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Steroid dimers—*In vitro* cytotoxic and antimicrobial activities

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ABSTRACT

The *in vitro* cytotoxic activity of previously synthesized steroid dimers with different spacer group (sulfide, trithiolane ring or phosphorotrithioate) and the substituent at C-17 position was tested for their possible effects against following human tumor cell lines: cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562) and two human breast cancer cell lines (MDA-MB-361 and MDA-MB-453). These compounds, applied at micromolar concentrations, exhibited cytotoxic activity of different intensity (compared with cisplatin as a control), modality and selectivity in these malignant cell lines. The best activity against all four cell cancer lines was exhibited by dimer-sulfides. All screened compounds exerted concentration-dependent cytotoxic activity against leukemia K562 cells. The compounds which exerted the most pronounced cytotoxic action exhibited notably higher cytotoxic activities against K562, HeLa and MDA-MB-453 cells in comparison to resting and PHA-stimulated PBMC, pointing to a significant selectivity in their antitumor actions. Examination of the mechanisms of cytotoxicity on leukemia K562 cells revealed pro-apoptotic action of each of the investigated compounds applied at concentrations $2IC_{50}$. The most prominent pro-apoptotic action was exhibited by dimer-sulfide of cholest-4-en-3-one. Furthermore, almost all of the tested compounds at IC_{50} concentrations induced G1 phase cell cycle arrest in K562 cells. Antimicrobial activity against Gram-positive, Gram-negative bacteria and fungal cells, and toxicity to brine shrimp *Artemia salina*, were evaluated. There was no antibacterial activity. The best antifungal activity was exhibited against *Saccharomyces cerevisiae* by dimers linked with trithiolane ring, indicating a selective activity of investigated compounds.

1. Introduction

One of the most fascinating challenges in modern organic chemistry is the design of structurally diverse and complex molecules which are useful for the study of important biological processes [1]. In many of them, symmetry plays a crucial role [2]. For instance, numerous proteins responsible for cell proliferation and differentiation exist as homodimers or become activated through dimerization as a key step in their respective signaling cascade [3]. For this reason, the synthesis of dimeric molecules (or bivalent ligands) capable, not only to interact with specific biologic receptors, but also to induce greater biological responses than the corresponding monomeric species have been developed.

Steroids are an important group of natural compounds widespread in almost all living organisms expressing various types of biological activity. Among them, steroid dimers form a significant group of pharmacologically active compounds that are predominantly biosynthesized by various marine organisms, and also synthesized in laboratories [4]. Dimerization of steroid skeleton renders some unique characteristics that are applicable to different areas. Dimeric steroids have micellar [5,6], detergent, and liquid–crystal properties [7], and have been used as catalysts for different types of organic reactions in which they play a key role in the rate enhancements from hydrophobic binding [8,9]. A number of dimeric steroids, *e.g.*, cephalostatins (homodimers) and ritterazines (heterodimers), are among the most potent natural cytotoxic agents [10–15]. These compounds exhibit an extraordinarily strong cytotoxic activity, with their most potent member cephalostatin 1 being 400-fold more active in *in vitro* testing than taxol, and therefore are one of the most powerful cytostatics ever to be tested by the National Cancer Institute [16]. Steroid dimers can also be used to create “molecular umbrella” for drug delivery

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[17–19]. To study the significance of steroid carrier on the antimalarial and antiproliferative activity *in vitro*, a number of cholic acid-based tetraoxane dimers were synthesized [20].

The classification of steroid dimers has been best described in the recently published book, *Steroid Dimers: Chemistry and Applications in Drug Design and Delivery* (2012) by Nahar and Sarker [4], in which the steroid dimers have been classified according to several criteria. First, they can be broadly classified into acyclic dimers (also known as linear dimers) and cyclic dimers. Acyclic dimers involving connections between A, B, C or D rings, or via C-19, direct or through spacers, form the major group of steroid dimers. In the cyclic steroid dimers, dimerization of steroids, direct or through spacers, leads to formation of new ring systems or macrocyclic structures, *e.g.*, cyclocholates or cholaphanes. Steroid dimers can also be classified as symmetrical and unsymmetrical dimers; when a dimer is composed of two identical steroid monomeric units, it is called a symmetrical dimer, and when two different monomeric steroid units are involved or two identical monomeric steroid units are joined in a way that there is no symmetry in the resulting dimer, the dimer is known as an unsymmetrical dimer. One other way of classifying steroid dimers is to divide them into natural and synthetic dimers. However, there are two more reviews, one by Li and Dias (1997) [21] and the other one by Nahar, Sarker and Turner (2007) [22] on steroid dimers covering their chemistry and applications.

The synthetic approaches reported so far, have led to the preparation of cyclic and acyclic steroidal dimers, by connection between two cyclopentanoperhydrophenanthrene skeletons (through A–A, B–B, C–C, D–D or A–D rings) [4,21–23]. The steroidal moieties could be directly linked [24,25], linked through spacer groups [26–30] and by connection through the steroidal side chains [31–33].

In continuation of our work on modified steroid compounds we have recently reported reactions of α,β-unsaturated steroidal ketones (several cholestane, androstane and pregnane carbonyl derivatives were chosen) with Lawesson's reagent (LR: 2,4-bis(*p*-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide) in which several sulfur and sulfur and phosphorus containing acyclic (linear) steroidal dimers were synthesized [34]. In all dimers obtained, two identical steroid molecules were joined via ring A–ring A connection through spacer groups. The structures of these dimeric steroids were unambiguously established from their analytical and spectral data (NMR spectroscopy). The conversion of the 4-en-3-one steroid A–ring system in starting molecules **1a–e** to the 3,5-diene system in dimers gave symmetrical 3,3′-sulfides **2a–e** and 3,3′-phosphorotrithioates **4b–e**. Dimers **3a–e** were obtained, by conversion of 4-en-3-one system to the 4-ene system, as mixtures of three possible isomers in approx. 8:1:1 ratio (deduced by comparing the peak areas of the H-4 in the corresponding ^{1}H NMR spectra) differing in the configuration at C-3 and C-3′, *i.e.*, by the position of trithiolane ring which linked two steroidal molecules. After several consecutive column chromatographies, diastereomerically pure major isomer was obtained. Unfortunately, all our efforts to get the other two isomers in pure form have failed (Fig. 1).

In the context mentioned above and as a continuation of our investigation of modified steroids as biologically active molecules [34–37], the goal of this study was to perform extensive investigation of *in vitro* cytotoxic activity of the previously synthesized steroid dimers **2a–e**, **3a–e** and **4b–e**. These compounds were tested against four human malignant cell lines: cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562) and two human breast cancer cell lines (MDA-MB-361 and MDA-MB-453). In addition, to assess the sensitivity of normal immunocompetent cells included in the antitumor immune response, the cytotoxicity of the most potent compounds **2a–c**, **2e** and **4b** were also tested against human peripheral blood mononuclear cells (PBMC)–both unstimulated and stimulated to proliferate by the mitogen phytohemagglutinin (PHA). The specific aim of this study was to get an insight into modalities of cytotoxic

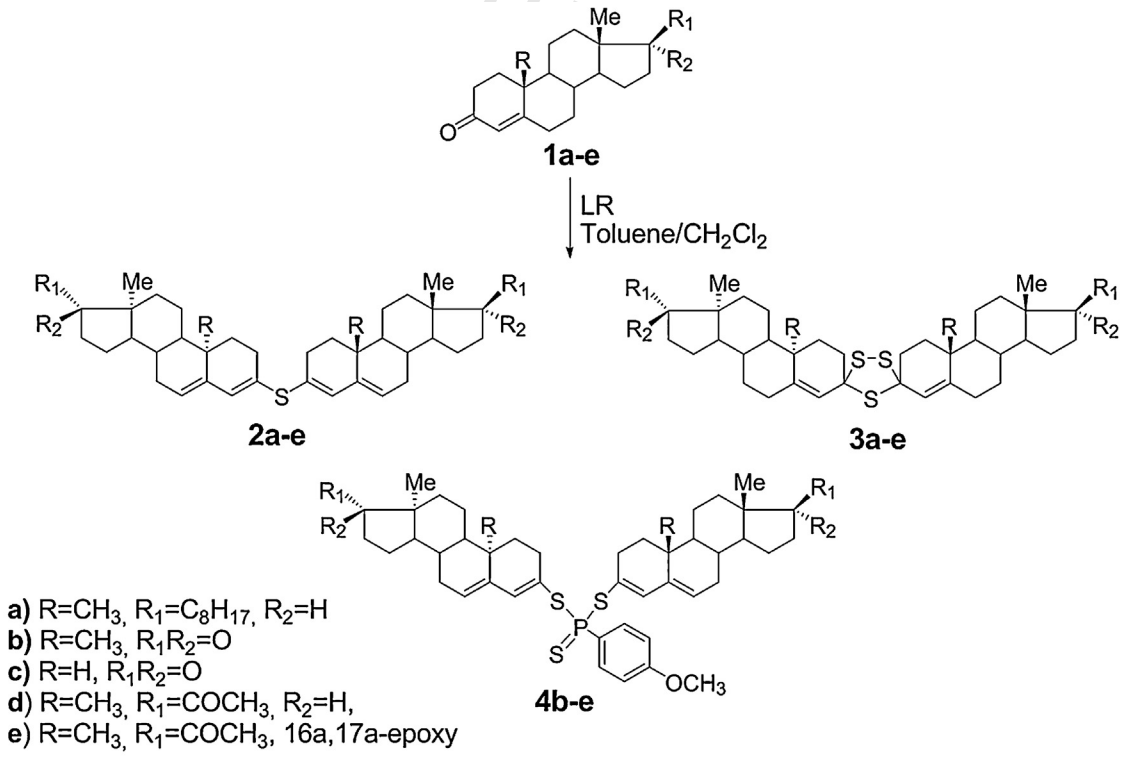

Fig. 1. Synthesis of steroid dimers **2a–e**, **3a–e** and **4b–e**.

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