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Synthesis and evaluation of new steroidal lactam conjugates with aniline mustards as potential antileukemic therapeutics

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ABSTRACT

Alkylating agents are still nowadays one of the most important classes of cytotoxic drugs, which display a wide range of therapeutic use for the treatment of various cancers. We have synthesized and tested four hybrid homo-azasteroidal alkylating esters for antileukemic activity against five sensitive to alkylating agents human leukemia cell lines *in vitro* and against P388 murine leukemia *in vivo*. Comparatively, melphalan and 3-(4-(bis(2-chloroethyl)amino)phenoxy)propanoic acid (POPAM) were also examined. All the homo-aza-steroidal alkylators showed relatively lower acute toxicity, very promising and antileukemic activity both *in vitro* and *in vivo*.

1. Introduction

Alkylating agents, classical nitrogen mustards, nitro-nitrosoureas soureas and non-classical alkylators, such as platinum complexes, are still one of the most important classes of cytotoxic drugs, which display a wide range of therapeutic use for the treatment of various cancers including Hodgkin's disease, non-Hodgkin's lymphoma, leukemias, lung, ovarian, breast cancer, etc [1]. Chlorambucil [2], melphalan [3] and mechlorethamine [4,5], are some of the alkylating agents (nitrogen mustards) that are currently in clinical use. Their healing properties are derived from their capacity to block the DNA replication by creating permanent and stable bonds (cross-links) between the double-stranded DNA chains [6]. These bonds can be internal (inter-strand cross-links), which are located in two antiparallel DNA strands or external (intra-strand cross-links), which are made between bases of the same strand of DNA [7].

Major disadvantage of using alkylating agents in cancer chemotherapy is their systemic toxicity and efficacy [6,8,9], as they are non-selective and kill both cancerous and healthy cells. In order to reduce toxicity, steroids have been used as carrier molecules to deliver the alkylators to a specific target tissue by interacting with steroid hormone receptors [10,11]. These conjugates improve the physicochemical properties of the drugs such as the lipophilicity and the solubility. Usually, the alkylating agent is attached on the steroid with an ester bond. Representative examples are the steroidal alkylating agents Estramustine (ester of estradiol and mechlorethamine, **(1)** and Prednimustine (ester of prednisolone and chlorambucil **(2)**, which are currently applied in cancer therapy on the treatment of prostate cancer and lymphoproliferative malignancies, respectively (Fig. 1) [12–14]. Homo-azasteroids, incorporating a lactam moiety (-NH-CO-) into steroidal ring/s, in their esterified forms have also been introduced as carrier molecules of alkylating agents with significant therapeutic efficacy in preclinical studies against leukemia and several other neo-plastic malignancies both *in vivo* and *in vitro* [15–23]. Herein, we report the synthesis and the evaluation of novel steroidal lactam derivatives of type **3**, which are conjugated with 3-(4-(bis(2-chloroethyl)amino)phenoxy)propanoic acid (POPAM) in the expectation of improving its biological activity.

The new homo-aza-steroidal alkylating esters of POPAM were designed with the ultimate goal of improving therapeutic efficacy of treatment of resistant tumors to classical and non-classical alkylating drugs. These hybrid compounds were aimed at: i) to link a well-balanced acting alkylator due to concerns of its toxicity and therapeutic effect with a modified aza-steroid which will assist with transportation of the alkylating moiety inside the cell and direct it to the targeted biomolecules in the nucleus, ii) to diminish

Abbreviations: POPAM, 3-(4-(bis(2-chloroethyl)amino)phenoxy)propanoic acid; L-PAM, L-phenylalanine mustard.

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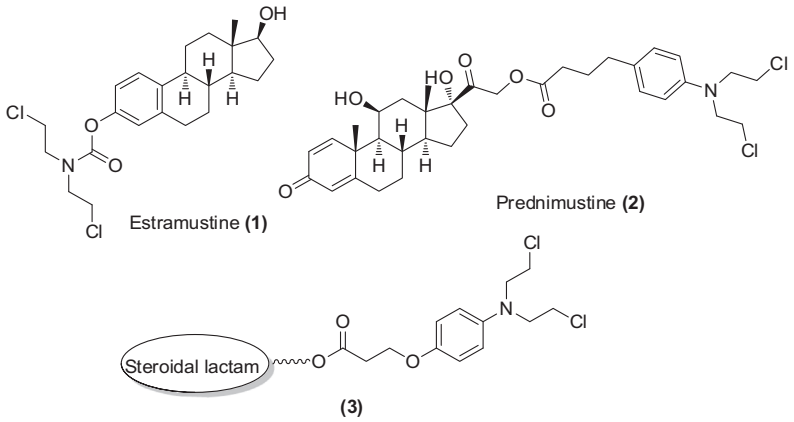


Fig. 1. Representative known steroidal alkylating agents **(1, 2)** and the general type of the steroidal lactam alkylators **(3)** studied in this work.

systemic toxicity and increase anticancer activity significantly resulting to improved therapeutic ratio, iii) to produce a multi-targeted antitumor effect and interfere with crucial biochemical molecular pathways and DNA repair mechanisms of the cancer cells such as the protein kinase C (PKC) signaling pathway [22,23].

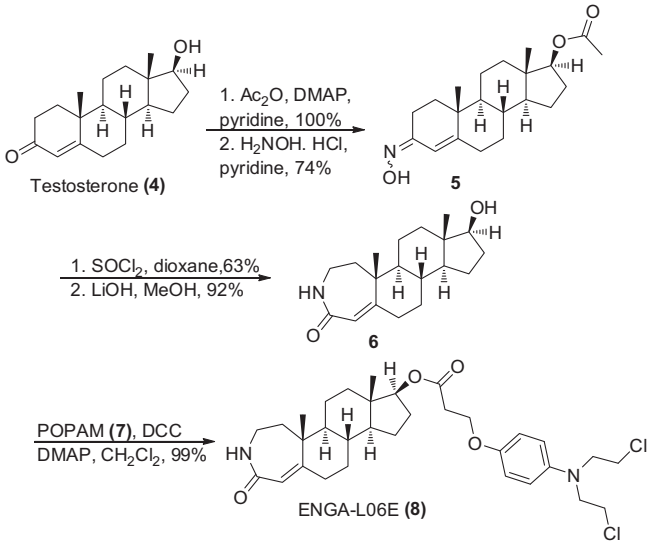
2. Results and discussion

Four new ester conjugates of steroidal lactams with POPAM were synthesized. The steroidal lactams (aza-homo steroids) used in the present study, carry one or more amide functionalities at the rings of the basic steroidal framework. It is known that such steroidal lactams can be synthesized from a ketosteroid via the corresponding oximes and Beckman rearrangement [24–26]. The first conjugate was prepared starting from testosterone **4** via the lactam **6** according to the known procedure of Catsoulacos and Camoutsis (Scheme 1) [27]. Testosterone was acetylated by acetic anhydride in pyridine and the corresponding acetate was condensed with hydroxylamine to give the *E*- and *Z*-oximes **5**. Next, the Beckman rearrangement of the mixture of **5** with $SOCl_2$ in dioxane was attempted. It is known that in a typical Beckman rearrangement the group *anti* to the OH group migrates from C to N. In the case of oximes **5** and in accordance to previously published data a single lactam **6** was isolated in 63% yield most likely due to *E/Z* isomerization prior to alkyl migration [25,28]. After deprotection of the acetyl moiety by basic hydrolysis (92%), the esterification of hydroxyl group was carried out by POPAM **(7)**, DMAP and DCC in CH_2Cl_2 yielding the desired conjugate ENGA-L06E **(8)** in quantitative yield.

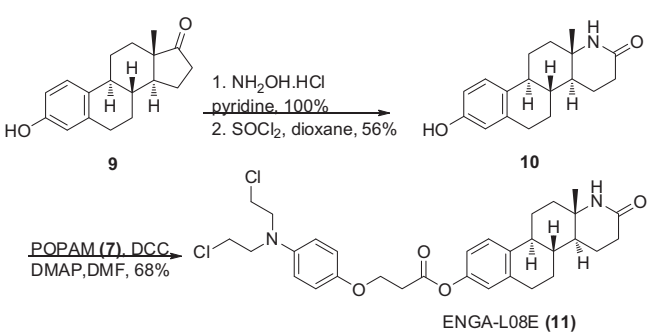
The second conjugate, ENGA-L08E **(11)** is a derivative of estrone **9** and was synthesized from the esterification of lactam **10** with acid **7** in the presence of DCC and DMAP in DMF (Scheme 2). Lactam 17*a*-aza-*D*-homoestrone **10** was synthesized according to the methodology of Liao and coworkers [29].

The other two steroidal lactam alkylators were prepared from adrenosterone via the novel monolactam **16** and dilactam **20**. The preparation of monolactam **16** was accomplished as presented in Scheme 3. Regioselective reduction of 17-keto group of the *D*-ring of adrenosterone was performed according to Morreal [30–32] with $NaBH_4$/MeOH to give the desired alcohol with traces of another product, which was used in the next step without further purification. Subsequent protection of hydroxyl group yielded 17-acetate **13** in 90% yield. Following the established route, compound **13** was selectively converted to oximes **14** after treatment with $NH_2OH.HCl$ in pyridine. Beckman rearrangement of the mixture of **15** gave the desired monolactam **16**, the structure of which was determined by comprehensive analyses of its ^1H and ^{13}C NMR, COSY, HSQC, and HMBC spectroscopic data. As shown in Fig. 2 key HMBC interactions of the protons H-2 with C-10, C-19 and C-3 indicate the NH group to be positioned between C-2 and C-3. This is further supported by the COSY correlations of NH with H-4 and H-2. Finally, deprotection of the hydroxyl group of **16** followed by esterification with acid **7** afforded conjugate ENGA-L07E **(17)** in 96% yield.

The bislactam alkylator **21** was prepared in a similar fashion by the synthetic route illustrated in Scheme 4. Oximes **18** were subjected to Beckmann rearrangement and the bislactam **19** was iso-



Scheme 1. Synthesis of ENGA-L06E **(8)**.



Scheme 2. Synthesis of ENGA-L08E **(11)**.