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An efficient approach to novel 17-5′-(1′,2′,4′)-oxadiazolyl androstenes via the cyclodehydration of cytotoxic *O*-steroidacylamidoximes, and an evaluation of their inhibitory action on 17α-hydroxylase/$C_{17,20}$-lyase

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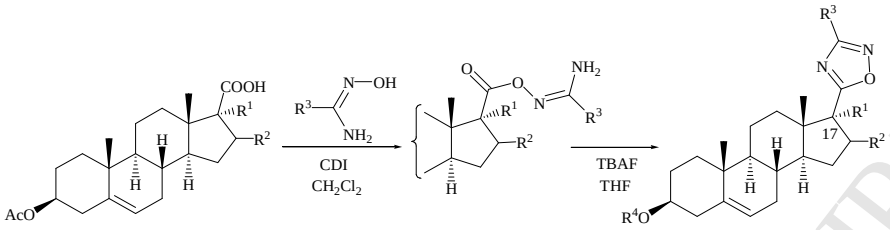
Graphical abstract



$R^1 = R^2 = H$
$R^1, R^2 =$ double bond

$R^3 = CH_3$, Ph, 4-CH_3-C_6H_4, 4-OMe-C_6H_4, 4-Br-C_6H_4
$R^4 =$ H, Ac

Highlights

Novel 17-exo-1,2,4-oxadiazolyl androstenes were synthetized in high yields

O-Acylation of different amidoximes with steroidal carboxylic acids were carried out

Cyclocondensations of *O*-acylamidoximes occurred under mild conditions

Enzyme-inhibitory and antiproliferative effects of the products were examined

An efficient approach to novel 17-5′-(1′,2′,4′)-oxadiazolyl androstenes via the cyclodehydration of cytotoxic *O*-steroidacylamidoximes, and an evaluation of their inhibitory action on 17α-hydroxylase/$C_{17,20}$-lyase

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Abstract

Novel 17-*exo*-oxadiazoles in the androst-5-ene series were efficiently synthesized in a two-step sequence via the corresponding *O*-acylamidoxime intermediates (obtained from steroidal 17-carboxylic acids and amidoximes in the presence of coupling reagent), which then underwent tetrabutylammonium fluoride-induced cyclocondensation under mild reaction conditions. The synthestized compounds were subjected to *in vitro* pharmacological studies to investigate their inhibitory effect on rat testicular $C_{17,20}$-lyase and their antiproliferative action on four malignant human adherent cell lines (HeLa, MCF7, A2780 and A431). One of the oxadiazolyl derivatives proved to exert significant enzyme-inhibitory action (IC_{50} = 0.60 □M), while some of the isolated *O*-acylated amidoxime intermediates displayed high cytotoxic activities on all examined cell lines, with IC_{50} values in the range 0.2~3.94 □M.

Keywords: steroids, *O*-acylamidoximes, antiproliferative activity, 1,2,4-oxadiazoles, $P450_{17}$□ inhibitor

1. Introduction

In consequence of the numerous synthetic possibilities available for the construction of different heterorings on the sterane core, and also of the differences in the ability of these derivatives to bind to their target receptors, leading to the manifestation of certain beneficial biological effects, research into steroidal compounds has recently focused on the syntheses and pharmacological investigations of heterocycle-containing compounds [1–3]. The most frequent chemical modifications are introduced at position 17 on ring D, where transformation of the extant functional group is facilitated. Cardiac glycosides, a large family of naturally occurring steroidal 17-*exo*-heterocycles present in both plants and animals, contain a five- or six-membered lactone ring at C-17 and a sugar moiety at C-3 of the sterane nucleus. The *cis* conformations of the skeletal rings A/B and C/D, together with the connected sugar moiety, are of significant pharmacological relevance as concerns their efficacy in the treatment of congestive heart failure and as anti-arrhythmic agents [4, 5]. Less well known, however, is the emerging role of this category of compounds for the prevention and/or treatment of cancer, as a result of which the 17-*exo*-heterocyclic steroids are now at the focus of scientific interest [6]. Attempts to modify the original aglycone cores in order to broaden the narrow therapeutic window typical for glycosides led to the conclusion that sex hormone-derived 17-*exo*-heterocycles involving a *trans* C/D ring junction exert an inhibitory effect on 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) rather than cardiotonic activity by blocking Na^+/K^+-ATPase [7]. $P450_{17\alpha}$ is a key regulatory enzyme in the androgen biosynthetic pathway and inhibitors of this protein can block male sex hormone synthesis at an early stage leading to their potential use in the medication of androgen-dependent diseases [8]. The first steroidal inhibitor, abiraterone, which is based on the structure of pregnenolone, one of the natural

substrates of $P450_{17\alpha}$, was approved in 2011 in the form of its 3β-acetate prodrug for the treatment of prostate cancer (Figure 1). The features responsible for the observed activity of abiraterone are the direct coordination of the lone pair of the nitrogen in the pyridyl ring at C-17 with the heme Fe and the interaction of the 3β-OH with asparagine 202 in the F helix in the active site of the enzyme [9]. The presence of the C16-17 double bond, however, also appears to be of crucial importance in its irreversible inhibitory effect on human $P450_{17\alpha}$ [8]. Besides abiraterone, a large number of 17-*exo*-heterocycles are known to induce efficient $P450_{17\alpha}$ inhibition: furanyl, thienyl [10, 11], oxazolyl, thiazolyl [12, 13], imidazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl [14, 15], pyrimidyl [16], and benzimidazolyl derivatives [17] were synthesized earlier and demonstrated to be more or less effective.

Figure 1.

Some 17-*exo*-heterocyclic steroids have also been reported to be involved in complex cell-signal transduction mechanisms in a hormone-receptor independent manner by the inhibition of angiogenesis, tubulin polymerization and the regulation of apoptotic pathways, and resulting in the selective control of human tumor but not normal cellular proliferation [18]. As such, they represent a promising form of targeted cancer chemotherapy.

As a continuation of our research on steroidal *exo*-heterocycles [19-24], our present goal was to introduce an 1,2,4-oxadiazole moiety at C-17, since the effects of this structural element on the biological activities of steroids have not yet been examined. 1,2,4-Oxadiazoles have often been used as hydrolysis-resistant bioisosteres of esters and amides in a number of biologically active synthetic compounds with widespread pharmacological effects [25], including anti-cancer activity [26–28]. The most common route for the construction of 1,2,4-

oxadiazoles is through the reaction of amidoximes with carboxylic acids in the presence of coupling reagents, e.g. dicyclohexylcarbodiimide (DCC) or 1,1'-carbonyldiimidazole (CDI), or with reactive acid chlorides, active carboxylic esters or anhydrides [29]. The *O*-acylation of amidoximes with carboxylic acid derivatives occurs under mild conditions [30], but the subsequent cyclodehydration of the intermediates to oxadiazoles generally requires long reaction times (5-12 h) in the presence of a strong base (NaH or NaOEt) or elevated temperatures (80-100 °C) for a weaker base (pyridine). The cyclization of *O*-acylamidoximes, however, can be promoted by tetrabutylammonium fluoride (TBAF) under mild conditions, since the fluoride ion acts as a strong base in a polar aprotic solvent, inducing both the ring-closure and dehydration steps of the cyclocondensation reaction [31].

Different routes were designed for the synthesis of 17-5′-(1′,2′,4′)-oxadiazolylandrost-5-enes structurally similar to abiraterone, so as to find reaction conditions ensuring high productivity. Furthermore, all compounds, including the *O*-acylated amidoxime intermediates, were screened *in vitro* by means of MTT assays for their antiproliferative activities against a panel of four human adherent cancer cell lines (HeLa, MCF7, A2780 and A431). The inhibitory effects of the heterocyclic products on rat testicular $C_{17,20}$-lyase were also determined by a radioligand incubation technique.

2. Results and Discussion

2.1. Synthetic studies

Steroidal 17-carboxylic acids (**3**, **5**) and their 3β-acetates (**4**, **6**) were synthesized from pregnenolone acetate (**1**) and pregnadienolone acetate (**2**) via the bromoform reaction and subsequent acetylation, using well-known literature methods (Scheme 1) [12, 32]. In order to investigate the reactivity of carboxylic esters against amidoximes, the previously reported methyl ester derivative **7** [33] was also produced via an alternative route from **3** in the

presence of $BF_3{\cdot}OEt_2$ in MeOH. However, the similar esterification of **5** could not be carried out, since C-16 is highly reactive in this case due to conjugation, and the addition of a methyl group to this position may also be feasible.

Scheme 1.

Amidoximes (**IIa–e**) were prepared by reacting aromatic nitriles (**Ia–d**) or acetonitrile (**Ie**) with hydroxylamine hydrochloride in basic media, using general synthetic prescriptions [27] (Scheme 2). The conventional 2-7-h heating period in aqueous EtOH resulted in the desired amidoximes (**IIa–e**) together with small amounts (10-15%) of amide (**IIIa–e**) impurity [34], but these by-products were easily removed during the work-up procedure or by column chromatography. Nevertheless, it is important to note that amide traces do not interfere in the reactions of amidoximes with carboxylic acid derivatives; the contaminated amidoxime merely has to be applied in excess. The substitution reactions were also carried out by using microwave (MW) irradiation, which shortened the reaction time to 15-20 min, but left the chemoselectivity unchanged.

Scheme 2.

Preliminary substitution experiments were carried out by reacting ester **7** with benzamidoxime (**IIa**) in the presence of K_2CO_3 [35]. No reaction was observed when the components were refluxed for 12 h [36], and MW irradiation either in solution or under solvent-free conditions [37] resulted in only low conversions. Apart from the moderate productivity, oxadiazolyl-Δ^{16}-androst-5-enes are otherwise not accessible by this method in consequence of the unavailability of suitable carboxylic esters.

The reactions of the more reactive acid chlorides (**8**, **9**) of **4** and **6** with benzamidoxime (**IIa**) were next investigated (Scheme 2). The steroidal carboxylic acids (**4**, **6**) were first converted to the acid chlorides (**8**, **9**) using oxalyl chloride and a catalytic amount of dimethylformamide (DMF) in CH_2Cl_2 [38]. After stirring at room temperature for 12 h, the excess of oxalyl chloride was distilled off, and 3 equivalents of benzamidoxime (**IIa**) in pyridine were added. It was necessary to heat the reaction mixtures for 7 h in order to achieve sufficient conversions because of the presence of the weakly basic pyridine, but the corresponding oxadiazoles (**12a**, **13a**) were obtained in yields of only 35 and 38% after purification by flash chromatography. The *O*-acylated amidoximes (**10a**, **11a**) formed as intermediates were converted to the corresponding heteroaromatic derivatives (**12a**, **13a**) under the conditions applied and therefore could not be isolated, although their formation was detected by TLC. When the transformations were repeated under MW irradiation, the reaction time was reduced to 30 min, but the yields of the products increased by only a few per cent. The application of a longer reaction time or a reagent excess did not have any effect on the conversions.

In the hope of using milder reaction conditions, but achieving better yields of the desired products, our attention turned to the reactions of carboxylic acids (**4**, **6**) and amidoximes (**IIa–e**) with the use of a coupling reagent. As concerns the relative effectiveness of DCC and CDI in DMF, better, but not complete conversion was observes on the TLC plates when carboxylic acid **4** was reacted with CDI. An investigation of the effects of other solvents (THF and CH_2Cl_2) revealed complete coupling with CDI in CH_2Cl_2 at room temperature. A similar solvent dependence was reported earlier for the reactions of CDI-activated carboxylic acids with acylhydrazides [39]. Subsequently, **4** was reacted with CDI in CH_2Cl_2, and benzamidoxime (**IIa**) was added to the mixture after completion of the coupling reaction (Scheme 2). The substitution was performed at room temperature to afford the corresponding

O-acylated derivative (**10a**) in a yield of 85% after purification. The application of temperatures higher than 30 °C resulted in decomposition of the CDI-activated carboxylic acid. The subsequent TBAF-induced cyclodehydration of **10a** furnished oxadiazole **12a** in an excellent yield within 1 h under mild conditions. Once the parameters appropriate for the efficient synthesis of 17-*exo*-oxadiazolyl androstenes had been found, similar reactions of **4** and **6** with different amidoximes (**IIa–e**) were carried out via the above-mentioned two-step protocol: both the *O*-acylated intermediates (**10a–e**, **11a–e**) and the heterocyclic derivatives (**12a–e**, **13a–e**) were obtained in excellent yields. For pharmacological studies, the 3β-OH analogs (**14a–e**, **15a–e**) of the cyclocondensation products (**12a–e**, **13a–e**) were synthesized by simple deacetylation.

The structures of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR measurements. The presence of the phenyl or *p*-substituted phenyl ring derived from the amidoximes (**IIa–d**) was demonstrated by the signals in the aromatic range of the ppm scale in (**10-15**)**a-d**, while an additional methyl signal was detected at around 2.0 ppm in the ^{1}H NMR spectra of **10e–15e**. The spectra of the *O*-acylamidoximes (**10a–e**, **11a–e**) and the related heterocyclic products (**12a–e**, **13a–e**) proved to be quite similar. The broad singlet of the amide protons at around 5.0 ppm was present in the spectra of the former compounds, but not in those of the cyclized products **12a–e** and **13a–e**. The chemical shifts of the protons close to the cyclocondensation reaction center differed somewhat in the related open-chain (**10a–e**, **11a–e**) and cyclic compounds (**12a–e**, **13a–e**), which is explained by the decreased shielding and therefore the downfield shift of these protons in the latter case, caused by the aromatic character of the oxadiazole ring.

2.2. Pharmacological studies

In recent years, there has been considerable interest in steroidal 17-*exo*-heterocycles in view of their potential use in the treatment of cancer either by blocking one of the key regulatory enzymes of androgen biosynthesis or by exerting direct antiproliferative effects on tumor cells. However, the 1,2,4-oxadiazole scaffold [40] and its saturated variants [41] have received less attention from both synthetic and pharmacological aspects. As several different compounds containing an 1,2,4-oxadiazole moiety have been reported to exert antiproliferative activity [26–28, 42], and the synthesized derivatives are structurally related to abiraterone, which is generally used as an effective $P450_{17\alpha}$ inhibitor in the medication of malignant prostate cancer, investigation of the synthesized compounds for cytotoxic efficacy and $P450_{17\alpha}$ enzyme inhibition appeared obvious. The inhibitory effects of **14a–e** and **15a–e** on rat testicular $C_{17,20}$-lyase were tested by means of a radiosubstrate incubation technique. Among the oxadiazoles, only **14e** and **15e** (containing a methyl group at position 3 of the heteroaromatic ring) exhibited noteworthy activity (Table 1). This is in good agreement with previous observations that bulky substituents on the heterocyclic moiety are less favorable in respect of $P450_{17\alpha}$ inhibition [43, 44]. Compound **15e**, with IC_{50} = 0.60 μM, inhibited the enzyme action to an extent roughly comparable with that of ketoconazole, whereas its potency lagged behind that of abiraterone. Oxadiazole **15a**, the saturated counterpart of **14a**, however, proved to be a poorer enzyme inhibitor, which demonstrates the importance of the C16-17 double bond, as expected.

Table 1

The antiproliferative activities of all the synthesized compounds, including the *O*-acylamidoximes, against the HeLa, MCF7, A2780 and A431 cell lines, were determined by

the microplate-based MTT colorimetric assay [46], in comparison with cisplatin as reference agent. The cell-proliferation inhibitory potencies of the investigated derivatives, expressed as IC_{50} and/or growth inhibition values (Table 2), revealed that the *O*-acylamidoxime derivatives (**10a–e**, **11a–e**) exerted consistently higher activities than those of the corresponding oxadiazoles (**12a–e**, **13a–e**). Moreover, the presence of the C16-17 double bond in **11a–e** generally led to an increase in potency relative to the saturated analogs (**10a–e**). Among the Δ^{16} derivatives, 17-acyl-*p*-methoxybenzamidoxime (**11c**) and 17-acylacetamidoxime (**11e**) exhibited outstanding cytotoxicity against all four cell lines, with IC_{50} values (at least in most cases) lower than those of cisplatin. The introduction of the various substituents R into the *O*-acylamidoxime residue had a greater effect on the antiproliferative action in **11a–e** than in **10a–e**. As concerns the oxadiazoles, the 3β-acetoxy derivatives (**12a–e**, **13a–e**) induced negligible cell-growth inhibition, irrespectively of the structure of their sterane skeleton, while their 3β-OH counterparts, especially those containing a saturated ring D (**14a–e**), proved to be more potent.

Table 2

3. Conclusions

In summary, novel types of 1,2,4-oxadiazoles in the androst-5-ene series were prepared from steroidal 17-carboxylic acids via a two-step pathway. After the determination of acceptable parameters for efficient conversions, various amidoximes were *O*-acetylated with CDI-activated carboxylic acids, and then subjected to TBAF-induced cyclodehydration. Both the substitution and ring-closure reaction steps proceeded under mild reaction conditions, to afford the *O*-acylamidoximes and then the 17-*exo*-heterocycles in good to excellent yields. The synthesized derivatives may deserve attention from a pharmacological aspect, since one of the heteroaromatic derivatives, **15e**, displayed significant *in vitro* inhibition of $C_{17,20}$-lyase. In contrast with the expected results, not the oxadiazoles, but their open-chain precursors (**10a–e**, **11a–e**) exerted *in vitro* cytotoxic activity against all four examined malignant cell lines which were higher than or comparable to that of the reference cisplatin. The nature of the substituent R had only a limited impact on the anticancer action in both the open-chain (**10a–e**, **11a–e**) and the cyclized products (**14a–e**, **15a–e**); the effects of differences in the sterane structure, i. e. the saturation of ring D were more substantial.

4. Experimental

Melting points (mp) were determined on an SMS Optimelt digital apparatus. Elemental analysis data were obtained with a Perkin Elmer CHN analyzer model 2400. The reactions under MW irradiation were carried out with a CEM Corporation Focused Microwave System, Model Discover SP. NMR spectra were recorded at room temperature with a Bruker DRX 500 instrument. Chemical shifts are reported in ppm (δ scale), and coupling constants (J) in Hz. For the determination of multiplicities, the *J*-MOD pulse sequence was used. Automated flow injection analyses were performed by using an HPLC/MSD system. The system comprised an Agilent 1100 micro vacuum degasser, a quaternary pump, a micro-well plate autoinjector and a 1946A MSD equipped with an electrospray ion (ESI) source operated in positive ion mode. The ESI parameters were: nebulizing gas N_2, at 35 psi; drying gas N_2, at 350 °C and 12 L/min; capillary voltage (V_{Cap}) 3000 V; and fragmentor voltage 70 V. The MSD was operated in scan mode with the mass range *m/z* 60−620. Samples (0.2 μL) were injected with automated needle wash directly into the solvent flow (0.3 mL/min) of MeCN/H_2O 70:30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software. All solvents were distilled immediately prior to use. Reagents and materials were obtained from commercial suppliers and were used without purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss): (A) CH_2Cl_2, (B) EtOAc/CH_2Cl_2 (2:98 v/v), (C) EtOAc/CH_2Cl_2 (5:95 v/v), (D) EtOAc/CH_2Cl_2 (10:90 v/v), (E) EtOAc/CH_2Cl_2 (40:60 v/v).

The spots were detected by 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: Merck silica gel 60, 40–63 μm.

4.1. Chemistry

*4.1.1. Synthesis of oxadiazoles **12a** and **13a** from steroidal carboxylic acids via acid chlorides*

3β-Acetoxyandrost-5-ene-17β-carboxylic acid (**4**, 288 mg, 0.80 mmol) or 3β-acetoxyandrost-5,16-diene-17-carboxylic acid (**6**, 287 mg, 0.80 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and oxalyl chloride (0.5 mL, 5.82 mmol) was added dropwise at 0 °C with constant stirring. After addition of a capillary drop of DMF, the mixture was stirred overnight at room temperature. The excess of oxalyl chloride was distilled off following the addition of toluene (2 × 10 mL) to the solution. The acid chloride (**8** or **9**) prepared *in situ* was dissolved in pyridine (20 mL), and benzamidoxime (**IIa**, 327 mg, 3 equiv.) was then added. The solution was refluxed for 7 h (method A) or irradiated with MW at 150 °C for 30 min (method B), then poured into a mixture of ice and cc. H_2SO_4 (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by flash chromatography with hexane/CH_2Cl_2 = 20:80 as eluent to furnish **12a** (129 mg, 35% – method A; 155 mg, 42% – method B) or **13a** (139 mg, 38% – method A; 161 mg, 44% – method B) as a white solid. The physical and spectral characterization of **12a** and **13a** is to be found in Sections *4.1.3.1* and *4.1.3.6*.

*4.1.2. General procedure for the O-acylation of amidoximes (**IIa–e**)*

3β-Acetoxyandrost-5-ene-17 β-carboxylic acid (**4**, 288 mg, 0.80 mmol) or 3β-acetoxyandrost-5,16-diene-17-carboxylic acid (**6**, 287 mg, 0.80 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and CDI (195 mg, 1.2 mmol) was added. The solution was stirred for 2 h at room temperature until completion of the coupling reaction, and 3 equiv. of benzamidoxime (**IIa**), substituted benzamidoxime (**IIb–d**) or acetamidoxime (**IIe**) was then added. After stirring for 2 h at room temperature, the mixture was evaporated and subjected to flash chromatography.

*4.1.2.1. O-3β-Acetoxyandrost-5-ene-17β-acylbenzamidoxime (**10a**)*

Compound **4** and benzamidoxime (**IIa**, 327 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **10a** was obtained as a white solid (325 mg, 85%), mp 177–179 °C, R_f = 0.38 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.79 (s, 3H, 18-H_3), 1.01 (m, 1H), 1.02 (s, 3H, 19-H_3), 1.14 (m, 2H), 1.28–1.43 (overlapping m, 3H), 1.45–1.62 (overlapping m, 5H), 1.73 (m, 1H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.04 (m, 2H), 2.25 (m, 2H), 2.49 (t, 1H, J = 9.4 Hz, 17-H), 4.60 (m, 1H, 3-H), 5.06 (s, 2H, NH_2), 5.38 (m, 1H, 6-H), 7.39 (m, 2H, 3′-H and 5′-H), 7.46 (m, 1H, 4′-H), 7.71 (d, 2H, J = 8.1 Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.4 (C-18), 19.3 (C-19), 20.9 (CH_2), 21.4 (Ac-CH_3), 23.9 (CH_2), 24.6 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 31.9 (CH), 36.6 (C-10), 37.0 (CH_2), 38.0 (CH_2), 38.3 (CH_2), 44.1 (C-13), 50.0 (CH), 54.3 (CH), 56.2 (CH), 73.8 (C-3), 122.3 (C-6), 126.7 (2C, C-2′ and C-6′), 128.6 (2C, C-3′ and C-5′), 130.9 (C-4′), 131.2 (C-1′), 139.7 (C-5), 156.1 (C-21), 170.5 (Ac-CO), 170.7 (C-20); ESI-MS 480 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{38}N_2O_4$ C 72.77; H 8.00. Found C 72.92; H 8.16.

*4.1.2.2. O-3β-Acetoxyandrost-5-ene-17β-acyl-p-toluamidoxime (**10b**)*

Compound **4** and *p*-toluamidoxime (**IIb**, 360 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **10b** was obtained as a

white solid (331 mg, 84%), mp 183–185 °C, R_f = 0.40 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.80 (s, 3H, 18-H_3), 1.01 (m, 1H), 1.02 (s, 3H, 19-H_3), 1.14 (m, 2H), 1.28–1.42 (overlapping m, 3H), 1.45–1.62 (overlapping m, 5H), 1.73 (m, 1H), 1.88 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.05 (m, 2H), 2.26 (m, 2H), 2.37 (s, 3H, 4′-CH_3), 2.49 (t, 1H, J = 9.4 Hz, 17-H), 4.60 (m, 1H, 3-H), 5.01 (bs, 2H, NH_2), 5.38 (m, 1H, 6-H), 7.20 (d, 2H, J = 8.0 Hz, 3′-H and 5′-H), 7.60 (d, 2H, J = 8.0 Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.4 (C-18), 19.3 (C-19), 20.9 (CH_2), 21.4 (2C, Ac-CH_3 and 4′-CH_3), 23.9 (CH_2), 24.6 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.0 (CH), 36.6 (C-10), 37.0 (CH_2), 38.0 (CH_2), 38.3 (CH_2), 44.1 (C-13), 50.0 (CH), 54.4 (CH), 56.2 (CH), 73.8 (C-3), 122.3 (C-6), 126.5 (2C, C-2′ and C-6′), 128.3 (C-1′), 129.3 (2C, C-3′ and C-5′), 139.7 (C-5), 141.1 (C-4′), 156.0 (C-21), 170.5 (Ac-CO), 170.7 (C-20); ESI-MS 494 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{40}N_2O_4$ C 73.14; H 8.18. Found C 72.95; H 8.24.

*4.1.2.3. O-3β-Acetoxyandrost-5-ene-17β-acyl-p-methoxybenzamidoxime (**10c**)*

Compound **4** and *p*-methoxybenzamidoxime (**IIc**, 399 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **10c** was obtained as a white solid (342 mg, 84%), mp 178–180 °C, R_f = 0.37 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.79 (s, 3H, 18-H_3), 1.01 (m, 1H), 1.02 (s, 3H, 19-H_3), 1.14 (m, 2H), 1.28–1.42 (overlapping m, 3H), 1.44–1.62 (overlapping m, 5H), 1.73 (m, 1H), 1.87 (m, 3H), 2.02 (s, 3H, Ac-H_3), 2.05 (m, 2H), 2.25 (m, 2H), 2.48 (t, 1H, J = 9.4 Hz, 17-H), 3.82 (s, 3H, 4′-OMe), 4.60 (m, 1H, 3-H), 5.01 (bs, 2H, NH_2), 5.37 (m, 1H, 6-H), 6.89 (d, 2H, J = 8.5 Hz, 3′-H and 5′-H), 7.64 (d, 2H, J = 8.5 Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.4 (C-18), 19.3 (C-19), 20.9 (CH_2), 21.4 (Ac-CH_3), 23.9 (CH_2), 24.6 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 31.9 (CH), 36.6 (C-10), 37.0 (CH_2), 38.0 (CH_2), 38.3 (CH_2), 44.1 (C-13), 50.0 (CH), 54.4 (CH), 55.3 (4′-OMe), 56.2 (CH), 73.8 (C-3), 113.9 (2C, C-3′ and C-5′), 122.3 (C-6), 123.4 (C-1′), 128.1 (2C, C-2′ and C-6′), 139.7 (C-5), 155.8 (C-21), 161.7 (C-4′), 170.5 (Ac-

CO), 170.8 (C-20); ESI-MS 532 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{40}N_2O_5$ C 70.84; H 7.93. Found C 71.01; H 8.09.

*4.1.2.4. O-3β-Acetoxyandrost-5-ene-17β-acyl-p-bromobenzamidoxime (**10d**)*

Compound **4** and *p*-bromobenzamidoxime (**IId**, 516 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 2:98 as eluent, **10d** was obtained as a white solid (384 mg, 86%), mp 165–168 °C, R_f = 0.43 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.79 (s, 3H, 18-H_3), 1.01 (m, 1H), 1.02 (s, 3H, 19-H_3), 1.14 (m, 2H), 1.29–1.41 (overlapping m, 3H), 1.44–1.62 (overlapping m, 5H), 1.74 (m, 1H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.05 (m, 2H), 2.24 (m, 2H), 2.48 (t, 1H, J = 9.3 Hz, 17-H), 4.59 (m, 1H, 3-H), 5.07 (bs, 2H, NH_2), 5.37 (m, 1H, 6-H), 7.53 (d, 2H, J = 8.5 Hz) and 7.58 (d, 2H, J = 8.5 Hz): 3′-H, 5′-H and 2′-H and 6′-H; ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.4 (C-18), 19.3 (C-19), 20.9 (CH_2), 21.4 (Ac-CH_3), 23.9 (CH_2), 24.6 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 31.9 (CH), 36.6 (C-10), 37.0 (CH_2), 38.0 (CH_2), 38.3 (CH_2), 44.2 (C-13), 50.0 (CH), 54.3 (CH), 56.2 (CH), 73.8 (C-3), 122.3 (C-6), 125.3 (C-4′), 128.2 (2C, C-2′ and C-6′), 130.1 (C-1′), 131.8 (2C, C-3′ and C-5′), 139.7 (C-5), 155.2 (C-21), 170.5 (Ac-CO), 170.7 (C-20); ESI-MS 559 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{37}BrN_2O_4$ C 62.48; H 6.69. Found C 62.62; H 6.58.

*4.1.2.5. O-3β-Acetoxyandrost-5-ene-17β-acylacetamidoxime (**10e**)*

Compound **4** and acetamidoxime (**IIe**, 179 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 20:80 as eluent, **10e** was obtained as a white solid (290 mg, 87%), mp 192–194 °C, R_f = 0.30 (ss E); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.74 (s, 3H, 18-H_3), 0.98 (m, 1H), 1.00 (s, 3H, 19-H_3), 1.11 (m, 2H), 1.25–1.34 (overlapping m, 3H), 1.41–1.58 (overlapping m, 5H), 1.70 (m, 1H), 1.83 (m, 4H), 1.95 (s, 3H, CH_3), 1.97

(m, 2H), 2.01 (s, 3H, Ac-H_3), 2.19 (m, 1H), 2.39 (t, 1H, J = 9.4 Hz, 17-H), 4.57 (m, 1H, 3-H), 4.80 (bs, 2H, NH_2), 5.36 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 17.0 (CH_3), 19.2 (C-19), 20.9 (CH_2), 21.4 (Ac-CH_3), 23.8 (CH_2), 24.5 (CH_2), 27.7 (CH_2), 31.7 (CH_2), 31.9 (CH), 36.6 (C-10), 36.9 (CH_2), 38.0 (CH_2), 38.3 (CH_2), 44.0 (C-13), 49.9 (CH), 54.2 (CH), 56.1 (CH), 73.8 (C-3), 122.2 (C-6), 139.7 (C-5), 154.8 (C-21), 170.5 (Ac-CO), 170.7 (C-20); ESI-MS 417 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{36}N_2O_4$ C 69.20; H 8.71. Found C 69.38; H 8.59.

*4.1.2.6. O-3β-Acetoxyandrost-5,16-diene-17-acylbenzamidoxime (**11a**)*

Compound **6** and benzamidoxime (**IIa**, 327 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **11a** was obtained as a white solid (351 mg, 92%), mp 190–193 °C, R_f = 0.36 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.01 (s, 3H, 18-H_3), 1.06 (s, 3H, 19-H_3), 1.07 (m, 1H), 1.14 (m, 1H), 1.47–1.76 (overlapping m, 8H), 1.86 (m, 2H), 2.02 (s, 3H, Ac-H_3), 2.07 (m, 2H), 2.33 (m, 3H), 4.60 (m, 1H, 3-H), 5.11 (s, 2H, NH_2), 5.38 (m, 1H, 6-H), 6.84 (m, 1H, 16-H), 7.38 (m, 2H, 3′-H and 5′-H), 7.45 (m, 1H, 4′-H), 7.71 (m, 2H, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.1 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH), 31.4 (CH_2), 32.1 (CH_2), 34.8 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.2 (C-13), 50.3 (CH), 56.4 (CH), 73.8 (C-3), 122.0 (C-6), 126.7 (2C, C-2′ and C-6′), 128.6 (2C, C-3′ and C-5′), 130.8 (C-4′), 131.2 (C-1′), 140.1 (C-5), 143.4 (C-16), 145.6 (C-17), 156.5 (C-21), 162.1 (C-20), 170.5 (Ac-CO); ESI-MS 478 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{36}N_2O_4$ C 73.08; H 7.61. Found C 73.19; H 7.82.

*4.1.2.7. O-3β-Acetoxyandrost-5,16-diene-17-acyl-p-toluamidoxime (**11b**)*

Compound **6** and *p*-toluamidoxime (**IIb**, 360 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 2:98 as eluent, **11b** was obtained as a white solid (334 mg, 85%), mp 189–192 °C, R_f = 0.38 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.02 (s, 3H, 18-H_3), 1.07 (s, 3H, 19-H_3), 1.08 (m, 1H), 1.14 (m, 1H), 1.47–1.76 (overlapping m, 8H), 1.86 (m, 2H),2.03 (s, 3H, Ac-H_3), 2.06 (m, 2H), 2.33 (m, 3H), 2.37 (s, 3H, 4′-CH_3), 4.61 (m, 1H, 3-H), 5.04 (bs, 2H, NH_2), 5.39 (m, 1H, 6-H), 6.84 (m, 1H, 16-H), 7.20 (d, 2H, J = 8.1 Hz, 3′-H and 5′-H), 7.61 (d, 2H, J = 8.1 Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.1 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (4′-CH_3), 30.2 (CH), 31.4 (CH_2), 32.2 (CH_2), 34.8 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.2 (C-13), 50.4 (CH), 56.4 (CH), 62.1 (CH), 73.8 (C-3), 122.0 (C-6), 126.5 (2C, C-2′ and C-6′), 128.3 (C-1′), 129.3 (2C, C-3′ and C-5′), 140.2 (C-5), 141.1 (C-17), 143.3 (C-16), 145.7 (C-4′), 156.4 (C-21), 162.1 (C-20), 170.5 (Ac-CO); ESI-MS 492 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{38}N_2O_4$ C 73.44; H 7.81. Found C 72.31; H 7.87.

*4.1.2.8. O-3β-Acetoxyandrost-5,16-diene-17-acyl-p-methoxybenzamidoxime (**11c**)*

Compound **6** and *p*-methoxybenzamidoxime (**IIc**, 399 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **11c** was obtained as a white solid (357 mg, 88%), mp 195–197 °C, R_f = 0.32 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.01 (s, 3H, 18-H_3), 1.06 (s, 3H, 19-H_3), 1.08 (m, 1H), 1.13 (m, 1H), 1.47–1.75 (overlapping m, 8H), 1.86 (m, 2H), 2.02 (s, 3H, Ac-H_3), 2.05 (m, 2H), 2.33 (m, 3H), 3.81 (s, 3H, 4′-OMe), 4.60 (m, 1H, 3-H), 5.05 (bs, 2H, NH_2), 5.38 (m, 1H, 6-H), 6.83 (bs, 1H, 16-H), 6.88 (d, 2H, J = 8.5 Hz, 3′-H and 5′-H), 7.66 (d, 2H, J = 8.5 Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.1 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH), 31.4 (CH_2), 32.1 (CH_2), 34.8 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.2 (C-13), 50.4 (CH), 55.3 (4′-OMe), 56.4 (CH), 73.8 (C-3), 113.9 (2C, C-3′ and C-

5′), 122.0 (C-6), 123.4 (C-1′), 128.1 (2C, C-2′ and C-6′), 140.1 (C-5), 143.3 (C-16), 145.7 (C-17), 156.3 (C-21), 161.7 (C-4′), 162.2 (C-20), 170.5 (Ac-CO); ESI-MS 530 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{38}N_2O_5$ C 71.12; H 7.56. Found C 71.26; H 7.40.

*4.1.2.9. O-3β-Acetoxyandrost-5,16-diene-17-acyl-p-bromobenzamidoxime (**11d**)*

Compound **6** and *p*-bromobenzamidoxime (**IId**, 516 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 5:95 as eluent, **11d** was obtained as a white solid (369 mg, 83%), mp 206–209 °C, R_f = 0.47 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.02 (s, 3H, 18-H_3), 1.07 (s, 3H, 19-H_3), 1.08 (m, 1H), 1.15 (m, 1H), 1.47–1.76 (overlapping m, 8H), 1.87 (m, 2H), 2.03 (s, 3H, Ac-H_3), 2.06 (m, 2H), 2.33 (m, 3H), 4.61 (m, 1H, 3-H), 5.08 (bs, 2H, NH_2), 5.39 (m, 1H, 6-H), 6.85 (bs, 1H, 16-H), 7.53 (d, 2H, J = 8.4 Hz) and 7.60 (d, 2H, J = 8.4 Hz): 2′-H, 6′-H and 3′-H, 5′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.1 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH), 31.4 (CH_2), 32.2 (CH_2), 34.8 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.2 (C-13), 50.4 (CH), 56.4 (CH), 73.8 (C-3), 122.0 (C-6), 125.4 (C-4′), 128.2 (2C, C-2′ and C-6′), 130.1 (C-1′), 131.9 (2C, C-3′ and C-5′), 140.2 (C-5), 143.7 (C-16), 145.5 (C-17), 155.5 (C-21), 162.0 (C-20), 170.5 (Ac-CO); ESI-MS 557 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{35}BrN_2O_4$ C 62.70; H 6.35. Found C 62.84; H 6.54.

*4.1.2.10. O-3β-Acetoxyandrost-5,16-diene-17-acylacetamidoxime (**11e**)*

Compound **6** and acetamidoxime (**IIe**, 179 mg) were used in the procedure described in section 4.1.2. After purification with EtOAc/CH_2Cl_2 = 30:70 as eluent, **11e** was obtained as a white solid (292 mg, 88%), mp 205–208 °C, R_f = 0.32 (ss E); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.97 (s, 3H, 18-H_3), 1.02 (m, 1H), 1.04 (s, 3H, 19-H_3), 1.12 (m, 1H), 1.39–1.50 (overlapping

m, 2H), 1.53–1.72 (overlapping m, 5H), 1.84 (m, 2H), 1.98 (s, 3H, CH_3), 2.00 (m, 2H), 2.02 (s, 3H, Ac-H_3), 2.29 (m, 4H), 4.58 (m, 1H, 3-H), 4.81 (bs, 2H, NH_2), 5.37 (m, 1H, 6-H), 6.78 (m, 1H, 16-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.1 (C-18), 17.0 (CH_3), 19.2 (C-19), 20.6 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH), 31.4 (CH_2), 32.1 (CH_2), 34.8 (CH_2), 36.7 (C-10), 36.8 (CH_2), 38.1 (CH_2), 46.0 (C-13), 50.3 (CH), 56.4 (CH), 73.8 (C-3), 122.0 (C-6), 140.1 (C-17), 143.3 (C-16), 145.7 (C-5), 155.2 (C-21), 162.2 (C-20), 170.5 (Ac-CO); ESI-MS 415 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{34}N_2O_4$ C 69.54; H 8.27. Found C 69.66; H 8.47.

*4.1.3. General procedure for the cyclocondensation of O-acylamidoximes (**10a–e**, **11a–e**)*

O-Acylamidoxime (**10a–e** or **11a–e**, 0.50 mmol) was dissolved in dry THF (5 mL) and TBAF (1M solution in THF, 0.05 mL) was added under a N_2 atmosphere. The solution was stirred for 1 h at room temperature, and then poured into water (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by flash chromatography.

*4.1.3.1. 3β-Acetoxy-17β-(5'-[3'-phenyl]-1',2',4'-oxadiazolyl)androst-5-ene (**12a**)*

10a (239 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with CH_2Cl_2 as eluent, **12a** was obtained as a white solid (214 mg, 93%), mp 131–134 °C, R_f = 0.40 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.65 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 1.05 (m, 1H), 1.17 (m, 1H), 1.28 (m, 1H), 1.40–1.66 (overlapping m, 7H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.08 (m, 2H), 2.19 (m, 1H), 2.33 (m, 2H), 2.46 (m, 1H), 2.96 (t, 1H, J = 9.5 Hz, 17-H), 4.61 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 7.47 (m, 3H, 3″-H, 4″-H and 5″-H), 8.09 (m, 2H, 2″-H and 6″-H), ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.3 (C-19),

20.8 (CH_2), 21.4 (Ac-CH_3), 24.7 (CH_2), 25.3 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.7 (C-10), 37.0 (CH_2), 37.6 (CH_2), 38.1 (CH_2), 45.3 (C-13), 48.5 (C-17), 49.9 (CH), 56.0 (CH), 73.8 (C-3), 122.2 (C-6), 127.2 (C-1″), 127.4 (2C, C-2″ and C-6″), 128.7 (2C, C-3″ and C-5″), 130.9 (C-4″), 139.8 (C-5), 167.9 (C-3′), 170.5 (Ac-CO), 180.9 (C-5′); ESI-MS 462 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{36}N_2O_3$ C 75.62; H 7.88. Found C 75.78; H 8.02.

*4.1.3.2. 3β-Acetoxy-17β-(5'-[3'-4''-tolyl]-1',2',4'-oxadiazolyl)androst-5-ene (**12b**)*

10b (246 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with CH_2Cl_2 as eluent, **12b** was obtained as a white solid (218 mg, 92%), mp 182–183 °C, R_f = 0.41 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 1.05 (m, 1H), 1.16 (m, 1H), 1.27 (m, 1H), 1.40–1.66 (overlapping m, 7H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.08 (m, 2H), 2.18 (m, 1H), 2.33 (m, 2H), 2.40 (s, 3H, 4″-CH_3), 2.45 (m, 1H), 2.94 (t, 1H, J = 9.5 Hz, 17-H), 4.61 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 7.27 (d, 2H, J = 8.1 Hz, 3″-H and 5″-H), 7.97 (d, 2H, J = 8.1 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.3 (C-19), 20.8 (CH_2), 21.4 (Ac-CH_3), 21.5 (4″-CH_3), 24.7 (CH_2), 25.3 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.7 (C-10), 37.0 (CH_2), 37.6 (CH_2), 38.1 (CH_2), 45.2 (C-13), 48.5 (C-17), 49.9 (CH), 56.0 (CH), 73.8 (C-3), 122.2 (C-6), 124.3 (C-1″), 127.3 (2C, C-2″ and C-6″), 129.4 (2C, C-3″ and C-5″), 139.8 (C-5), 141.2 (C-4″), 168.0 (C-3′), 170.5 (Ac-CO), 180.7 (C-5′); ESI-MS 498 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{38}N_2O_3$ C 75.92; H 8.07. Found C 76.04; H 7.97.

*4.1.3.3. 3β-Acetoxy-17β-(5'-[3'-4''-methoxyphenyl]-1',2',4'-oxadiazolyl)androst-5-ene (**12c**)*

10c (254 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with hexane/CH_2Cl_2 = 20:80 as eluent, **12c** was obtained as a white solid (231 mg, 94%), mp 193–196 °C, R_f = 0.43 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H,

18-H_3), 1.02 (s, 3H, 19-H_3), 1.05 (m, 1H), 1.17 (m, 1H), 1.26 (m, 1H), 1.39–1.67 (overlapping m, 7H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.08 (m, 2H), 2.17 (m, 1H), 2.33 (m, 2H), 2.44 (m, 1H), 2.93 (t, 1H, *J* = 9.5 Hz, 17-H), 3.86 (s, 3H, 4″-OMe), 4.62 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 6.97 (d, 2H, *J* = 8.5 Hz, 3″-H and 5″-H), 8.02 (d, 2H, *J* = 8.5 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.2 (C-18), 19.3 (C-19), 20.8 (CH_2), 21.4 (Ac-CH_3), 24.6 (CH_2), 25.2 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.0 (CH), 36.7 (C-10), 37.0 (CH_2), 37.6 (CH_2), 38.1 (CH_2), 45.2 (C-13), 48.5 (C-17), 49.9 (CH), 55.3 (4″-OMe), 56.0 (CH), 73.8 (C-3), 114.1 (2C, C-3″ and C-5″), 119.7 (C-1″), 122.2 (C-6), 129.0 (2C, C-2″ and C-6″), 139.8 (C-5), 161.7 (C-4″), 167.6 (C-3′), 170.5 (Ac-CO), 180.6 (C-5′); ESI-MS 514 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{38}N_2O_4$ C 73.44; H 7.81. Found C 73.62; H 7.96.

*4.1.3.4. 3β-Acetoxy-17β-(5′-[3′-4″-bromophenyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**12d**)*

10d (279 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with hexane/CH_2Cl_2 = 20:80 as eluent, **12d** was obtained as a white solid (245 mg, 91%), mp 190–192 °C, R_f = 0.47 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 1.06 (m, 1H), 1.17 (m, 1H), 1.27 (m, 1H), 1.39–1.66 (overlapping m, 7H), 1.87 (m, 3H), 2.03 (s, 3H, Ac-H_3), 2.07 (m, 2H), 2.18 (m, 1H), 2.33 (m, 2H), 2.44 (m, 1H), 2.95 (t, 1H, *J* = 9.5 Hz, 17-H), 4.61 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 7.60 (d, 2H, *J* = 8.0 Hz, 2″-H and 6″-H), 7.96 (d, 2H, *J* = 8.0 Hz, 3″-H and 5″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.3 (C-19), 20.8 (CH_2), 21.4 (Ac-CH_3), 24.7 (CH_2), 25.3 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.7 (C-10), 37.0 (CH_2), 37.6 (CH_2), 38.1 (CH_2), 45.3 (C-13), 48.5 (C-17), 49.9 (CH), 56.0 (CH), 73.8 (C-3), 122.1 (C-6), 124.4 and 126.1: C-1″ and C-4″, 128.9 (2C, C-2″ and C-6″), 132.0 (2C, C-3″ and C-5″), 139.8 (C-5), 167.2 (C-3′), 170.5 (Ac-CO), 181.2 (C-5′); ESI-MS 541 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{35}BrN_2O_3$ C 64.56; H 6.54. Found C 64.74; H 6.71.

*4.1.3.5. 3β-Acetoxy-17β-(5′-[3′-methyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**12e**)*

10e (208 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with EtOAc/CH_2Cl_2 = 2:98 as eluent, **12e** was obtained as a white solid (185 mg, 93%), mp 163–166 °C, R_f = 0.43 (ss B); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.59 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.03 (m, 1H), 1.15 (m, 1H), 1.24 (m, 1H), 1.35–1.64 (overlapping m, 7H), 1.85 (m, 3H), 2.02 (s, 3H, Ac-H_3), 2.05 (m, 2H), 2.12 (m, 1H), 2.32 (m, 3H), 2.37 (s, 3H, 3′-CH_3), 2.86 (t, 1H, J = 9.5 Hz, 17-H), 4.60 (m, 1H, 3-H), 5.38 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 11.6 (3′-CH_3), 13.2 (C-18), 19.3 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 24.6 (CH_2), 25.2 (CH_2), 27.7 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.6 (C-10), 37.0 (CH_2), 37.6 (CH_2), 38.1 (CH_2), 45.1 (C-13), 48.4 (C-17), 49.9 (CH), 56.0 (CH), 73.8 (C-3), 122.1 (C-6), 139.8 (C-5), 166.6 (C-3′), 170.5 (Ac-CO), 180.6 (C-5′); ESI-MS 399 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{34}N_2O_3$ C 72.33; H 8.60. Found C 72.51; H 8.54.

*4.1.3.6. 3β-Acetoxy-17-(5′-[3′-phenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**13a**)*

11a (238 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with hexane/CH_2Cl_2 = 20:80 as eluent, **13a** was obtained as a white solid (216 mg, 94%), mp 168–170 °C , R_f = 0.47 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.08 (s, 3H) and 1.10 (s, 3H): 18-H_3 and 19-H_3, 1.15 (m, 2H), 1.51–1.81 (overlapping m, 7H), 1.89 (m, 2H), 2.04 (s, 3H, Ac-H_3), 2.08 (m, 1H), 2.18 (m, 1H), 2.35 (m, 2H), 2.43 (m, 1H), 2.57 (m, 1H), 4.62 (m, 1H, 3-H), 5.41 (m, 1H, 6-H), 6.98 (m, 1H, 16-H), 7.48 (m, 3H, 3″-H, 4″-H and 5″-H), 8.11 (m, 2H, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 32.6 (CH_2), 34.7 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.8 (C-13), 50.3 (CH), 56.6 (CH), 73.8 (C-3), 122.0 (C-6), 127.1 (C-1″), 127.4 (2C, C-2″ and C-6″), 128.7 (2C, C-3″ and C-5″), 130.9 (C-4″), 139.8

(C-5), 140.2 (C-17), 141.4 (C-16), 168.4 (C-3′), 170.5 (Ac-CO), 173.2 (C-5′); ESI-MS 481 $[M+Na]^+$; Anal. Calcd. for $C_{29}H_{34}N_2O_3$ C 75.95; H 7.47. Found C 76.08; H 7.65.

*4.1.3.7. 3β-Acetoxy-17-(5′-[3′-4″-tolyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**13b**)*

11b (245 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with EtOAc/CH_2Cl_2 = 2:98 as eluent, **13b** was obtained as a white solid (217 mg, 92%), mp 152–155 °C, R_f = 0.47 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.07 (s, 3H) and 1.10 (s, 3H): 18-H_3 and 19-H_3, 1.17 (m, 2H), 1.51–1.79 (overlapping m, 7H), 1.88 (m, 2H), 2.04 (s, 3H, Ac-H_3), 2.08 (m, 1H), 2.16 (m, 1H), 2.35 (m, 2H), 2.41 (s, 3H, 4″-CH_3), 2.43 (m, 1H), 2.56 (m, 1H), 4.62 (m, 1H, 3-H), 5.41 (m, 1H, 6-H), 6.96 (m, 1H, 16-H), 7.27 (d, 2H, J = 8.1 Hz, 3″-H and 5″-H), 7.99 (d, 2H, J = 8.1 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125MHz): δ_C 16.0 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 21.5 (4″-CH_3), 27.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 32.6 (CH_2), 34.7 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.8 (C-13), 50.3 (CH), 56.6 (CH), 73.8 (C-3), 122.0 (C-6), 124.4 (C-1″), 127.4 (2C, C-2″ and C-6″), 129.4 (2C, C-3″ and C-5″), 139.8 (C-5), 140.2 (C-17), 141.1 (C-4″), 141.2 (C-16), 168.4 (C-3′), 170.5 (Ac-CO), 173.0 (C-5′); ESI-MS 496 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{36}N_2O_3$ C 76.24; H 7.68. Found C 76.39; H 7.65.

*4.1.3.8. 3β-Acetoxy-17-(5′-[3′-4″-methoxyphenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**13c**)*

11c (253 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with CH_2Cl_2 as eluent, **13c** was obtained as a white solid (227 mg, 93%), mp 162–164 °C, R_f = 0.33 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.07 (s, 3H) and 1.10 (s, 3H):

18-H_3 and 19-H_3, 1.16 (m, 2H), 1.53–1.78 (overlapping m, 7H), 1.87 (m, 2H), 2.04 (s, 3H, Ac-H_3), 2.06 (m, 1H), 2.17 (m, 1H), 2.35 (m, 2H), 2.40 (m, 1H), 2.54 (m, 1H), 3.86 (s, 3H, 4″-OMe), 4.61 (m, 1H, 3-H), 5.41 (m, 1H, 6-H), 6.97 (m, 3H, 16-H, 3″-H and 5″-H), 8.04 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.2 (C-19), 20.7 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 32.6 (CH_2), 34.7 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.8 (C-13), 50.3 (CH), 55.3 (4″-OMe), 56.6 (CH), 73.8 (C-3), 114.1 (2C, C-3″ and C-5″), 119.7 (C-1″), 122.0 (C-6), 129.0 (2C, C-2″ and C-6″), 139.8 (C-5), 140.2 (C-17), 141.2 (C-16), 161.7 (C-3′), 168.1 (C-4″) 170.5 (Ac-CO), 173.4 (C-5′); ESI-MS 511 $[M+Na]^+$; Anal. Calcd. for $C_{30}H_{36}N_2O_4$ C 73.74; H 7.43. Found C 73.56; H 7.61.

*4.1.3.9. 3β-Acetoxy-17-(5′-[3′-4″-bromophenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**13d**)*

11d (278 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with CH_2Cl_2 as eluent, **13d** was obtained as a white solid (255 mg, 95%), mp 179–181 °C, R_f = 0.51 (ss A); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.07 (s, 3H) and 1.10 (s, 3H): 18-H_3 and 19-H_3, 1.17 (m, 2H), 1.50–1.80 (overlapping m, 7H), 1.89 (m, 2H), 2.04 (s, 3H, Ac-H_3), 2.08 (m, 1H), 2.17 (m, 1H), 2.35 (m, 2H), 2.42 (m, 1H), 2.54 (m, 1H), 4.62 (m, 1H, 3-H), 5.41 (m, 1H, 6-H), 6.98 (m, 1H, 16-H), 7.61 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H), 7.98 (d, 2H, J = 8.4 Hz, 3″-H and 5″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.2 (C-19), 20.6 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 32.7 (CH_2), 34.6 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.8 (C-13), 50.3 (CH), 56.6 (CH), 73.8 (C-3), 122.0 (C-6), 125.5 and 126.2: C-1″ and C-4″, 129.0 (2C, C-2″ and C-6″), 132.0 (2C, C-3″ and C-5″), 139.6 (C-5), 140.2 (C-17), 141.8 (C-16), 167.7 (C-3′), 170.5 (Ac-CO), 173.4 (C-5′); ESI-MS 538 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{33}BrN_2O_3$ C 64.80; H 6.19. Found C 64.98; H 6.31.

*4.1.3.10. 3β-Acetoxy-17-(5′-[3′-4″-methyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**13e**)*

11e (207 mg) was used for the reaction in the procedure described in section 4.1.3. After purification with CH_2Cl_2 as eluent, **13e** was obtained as a white solid (180 mg, 91%), mp 92–94 °C, R_f = 0.49 (ss B); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.02 (s, 3H) and 1.07 (s, 3H): 18-H_3 and 19-H_3, 1.15 (m, 2H), 1.45–1.78 (overlapping m, 7H), 1.87 (m, 2H), 2.03 (s, 3H, Ac-H_3), 2.05 (m, 1H), 2.13 (m, 1H), 2.34 (m, 2H), 2.39 (s, 3H, 3′-CH_3), 2.42 (m, 2H), 4.61 (m, 1H, 3-H), 5.39 (m, 1H, 6-H), 6.88 (m, 1H, 16-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 11.7 (3′-CH_3), 16.0 (C-18), 19.2 (C-19), 20.6 (CH_2), 21.4 (Ac-CH_3), 27.7 (CH_2), 30.2 (CH), 31.4 (CH_2), 32.6 (CH_2), 34.6 (CH_2), 36.8 (C-10), 36.9 (CH_2), 38.1 (CH_2), 46.7 (C-13), 50.3 (CH), 56.6 (CH), 73.8 (C-3), 122.0 (C-6), 139.7 (C-17), 140.2 (C-5), 141.2 (C-16), 167.1 (C-3′), 170.5 (Ac-CO), 173.0 (C-5′); ESI-MS 397 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{32}N_2O_3$ C 72.70; H 8.13. Found C 72.84; H 8.21.

*4.1.4. General procedure for the synthesis of oxadiazoles **14a–e** and **15a–e***

Compound **12a**–**e** or **13a–e** (0.30 mmol) was dissolved in MeOH (10 mL), and KOH (50 mg, 0.89 mmol) was added. The solution was stirred at room temperature for 8 h, diluted with water and neutralized with dilute HCl. The precipitate that formed was filtered off, washed with water and dried.

*4.1.4.1. 3β-Hydroxy-17β-(5′-[3′-phenyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**14a**)*

Substrate: **12a** (138 mg). **14a** was obtained as a white solid (116 mg, 92%), mp 184–186 °C, R_f = 0.34 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.65 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.03 (m, 1H), 1.12 (m, 1H), 1.27 (m, 1H), 1.40–1.70 (overlapping m, 7H), 1.85 (m, 3H), 2.08 (m, 2H), 2.13–2.33 (overlapping m, 3H), 2.46 (m, 1H), 2.95 (t, 1H, J = 9.5 Hz, 17-H), 3.53 (m, 1H, 3-H), 5.37 (m, 1H, 6-H), 7.47 (m, 3H, 3″-H, 4″-H and 5″-H), 8.09 (m, 2H, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.4 (C-19), 20.8 (CH_2), 24.7 (CH_2),

25.3 (CH_2), 31.6 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.6 (C-10), 37.3 (CH_2), 37.7 (CH_2), 42.2 (CH_2), 45.3 (C-13), 48.6 (C-17), 50.0 (CH), 56.1 (CH), 71.7 (C-3), 121.2 (C-6), 127.1 (C-1″), 127.4 (2C, C-2″ and C-6″), 128.7 (2C, C-3″ and C-5″), 130.9 (C-4″), 140.9 (C-5), 167.9 (C-3′), 180.9 (C-5′); ESI-MS 419 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{34}N_2O_2$ C 77.48; H 8.19. Found C 77.60; H 8.25.

*4.1.4.2. 3β-Hydroxy-17β-(5′-[3′-4″-tolyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**14b**)*

Substrate: **12b** (142 mg). **14b** was obtained as a white solid (121 mg, 93%), mp 162–164 °C, R_f = 0.33 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 1.03 (m, 1H), 1.11 (m, 1H), 1.27 (m, 1H), 1.39–1.69 (overlapping m, 7H), 1.85 (m, 3H), 2.08 (m, 2H), 2.13–2.33 (overlapping m, 3H), 2.40 (s, 3H, 4″-CH_3), 2.44 (m, 1H), 2.94 (t, 1H, J = 9.5 Hz, 17-H), 3.53 (m, 1H, 3-H), 5.37 (m, 1H, 6-H), 7.27 (d, 2H, J = 8.1 Hz, 3″-H and 5″-H), 7.97 (d, 2H, J = 8.1 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.4 (C-19), 20.8 (CH_2), 21.5 (4″-CH_3), 24.7 (CH_2), 25.3 (CH_2), 31.6 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.6 (C-10), 37.3 (CH_2), 37.7 (CH_2), 42.2 (CH_2), 45.3 (C-13), 48.6 (C-17), 50.0 (CH), 56.1 (CH), 71.7 (C-3), 121.2 (C-6), 124.3 (C-1″), 127.3 (2C, C-2″ and C-6″), 129.4 (2C, C-3″ and C-5″), 140.9 and 141.2: C-5 and C-4″, 167.9 (C-3′), 180.7 (C-5′); ESI-MS 433 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{36}N_2O_2$ C 77.74; H 8.39. Found C 77.86; H 8.27.

*4.1.4.3. 3β-Hydroxy-17β-(5′-[3′-4″-methoxyphenyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**14c**)*

Substrate: **12c** (147 mg). **14c** was obtained as a white solid (128 mg, 95%), mp 155–158 °C, R_f = 0.45 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 1.03 (m, 1H), 1.10 (m, 1H), 1.25 (m, 1H), 1.40–1.66 (overlapping m, 7H), 1.85 (m, 3H), 2.03–2.45 (overlapping m, 6H), 2.92 (t, 1H, J = 9.5 Hz, 17-H), 3.53 (m, 1H, 3-H), 3.85 (s, 3H, 4″-OMe), 5.36 (m, 1H, 6-H), 6.97 (d, 2H, J = 8.5 Hz, 3″-H and 5″-H), 8.02 (d, 2H, J =

8.5 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.2 (C-18), 19.4 (C-19), 20.8 (CH_2), 24.7 (CH_2), 25.2 (CH_2), 31.6 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.6 (C-10), 37.2 (CH_2), 37.7 (CH_2), 42.2 (CH_2), 45.2 (C-13), 48.5 (C-17), 50.0 (CH), 55.3 (4″-OMe), 56.1 (CH), 71.7 (C-3), 114.1 (2C, C-3″ and C-5″), 119.6 (C-1″), 121.2 (C-6), 129.0 (2C, C-2″ and C-6″), 140.9 (C-5), 161.7 (C-4″), 167.6 (C-3′), 180.6 (C-5′); ESI-MS 449 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{36}N_2O_3$ C 74.97; H 8.09. Found C 75.14; H 8.24.

*4.1.4.4. 3β-Hydroxy-17β-(5′-[3′-4″-bromophenyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**14d**)*

Substrate: **12d** (162 mg). **14d** was obtained as a white solid (140 mg, 94%), mp 172–174 °C, R_f = 0.39 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.64 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 1.03 (m, 1H), 1.11 (m, 1H), 1.25 (m, 1H), 1.39–1.68 (overlapping m, 7H), 1.85 (m, 3H), 2.07 (m, 2H), 2.13–2.32 (overlapping m, 3H), 2.44 (m, 1H), 2.94 (t, 1H, J = 9.5 Hz, 17-H), 3.53 (m, 1H, 3-H), 5.36 (m, 1H, 6-H), 7.60 (d, 2H, J = 8.0 Hz, 2″-H and 6″-H), 7.96 (d, 2H, J = 8.0 Hz, 3″-H and 5″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 13.3 (C-18), 19.4 (C-19), 20.8 (CH_2), 24.7 (CH_2), 25.3 (CH_2), 31.6 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.6 (C-10), 37.3 (CH_2), 37.7 (CH_2), 42.2 (CH_2), 45.3 (C-13), 48.5 (C-17), 50.0 (CH), 56.1 (CH), 71.7 (C-3), 121.2 (C-6), 125.4 and 126.1: C-1″ and C-4″, 128.9 (2C, C-2″ and C-6″), 132.0 (2C, C-3″ and C-5″), 140.9 (C-5), 167.2 (C-3′), 181.2 (C-5′); ESI-MS 261; Anal. Calcd. for $C_{27}H_{33}BrN_2O_2$ C 65.19; H 6.69. Found C 65.31; H 6.85.

*4.1.4.5. 3β-Hydroxy-17β-(5′-[3′-methyl]-1′,2′,4′-oxadiazolyl)androst-5-ene (**14e**)*

Substrate: **12e** (120 mg). **14e** was obtained as a white solid (93 mg, 87%), mp 152–155 °C, R_f = 0.32 (ss D); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 0.58 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 1.02 (m, 1H), 1.22 (m, 1H), 1.24 (m, 1H), 1.32–1.83 (overlapping m, 10H), 2.02 (m, 2H), 2.10 (m, 1H), 2.29 (m, 3H), 2.37 (s, 3H, 3′-CH_3), 2.85 (t, 1H, J = 9.5 Hz, 17-H), 3.51 (m, 1H, 3-H),

5.35 (m, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 11.6 (3′-CH_3), 13.2 (C-18), 19.4 (C-19), 20.8 (CH_2), 24.6 (CH_2), 25.2 (CH_2), 31.6 (CH_2), 31.8 (CH_2), 32.1 (CH), 36.5 (C-10), 37.2 (CH_2), 37.6 (CH_2), 42.2 (CH_2), 45.2 (C-13), 48.4 (C-17), 50.0 (CH), 56.1 (CH), 71.6 (C-3), 121.2 (C-6), 140.9 (C-5), 166.6 (C-3′), 180.6 (C-5′); ESI-MS 357 $[M+H]^+$ 356.50; Anal. Calcd. for $C_{22}H_{32}N_2O_2$ C 74.12; H 9.05. Found C 74.26; H 8.93.

*4.1.4.6. 3β-Hydroxy-17-(5′-[3′-phenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**15a**)*

Substrate: **13a** (138 mg). **15a** was obtained as a white solid (117 mg, 94%), mp 182–185 °C, R_f = 0.33 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.09 (s, 6H): 18-H_3 and 19-H_3, 1.11 (m, 2H), 1.48–1.87 (overlapping m, 9H), 2.07 (m, 1H), 2.18 (m, 1H), 2.26 (m, 1H), 2.33 (m, 1H), 2.43 (m, 1H), 2.56 (m, 1H), 3.54 (m, 1H, 3-H), 5.38 (m, 1H, 6-H), 6.98 (m, 1H, 16-H), 7.48 (m, 3H, 3″-H, 4″-H and 5″-H), 8.11 (m, 2H, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.3 (C-19), 20.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 31.6 (CH_2), 32.7 (CH_2), 34.7 (CH_2), 36.7 (C-10), 37.1 (CH_2), 42.3 (CH_2), 46.8 (C-13), 50.4 (CH), 56.7 (CH), 71.7 (C-3), 121.0 (C-6), 127.2 (C-1″), 127.4 (2C, C-2″ and C-6″), 128.7 (2C, C-3″ and C-5″), 130.9 (C-4″), 139.8 (C-5), 141.3 (C-17), 141.5 (C-16), 168.4 (C-3′), 173.2 (C-5′); ESI-MS 417 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{32}N_2O_2$ C 77.85; H 7.74. Found C 78.01; H 7.86.

*4.1.4.7. 3β-Hydroxy-17-(5′-[3′-4″-tolyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**15b**)*

Substrate: **13b** (142 mg). **15b** was obtained as a white solid (120 mg, 93%), mp 186–188 °C, R_f = 0.34 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_C 1.08 (s, 3H) and 1.09 (s, 3H): 18-H_3 and 19-H_3, 1.11 (m, 2H), 1.48–1.88 (overlapping m, 9H), 2.07 (m, 1H), 2.18 (m, 1H), 2.26 (m, 1H), 2.33 (m, 1H), 2.41 (s, 3H, 4″-CH_3), 2.43 (m, 1H), 2.56 (m, 1H), 3.54 (m, 1H, 3-H), 5.38 (m, 1H, 6-H), 6.97 (m, 1H, 16-H), 7.28 (d, 2H, J = 8.1 Hz, 3″-H and 5″-H), 8.00 (d, 2H, J =

8.1 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.3 (C-19), 20.7 (CH_2), 21.5 (4″-CH_3), 30.3 (CH), 31.5 (CH_2), 31.6 (CH_2), 32.6 (CH_2), 34.7 (CH_2), 36.7 (C-10), 37.1 (CH_2), 42.3 (CH_2), 46.8 (C-13), 50.4 (CH), 56.7 (CH), 71.7 (C-3), 121.0 (C-6), 124.4 (C-1″), 127.4 (2C, C-2″ and C-6″), 129.4 (2C, C-3″ and C-5″), 139.8 (C-5), 141.2 (2C, C-17 and C-4″), 141.3 (C-16), 168.4 (C-3′), 173.0 (C-5′); ESI-MS 431 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{34}N_2O_2$ C 78.10; H 7.96. Found C 77.92; H 8.14.

4.1.4.8. 3β-Hydroxy-17-(5′-[3′-4″-methoxyphenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene ***(15c)***

Substrate: **13c** (147 mg). **15c** was obtained as a white solid (127 mg, 95%), mp 172–174 °C, R_f = 0.39 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.07 (s, 3H) and 1.08 (s, 3H): 18-H_3 and 19-H_3, 1.11 (m, 2H), 1.51–1.87 (overlapping m, 9H), 2.06 (m, 1H), 2.17 (m, 1H), 2.28–2.33 (m, 2H), 2.42 (m, 1H), 2.55 (m, 1H), 3.54 (m, 1H, 3-H), 3.86 (s, 3H, 4″-OMe), 5.37 (m, 1H, 6-H), 6.97 (m, 3H, 16-H, 3″-H and 5″-H), 8.04 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.3 (C-19), 20.7 (CH_2), 30.3 (CH), 31.5 (CH_2), 31.6 (CH_2), 32.6 (CH_2), 34.7 (CH_2), 36.7 (C-10), 37.1 (CH_2), 42.3 (CH_2), 46.8 (C-13), 50.4 (CH), 55.3 (4″-OMe), 56.7 (CH), 71.6 (C-3), 114.1 (2C, C-3″ and C-5″), 119.7 (C-1″), 121.0 (C-6), 129.0 (2C, C-2″ and C-6″), 139.8 (C-5), 141.2 (C-16), 141.3 (C-17), 161.7 (C-3′), 168.1 (C-4″), 173.4 (C-5′); ESI-MS 448 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{34}N_2O_3$ C 75.31; H 7.67. Found C 75.56; H 7.77.

4.1.4.9. 3β-Hydroxy-17-(5′-[3′-4″-bromophenyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene ***(15d)***

Substrate: **13d** (161 mg). **15d** was obtained as a white solid (138 mg, 93%), mp 176–179 °C, R_f = 0.37 (ss C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.07 (s, 3H) and 1.08 (s, 3H): 18-H_3 and

19-H_3, 1.11 (m, 2H), 1.49–1.87 (overlapping m, 9H), 2.06 (m, 1H), 2.18 (m, 1H), 2.24–2.34 (m, 2H), 2.43 (m, 1H), 2.54 (m, 1H), 3.54 (m, 1H, 3-H), 5.37 (m, 1H, 6-H), 6.98 (m, 1H, 16-H), 7.61 (d, 2H, J = 8.4 Hz, 2″-H and 6″-H), 7.98 (d, 2H, J = 8.4 Hz, 3″-H and 5″-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.0 (C-18), 19.3 (C-19), 20.7 (CH_2), 30.3 (CH), 31.4 (CH_2), 31.6 (CH_2), 32.7 (CH_2), 34.7 (CH_2), 36.7 (C-10), 37.1 (CH_2), 42.3 (CH_2), 46.8 (C-13), 50.4 (CH), 56.6 (CH), 71.6 (C-3), 121.0 (C-6), 125.5 and 126.1: C-1″ and C-4″, 128.9 (2C, C-2″ and C-6″), 132.0 (2C, C-3″ and C-5″), 139.6 (C-5), 141.3 (C-17), 141.8 (C-16), 167.7 (C-3′), 173.4 (C-5′); ESI-MS 261; Anal. Calcd. for $C_{27}H_{31}BrN_2O_2$ C 65.45; H 6.31. Found C 65.63; H 6.49.

*4.1.4.10. 3β-Hydroxy-17-(5′-[3′-methyl]-1′,2′,4′-oxadiazolyl)androst-5,16-diene (**15e**)*

Substrate: **13e** (119 mg). **15e** was obtained as a white solid (95 mg, 89%), mp 141–143 °C, R_f = 0.31 (ss D); ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 1.02 (s, 3H) and 1.06 (s, 3H): 18-H_3 and 19-H_3, 1.10 (m, 2H), 1.44–1.77 (overlapping m, 7H), 1.86 (m, 2H), 2.05 (m, 1H), 2.14 (m, 1H), 2.25 (m, 1H), 2.31 (m, 1H), 2.39 (s, 3H, 3′-CH_3), 2.42 (m, 2H), 3.52 (m, 1H, 3-H), 5.37 (m, 1H, 6-H), 6.88 (m, 1H, 16-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 11.7 (3′-CH_3), 16.0 (C-18), 19.3 (C-19), 20.6 (CH_2), 30.3 (CH), 31.2 (CH_2), 31.6 (CH_2), 32.6 (CH_2), 34.6 (CH_2), 36.7 (C-10), 37.1 (CH_2), 42.2 (CH_2), 46.7 (C-13), 50.4 (CH), 56.7 (CH), 71.6 (C-3), 121.0 (C-6), 141.2 (2C, C-5 and C-17), 141.3 (C-16), 167.1 (C-3′), 173.0 (C-5′). ESI-MS 355 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{30}N_2O_2$ C 74.54; H 8.53. Found C 74.72; H 8.59.

4.2. Pharmacology

4.2.1. Determination of $C_{17,20}$-lyase activity and its inhibition in the rat testis

The extents of the inhibitory effects exerted on the activity of $C_{17,20}$-lyase by the newly synthesized oxadiazolyl steroids (**14a–e**, **15a–e**) were determined via an *in vitro* radiosubstrate incubation method described earlier [21, 43]. In brief, adult Wistar rat testicular tissue was homogenized with an Ultra-Turrax in 0.1 M HEPES buffer (pH = 7.3) containing 1 mM EDTA and 1 mM dithiotreitol. Aliquots of this homogenate were incubated in 200 μL final volume at 37 °C for 20 min in the presence of 0.1 mM NADPH. 1 μM [^{3}H]17-hydroxyprogesterone was added to the incubate in 20 μL of a 25 v/v% propylene glycol solution. Test compounds were applied at 50 μM and introduced in 10 μL of DMSO. (These organic solvent contents did not reduce the enzyme activity substantially.) Control incubates without test substances, and incubates with the reference compound ketoconazole and abiraterone were also prepared in every series. Following incubation, the androst-4-ene-3,17-dione formed and the 17-hydroxyprogesterone remaining were isolated through extraction and TLC. $C_{17,20}$-lyase activity was calculated from the radioactivity of the androst-4-ene-3,17-dione obtained. At least two experiments were performed with each test compound and the standard deviations of the mean enzyme activity results were within ± 10%. IC_{50} values were determined for the more active inhibitors. In this case, the conversion was measured at 5 or 6 different concentrations of the test compound. IC_{50} results were calculated by linear regression analysis following a logit-log transformation of the data, and the standard deviations were determined from the fitted lines.

4.2.2. Determination of antiproliferative effects

Human cancer cell lines (Hela, MCF-7, A2780 and A431, isolated from cervical adenocarcinoma, breast adenocarcinoma, ovarian cancer and skin epidermoid carcinoma, respectively) were maintained in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential aminoacids and an antibiotic–antimycotic mixture. All cell lines were purchased from the European Collection of Cell Cultures (Salisbury, UK) and grown in a humidified atmosphere of 5% CO_2 at 37 °C. For pharmacological investigations, 10 mM stock solutions of the tested compounds were prepared with dimethyl sulfoxide. The highest applied dimethyl sulfoxide concentration of the medium (0.3%) did not have any substantial effect on the proliferation of the cells. All the chemicals, if otherwise not specified, were purchased from Sigma–Aldrich Ltd. (Budapest, Hungary). Cells were seeded onto 96-well plates at a density of 5000 cells/well and allowed to stand overnight, after which the medium containing the tested compound was added. After a 72-h incubation, viability was determined by the addition of 20 μL of MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) solution (5 mg/mL). The precipitated formazan crystals were solubilized in dimethyl sulfoxide and the absorbance was determined at 545 nm with an ELISA reader [45]. Two independent experiments were performed with 5 parallel wells; cisplatin, an agent administered clinically in the treatment of certain gynecological malignancies, was used as a positive control. All tested agents were screened at 10 and 30 μM first and when the lower concentration resulted in higher than 50% growth inhibition, and therefore an IC_{50} value around 10 μM could be expected on any of the cell lines, the assay

was repeated with a set of dilutions. Sigmoidal dose–response curves were fitted to the measured data. Calculations of IC_{50} values were performed by means of GraphPad Prism 4.0 (GraphPad Software; San Diego, CA, USA).

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Supplementary data

Supplementary data related to this article can be found at

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Legends for Figures and Schemes

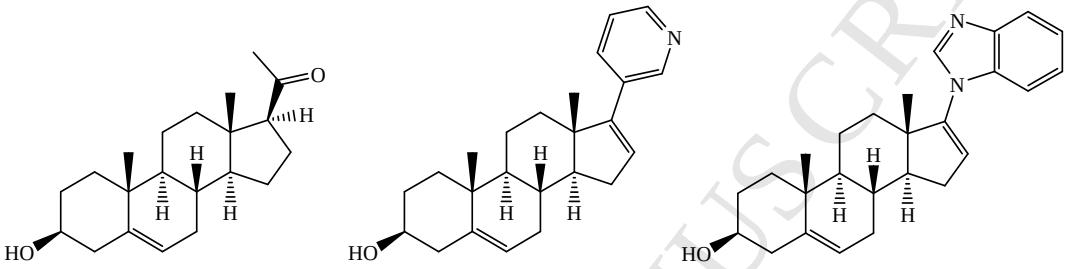
Figure 1. The natural substrate and two effective synthetic inhibitors of $P450_{17\alpha}$

Scheme 1. Synthesis of 17-carboxylic acids and an ester in the androstane series.

Reagents and conditions: (*i*) Br_2, NaOH, H_2O, dioxane, 0 °C → rt, 3 h then Na_2SO_3, reflux, 15 min; (*ii*) Ac_2O, pyridine, 8 h, rt; (*iii*) $BF_3{\cdot}OEt_2$, MeOH, 65 °C, 3 h.

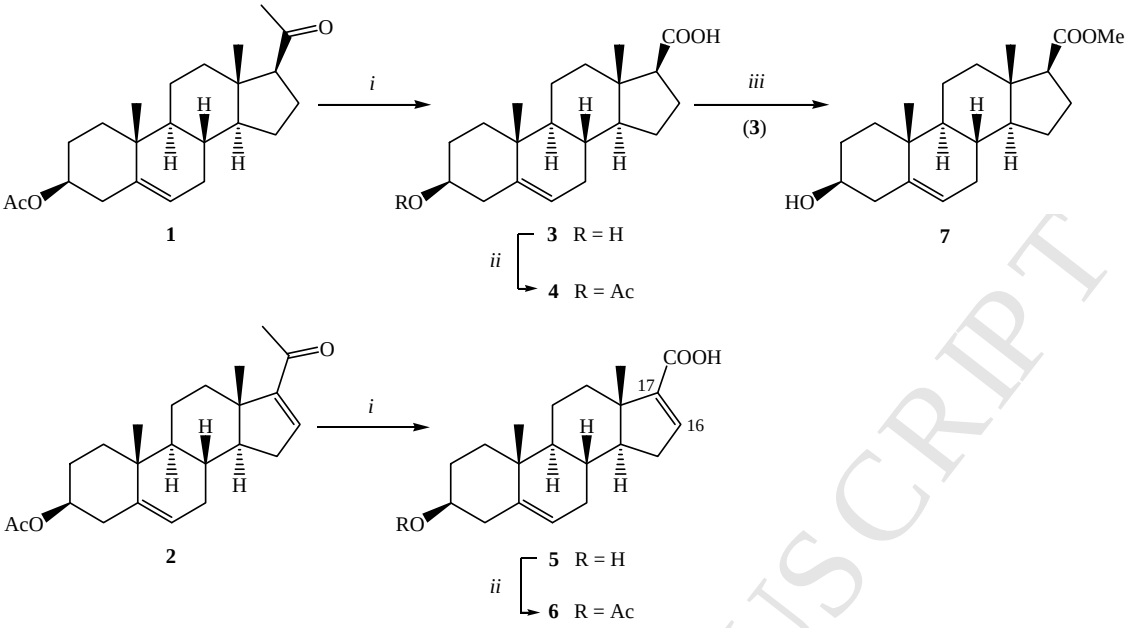
Scheme 2. Synthesis of steroidal 17-5′-(1′,2′,4′)-oxadiazoles

Reagents and conditions: (*i*) $NH_2OH{\cdot}HCl$, NaOH, EtOH, H_2O, reflux for 2-7 h or MW, 110 °C, 200 W, 15-20 min; (*ii*) oxalyl chloride, cat. DMF, CH_2Cl_2, 12 h, 0 °C → rt; (*iii*) pyridine, **IIa**, 115 °C for 7 h (method A) or MW, 150 °C, 250 W, 30 min (method B); (*iv*) CDI, CH_2Cl_2, rt, 2 h, then **IIa–e**, rt, 2 h; (*v*) TBAF, THF, N_2 atm, rt, 1 h; (*vi*) KOH, MeOH, rt, 8 h.

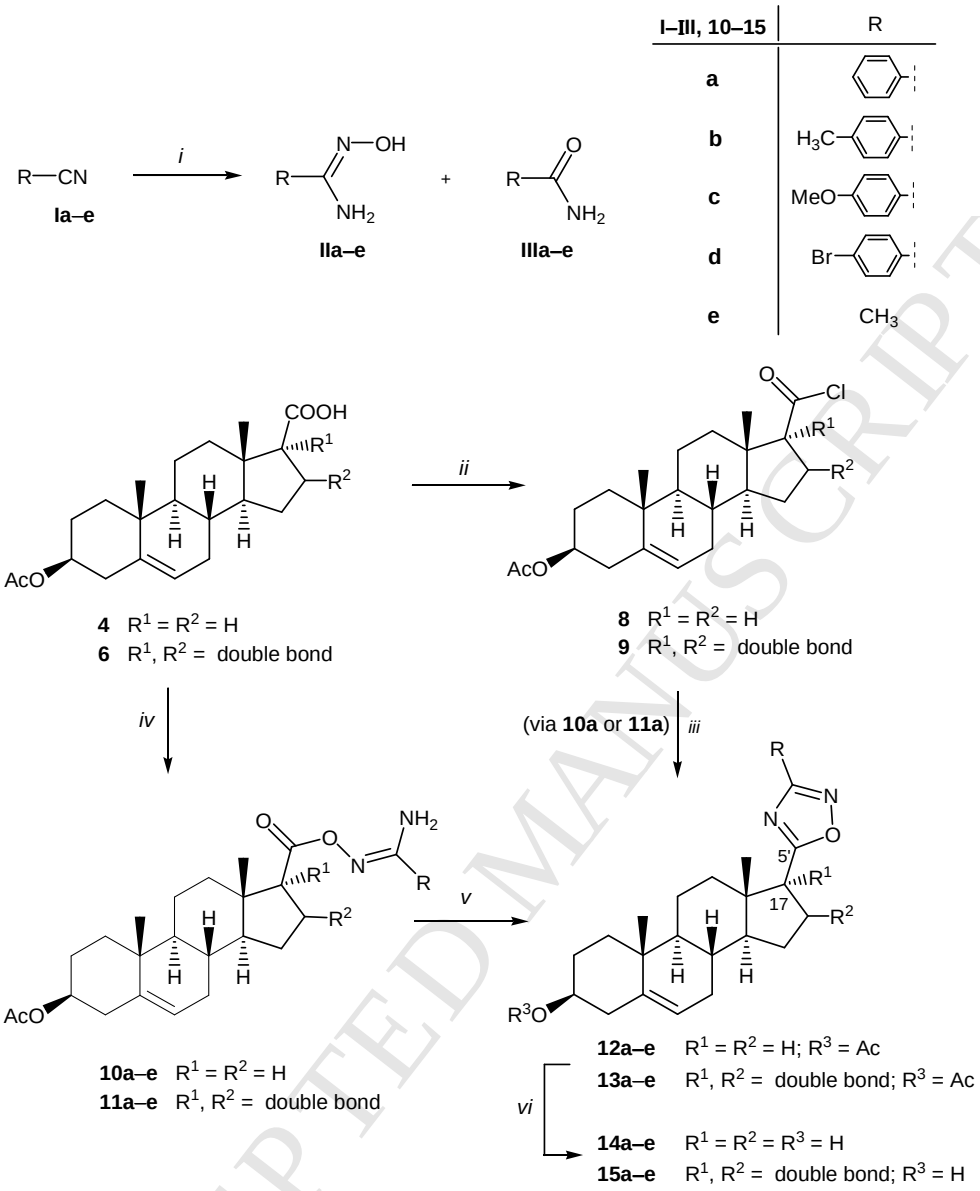


pregnenolone abiraterone galeterone

Figure 1.

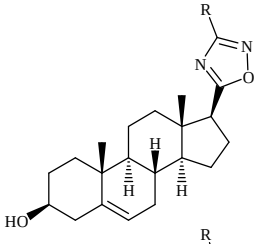
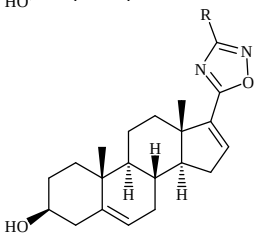


Scheme 1.



Scheme 2.

Table 1. Inhibition of rat testicular $C_{17,20}$-lyase by steroidal oxadiazoles

	Compd	R	Relative conversion[a] (%)	IC_{50} (μM)
	**14a**	Ph	n.i.[b]	-
	14b	*p*-CH_3-C_6H_4	84±4	-
	14c	*p*-OMe-C_6H_4	n.i.	-
	14d	*p*-Br-C_6H_4	n.i.	-
	14e	CH_3	30±4	-
	**15a**	Ph	n.i.	-
	15b	*p*-CH_3-C_6H_4	n.i.	-
	15c	*p*-OMe-C_6H_4	n.i.	-
	15d	*p*-Br-C_6H_4	87±4	-
	15e	CH_3	-	0.60±0.19
	KTZ[c]	-	-	0.32±0.02
	ABT[c]	-	-	0.0125±0.0015

[a]measured in the presence of 50 μM of the compound tested; control incubation with no inhibition is taken as 100%

[b]no inhibition

[c]KTZ: ketoconazole; ABT: abiraterone (reference compounds)

Table 2. Cytotoxic activity of *O*-acylamidoximes and oxadiazoles in the androstene series

Compd	Con. μM	HeLa		MCF7		A2780		A431	
		Inhibition % (± SEM)	IC_{50}[a] (μM)	Inhibition % (± SEM)	IC_{50} (μM)	Inhibition % (± SEM)	IC_{50} (μM)	Inhibition % (± SEM)	IC_{50} (μM)
10a	10	74.8 (± 1.4)	8.84	42.1 (± 1.6)	11.06	95.9 (± 0.3)	2.20	16.4 (± 0.4)	13.94
	30	94.3 (± 0.4)		78.8 (± 1.1)		96.2 (± 0.5)		65.7 (± 0.6)	
10b	10	61.5 (± 1.1)	8.22	43.7 (± 1.4)	12.12	95.8 (± 0.2)	2.47	16.5 (± 1.1)	>30
	30	89.7 (± 1.0)		76.0 (± 0.6)		96.1 (± 0.3)		43.1 (± 1.0)	
10c	10	67.9 (± 2.7)	7.75	46.8 (± 2.2)	10.26	95.9 (± 0.2)	1.32	44.6 (± 2.4)	10.77
	30	93.8 (± 0.6)		82.0 (± 0.5)		95.8 (± 0.5)		83.0 (± 1.9)	
10d	10	66.6 (± 0.9)	8.07	65.1 (± 1.6)	9.39	91.2 (± 1.9)	2.76	55.1 (± 2.0)	8.40
	30	90.0 (± 0.8)		83.6 (± 1.1)		95.6 (± 0.3)		62.9 (± 0.6)	
10e	10	96.3 (± 0.2)	6.00	49.0 (± 1.0)	10.23	96.8 (± 0.2)	0.34	44.7 (± 1.6)	10.98
	30	96.3 (± 0.3)		78.6 (± 0.7)		96.7 (± 0.1)		88.7 (± 0.9)	
11a	10	95.2 (± 0.2)	5.56	80.5 (± 1.1)	4.74	95.6 (± 0.2)	0.69	70.0 (± 2.7)	5.27
	30	95.8 (± 0.2)		91.3 (± 0.8)		95.8 (± 0.2)		79.3 (± 2.5)	
11b	10	60.3 (± 0.8)	8.75	58.3 (± 0.8)	5.60	71.9 (± 1.2)	2.80	56.7 (± 0.4)	7.90
	30	61.2 (± 0.3)		65.8 (± 0.7)		86.4 (± 0.9)		63.7 (± 0.6)	
11c	10	95.5 (± 0.3)	3.86	83.6 (± 0.5)	3.52	96.1 (± 0.2)	0.95	83.5 (± 1.5)	1.78
	30	96.1 (± 0.2)		84.1 (± 0.6)		95.9 (± 0.1)		83.0 (± 0.7)	
11d	10	73.2 (± 0.5)	7.17	77.2 (± 0.5)	6.00	95.6 (± 0.4)	2.30	54.8 (± 0.8)	8.71
	30	95.7 (± 0.1)		92.5 (± 0.8)		96.5 (± 0.2)		72.1 (± 1.0)	
11e	10	96.4 (± 0.3)	3.22	62.0 (± 1.9)	3.94	95.6 (± 0.3)	0.22	66.0 (± 1.0)	3.71
	30	96.0 (± 0.3)		89.3 (± 0.7)		95.2 (± 0.2)		93.3 (± 0.4)	
12a	10	<20[c]	n.d.[b]	<20	n.d.	<20	n.d.	40.3 (± 1.0)	n.d.
	30	49.0 (± 0.5)		43.2 (± 1.3)		44.9 (± 2.2)		59.1 (± 0.4)	
12b	10	<20	n.d.	<20	n.d.	<20	n.d.	<20	n.d.
	30	<20		<20		<20		<20	
12c	10	<20	n.d.	<20	n.d.	28.2 (± 2.7)	n.d.	<20	n.d.
	30	44.7 (± 1.2)		43.2 (± 0.9)		52.8 (± 2.1)		25.6 (± 1.5)	
12d	10	<20	n.d.	<20	n.d.	<20	n.d.	<20	n.d.
	30	<20		<20		20.4 ± 2.0		<20	
12e	10	31.6 (± 1.8)	n.d.	20.0 (± 0.9)	n.d.	34.3 (± 0.8)	n.d.	<20	n.d.
	30	52.7 (± 1.4)		30.6 (± 1.4)		53.0 (± 1.0)		28.1 (± 2.8)	
13a	10	<20	n.d.	28.3 (± 1.4)	n.d.	<20	n.d.	<20	n.d.
	30	33.9 (± 0.5)		39.0 (± 1.0)		25.7 (± 1.7)		20.3 (± 2.0)	
13b	10	27.7 (± 1.0)	n.d.	<20	n.d.	<20	n.d.	<20	n.d.
	30	20.7 (± 1.1)		<20		23.1 (± 1.4)		<20	
13c	10	35.0 (± 1.2)	n.d.	<20	n.d.	33.8 (± 2.2)	n.d.	<20	n.d.
	30	52.9 (± 1.3)		33.0 (± 0.8)		57.7 (± 0.9)		29.9 (± 0.9)	
13d	10	<20	n.d.	<20	n.d.	<20	n.d.	<20	n.d.
	30	<20		<20		<20		<20	
13e	10	<20	n.d.	<20	n.d.	32.6 (± 1.5)	n.d.	<20	n.d.
	30	34.4 (± 2.5)		57.5 (± 2.8)		80.4 (± 0.4)		<20	
14a	10	70.8 (± 0.6)	4.82	40.5 (± 1.1)	>30	30.7 (± 1.5)	>30	74.0 (± 0.2)	4.52
	30	72.7 (± 0.8)		43.8 (± 2.6)		35.5 (± 2.4)		76.3 (± 0.4)	
14b	10	70.1 (± 0.6)	5.04	39.6 (± 1.8)	>30	<20	>30	73.6 (± 0.2)	4.64
	30	71.9 (± 0.4)		38.3 (± 1.6)		38.0 (± 2.6)		74.0 (± 0.3)	
14c	10	72.7 (± 0.4)	2.24	61.5 (± 1.8)	5.65	37.0 (± 0.8)	>30	74.5 (± 0.2)	2.17
	30	79.2 (± 0.4)		63.0 (± 1.4)		44.4 (± 0.6)		79.6 (± 0.2)	
14d	10	63.4 (± 0.9)	6.58	43.8 (± 1.2)	>30	<20	>30	71.3 (± 0.4)	5.76
	30	65.7 (± 0.2)		49.5 (± 1.9)		<20		73.3 (± 0.5)	
14e	10	23.2 (± 1.4)	n.d.	26.8 (± 2.4)	n.d.	35.3 (± 0.9)	n.d.	42.6 (± 2.2)	n.d.
	30	78.7 (± 1.0)		77.7 (± 1.0)		59.7 (± 1.3)		78.4 (± 0.8)	
15a	10	23.4 (± 1.9)	n.d.	37.1 (± 1.5)	n.d.	<20	n.d.	<20	n.d.
	30	73.7 (± 0.3)		81.1 (± 0.6)		78.8 (± 1.0)		94.7 (± 0.4)	
15b	10	20.8 (± 1.5)	n.d.	28.8 (± 2.1)	n.d.	<20	n.d.	33.3 (± 1.3)	n.d.
	30	53.7 (± 0.5)		63.0 (± 2.0)		49.9 (± 1.8)		80.5 (± 2.2)	
15c	10	63.6 (± 0.7)	7.94	59.4 (± 1.4)	8.60	42.5 (± 1.3)	13.24	63.9 (± 1.0)	7.72
	30	87.1 (± 0.9)		85.5 (± 0.4)		60.7 (± 0.9)		88.2 (± 1.6)	
15d	10	37.4 (± 1.6)	n.d.	42.5 (± 2.6)	n.d.	39.9 (± 1.6)	n.d.	45.6 (± 1.2)	n.d.

15e	30	91.9 (± 0.4)	n.d.	92.3 (± 1.1)	n.d.	77.0 (± 0.9)	n.d.	93.7 (± 0.4)	n.d.
	10	<20		23.5 (± 2.5)		21.2 (± 0.9)		28.3 (± 0.8)	
	30	29.6 (± 0.7)		49.4 (± 2.5)		41.1 (± 1.8)		25.8 (± 1.2)	
CP[d]	10	42.6 (± 2.3)	12.43	53.0 (± 2.3)	9.63	83.6 (± 1.2)	1.30	88.6 (± 0.5)	2.84
	30	99.9 (± 0.3)		86.9 (± 1.3)		95.0 (± 0.3)		90.2 (± 1.8)	

[a] IC_{50} values were determined when the tested compound elicited at least 50% growth inhibition at 10 μM against any of the cell lines used. The presented values are from two independent determinations with five parallel wells; standard deviation < 15%.

[b] n.d.: not determined.

[c] inhibition values < 20% are not presented.

[d] cisplatin (reference compound).

Supplementary Material for

An efficient approach to novel 17-5′-(1′,2′,4′)-oxadiazolyl androstenes via the cyclodehydration of cytotoxic *O*-steroidacylamidoximes, and an evaluation of their inhibitory action on 17α-hydroxylase/$C_{17,20}$-lyase

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