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Synthesis, crystal structure, spectroscopic properties and potential anti-cancerous activities of four unsaturated bis-norcantharimides

Shuang-Shuang Cheng [a], Yan Shi [a], Xiao-Na Ma [a], Dian-Xiang Xing [a], Lian-Dong Liu [b], Yun Liu [a], Yun-Xue Zhao [c], Qi-Cheng Sui [a], Xue-Jie Tan [a, *]

[a] School of Chemistry and Pharmaceutical Engineering, Qilu University of Technology, Jinan, Shandong Province, 250353, PR China
[b] College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan, Shandong Province, 250014, PR China
[c] Department of Pharmacology, School of Medicine, Shandong University, Jinan, Shandong Province, 250012, PR China

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ABSTRACT

Four unsaturated norcantharimide (**UNCI**) dimers were synthesized and characterized by elemental analysis, ESI-QTOF-MS, FT/IR, UV–Vis, ^{1}H and ^{13}C NMR as well as single crystal X-ray diffraction. In addition, theoretical studies have been investigated to compare with the experimental findings. Introduction of various lengths of single bond link chains provides high conformational flexibility and thus unusual molecular and crystal structures for dimers. Two of the four dimers twist into helicate, but crystallize into centrosymmetric lattice; one adopts approximately centrosymmetric conformer, but packs into non-centrosymmetric polar space group ($P2_1$). Moreover, in vitro cytotoxic activities of four **UNCI** dimers and their corresponding saturated **NCI** dimers were evaluated. All four **UNCI** dimers are inactive and one **NCI** dimer shows modest cytotoxicity. These findings were compared with the relevant results in literature. It is found that the antitumor properties of **UNCI**/**NCI** dimers depend mainly on the length of link chains (the longer chain, the higher therapeutic efficacy) and have relationship with the double bond, which requires more experimental support.

1. Introduction

As archetypal small molecule protein phosphatase inhibitors [1], cantharidin (**CAN**) and norcantharidin (a demethylated form of cantharidin, also called demethylcantharidin, so abbreviated as **DMC**, Scheme 1) have been used worldwide as an anticancer agent since 1264 for the treatment of hepatoma, leukemia, pancreatic cancer, colon cancer, oral carcinoma, bladder cancer, breast cancer, lung cancer and digestive tract tumors [2]. Their ability to act against multidrug-resistant cells makes it an ideal compound for individualized cancer treatment [3]. Similarly, **CAN** possesses cytotoxicity to a series of normal cells, including gastrointestinal tract, urethra and kidney [2d], which delayed their use in the pharmaceutical industry. However, the organic chemistry has provided new and more potent derivatives with high activity against protein phosphatase enzyme and less toxicity profiles.

During the last five decades, thousands of analogues and derivatives have been synthesized and thoroughly investigated [2e], including **DMC**-platinum complexes [2b,2c,4], (nor)cantharimide series (abbreviated as **CAI/NCI**, Scheme 1) [2m,5], anhydride ring-opened series (especially those with only one free carboxylate) [1g,6] and so on. These analogues have demonstrated all kinds of antitumor activities and each has its own specific activity. Due to the possibility to incorporate any kinds of substituent in the nitrogen, as well as essentially equipotent inhibitory activity of the serine/threonine protein phosphatases 1 and 2A (PP1 and PP2A) with **CAN** (more potent than **DMC**) [7], **CAI/NCI** series show higher anticancer activities, and have been shown to inhibit xanthenes oxidase and to have antiplatelet effects on thrombin, arachidonic acid, collagen, and platelet-activating factor-induced aggregation [8]. So, derivatives of modified **CAI/NCI** are potentially useful as anticancer agents.

As we know, the type of heteroatoms in the bridge and in the anhydride cycle are very important, but, the presence of double bond (5,6-ene) has little effect on activity [5d,7,9]. 5,6-dehydrocantharidin (the unsaturated analogue of CAN, abbreviated as UCAN) and CAN have similar inhibition of PP2A [2e]. 5,6-dehydronorcantharidin (the unsaturated analogue of DMC,

* Corresponding author.
E-mail address: tanxuejie@163.com (X.-J. Tan).

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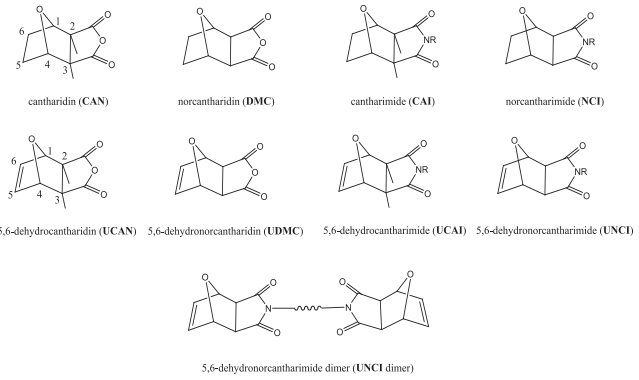
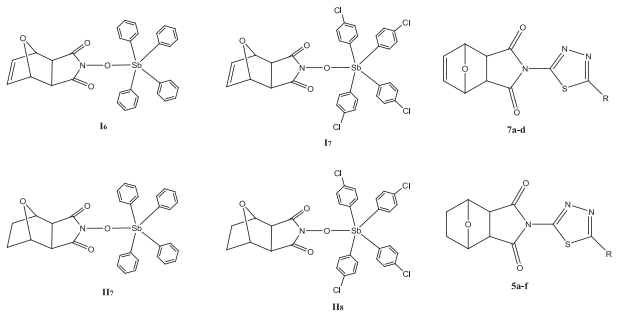


Scheme 1. Some important core structures of cantharidine analogues.

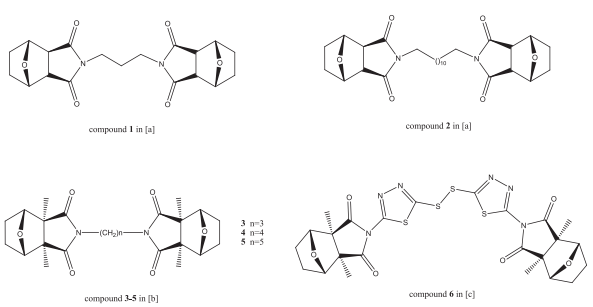
abbreviated as UDMC) and DMC still have similar inhibition of PP1, PP2A and PP2B [1f]. This suggests that UCAN and UDMC show similar anti-cancer and protein phosphatase activity as that of CAN and DMC (others think that the saturation of C5–C6 bond appears to affect the inhibitory activity, but not so crucial [5d]). More importantly, UCAN and UDMC are so easily synthesizable that they can often be used as the starting material in the synthesis of CAN and DMC [10]. Then, what's the difference between 5,6-dehydronorcantharimide (the unsaturated analogue of NCI, abbreviated as UNCI) and NCI? Surprisingly, the derivatives based on UNCI have hardly been explored in the literature, much less than that of UCAN and UDMC analogues. Wang et al. found that the arylantimony derivatives based on NCI and UNCI have similar in vitro antitumor activities [11]. For example, the complexes I_6, I_7, and II_7, II_8 in their paper have very high and similar antitumor activities against some cancer cells (Scheme 2). Li et al. [12] have investigated the antiproliferative activities of ten UNCI and NCI derivatives (Scheme 2, 5a-5f, 7a-7d), which displayed moderate and similar inhibitory activities against A549 and PC-3 cell lines with the IC50 values > 250.0 μmol/l (the IC50 of DMC were 44.8 and 201.0 μmol/l in their experiments). In one word, since UNCI and its analogues not only have simple chemical structures and less toxicity, but also retain the antitumor activities, is it possible for them to provide enormous possibilities for the science and industry of antitumor medicine? These uncertainties encourage us for further investigations related to these issues. We hope that these chemical modifications in the structure of UNCI could be a real and rapid way in developing new drug candidates.

On the other hand, the dimer structure is ubiquitous in natural products and dimeric molecules would be expected to show enhanced receptor affinity relative to their corresponding monomeric counterparts [13]. Dimeric compounds have been synthesized and studied for the treatment of cancer, HIV, Alzheimer, malaria and various parasitic diseases [14]. McCluskey et al. [15] reported the synthesis and anticancer activities of two **NCI** dimers (Scheme 3, compounds **1** and **2**), which displayed the highest levels of cytotoxicity against a series of cell lines among 35 **NCI** derivatives that they synthesized. In addition, Noda et al. [16] isolated three **CAI** dimers (Scheme 3, compounds **3** to **5**) from the Chinese blister beetle, *Mylabris phalerate* PALLAS (Meloidae). Their structures were determined based on spectroscopic and chemical evidence. But their cytotoxic activities were not demonstrated and have not been reported up to the present. The fourth example of **CAI** dimer is (3aR,3'aR,4S,4′S,7R,7′R,7aS,7'aS)-rel- 2,2'-[dithiobis(1,3,4-thiadiazole-5,2-diyl)]bis[hexahydro-3a,7a-dimethyl- 4,7-epoxy-1H-isoindole-1,3(2H)-dione (Scheme 3, compound **6**) reported by Kok et al. [17]. The compound showed cytotoxic potential on the entire four cancer cell lines examined. The cytotoxic pattern of the dimer on carcinoma cell lines was similar to that of similar single state. The major difference between them was observed in KG1a, where the dimer was still effective at 12.5 μg/ml but a higher concentration was required for that of similar single state. Compared with these **NCI** and **CAI** dimers, we are unaware, however, of any studies about the detailed structure and potential biological activities based on **UNCI** dimmers.

Another blank area is the crystal structure of the dimers. There have been no reports about the crystal structure of any **NCI**/**CAI** dimers. It is known that most small molecule drugs (>90%) are delivered in crystalline form [18] and at least half of marketed solid chemical drug substances exhibit polymorphism [19]. Meanwhile, medicinal chemistry requires robust reliable structures to accurately position key pharmacophoric units in the correct chemical space. It is this positioning that gives rise ultimately to the desired biological activity. To obtain a better understanding of the solid-state properties of these substances, it is necessary to identify and characterize crystal structures, and even to perform a polymorphic screening and physiochemical properties characterization, on potential drug candidates. This will help in eventually selecting a suitable form for further development and manufacturing. As part of our ongoing project studying novel unsaturated analogues of **DMC**/**NCI**, we have recently obtained systematical studies on a novel silver and singly protonated **UDMC** complex [20]. We now present our studies on the synthesis and thorough spectral and X-ray crystallographic characterization of four **UNCI** dimers (shown in Scheme 4). **UDMC-DETA** and **UDMC-TETA** are new; the rest two dimers have appeared before [21], but neither detailed structure information nor any properties have been reported. It is a substantial challenge to identify all these dimers' single-crystal structures because they are usually isolated as thin powders, which showed no tendency to crystallize. At last, all dimers are evaluated for their in vitro cytotoxic activity against



Scheme 2. Two sets of similar compounds with similar antitumor activities reported in Refs. [11] and [12]. Their name abbreviations are maintained as in the original report.



Scheme 3. The structures of some (nor)cantharimide (**NCI**/**CAI**) dimers reported in literature [15–17].