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Review

Steroid-linked nitrogen mustards as potential anticancer therapeutics: A review

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ABSTRACT

Nitrogen mustards, an important class of drugs for cancer therapy, are known as DNA alkylating agents. The nitrogen mustards are highly reactive and, as a consequence, lack of selectivity and produce several adverse side effects. In order to minimize these undesirable effects, the attachment of nitrogen mustards to a steroidal hormone with affinity for its receptor can lead to highly selective and less toxic antineoplastic therapeutics. This review will focus on the design, synthesis and evaluation of such steroid-nitrogen mustard hybrids as antineoplastic agents. Among these compounds, modified steroids with aromatic nitrogen mustards linked by an ester function were found to have better DNA alkylating properties, improved selectivity as well as low toxicity.

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Contents

Abbreviations: ADR, adriamycin; BCNU, carmustine; ACA, aminocinnamic acid; CHL, chlorambucil or *para*-[*N*,*N*-bis(2-chloroethyl)amino]phenylbutyric acid; CNC, chloronitrocarbamoyl; CP, cisplatin; DTIC, dacarbazine; DnsEM, dansyl estramustine; EM, estramustine; EMP, estramustinephosphate; ER, estrogen receptor; EoM, estromustine; HASE, homo-aza-steroidal alkylating ester; ILS, increase in lifespan; LDL, low-density lipoproteine; rLDL, reconstituted-LDL; MNU, methylnitrosourea; MN, micronuclei; 4-Me-CABA, *para*-methyl-*meta*-[*N*,*N*-bis(2-chloroethyl)amino]benzoic acid; PHE, *para*-[*N*,*N*-bis(2-chloroethyl)amino]phenylacetic acid; PHO, *para*-[*N*,*N*-bis(2-chloroethyl)amino]phenoxy acetic acid; PRIs, proliferation rate indices; PC, prostate cancer; RBA, relative binding affinity; MeCCNU, semustine; SMART, somatic mutation and recombination test; SCEs, sister chromatid exchanges.

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1. Introduction

Several studies demonstrate that steroid hormones, including their agonists and antagonists, generate beneficial and, often excellent results in the prevention and treatment of many types of cancers. There are steroid hormones used in clinics for the treatment of hormono-dependent cancers such as breast, prostate, ovarian, endometrium. Many steroids are also used for the treatment of cancer symptoms [1,2]. This was the result of the discovery of steroid hormone receptors and the role of hormones themselves in the development and progression of several types of cancers. The implication of steroid hormones was found mainly in breast cancer, prostate cancer, ovarian and endometrial cancers, gastrointestinal cancers, small cell lung cancer and meningiomas [3–13]. This led to the notion of a receptor-mediated chemotherapy. Consequently, many researchers developed various steroid-cytotoxic hybrid molecules (or conjugates) to target hormone-dependent diseases [14]. The goal of such a drug-design was obviously to deliver the conjugate more precisely to cancer cells thereby, decreasing the known adverse side effects associated with chemotherapy.

Nowadays, many of the most effective drugs used for chemotherapy against cancer have in common the potential to alkylate DNA, RNA, and several proteins [15,16]. Alkylation of DNA is the main lesion leading to anticancer activity. The nitrogen mustards are that type of drugs that show very potent antineoplastic effects. They are the first and most comprehensively studied DNA interstrand cross-linking agents. These anticancer drugs are used effectively in myelogenic leukemia, Hodgkin disease, lung, testicle, ovarian and breast cancers, as well as in several lymphomas [17]. However, their uses are hampered by their lack of selectivity, high toxicity and the potential development of resistance to the drug. The undesirable side effects and development of resistance are, in fact, the main obstacles of most existing anticancer therapies.

It is known that combination therapy that uses different types of anticancer drugs can improve the effectiveness and reduce toxicities. In addition, the combination of an anticancer molecule with a steroidal skeleton with affinity toward its cognate receptor can be more selective and less toxic than the classic non-targeted approach [18–20]. Several studies of different hormone-linked antineoplastic agents showed highly effective results in receptor positive tumors *in vivo* [21,22]. Among these conjugated molecules, the antitumor steroid hormone-nitrogen mustard combination was found to be quite successful [23]. It is expected that a lipophilic steroid carrier molecule would aid transport the nitrogen mustard moiety more efficiently to specific target tissues. So, the simple blend of nitrogen mustard with a steroid improves its anticancer activity and reduces its toxic effects by simply increasing its lipophilicity as well as by altering its physicochemical properties. In fact, the steroid-nitrogen mustard conjugates can more easily and efficiently penetrate the lipid bi-layer membranes of the cells and reach its site of action; the nucleus [24,25].

In order to further study the mechanism of action and interactions within the receptor of the steroid-nitrogen mustard hybrids, closely related analogs were made. These studies have shown that some hybrid derivatives are very effective in receptor positive tumors *in vivo* and are even superior to simple mixtures of unlinked alkylating agents combine with hormone [26,27]. Moreover, it was observed that enhanced DNA alkylation in tumor tissue and lower toxicity was achieved with the hybrid anticancer agents [28]. It was found that dehydroepiandrosterone (DHEA)-nitrogen mustard hybrid showed no effect where as modified steroids skeleton bearing the same alkylating agent were proved more efficient *in vitro* and *in vivo*. Several studies showed that the modified steroids increased activity was due to the interactions of similar functional groups that exist in the target protein (or receptor) [29,30]. Kapou et al. showed that small functional modifications on the steroidal part of complex molecules, made of an alkylating moiety and a steroid, gave compounds with enhanced biological activity [31]. The modified steroids-nitrogen mustards hybrids showed enhanced antileukemic effects. This suggests a determinant role of the steroid moiety itself on the mechanism of action.

Over the years, many nitrogen mustard analogs have been synthesized with the aim of improving selectivity and reducing their toxic side effects. However, only a handful of these analogs, such as chlorambucil (CHL) and melphalan, are used in clinics today [32–37]. It was shown that the addition of an amide (—NHCO—) function into the steroid skeleton was beneficial for biological activity as compared to the unmodified steroids. So chlorambucil esters and other analogs esters of modified steroid were much more effective antileukemic drugs. Structure activity relationship (SAR) studies recognized the value of an amide group incorporated on the steroidal portion of these compounds in the form of a D-lactam [29] or as a 17β-acetamido substituent [38,39]. Furthermore, it was shown that the insertion of a keto group in the B steroidal ring produced highly potent compounds. It was recognized that this structural modification is crucial for the design of useful and active analogs [40,41].

This review will focus on the design, synthesis and structure activity relationship studies of steroid-linked nitrogen mustard as anticancer therapeutics. Here, we report several examples of steroid-linked nitrogen mustard analogs with, either unmodified (Section 1) or modified (Section 2) steroid skeletons.

2. Nitrogen mustard with unmodified steroidal skeleton

2.1. Nitrogen mustard with androgenic steroidal skeletons

Jones et al. reported the synthesis and the evaluation of various 17α- and 17β-N(CH_2CH_2Cl)$_2$ androstane compounds (**1**–**6**), Fig. 1. The most reliable synthetic route involved diethanolamine (—N(CH_2CH_2OH)$_2$) derivatization [42].

The synthetic methodologies are shown in Scheme 1. 5α-androstan-17β-ol (**7**) [43] was converted to 17β-bis(2-hydroxyethyl) amino-5α-androstane (**8**) (60% yield) by a four step reaction sequence [23,44]. 17α-Precursor (**9**) was obtained in 41%, overall yield from **7** [42]. 17β-Mustard **3** was obtained with 50% yield using carefully purified $SOC1_2$ (in the presence of triphenyl phosphate [45]). The reaction of 17α-precursor **9** with the same reagent afforded 17α-bis(2-chloroethyl)-amino-5α-androstane (**4**) in 48% yield. Compounds **3** and **4** were also obtained by chlorination of **8** and **9** with neat, freshly distilled $POCl_3$ in 50–55% yield. $POCl_3$ was the best reagent for the preparation of **3** and **4** as did not require rigorous purification of the chlorinating agent and solvent, the reaction mixtures are more stable and isolation/purification of the mustards are much simpler than for the $SOCl_2$ reagent.

For preparation of Δ^4-3-keto mustards **1** and **2**, they used the same reaction which was successful for the synthesis of mustards **3** and **4** but it did not work for the preparation of the C-3 oxygenated and unsaturated mustards **1** and **2**. Instead, chlorination of the epimeric diols (**11** and **12**) obtained from **10**, to give the desired androst-4-en-3-one mustards **1** and **2**, respectively, were done with mesylchloride (Scheme 2). Bis(2-hydroxyethyl)amine (**14**) was obtained in 70% yield from 17β-amino-5α-androstan-3-ol (**13**) and treatment of the triol (**14**) with $POCl_3$, led to the formation of **6** in good yield (Scheme 3). Contrarily, the chlorination of **14** with purified $SOC1_2$ gave instead the trichloro derivative (**5**) in 25% yield. From biological results most of the steroid-mustards proved to be inactive. They did not show any cytotoxic activity when tested against DMBA-induced mammary tumors in Sprague-Dawley rats. This could be due to the lack of solubility and poor bioavailability of the nitrogen mustard derivatives.