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Research paper

Novel anticancer hybrids from diazen-1-ium-1,2-diolate nitric oxide donor and ROS inducer plumbagin: Design, synthesis and biological evaluations

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ABSTRACT

High levels of both nitric oxide (NO) and reactive oxygen species (ROS) could act as pro-apoptotic signals in cancerous cells. In this study, we conjugated diazeniumdiolates (NONOates), an important class of NO donors, with a natural occurring plumbagin (PL) which is primarily an excellent ROS inducer. Herein, a total of 12 novel plumbagin/NONOate hybrids have been synthesized and evaluated for their inhibitory effects on a panel of human cancer cell lines (MDA-MB-231, A549, HepG2 and HCT-116 cells) and two normal human cells (HK-2 and WRL-68 cells). Among them, compounds **10a** and **10b** demonstrated superior potencies compared to their parent compound (IC_{50} values of 3.48–6.68 μM) against the above cancer cell lines but weak inhibitory effects on normal cells. In concordance with their selective cytotoxicities, **10a** and **10b** released higher level of NO in cancer cells than normal cells. Besides, the potent compound **10a** induced apoptosis of A549 cells in a concentration-dependent manner and resulted in more ROS generation compared with the parent compound plumbagin.

1. Introduction

Nitric oxide (NO), a cardinal signaling molecule, has emerged as a mediator in many physiological and pathological processes [1]. It has been shown that angiogenesis, proliferation and metastasis can normally be stimulated or enhanced by lower levels of NO (<100 nM). While higher concentrations of NO depress cancer progression by inducing apoptosis, sensitizing tumors to chemo-, radio-, or immunotherapy, reversing resistance to chemotherapy, and retarding the angiogenic and metastatic cascades [2,3]. Accordingly, numerous NO-based anti-cancer agents have been developed for the potential application for cancer therapy [4]. As with NO, the cellular effects of reactive oxygen species (ROS) are also concentration dependent [5]. Noticeably, most malignant cells possess under varied contexts inherently higher ROS level compared to normal cells [6,7]. This difference in the redox state between cancer cells and non-cancer cells allows ROS inducing compounds may elicit effective but selective cytotoxic effects against cancer cells [8,9]. And given that ROS has been involved in regulation of NO synthases (NOSs) expressions and NO-mediated signaling, and NO donors pretreatment in some types of cancer cells have in turn been more sensitive to ROS inducing drugs such as cisplatin and doxorubicin, coadministration of NO donor with ROS inducers as medication for cancer treatment is of particular interest in recent years [10–13].

Diazeniumdiolates (NONOates) as an important class of NO donors, have abilities to release high levels of NO (two molecules of NO from per molecule of diazeniumdiolate) under physiological conditions (pH 7.4, 37 °C) [14]. As the benefit of this excellent performance, the utility of diazeniumdiolates has been an attractive application for designing antitumor drugs candidates [15–17].

The 1,4-naphthoquinone-based compound, plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) was isolated from *Plumbago* species, which have been extensively used for the treatment of rheumatic arthralgia, abscess and scrofula in Traditional Chinese Medicines (TCMs) [18]. Due to its attractive antitumor activity, it has been attracting a rising attention from cancer biologists. Although its exact mechanism of action varies in different systems, the most prominent role is as a ROS inducer which overcome the

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antioxidant threshold limit of the cancer cells, thus damaging cellular components to cause cell death [19,20].

On the basis of the above-mentioned studies and our own previous work on the modification of natural product plumbagin [21], we decided to introduce varying carboxylic acid side chains into the C-3 position of plumbagin, and then designed and synthesized a series of novel plumbagin/NO donor hybrids containing diazeniumdiolates with the aim to discover the promising derivatives with improved efficiency, selectivity and safety compared with their parent compound. Herein, a total of 12 plumbagin/NO donor hybrids (**9a**-**10c, 11a**-**16a**) have been synthesized with their structure determined by ^{1}H NMR, ^{13}C NMR and ESI/HRMS. Their *in vitro* cytotoxicities, intracellular level of NO and ROS production, and preliminary mechanism underlying their anticancer actions were also investigated.

2. Results and discussion

2.1. Chemistry

The synthesis of the O^2-protected diazeniumdiolates is depicted in Scheme 1. The diazeniumdiolate sodium salts (**3a**-**3c**), were synthesized according to a modified procedure reported by Lehmann [22], and then reacted with chloromethyl methyl sulfide and subsequent treatment with sulfuryl chloride to furnish chloromethyl NONOate (**5a**-**5c)** [23].

For the purpose of biological evaluation (see below). We also performed derivatives (**6a**-**6c)** by the stepwise synthesis of **3a-3c** with bromides with different alkyl chain length (m = 2, 3, 4), to investigate metabolic capabilities of various ester bonds in the presence of intracellular esterases.

The 1,4-dihydronaphthalenyl carboxylic acids (**8a** and **8b**) were prepared by combining plumbagin (**1**) with varying length of dicarboxylic acids (**7**) through oxidative decarboxylation and Kochi-Anderson addition following the procedure of Salmon-Chemin et al. [24].

The plumbagin/NONOate hybrids (**9a-10c**, **11a-16a**) were synthesized in moderate yields by condensation of semisynthesized plumbagin analogues (**8a** and **8b**) with intermediates (**5a-6c**). As shown in Scheme 2.

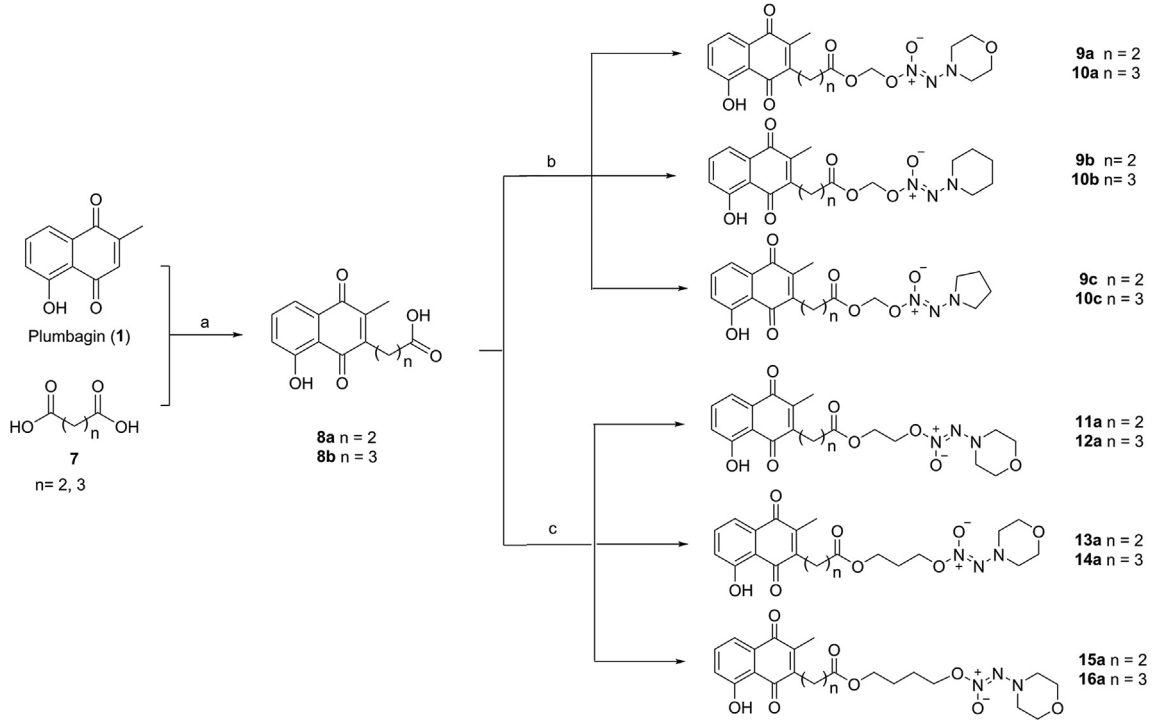
2.2. In vitro cytotoxic activity

We first evaluated the preliminary inhibitory effects of plumbagin (**1**) and compounds **9a**-**10c** on three tumor cell lines (MDA-MB-231, HepG2 and A549 cells) by MTT assay at 10 μM, As shown in Table 1, compound **10a** and **10b** showed inhibitory activity (>75% for all tested cell lines) superior to plumbagin (58.9−73.8%).

Given that various length of the linker in most case had certain differences to inhibitory activities, we also detected inhibitory activities of compounds **11a**-**16a** with the same secondary amine in the diazeniumdiolate moiety and different length of the carboxylic acids and diol linkers. While it was found that except **11a** which showed modest inhibitory activity (30−35%), five other compounds displayed compromised potency (<30% for all tested cell lines).

To comprehensively evaluate the cytotoxicities of these potent compounds. We further selected the representative panel of human cancer cell lines such as MDA-MB-231 (breast), A549 (lung), HepG2 (liver) and HCT-116 (colon) as a model for their anticancer activities. The human renal tubular epithelial cell line HK-2 and normal human liver cell line WRL-68 were also chosen to determine their nephrotoxicity and liver toxicity *in vitro*. As seen from the results summarized in Table 2, compounds **10a** and **10b** displayed the superior potent activities (IC_{50} values of 3.48−6.68 μM) in all the cancer cell lines after incubation for 48 h. Noticeably, they had weak inhibitory effects on normal HK-2 and WRL-68 cells, indicating their effective and selective cytotoxicities against cancer cells.

Structure and activity relationships (SARs) revealed that



Scheme 1. Reagents and conditions (a) NO, 40 psi, MeOH/Ether, NaOMe (1 eq), r. t., 24 h (b) $ClCH_2SCH_3$ (1 eq), DMF, K_2CO_3 (0.5 eq), r. t., 3 h (c) $Br(CH_2)_mBr$ (1.1 eq), Na_2CO_3(0.5 eq), dry DMF, 0 °C, 3 h (d) SO_2Cl_2(1.2 eq), CH_2Cl_2, 0 °C, 3 h.