Synthesis and Preliminary Screening of Novel A- and D-Ring Modified Steroids as Aromatase Inhibitors

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Abstract: Estrogens are responsible for the growth of hormone-dependant breast cancer. Regulation of estrogen biosynthesis is considered as a potential therapeutic strategy for the control of breast cancer. The enzyme aromatase catalyzes conversion of androgens into estrogens in the last step of estrogen biosynthesis. Inhibition of aromatase is adopted as an efficient approach for the prevention and treatment of breast cancer. Many steroidal and nonsteroidal aromatase inhibitors have been developed and are used clinically for breast cancer therapy. In this report, it has been tried to incorporate some structural features (six membered lactam ring) of nonsteroidal aromatase inhibitors like aminoglutethimide and rogletimide into the A-/D-ring of the steroid nucleus with certain additional features responsible for binding to the enzyme aromatase. Some ring-A [17β-hydroxy-4-(4-substitutedphenyl)-4-aza-5-androsten-3-one] and ring-D modified steroidal compounds [4-substituted-17a-aza-*D*-homo-4-androstene-3,17-dione, 4-substituted-17-aza-*D*-homo-4-androstene-3,16,17a-trione, 4-substituted-17a-methyl-17a-aza-*D*-homo-4-androsten-3-one] were synthesized and screened for binding to the aromatase enzyme. None of the synthesized compounds showed promising aromatase inhibiting activity leading to the conclusion that structurally the original androstane ring skeleton should not be tampered with, for obtaining good aromatase inhibiting activity.

Keywords: A-/D-Heterosteroids, Androstanes, Aromatase, Aromatase Inhibitors, Azasteroids, Breast cancer.

1. INTRODUCTION

Breast cancer, the most common form of cancer diagnosed in women, remains the second most common cause of death in women in the Western world despite advances made in its treatment. Generally considered to be the disease of older women, 22 % of the breast cancer cases occur in females below the age of 50 [1]. More than one million women develop breast cancer each year worldwide with nearly half of these diagnoses occurring in the Unites States and Europe. Still worse is the fact that nearly 40 % of these women die of their disease [2]. Approximately two-thirds of postmenopausal breast cancer patients have estrogen-dependent breast cancer, which contains estrogen receptors (ERs) and requires estrogens for tumor growth [3].

The estrogen synthase (aromatase) enzyme system is responsible for the biosynthesis of estrogens in humans. Though estrogens are vital for normal growth and development, but they also promote the growth of certain breast cancers [4]. Approximately 30-50 % of breast cancers are considered to be hormone-dependent. Consequently, regulation of estrogen biosynthesis has advanced as a potential therapeutic strategy, resulting into development of active-site inhibitors having potential for the control of breast cancer. Aromatase is a cytochrome P-450 enzyme complex responsible for the conversion of androgens to estrogens. Aromatase catalyzes conversion of androgens into estrogens in the last step of estrogen biosynthesis [5]. Aromatase inhibitor testolactone [6] (**1**), is a leading irreversible aromatase inhibitor of very low potency. Aminoglutethimide **(4**) and 4-hydroxyandrostenedione **(2**) have demonstrated therapeutic effectiveness in the treatment of hormone-dependent breast tumors in both animals [7,8] and humans [9-13]. Compounds that inhibit the enzyme aromatase, such as exemestane [14] **(3**), rogletimide [15] (**5**), anastrozole [16] (**6**), letrozole [17] **(7**), vorozole [18] (**8**) and many other steroidal and nonsteroidal compounds have applications in the treatment of advanced estrogen-dependent breast cancer [18]. Inhibition of aromatase is an efficient approach for the prevention and treatment of breast cancer [19].

Potent steroidal aromatase inhibitors are mainly the C-1, C-2, C-4, C-6, C-19 substituted and C-2 and C-19 bridged steroidal compounds. New analogs of formestane have been synthesized and their biological activity investigated in an attempt to find new aromatase inhibitors and to gain insight into their structure-activity relationships (SAR) [20, 21].

In the steroids, effective aromatase enzyme inhibition is observed in compounds having androstane skeleton possessing an A/B trans ring junction and a ketone functionality at the C-3 position [3], while nonsteroidal inhibitors have a suitably positioned hetero atom to interact strongly with heme iron. With the exception of testolactone (**1**) D-modified steroids have hardly received any research attention

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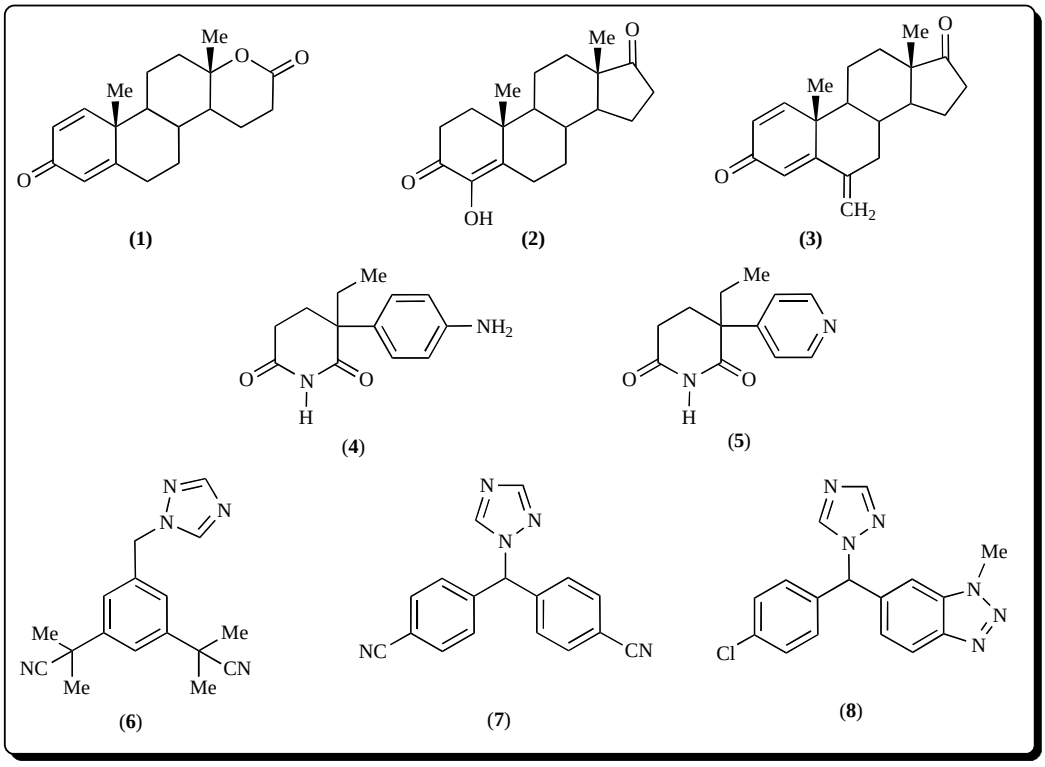


as aromatase inhibitors. Aiming to inhibit aromatase activity, we planned to synthesize some A- and D-ring modified steroidal compounds and to perform preliminary screening for their aromatase inhibiting activity.

Aminoglutethimide (**4**) and rogletimide (**5**) have amide nitrogen in the six membered ring. To strike a structural resemblance between these derivatives (**4**, **5**) and the steroidal skeleton, it was thought to incorporate nitrogens in both A- and D-rings independently in the steroidal skeleton as amide/imide functions. Such a structural change in the steroidal system could cause higher binding of the modified steroidal derivatives to the aromatase enzyme with retention of selectivity of steroids for the enzyme.

2. EXPERIMENTAL

2.1. General

Melting points were determined using a VEEGO make microprocessor-based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA T FT-IR spectrophotometer using KBr discs. NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as internal standard for ^1H NMR. Chemical shift values are mentioned in δ, ppm. Chromatographic separations were performed on silica gel columns. The microanalyses for C, H and N were performed on Thermo Scientific FLASH 2000 organic elemental analyzer. The progress of all reactions was monitored by TLC on 2 cm x 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV (254 nm) and iodine vapours. The term "dried" refers to the use of anhydrous sodium sulfate. All reagents used were of analytical reagent grade.

2.1. Chemical

2.2.1. 17β-Hydroxy-4-aza-5-androsten-3-one [22] (10a)

17β-Hydroxy-4-nor-5-oxo-3,5-seco-3-androstanoic acid [23] (**9**) (0.5 g, 1.62 mM) was heated with urea (0.19 g, 3.24 mM) in ethylene glycol at 150 °C under nitrogen atmosphere for 2 hrs with constant stirring. The reaction mixture was poured into water (50 ml), acidified with conc. hydrochloric acid (5 ml) and stirred for 15 min. The crude product was filtered out and dried. The solid so obtained was crystallized from methanol to afford compound (**10a**) [22] (0.31 g, 65 %), m.p. 285-88 °C (Lit; [22] 289-91 °C). UV (MeOH): 231 (log ε 3.33). IR (KBr): 3197, 1666, 1623, 1551, 1386, 1199 and 1056. NMR: 8.38 (s, 1H), 4.87-4.89 (m, 1H), 3.58-3.62 (t, 1H), 1.09 (s, 3H) and 0.76 (s, 3H).

General Procedure for 17β-hydroxy-4-(4-substituted-phenyl)-4-aza-5-androsten-3-ones

Compound (**9**) (1.62 mM) was heated with different aromatic amines (2.4 mM) in presence of DMAP (25 mg) as catalyst at 160 °C under nitrogen atmosphere for 2 hrs. The reaction mixture was poured into water (50 ml) acidified with conc. hydrochloric acid (5 ml) and stirred for 15 min. The crude product was filtered out, dried and purified by passing through a silica gel column using hexane-ethyl acetate, (9:1) as an eluent. The solid so obtained was crystallized from methanol to afford the desired compound.

2.2.2. 17β-Hydroxy-4-phenyl-4-aza-5-androsten-3-one (10b)

Yield (0.19 g, 32 %), m.p. 263-65 °C. UV (MeOH): 229 (log ε 3.23). IR (KBr): 3457, 1641, 1387 and 1235. NMR: 7.33-7.37 (m, 2H), 7.23-7.27 (m, 1H), 7.00-7.03 (m, 2H), 4.30-4.32 (m, 1H), 3.57-3.61 (t, 1H), 2.65-2.9 (m, 2H), 1.21 (s, 3H) and 0.72 (s, 3H). Calculated for $C_{24}H_{31}NO_2$: C 78.87,

H 8.55, N 3.83. Found: C 78.02, H 8.71, N 3.54. MS: m/z 367.1 (M^+).

2.2.3. 17β-Hydroxy-4-(4-fluorophenyl)-4-aza-5-androsten-3-one (10c)

Yield (0.23 g, 38 %), m.p. 266-68 °C. UV (MeOH): 228 (log ε 3.37).IR (KBr): 3479, 1642, 1504, 1398 and 1209. NMR: 6.96-7.06 (m, 4H), 4.35-4.42 (m, 1H), 3.62-3.71 (t, 1H), 2.57-2.62 (m, 2H), 1.19 (s, 3H) and 0.72 (s, 3H). Calculated for $C_{24}H_{30}FNO_2$: C 75.17, H 7.88, N 3.65. Found: C 74.89, H 7.50, N 3.85. MS: m/z 384.1 (M^+).

2.2.4. 17β-Hydroxy-4-(4-tolyl)-4-aza-5-androsten-3-one (10d)

Yield (0.2 g, 35 %), m.p. 214 -17 °C. UV (MeOH): 229 (log ε 3.35). IR (KBr): 3199, 1675, 1651, 1510, 1390, 1217 and 804. NMR: 7.21-7.23 (d, 2H), 6.94-6.97 (d, 2H), 4.40-4.42 (m, 1H), 3.63-3.68 (t, 1H), 2.36 (s, 3H), 1.24 (s, 3H) and 0.79 (s, 3H). Calculated for $C_{25}H_{33}NO_2$: C 79.11, H 8.76, N 3.69. Found: C 79.43, H 8.39, N 3.96. MS: m/z 380.1 (M^+).

2.2.5. 4-Hydroxy-17a-aza-D-homo-4-androstene-3,17-dione [24] (13)

The epoxide (**12**) was prepared as per the reported method [24,25]. To a chilled solution of epoxide (**12**) (0.6 g, 1.9 mM) in glacial acetic acid (6 ml) a chilled mixture of conc. sulphuric acid (0.8 ml, 11.4 mM) in glacial acetic acid (2.0 ml) was added with occasional shaking and the reaction mixture was allowed to stand for 18 hrs at 0-5 °C. The reaction mixture was poured into water (100 ml) and basified with potassium hydroxide solution (5 %). The resulting alkaline mixture was acidified with glacial acetic acid followed by neutralization with excess of sodium bicarbonate solution (5 %) and extracted with chloroform (3 x 25 ml). the combined chloroform extract was washed with sodium bicarbonate solution (5 %), dried and the solvent recovered to afford crude product which was crystallized from methanol to obtain crystals of compound [24] (**13**) (0.3 g, 50 %), m.p. 262-65°C (Lit; [24] 264-66 °C). UV (MeOH): 276 (log ε 3.19). (Alk. MeOH): 315 (log ε 4.1).IR (KBr): 3458, 3183, 1681, 1391, 1238, 1163 and 964. NMR: 6.05 (s, 1H), 1.13 (s, 3H) and 1.10 (s, 3H). MS: m/z 318.0 (M^+).

2.2.6. 4-Phenylthia-17a-aza-D-homo-4-androstene-3,17-dione (14)

Thiophenol (0.250 g, 4.4 mM) was stirred with sodium hydride (0.105 g, 4.4 mM) in dry dioxane (5.0 ml) under nitrogen atmosphere at 20 °C. A solution of the epoxide [24] (**12**) (0.7 g, 2.21 mM) in dry dioxane (5.0 ml) was added slowly to the above solution with stirring. The reaction mixture was stirred for 6 hrs at room temperature, poured into water (100 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and the solvent removed to afford the crude product. The crude product so obtained was purified by passing through a column of silica gel eluting with hexane first to remove excess thiophenol and then with hexane-ethyl acetate (9:1). The solid so obtained was crystallized from methanol to afford yellowish crystals of the desired compound (**14**) (0.6 g, 68 %), m.p. 222-24 °C. UV (MeOH): 249 (log ε 3.91). IR (KBr): 3195, 1678, 1654, 1396, 1232, 1157, 1068, 743, 465 and 427. NMR: 7.08- 7.22 (m, 5H)**,** 6.05 (b, 1H)**,** 1.30 (s, 3H) and 1.21 (s, 3H). Calculated for $C_{25}H_{31}NO_2S$: C 73.31, H 7.63, N 3.42. Found: C 73.54, H 7.91, N 3.29. MS: m/z 410.1 (M^+).

2.2.7. 4-(4-Aminophenylthia)-17a-aza-D-homo-4-androstene-3,17-dione (15)

p-Aminothiophenol (0.3 g, 2.4 mM) was stirred with sodium hydride (0.056 g, 2.4 mM) in dry dioxane (5.0 ml) under nitrogen atmosphere at 20°C. A solution of the epoxide [24] (**12**) (0.5 g, 1.6 mM) in dry dioxane (3.0 ml) was added slowly to the above solution with stirring. The reaction mixture was stirred for 8 hrs at room temperature. The reaction mixture was poured into water (100 ml), acidified with hydrochloric acid and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and solvent recovered to afford the crude product. The crude product so obtained was purified by passing through a column of silica gel eluting first with chloroform and then with chloroform-methanol (9.5: 0.5). The solid so obtained was crystallized from methanol to afford yellowish crystals of compound (**15**) (0.2 g, 30 %), m.p. 210-13 °C. UV (MeOH): 260 (log ε 4.3). IR (KBr): 3444, 1653, 1494, 1395, 1159, 820, 750 and 519. NMR: 7.06-7.09 (d, 2H), 6.54-6.55 (d, 2H), 6.29 (b, 1H), 1.25 (s, 3H) and 1.24 (s, 3H). Calculated for $C_{25}H_{32}N_2O_2S$: C 70.72, H 7.60, N 6.60. Found: C 70.35, H 7.69, N 6.93. MS : m/z 425.1 (M^+).

2.2.8. 4ξ,5-Oxido-17-aza-D-homo-5ξ-androstane-3,16,17a-trione (20)

Cold solutions of sodium hydroxide (20 %, 1.5 ml, 7.6 mM) and hydrogen peroxide (30 %. 2 ml, 1.74 mM) were added simultaneously drop by drop during stirring to a chilled solution of 16,17a-dioxo-17-aza-*D*-homo-4-androsten-3-one [26] (**19**) (1.0 g, 3.17 mM) in methanol (25 ml). The reaction mixture was allowed to stand for 3 hrs at 0 °C, diluted with water (200 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and the solvent recovered to afford the crude product 4ξ,5-oxido-17-aza-*D*-homo-5ξ-androstane-3,16,17a-trione (**20**) (0.6 g, 60 %), which was used as such for the next step without purification. UV (MeOH): Transparent at 239. IR (KBr): 3384, 1689, 1444, 1373, 1275 and 1054.

2.2.9. 4-Hydroxy-17-aza-D-homo-4-androstene-3,16,17a-trione (21)

The reaction was performed as described under compound (**13**) using 4ξ,5-oxido-17-aza-*D*-homo-5ξ-androstane-3,16,17a-trione (**20**) (1.0 g, 3.0 mM). The reaction mixture was poured into water (100 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with sodium bicarbonate solution (5 %), dried and solvent recovered to afford a crude product which was crystallized from methanol to afford 4-hydroxy-17-aza-*D*-homo-4-androstene-3,16,17a-trione (**21**) (0.5 g, 50 %), m.p 240-45 °C. UV (MeOH): 276 (log ε 3.58), (Alk. MeOH): 315 (log ε 3.53). IR (KBr): 3390, 3298, 1688, 1658, 1433, 1384, 1280 and 1031. NMR: 7.62 (b, 1H; exchanged with D_2O)**,** 6.03 (s, 1H; exchanged with D_2O)**,** 1.16 (s, 3H) and 1.12 (s, 3H). Calculated for $C_{19}H_{25}NO_4$: C 68.86, H 7.60,

N 4.23. Found: C 68.43, H 7.46, N 4.61. MS: m/z 332.1 (M^+).

2.2.10. 16,17a-Dioxo-4-phenylthia-17-aza-D-homo-4-androsten-3-one (22)

The reaction was performed as described under compound (**14**) using epoxide (**20**) (0.5 g, 1.5 mM). The reaction mixture was stirred for 3 hrs at room temperature, poured into water (100 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and the solvent removed to afford the crude product. The crude product so obtained was purified by passing through a column of silica gel eluting with hexane first to remove excess thiophenol and then with hexane-ethyl acetate (9:1). The solid so obtained was crystallized from methanol to afford yellow crystals of 16,17a-dioxo-4-phenylthia-17-aza-*D*-homo-4-androsten-3-one (**22**) (0.3 g, 68 %), m.p 125-27 °C. UV (MeOH): 250 (log ε 3.77). IR (KBr): 3229, 1695, 1686, 1580, 1437, 1276, 1113 and 1024. NMR: 7.63 (b, 1H; exchanged with D_2O)**,** 7.12-7.19 (d, 2H)**,** 7.01-7.05 (m, 3H)**,** 1.17 (s, 3H) and 0.86 (s, 3H). Calculated for $C_{25}H_{29}NO_3S$: C 70.80, H 6.90, N 3.31. Found: C 70.42, H 7.11, N 3.63. MS: m/z 424.1 (M^+).

2.2.11. 4-(4-Aminophenylthia)-17-aza-D-homo-4-androstene-3,16,17a-trione (23)

The reaction was performed as described under compound (**15**) using epoxide (**20**) (0.5 g, 1.58 mM).The reaction mixture was poured into water (100 ml), acidified with hydrochloric acid (5 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and the solvent recovered to afford the crude product. The crude product so obtained was purified by passing through a column of silica gel eluting first with chloroform and then with chloroform-methanol (9:1). The solid so obtained was crystallized from methanol to afford yellowish crystals of 4-(4-aminophenylthia)-17-aza-*D*-homo-4-androstene-3,16,17a-trione (**23**) (0.28 g, 59.66 %), m.p 120-25 °C. UV (MeOH): 258 (log ε 3.07). IR (KBr): 3531, 3243, 1694, 1373, 1267, 1064 and 829. NMR: 7.21-7.23 (d, 2H), 6.95-6.97 (d, 2H), 1.25 (s, 3H) and 0.82 (s, 3H). Calculated for $C_{25}H_{30}N_2O_3S$: C 68.46, H 6.89, N 6.39. Found: C 68.15, H 6.71, N 6.23. MS: m/z 456.6 (M+ 18).

2.2.12. 4-Hydroxy-17a-methyl-17a-aza-D-homo-4-androsten-3-one [24] (26)

To a chilled solution of 17a-methyl-4ξ,5-oxido-17a-aza-*D*-homo-5ξ-androstan-3-one [24,25] (**25**) (0.7 g, 2.0 mM) in glacial acetic acid (6 ml) a chilled mixture of conc. sulphuric acid (1.4 ml, 14.0 mM) in glacial acetic acid (2.0 ml) was added with occasional shaking and the reaction mixture was allowed to stand for 18 hrs at 0-5 °C. The reaction mixture was poured into water (100 ml) and basified with potassium hydroxide solution (5 %). The resulting alkaline mixture was acidified with glacial acetic acid followed by neutralization with excess of sodium bicarbonate solution (5 %) and extracted with chloroform (3 x 25 ml). The combined chloroform extract was washed with sodium bicarbonate solution (5 %), dried and the solvent recovered to afford crude product which was crystallized from methanol to obtain crystals of 4-hydroxy-17a-methyl-17a-aza-*D*-homo-4-androsten-3-one [24] (**21**) (0.31 g, 52 %), m.p. 227-30 °C (Lit; [24] 227-29 °C). UV (MeOH): 274 (log ε 4.18), (Alk. MeOH): 334 (log ε 4.46). IR (KBr): 3431, 1672, 1620, 1369, 1163, 1089 and 987. NMR: 6.05 (s, 1H)**,** 1.14 (s, 3H) and 0.88 (s, 3H). MS: m/z 318.1 (M^+).

2.2.13. 17a-Methyl-4-phenylthia-17a-aza-D-homo-4-androsten-3-one (27)

The reaction was performed as described under compound (**14**) using 17a-methyl-4ξ,5-oxido-17a-aza-*D*-homo-5ξ-androstan-3-one [24] (**25**) (0.4g, 1.3 mM). The reaction mixture was stirred for 6 hrs at room temperature, poured into water (100 ml) and extracted with dichloromethane (4 x 25 ml). The combined extract was washed with water, dried and the solvent removed to afford the crude product. The crude product so obtained was purified by passing through a column of silica gel eluting with hexane first to remove excess thiophenol and then with hexane-ethyl acetate (9:1). The solid so obtained was crystallized from methanol to afford yellowish crystals of desired compound (**27**) (0.2 g, 52 %), m.p. 173-75 °C. UV (MeOH): 251 (log ε 4.03). IR (KBr): 1689, 1562, 1474, 1378, 1282 1106, 746, 808 and 693. NMR: 7.00-7.19 (m, 5H)**,** 1.19 (s, 3H) and 1.00 (s, 3H). Calculated for $C_{26}H_{35}NOS$: C 76.24, H 8.61, N 3.42. Found: C 76.38, H 8.55, N 3.64. MS: m/z 410.1 (M^+).

2.3. Biological

2.3.1. Human Placental Microsomal Aromatase Assay

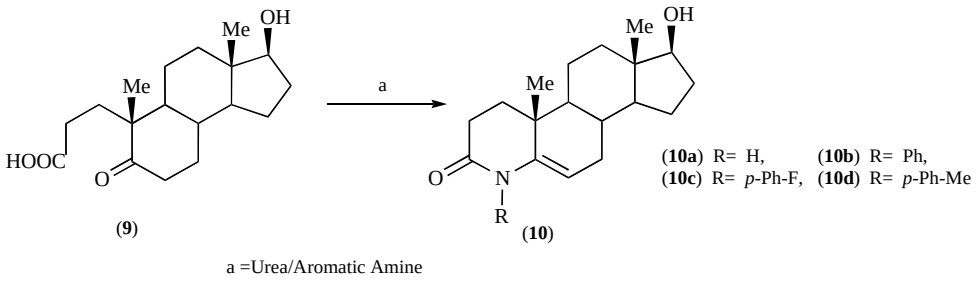
The synthesized compounds were screened for aromatase inhibiting activity in human placental microsomal assay. As human term placenta is a rich source of aromatase enzyme, the assay is a measure of test compounds to bind aromatase enzyme in presence of natural substrates testosterone and androstenedione.

2.3.1.2. Enzyme Preparation

The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue as per the procedure described by Thompson and Siiteri [29]. The isolated microsomes were suspended in minimum volume of phosphate buffer (0.05 M, pH 7.4, 20 %). Additionally DTT (Dithiothreitol, 10 mM) and EDTA (1 mM) were added to protect the enzyme from degradation. The enzyme preparation was stored at -70°C.

2.3.1.2. Aromatase Inhibition Assay

The assay was performed by measuring the ^3H_2O formed from [1β-^{3}H] androstenedione during aromatization [30]. Each incubation tube contained [1β-^{3}H] androstenedione (0.08 μCi), unlabeled androstenedione (485 nM), NADP (2 mM), glucose-6-phosphate (20 mM), glucose-6-phosphate dehydrogenase (0.4 units), and the inhibitor (0.05 nM) in phosphate buffer (pH 7.4). The test compounds had been dissolved in DMSO and diluted with buffer. The final DMSO concentration in the control and inhibitor incubation was 2%. Each tube was preincubated for 5 min at 30 °C in a shaking water bath. Microsomal protein (0.1 mg) was added to start the reaction. The total volume for each incubation was 0.2 ml. The reaction was terminated by withdrawing 100 μl aliquots at 0, 7, 14, and 21 min and pipetting them into



Scheme 1.

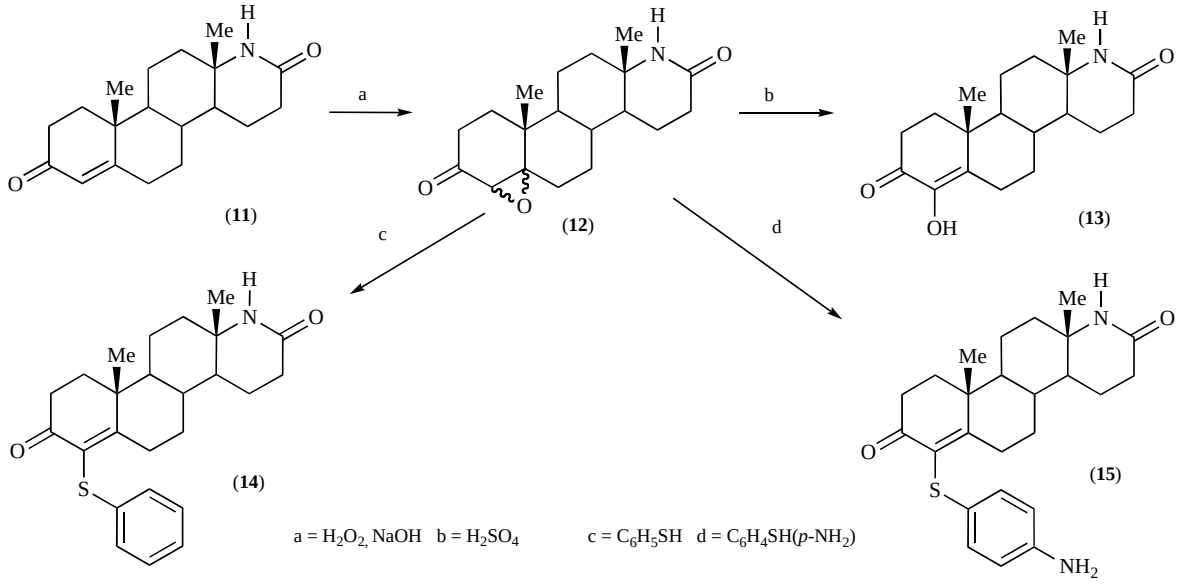
200 µl of a cold mercuric chloride solution (1 mM). After addition of an aqueous dextran-coated charcoal (DCC) suspension (200 µl, 2%), the vials were shaken for 20 min and centrifuged at 1500 x g for 5 min to separate the charcoal-adsorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture using Perkin Elmer-Wallac β-Counter.

3. RESULTS AND DISCUSSION

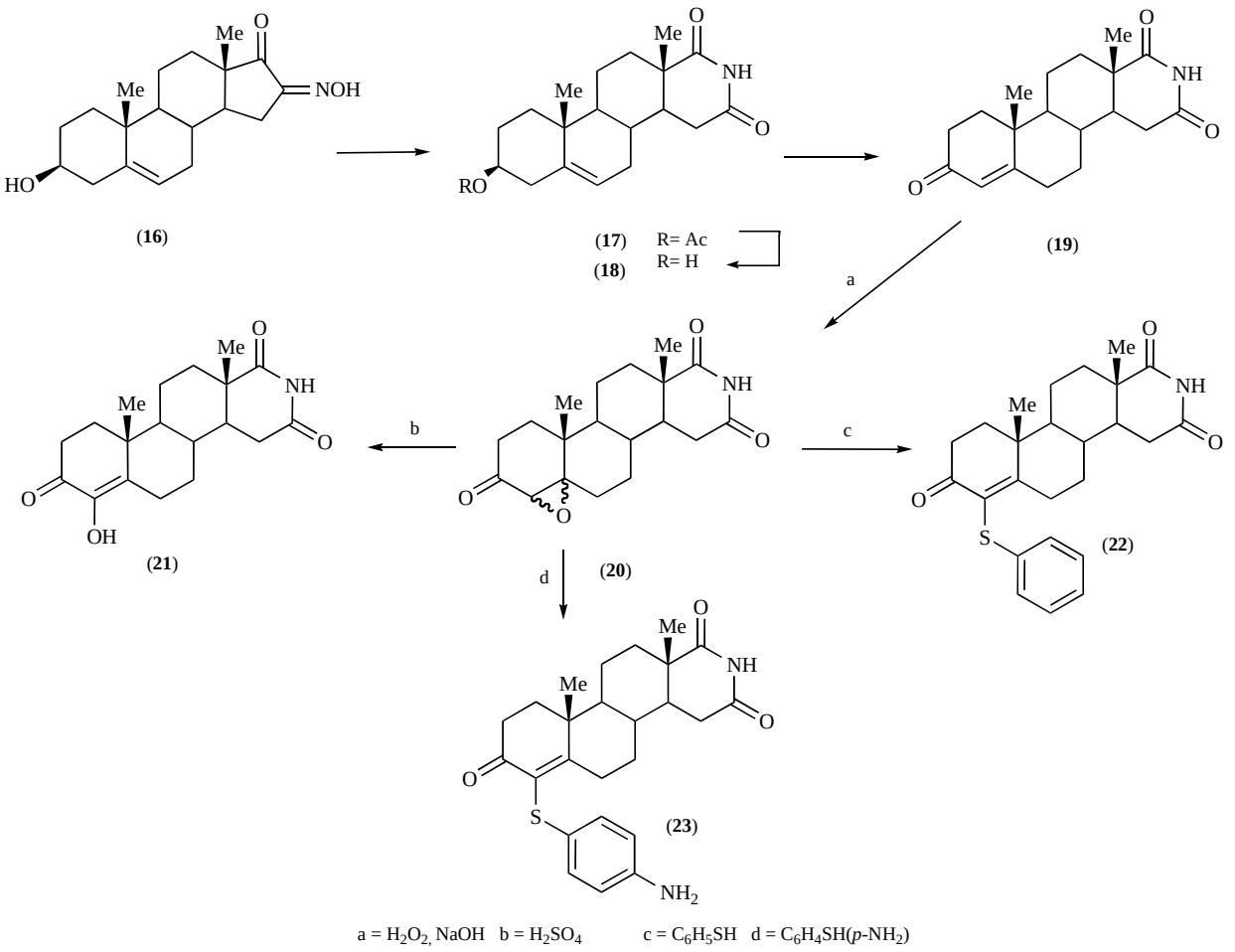
3.1. Chemical

For the synthesis of A-ring modified steroidal compounds 17β-hydroxy-5-oxo-*A*-nor-3,5-seco-3-androstanoic acid [23] (**9**) was used as the starting material for the synthesis of 4-aza-5-ene derivatives of androstane as shown in Scheme **1**. Compound (**9**) was condensed with urea in ethylene glycol at 150 °C to afford 17β-hydroxy-4-aza-5-androsten-3-one [22] (**10a**). [IR: 3197 (N-H str) and 1666 cm^{-1} (4-C=O lactam). PMR δ: 8.38 (s, 1H; 4-N-*H*), 4.87-4.89 (m, 1H; 6-C*H*) and 3.58-3.62 (t, 1H; 17α-C*H*)]. Fusion of compound (**9**) with aniline in presence of dimethylaminopyridine (DMAP) as catalyst at 160 °C under nitrogen atmosphere, afforded 17β-hydroxy-4-phenyl-4-aza-5-androsten-3-one (**10b**). [IR: 3457 (17- OH) and 1641 cm^{-1} (3-C=O). PMR: δ 7.33-7.37 (m, 2H; Ar-C*H*), 7.23-7.27 (m, 1H; Ar-C*H*), 7.00-7.03 (m, 2H; Ar-C*H*), 4.30-4.32 (m, 1H; 6-C*H*) and 3.57-3.61 (t, 1H; 17α-C*H*)]. When compound (**9**) was treated with *p*-fluoroaniline in presence of DMAP, it yielded 17β-hydroxy-4-(4-fluorophenyl)-4-aza-5-androsten-3-one (**10c**). [IR: 3479 (17-OH) and 1642 cm^{-1} (4-C=O lactam). It offered characteristic NMR signals at δ 6.96-7.06 (m, 4H) for aromatic protons]. When compound (**9**) was cyclized with *p*-toluidine it offered 17β-hydroxy-4-(4-tolyl)-4-aza-5-androsten-3-one (**10d**). [IR: 3476 (17- OH) and 1642 cm^{-1} (3-C=O). PMR: δ 7.21-7.23 (d, 2H; Ar-*H*), 6.94-6.97 (d, 2H; Ar-*H*), 4.40-4.42 (m, 1H; 6-C*H*), 3.63-3.68 (t, 1H; 17-*H*) and 2.36 (s, 3H; Ar-CH_3)].

For the synthesis of D-ring modified steroids two different schemes (Schemes **2** & **3**) were followed. For Scheme **2** 17a-aza-*D*-homo-4-androstene-3,17-dione [24] (**11**) was used as a starting material. Various D-ring modified 4-androstene steroids were synthesized as depicted in Scheme **2**. Treatment of the enone (**11**) with alkaline hydrogen peroxide afforded the epoxide, 4ξ,5-oxido-17a-aza-*D*-homo-5ξ-androstane-3,17-dione (**12**), which upon acid treatment offered the 4-hydroxy-17a-aza-*D*-homo-4-androsten-3,17-dione [24] (**13**). The epoxide (**12**), when stirred with thiophenol in dry dioxane with sodium hydride afforded the 4-phenylthia-17a-aza-*D*-homo-4-androstene-3,17-dione (**14**). [IR: 3195 (secondary-N-H), 1678 (-C=O) and 1654 (17-C=O) cm^{-1}. PMR: δ 7.08-7.22 (m, 5H; Ar-*H*) and 6.05 (b, 1H; N*H*)]. The oxirane (**12**) when stirred with *p*-aminothiophenol in dry dioxane with sodium hydride



Scheme 2.



Scheme 3.

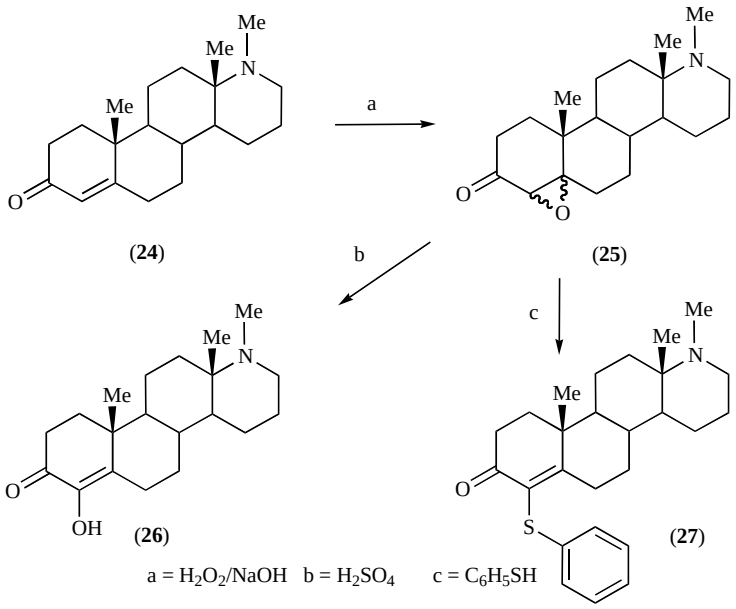
afforded the targeted 4-(4-aminophenylthia)-17a-aza-*D*-homo-4-androstene-3,17-dione (**15**). [IR: 3444 (-N-H) and 1653 cm^{-1} (-C=O). PMR: δ 7.06-7.09 (d, 2H; Ar-*H*), 6.54-6.55 (d, 2H; Ar-*H*), 6.29 (b, 1H; N*H*), 1.25 (s, 3H) and 1.24 (s, 3H)].

It was planned to synthesize 17-aza derivatives with substitution at position 4. Beckmann's rearrangement of 3β-hydroxy-16-oximino-5-androsten-17-one [27] (**16**) was effected with acetic anhydride and acetic acid to afford 3β-acetoxy-17-aza-*D*-homo-5-androstene-16,17a-dione [28] (**17**). Alkaline hydrolysis and Oppenauer oxidation of compound (**17**) gave 16,17a-dioxo-17-aza-*D*-homo-4-androsten-3-one [26] (**19**). The enone (**19**) on treatment with alkaline hydrogen peroxide afforded the epoxide (**20**) which shows no absorption at 240 nm in its UV spectrum due to the absence of α,β-unsaturated system. Epoxide (**20**) upon acidic treatment afforded 4-hydroxy-17-aza-*D*-homo-4-androstene-3,16,17a-trione (**21**). [UV (MeOH): 276 (log ε 3.58), (Alk. MeOH): 315 (log ε 3.53). IR: 3390 (-OH) and 1688 cm^{-1} (3-C=O). PMR: δ 7.62 (b, 1H; -NH), 6.03 (s, 1H; OH)]. When the epoxide (**20**) was stirred with thiophenol in basic medium, it afforded 16,17a-dioxo-4-phenylthia-17-aza-*D*-homo-4-androsten-3-one (**22**). [IR: 1695 (3-C=O) and 1686 cm^{-1} (16, 17-C=O). PMR: δ 7.63 (b, 1H; -NH), 7.12-7.19 (d, 2H; -Ar-H), 7.01-7.05 (m, 3H; -Ar-H)]. When the epoxide (**20**) was treated with *p*-aminothiophenol and sodium hydride at room temperature it gave 4-(4-aminophenylthia)-17-aza-*D*-homo-4-androstene-3,16,17a-trione (**23**). [IR: 1694 cm^{-1} (3-C=O). PMR: δ 7.21-7.23 (m, 2H; Ar-2H), 6.95-6.97 (m, 2H; Ar-2H)].

It was also planned to have basic nitrogen in the D-ring in place of the acidic amide. Scheme **4** was followed for this purpose. 17a-Methyl-17a-aza-*D*-homo-4-androsten-3-one [24] (**24**) **o**n treatment with alkaline hydrogen peroxide afforded the epoxide (**25**). Treatment of the oxirane (**25**) with conc. sulphuric acid in glacial acetic acid gave 4-hydroxy-17a-methyl-17a-aza-*D*-homo-4-androsten-3-one (**26**). IR: 3431 (-OH) and 1672 (3-C=O) cm^{-1}, PMR: δ 6.05 (s, 1H; O*H*)]. The epoxide (**25**) was stirred with thiophenol in dry dioxane and sodium hydride under inert environment at room temperature to afford 17a-methyl-4-phenylthia-17a-aza-*D*-homo-4-androsten-3-one (**27**). [IR: 1689 (3-C=O) cm^{-1}. PMR: δ 7.00-7.19 (m, 5H; Ar-*H*)].

3.2 Biological

All of the synthesized compounds (**10a-10d, 13-15, 20-23, 26 and 27**) were evaluated for their aromatase inhibiting activity. The assay was performed by monitoring the enzyme activity by measuring the ^3H_2O formed from [1β-^{3}H] androstenedione during aromatization. The activity profile of the compounds is indicated in Table **1**. Rings A and D in



Scheme 4.

compounds (**10, 13-15, 20-23**) were modified to make them look structurally similar to nonsteroidal compounds (**4, 5**). This change was effected with a view so that such structural modification might cause a change in binding mode affinity of these compounds to aromatase enzyme. As evident from the results given in Table **1**, these compounds lost their binding affinity almost completely, to the enzyme. Restoration of basicity to the nitrogen atom in compounds (**26** and **27**) did not cause any impact as far as binding affinity to the enzyme was concerned.

Table 1. Biological Activity (% Inhibition of Aromatase Enzyme) of Compounds

Compd.	Conc. µM	% Inhibition
10a	5.0	0.9±1.6
10b	5 .0	0.6±1.0
10c	5.0	4.6±4.4
10d	5 .0	2.3±2.3
13	0.5	0.0±0.0
14	0.5	0.3±0.5
15	0.5	0.7±1.2
20	0.5	4.9±3.3
21	0.5	2.0±2.8
22	5.0	11.0±1.5
23	5 .0	2.3±1.8
26	5.0	0.0±0.1
27	5 .0	0.0±0.0
Exemestane	5.0	96.1±2.2

In our earlier report [21] we have reported some ring-A substituted compounds having the original androstane ring skeleton intact. Some of these compounds showed good aromatase inhibiting properties. In the present study, it has been observed that whenever a nitrogen atom (with or without basicity) has been introduced into the androstane ring skeleton by replacement of carbon at C-4, C-16 and C-17 positions, total loss of binding affinity to the enzyme resulted. This clearly shows that in the newly synthesized compounds the original androstane skeleton must be maintained for binding to the aromatase enzyme. Any future designing process for the development of potential aromatase inhibitors must take into consideration the fact that any tampering of the original androstane ring skeleton would most likely result in drastic reduction/abolition of aromatase inhibiting activity.

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