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Synthesis and structure evaluation of a novel cantharimide and its cytotoxicity on SK-Hep-1 hepatoma cells

Stanton Hon Lung Kok,[a,b] Chung Hin Chui,[a,b,c] Wing Sze Lam,[a,b] Jien Chen,[a,b] Fung Yi Lau,[c] Raymond Siu Ming Wong,[c] Gregory Yin Ming Cheng,[c] Paul Bo San Lai,[d] Thomas Wai Tong Leung,[e] Michael Wing Yiu Yu,[a,b] Johnny Cheuk On Tang[a,b,*] and Albert Sun Chi Chan[a,b,*]

[a]*Lo Ka Chung Centre for Natural Anti-Cancer Drug Development, Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, PR China*
[b]*State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Shenzhen, PR China*
[c]*Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, PR China*
[d]*Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, PR China*
[e]*Oncology Center, The Hong Kong Sanatorium and Hospital, Hong Kong, PR China*

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Abstract—A remarkable control of the potency of cantharimide is described based on the electronic properties of functional group and it exhibits a relatively less toxic effect to the non-malignant hematological disorder bone marrow cells.

Cantharidin, *exo*,*exo*-2,3-dimethyl-7-oxobicyclo[2.2.1] heptane-2,3-dicarboxylic acid anhydride, is an active ingredient isolated from the Chinese blister beetles *Mylabris phalerata* or *M. cichorii*.[1] The dried bodies of these beetles are sold in China and have been used by Chinese as a natural remedy for the past 2000 years. Cantharidin has been reported to be active against various human cancers,[1] in particular to hepatocarcinoma; however, its severe renal toxicity limits the development as chemotherapeutic agents.[2] Although numerous physiological and biochemical studies were made in order to find the cause of growth inhibition and cell death, the critical molecular mechanism of action and pathways remain unclear.

Synthetic norcantharidin (the demethylated cantharidin analogue)[3–9] and cantharimide (the anhydride oxygen atom replaced by nitrogen atom) derivatives illustrate their clinical potential as a result of similar growth inhibition activity as cantharidin having a strong suppression of renal and gastrointestinal toxicity[2] (Fig. 1).

2-Aminobenzothiazole derivatives have long been recongnized as therapeutic active skeletons which are useful for making antitumor agents,[10–15] neurotransmission blocker,[16–18] calmodulin(CaM) antagonists,[19] and neuroprotective agents,[20,21] and other biological activities.[22] For example, 2-(4-aminophenyl)benzothiazoles (Fig. 2) represent a novel class of potent and selective antitumor agents.[10,11,13] Our earlier reports demonstrated that cantharimide analogues bearing 2-aminobenzothiazole moiety were effective to minimize the original cytotoxicity whilst maintaining potency toward cancer cell lines in vitro.[23,24]

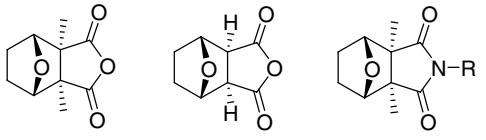

Figure 1. Cantharidin and its derivatives.

Keywords: Cantharimide; 2-Aminobenzothiazole; Neoplasm.
* Corresponding authors. Tel.: +852 3400 8727; fax: +852 2364 9932; e-mail addresses: bccotang@inet.polyu.edu.hk; bcachan@inet.polyu.edu.hk

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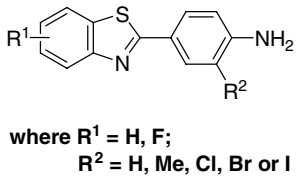
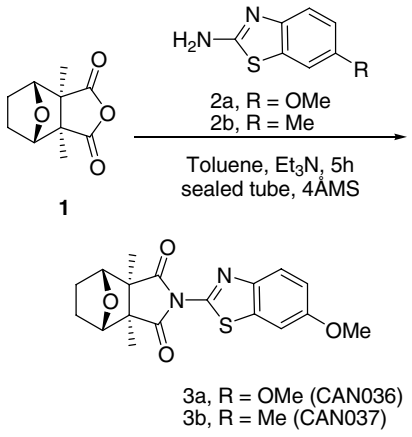


Figure 2. 2-(4-Aminophenyl)benzothiazole and its derivatives.

Based on the principal chemical structure of cantharidin, we have synthesized a number of its family analogues and named as CAN series. After obtaining a family of cantharidin analogues, we further screened its possible cytotoxic activity using a hepatocellular carcinoma cell line SK-Hep-1. Two of those synthetic samples were selected and screened to show a significant cytotoxic response on hepatoma cell line according to their striking differences in electronic properties and their potencies, which were comparable to that of cantharidin.

In this paper, we report the first synthesis of cantharimide **3a** (CAN036) and a known **3b** (CAN037)[12] derived from cantharidin (CAUTION, avoid skin contact and inhalation) and 2-aminobenzothiazole derivatives, using the standard 'one-pot' condensation reaction, enabling the multiple-parallel synthesis of cantharimide libraries (Scheme 1). Thus, condensation of the 2-aminobenzothiazole with cantharidin **1** in the presence of toluene:triethylamine mixture (3:1) gave the corresponding imide in moderate to high yield. The 2-aminobenzothiazole derivatives are either commerically avaliable or prepared following a standard protocol from aniline.[25] In addition, we report the effect of two cantharimides **3a** and **3b** on the growth of a hepatoma cell line SK-Hep-1 in vitro using a standard MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium]) assay.

We studied the possible cytotoxic activity of cantharidin analogues by means of MTS assay. As shown in Figure 3, **3a** (i.e., CAN036) showed a comparable MTS_{50} activity (50% of MTS reduction ability by the



Scheme 1. Condensation of anhydride with 2-aminobenzothiazole derivatives.

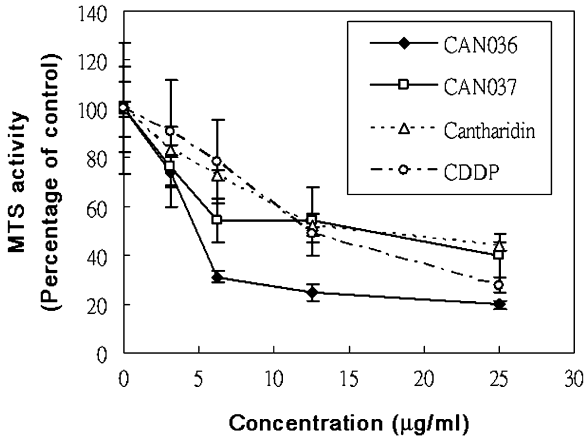


Figure 3. MTS activities of **3a** (CAN036) and **3b** (CAN037) on SK-Hep-1 cell after 48 h. Results are shown as means ± standard derivation from triplicate tests. It is a representative result from three independent experiments and similar results obtained.

chemical treated cell as compared with control, 3–6 μg/mL) as cantharidin (MTS_{50} = 6.25–12.5 μg/mL) and cisplatinum on SK-Hep-1 hepatocellular carcinoma cell line (MTS_{50} = 6.25–12.5 μg/mL).[24] In contrast, **3b** bearing methyl group illustrated a weaker activity against cancer cell line (MTS_{50} = 12.5–25 μg/mL). This is the first important characteristic of the cantharidin-mimics that its relative reactivity toward anticancer activity may be controlled by the introduction of electron withdrawing group at 6-position. In fact, the magnitude of this effect was found to be approximately four times stronger on SK-Hep-1. According to our preceding studies on 6-OCF_3 analogue, a similar observation was found in which a direct relationship between electron withdrawing group and cytotoxic potency provides a general trend of finding the most potent analogues in the cantharimide series.[23] Apoptotic feature including cell shrinkage could readily be observed in SK-Hep-1 cell after incubating with CAN036. Similar morphological change could also be identified in other human solid tumor cell lines including the LNCaP prostate carcinoma and the MCF-7 breast cancer cells (Fig. 4).

In order to confirm that apoptosis was induced in the majority of SK-Hep-1 cells after treating with CAN036 and CAN037, we measured their caspase 3 and TUNEL activities. As shown in Figure 5, both of caspase 3 and TUNEL activities were significantly elevated, especially when the concentration of both compounds was at 25 μg/mL. Since caspase 3 is the central machinery for apoptosis while DNA fragmentation is the main description for programmed cell death, we believed that apoptotic pathway was recruited.

Since bone marrow suppression and gastrointestinal and urinary tract toxicity are still important side effects of cantharidin, we further tested their toxicity on non-malignant hematological disorder bone marrow (Fig. 6). Morphologically, both CAN036 and CAN037 showed cytotoxic action on SK-Hep-1 cells by inducing loss of colony formation potential and cell shrinkage after

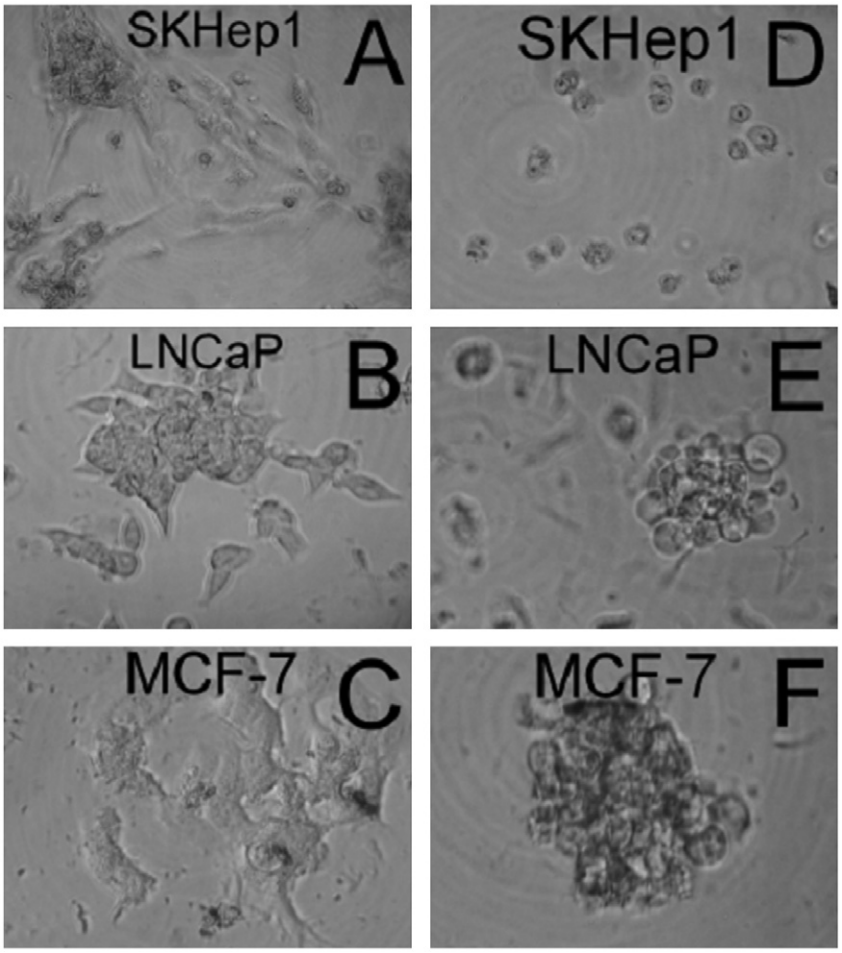


Figure 4. Morphological change of SK-Hep-1, LNCaP, and MCF-7 cancer cells (A, B, and C) incubated with 0.1% DMSO as control; (D, E, and F) incubated with 25 μg/mL (0.1% DMSO) of **3a** (CAN036) for 48 h.

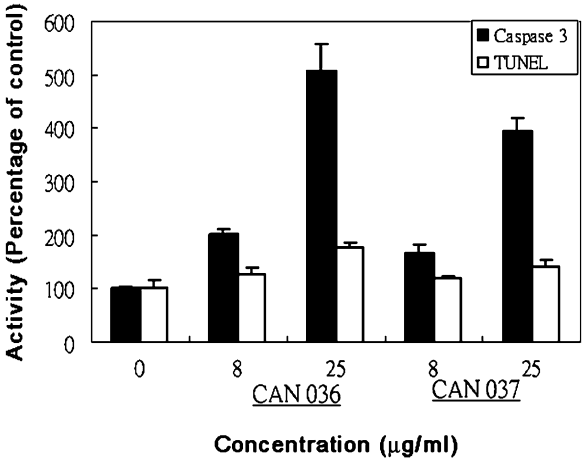


Figure 5. Caspase 3 and TUNEL activity assays to study the possible apoptotic potential of both **3a** (CAN036) and **3b** (CAN037), respectively.[27] For both assays, results are shown as means ± standard derivation from triplicate tests. It is a representative result from three independent experiments and similar results obtained.

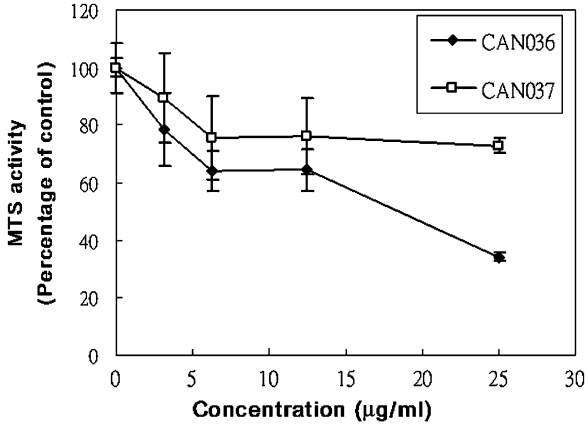


Figure 6. MTS activity of CAN036 and CAN037 on non-malignant hematological disorder bone marrow after 48 h. Results are shown as means ± standard derivation from triplicate tests.

48-h of incubation. However, bone marrow samples were found to be less sensitive to CAN037 that was consistent to our expectation. Although the CAN036 exhibits a relatively high toxicity to normal bone marrow sample, the MTS_{50} shows a 2- to 4-fold less sensitivity than cantharidin. In other words, the results of our study of the comparative cytotoxic properties of the electron withdrawing group at 6-position alongside the corresponding electron donating analogue showed that

CAN036 exhibits the most potent cytotoxic activity with a high selectivity to tumor cells. This fundamental relationship should prove its usefulness in the design of new analogues possessing further enhanced properties. A similar dose-dependent relationship of cantharidin and CAN037 on SK-Hep-1 was also observed.

Here we demonstrate that CAN036 could induce cytotoxicity on SK-Hep-1 hepatoma cell in a dose-dependent manner. However, investigation for the mechanism of a potential drug including the understanding of its signal transduction pathway is considered to be clinically significant.[26] In addition to caspase 3 activation, our preliminary data suggest that cell cycle arrest is involved. This may also explain why the non-malignant hematological disorder bone marrow sample was less sensitive to the cytotoxic response of CAN036 since in the bone marrow sample, there would be fewer stem cells which are being actively involved in cell cycle when compared with those of the cancer stem cell even the generation time of bone marrow cells was comparable to that of cancer cells.

In conclusion, CAN036 was first synthesized to show antitumor effect as a cantharidin-mimic and it showed a dose-dependent inhibition of tumor cell proliferation over the range from 3 to 6 μg/mL. Lack of selectivity is one of the limitations of antitumor drugs and it is a common misunderstanding of some researchers seeking only the most potent drug without consideration for the toxicity of non-malignant cells. The inherent resistance of tumor cells remains another critical problem of treating malignancy. Thus, the development of new antitumor agents relies on the balance between their cytotoxicity on the cancer and non-malignant cells. In terms of MTS activity, our initial SAR studies demonstrated that the introduction of electron withdrawing group of 2-aminobenzothiazole at 6-position was well designed to enhance the cytotoxicity to cancer cells while reducing the non-malignant cell toxicity and it would be a beneficial effect of molecularly targeted chemotherapy. The facile one-step coupling of cantharidin with 2-aminobenzothiazole for the synthesis of small molecule inhibitors opens a new regimen for the treatment for cancers of varying etiologies.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.039.

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