_3), 3.00 and 3.06 (both equivalent to 1H), and 8.43 ppm (br, 1H; exch D_2O); MS *m/z* 331 ($M^{+\cdot}$). Anal $C_{19}H_{25}NO_4$.

17-Aza-D-homo-4-hydroxy-4-androstane-3,16,17a-trione **7**
To a stirred solution of **6** (0.5 g) in glacial acetic acid (10 ml) was added sulfuric acid (0.4 ml) in glacial acetic acid (2.5 ml), at room temperature. The reaction mixture was kept at room temperature for 20 h, poured into ice-cold water (500 ml), and extracted with ethyl acetate (4 x 50 ml). The combined ethyl acetate extract was washed with sodium bicarbonate solution (5%), water, dried, and the solvent was removed under reduced pressure to give a brown solid residue which was chromatographed over a column of neutral alumina (10 g). Elution with chloroform/methanol (9:1) and removing the solvent under reduced pressure gave a solid residue which was crystallised from methanol to afford **7** (0.2 g, 40%); mp 194–196°C; UV_{max} (MeOH): 276 nm (log ε 3.90); (0.1 N KOH/MeOH): 315 nm (log ε 3.63); IR (KBr) ν 3385, 3205, 1725, 1695, 1660, 1384, 1277, 1166, 1104 and 864 cm^{-1}; ^{1}H-NMR ($CDCl_3$); δ 1.18 (s, 3H, 19-CH_3), 1.22 (s, 3H, 18-CH_3), 6.20 (s, 1H, exch D_2O, 4-OH) and 8.43 ppm (br, 1H; exch D_2O, NH); MS *m/z* 331 ($M^{+\cdot}$). Anal $C_{19}H_{25}NO_4$.

17-Methyl-17-aza-D-homo-4-androstene-3,16,17a-trione **8**
To a stirred mixture of 17-aza-D-homo-4-androstene-3,16,17a-trione **5** (0.5 g), anhydrous potassium carbonate (0.25 g), and anhydrous ethyl methyl ketone (30 ml), was added methyl iodide (0.5 ml) at 30°C. After 30 min the reaction mixture was refluxed for 4 h, cooled, and filtered. The solvent was removed under reduced pressure to give an oily residue which was crystallised from ethyl acetate to afford **8** (0.25 g, 47.87%); mp 160–162°C; UV_{max} (MeOH): 239 nm (log ε 4.31); IR (KBr) ν 2882, 1724, 1665, 1415, 1357, 1290, 1099, 1030 and 860 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.23 (s, 6H, 18-CH_3 and 19-CH_3), 3.16 (s, 3H, N-CH_3), and 5.73 (s, 1H). Anal $C_{20}H_{27}NO_3$.

17-Amino-16,17a-dioxo-17-aza-D-homo-5-androsten-3β-ol **9**
16,17a-Dioxo-17-aza-D-homo-5-androsten-3β-yl acetate **3** (1.0 g) was stirred with hydrazine hydrate (25 ml) for 12 h under a nitrogen atmosphere at room temperature. The solvent was removed under reduced pressure to give a white solid residue which was then refluxed with dry xylene for 3 h. The solvent was recovered under reduced pressure and the product washed with petroleum ether (60–80°C), aqueous sodium bicarbonate solution (5%) and water, and it was crystallised from aqueous ethanol to afford **9** (0.6 g, 64.87%); mp 206–208°C; IR (KBr) ν 3597, 3390, 1730, 1675, 1380, 1250, 1175, 1065 and 985 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.03 (s, 3H, 19-CH_3), 1.20 (s, 3H, 18-CH_3), 4.96 (br, 2H, exch D_2O), and 5.40 ppm (m, 1H). Anal $C_{19}H_{28}N_2O_2$.

Aromatase assay in human placental microsomes

Aromatase assays on compounds were carried out by MG Rowlands, at the Institute of Cancer Research Royal Cancer Hospital, Drug Development Section, CRC Laboratories, UK, according to a previously described procedure [12].

Acknowledgments

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