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Steroidal pyrazolines and pyrazoles as potential 5α-reductase inhibitors: Synthesis and biological evaluation

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ABSTRACT

Taking pregnenolone as the starting material, two series of pyrazolinyl and pyrazolyl pregnenolones were synthesized through different routes. The synthesis of the analogs of both series is multistep and proceeds in good overall yields. While the key step in the synthesis of pyrazolinyl pregnenolones is the heterocyclization of benzylidine derivatives (**3**) in presence of hydrazine hydrate, it is the condensation of 3β-hydroxy-21-hydroxymethylidenepregn-5-en-3β-ol-20-one (**5**) with phenylhydrazine in the synthesis of pyrazolyl derivatives. Compounds of both the series were tested for their 5α-reductase inhibitory activities. Amongst all the compounds screened for their 5α-reductase inhibitory activities, compound **4b**, **4c** and **6b** were found to be the most active.

1. Introduction

Steroids represent a pharmacologically active class of molecules associated with variety of physiological functions. Steroidal derivatives in which ring D is modified with heterocyclic rings have been of great pharmaceutical interest [1]. Steroids as well as their derivatives have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular [2], autoimmune diseases [3], brain tumors, breast cancer, prostate cancer, osteoarthritis, etc. [4]. The promise of using steroids for development of lead molecules lies in the regulation of a variety of biological processes by these molecules and being a fundamental class of signaling molecules [5]. Though steroids 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. Unfortunately 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 [6,7]. It thus necessitates the development of new drugs against this dreadful disease which remains the primary focus of various research groups throughout the world. Emerging new molecularly defined targets, such as 5α-reductase (5AR), are being actively explored for treatment of cancer.

The enzyme steroid 5α-reductase (5AR) catalyses the NADPH-dependant reductive conversion of testosterone to dehydrotestosterone. The higher 5AR activity leads to increased levels of dihydrotestosterone in the peripheral tissues, which is implicated in the pathogenesis of prostate cancer, acne and male pattern baldness [8]. However the deficiency of 5AR in males results in an incomplete differentiation of external genitalia at birth [9]. It has been established that there are two genes encoding two distinct isozymes of 5AR that are differentially expressed in human tissues and are referred to as type I 5AR (5AR1) and type II 5AR (5AR2) [10]. While the former is expressed predominantly in the skin and liver, the later is expressed mainly in prostate, seminal vesicles, liver and epididymis [11]. A number of steroidal [12] and non-steroidal [13] inhibitors have been tested against 5AR. The two most important steroid based 5AR inhibitors are Finasteride (PROSCAR, Merck) and dutasteride (Avodart, GlaxoSmithKline). Finasteride, a type II-selective inhibitor, was the first 5AR inhibitor approved in the United States for the treatment of prostate cancer and benign prostatic hyperplasia (BPH). Dutasteride has no selectivity and acts as an inhibitor against type I and type II 5AR. However both the approved drugs suffer from serious side effects such as erectile dysfunction, abnormal ejaculation, impotence, abnormal sexual function, decreased sexual desire, gynecomastia etc. [14]. Recent literature precedents indicate various steroidal

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D-ring heterocyclic derivatives as inhibitors of 5AR and 17α-hydroxylase/C_{17-20}-lyase of which 17-imidazolyl, pyrazolyl, pyrazolinyl, isoxazolyl, oxazolyl and thiazolyl derivatives are very potent [24]. Taking inputs from these literature precedents to obtain the skeleton structure required for 5AR inhibitory activity, we, in continuation of our research program directed towards the development of steroid based lead molecules [15], designed synthesis of two series of novel pyrazolinyl and pyrazolyl analogs from pregnenolone. Further, as it is well established that 17β-heterocycles are more active towards the 5AR inhibition [24], we also designed the heterocycles on the same lines with β-orientation at the 17-position. All the synthesized pregnenolone derivatives were evaluated for their 5AR inhibitory activity. It was observed that compounds **4b**, **4c** and **6b** exhibit excellent activities and are the most potent of all the screened compounds.

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 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 pyrazoline derivatives (*4a*–*4j*)

To a solution of pregnenolone 1 (0.316 g, 1 mmol, 1 eq.) in ethanol (10 ml) was added a conc. aq. solution of KOH (2 eq.). Then aldehyde 2 (1.2 eq.) was charged into the reaction mixture to get the corresponding benzylidine derivative 3. After completion, the reaction mixture was precipitated with water. The precipitate was filtered, dried and recrystallized from EtOAc:Hexane to give product as solid white powder. It is to be mentioned that when non-aromatic aldehydes were used, the product was formed in a very minor quantity and that too not stable enough at ambient conditions. Thus the study was restricted to the use of aromatic aldehydes only. The condensation product 3 (1.0 g, 2.4 mmol) was refluxed in ethanol in the presence of hydrazine hydrate (0.24 g, 4.8 mmol) so as to yield the desired pyrazolines. However the products thus obtained were very unstable and they decomposed even at ambient temperature conditions probably because of the inherent instability associated with pyrazolines. The solvent thus used was replaced by acetic acid so as to ensure the formation of N-acetyl pyrazoline **4** (0.99 g, 2.2 mmol, 90%) which was highly stable. The product was precipitated by charging the reaction mass into excessive amounts of ice-cold water. After filtration under suction, the product was obtained in high yields as colorless powder which was later dried in vaccuo. However we observed a diastereomeric mixture of pyrazolines which was separated through the method discussed in the experimental section to yield the desired isomer. The same procedure was followed for the synthesis of all other analogs. Spectral data of various compounds is given as under (Most of the peaks due to steroidal skelton were merged and could not be differentiated. Thus δ values of only those peaks that distinguish the product and could easily be differentiated are reported).

*2.2.1.1. 1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-phenylpyrazol-1-yl) ethanone (**4a**).* Colourless solid powder. Yield 76%. M.p: 194–197 °C. $[\alpha]_D^{25}$ −85.9 (*c* 1 in $CHCl_3$). IR (KBr, cm^{-1}): 3384, 2926, 1717, 1646, 1404, 1042, 699. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.67 (s, 3H), 1.06 (s, 3H), 1.82–1.90 (m, 6H), 2.17 (s, 3H), 2.65 (t, 1H, *J* = 8.8), 2.79 (m, 2H), 3.26 (m, 1H), 3.49 (m, 1H), 5.33 (s, 1H), 5.44 (m, 1H), 7.15 (d, 2H, *J* = 6.5), 7.22–7.32 (m, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.85, 20.85, 22.40, 23.32, 25.83, 31.13, 33.04, 33.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 160.63, 164.62. ESI-MS: 483 (M^++Na). Anal. Calcd. for $C_{30}H_{40}N_2O_2$: C, 78.22; H, 8.75; N, 6.08; found C, 78.47; H, 8.83; N, 6.21.

*2.2.1.2. 1-(5-(3-Fluorophenyl)-4,5-dihydro-3-((10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl) pyrazol-1-yl)ethanone (**4b**).* Colourless solid. Yield 79%. M.p: 168–171 °C. $[\alpha]_D^{25}$ −40.2 (*c* 1 in $CHCl_3$). IR (KBr, cm^{-1}): 3408, 2936, 1718, 1448, 1021, 756. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.67 (s, 3 H), 1.06 (s, 3H), 1.82–1.90 (m, 6H), 2.15 (s, 3H), 2.67 (t, 1H, *J* = 8.8), 2.77 (m, 2H), 3.26 (m, 1H), 3.35 (m, 1H), 5.30 (s, 1H), 5.39 (m, 1H), 6.80–6.91 (m, 2H), 7.30–7.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.24, 18.40, 19.95, 20.82, 23.58, 28.60, 30.59, 31.03, 35.54, 36.27, 37.38, 41.23, 45.07, 50.69, 55.38, 57.66, 70.64, 111.11, 113.16, 120.30, 129.37,139.86, 143.79, 159.69, 164.58, 167.71. ESI-MS: 479 (M^++H). Anal. Calcd. for $C_{30}H_{39}FN_2O_2$: C, 75.28; H, 8.21; N, 5.85; found C, 75.43; H, 8.03; N, 6.04.

*2.2.1.3. 1-(5-(4-Fluorophenyl)-4,5-dihydro-3-(10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl) pyrazol-1-yl)ethanone (**4c**).* Colourless solid powder. Yield 82%. M.p: 222–225 °C. $[\alpha]_D^{25}$ −58.0 (*c* 1 in $CHCl_3$). IR (KBr, cm^{-1}): 3375, 3166, 2928, 1721, 1404, 1042, 756. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.65 (s, 3 H), 1.05 (s, 3H), 1.82–1.90 (m, 6H), 2.17 (s, 3H), 2.76 (m, 2H), 3.26 (m,1H), 3.35 (m, 1H), 5.35 (s, 1H), 5.37 (m, 1H), 7.04 (d, 2H, *J* = 8.6), 7.14 (d, 2H, *J* = 8.4). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.34, 19.40, 19.65, 20.82, 23.58, 28.60, 30.59, 31.03, 35.54, 36.27, 38.38, 41.23, 42.91, 45.07, 51.69, 56.38, 57.66, 70.64, 111.11, 113.16, 120.30, 129.37,139.86, 143.79, 159.15, 162.71, 164.56, 167.71. ESI-MS: 479 (M^++H). Anal. Calcd. for $C_{30}H_{39}FN_2O_2$: C, 75.28; H, 8.21; N, 5.85; found C, 75.51; H, 8.05; N, 6.09.

*2.2.1.4. 1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-p-tolylpyrazol-1-yl)ethanone (**4d**).* Greyish powder. Yield 79%. M.p: 230–233 °C. $[\alpha]_D^{25}$ −33.3 (*c* 1 in $CHCl_3$). IR (KBr, cm^{-1}): 3416, 2936, 1719, 1642, 1455, 1087, 756. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.61 (s, 3 H), 1.08 (s, 3H), 1.82–1.92 (m, 6H), 2.05 (s, 3H), 2.22 (s, 3H), 2.67 (t, 1H, *J* = 8.8), 2.77 (m, 2H), 3.40 (m, 1H), 3.48 (m, 1H), 5.36 (m, 2H), 7.80 (d, 4H, *J* = 6.3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.87, 20.54, 22.46, 23.38, 25.83, 31.13, 33.04, 33.19, 33.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.38, 47.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 161.65, 162.53. ESI-MS: 497 (M^++Na). Anal. Calcd. for $C_{31}H_{42}N_2O_2$: C, 78.44; H, 8.92; N, 5.90; found C, 78.67; H, 8.73; N, 6.13.

*2.2.1.5. 1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-o-tolylpyrazol-1-yl) ethanone (**4e**).* Colourless