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Research Article

Synthesis and Characterization of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one as 5α-Reductase Inhibitors, antimicrobial and antioxidant activities

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ABSTRACT
The study reports the convenient and efficient synthesis of several new analogues of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one (**3a**-**3e**) from commercially available Diosgenin as the starting material. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry. We herein report the 5*α*-reductase inhibitory, antimicrobial and antioxidant activity of these synthesized analogues in comparison to the reference drugs. The results from these experiments indicate that compound 3*β*-(2,4-Dinitrobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**3d**) was found to be most promising analogue against 5*α*-reductase enzyme along with antimicrobial and antioxidant activity while, analogue 3β-(4-Hydroxybenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**3c**) found to be least active. The detailed 5α-reductase inhibitory, antimicrobial and antioxidant activities of the synthesized compounds were reported in this communication.

Keywords: 5α-Reductase inhibitor, Antimicrobial, Antioxidant, Dutasteride, Ciprofloxacin, Voriconazole

INTRODUCTION

Steroids perform several fundamental functions in the living systems; therefore, form the basis of many important discoveries in the field of medicinal chemistry [1]. It is well known that modified steroids play a vital role in the development of living organisms throughout their life cycle because of their ability to affect some of the essential biological functions [2-4]. Although, various modifications of steroids such as derivatization, cyclization and heterocyclization have been tried but as far the literature precedents are concerned, only small efforts made towards the efficient synthesis and simultaneous biological analysis of aromatic ring substituted steroids [5-6]. A steroid containing substituted aromatic ring condensed with cyclopentanophenanthrene scaffold has achieved significant importance in the view of their diverse and interesting biological activities [7-8]. It is not surprising that aromatic ring substituted steroids have become an area of interest not only for a chemist but also for pharmacologists and physicians as well [9-12].

Recent studies revealed that incorporation of substituted aromatic rings enhances biological activities of steroidal molecules as 5*α*-reductase inhibitor, antimicrobial and antioxidant activities [13-16]. Moreover, the treatment of infectious diseases still remains an important and challenging problem. The clinical use of drugs has been limited by their relative high risk of toxicity, microbial resistance or pharmacokinetic deficiencies. A major research emphasis to counter this growing problem is the development of novel 5*α*-reductase inhibitors, antimicrobials and antioxidant agents. The incorporation of hydrophobic units to steroidal nucleus increases its ability to interact with cell membranes and pass through them [17-18].

5α-reductase inhibitors are a group of drugs with anti-androgenic activity, used in treatment of benign prostatic hyperplasia and androgenic alopecia [19]. Testosterone is converted to dihydrotestosterone by this enzyme. The hyperplasia of the prostate gland has been associated with high level of serum 5α-dihydrotestosterone. The product of 5α-reductase accumulates into the nuclei of responsive cells and binds to androgen receptors such as those of human prostate [20-21]. Two types of 5α-reductase enzyme are identified: type 1 and type 2, each encoded by a different genes, which have been characterized in several species. The type 1 is present in skin and hair follicles and acts at basic and neutral pH whereas type 2 is present in basal and stromal cells of prostate which act at acidic pH. Both enzymes are having hydrophobic pocket made up of Cys, Leu and Val. These enzymes are also having an electrophilic site which binds to the enone system of testosterone and convert it into DHT [22-25].

In this communication, we studied the effect of incorporation of aromatic ring by amide formation at the 3*β* amino group of the steroidal nucleus in terms of the 5*α*-reductase inhibitory activity alongside the antimicrobial and antioxidant activity. Therefore, we have speculated that similar compounds with six membered lactam ring D and further modifications on ring A could potentially be good 5*α*-reductase inhibitors, antimicrobials and

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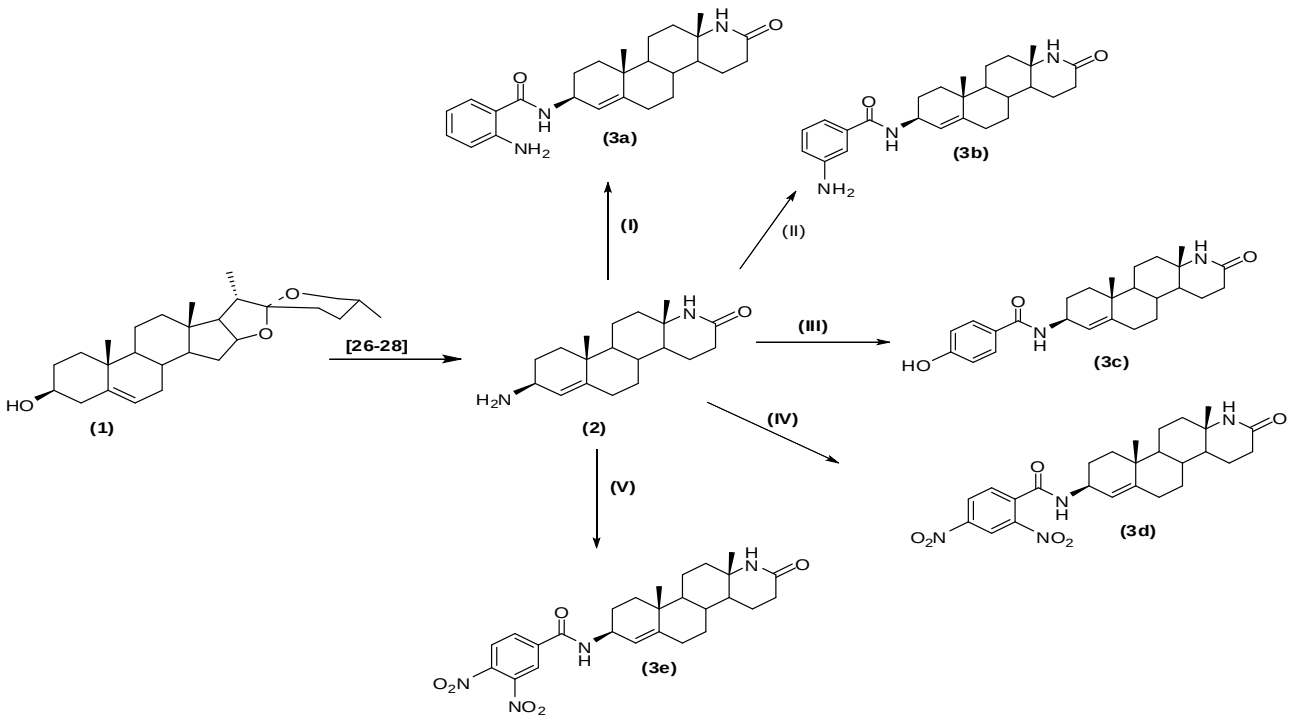

Reagents and conditions: (I) 2-Aminobenzoic acid, Dicyclohexylcarbodiimide, DCM stir 23 h, 35°C (II) 3-Aminobenzoic acid, Dicyclohexylcarbodiimide, DCM stir 25 h, 35°C (III) 4-Hydroxybenzoic acid, Dicyclohexylcarbodiimide, DCM stir 27 h, 35°C (IV) 2,4-Dinitrobenzoic acid, Dicyclohexylcarbodiimide, DCM, stir 33 h, 35°C (V) 3,4-Dinitrobenzoic acid, Dicyclohexylcarbodiimide, DCM stir 37 h, 35°C.

antioxidant agents. In view of these findings, in this communication we have reported the synthesis of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one and evaluated them for their 5α-reductase inhibitory, antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates kiesel gel 0.25 mm, 60 GF_{254}, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by iodine vapors/ultraviolet light as visualizing agent. Infrared (IR) spectra were recorded on NICOLET-380 FT-IR spectrophotometer using KBr pellets. ^{1}H-NMR spectra (δ, ppm) were recorded on Brucker AC-400 F, 400 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C-NMR spectra were recorded in DMSO-d_6 solution on a Bruker Advance II 400 spectrometer at 100 MHz using tetramethylsilane as the internal reference. Mass spectra were recorded on a Shimadzu GCMSQP 1000 EX aparatus. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Loba Chemie and Sigma Aldrich.

Chemistry: Compounds (3a-3e) were obtained from 3*β*-Amino-17*a*-aza-*D*-homo-4-androsten-17-one (2) that was synthesized from commercially available (25*R*)-5-spirosten-3β-ol (1), also known as Diosgenin, according to literature [26-28], as shown in Scheme 1. Amides (3a-3e) of 3*β*-Amino-17*a*-aza-*D*-homo-4-androsten-17-one (2) were prepared by treating it with different substituted aromatic acids in dichloromethane along with dicyclohexylcarbodiimide to give desired 3*β*-substituted amides of 17*a*-Aza-*D*-homo-4-androsten-17-one (3a-3e). Dicyclohexylurea (DCU) was obtained as by product which was filtered off and completion of reactions were confirmed with the help of TLC. All the synthesized analogues (3a-3l) were subjected to column chromatography for the isolation of pure compounds.

General procedure for the synthesis of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one compounds (3a-3e): A solution of 3*β*-Amino-17*a*-aza-*D*-homo-4-androsten-17-one (2) (0.5g) and dicyclohexylcarbodiimide (DCC) (0.34 g) in anhydrous dichloromethane (30.0 mL) was stirred to which substituted aromatic acid (0.0033 mol) was added slowly. Stirring was continued at room temperature till reaction completion which was confirmed by TLC. The precipitates of dicyclohexylurea (DCU) were filtered and the remaining solvent was removed under vacuum. The residue obtained was crystallized from ethyl acetate to yield 3*β*-substituted amides of 17*a*-Aza-*D*-homo-4-androsten-17-one.

3*β*-(2-Aminobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (3a): Molecular formulae $C_{26}H_{35}N_2O_3$, yield 55.9%, mp 180-182°C. IR (KBr) cm-1: 3358 (NH), 3345, 3187 (NH_2), 2964 (CH str), 1682 (CONH lactam), 1657

Table 1. IC_{50} Values of Synthesized Compounds

Comp.	*In vitro bioactivity* (IC_{50}, µM)		
	Human type-1 5α-R (DU-145 cells)	Human type-1 5α-R (PC-3cells)	Human type-2 5α-R (Human prostate homogenates)
3a	43.723 ± 0.763	55.877 ± 0.319	58.877 ± 1.284
3b	35.746 ± 0.535	39.864 ± 0.896	43.866 ± 0.138
3c	133.183 ± 0.219	115.417 ± 0.518	95.559 ± 0.977
3d	15.606 ± 0.863	17.616 ± 0.183	21.876 ± 0.348
3e	19.096 ± 0.214	23.857 ± 0.419	25.189 ± 0.988
Dutasteride	33.203 ± 0.505	37.756 ± 0.935	35.750± 1.517

(CONH), 1628 (C=C), 1550 (C=C Ar), 1160 (C-N). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 0.93 (*s*, 3H, 18-*CH_3*), 1.23 (*s*, 3H, 19-*CH_3*), 1.31-2.25 (*m*, 19H, steroidal ring), 3.88 (*m*, 1H, *3α-H*), 4.25 (*br s*, 1H, 4-vinylic), 5.15 (s, 2H, *NH2*), 6.23 (s, 1H, *CONH* lactam), 7.18 (*d*, 2H, ArH, J = 8.5), 7.49 (*d*, 2H, ArH, *J* = 8.5), 8.35 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*): δ 170.84 (C=O lactam), 169.75 (C=O), 148.77 (C'-2), 147.53 (C-5), 133.91 (C'-4), 127.34 (C'-6), 116.85 (C'-1), 116.77 (C-4), 117.81 (C'-5), 115.43 (C'-3), 54.29 (C-13), 53.75 (C-9), 47.18 (C-14), 44.39 (C-3), 39.85 (C-12), 36.78 (C-10), 36.53 (C-8), 34.19 (C-1), 32.18 (C-6), 31.81 (C-16), 31.66 (C-7), 25.17 (C-2), 21.85 (C-19), 20.39 (C-11), 20.16 (C-15), 19.43 (C-18). Anal.: calcd.: C 74.07, H 8.37, N 9.97. Found: C 74.04, H 8.38, N 9.99. MS (ESI) m/z = 422 (M+1).

3*β*-(3-Aminobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (3b): Molecular formula $C_{26}H_{35}N_2O_3$, Yield 53.4%, mp 181-183°C. IR (KBr) cm^{-1}: 3369 (NH), 3337, 3183 (NH_2), 2955 (CH str), 1669 (CONH lactam), 1645 (CONH), 1611 (C=C), 1516 (C=C Ar). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 0.86 (*s*, 3H, 18-*CH_3*), 1.14 (*s*, 3H, 19-*CH_3*), 1.18-2.09 (*m*, 19H, steroidal ring), 3.50 (*m*, 1H, *3α-H*), 4.15 (*br s*, 1H, 4-vinylic), 5.37 (s, 2H, *NH_2*), 5.96 (s, 1H, *CONH* lactam), 6.64 (*d*, 1H, ArH, *J* = 8.5), 7.07 (*s*, 1H, ArH), 7.32 (*d*, 1H, ArH, *J* = 8.5), 7.49 (*d*, 1H, ArH, *J* = 8.7), 8.35 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*): δ 170.84 (C=O lactam), 167.54 (C=O), 117.43 (C'-4), 148.69 (C-5), 148.45 (C'-3), 139.92 (C'-5), 134.85 (C'-1), 116.71 (C'-6), 115.81 (C-4), 110.87 (C'-2), 54.59 (C-13), 53.72 (C-9), 47.81 (C-14), 44.16 (C-3), 40.18 (C-12), 37.45 (C-10), 36.25 (C-8), 34.18 (C-1), 32.85 (C-6), 31.85 (C-16), 31.65 (C-7), 25.15 (C-2), 21.94 (C-19), 21.17 (C-11), 20.18 (C-15), 19.35 (C-18). Anal.: calcd.: C 74.07, H 8.37, N 9.97. found: C 74.05, H 8.38, N 9.98. MS (ESI) m/z = 422 (M+1).

3*β*-(4-Hydroxybenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (3c)*:* Molecular formulae $C_{26}H_{34}N_2O_3$, yield 47.9%, mp 175-177°C. IR (KBr) cm-^{1}: 3315 (OH), 2937 (CH str), 1682 (CONH lactam), 1677 (CONH), 1625 (C=C), 1513 (C=C Ar). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 0.97 (*s*, 3H, 18-*CH_3*), 1.17 (*s*, 3H, 19-*CH_3*), 1.24-1.98 (*m*, 19H, steroidal ring), 3.57 (*m*, 1H, *3α-H*), 3.92 (*br s*, 1H, 4-vinylic), 6.89 (s, 1H, *CONH* lactam), 7.25 (*d*, 2H, ArH, *J* = 8.4), 7.53 (*d*, 2H, ArH, *J* = 7.7), 7.74 (s, 1H, OH), 8.71 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*): δ 170.57 (C=O lactam), 167.51 (C=O), 161.94 (C'-4), 147.58 (C-5), 128.94 (C'-2), 127.92 (C'-6), 125.81 (C'-1), 115.89 (C'-3), 115.75 (C-4), 113.33 (C'-5), 54.57 (C-13), 53.74 (C-9), 48.26 (C-14), 45.19 (C-3), 40.29 (C-12), 37.44 (C-10), 36.51 (C-8), 34.19 (C-1), 33.17 (C-6), 31.83 (C-16), 31.65 (C-7), 24.18 (C-2), 21.94 (C-19), 21.19 (C-11), 20.11 (C-15), 19.27 (C-18). Anal.: calcd.: C 73.90, H 8.11, N 6.63. found: C 73.93, H 8.12, N 6.59. MS (ESI) m/z = 424 (M+1).

3*β*-(2,4-Dinitrobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (3d): Molecular formula $C_{26}H_{32}N_4O_6$, Yield 53.2%, mp 177-179°C. IR (KBr) cm^{-1}: 3324 (NH str), 2931 (CH str), 1684 (CONH lactam), 1655 (CONH), 1603 (C=C), 1556 (C=C Ar), 1537, 1346 (NO_2). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 0.87 (*s*, 3H, 18-*CH_3*), 1.23 (*s*, 3H, 19-*CH_3*), 1.27-2.29 (*m*, 19H, steroidal ring), 3.65 (*m*, 1H, *3α-H*), 4.26 (*br s*, 1H, 4-vinylic), 5.90 (s, 1H, *CONH* lactam), 7.53 (*d*, 2H, ArH, *J* = 8.3), 8.53 (s, 1H, *CONH*), 9.03 (*s*, 1H, ArH). ^{13}C-NMR (100 MHz, DMSO-*d_6*): δ 171.89 (C=O lactam), 166.73 (C=O), 152.39 (C'-4), 147.55 (C-5), 146.18 (C'-2), 137.63 (C'-1), 130.17 (C'-5), 129.84 (C'-6), 118.56 (C'-3), 115.57 (C-4), 54.55 (C-13), 53.74 (C-9), 47.24 (C-14), 45.38 (C-3), 39.84 (C-12), 37.17 (C-10), 36.41 (C-8), 34.29 (C-1), 32.88 (C-6), 31.93 (C-16), 31.65 (C-7), 24.19 (C-2), 21.85 (C-19), 21.37 (C-11), 20.11 (C-15), 19.33 (C-18). Anal.: calcd.: C 62.89, H 6.50, N 11.28. found: C 62.91, H 6.49, N 11.27. MS (ESI) m/z = 498 (M+1).

3*β*-(3,4-Dinitrobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (3e): Molecular formula $C_{26}H_{32}N_4O_6$, Yield 51.2%, mp 191-193°C. IR (KBr) cm^{-1}: 3321 (NH str), 2932 (CH str), 1696 (CONH lactam), 1655 (CONH), 1626 (C=C), 1545 (C=C Ar), 1538, 1342 (NO_2). ^{1}H NMR (400 MHz, DMSO-*d_6*): δ 0.99 (*s*, 3H, 18-*CH_3*), 1.21 (*s*, 3H, 19-*CH_3*), 1.23-2.21 (*m*, 19H, steroidal ring), 3.52 (*m*, 1H, *3α-H*), 4.23 (*br s*, 1H, 4-vinylic), 5.88 (*s*, 1H, *CONH* lactam), 8.73 (*d*, 2H, ArH, *J* = 7.5), 9.11 (*s*, 1H, ArH), 9.27 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*): δ 171.86 (C=O lactam), 167.53 (C=O), 147.81 (C'-4), 147.53 (C-5), 144.11 (C'-3), 137.65 (C'-1), 134.71 (C'-6), 124.93 (C'-5), 119.83 (C'-2), 115.86 (C-4), 54.63 (C-13), 53.77 (C-9), 47.29 (C-14), 45.44 (C-3), 39.19 (C-12), 37.45 (C-10), 36.58 (C-8), 33.17 (C-1), 32.71 (C-6), 31.94 (C-16), 31.46 (C-7), 24.19 (C-2), 21.79 (C-19), 21.31 (C-11), 20.15 (C-15), 19.45 (C-18). Anal.: calcd.: C 62.89, H 6.50, N 11.28. found: C 62.93, H 6.48, N 11.26. MS (ESI) m/z = 498 (M+1).

5α-reductase inhibitory activity: Synthesized compounds were evaluated for their 5α-reductase *in vitro* inhibitory activity as per reported procedure [29] by calculating IC_{50} values against different cell lines such as DU-145, PC-3

Table 2. MIC, MBC and MFC Results of Tested Compounds

Comp.	*B. subtilis*		*S. aureus*		*P. aeruginosa*		*E. coli*		*C. albicans*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
3a	12.5	25	6.25	12.5	12.5	25	12.5	25	12.5	25	6.25	12.5
3b	6.25	12.5	12.5	25	12.5	25	6.25	12.5	12.5	25	12.5	25
3c	25	50	25	50	50	100	25	50	25	50	25	50
3d	3.12	6.25	3.12	6.25	3.12	6.25	3.12	6.25	3.12	6.25	3.12	6.25
3e	3.12	6.25	6.25	12.5	3.12	6.25	3.12	6.25	6.25	12.5	3.12	6.25
Ciprofloxacin	6.12	12.5	6.12	12.5	6.12	12.5	6.12	12.5	-	-	-	-
Voriconazole	-	-	-	-	-	-	-	-	6.12	12.5	6.12	12.5

(human type 1 enzyme) and human prostate homogenate (human type 2 enzyme) using dutasteride as a standard drug as shown in (Table 1). PC-3 and DU-145 and human prostate homogenate were purchased from NCCS, Pune (India) and RPMI-1640 Himedia.

Antimicrobial activity*:* The newly synthesized compounds (3a-3e) were screened for their antimicrobial activity as per reported procedure [30-33]. In case of antibacterial activity *Bacillus subtilis* (MTCC 96), *Staphylococcus aureus* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 2453) and *Escherichia coli* (MTCC 40) strains were used and ciprofloxacin used as standard drug whereas for antifungal activity *C. albicans* (MTCC 8184) and *Aspergillus niger* (MTCC 8189) strains were used and voriconazole drug as standard agent. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) were shown in Table-2.

Antioxidant activity: Antioxidant Activity was measured in terms of hydrogen peroxide scavenging activity as per reported procedure [34]. Solution was prepared in phosphate buffer (pH 7.4), 40 nM of hydrogen peroxide. Synthesised compounds were treated at different concentrations (100, 300, 500 µg/ ml) of hydrogen peroxide solution as shown in table 3.

RESULT AND DISCUSSION

Synthesized compounds were checked by different techniques such as TLC, melting point and structures were confirmed by spectral techniques (IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry): IR spectra of all compounds (**3a-3e**) showed absorption band at around 3315-3369, 2931-2964, 1669-1696, 1645-1677, 1603-1628 and 1513-1556 cm^{-1} regions, conforming the presence of NH, CH, CONH (lactam), CONH, C=C, C=C Ar, respectively. Synthesized derivatives showed ^{1}H NMR spectra which was verified on the basis of chemical shifts, multiplicities and coupling constants. The spectra of most compounds determined the characteristic 3H protons of 18-CH_3 at 0.86-0.99 ppm, 3H protons of 19-CH_3 at δ 1.14-1.23 ppm, 19H protons of steroidal ring were found at around δ 1.18-2.29 ppm, 1H proton of *3α-H* at δ 3.50-3.88 ppm, 1H 4-vinylic proton were at δ 3.92-4.26 ppm, CONH (lactam) proton were around δ 5.88-6.89 ppm and characteristic proton of CONH found around δ 8.35-9.27 ppm. ^{13}C-NMR spectra of compounds has characteristic signals appeared at around *CONH* lactam δ 170.57-171.89, *CONH* at δ 166.73-169.75, C-19 at δ 21.79-21.94 and C-18 at δ 19.27-19.45 ppm.

In this study, the active molecules have been derivatized with different substituents, to investigate the pharmacophoric elements, responsible for better activity. In our new synthetic scheme design, the introduction of substituted aromatic rings has shown minimal to potent 5*α*-reductase inhibition, antimicrobial and antioxidant activity. It has been observed that the introduction of substituted aromatic rings to the steroidal nucleus has marked effect in its biological activity. The 5α-reductase inhibitory activity of all the newly synthesized compounds (**3a**-**3e**) and dutasteride *in vitro* was determined by using prostate cancer cell line such as DU-145, PC-3 (type-1) and human prostate homogenates (type-2) as shown in Table 1. Most of the newly synthesized compounds displayed prominent 5*α*-reductase inhibitory activity. In order to study the influence of substituted aromatic rings on 5*α*-reductase enzyme the biological activity has been carried out.

From the result, it was concludes that 5*α*-reductase inhibitory activity could be increase by increasing the lipophilicity. So, the structural modifications were performed with the aim to enhance the lipophilicity of steroidal molecule for optimal biological response. Compound 3*β*-(2,4-Dinitrobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**3d**) showed maximum inhibitory potential in the *in vitro* assay (least IC_{50}) of 15.60 µM (DU-145), 17.61 µM (PC-3) type 1 and 21.87 µM type 2 with dual inhibitory activity against both type I and type II isozymes as compared to reference drug dutasteride with IC_{50} 33.20 µM (DU-145), 37.75 µM (PC-3) and 35.75 µM type 2. Compound 3*β*-(3, 4-Dinitrobenzamido)-17*a*-aza-*D*-

homo-4-androsten-17-one (**3e**) was found to another promising analogue with IC_{50} values 19.09 µM (DU-145), 23.85 µM (PC-3) type 1 and 25.18 µM type 2. So, it was assumed from upper findings that NO_2 substituted analogues would be sympathetic for inhibiting both type-1 and type-2 isozyme. While the compound 3*β*-(3-Aminobenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**3b**) was found to be mediocre inhibitor with IC_{50} values 35.74 µM (DU-145), 39.86 µM (PC-3) type 1 and 43.86 µM type 2. Curiously, it has been observed that the compound with OH substituted analogue 3*β*-(4-

Table 3. Hydrogen Peroxide Scavenging activity of Synthesized Compounds

Comp.	Scavenging of hydrogen peroxide at different concentration (%)		
	100 µg	300 µg	500 µg
3a	49.24	51.62	53.58
3b	53.76	55.17	57.34
3c	39.58	42.65	43.66
3d	64.15	69.69	73.21
3e	61.24	63.46	67.65
Ascorbic Acid	51.47	53.45	55.38

Hydroxybenzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**3c**) was found to be least potent with IC_{50} values 133.18 µM (DU-145), 115.41 µM (PC-3) type-1 and 95.55 µM type 2. At last, it was observed that presence of NO_2 group showed maximum inhibitory activity whereas OH substituted analogue would result into decrease in activity. Newly synthesized compounds (3a-3p) were also evaluated for their *in vitro* antimicrobial activity against Gram-positive bacteria *B. subtilis*, *S. aureus* Gram-negative bacteria *P. aeruginosa*, *E. coli* and fungal strain *C. albicans* and *A. niger*. The compound (**3d**) displayed excellent activity against *B. subtilis*, *S.* aureus, *P. aeruginosa* and *E. coli* with MIC 3.12 µg/mL and MBC 6.25 µg/mL whereas analogue (**3e**) having MIC 3.12 µg/mL and MBC 6.25 µg/mL against *B. subtilis*, *P. aeruginosa* and *E. coli* and MIC 6.25 µg/mL, MBC 12.5 µg/mL (*S.* aureus). The analogue (**3b**) reveals moderate activity with MIC 6.25µg/mL, MBC 12.5 µg/mL (*B. subtilis, P. aeruginosa*), MIC 12.5 µg/mL, MBC 25 µg/mL (*S. aureus, E. coli*). Astonishingly the OH substituted analogue (**3c**) was found to be slightest active against most of the bacterial strain with MIC 25 µg/mL, MBC 50 µg/mL (*B. subtilis, S. aureus, E. coli*), MIC 50 µg/mL, MBC 100 µg/mL (*P. aeruginosa*). The antifungal screening demonstrate that the analogue (**3d**) was found to be the most promising analogue having MIC 3.12 µg/mL, MFC 6.25 µg/mL (*C. albicans* and *A. niger*) followed by compound (**3e**) with MIC 6.25 µg/mL, MFC 12.5 µg/mL (*C. albicans*), MIC 6.25 µg/mL, MFC 12.5 µg/mL (*A. niger*). The derivative (**3a**) was found to be intermediately acting with MIC 12.5 µg/mL, MFC 25 µg/mL (*C. albicans*), MIC 6.25 µg/mL, MFC 12.5 µg/mL (*A. niger*). Among all the newly synthesized analogues the compound (**11c**) was found to be least active with MIC 25 µg/mL, MFC 50 µg/mL (*C. albicans* and *A. niger*). So, the presence of electron withdrawing substituents NO_2 increases the biological response while electron donating substituent OH devoid of biological activity.

The evaluation of antioxidant activities was carried out by the method of scavenging of hydrogen peroxide in comparison to standard compounds. From all the synthesized compounds analogues (**3d**) with NO_2 moiety was the most active with scavenging of hydrogen peroxide of 73.21% at 500 µg/mL concentration, followed by compound (**3e**) having hydrogen peroxide scavenging ability 67.65% at 500 µg/mL. The compound (**3c**) was found to be least active with 43.66% at 500 microgram per mL hydrogen peroxide scavenging ability. A close examination of the structures of the newly synthesized active analogues revealed that, their 5*α*-reductase inhibitory, antimicrobial and antioxidant activity is strongly bound to the nature and position of the substituent attached to steroidal ring. In general, it could be clearly recognized that potential biological analogues activity was encountered with NO_2 substituted derivatives i.e. (**3d** and **3e**). It might be due to nitro substituted aromatic rings to the steroidal nucleus supports its therapeutic activity.

CONCLUSION

This study reports the synthesis of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one analogues (**3a-3e**) and was well characterized by spectral analysis. Many promising substances have been discovered or developed but till now no commercial drugs have evolved over the last decades of research in the field of steroid having 5*α*-reductase inhibitory, antimicrobial and antioxidant activity. The main aim of the present investigation is to synthesize and investigate the 5*α*-reductase inhibitory, antimicrobial and antioxidant activity of newly synthesized compounds, with the hope of discovering new leads structure serving as potential agents. From entire study it was establish that compound (**3d**) prove to be a most prominent molecule having response against 5*α*-reductase enzyme along with antimicrobial and antioxidant activity while, analogue (**3c**) found to be least active. So, it was concluded that in the series of newly synthesized analogues of 17a-aza -D-homo-4-androsten-17-one, its biological activity has been greatly enhanced in some of the compounds due to nature of substituent at C-3 position of main steroidal moiety responsible for better interaction of enzyme with the compound and altered lipophilicity. Therefore the present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent and selective agents which were active against 5*α*-reductase and resistant strain for the treatment of microbial infections along with potent antioxidant activity.

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