European Journal of Medicinal Chemistry 74 (2014) 451–460

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Probing the selective antitumor activity of 22-oxo-26-selenocyanocholestane derivatives

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ARTICLE INFO

Article history:
Received 13 July 2013
Received in revised form
29 December 2013
Accepted 30 December 2013
Available online 10 January 2014

Keywords:
22-Oxo-26-selenocyanocholestanes
Cervicouterine cancer cells
Antiproliferative activity
Cytotoxicity
Apoptosis

ABSTRACT

Diverse steroidal compounds have shown antiproliferative activity on certain tumor cell lines; however, their complete role on cancer cells has not been extensively established since the research is quite recent. Hence, deeper study in this field is required. Due to the importance of selenium in animal and human health; herein, we report the synthesis, characterization, and biological evaluation of two novel 22-oxo-26-selenocyanocholestanic steroids on cervicouterine cancer cells and non-tumor cells. The title compounds were straightforward prepared from diosgenin and hecogenin in excellent overall yields. We determined their effect on cell proliferation on HeLa, CaSki, and ViBo cell cultures. Their cytotoxic effect on tumor cells, as well as on peripheral blood lymphocytes was also evaluated. The increase in the expression of active caspase-3 along with the fragmentation of DNA confirm that the new 22-oxo-26-selenocyanocholestane frameworks potentiate apoptosis in tumor cells. The antiproliferative activity on tumor cells affects to some extent the proliferative potential of peripheral blood lymphocytes, so an immunosuppressive effect has also been established. The novel 22-oxo-26-selenocyanocholestane compounds show selective antitumor activity and therefore are promising lead candidates for further *in vivo* evaluation.

1. Introduction

Steroids constitute a group of structurally related compounds widely distributed in the animal, plant, and fungal kingdoms [1]. The medicinal chemistry of steroids has generated a large variety of structures and most of them have showed diverse biological activities [2]. This particular group of natural products has been extensively studied in the development of new drugs for the treatment of infections, diabetes, tuberculosis, hormonal disorders, and cancer, among others [3]. Either natural or synthetic steroidal compounds are involved in a wide variety of biological processes, and their synthesis and biological evaluation is of interest [4].

In general, the pharmacophoric points contained in steroidal structures and their intrinsic properties itself play a very important role in the biological activity. It is of interest, therefore, to search for new variants of biologically active steroid derivatives with enhanced selective activity [5]. Previously, we reported the synthesis and selective anticancer activity of 22-oxocholestanic compounds derived from diosgenin (**1**) and hecogenin (**2**) [6–8]. The 22-oxocholestanic side-chain has shown to be a very important pharmacophoric point (Scheme 1); for instance, it is present in a family of natural occurring steroidal glycosides isolated from the *Ornithogalum saundersiae*, which have proved to be potent antitumor agents [9–11].

Pharmacological research has proved that the biological activities of molecules such as triterpenes and steroids are intensely associated with their conformation features and key functional groups [12,13]. Structure–activity relationship studies are useful for modifying, designing or synthesizing novel analogs of substances to treat tumors and other diseases. Thus, the latter studies led us to prepare 22-oxocholestanic derivatives containing additional heteroatoms such as selenium, which has been attached to the side chain at C-26. Selenium is a promising antioxidant agent currently being evaluated as a cancer prevention promoter [14]; it represents an important trace element, essential to human health, which is present in both organic (e.g. selenocysteine and selenomethionine)

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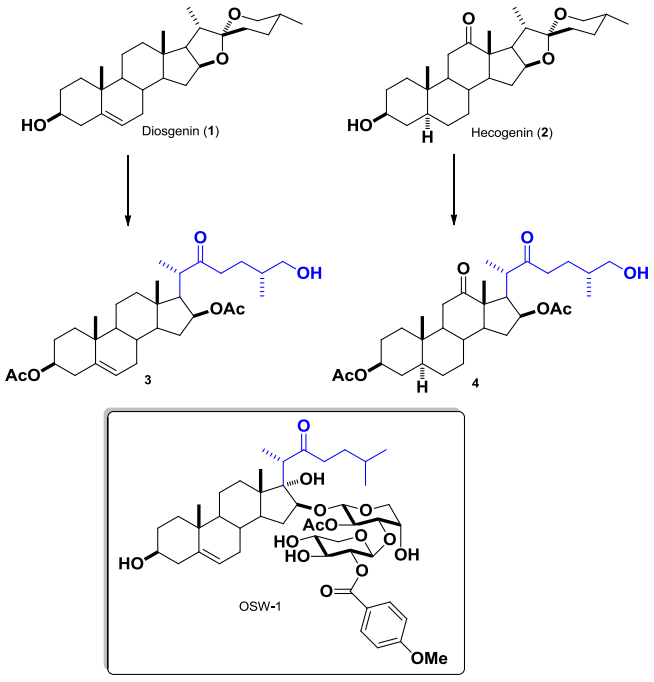
http://dx.doi.org/10.1016/j.ejmech.2013.12.059



Scheme 1. The 22-oxocholestanic pharmacophoric moiety in natural and synthetic steroids.

and inorganic forms (e.g. selenite and selenate). This chalcogen is an essential constituent of extracellular and cellular metalloenzymes, glutathione peroxidase, thioredoxin reductase and other selenoproteins. Furthermore, it has been reported that compounds that contain heteroatoms (such as selenium) increase their antiproliferative properties [15,16]. Several authors have reported features around the mechanism of the selenium in anticancer activity. For instance, it has been reported that selenium (in its different forms, *vide supra*) exerts antitumor effects by inducing autophagic cell death and apoptosis mediated by Reactive Oxygen Species (ROS) [17]. It has been reported too, that selenium acts as a chemopreventive agent and as a prodrug [18,19]. The present study deals with the synthesis and full characterization of 22-oxo-26-selenocyanocholestane derivatives obtained from sapogenins **1** and **2**. We probed their selective anticancer activity through a series of assays as follows: determination of the antiproliferative activity and the cytotoxic activity on tumor and non-tumor cells. Besides, cell morphology evaluations, cell cycle analysis, and apoptosis assays were performed.

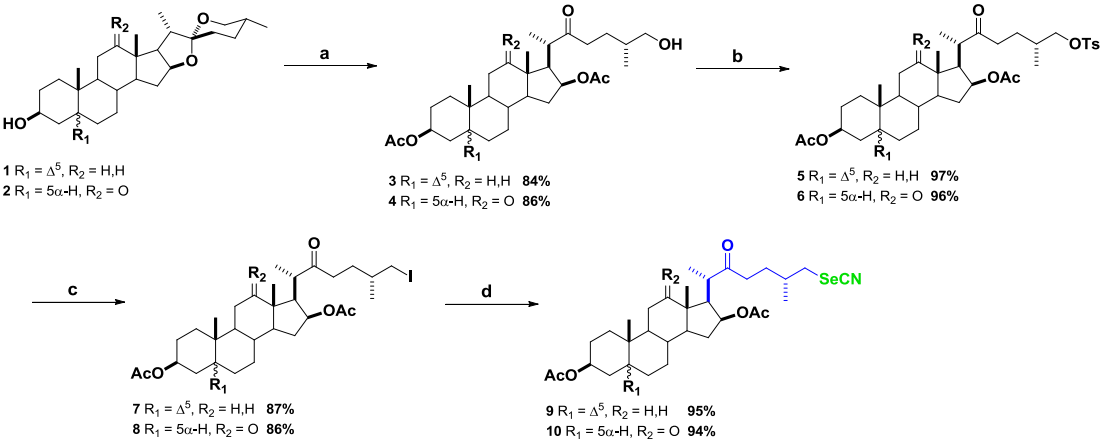
2. Results and discussion

2.1. Chemical synthesis and characterization

We previously reported the synthesis of 22-oxo-26-hydroxycholestanes (**3**–**4**) from diosgenin and hecogenin, by means of a spiroketal acetolysis promoted by $BF_3 \cdot OEt_2$ [6]. This time, the selenium containing compounds were prepared following a straightforward synthetic strategy employing the above-mentioned methodology (Scheme 2). The 26-hydroxyl groups of derivatives **3** and **4** were tosylated under standard conditions (compounds **5** and **6**) followed by the Finkelstein reaction in order to obtain the corresponding iodinated steroids **7** and **8**, according to the procedures reported by Uhle [20]. This pathway gave the desired precursors (**7** and **8**) in nearly quantitative yields. It was noted that, because of the mildness and cheapness of the procedure, the Finkelstein reaction was chosen over the direct halogenation of the alcohols with *N*-halosuccinimides; besides, excellent yields were obtained with the former. The reaction of **7** and **8** with potassium selenocyanate (KSeCN) in refluxing THF gave the corresponding target 26-selenocyanocholestanes (**9** and **10**, Scheme 2) in excellent yields (95 and 94% respectively).

Since the selenocyano group behaves as a *pseudohalide* ion (high nucleophilicity), it readily yielded the desired compounds without by-products [21]. It is well known that the selenocyanate ion can be isomerized to the corresponding isoselenocyanate group, which is also a good nucleophile, such derivatives could have been obtained and identified by NMR; but in any case isoselenocyanate by-products were detected. The nucleophilic substitution with KSeCN was also performed over the tosylates **5** and **6** but moderate yields below 50% were obtained instead. Both target compounds demonstrated high stability and were fully characterized. A combination of COSY, HSQC, and HMBC experiments was used to complete the ^{1}H- and ^{13}C NMR assignment of **9** and **10**. The mass spectra of the selenocompounds yield a characteristic molecular ion isotope pattern.

Briefly, the ^{1}H NMR data of compound **9** shows the characteristic signal for the vinyl proton at C-6 at 5.36 ppm. The *pro-R* and *pro-S* protons at C-26 were observed at 3.11 and 2.93 ppm with $J_{gem} = 12.1$ Hz, $J_{26a,25} = 5.4$ Hz, and $J_{26b,25} = 7.2$ Hz; for compound **10**



Scheme 2. Synthesis of the 22-oxo-26-selenocyanocholestane derivatives from diosgenin and hecogenin. Reagents and conditions: (a) Ac_2O, $BF_3 \cdot OEt_2$, 0 °C; (b) *p*-TsCl, DMAP, CH_2Cl_2, rt; (c) NaI, acetone, reflux; (d) KSeCN, THF, reflux.