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Research paper

Design and synthesis of novel hydroxyanthraquinone nitrogen mustard derivatives as potential anticancer agents via a bioisostere approach

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ABSTRACT

A series of hydroxyanthraquinones having an alkylating N-mustard pharmacophore at 1′-position were synthesized via a bioisostere approach to evaluate their cytotoxicity against four tumor cell lines (MDA-MB-231, HeLa, MCF-7 and A549). These compounds displayed significant in vitro cytotoxicity against MDA-MB-231 and MCF-7 cells, reflecting the excellent selectivity for the human breast cancer. Among them, compound **5k** was the most cytotoxic with IC_{50} value of 0.263 nM and is more potent than DXR (IC_{50} = 0.294 nM) in inhibiting the growth of MCF-7 cells. The excellent cytotoxicity and good selectivity of compound **5k** suggest that it could be a promising lead for further design and development of anticancer agents, especially for breast cancer.

1. Introduction

Nitrogen mustards (N-mustards) are frequently used anticancer agents in the clinic, such as cyclophosphamide, chlorambucil and melphalan [1]. However, the severe host toxicity and drug resistance associated with these drugs continue to present serious impediments in cancer chemotherapy. Moreover, the efficiency of these drugs is limited by low affinity for DNA and rapid quenching via hydrolysis prior to DNA alkylation [2–4]. Many N-mustard structural modifications have been made during the last 40 years in order to increase the target affinity. In this regard, medicinal chemists have focused on changing the carrying parts for N-mustards by linking the N-mustard residue to DNA-binding molecules [5–12] (Fig. 1). However, up to date, despite the fact that many carriers have been investigated, the majority of them focused on benzene derivatives and new clinical candidates with substantially enhanced activities remain elusive.

The anthracyclines (doxorubicin, daunorubicin, idarubicin, epirubicin, and the anthraquinone mitoxantrone) are one of the most effective anticancer agents for many types of tumors [13]. Although newer drugs have emerged continually in recent years, the fact that anthracyclines remain a first-line treatment for cancer underscores its sustained relevance as an anticancer drug. Based on the proven efficacy of anthracyclines and related compounds in antitumor application, their molecular motifs may serve as a good platform for structural modification to enhance potency in other types of cytotoxic agents. A unique feature of anthracyclines is the presence of a 1,4-dihydroxyanthraquinone moiety that may play a key role both in the DNA binding and cell or tissue bioavailability [14–16]. Considering the importance of the 1,4-dihydroxyanthraquinone moiety in anticancer drugs and biologically active natural products [17–19], the combination of a 1,4-dihydroxyanthraquinone structure with an N-mustard moiety could lead to novel compounds possessing potent anticancer properties. The anthraquinone portion provides a potential substrate for DNA intercalation [20,21] and bioreductive activation [22,23], and the alkylating group imparts cytotoxic activity. In this regard, some chrysophanol and emodin-nitrogen mustard conjugates have been synthesized and their anticancer activity against Leukemia L1210 and HL-60 cells were evaluated by Watanabe's

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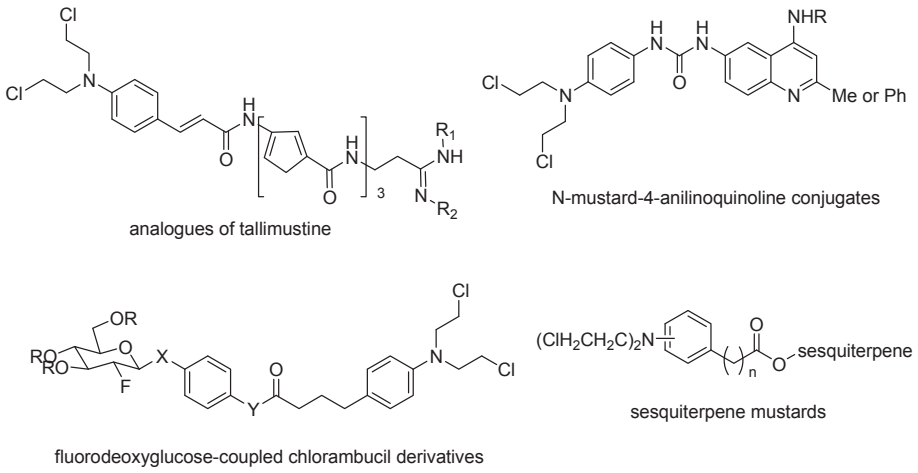

Fig. 1. Selected examples of N-mustards with a benzene derivative carrier.

group [24]. Thereafter the synthesis of anthraquinone–-chlorambucil conjugates and their antitumor activity have been reported by Ahn's group [25].

Inspired by these results and based on bioisosterism, we proposed to replace the ether bridge at C-7 of doxorubicin (DXR) with a nitrogen atom in anticipation that the resultant molecules possessing two pharmacophores (hydroxyanthraquinone and N-mustard) with one molecule may exhibit potent antitumor activity while decreasing the drug resistance to certain extent (Fig. 2). The ether bridge of DXR between the anthracyclinone moiety and the aminosugar was thought to be important for its activity [26]. Bioisosteric replacement of functional groups is a frequently employed technique in drug discovery for the generation of more potent and more selective analogues of lead structures [27]. On the other hand, with mitoxatrone and bisantrene in clinical use for the therapy of cancer, the nitrogen atom and bisethylamine moiety seemed to represent an important factor for their high therapeutic indices. In this work, with the goal to develop potent hydroxyanthraquinones with antitumor activity and as a part of our ongoing interest in the synthesis of novel hydroxyanthraquinone compounds and biological evaluation [28–31], we combined these features into one molecule to mimic the structure of DXR and mitoxantrone. Herein, we report our studies on the design and synthesis of a new series of hydroxyanthraquinone N-mustard derivatives and demonstrate their biological activities.

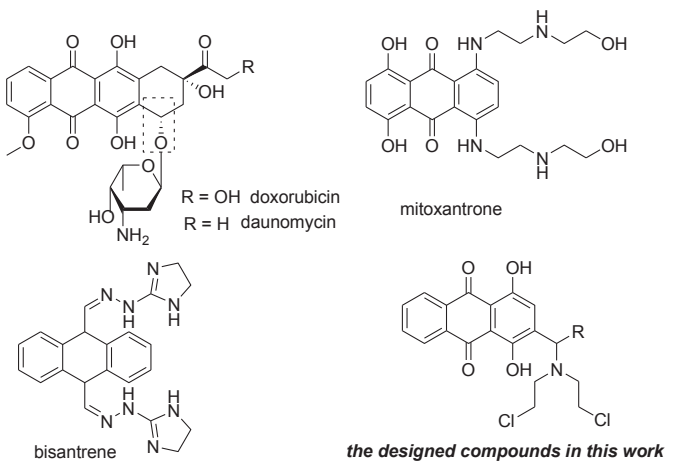

Fig. 2. The structures of representative anthracyclines and the newly designed compounds.

2. Results and discussion

2.1. Chemistry

The synthetic route for the hydroxyanthraquinone bearing an alkylating N-mustard residue on 1′-position is shown in Scheme 1. The commercially available starting material hydroxyanthraquinone **1** was converted to compound **2** via a modified Marschalk reaction according to the procedure previously developed in our laboratory [29]. Chlorination of **2** with thionyl chloride at 50 °C led to the intermediate **3**, which was directly used in the next step without the need for purification. Subsequently, condensation of diethanolamine with intermediate **3** in dichloromethane at room temperature gave intermediate **4**. There was no need to purify the crude product after workup in this step and the crude product can be directly used in the next step. A final chlorination of intermediate **4** with thionyl chloride afforded the target compounds **5a-t**. This chlorination was successfully performed by using the same condition for the synthesis of intermediate **3**. After completion of the reaction, the desired products were easily isolated in pure form by column chromatography. It should be noted that through all the reactions for the synthesis of the target compounds, the protection of hydroxyl groups was not required. All the newly synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR as well as high resolution mass spectrum (HR-MS).

2.2. Antitumor activity

The in vitro antitumor activities of the anthraquinone N-mustards **5a-t** were evaluated against the MDA-MB-231 (triple negative breast cancer), HeLa (cervical cancer), MCF-7 (ER^{+} breast cancer), and A549 (non-small cell lung cancer) cell lines and were compared with the cytotoxic activity of DXR and chlorambucil, chosen as reference drugs in this assay. The inhibitory activities (IC_{50}) of the tested compounds are shown in Table 1.

As shown in Table 1, almost all the synthesized target compounds are more potent than chlorambucil against MDA-MB-231, HeLa, and MCF-7 cell lines. For instance, compound **5i** showed a ca. 900-fold increase in its inhibitory activity against MDA-MB-231 cells compared to chlorambucil; compound **5q** was 10-fold more active against HeLa cells than chlorambucil; compound **5k**