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Research paper

Diosgenin-based thio(seleno)ureas and triazolyl glycoconjugates as hybrid drugs. Antioxidant and antiproliferative profile

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ABSTRACT

The stereoselective preparation of diosgenin-derived thio(seleno)ureas and glycomimetics bearing a 1,2,3-triazolyl tether on C-3 has been accomplished. The key steps in the synthetic pathway are the incorporation of an amino moiety and its further transformation into thio- and selenoureas, and also a *click chemistry* reaction involving a propargyl residue and an azido moiety to afford carbohydrate-derived 1,2,3-triazoles; subsequent BF_3-promoted acetolysis of the spiranic moiety afforded the corresponding 22-oxocholestanic structure.

The *N*-phenyl selenourea, an hitherto unknown steroidal derivative, turned out to be a potent ROS scavenger, in particular against free radicals (EC_{50} = 29.47 ± 2.33 μM, DPPH method), and as a glutathione peroxidase mimic in the elimination of H_2O_2 ($t_{1/2}$ = 4.8 min, 1% molar ratio). 22-Oxocholestane structures bearing a C-3 azido, propargyl, thioureido, and particularly selenoureido moiety behaved as strong antiproliferative agents against HeLa cells (IC_{50} 1.87–11.80 μM). *N*-phenyl selenourea also exhibited IC_{50} values lower than 6.50 μM for MDA-MB-231, MCF-7 and HepG2 cancer cells; apoptosis was found to be involved in its mode of action. Such compound was also capable of efficiently eliminating ROS endogenously produced by HeLa cells. Antiproliferative properties of thioxo and selenoxo derivatives were stronger than diosgenin.

1. Introduction

Cancer, an extraordinarily complex and multifactorial pathology leading to the aberrant proliferation of abnormal cells, is currently a major concern in public health-care systems, as it is one of the main causes of morbidity and mortality worldwide [1]. Although a vast arsenal of cytotoxic agents, either naturally-occurring [2], synthetic [3] or semisynthetic [4] ones and sophisticated therapies [5], have been developed so far, no satisfactory treatments have been developed for many cases yet. High toxicity [6] and chemoresistance [7] are key points to be faced in classical anticancer therapies. Therefore, the design of novel families of chemotherapeutic agents featured with enhanced activities, improved selectivity to ameliorate their severe side-effects, different action mechanisms and cellular targets [8] is a pivotal task in Medicinal Chemistry and pharmaceutical industry.

In this context, stereoidal derivatives, either isolated from nature [9] or semisynthetic [10], play a predominant role in the treatment of malignancies; they are considered as lead compounds

Abbreviations: BHT, butylated hydroxytoluene, 2,6-di-*tert*-butyl-4-methylphenol; CM-H_2DCFDA, 5-(and-6) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester; CuAAC, copper-catalyzed azide-alkyne cycloaddition; DIPEA, diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DPPH, 2,2-diphenyl-1-picrylhydrazyl, free radical; DTT, dithiothreitol; EC_{50}, half maximal effective concentration; GPx, glutahione peroxidase; IC_{50}, half inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RNS, reactive nitrogen species; ROS, reactive oxygen species; SEM, standard error of the mean.

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in the discovery of novel anticancer drugs and also as probes for elucidating cancer molecular mechanisms [11]; sex hormones like androgens and estrogens have been successfully applied as adjuvants in neoplastic diseases. Their notable activities in cancer disease have stimulated researchers to undergo a plethora of chemical modifications involving both, the tetracyclic steroid backbone, and also the pending side-chain to give rise to enzymatic inhibitors (e.g. sulfatase, aromatase, hydroxysteroid dehydrogenase), antiestrogens, antiprogestins and cytotoxic agents [12].

Herein we have focused on diosgenin, the aglycon of dioscin, a spirostanic saponin containing a trisaccharide unit isolated from the tuberous roots of yam (*Dioscorea* sp.). Both, dioscin and diosgenin are phytochemicals featured with relevant reported biological properties including antiviral, anti-inflammatory, antidiabetic or anticancer activities [13], among others. In particular, diosgenin has been used as a starting material for the synthesis of steroidal drugs, useful in the treatment of leukemia and colon cancer, and also as contraceptives (Fig. 1) [13b].

2. Results and discussion

2.1. Chemistry

Our main target has been the functionalization at the C-3 position of diosgenin with a chalcogen (S or Se)-containing motif [14] or with a 1,2,3-triazolyl scaffold [15], in order to obtain hybrid structures with two biologically-active pharmacophores, the steroidal residue and the substituent at C-3. The use of dual and multitarget pharmacophores is currently a leading strategy in the design of novel antiproliferative agents [16]. Such approach emerged as an attempt to overcome the limitations of the combination therapy (the use of cocktails of drugs) treating unresponsive patients [17].

Furthermore, the incorporation of functionalities capable of participating in redox processes into drug structures can afford strong antioxidants useful in the control of cellular oxidative stress [18], the unbalanced pro-oxidant/antioxidant situation that provokes deleterious effects such as cellular aging, cardiovascular or neurodegenerative diseases, among other disorders [19]. Furthermore, oxidative stress has been related to the initial stages of numerous cancer processes [20].

We have accomplished the incorporation of thio- and selenoureas at C-3 position of diosgenin, as unprecedented steroidal families. For this purpose, tosylated derivative **2** was accessed starting from commercially-available diosgenin **1**, upon treatment with TsCl in Py, in the presence of a catalytic amount of DMAP (Scheme 1). Compound **2** exhibited high inherent instability, what precluded its chromatographic purification; therefore its isolation involved fast liquid–liquid extractions with cold solutions and removal of the solvent at rt.

Nucleophilic displacement of the tosylate group with NaN_3 in DMF afforded the azido derivative **3** (52% yield), with α configuration at C-3; replacement of the tosyloxy group with a mesyloxy leaving group did not provide any improved yields. ^{1}H NMR spectrum of **3** showed a deshielding of H-3 when compared with starting material **1** (3.81 *vs*. 3.52 ppm); furthermore, a narrower signal of H-3 in the azido derivative was also observed, which is compatible with two axial/equatorial and two equatorial/equatorial couplings, and thus, with the disappearance of axial/axial coupling constants, resulting from the inversion of the configuration at C-3.

The synthesis of **4**, with a 22-oxocholestanic structure, was accomplished by using a BF_3-promoted acetolysis of the spiranic moiety, using the same conditions previously reported [21] for structurally-related derivatives. The formation of targeted C-26-hydroxylated compound is strongly dependent on the reaction conditions, in particular on reaction time (9 min) and temperature (0 °C). More prolonged reaction times provoke the acetylation on the C-26 position, resulting in a more complicated chromatographic purification; furthermore, the ratio of furostanic and epoxycholestanic derivatives, obtained as by-products, is increased with the temperature, thus, leading to reduced yields of the desired derivative [22].

TLC's showed also the formation of a minor and non-identified product with a similar R_f value as **4**; previous results found for some analogs suggest that such kind of compound might be the C-20 epimer, resulting from an acid-promoted enolization of the carbonyl group on C-22. Careful chromatographic purification afforded pure **4** in a 52% yield. The chemoselective opening of rings E and F and the subsequent formation of the 22-oxocholestanic skeleton was fully demonstrated by NMR spectroscopy; thus, H-16, on a carbon bearing an acetoxy group, and H-20, on the α-position of the carbonyl group, underwent a significant deshielding (0.57 and 1.08 ppm, respectively) compared with starting compound **3**. It is also remarkable the change in H-26 protons, as they appear as two diastereotopic signals for spiranic derivative **3**, whereas for derivative **4**, upon the ring-opening of the spiranic motif, they showed a single signal, which is compatible with a higher conformational flexibility. Moreover, in the ^{13}C NMR spectrum, the signals assigned to the new ketone and acetate groups (213.7 and 169.8 ppm, respectively) also support the proposed structure for **4**.

Hydrogenolysis of the azido moiety of **4** using $Pd(OH)_2/C$ as catalyst in short reaction times (2 h) afforded the corresponding amino derivative **5** without affecting the alkene functionality;

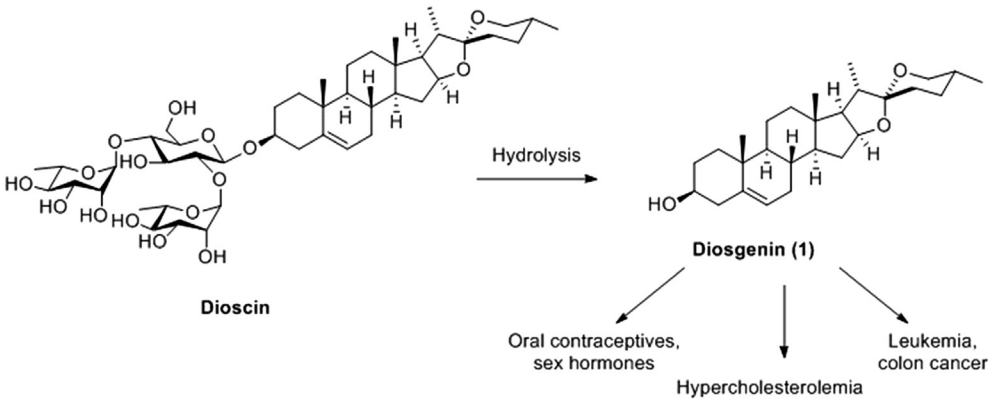

Fig. 1. Structure of dioscin and diosgenin.