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Synthesis of methoxycarbonylpyrazolylandrostene derivatives, and their potential inhibitory effect on androgen biosynthesis and cell proliferation

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ABSTRACT

The Claisen condensations of 3β-acetoxypregn-5-en-20-one (**1**) and 3β-acetoxypregna-5,16-diene (**7**) with dimethyl oxalate are known to lead to 3β-hydroxy-21-methoxalylpregn-5-en-20-one (**2**) and 3β-hydroxy-21-methoxalylpregna-5,16-dien-20-one (**8**), respectively. The reactions of **2** with *p*-substituted phenylhydrazines afford pyrazol-5-yl derivatives (**5**) as main, and 3-yl regioisomers (**4**) as minor products. The corresponding reactions of 16-ene analogue **8** afford only pyrazol-5-yl regioisomer **9**. Oppenauer oxidation of the pyrazolyl compounds yields the corresponding Δ^4-3-ketosteroids. We investigated the antiandrogenic effects of new methoxycarbonylpyrazolyl compounds through determination of their *in vitro* inhibition of the activities of rat testicular $C_{17,20}$-lyase, Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) and 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3). A Δ^5-3β-hydroxy compound in the D-ring-saturated androst-5-ene series bearing an unsubstituted phenyl group on the pyrazolyl heterocycle (**5a**) proved to be a potent inhibitor of Δ^5-3β-HSD. The 4-methoxyphenyl derivative (**5e**) and the 3-oxo counterpart (**6a**) of **5a** also displayed substantial inhibition. The other tested compounds exerted only weak inhibitory action against the enzymes investigated. The newly synthetized compounds were evaluated *in vitro* by means of MTT assays for antiproliferative activity against Hela (cervical carcinoma), A431 (skin epidermoid carcinoma) and MCF7 (breast adenocarcinoma) cells. In all four groups (3β-hydroxy- and 3-ketosteroids with saturated or unsaturated ring D), the most potent analogs contain a 4-tolyl or 4-methoxyphenyl group. Compound **5d** exhibited substantial antiproliferative action against the three cell lines investigated, whereas **9d** inhibited the growth of Hela cells markedly. The most noteworthy inhibition was exerted by **6a** against A431 cells.

1. Introduction

Prostatic cancer (PC) is a life-threatening disease and a major cause of cancer-related mortality in developed countries. PC is hormone-dependent in a majority of the cases and can be controlled by androgen ablation. 17-(3-Pyridyl)androsta-5,16-dien-3β-ol (Abiraterone) blocks testosterone synthesis by inhibiting 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) and is successfully applied in anticancer therapy. Efficacy of Abiraterone inspired numerous studies concerning the synthesis of *exo*-heterocyclic steroids with potential 17α-hydroxylase/$C_{17,20}$-lyase inhibitory activity [1]. Oxazolyl [2,3], pyrazolyl [4], triazolyl, tetrazolyl [5], imidazolyl [4,5], isoxazolyl [4] and thiazolyl [2,6] derivatives have recently been synthetized and reported to be potent $C_{17,20}$-lyase inhibitors. Furthermore, various 17β-*exo*-heterocyclic steroids are known to exert hormone-independent antiproliferative activity via the inhibition of angiogenesis, the disruption of tubulin polymerization or the upregulation of apoptotic pathways [7]. Potential antihormonal/antiandrogen or antiproliferative effects make heterocyclic steroids desirable target compounds in modern medicinal chemistry. Androstane derivatives containing a pyrazoline ring (both ring D-fused and 17β-substituted) have been synthetized and investigated for their antiproliferative activities [8–10]. Solanidine analogs containing pyrazoline and pyrazolidine rings have been reported to

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be potent against the HL-60 leukemia cell line [11]. We earlier described the synthesis of 17β-*N*-phenyl pyrazolyl- and 17β-*C*-phenyl pyrazolinylandrostenes, and investigated their inhibitory activities against $C_{17,20}$-lyase *in vitro* [12].

As a continuation of our research program, we report here on the introduction of new structural elements into the previously described set of steroidal pyrazoles [12]: a Δ^{16}-double bond, and a methoxycarbonyl group attached to the pyrazole ring. We investigated the influence of the methoxycarbonyl group on the cyclization process, which in principle can afford a mixture of 3′- and 5′-pyrazolyl regioisomers. We studied potential antisteroidogenic effects: the inhibition of 17α-hydroxylase/$C_{17,20}$-lyase ($P450_{17\alpha}$), Δ^5-3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (Δ^5-3β-HSD) and 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), enzymes present in the rat testis. We were additionally investigated the antiproliferative effects of the new synthetized steroids. Although nonsteroidal pyrazole carboxylates with antiproliferative activities are known in the literature [13,14], steroidal methoxycarbonylpyrazoles with such biological properties have not yet been reported.

2. Experimental

2.1. General

Melting points (mp-s) were determined on a Kofler block and are uncorrected. Specific rotations were measured in $CHCl_3$ (*c* 1) at 20 °C with a POLAMAT-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Elementary analysis data were determined with a Perkin-Elmer CHN analyzer model 2400. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss): (A) acetone/toluene/hexane (30:35:35, v/v/v), (B) ethyl acetate/dichloromethane (20:80, v/v). The spots were detected after spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm. All solvents were distilled prior to use. NMR spectra were recorded on a Bruker DRX 500 instrument at 500 (^{1}H NMR) or 125 MHz (^{13}C NMR). Chemical shifts are reported in ppm (δ scale), and coupling constants (*J*) in Hertz. For the determination of multiplicities, the *J*-MOD pulse sequence was used.

2.2. Preparation of 3β-hydroxy-21-methoxalylpregn-5-en-20-one (**2**)

3β-Acetoxypregn-5-en-20-one (**1**) 7.12 g (20 mmol) was dissolved in 100 ml benzene, and 4.75 g (40 mmol) dimethyl oxalate and 2.16 g (40 mmol) $NaOCH_3$ were then added. The mixture was stirred at room temperature for 5 h. The resulting suspension was diluted with ethyl acetate, and 8 ml cc. HCl was added. The organic phase was washed with water, dried with Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from acetone to afford **2** (6.80 g, 84%), mp 170–172 °C (Ref. [15]: mp 172–173.5 °C). R_f = 0.39 (ss A), 0.54 (ss B); $[\alpha]_D^{20}$ + 47 (*c* 1 in $CHCl_3$). (Found C, 71.53; H, 8.42. $C_{24}H_{34}O_5$ requires C, 71.61; H, 8.51%.) ^{1}H NMR (δ, ppm, $CDCl_3$): 0.64 and 0.99 (s, 3H, 18-H_3, and s, 3H, 19-H_3), 2.54 (t, 1H, *J* = 8.8 Hz, 17-H), 3.51 (m, 1H, 3-H), 3.89 (s, 3H, $COOCH_3$), 5.34 (d, 1H, *J* = 4.5 Hz, 6-H), 6.32 (s, 1H, 21-H).

2.3. Preparation of 3β-hydroxy-21-methoxalylpregna-5,16-dien-20-one (**8**)

5.84 g (40 mmol) dimethyl oxalate was dissolved in 20 ml dry pyridine, and the solution was added to 4 g $NaOCH_3$ under NaCl/ice cooling and stirring. 4 g (11.2 mmol) 3β-acetoxy-pregn-5-en-20-one (**7**) in 50 ml pyridine solution was then added dropwise. After the addition, the mixture was stirred for a further 1 h, and next poured into ice/150 ml cc. HCl. The precipitate that formed was filtered off, washed with water several times and dried to afford **8** (4.1 g, 91%), mp 181–183 °C (Ref. [15]: mp 183–185 °C). R_f = 0.34 (ss A); $[\alpha]_D^{20}$ − 35 (*c* 1 in $CHCl_3$). (Found C, 72.14; H, 8.23. $C_{24}H_{32}O_5$ requires C, 71.97; H, 8.05%.) ^{1}H NMR (δ, ppm, $CDCl_3$): 0.98 and 1.04 (s, 3H, 18-H_3, and s, 3H, 19-H_3), 3.53 (m, 1H, 3-H), 3.89 (s, 3H, $COOCH_3$), 5.34 (s, 1H, 6-H), 6.67 (s, 1H, 21-H), 6.91 (s, 1H, 16-H).

2.4. General procedure for the preparation of 17β-[5-methoxycarbonyl-1-phenyl- or -1-(4-subst.-phenyl)-1H-pyrazol-3-yl]androst-5-en-3β-ol (**4a**–**c**), 17β-[3-methoxycarbonyl-1-phenyl- or -1-(4-subst.-phenyl)-1H-pyrazol-5-yl]androst-5-en-3β-ol (**5a**–**e**), and 17-[3-methoxycarbonyl-1-phenyl- or -1-(4-subst.-phenyl)-1H-pyrazol-5-yl]androsta-5,16-dien-3β-ol (**9a**–**e**)

Compound **2** or **8** (6 mmol, 2.42 or 2.40 g) and KOAc (1.2 g, 12 mmol) were dissolved in glacial acetic acid (75 ml), and phenylhydrazine hydrochloride (**3a**) or one of its *p*-substituted derivatives (**3b**–**e**) (1.1 equivalent) was added. The reaction mixture was stirred at room temperature for 6 h, and then poured into ice-cold water (500 ml). The precipitate that formed was filtered off and washed with water. The residue obtained was dissolved in dichloromethane and chromatographed on silica gel, initially starting with dichloromethane/hexane (3:1, v/v) as eluent, followed by dichloromethane and ethyl acetate/dichloromethane (5:95, v/v) to afford **4a**–**c** and **5a**–**e** or **9a**–**e**.

2.4.1. 17β-[5-Methoxycarbonyl-1-phenyl-1H-pyrazol-3-yl]androst-5-en-3β-ol (**4a**) and 17β-[3-methoxycarbonyl-1-phenyl-1H-pyrazol-5-yl]androst-5-en-3β-ol (**5a**)

The resulting crude product was chromatographed on silica gel to yield pure **4a** (187 mg, 6.6%), mp 130–133 °C. R_f = 0.48 (ss A); $[\alpha]_D^{20}$ − 32 (*c* 1 in $CHCl_3$). (Found C, 76.08; H, 8.15. $C_{30}H_{38}N_2O_3$ requires C, 75.92; H, 8.07%.) ^{1}H NMR (δ, ppm, $CDCl_3$): 0.60 and 1.02 (s, 3H, 18-H_3, and s, 3H, 19-H_3), 2.78 (t, 1H, *J* = 9.8 Hz, 17-H), 3.51 (m, 1H, 3-H), 3.77 (s, 3H, $COOCH_3$), 5.36 (d, 1H, *J* = 5.0 Hz, 6-H), 6.84 (s, 1H, 4′-H), 7.38–7.45 (overlapping multiplets, 5H, 2″-, 3″-, 4″-, 5″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.1 (C-18), 19.4 (C-19), 20.8, 24.6, 26.4, 31.6, 31.9, 32.3, 36.6, 37.3, 37.7, 42.3, 43.8, 49.9, 50.3, 51.8 ($COOCH_3$), 56.2, 71.7 (C-3), 111.6 (C-4′), 121.4 (C-6), 125.9 and 128.4 (4C, C-2″, C-3″, C-5″ and C-6″), 128.2 (C-4″), 132.9, 140.4, 140.9 (C-5), 153.6, 159.8 ($COOCH_3$). Continued elution resulted in **5a** (2.6 g, 90%), mp 203–206 °C. R_f = 0.41 (ss A); $[\alpha]_D^{20}$ − 106 (*c* 1 in $CHCl_3$). (Found C, 75.84; H, 8.27. $C_{30}H_{38}N_2O_3$ requires C, 75.92; H, 8.07%.) ^{1}H NMR (δ, ppm, $CDCl_3$): 0.69 and 0.95 (s, 3H, 18-H_3, and s, 3H, 19-H_3), 2.77 (t, 1H, *J* = 9.8 Hz, 17-H), 3.46 (m, 1H, 3-H), 3.92 (s, 3H, $COOCH_3$), 5.30 (d, 1H, *J* = 5.0 Hz, 6-H), 6.83 (s, 1H, 4′-H), 7.35 (d, 2H, *J* = 7.5 Hz, 2″ and 6″-H), 7.45 (overlapping multiplets, 3H, 3″-, 4″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.3 (C-18), 19.3 (C-19), 20.7, 24.4, 29.6, 31.6, 31.7, 32.3, 36.5, 37.2, 37.3, 42.2, 44.3, 47.0, 49.9, 51.9 ($COOCH_3$), 56.0, 71.6 (C-3), 108.1, 121.2 (C-6), 127.1 and 128.9 (5C, C-2″, C-3″, C-4″, C-5″ and C-6″), 139.5, 140.8, 143.1, 146.5, 163.1 ($COOCH_3$).

2.4.2. 17β-[1-(4-Chlorophenyl)-5-methoxycarbonyl-1H-pyrazol-3-yl]androst-5-en-3β-ol (**4b**) and 17β-[1-(4-chlorophenyl)-3-methoxycarbonyl-1H-pyrazol-5-yl]androst-5-en-3β-ol (**5b**)

The resulting crude product was chromatographed on silica gel to yield pure **4b** (160 mg, 5.2%), mp 109–112 °C. R_f = 0.50 (ss A); $[\alpha]_D^{20}$ − 38 (*c* 1 in $CHCl_3$). (Found C, 70.94; H, 7.26. $C_{30}H_{37}ClN_2O_3$ requires C, 70.78; H, 7.33%.) ^{1}H NMR (δ, ppm, $CDCl_3$): 0.58 and 1.01 (s, 3H, 18-H_3, and s, 3H, 19-H_3), 2.76 (t, 1H, *J* = 9.8 Hz, 17-H), 3.52 (m, 1H, 3-H), 3.79 (s, 3H, $COOCH_3$), 5.37 (d, 1H, *J* = 5.0 Hz,