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Efficient access to novel androsteno-17-(1′,3′,4′)-oxadiazoles and 17β-(1′,3′,4′)-thiadiazoles *via* *N*-substituted hydrazone and *N*,*N*′-disubstituted hydrazine intermediates, and their pharmacological evaluation *in vitro*

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ABSTRACT

A series of novel 17-*exo*-oxadiazoles and -thiadiazoles in the Δ^5 androstene series were efficiently synthesized from pregnenolone acetate and pregnadienolone acetate *via* multistep pathways. 17β-(1′,3′,4′)-Oxadiazoles were obtained in high yields by the phenyliodonium diacetate-induced oxidative ring closure of semicarbazone and *N*-acylhydrazones derived from 3β-acetoxy- and 3β-hydroxyandrost-5-ene-17β-carbaldehydes. For the synthesis of analogous Δ^{16}-17-oxadiazolyl derivatives, *N*,*N*′-disubstituted hydrazine intermediates were prepared from 3β-acetoxyandrosta-5,16-diene-17-carboxylic acid, which then underwent cyclodehydration in the presence of $POCl_3$. The cyclization of steroidal *N*,*N*′-diacylhydrazines containing a saturated ring D with the Lawesson reagent afforded 17β-(1′,3′,4′)-thiadiazoles in good yields. Most of the products were subjected to deacetylation in basic media in order to enlarge the compound library available for pharmacological studies. All of these derivatives were screened *in vitro* for their antiproliferative effects against four malignant human adherent cell lines (HeLa, A2780, MCF7 and A431) by means of the MTT assay. The 3β-hydroxy derivatives of the newly-synthesized 17-*exo*-heterocycles were tested *in vitro* to investigate their inhibitory effects on rat testicular $C_{17,20}$-lyase. One of the 1,3,4-oxadiazolyl derivatives proved to exert noteworthy enzyme-inhibitory action, with an IC_{50} (0.065 μM) of the same order of magnitude as that of abiraterone.

1. Introduction

Thanks to the dual pharmacological importance of 17-*exo*-heterocyclic androstanes, research toward the development of such compounds is still at the focus of scientific interest. Some of these derivatives can be of great relevance as potential 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) inhibitors, while other analogs are known to exhibit direct antiproliferative effects on cancer cell lines of diverse origins by promoting a disturbance in the cell-cycle and the upregulation of apoptotic pathways.

$P450_{17\alpha}$, a key regulatory enzyme of androgen hormone biosynthesis possessing both 17α-hydroxylase and $C_{17,20}$-lyase activities, is responsible for the two-step conversion of pregnenolone to dehydroepiandrosterone and of progesterone to androstenedione in both the testes and the andrenal glands. Since the products of the first catalyzed reaction, *i.e.* the 17α-hydroxylation of the pregnane precursors, are common intermediates for the synthesis of androgens and glucocorticoids, it has been suggested that a clinically relevant inhibitor for androgen ablation should ideally inhibit only the $C_{17,20}$-lyase activity of the enzyme [1]. However, most of the steroidal inhibitors, such as abiraterone (17-[3-pyridyl] androsta-5,16-dien-3β-ol), which was approved in 2011 for the medication of prostate carcinoma, are not selective enough necessitating their application in combination with prednisone. Nevertheless, a direct indicator of the suitability of a compound for the treatment of androgen-dependent diseases is its ability to block the $C_{17,20}$-lyase activity of this metalloprotein, which is directly involved in the formation of androgens [1]. Considerable attention has therefore been focused in recent decades on the synthesis and

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pharmacological studies of semisynthetic steroidal 17-*exo*-heterocyclic compounds, and a number of derivatives with a triazolyl, tetrazolyl [2], pyrazolyl, imidazolyl, isoxazolyl [3], oxazolinyl [4], oxazolyl [5] or thiazolyl [5,6] ring at position C-17 have proved to be efficient inhibitors of $C_{17,20}$-lyase. It was demonstrated on the basis of structure–activity relationships that a potent inhibitor should possess certain structural features. They are all based on the structure of the natural substrates of $P450_{17\alpha}$, but the sterane core has been modified chemically, particularly by the introduction of five- or six-membered heterocycles containing oxygen, nitrogen or sulfur atoms with lone electron pairs at position C-17, in order to increase the ability of the molecules to coordinate with the heme iron at the active site of the enzyme. Moreover, the presence of a nitrogen atom at either position 3′ or 4′ relative to the nitrogen or carbon atom through which the heteroring is connected to the sterane skeleton and the presence of the 16,17-double bond seem to be important for potent inhibition. A hydroxy or keto function at C-3 can also alter the interaction by forming hydrogen-bonds with arginine, glutamine/glutamate or water within the protein binding pocket [1]. An important advance in this context was reported by DeVore et al., who clarified the binding mode of the substrate to the active site by X-ray studies on the complexes of abiraterone and TOK-001, another clinically-relevant steroidal inhibitor with human $P450_{17\alpha}$, respectively [7].

On the other hand, five-membered heterocycles containing two nitrogen and one oxygen or sulfur atoms have been reported to exhibit a broad spectrum of biological activities [8]; among them, 2,5-disubstituted 1,3,4-oxadiazoles are known to exhibit antimicrobial [9], anti-inflammatory [10], anticancer [11] and antiviral [12] activities. One of the best-known procedures for the preparation of 1,3,4-oxadiazoles involves the cyclodehydration of *N*,*N*′-diacylhydrazines in the presence of a dehydrating agent, such as phosphorus oxychloride [13], polyphosphoric acid [14], PPh_3/CX_4 (X = Cl, Br or I) [15] or ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) [16]. Otherwise, the 1,2-diacylhydrazine intermediates are also challenging structures in pharmacophore research, thanks to their anti-HIV [17] or fungicidal [18] properties. Nonsteroidal diacylhydrazines are also well-known insect growth regulators [19]. Another synthetic strategy for access to 1,3,4-oxadiazoles is the oxidative cyclization of aldehyde *N*-acylhydrazones with different oxidizing agents, *e.g.* ceric ammonium nitrate [20], potassium permanganate [21], tetravalent lead compounds [22], hypervalent iodine-containing reagents [23], and others. *N*-Acylhydrazones are also frequently applied as ligands in coordination chemistry for the formation of potentially bioactive transition metal complexes [24]. Compounds containing this moiety have been reported to exert pharmacological activities [25], such as antitumor [26] or antibacterial [27] effects.

Symmetrical and unsymmetrical 1,3,4-thiadiazoles are also of great interest from the aspect of drug development, since various compounds possessing this structural motif exhibit an antibacterial, antifungal, anti-inflammatory, anticancer, antiviral or anticonvulsant effect [28]. A widely applied method for construction of the 1,3,4-thiadiazole ring is the thionation of *N*,*N*′-diacylhydrazines with the Lawesson reagent (LR), followed by spontaneous cyclization and dehydrosulfurization [29]. This procedure has numerous advantages over other methods, *e.g.* mild reaction conditions, good yields, the lack of by-products, a readily handled thionation agent, *etc*.

Only a few examples have been published of compounds in which a 1,3,4-oxa- or 1,3,4-thiadiazole heteroring is attached to the sterane skeleton [30,31]. In view of the medicinal relevance of these structural building blocks, and the potential $C_{17,20}$-lyase-inhibitory effect of 17-*exo*-heterocyclic androstanes, and especially those containing smaller substituents on their heterorings, our present goal was to introduce such heterocycles at position 17 of the androst-5-ene and androsta-5,16-diene skeletons. Since some 17-*exo*-heterocyclic steroids [32,33], including analogous steroidal 17-(1′,2′,4′)- and 17-(1′,3′,4′)-oxadiazoles [34,35], have been reported to exhibit cytotoxic effects by inducing cell cycle blockade and apoptosis, it was planned to carry out not only inhibition tests on rat testicular $C_{17,20}$-lyase, but also *in vitro* antiproliferative assays on cancer cell lines of diverse origins. The nature of the substituent on the heteroring may have an impact on the anticancer action and may therefore reveal useful structure–activity relationships, although the detailed mechanisms of action and the exact target in these cases are not clear.

2. Results and discussion

2.1. Synthetic studies

The syntheses and antiproliferative properties of some 17β-1′,3′,4′-oxadiazoles in the androstane series have recently been reported [35]. The previous synthetic method involving the oxidative cyclization of *N*-acylhydrazones was extended to the preparation of further derivatives. The *N*-acylhydrazones **5a**–**c** and **6a**–**c** were obtained by the condensation of 3β-hydroxyandrost-5-ene-17β-carbaldehyde (**2**) and its 3-acetylated analog **3** with semicarbazide **4a** or acyl hydrazines **4b** or **4c**, respectively (Table 1). The reactions were carried out by both conventional heating (CH) and microwave (MW) irradiation under the experimental conditions described earlier [35]. The nature of the functional group at C-3 had a slight influence on the yields of the products, since **6a**–**c** were isolated in lower yields (entries 4–6) as compared with **5a**–**c** (entries 1–3) in consequence of the lower solubility of **3** than that of its 3β-hydroxy analog **2** in EtOH. Under MW conditions, the reaction times were reduced significantly presumably due to the higher temperature, while the yields could be improved to only a moderate degree. For the syntheses of **5a** and **6a**, NaOAc was used as base in order to liberate the semicarbazide **4a** from its hydrochloride salt. The hydrazones were considered to form as (*E*) isomers [35] and proved to be quite stable in the cases of **5a**, **5c**, **6a** and **6c**, while the methyl-substituted derivatives **5b** and **6b** were subjected to subsequent cyclization directly after purification in consequence of their lower stability.

During the phenyliodonium diacetate (PIDA)-induced oxidative ring closure [36] of **6a** in CH_2Cl_2 at room temperature, **8a** was isolated in only moderate yield in comparison with the similar reaction of **6c** to **8c**, which can be attributed to the more polar character and hence the lower solubility of **6a** in the applied solvent (Scheme 1). Although the acylhydrazone **5c** underwent similar cyclization in the presence of PIDA to afford **7c**, **5a** was transformed to undesirable products instead of undergoing conversion to **7a**. However, **7a** and **7c** could also be synthesized in high yields by the deacetylation of **8a** and **8c** in alkaline MeOH.

Surprisingly, the analogous oxidative cyclization of **5b** or **6b** led to two different products: the expected 1,3,4-oxadiazole **7b** or **8b** was produced in a yield of only about 20%, with the major formation of an open-chain *N*,*N*-diacetylated compound **9** or **10** (Scheme 2). Similar PIDA-induced *N*-acetylation of an aldehyde hydrazone was reported earlier through nitrilimine formation followed by 1,3-electrophilic addition to AcOH and subsequent rearrangement of an acyl group to afford *N*,*N*′-diacetylhydrazine [37,38]. The lower stability and the different behavior of **5b** and **6b** under oxidative conditions may be explained by the lack of the extended conjugation present in the amino- (**5a** and **6a**) and 4-pyridyl- (**5c** and **6c**) substituted derivatives. In order to determine the structure of **10**, the ^1H and ^{13}C NMR spectra of **6b**, **8b** and **10** were compared. In the ^1H NMR spectrum of **10**, the 20-H signal of the starting compound