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# Thiohydrazides in the Synthesis of Functionalized Extranuclear Heterosteroids †

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**Abstract:** Heterocyclic derivatives of hormones have attracted great interest as a privileged scaffold for drug discovery due to their outstanding biological activity. A number of them are potent anticancer agents which are used in the chemotherapy of breast and prostate cancers. Here, the data obtained by the authors in the field of studying functionalized thiohydrazides as simple "versatile agents" for the installation of heterocyclic moiety to the steroid core are summarized. Namely, a flexible synthetic approach to unknown pyrazolines, 1,3,4-thiadiazole, thiadiazine, and pyridazine derivatives of steroids with selective control of heterocyclization patterns are discussed. Steroidal 1,3,4-thiadiazoles were obtained via the oxidative heterocyclization of oxamic acid thiohydrazides with 16-hydroxymethylidene-$\Delta^{1,3,5(10)}$-estratrieno-17-one. An extension of this reaction to steroidal α,β-unsaturated ketones resulted in androst-5-ene-[17,16d]-pyrazolines. Spiro-androstene-17,6′[1′,3′,4′]thiadiazines were exclusively synthesized employing 16β,17β-epoxypregnenolone. Using 21-bromopregna-5,16-dien-20-one as a substrate, 17-[1′,3′,4′]thiadiazine-substituted androstenes were prepared. 18-Nor-5α-androsta-2,13-diene[3,2-d]pyridazines, androsta-2-ene[3,2-d]pyridazines and $\Delta^{1,3,5(10)}$-estratrieno[16,17-d]pyridazines were synthesized via two steps involving the Vilsmeier–Haack reaction of enolizable steroidal ketones, giving chlorovinyl aldehydes, followed by the imination of the former with oxamic acid thiohydrazides. The antiproliferative activity of the synthesized compounds against breast and prostate cancer cell lines, along with lead compounds' in-depth characterization, are included. The lead compounds were found to have potent selectivity and, in some cases, a significant effect on the signaling pathways in parental and 4-hydroxytamoxifen-resistant cells.

**Keywords:** steroids; heterocycles; thiohydrazides; anticancer activity



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## 1. Introduction

The heterocyclic derivatives of steroids have gained significant attention in pharmaceutical research as a promising framework for drug discovery [1,2]. For example, abiraterone, 17-pyridine-bearing androsta-5,16-dien-3-ol, is currently used in clinic for the treatment of advanced prostate cancers [3]. Synthetic androgen danazol, also known as isoxazolethisterone, is marked for the treatment of endometriosis and premenstrual syndrome [4]. Dutasteride, bearing 5,6-dihydro-2H-pyridin-2-one motif, is a potent 5α-reductase inhibitor used as a part of benign prostatic hyperplasia therapy [5]. The continually growing demand for the development of new therapeutic agents with improved selectivity and reduced side effects for the treatment of a variety of diseases requires the synthesis of heterocyclic derivatives of steroids with high levels of molecular diversity.

A number of excellent reviews on synthetic strategies directed towards the synthesis of heterosteroids have emerged in recent years [6,7]. Some of them focused on heterocycles [8], including fused-thiazoles [9], pyrazolines [10], bicyclic pyridines [11], and pyrimidines [12]. Other highlight the advances of steroid D-ring modification [13,14] or advances in the synthesis of steroidal conjugates and dimers [15–17]. The use of a click chemistry [18] and transition metal species [19] in the synthesis of heterosteroids has also been considered. Here, we summarize our achievements in the synthesis of heterocyclic derivatives of steroids based on oxamic acid thiohydrazides as polyheteroatom reagents. These reagents are readily available from α-chloroacetamides [20], and enable various five- and six-membered *N*,*O*- and *S*-heterocycles' construction [21–26]. The use of oxamic acid thiohydrazides as a reagent for the modification of steroids has never been systematically analyzed. The antiproliferative activity of the synthesized compounds against breast and prostate cancer cell lines, as well as a detailed characterization of the lead compounds, are included.

## 2. Results and Discussion

Our interest in the functionalized heterocyclic derivatives of steroids [27–33] resulted in based on oxamic acid thiohydrazides flexible strategy for the installation of a heterocyclic pendant at the steroid core, initially containing a carbonyl group (Figure 1). Intermediate hydrazones derived from oxamic acid thiohydrazides and steroids due to tautomerization and cis/trans isomerization can undergo oxidative aromatization, electrocyclic reactions, SH- and NH-nucleophilic cyclizations, as discussed below.

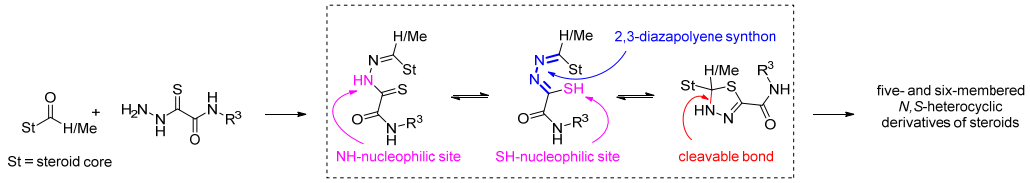
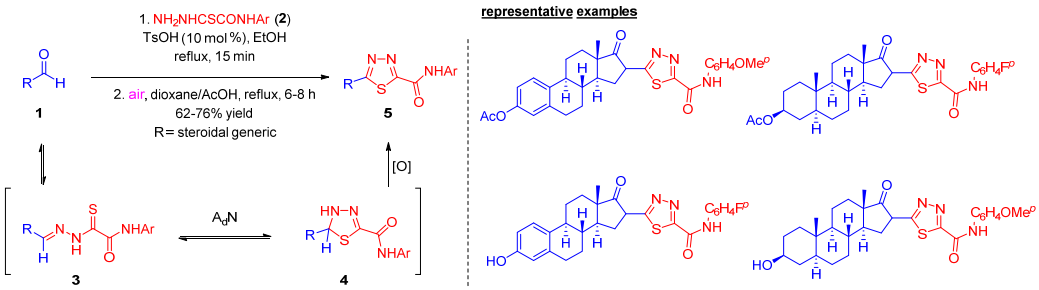



**Figure 1.** Reactivity of hydrazones derived from oxamic acid thiohydrazides and steroids.

### *2.1. Steroidal 1,3,4-thiadiazoles*

A frequently observed reaction of thiohydrazide-derived hydrazones when subjected to oxidative conditions is the occurrence of an intramolecular cyclization. This process involves the attack of a S-nucleophile on the electrophilic C=N bond, resulting in the formation of 1,3,4-thiadiazoles [24]. Having observed a seemingly general transformation pattern for hydrazones of oxamic acid thiohydrazides derived from steroids, we elaborated an efficient method for the synthesis of steroidal carbamoyl-1,3,4-thiadiazoles [34]. Reactions of thiohydrazides 2 with 16-hydroxymethylidene-5α-androstan-17-one and 16-hydroxymethylidene-$\Delta^{1,3,5(10)}$-estratrieno-17-one 1 provided the corresponding 1,3,4-thiadiazoles derivative 5 (Scheme 1).



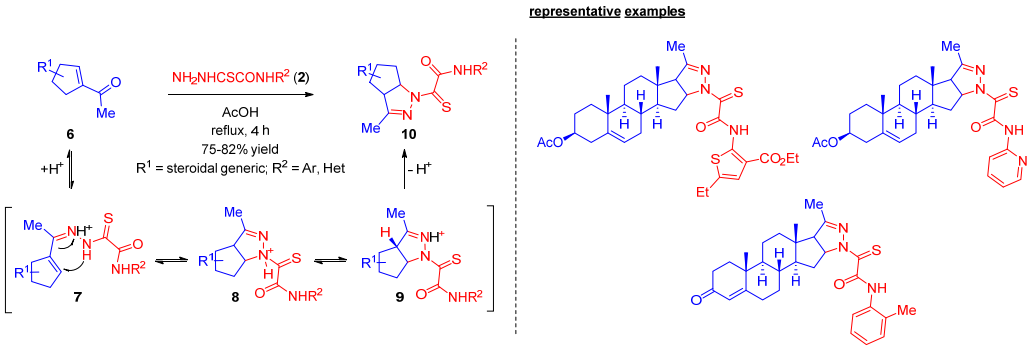

**Scheme 1.** Heterocyclization toward 1,3,4–thiadiazoles.

The reaction proceeds via an AdN-type cyclization of the thione isomer intermediate **3** to form cyclic thiadiazoline **4**, which subsequently undergoes aromatization (oxidative, if

X = H) leading to the formation of thiadiazoles **5**. The efficiency of air as an oxidant in the reaction involving glacial acetic acid has been observed.

### *2.2. Steroidal Pyrazolines*

This reaction can be further extended to steroidal α,β-unsaturated ketones **6**, resulting in the selective formation of androst-5-ene-[17,16d]-pyrazolines **10** (Scheme 2) [35–37]. The reaction conditions employed are comparable to those documented in the literature for the preparation of thiadiazoles [24] except for heating in pure glacial acetic acid. This slight alteration in circumstances promotes the development of pyrazolines **10**.

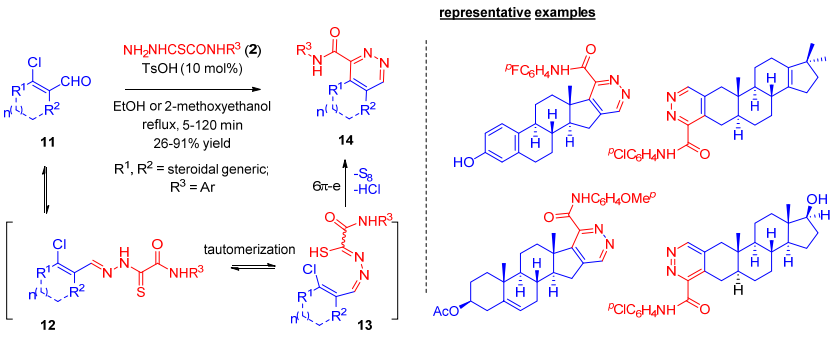



**Scheme 2.** Synthesis of androst–5–ene–[17,16d]–pyrazolines.

The proposed mechanism suggests that the initial step of the reaction involves the formation of hydrazones, as mentioned earlier. However, when these hydrazones are in their protonated form (referred to as form **7**), they undergo NH-nucleophilic addition to an activated double bond, leading to the synthesis of pyrazolines **8**. After the 1,3[H] shift and deprotonation of intermediates **9**, pyrazolines **10** are formed. The heterocyclization process was observed to be more favorable for oxamic acid thiohydrazides that have electron-donating substituents at the aryl group (yields ranged from 75 to 82%).

### *2.3. Steroidal Pyridazines*

In addition, it has been shown that the reaction of the oxamic acid thiohydrazides **2** with steroidal β-chlorovinyl aldehydes **11** under acidic conditions results in annulated steroidal pyridazines **14** (Scheme 3) [38]. The developed method was marked by significant efficiency in synthesizing derivatives of the androstene and estrane series, which incorporate pyridazine motifs fused to the A and D rings of the steroid core. The reaction is believed to occur via cascade imination/6π-electrocyclization of hydrazone thiol tautomers **13** to provide the pyridazines **14** [39].

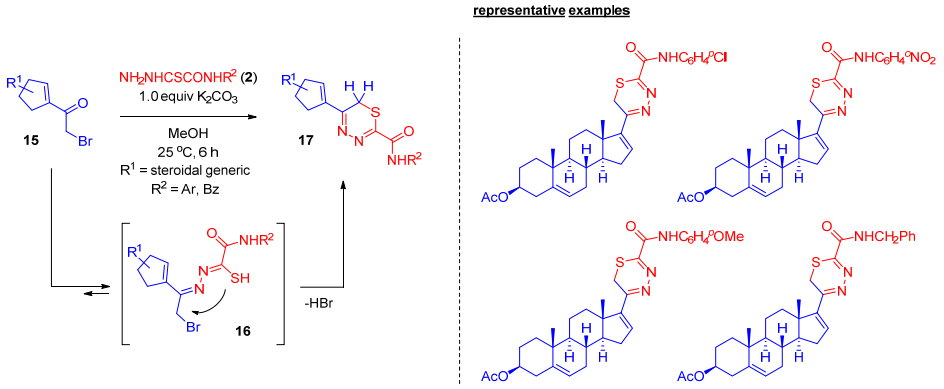



**Scheme 3.** Cyclization towards steroidal pyridazines.

### *2.4. Steroidal 1,3,4-thiadiazines*

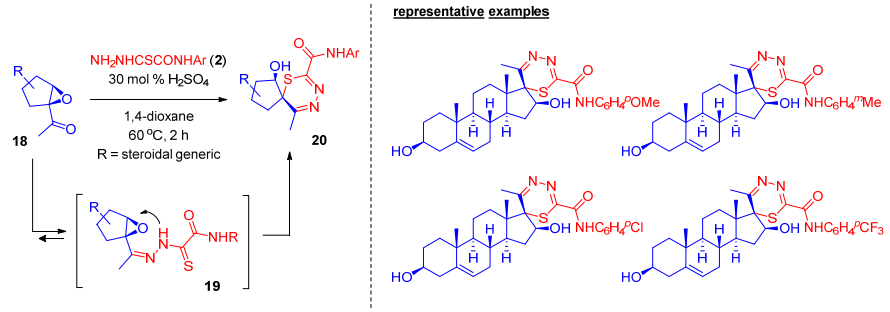
Reactions of oxamic acid thiohydrazides **2** with steroids bearing α-bromoketone moiety resulted in steroidal 1,3,4-thiadiazines with high chemoselectivity [40]. Namely,

the reaction of 21-bromopregna-5,16-dien-20-one **15** with oxamic acid thiohydrazides **2** under mild basic conditions affords 17-(6′*H*-1′,3′,4′-thiadiazine-2′-carboxamide)androst-5,17-dienes **17** in virtually quantitative yields (Scheme 4).




**Scheme 4.** Synthesis of 1′,3′,4′-thiadiazine androst-5,17-dienes.

The spiro-androstene-17,6′[1′,3′,4′]thiadiazines **20** were exclusively synthesized through the reaction of 16*β*,17*β*-epoxypregnenolone **18** with oxamic acid thiohydrazides **2** in the presence of sulfuric acid (Scheme 5) [40]. The relative spatial arrangement of the electrophilic reaction site and the carbonyl moiety in the steroid structure defines the reaction product. In both heterocyclizations, the key step is the nucleophilic attack of the hydrazone *SH*-group at electrophilic reaction sites at the steroid core (see intermediates **16** and **19**).




**Scheme 5.** Synthesis of 16*β*-hydroxyspiro-androsteno-17,6′[1,3,4]thiadiazines.

## *2.5. Antiproliferative Activity*

The above-described heterocyclic derivatives of steroids showed significant antiproliferative activity. Overall lead compounds **I** and **II**-pyridazines annulated with 17β-hydroxy-5α-androsta-2-ene at A-ring and 3β-acetoxyandrost-5-ene at D-ring exhibited selectivity against the hormone-dependent human breast cancer cell line, along with higher cytotoxicity than the cisplatin reference drug (Figure 2) [38].

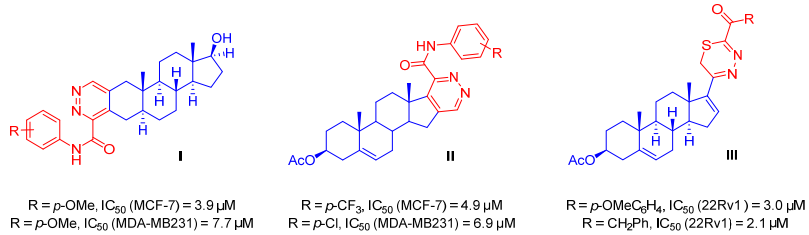



**Figure 2.** Cytotoxic effects of steroidal pyridazines and 1,3,4-thiadiazines.

17-[1′,3′,4′]Thiadiazine-substituted androstenes with general formula **III** (Figure 2) were lead compounds against human androgen receptor-positive prostate cancer cells

22Rv1 [40]. They showed micromolar values of $IC_{50}$ with antiproliferative potency higher than those for bicalutamide-reference drug. Compound **III**-induced 22Rv1 cell death was shown to be associated with the modulation of the AR, ERK 1/2, NF-κB, and PARP pathways. Thus, compounds **I–III** are of great interest for more in-depth study, and may be considered as a candidates for future anticancer drug design, in particular against hormone-receptor-positive breast and prostate cancers.

## 3. Conclusions

The development of synthetic methods based on simple intermediates leading to a variety of structurally diverse heterocyclic derivatives of natural products has been a focus for medicinal and organic chemists for years. The use of oxamic acid thiohydrazides as precursors for the formation of hydrazones from steroids has led to a range of heterocyclic derivatives of steroids in a highly chemoselective manner. Carbamoyl-substituted steroidal thiadiazoles, thiadiazines, pyrazolines, and pyridazines were obtained from readily available starting materials under mild conditions by tuning the hydrazones' reactivity profiles. Data on the biological activity of these compounds could enable the further exploration of the therapeutic potential of heterocyclic derivatives of steroids. First of all, this is relevant for developing novel and highly effective anticancer drugs for the treatment of breast and prostate cancers.

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