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Research Article

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

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ABSTRACT
The study reports the convenient and efficient synthesis of several new analogues of 3β-substituted amides of 17a-aza-D-homo-4-androsten-17-one (11a-11d) 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-Chlorophenyl)acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11b) was found to be most promising analogue against 5*α*-reductase enzyme along with antimicrobial and antioxidant activity while, analogue 3*β*-(2-(4-Methoxyphenyl)acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11d) found to be least active. The detailed 5α-reductase inhibitors, antimicrobial and antioxidant activities of the synthesized compounds were reported in this communication.

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

INTRODUCTION

Steroids represent an important class of natural as well as synthetic drugs because of their ability to penetrate cells and perform some of the most fundamental biological functions. Medicinal chemists demonstrate that minor changes in the steroid nucleus can cause extensive change in biological activity and this became the basis of many important discoveries in the medicinal chemistry[1-4]. Naturally occurring steroid nuclei have been modified in several ways with the aim of finding more active compounds, free from undesirable or harmful side effects, and of recognizing the structural and stereochemical features required for the display of specific, selective physiological activity[5]. A steroid containing substituted aromatic ring condensed with cyclopentanophenanthrene scaffold has achieved significant importance in the view of their diverse and interesting biological activities[6,7]. This is pertinently true for the rational semi synthetic approach to modify steroidal nucleus. It is recognized that various advantages are associated with steroid based chemotherapeutics and addition of substituted aromatic rings to steroids usually led to change in their physiological properties that grounds new interesting biological activities[8].

Over the past few decades, the development of aromatic ring substituted steroid derivatives has become one of the major goals of steroidal chemist. Recent studies revealed that incorporation of substituted aromatic rings enhances biological activities of steroidal molecules as 5*α*-reductase inhibitor, antimicrobial and anabolic drugs[9,10]. Moreover, the treatment of infectious diseases still remains an important and challenging problem. The clinical use of these agents 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. Therefore, they can improve transport and hybridization properties of such aromatic steroid molecules[11,12]. Incorporation of the hydrophobic unit at 3β position of the steroidal nucleus is one of the best possibilities which ultimately increase the ability to interact with cell membrane and show its desired function in our body[13,14].

5α-reductase inhibitors are a group of drugs with anti-androgenic activity, used in treatment of benign prostatic hyperplasia and androgenic alopecia[15]. The 5α-reductase enzyme converts Δ4-3-ketosteroids to 5α-3-ketosteroids in androgen dependent tissues. 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 animal prostate[16,17].

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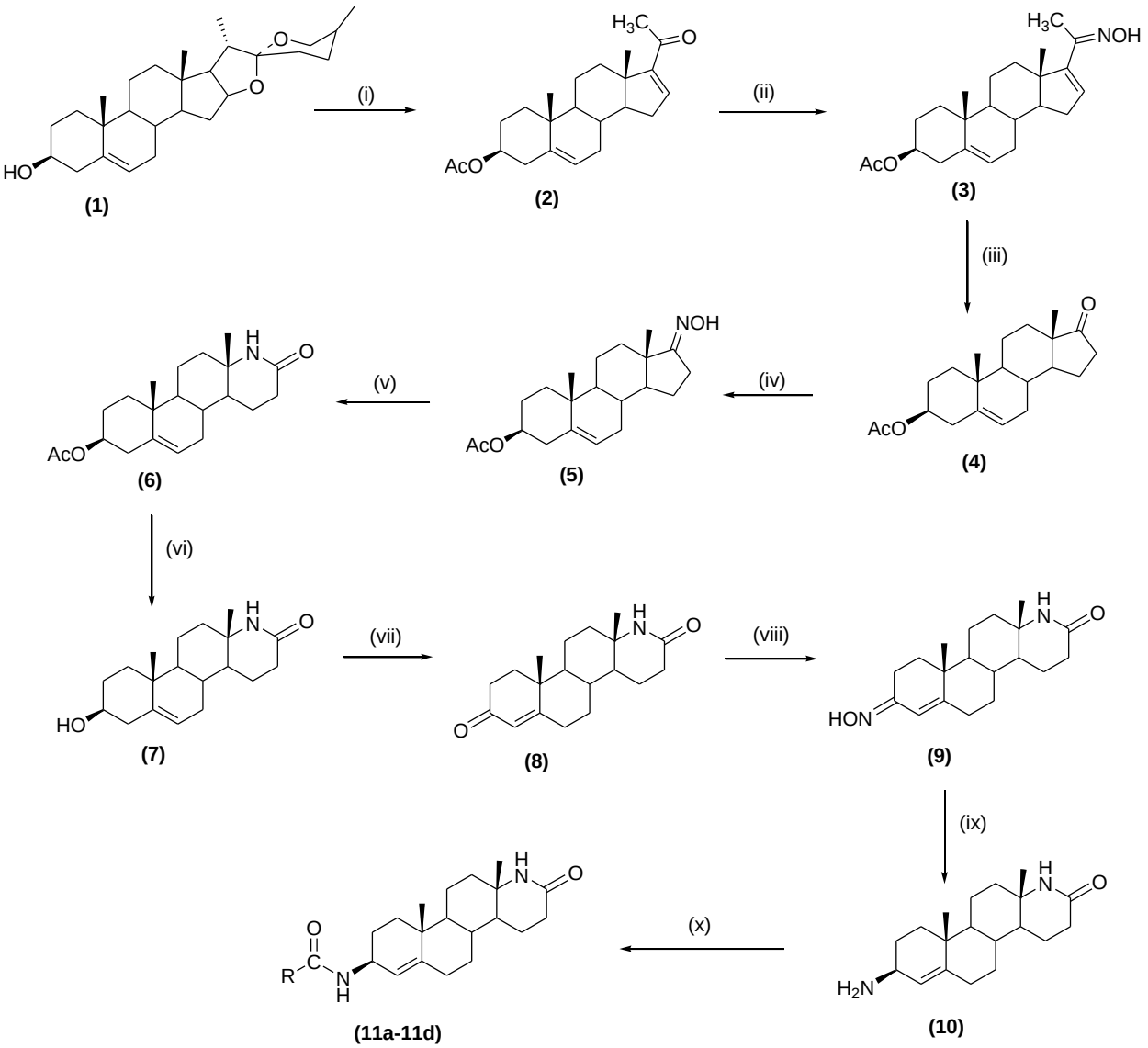
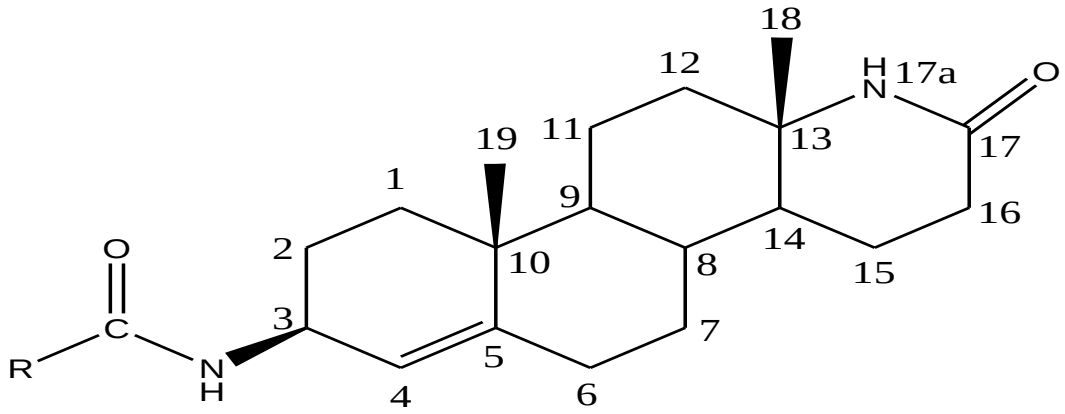
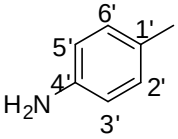
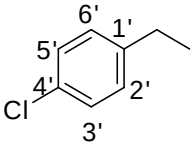
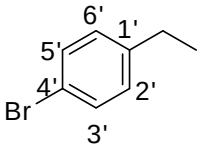
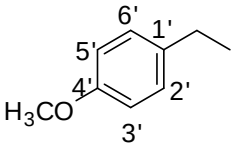


Scheme 1 Reagents and conditions: (i) $(CH_3CO)_2O$, CrO_3, pyridine, CH_3NH_2.HCl, reflux 8 h (ii) NH_2OH.HCl, pyridine, reflux 2 h, 85°C (iii) $POCl_3$, Pyridine, HCl, stir 1 h, 0°C (iv) NH_2OH.HCl, CH_3COONa, reflux 3 h (v) $SOCl_2$, benzene, stir 17 min, 15°C (vi) KOH, CH_3OH reflux 2 h (vii) $Al(O\text{-}iPr)_3$, cyclohexanone, dioxan, toluene, reflux 5 h (viii) NH_2OH.HCl, CH_3COONa, reflux 5 h (ix) Zn-Cu couple, C_2H_5OH, reflux 17 h (x) Dicyclohexylcarbodiimide, DCM, substitued acids, stir 48 h, 35°C.



Two types of 5α-reductase enzymes are type 1 and type 2. 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[18-21]. Both enzymes are having hydrophobic pocket made up of Cys,

Table 1. *In vitro* activity of 3*β*-substituted amides of 17*a*-Aza-*D*-homo-4-androsten-17-one (11a-11d)

Compounds	R	In vitro bioactivity[a] (IC_{50}μM) Human type-1 5*α*- Reductase		Human type-2 5*α*-R (Human prostate homogenates)[d]
		(DU-145 cells)[b]	(PC-3cells)[c]	
11a		42.782 ± 0.219	38.810 ± 0.518	22.382 ± 0.977
11b		31.294 ± 0.639	23.021 ± 0.521	26.345 ± 0.199
11c		30.871 ± 1.625	27.468 ± 0.324	28.227 ± 0.211
11d		46.946 ± 1.455	48.786 ± 0.193	42.758 ± 0.275
Dutasteride	-	33.203 ± 0.505	37.756 ± 0.935	35.750 ± 1.517

[a]The Effect of IC_{50} values of synthesized compounds on both 5α-reductase (type-1 and type-2) [b,c]Human Prostatic carcinoma (DU-145, PC-3 cell line expressing type-1 isozyme) [d]Human Prostate homogenate, pH 5.5: mainly type-2 isozyme is active.

Leu and Val. These enzymes are also having an electrophilic site which binds to the enone system of testosterone and convert it into DHT.

In this communication, we studied the effect of incorporation of aromatic ring or functional group 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 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.

Experimental: 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 (Thin Layer Chromatography) and the spots were visualized by iodine vapours/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 400 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C-NMR spectra were recorded on in (Dimethylsulphoxide) DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 100 MHz using tetramethylsilane as the internal reference. Mass spectra were recorded on a Shimadzu GCMSQP 1000 EX apparatus. The necessary chemicals were purchased from Sigma Aldrich.

Chemistry: Compounds (11a-11d) were obtained from 3β-Amino-17a-aza-D-homo-4-androsten-17-one (10) that was synthesized from commercially available 3β(25*R*) spirosten-5-ene-ol (1), also known as Diosgenin, according to literature Scheme 1[22-25]. Amides (11a-11d) of 3β-Amino-17a-aza-D-homo-4-androsten-17- one (10) 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 (11a-11d).

General procedure for the synthesis of 3*β*-substituted amides of 17*a*-aza-*D*-homo-4-androsten-17-one compounds (11a-11d)

A solution of 3*β*-Amino-17*a*-aza-*D*-homo-4-androsten-17-one (**10**) (0.5g) and dicyclohexylcarbodiimide (DCC) (0.34 g) in anhydrous dichloromethane (30.0 mL) was stirred to which substituted acid (0.0033 mol) was added slowly. Stirring was continued at room temperature till reaction completion which was confirmed by TLC. The

Table 2 Antibacterial and antifungal activity of compounds (11a-11d)

Compounds	Gram positive bacteria		Gram negative bacteria		Fungal strain	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
11a	1.56	3.12	1.56	1.56	0.78	1.56
11b	0.78	0.78	0.78	0.78	0.78	0.78
11c	0.78	1.56	0.78	0.78	1.56	1.56
11d	25	50	25	25	50	50
Ciprofloxacin[e]	6.25	6.25	6.25	6.25	-	-
Voriconazole[f]	-	-	-	-	6	25

[e]Ciprofloxacin was used as standard drug for antibacterial activity and [f]voriconazole as standard for antifungal activity

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 (11a-11d)

3*β*-(4-Amino-benzamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11a)

Molecular formula $C_{26}H_{35}N_3O_2$, yield 52.6%, mp 181-184°C. IR (KBr) cm^{-1}: 3289 (NH), 3357, 3185 (NH_2), 2955 (CH str), 1675 (CONH lactam), 1639 (CONH), 1608 (C=C), 1548 (C=C Ar). ^{1}H NMR (400 MHz, $CDCl_3$): 0.86 (*s*, 3H, 18-*CH_3*), 1.14 (*s*, 3H, 19-*CH_3*), 1.17-2.68 (*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*, 2H, ArH, *J* = 8.5), 7.43 (*d*, 2H, ArH, *J* = 8.5), 8.27 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-d6) δ: 170.19 (C=O lactam), 166.15 (C=O), 150.58 (C'-4), 147.63 (C-5), 131.87 (C'-6), 131.52 (C'-2), 123.49 (C'-1), 115.81(C-4), 113.89 (C'-5), 113.44 (C'-3), 54.86 (C-9), 54.58 (C-13), 47.18 (C-14), 44.12 (C-3), 39.82 (C-12), 36.82 (C-10), 35.74 (C-8), 33.93 (C-1), 32.16 (C-6), 31.75 (C-7), 31.18 (C-16), 24.66 (C-2), 21.94 (C-19), 21.14 (C-11), 20.88 (C-15), 19.27 (C-18). MS (ESI) m/z = 422 (M+1).

3*β*-(2-(4-Chlorophenyl) acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11b)

Molecular formula $C_{27}H_{35}ClN_2O_2$, yield 61.7%, mp 139-141°C. IR (KBr) cm^{-1}: 3264 (NH str), 2930 (CH str), 1687 (CONH lactam), 1664 (CONH), 1617 (C=C), 1534 (C=C Ar), 734 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): 1.13 (*s*, 3H, 18-*CH_3*), 1.25 (*s*, 3H, 19-*CH_3*), 1.27-1.96 (*m*, 19H, steroidal ring), 3.66 (*m*, 1H, *3α-H*), 3.74 (*s*, 2H, CH_2, Ar-CH_2-CO), 3.95 (*br s*, 1H, 4-vinylic), 6.77 (s, 1H, *CONH* lactam), 7.19 (*d*, 2H, ArH, *J* = 8.4), 7.29 (*d*, 2H, ArH, *J* = 7.7), 7.43 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*) δ:171.82 (C=O lactam), 169.63 (C=O), 147.53 (C-5), 132.87 (C'-4), 132.74 (C'-1), 129.76 (C'-2), 129.19 (C'-3), 128.91 (C'-6), 128.34 (C'-5), 115.69 (C-4), 54.17 (C-13), 53.72 (C-9), 47.56 (C-14), 43.28 (C-3), 42.82 (CH_2), 39.21 (C-12), 37.45 (C-10), 36.52 (C-8), 33.14 (C-1), 32.12 (C-6), 31.55 (C-7), 31.11 (C-16), 24.14 (C-2), 20.94 (C-19), 20.79 (C-11), 19.88 (C-15), 19.43 (C-18). MS (ESI) m/z = 456 (M+1).

3*β*-(2-(4-Bromophenyl) acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11c)

Molecular formula $C_{27}H_{35}BrN_2O_2$, yield 51.8%, mp 147-149°C. IR (KBr) cm^{-1}: 3285 (NH str), 2933 (CH str), 1697 (CONH lactam), 1654 (CONH), 1616 (C=C), 1544 (C=C Ar), 617 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$): 1.12 (*s*, 3H, 18-*CH_3*), 1.19 (*s*, 3H, 19-*CH_3*), 1.25-1.95 (*m*, 19H, steroidal ring), 3.65 (*m*, 1H, *3α-H*), 3.76 (*s*, 2H, CH_2, Ar-CH_2-CO), 3.93 (*br s*, 1H, 4-vinylic), 6.19 (s, 1H, *CONH* lactam), 6.73 (*d*, 2H, ArH, *J* = 8.4), 7.17 (*d*, 2H, ArH, *J* = 8.4), 7.23 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*) δ: 170.64 (C=O lactam), 169.35 (C=O), 147.14 (C-5), 134.42 (C'-1), 132.19 (C'-3), 131.26 (C'-5), 129.55 (C'-6), 129.34 (C'-2), 121.57 (C'-4), 115.75 (C-4), 54.28 (C-13), 52.54 (C-9), 47.53 (C-14), 43.68 (C-3), 42.71 (CH_2), 39.63 (C-12), 36.49 (C-10), 35.57 (C-8), 33.27 (C-1), 32.14 (C-6), 30.83 (C-7), 30.19 (C-16), 24.11 (C-2), 21.84 (C-19), 20.56 (C-11), 19.85 (C-15), 19.45 (C-18). MS (ESI) m/z = 500 (M+1).

3*β*-(2-(4-Methoxyphenyl) acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (11d)

Molecular formula $C_{28}H_{38}N_2O_3$, yield 57.2%, mp 135-137°C. IR (KBr) cm^{-1}: 3258 (NH str), 2928 (CH str), 1685 (CONH lactam), 1654 (CONH), 1636 (C=C), 1560 (C=C Ar), 1240 (C-O). ^{1}H NMR (400 MHz ,$CDCl_3$): 0.79 (*s*, 3H, 18-*CH_3*), 1.37 (*s*, 3H, 19-*CH_3*), 1.44-2.12 (*m*, 19H, steroidal ring), 3.55 (*m*, 1H, *3α-H*), 3.69 (*s*, 2H, CH_2, Ar-CH_2-CO), 3.85 (s, 3H, OCH_3), 3.93 (*br s*, 1H, 4-vinylic), 6.42 (s, 1H, *CONH* lactam), 6.93 (*d*, 2H, ArH, *J* = 8.4), 7.39 (*d*, 2H, ArH, *J* = 8.4), 7.88 (*s*, 1H, *CONH*). ^{13}C-NMR (100 MHz, DMSO-*d_6*) δ: 170.84 (C=O lactam), 169.76 (C=O), 158.56 (C'-4), 147.55 (C-5), 129.65 (C'-2), 129.43 (C'-6), 126.92 (C'-1), 115.87 (C-4), 113.88 (C'-5), 113.62 (C'-3), 55.13 (OCH_3), 54.56 (C-13), 52.78 (C-9), 47.15 (C-14), 43.37 (C-3), 42.87 (CH_2), 39.43 (C-12), 36.47 (C-10), 36.14 (C-8), 33.15 (C-1), 32.18 (C-6), 30.64 (C-7), 30.19 (C-16), 24.18 (C-2), 21.61 (C-19), 20.83 (C-11), 19.88 (C-15), 19.43 (C-18). MS (ESI) m/z = 452 (M+1).

5α-reductase inhibitory activity: 5α-reductase inhibitory activity was carried out on newly synthesized analogues against DU-145, PC3 (human type I enzyme) and human prostate homogenate (human type II enzyme) using dutasteride as a standard drug as shown in Table 1. PC-3, DU-145 and human prostate homogenate were purchased from NCCS (National Center for Cell Science), Pune India and RPMI-1640 Himedia. Appropriate culture media supplemented with 10% inactivated foetal bovine serum, 100 U/mL penicillin and 100µl/mL streptomycin incubated at 37 ºC and 5% CO_2 in humidified incubator is used for maintaining cell lines. Cells were then subcultured by trypsinization with trypsin solution under sterile condition after achieving 80% confluence. Before 24 h of testing the cells were seeded in 96 well plates at the concentration of 3000 cells/ well in 100µl of the medium afterward the cells were incubated for overnight in

Table 3 MBC and MFC results of synthesized analogues (11a-11d)

Compounds	Bacterial strain MBC[g]				Fungal strain MFC[h]	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
11a	12.5	6.25	6.25	12.5	6.25	12.5
11b	3.12	3.12	6.25	3.12	6.25	3.12
11c	6.25	12.5	6.25	3.12	6.25	3.12
11d	25	50	12.5	12.5	50	25
Ciprofloxacin	12.5	12.5	12.5	12.5	-	-
Voriconazole	-	-	-	-	12.5	50

[g]MBC (µg/mL) = minimum bactericidal concentration, i.e., the lowest concentration to completely kill bacteria. [h]MFC (µg/mL) = minimum fungicidal concentration, i.e., the lowest concentration to completely kill the fungi.

Table 4 Showing hydrogen peroxide scavenging activity of synthesized compounds (11a-11d)

Compounds	Scavenging of hydrogen peroxide at different concentration (%)		
	100 µg	300 µg	500 µg
11a	50.53	50.78	53.29
11b	57.02	60.92	63.67
11c	53.15	54.65	56.77
11d	42.13	44.82	47.54
Ascorbic Acid[i]	51.47	53.45	55.38

[i]Ascorbic Acid as standard for hydrogen peroxide scavenging activity at different concentrations of 100µg, 300µg, 500µg along with synthesised compounds.

triplicate with varying concentration of compounds ranging from 1-100 µm/ mL with dutasteride as standard and then each well was replaced by 2 µl of 3-(4, 5-dimethylthiazol-2-yl) 2, 5 diphenyltetrazolium bromide (MTT) solution (5 mg/mL) after 3 days of incubation and kept in an incubator for 3 h. It was determined on the basis of mitochondrial reduction of MTT to Formazan crystals in dimethyl sulfoxide (DMSO) the relative percentage of metabolically active cells and untreated controls was determined. Stock solution and other dilutions (1µM/mL, 10µM/mL, 50µM/mL and 100µM/mL) were prepared in DMSO and the inhibitory concentration (IC50) of samples was calculated by measuring their spectrophotometric absorbance with the help of microplate reader (BIORAD) at 570/ 630 nm. IC50 value was calculated for the compounds at different concentrations and all the procedures were carried out thrice. All data was expressed in terms of S.D and mean value. Wherever appropriate, the data was also subjected to unpaired two tailed student's t test. A value of $p < 0.05$ was considered as significant[26].

Antibacterial activity: All the newly synthesized compounds (11a-11d) were screened against *Bacillus subtilis* (MTCC 96), *Staphylococcus aureus* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 2453) and *Escherichia coli* (MTCC 40) were used and ciprofloxacin drug as standard agent. Broth dilution technique was used for the determination of minimum inhibitory concentrations (MICs), which contained logarithmic serially two fold diluted amount of the test compound and control were inoculated with approximately 5 x 105 c.f.u. of actively dividing bacteria cells and after that cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) were shown in Table-2. The minimum bactericidal concentration (MBC), was calculated by taking 0.1 mL volume from each tube and spread on agar plates and after 18-24 h of incubation at 35 °C the number of c.f.u was counted and MBC was calculated which is known as the lowest drug concentration at which 99.9% of the inoculum was killed[26,27]. The minimum bactericidal concentrations were shown in Table-3.

Antifungal activity: The newly synthesized compounds (11a-11d) were screened for their antifungal activity against *Candida albicans* (MTCC 8184) and *Aspergillus niger* (MTCC 8189) were used and voriconazole drug as standard agent. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6 x 104 -6 x 104 c.f.u./mL. The cultures were incubated at 35 °C for 48 h and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC) were shown in Table-2. To obtain the minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 48 h of incubation at 35 °C MFC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed[28,29]. The minimum fungicidal concentrations were given in Table-3.

Antioxidant activity: Hydrogen peroxide scavenging activity was used to determine antioxidant activity. The phosphate buffer (pH 7.4) was used to prepare (40 mM) solution of hydrogen peroxide and then all the synthesized compounds having different concentrations (100, 300, and 500 µg/mL) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen

peroxide. The percentage scavenging of hydrogen peroxide of the synthesized compounds and the standard compounds were calculated using the following formula: Percentage scavenging [H2O2] = [(A0 - A1)/ A0] x 100, where A0 was the absorbance of the blank, and A1 was the absorbance in the presence of the sample and standards[30]. The percentage scavenging of hydrogen peroxide by the synthesized compounds at 100, 300 and 500 µg/mL concentration were absorbed and results are summarized in Table 4.

RESULTS AND DISCUSSION

Synthesised compounds were evaluated by different techniques such as TLC, M.P, (IR, ^{1}H NMR, ^{13}C NMR spectroscopy and Mass spectrometry). IR of all synthesised compounds (11a-11d) showed absorption bond at around 3258-3389 cm^{-1} (NH stretch), 2828-2955 cm^{-1} (CH), 1675-1697 cm^{-1} (CONH Lactam), 1639-1664 cm^{-1} (CONH), 1608-1636 cm^{-1} (C=C), 1534-1560 (C=C Ar). ^{1}H NMR spectrum which was verified on the basis of chemical shifts, multiplicities and coupling constants. The ^{1}H spectra of synthesised compounds were determined having 3H proton of 18-CH_3 at δ 0.79-1.13 ppm, 3 proton of 19-*CH_3* at δ 1.14-1.37, 1.17-2.68 (19 H steroidal ring), 1H proton of *3α-H* at δ 3.50-3.66, 1H 4-vinylic proton were at δ 3.93-4.15, CONH (lactam) proton were around δ 5.96-6.77 and characteristic proton of CONH found around δ 7.23-8.27. ^{13}C-NMR spectra of compounds has characteristic signals appeared at around *CONH* lactam δ 170.19-171.82, *CONH* at δ 166.15-169.76, C-19 at δ 20.94-21.94 and C-18 at δ 19.27-19.45.

From the results, it has been shown that compound 3*β*-(2-(4-Chlorophenyl)acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one **(11b)** showed the maximum inhibitory potential in the *in vitro* assay (lower IC_{50}) of 31.29 µM (DU-145), 23.02 µM (PC-3) (type-1) and 26.34 µM (type 2) and it was found to be dual acting against both type-1 and type-2 isozymes followed by compound 3*β*-(2-(4-Bromophenyl)acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one (**11c**) with IC_{50} of 30.87 µM (DU-145), 27.46 µM (PC-3) (type-1) and 28.22 µM (type 2) as compared to reference drug dutasteride with IC_{50} values 33.20 µM (DU-145), 37.75 µM (PC-3) (type-1) and 33.75 µM (type-2). Enhanced activity of compound **(11b** and **11c)** can be explained on the basis of lipophilicity of these compounds which enhances the interaction with enzyme sites which forms more readily a steroid enzyme activated complex. Cl and Br group substituted analogue is more lipophilic and found to be active for both enzymes. Compound 3*β*-(4-Amino-benzamido)-17*a*-aza-*D*-homo-4-androsten-17-one **(11a)** showed moderate inhibitory activity having IC_{50} values 42.78 µM (DU-145), 38.81 µM (PC-3) (type-1) and 22.38 µM (type-2). Due to presence of NH_2 moiety increase the polarity of the molecule and decreases the binding of hydrophobic pocket of the enzyme with the molecule. Moreover, it has been observed that the compounds with -*OCH_3* substitution 3*β*-(2-(4-Methoxyphenyl)acetamido)-17*a*-aza-*D*-homo-4-androsten-17-one **(11d)** was found to be least potent with IC_{50} values 46.94 µM (DU-145), 48.78 µM (PC-3) (type-1) and 42.75 µM (type-2).

Newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *B. subtilis, S. aureus, P. aeruginosa* and *E. coli* and antifungal activity against *C. albicans* and *A. niger*. The investigation of antimicrobial screening data revealed that all the tested compounds showed moderate to good antimicrobial activity. The compound **(11b)** displayed an excellent activity against Gram-positive bacteria *B. subtilis* and *S. aureus* with MIC 0.78 µg/mL and MBC 3.12 µg/mL and it also showed prominent activity against Gram-negative bacteria *P. aeruginosa* and *E. coli* with MIC 0.78 µg/mL, MBC 6.25 µg/mL and MIC 0.78 µg/mL, MBC 3.12 µg/mL.

Compound **(11c)** was found to be another promising analogue against *B. subtilis* with MIC 0.78 µg/mL, MBC 6.25 µg/mL, *S. aureus* having MIC 1.56 µg/mL, MBC 12.5 µg/mL, *P. aeruginosa* and *E. coli* with MIC 0.78 µg/mL, MBC 6.25 µg/mL and MIC 0.78 µg/mL, MBC 3.12 µg/mL. So, it was recommended that *chloro* and *bromo* substituted analogues would appear to be favorable for potent antibacterial activity; this could be justified as the presence of lipophilic group was responsible for high potency of these compounds. The analogue (11a) exhibited moderate activity with MIC 1.56 µg/mL, MBC 12.5 µg/mL (*B. subtilis*), MIC 3.12 µg/mL, MBC 6.25 µg/mL (*S. aureus*), MIC 1.56 µg/mL, MBC 6.25 µg/mL (*P. aeruginosa*) and MIC 1.56 µg/mL, MBC 12.5 µg/mL (*E. coli*). The analogue (11d) was found to be least active against most of the bacterial strains with MIC 25 µg/mL, MBC 50 µg/mL (*B. subtilis*), MIC 50 µg/mL, MBC 100 µg/mL (*S. aureus*), MIC 25 µg/mL, MBC 50 µg/mL (*P. aeruginosa*) and MIC 25 µg/mL, MBC 50 µg/mL (*E. coli*).

The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition as compared to standard drug voriconazole. Among the screened analogues the compound (11b) exhibit highest antifungal activity against both fungal strains with MIC 0.78 µg/mL, MFC 6.25 µg/mL (*C. albicans)* and MIC 0.78 µg/mL, MFC 3.12 µg/mL (*A. niger)*. Analogue (11a) have shown mediocre inhibition with MIC 0.78 µg/mL, MFC 6.25 µg/mL (*C. albicans)* and MIC 1.56 µg/mL, MFC 12.5 µg/mL (*A. niger)*. The compound (11d) was found to be least active with MIC 50 µg/mL, MFC 100 µg/mL (*C. albicans* and *A. niger)*. It was justified that the presence of electron donating group like -*OCH_3* substituted analogues were devoid of antifungal activity.

Antioxidant activity of all newly synthesised compounds were evaluated in comparison to ascorbic acid was taken as standard. Compound (**11b**) was found to be the most active with scavenging of hydrogen peroxide of 63.67 at 500 µg/ml concentration, followed by compound (11d) having least activity as it is having methoxy group with hydrogen peroxide scavenging of 47.54 at 500 µg/ml concentration as compared to ascorbic acid having hydrogen peroxide scavenging 55.38 at 500 µg/ml concentration respectively. So, these data also revealed that presence of electron withdrawing groups are responsible for showing maximum activity and electron

donating group are responsible for least potent antioxidant activity.

CONCLUSION

This study reports the synthesis of 3*β*-substituted amides of *17a-Aza-D-homo-4-androsten-17-one* analogues (**11a-11d**) 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 steroids having both 5*α*-reductase inhibitory, antimicrobial and antioxidant activity. It was found that the analogue (11b and 11c) have shown prominent activity against 5*α*-reductase enzyme, microbial strain and it also act as antioxidant agents, these analogues were active due to presence of electronegative atom *Cl* and *Br* substituents. Moreover the analogue (11a) was the found to be the moderately acting and (11d) was found to be least active against 5α-reductase enzyme, microbial strains and antioxidant activity. So, it is concluded that the presence of electron withdrawing group substituted analogue tend to increase the biological response and electron donating group substituted analogue reduces the same. These new therapeautic agents could be considered as lead molecule for the future development of drugs which could be used for the treatment of benign prostatic hyperplasia and microbial infection.

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