Bioorganic Chemistry **28,** 266–272 (2000)
doi:10.1006/bioo.2000.1178, available online at http://www.idealibrary.com on IDEAL®

A Simple Procedure for Preparation of *N*-Thiazolyl and *N*-Thiadiazolylcantharidinimides and Evaluation of Their Cytotoxicities against Human Hepatocellular Carcinoma Cells

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Received January 28, 2000

We made an effort to prepare effective cantharidinimides by heating the reactants **1** and **2a–j** to 200°C with toluene and triethylamine to provide 10 *N*-thiazolyl- and *N*-thiadiazolylcantharidinimides **3a–j** in high yields of 48–91%. All of the synthetic compounds were tested for their capability to suppress growth of the human hepatocellular carcinoma cell lines, SK-Hep-1 and Hep 3B. The results showed that compound **3f** was the most potent, and it was more cytotoxic than cantharidin.

Key Words: cantharidin; *N*-thiazolylcantharidinimide; *N*-thiadiazolylcantharidinimide; human hepatocellular carcinoma cell; cytotoxicity.

INTRODUCTION

Cantharidin **1** is found in *Mylabris caraganae* and various other insects. In clinical studies it has been shown to possess antitumor and antihepatoma properties. It is reported to have extremely high potency as well as showing toxic properties (*1–3*), which makes it useless in the clinic. It is used as a standard in research confined to vetertinary medicine due to it’s irritant and vesicating effects. In a search for less toxic analogues of cantharidin or cantharidinimide derivatives, a slightly modified structure has been synthesized in an analogous manner (*4*). Cantharidin **1** can undergo a ring-opening reaction to become dicarboxylic acid and can be prepared as a series of imides by heating with primary amine. The formation of products of the *N*-aliphatic imides is more rapid than that of aromatic imides (*5*). The present study shows that the characters of amine basicity and chosen temperature are crucial, and the characters of the group and their position on the aromatic ring also influence yields. In order to obtain novel types of related imides and to study the scope of these synthetic reactions, the same technique was applied to the reaction of compound **1** with thiazolylamine or thiadiazolylamine in a high-pressure tube with dry toluene and TEA (Triethylamine) heated to ca. 200°C. This method gave good yields after evaporation and

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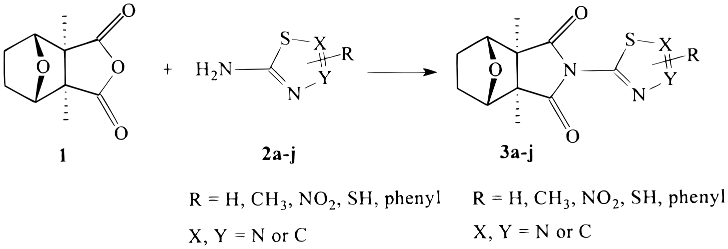


SCHEME 1.

purification by silica gel column chromatography and recrystallization in methanol. (Scheme 1).

RESULTS AND DISCUSSION

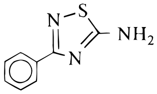
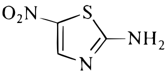
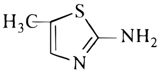
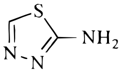
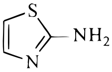
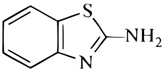
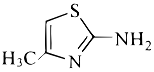
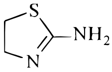
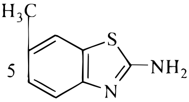
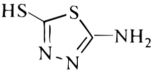
As shown in Table 1, the *N*-thiazolyl- and *N*-thiadiazolylcantharidinimides **3a–3j** could be prepared by means of the pressure technique synthesis. The yields vary from 48% to 91% and show a trend compatible with expected basicity, and characters of the thiazolyl and thiadiazolyl ring groups influencing compound **2**. High yields were obtained for **3a** to **3d**. The NH_2 basicities of aminothiazolylcantharidinimides and aminothiadiazolylcantharidinimides are unknown but will be slightly different between one of corresponding aminothiazols which has an electron deficiency of the thiazol and thiadiazol rings. Variations in yields of **3a, 3g**, and **3j** may perhaps reflect inductive electron donation and electron withdrawal by the thiadiazolyl ring, since an inductive effect will inversely increase with distance between the three nitrogen atoms and sulfur atom. The results obtained with **3b, 3e, 3d, 3e, 3f**, and **3h**, however, strongly confirm the influence of amine nucleophilicity and their basicities, and the characters of functional group position on the ring. Compound **2f** exerted the most electron-withdrawing capability with resonance and induction effects, and the formation of cantharidinimide appeared to become more difficult. It should be noted that the more conjugated character, the higher the yield that would be obtained, as is seen in **3c** $>$ **3i**. The preparative technique was also influenced by other factors that can cause strong variations in the results. The formation of cantharidinimides might be expected via ring opening and dehydrated reaction steps and hence the reaction temperature was also a crucial factor in this formation.

The potential cytotoxicity of the prepared cantharidinimides was investigated against hepatocellular carcinoma cell lines, Hep 3B (*6*) and SK-Hep-1 (*7*) and evaluated using MTT cell viability assays (Table 2). It has been shown that viable cell numbers correlate with optical density as determined by the MTT assay (*8,9*).

Being comparable in cytotoxicity to cantharidin, the IC_{50} values of all of the cantharidinimide derivatives (**3a–j**) were 0.6 to 900 μM, and of cantharidin were 2 to 4 μM and of **C-N** (Cantharidinimide) and **C-M** (*N*-Methylcantharidinimide) were completely inactive up to the highest concentration tested (2000 μM). Since **C-M** has been produced as an antihepatoma drug in China (*10*), the reason that it was noncytotoxic to the tested hepatoma cell lines was unknown. The lack of activity for

TABLE 1

Preparation of Cantharidinimide Derivatives (**3a–3j**)[a]

1		**2a**	**3a** (91)	6		**2f**	**3f** (69)
2		**2b**	**3b** (89)	7		**2g**	**3g** (60)
3		**2c**	**3c** (87)	8		**2h**	**3h** (55)
4		**2d**	**3d** (86)	9		**2i**	**3i** (54)
5		**2e**	**3e** (69)	10		**2j**	**3j** (48)

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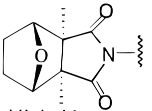
[a] **3a–3j**: Cantharidinimides.

[b] **2a–2j**: Amines.

[c] The yields obtained after purification by chromatography on silical gel.



Cantharidinimide

C-N suggested that the presence of a thiazole or thiadiazole moiety is probably important for the cytotoxic properties of this series. The IC_{50} values of thiazolylcantharidinimides decreased in the order **3b** $>$ **3d** $\approx$ **3e** $>$ **3c** $>$ **3h** $\approx$ **3i** $>$ **3f**. In this study, the only compound showing higher cytotoxicity than cantharidin was **3f** in which a nitrosubstituent was introduced on the 5′-position of thiazole group of **3c**; while compound with a methyl substituent at 5′- or 4′-position of thiazole group of **3c** reduced the cytotoxic activity and the position of methyl- also affected the biological activity, it produced three- to five-fold difference effects on the cell (**3b** vs **3d**). The saturation of the 4′-, 5′-double bond of thiazole group led to a four-fold increased in cytotoxicity against tumor cell lines tested (**3i** vs **3c**). The result showed that the presence of electron withdrawing substituents (**3h**, **3i**, and **3f**) markedly enhanced cytotoxicity (**3b**, **3d**, and **3e**).