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Design and synthesis of D-ring steroidal isoxazolines and oxazolines as potential antiproliferative agents against LNCaP, PC-3 and DU-145 cells

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ABSTRACT

Two series of novel steroidal isoxazolines and oxazolines were synthesized through different routes from dehydroepiandrosterone acetate and pregnenolone acetate, respectively. The synthesis of the analogs of both series is multistep and proceeds in good overall yields. While the key step in the synthesis of former is the cycloaddition of aromatic nitrile oxides across α,β-unsaturated olefins, it is the condensation of α,β-azidoalcohols with aromatic aldehydes in the later. Compounds of both the series were tested for their cytotoxic activities against LNCaP, PC-3 and DU-145 prostate cancer cell lines. Amongst all the compounds of both the series screened for their prostate cancer activity, compound **6a**, **6e** and **12a** are the most active especially against **LNCaP** and **DU-145** cancer cell lines.

1. Introduction

Steroids represent a pharmacologically active class of molecules associated with variety of physiological functions. Steroids as well as their derivatives have been found to have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular [1], autoimmune diseases [2], brain tumors, breast cancer, prostate cancer, osteoarthritis, etc. [3]. The promise of using steroids for development of lead molecules lies in their regulation of a variety of biological processes and being a fundamental class of signaling molecules [4]. Though steroid and steroid based molecules have been used as active pharmaceutical agents against various diseases, there has recently been a surge in the exploitation of these molecules against cancer. Despite the recent advances in the early diagnosis, prevention and therapy, cancer still remains a challenge as it affects millions of people world over and is one of the leading causes of death [5,6].

Prostate cancer is the second most common cancer worldwide and in the absence of any effective treatments available, this disease remains to be a challenge for researchers across the globe. Recently a large no of steroidal derivatives containing five- or six- membered 17β-*exo*-heterocycles (preferably nitrogen containing) have been found to cause the inhibition of 17α-hydroxylase/C_{17-20}-lyase ($P450_{17\alpha}$) which can block adrenal androgen synthesis at an early stage and may therefore be useful in the treatment of prostatic carcinoma [7]. The preliminary structure activity relationship (SAR) reveals that such activity is related to the presence of nitrogen in the heterocyclic moiety on ring D, with the nitrogen coordinating with the heme iron atom at the active site of the enzyme [8]. Taking inputs from these literature precedents, we, in continuation of our program toward the development of steroid based lead molecules [9], designed synthesis of two series of novel isoxazoline and oxazoline analogs from dehydroepiandrosterone and pregnenolone respectively. The compounds of both the series were evaluated for their antiproliferative activity toward PC-3, DU-145 (androgen-independent) and LNCaP (androgen-dependent) prostate cancer cell lines. It was observed that compounds **6a**, **6e** and **12a** exhibit excellent cytotoxicity especially against LNCaP and DU-145 cell lines.

2. Experimental

2.1. General methods

Melting points were recorded on Buchi Melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22

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instrument. NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

2.2. Chemical synthesis

2.2.1. General procedure for the synthesis of compounds **6a–f**

The preparation of compound **5** is already reported in the literature [10]. To a solution of compound **5** (0.10 g, 0.30 mmol) in THF was added p-methyl phenyl nitrile oxide (0.092 g, 0.60 mmol, generated in situ from the corresponding chlorooxime in presence of Et_3N). The mixture was stirred for 1hr at 0 °C and then at room temperature for further 5 h. The reaction mixture was then filtered and extracted with ethyl acetate (3 × 10 mL). The organic solvent was dried over Na_2SO_4 and then evaporated in vacuo. Purification was performed over silica gel (100–200 mesh; elution, hexane: EtOAc) to yield the cycloaddition product **6a** (0.114 g, 0.24 mmol, 83% yield). Though the cycloaddition could conceptually lead to the formation of two regioisomers, we fortunately got the reported regioisomer as the major product. The regiochemistry was assigned on the basis of NMR chemical shift values of the methylene protons in the isoxazoline ring which differ markedly for the two regioisomers [16]. The stereochemistry at C-17 was assigned on the basis of reported literature precedents [7]. The spectral details of various such analogs (**6a–f**) are given as follows (most of the peaks belonging to steroidal skeleton were merged and could not be differentiated. Thus δ values of only those peaks that could easily be differentiated are reported):

*2.2.1.1. 3β-Hydroxy-5-pregnene-17-(3-(p-methyl-phenyl)-4′,5′-dihydroisoxazole carboxaldehyde (**6a**).* Yield: 83%, mp 170 °C; $[\alpha]_D^{20}$ + 6.5 (*c* 0.20, $CHCl_3$); IR (KBr): 3500 (OH), 2700 (—CHO) cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): 0.78 and 1.05 (2s, angular CH_3), 2.38–2.40 (s, 3H, CH_3), 3.18 (d, ^1H, *J* = 17.2), 3.42–4.32 (m, 1H, —CHO—), 3.68 (d, 1H, *J* = 17.2), 5.36 (br, 1H), 7.21(d, 2H, *J* = 8.04), 7.56 (d, 2H, *J* = 8.04), 9.66 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 15.5, 20.9, 22.3, 24.0, 28.9, 30.5, 30.7, 32.2, 36.2, 39.0, 41.3, 46.4, 54.2, 58.7, 71.7, 75.7, 76.2, 82.4, 109.9, 120,7, 125.5, 126.0, 128.4, 137.9, 152.6, 161.1, 200.3; MS [ESI, 484 (M^+ + Na)], Anal. Calcd. for $C_{30}H_{39}NO_3$, C, 78.05; H, 8.52; N, 3.30. Found C, 78.02; H, 8.47; N, 3.33,

*2.2.1.2. 3β-Hydroxy-5-pregnene-17-(3-(p-chloro-phenyl)-4′,5′-dihydroisoxazole carboxaldehyde (**6b**).* Yield: 81%, mp 175–176 °C; $[\alpha]_D^{20}$ + 10.1 (*c* 0.20, $CHCl_3$); IR (KBr): 3497, 2857, 2711, 1679. cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): 0.79 and 1.04 (s, angular CH_3), 3.18 (d, 1H, *J* = 16.7), 3.50 (m, 1H, —CHO—), 3.67 (d, 1H, *J* = 16.7), 5.35 (br, 1H), 7.08 (d, 2H, *J* = 7.90), 7.55 (d, 2H, *J* = 7.90), 9.63 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 14.13, 19.44, 20.69, 22.73, 23.88, 29.76, 31.56, 31.75, 36.56, 37.18, 37.26, 38.83, 42.27, 42.48, 49.98, 53.88, 56.10, 71.75, 94.74, 115.90, 116.07, 121.33, 125.37, 128.78, 140.90, 156.65 and 200.29; MS [ESI, 483 (M^+ + H)], Anal. Calcd. for $C_{29}H_{36}ClNO_3$, C, 72.26; H, 7.53; N, 2.91. Found C, 72.41; H, 7.32; N, 2.77.

*2.2.1.3. 3β-Hydroxy-5-pregnene-17-(3-(anthracen-1-yl)-4′,5′-dihydroisoxazole carboxaldehyde) (**6c**).* Yield: 80%, mp 197–199 °C; $[\alpha]_D^{20}$ + 14.1 (*c* 0.20, $CHCl_3$); IR (KBr): 3505, 2865, 2704, 1662 cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): δ 0.80 and 1.08 (s, angular CH_3), 3.39 (d, 1H, *J* = 15.6), 3.49 (m, 1H, —CHO—), 3.84 (d, 1H, *J* = 15.6), 5.33 (br, 1H), 7.50 (m, 4H), 7.85 (d, 2H, *J* = 8.9), 8.05 (d, 2H, *J* = 8.9), 8.54 (m,1H), 9.83 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5, 201.9, 22.3, 24.0, 28.9, 30.5, 38.7, 32.2, 36.2, 39.0, 41.3, 46.4, 54.2, 58.7, 71.7, 75.7, 76.2, 82.4, 109.9, 120.7, 122.32, 115.05, 126.5, 126.0, 128.4, 138.5, 152.6, 162.1, 199.9.; MS [ESI, 548 (M^+ + H)], Anal. Calcd. for $C_{37}H_{41}NO_3$, C, 81.14; H, 7.54; N, 2.56. Found C, 81.29; H, 7.67; N, 2.48.

*2.2.1.4. 3β-Hydroxy-5-pregnene-17-(3-(p-fluoro-phenyl)-4′,5′-dihydroisoxazole carboxaldehyde (**6d**).* Yield: 77%, mp 186–188 °C; $[\alpha]_D^{20}$ + 12.2 (*c* 0.20, $CHCl_3$); IR (KBr): 3500, 2857, 2703, 1683 cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): 0.78 and 1.05 (s, angular CH_3), 3.18 (d, 1H, *J* = 17.4), 3.42–4.32 (m, 1H, —CHO—), 3.68 (d, 1H, *J* = 17.4), 5.35 (br, 1H), 7.23 (d, 2H, *J* = 8.0), 7.54 (d, 2H, *J* = 8.0), 9.67(s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 15.8, 20.9, 22.3, 24.0, 28.9, 30.5, 30.7, 32.2, 36.2, 39.0, 41.3, 46.4, 54.2, 58.7, 71.7, 75.7, 76.2, 82.6, 109.6, 120.7, 126.5, 126.0, 129.4, 138.9, 151.6, 162.1, 200.4; MS [ESI, 488 (M^+ + Na)], Anal. Calcd. for $C_{29}H_{36}FNO_3$, C, 74.81; H, 7.79; N, 3.01. Found C, 74.99; H, 7.62; N, 3.29.

*2.2.1.5. 3β-Hydroxy-5-pregnene-17-(3-(p-methoxy-phenyl)-4′,5′-dihydroisoxazole carboxaldehyde (**6e**).* Yield: 82%, mp 163–164 °C; $[\alpha]_D^{20}$ + 16.2 (*c* 0.20, $CHCl_3$); IR (KBr): 3500, 2858, 2705, 1682 cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): 0.77 and 1.08 (s, angular CH_3), 3.19 (d, 1H, *J* = 17.0), 3.49–3.56 (m, 1H, —CHO—), 3.69 (d, 1H, *J* = 17.0), 3.86 (s, 3H), 5.35 (br, 1H), 6.94 (d, 2H, *J* = 7.6), 7.72 (d, 2H, *J* = 7.6), 9.67 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 14.4, 21.9, 22.3, 24.0, 29.9, 30.5, 30.7, 32.5, 36.4, 39.5, 41.4, 46.4, 54.2, 58.7, 71.8, 75.7, 76.2, 84.4, 109.9, 120.7, 125.5, 126.0, 128.4, 137.9, 152.6, 161.1, 200.6; MS [ESI, 500.1 (M^+ + Na)], Anal. Calcd. for $C_{30}H_{39}NO_4$, C, 75.44; H, 8.23; N, 2.93. Found C, 75.63; H, 8.47; N, 2.61.

*2.2.1.6. 3β-Hydroxy-5-pregnene-17-(3-(o-nitro-phenyl)-4′,5′-dihydroisoxazole carboxaldehyde) (**6f**).* Yield: 78%, mp 188–191 °C; $[\alpha]_D^{20}$ + 6.4 (*c* 0.20, $CHCl_3$); IR (KBr): 3500, 2856, 2700, 1675 cm^{-1}, ^1H NMR (δ, ppm, $CDCl_3$): 0.78 and 1.05 (s, angular CH_3), 3.28 (d, 1H, *J* = 17.1), 3.42–4.47 (m, 1H, —CHO—), 3.54 (d, 1H, *J* = 17.1), 5.35 (br, 1H), 7.46 (d, 1H, *J* = 6.89), 7.66 (m, 2H), 8.65 (d, 1H, *J* = 6.89), 9.66 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 14.01, 19.38, 20.64, 21.63, 22.64, 23.88, 25.36, 29.31, 30.91, 31.50, 36.52, 37.22, 38.77, 39.22, 42.24, 49.94, 53.52, 56.07, 71.69, 121.30, 124.84, 130.80, 131.12, 133.52, 199.71; MS [ESI, 493 (M^+ + H)], Anal. Calcd. for $C_{29}H_{36}N_2O_5$, C, 70.71; H, 7.37; N, 5.69. Found C, 70.94; H, 7.54; N, 5.92.

2.2.2. General procedure for the synthesis of compounds **12a–g**

Compound **11** [11] (2.4 g, 6 mmol) was dissolved in dry CH_2Cl_2 (50 ml) and treated with appropriate aromatic aldehydes (1.1 equivalent). The mixture was cooled to 0 °C followed by the dropwise addition of $BF_3{\cdot}OEt_2$ (50%) (12 mmol, 1.65 ml) which was accompanied by evolution of gas. The reaction mixture was stirred at room temperature for 6 h. After the disappearance of starting material as monitored by TLC, saturated $NaHCO_3$ solution was added and the mixture was stirred until bubbling ceased. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified by chromatography on silica gel with hexane/CH_2Cl_2 (30:70, v/v) to give the 3β-acetylated oxazoline derivatives. Deacetylation was performed using methanolic $NaOCH_3$ to give the product **12a–g** (4–4.5 mmol, 67–75% yield) in the pure form after purification by chromatography on silica gel using ethylacetate/CH_2Cl_2. The spectral details of various such analogs (**12a–g**) are given as follows (most of the peaks belonging to steroidal skeleton were merged and could not be differentiated. Thus δ values of only those peaks that could easily be differentiated are reported):

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*2.2.2.1. (5'R)-17β-[2-Phenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12a**).* Yield: 73%, mp 214–215 °C; $[\alpha]_D^{20}$ – 53 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.87 (s, 3H), 1.04 (s, 3H), 3.51 (m, 1H), 3.63 (dd, 1H, *J* = 14.5 Hz and *J* = 8.0 Hz), 4.05 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.65 (m, 1H), 5.34 (d, 1H, *J* = 5.0 Hz), 7.39 (t, 2H, *J* = 7.5 Hz), 7.46 (t, 1H, *J* = 7.5 Hz), 7.92 (d, 2H, *J* = 7.5 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.8, 24.7, 31.7, 31.7, 31.8, 31.9, 36.6, 37.3, 38.2, 39.0, 42.3, 42.7, 50.3, 55.0, 56.0, 59.7, 71.6, 81.7, 121.4, 128.1, 128.3, 131.1, 132.3, 140.9, 164.1; MS [ESI, 420 ($M^+ + H$)], Anal. Calcd. for $C_{28}H_{37}NO_2$, C, 80.15; H, 8.89; N, 3.34. Found C, 80.87; H, 8.92; N, 3.33.

*2.2.2.2. (5'R)-17β-[2-(4-Nitrophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12f**).* Yield: 70%, mp 237–239 °C; $[\alpha]_D^{20}$ – 46 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.88 (s, 3H), 1.06 (s, 3H), 3.52 (m, 1H), 3.69 (dd, 1H, *J* = 14.6 Hz and *J* = 8.1 Hz), 4.11 (dd, 1H, *J* = 14.1 Hz and *J* = 9.6 Hz), 4.73 (dd, 1H, *J* = 17.9, 8.9), 5.35 (d, 1H, *J* = 5.0 Hz), 8.09 (d, 2H, *J* = 8.2 Hz), 8.26 (d, 2H, *J* = 8.2 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.7, 24.7, 31.6, 31.8, 31.7, 31.9, 36.6, 37.3, 39.1, 42.3, 42.7, 50.2, 55.0, 56.0, 59.9, 71.6, 82.5, 121.3, 123.5, 129.1, 132.2, 133.8, 140.9, 149.2, 162.3; MS [ESI, 487 ($M^+ + Na$)], Anal. Calcd. for $C_{28}H_{36}N_2O_4$, Found C, 72.39; H, 7.81; N, 6.03, Found C, 72.37; H, 7.39; N, 6.06.

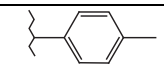
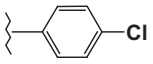
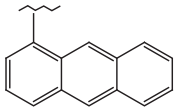
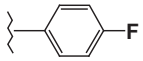
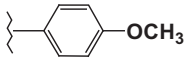
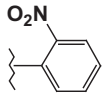
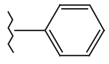
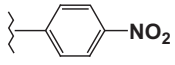
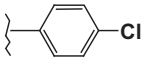
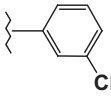
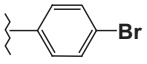
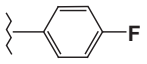
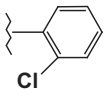
*2.2.2.3. (5'R)-17β-[2-(4-Chlorophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12c**).* Yield: 65%, mp 215–217 °C; $[\alpha]_D^{20}$ – 48 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.88 (s, 3H), 1.05 (s, 3H), 3.50 (m, 1H), 3.63 (dd, 1H, *J* = 14.5 Hz and *J* = 8.0 Hz), 4.05 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.66 (m, 1H), 5.35 (d, 1H, *J* = 5.0 Hz), 7.38 (t, 2H, *J* = 8.5 Hz), 7.86 (dd, 2H, *J* = 8.5 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.8, 24.7, 31.7, 31.7, 31.9, 32.3, 36.6, 37.3, 39.3, 42.7, 42.8, 50.3, 55.0, 56.0, 59.7, 71.7, 81.1, 121.4, 126.7, 128.6, 129.4, 131.7, 137.3, 140.9, 163.3; MS [ESI, 454 ($M^+ + H$)], Anal. Calcd. for $C_{28}H_{36}ClNO_2$, C, 74.07; H, 7.99; N, 3.08. Found C, 74.09; H, 8.01; N, 3.05.

*2.2.2.4. (5'R)-17β-[2-(3-Chlorophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12d**).* Yield: 71%, mp 232–234 °C; $[\alpha]_D^{20}$ – 53 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.86 (s, 3H), 1.04 (s, 3H), 3.51 (m, 1H), 3.63 (dd, 1H, *J* = 14.5 Hz and *J* = 8.0 Hz), 4.05 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.66 (m, 1H), 5.35 (d, 1H, *J* = 5.0 Hz), 7.33 (t, 1H, *J* = 8.0 Hz), 7.43 (d, 1H, *J* = 8.0 Hz), 7.81 (d, 1H, *J* = 8.0 Hz), 7.92 (s, 1H); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.7, 24.7, 31.7, 31.7, 31.9, 36.6, 37.3, 39.0, 42.3, 42.7, 50.3, 55.0, 56.0, 59.7, 71.7, 82.1, 121.4, 126.7, 128.3, 129.5, 129.9, 134.3, 137.2, 140.9, 163.1; MS [ESI, 454 ($M^+ + H$)], Anal. Calcd. for $C_{28}H_{36}ClNO_2$, C, 74.07; H, 7.99; N, 3.08. Found C, 74.06; H, 8.01; N, 3.06.

*2.2.2.5. (5'R)-17β-[2-(4-Bromophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12e**).* Yield: 67%, mp 202–203 °C; $[\alpha]_D^{20}$ – 37 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.87 (s, 3H), 1.04 (s, 3H), 3.50 (m, 1H), 3.62 (dd, 1H, *J* = 14.5 Hz and *J* = 8.0 Hz), 4.04 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.65 (m, 1H), 5.35 (d, 1H, *J* = 5.0 Hz), 7.54 (d, 2H, *J* = 7.5 Hz), 7.78 (d, 2H, *J* = 7.5 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.8, 24.7, 31.7, 31.7, 31.8, 31.9, 36.6, 37.3, 39.3, 42.3, 42.7, 50.3, 55.0, 56.0, 59.8, 71.7, 82.1, 121.4, 125.7, 127.1, 129.4, 131.6, 132.3, 140.9, 164.2; MS [ESI, 499 ($M^+ + H$)], Anal. Calcd. for $C_{28}H_{36}BrNO_2$, C, 67.46; H, 7.28; N, 2.81. Found C, 67.45; H, 7.26; N, 2.82.

*2.2.2.6. (5'R)-17β-[2-(4-Fluorophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12f**).* Yield: 73%, mp 233–235 °C; $[\alpha]_D^{20}$ – 62 (*c* 1, $CHCl_3$); ^1H NMR (δ, ppm, $CDCl_3$): 0.86 (s, 3H), 1.04 (s, 3H), 3.51 (m, 1H), 3.62 (dd, 1H, *J* = 14.5 Hz and *J* = 8.0 Hz), 4.03 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.67 (m, 1H), 5.34 (d, 1H, *J* = 5.0 Hz), 7.07 (t, 2H, *J* = 8.5 Hz), 7.92 (dd, 2H, *J* = 8.0 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.7, 19.4, 20.9, 23.8, 24.7, 31.7, 31.7, 31.9, 36.6, 37.3, 39.2, 42.3, 42.7, 50.3, 55.0, 56.0, 59.6, 71.7, 81.6, 115.3, 121.4,

Table 1
IC_{50} values (μM) of steroidal isoxazolines (**6a–f**) and steroidal oxazolines (**12a–g**) against human prostate cancer cell lines.

Entry	LNCaP	PC-3	DU-145	-Ar
6a	2.29 ± 0.03	12.36	5.73 ± 0.02	
6b	8.21	11.51	4.63	
6c	3.23 ± 0.04	7.38	14.84	
6d	5.25 ± 0.03	ND	6.84	
6e	2.32 ± 0.04	5.69 ± 0.02	5.11 ± 0.03	
6f	16.32	13.57	11.40	
12a	3.24 ± 0.02	10.11	3.10 ± 0.03	
12b	26.38	24.28	21.91	
12c	13.46	33.75	32.90	
12d	4.32 ± 0.03	7.62	11.34	
12e	3.15 ± 0.02	ND	14.84	
12f	10.34	12.69	16.99	
12g	11.23	ND	12.60	
Finasteride	14.53	17.83	13.53	–

ND = not determined.
Prostate cancer cell lines: LNCaP (androgen dependant), PC-3 and DU-145 (androgen independent).

124.4, 130.1, 128.3, 131.1, 132.3, 140.9, 165.6; MS [ESI, 460 (M^+ + Na)], Anal. Calcd. for $C_{28}H_{36}FNO_2$, C, 76.85; H, 8.29; N, 3.20. Found C, 76.86; H, 8.27; N, 3.23.

*2.2.2.7. (5'R)-17β-[2-(2-Chlorophenyl)-4,5-dihydrooxazol-5-yl]androst-5-en-3β-ol (**12g**).* Yield: 69%, mp 164–167 °C; $[\alpha]_D^{20}$ − 56 (*c* 1, $CHCl_3$); ^{1}H NMR (δ, ppm, $CDCl_3$): 0.82 (s, 3H), 1.01 (s, 3H), 3.50 (m, 1H), 3.68 (dd, 1H, *J* = 14.5 Hz and *J* = 8.5 Hz), 4.13 (dd, 1H, *J* = 14.5 Hz and *J* = 9.5 Hz), 4.64 (m, 1H), 5.34 (d, 1H, *J* = 5.0 Hz), 7.28 (t, 1H, *J* = 8.0 Hz), 7.34 (d, 1H, *J* = 7.5 Hz), 7.43 (d, 1H, *J* = 7.5 Hz), 7.75 (d, 1H, *J* = 7.5); ^{13}C NMR (δ, ppm, $CDCl_3$): 12.6, 19.4, 20.9, 23.7, 24.7, 31.7, 31.7, 31.9, 36.6, 37.3, 39.0, 42.3, 42.6, 50.3, 55.0, 56.0, 60.3, 71.7, 81.6, 121.4, 126.7, 127.7, 130.7, 131.2, 131.3, 132.4, 140.9, 162.7; MS [ESI, 477 (M^+ + Na)], Anal. Calcd. for $C_{28}H_{36}ClNO_2$, C, 74.07; H, 7.99; N, 3.08. Found C, 74.08; H, 8.01; N, 3.05.

2.3. Cell culture and bio-assays

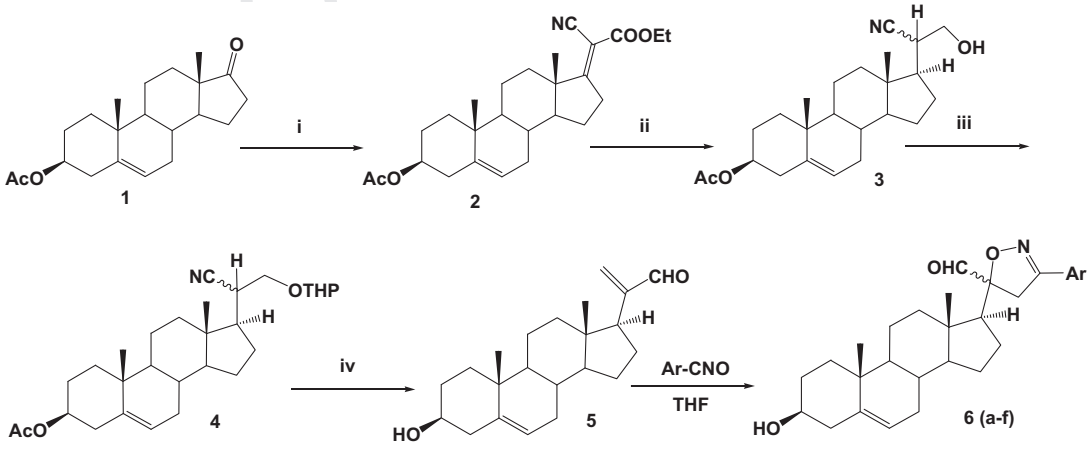
The human prostate cancer cell lines used for the test were LNCaP, PC-3 and DU-145. All these cancer cell lines were obtained from National cancer institute (NCI), biological testing branch, Federick Research and Development centre, USA. Cellular viability in the presence and absence of experimental agents was determined using the standard Sulforhodamine B assay. Briefly, cells in their log phase of growth were harvested, counted and seeded (10^4 cells/well in 100 μL medium) in 96-well microtitre plates. After 24 h of incubation at 37 °C and 5% CO_2 to allow cell attachment, cultures were treated with varying concentrations (10^{-9}–10^{-4} M) of experimental agents i.e., the steroidal analogs kept in six series of tubes. Four replicate wells were set up for each experimental condition. Test samples were left in contact with the cells for 48 h under same conditions. Thereafter, cells were fixed with 50% chilled trichloroacetic acid (TCA) and kept at 4 °C for 1 h, washed and air dried. Cells were stained with Sulforhodamine B dye. The adsorbed dye was dissolved in Tris-Buffer and plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was calculated by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated. Finally the IC_{50} values (Table 1) were calculated using Sigma Plot software. Finasteride was used as a positive control. The different steroidal derivatives (test material) were dissolved in a mixture of DMSO:Water (1:1) and then introduced into the medium containing the cancer cell lines.

2.4. Results and discussion

In spite of the tremendous pharmacological potential of the heterocyclic analogs of D-ring modified steroids [12], there are limited literature precedents available for their efficient synthesis and evaluation as potential pharmacological agents. This is especially true of the five membered heterocyclic oxazoline and isoxazoline analogs which are pharmacologically very interesting species [13]. Though there are reports available about the synthesis of isoxazole and oxazole analogs condensed with the steroid skeleton [14], same is not true for the synthesis of such analogs in the side chain at ring D. Thus we, in continuation of our interest in developing pharmacologically active steroidal D-ring heterocyclic analogs, herein report the efficient synthesis of novel side chain D-ring isoxazoline and oxazoline derivatives of 17-androstanes and pregnenolone respectively. The synthetic strategies are discussed below.

2.4.1. Synthesis of isoxazoline derivatives **6 (a–f)**

The starting ketone **1** upon condensation with ethyl cyanoacetate in boiling toluene, in the presence of ammonium acetate was transformed to Knoevenagel adduct **2** presumably as a mixture of *E* and *Z* isomers (89% yield) after purification using silica-gel column chromatography. The condensation product was reduced with an excess of sodium borohydride in methanol to the saturated alcohol **3** (97% yield). Hydroxy group in compound **3** was then protected as its tetrahydropyranyl (THP) ether and the derivative **4** was subjected to reduction with an excess of neat diisobutylaluminum hydride (DIBAL) for prolonged period in toluene at −78 °C resulting in the formation of two products as indicated by the TLC. After purification and spectral analysis of the products, it was seen that the major product was the required one i.e. **5** formed in almost 50% yield. The intermediate **5** served as an activated olefin having a great potential for the construction of large number of carbocyclic and heterocyclic analogs across ring D preferably through dipolar cycloadditions. The same was done for the preparation of isoxazoline derivatives **6 (a–f)** by employing the cycloaddition of aromatic nitrile oxides across the olefin **5**. Though the cycloaddition could conceptually lead to the formation of two regioisomers, we fortunately obtained only one regioisomer as the sole isolable product. The regiochemistry was assigned on the basis of NMR chemical shift values of the methylene protons



Scheme 1. Synthesis of the D-ring Dehydroepiandrosterone isoxazolines. Reagents and conditions: (i) $NCCH_2COOEt$, AcOH, $AcNH_4$/toluene (90%); (ii) $NaBH_4$/MeOH (98%); (iii) DHP/p-TSA/DCM (95%); (iv) DIBAL neat/toluene/−78 °C (50%).

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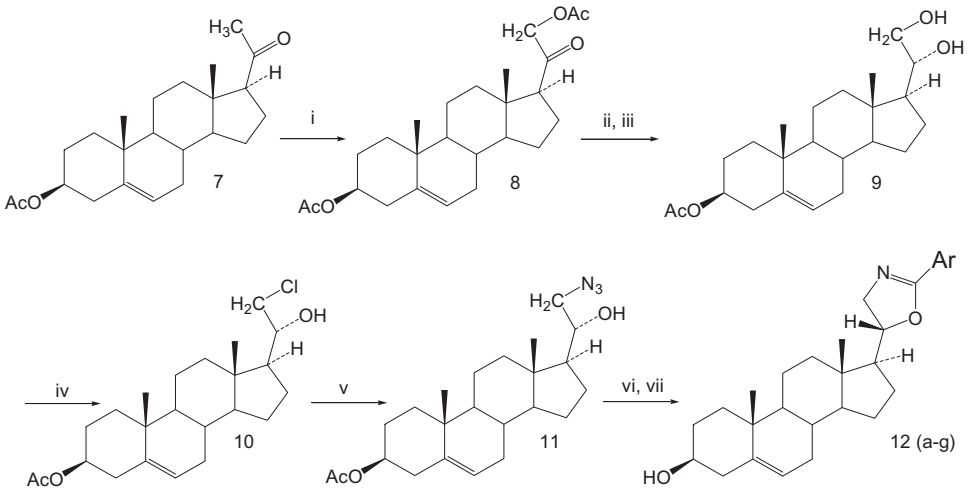

Scheme 2. Synthesis of D-ring substituted pregnenolone oxazolines. Reagents and conditions: (i) Pb $(OAc)_4$, $BF3.OEt_2$, MeOH; (ii) KBH_4, MeOH, rt; (iii) Al_2O_3, MW; (iv) $P(Ph)_3$, CCl_4, reflux; (v) NaN_3, DMF, 90 °C; (vi) aromatic aldehydes, CH_2Cl_2, $BF3{\cdot}OEt_2$, rt, (vii) NaOH, MeOH, rt;

in the isoxazoline ring which differ markedly for the two regioisomers [16]. The stereochemistry at C-17 was assigned on the basis of reported literature precedents [7]. The stereochemistry of the hydroxyl at C-3 was not changed during any stage of the synthesis (Scheme 1).

2.4.2. Synthesis of the oxazoline derivatives ***12 (a–g)***

For the synthesis of oxazolines, we followed an efficient strategy earlier described by Wolfing et al. [11]. This strategy involves a one pot conversion of aldehydes to oxazolines upon reaction with α,β-azidoalcohols. For this we chose 3β-acetoxypregn-5-en-20-one (**7**) as the starting material. Oxidation with $Pb(OAc)_4$ in the presence of $BF_3.OEt_2$ furnished 3β,21-diacetoxypregn-5-en-20-one (**8**). Reduction with KBH_4 gave two compounds, (20R)-3β,21-diacetoxypregn-5-en-20-ol and its 20S epimer, latter in a very small quantity. The required pure epimer was obtained by flash chromatography. Selective deacetylation on alkaline alumina was carried out by an earlier developed method to obtain the dihydroxy derivative **9** [11]. Chlorination of **9** in the Appel reaction [15] produced the (20R)-3β-acetoxy-21-chloropregn-5-en-20-ol **(10)**. Nucleophilic exchange with NaN_3 in dimethylformamide led to the required (20R)-3β-acetoxy-21-azidopregn-5-en-20-ol **(11)**. Reaction of the α,β-azidoalcohol **11** with appropriately substituted aromatic aldehydes activated by $BF_3{\cdot}OEt_2$ as Lewis acid catalyst, proceeded cleanly to give the corresponding acetylated product which upon deacetylation in presence of methanolic $NaOCH_3$ yielded the deacetylated D-ring steroidal oxazolines **12 (a–g)** (Scheme 2).

The *in vitro* cytotoxicity studies of various steroidal isoxazoline and oxazoline derivatives revealed that these derivatives are cell specific as these were found to be active mostly against the **LNCaP** (androgen dependant) compared to **PC-3** and **DU-145** (androgen independent) prostate cancer cell lines. As compounds **6a**, **6e** and **12a** were found to be more active than other analogs, it can be assumed that electron donating groups have an effect over the activity.

2.4.3. Biology

The following table gives the cancer cell inhibitory data obtained after treating different cancer cell lines with test doses of different D-ring substituted steroidal isoxazoline **(6a–f)** and oxazoline **(12a–g)** derivatives and the values are reported in terms of **IC_{50}**.

It is clear from the IC_{50} values, that the compounds **6a, 6e and 12a** showed significant cytotoxic activity especially against LNCaP and DU-145 prostate cancer cell lines. It is evident from the data that the nature of substituent on the aromatic ring influences the relative cytotoxicity which can be attributed to their differences in either the bioavailability or the protein binding properties. As already discussed it can be assumed that the electron donating groups such as methyl- and methoxy- increase the *in vitro* cytotoxicity. Overall the cytotoxicity of these compounds is cancer cell specific as evidenced by the IC_{50} values.

3. Conclusions

A series of novel D-ring substituted isoxazoline and oxazoline derivatives of dehydroepiandrosterone and pregnenolone respectively were synthesized and screened for anticancer activity against a panel of human prostate cancer cell lines. From the data it was found that all the compounds are having promising anticancer activity especially against **LNCaP** and **DU-145** cell lines and the compound **6e** was found to be the most active in this study.

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