A Simple Procedure for Preparation of *N*-Thiazol, Thiadiazol, Pyridyl and Sulfanylamidocantharidinimines Analogues and Evaluation of Their Cytotoxicities against Human HL-60, MCF7, Neuro-2a and A549 Carcinoma Cell

Ing-Jy Tseng,^a Shioh-Yunn Sheu,^b Ying-Tzu Chen,^c Chu-Yun Huang,^c Ching-Tung Lin,^e and Pen-Yuan Lin^{*d}

^aSchool of Geriatric Nursing and Care Management, Taipei Medical University; ^bDepartment of Pharmaceutical Analysis, School of Pharmacy, Taipei Medical University; ^cDepartment of Clinical Pharmacy, School of Pharmacy, Taipei Medical University; ^dDepartment of Pharmaceutical Sciences, School of Pharmacy, Taipei Medical University; 250 Wu-Hsing St., 11031 Taipei, Taiwan; and ^eDepartment of Chemistry, Tam-Kang University; 151 Yingzhuan Rd. Danshui Dist, 25137 New Taipei City, Taiwan. Received May 7, 2012; accepted August 3, 2012

The lab made an effort to prepare some biological active cantharidinimines by heating the reactant **1** and **2a–g**, **5h–i** and **7j–r** amines to suitable temperature with ethanol to provide 18 *N*-thiazolyl-, sulfanyl-, aminopyridyl-, bromopyridyl-, alkylpyridyl- and hydroxypyridylcantharidinimines **3a–g**, **4a–c**, **6h–i** and **8j–r** in yield of 4–77% (Chart 1). These cantharidinimine derivatives were tested for their capabilities to suppress growth of the human carcinoma cell lines, HL-60, MCF7, Neuro-2a and A549, because the incidence rate is more prominent in Asian countries than western countries. Compounds **3c–d** and **6h–i** were found to have some antitumor activity in HL-60 but less activity in MCF cell and compounds **8j–l** displayed some inhibition effects to A549 cell line, but less effect to Neuro-2a cell line. Compounds **8m–r** had no cytotoxic effect against both cell lines. The cytotoxic effects of these cantharidinimine compounds seemed to be better than the cantharidinimide compounds which we had mentioned several years ago.

Key words cantharidin; *N*-azayl-thiadiazolylcantharidinimine; cytotoxicity; sulfanyl-thiadiazolylcantharidinimine; thiazolyl-thiadiazolylcantharidinimine

Cantharidin **1** is found in *Mylabris caraganae* and various other insects and shows extremely high vesicant potency and toxic properties.^{1–4)} Recent reports have indicated that the action of cantharidin in mice is associated with binding to phosphorylase 2A in liver cytosol and inhibition of its phosphorylase phosphatase activity.^{5–9)} In our previous studies,¹⁰⁾ increasing the solubility of the title compounds decreased the toxicity while maintaining the biological activities. *N*-Thiazolyl, sulfanylcatharidinimides with a better solubility showed high cytotoxicity against human carcinoma cell lines. The present study shows that characters of amine basicity and the chosen temperature are crucial. Furthermore the characters of the groups and their position on the aromatic or azalyl ring also influence yields. In order to obtain novel types of related imines and study the scope of these synthetic reactions, the same technique was applied to the reaction of compounds **1** with thiazolyl, thiadiazolyl, sulfanyl, or azalylamines in high-pressure tube with dry ethanol and heated to ca. 140°C. This temperature and reaction time influence the product yields. The method might give good yields after evaporated and recrystallization in methanol (Table 1). In our previous research,¹¹⁾ we synthesized many cantharidinimide derivatives by modification with aliphatic, azalyl, thiazolyl, thiadiazolyl and pyridyl groups. Those compounds exhibited a certain degree of cytotoxic activity on HL-60, Hep3B and HepG2,^{3–5)} NUGC (Human Gastric carcinoma cells), HONZ-1 (Human Nasopharyngeal carcinoma cells)^{11–16)}. All of these compounds contained an imide group. Thus, we use this feature to synthesize different imine derivatives to study effects of anti-tumor therapy and cytotoxicity as carcinoma cells inhibitors

and therapy sources.

Results and Discussion

With the high-pressure technique, we obtained cantharidinimines (after recrystallization from methanol) in yields of 4–77%, as listed in Table 1. The highest yields were obtained from dimethylpyridyl **7q** as well as thiazoylamines **2a–e**. The NH₂ basicities of aminothiazolyl-, aminosulfanyl- and pyridylcantharidinimine are unknown but will be slightly different between one of corresponding aminothiazoyl-, aminosulfanyl- and pyridylamine. There is an electron deficiency of the thiazol, sulfanylamide, aza and aminopyridine rings. Compounds **2h–i** which had the sulfanyl and pyridyl ring exerted the most electron withdrawing capability with resonance and induction effects. The formation of cantharidinimine appeared to become more difficult hence showed the very low yields, **6h** and **6i**. We have noted in our previous works that the more conjugated character, the higher the yield obtained. Withdrawing functional groups on the aza ring always gave a lower yield than the electron donating group. Compounds **4a–c** were obtained from the reduction of compounds **3a–c** by using reduced reagent, NaBH₄ with ethanol at room temperature and refluxed to give yields 28%, 14% and 2%. Their NMR values displayed the singlet signal at δ 5.78, 5.80 and 5.87 for one H (reduced hydrogen) and IR showed the absorption of reduced of imines and C=O at 1700, 3492 cm^{–1} for **4a**, at 1701, 3440 cm^{–1} for **4b** and at 1692, 3417 cm^{–1} for **4c**, and all of their mass spectra were obtained on a Joel JMX-DX300 for high resolution spectrometer. The variable yields of compounds **2d–g**, **2h–i**, **8j–r**, which had parent aryl, azalyl, sulfanyl and pyridyl compounds might reflect the inductive electron negative, bulky steric, or the anhydride oxygen linkage electrical

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* To whom correspondence should be addressed. e-mail: lpy0620@tmu.edu.tw

Chart 1

density effect influencing the yields.

The results of these yields strongly confirm the influence of amine nucleophilicity and basicity. The potential cytotoxicities of prepared cantharidinimines were investigated against human myeloid leukemia cell (HL-60), human breast adenocarcinoma (MCF7, from ATCC), rat neuroblastoma (Neuro-2a, N₂a, from ATCC) and human lung carcinoma (A549, from ATCC) lines and evaluated. Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay (Table 2) for cytotoxicity tests to show cantharidin and its cantharidinimine analogues listed in Table 1, **3a–g**, **6h–i**, **8j–r**. Cantharidin **1** was more toxic and exhibited greater cytotoxicity. While synthesized cantharidinimines were less toxic but also exhibited inhibitory effects. Being comparable in cytotoxicity to cantharidin, the IC₅₀ values of all of the cantharidinimine derivatives **3a–8r** were 6.0 to 100 μM, and of cantharidin were 4.6–82.8 μM and of **3a–c** and **3g** were completely inactive up to the high concentration tested (100 μM). Compounds **3d–f** have thiadiazolyl functional group showed a higher cytotoxicity than **3a–c** and **3g**. Compounds **3d** and **3f** had azoyl ring structure that gave the same cytotoxicities. Sulfanilamide derivatives with two methyl substitutes, **6h–i**, showed high cytotoxicities to HL-60 cell, **6h** displayed more cytotoxicity than **6i**, with a different position of pyridyl ring. Unfortunately they all showed inactivities up to the MCF7 cell line. Compounds **8j–r** had amino substitutes and pyridyl group which might show that the presence of electron donating substitute, **8l**, **8m**, **8n** and **8r** enhanced cytotoxicities remarkably. Compounds **8j–l** showed cytotoxic activity to A549 cell line but displayed no activity toward Neuro-2a cell line. It might have some effects for other cell lines and the

less cytotoxicity might be used to become as prodrug references. Compared to our previous works, we noticed that these cantharidinimine derivatives had more cytotoxic effects than cantharidinimides, especially in the presence of sulfanilamide groups, and the diamino derivatives of cantharidinimine also showed the activities better than its cantharidinimide derivatives. We will continue this works in the future.

Experimental

Test Samples Chinese blister beetles were extracted with a water–ethanol ratio of 1:1 solution, filtered with celite, purified by chromatography on silica gel, and then recrystallized with ethanol to give cantharidin **1**. Compound **3–8** were prepared from cantharidin and primary amines in the presence of triethylamine in ethanol in a high-pressure tube. IR spectra were recorded on a Thermo Mattson IR300 spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer in pyridin-*d*₅ solution with respect to the corresponding solvent as the internal standard. ¹H- and ¹³C-NMR spectra were measured at 500 and 125 MHz, respectively. NMR experiments included ¹H–¹H correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), ¹H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC). All chemical shifts were expressed in ppm (δ). Multiplicities of all carbon signals were verified through DEPT experiments. Mass spectra were measured on a Finnigan/Thermo Quest MAT 95XL, JEOL JMX-HX 110 and JEOL JMX-DX 300 for high or low resolution spectrometers.¹⁴⁾ For the chromatographic analysis Merck Silica Gel 60 (230–400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased

Table 1. Preparation of Cantharidinimines

1		3a (36) ^b	6		3f (47)
	2a ^a			2f	
2		3b (39)	7		3g (10)
	2b			2g	
3		3c (20)	8		6h (6)
	2c			5h	
4		3d (6)	9		6i (3)
	2d			5i	
5		3e (4)			
	2e				
10		8j (19)	11		8k (22)
	7j			7k	
13		8m (13)	14		8n (8)
	7m			7n	
15		8o (11)			
	7o				
16		8p (34)	17		8q (77)
	7p			7q	
18		8r (24)			
	7r				

^a2a–2g, 5h–i, 7j–r: amines. 3a–g, 6h–i, 8j–r: cantharidinimines ^bThe yields obtained after purification by chromatography on silica gel. ^cImine linkage position

Cantharidinimine

Table 2. Cytotoxicity of Cantharidin 1, *N*-Thiazol-, *N*-Thiadiazol-, and Sulfanylcatharidinimines in Human HL-60, MCF7, Nuro-2a, A549 Carcinoma Cells

Cell line	IC ₅₀ (μM) ^{a)}									
	1	3a	3b	3c	3d	3e	3f	3g	6h	6i
HL-60	7.2	>100 ^{b)}	>100	>100	17.7	41.9	18.1	>100	6	35.9
MCF7	82.8	>100	>100	>100	>100	>100	>100	100	>100	>100
	1	8j	8k	8l	8m	8n	8o	8p	8q	8r
Nuro-2a	70.4	>100	>100	>100	>100	>100	>100	>100	>100	>100
A549	4.57	21.6	21.1	31.8	>100	>100	>100	>100	>100	>100

^{a)} IC₅₀ was calculated after 48 h of continuous drug exposure. Values are the mean of three to four experiments with coefficients of variation of 5–10%. ^{b)} No indication of the cytotoxicity was considered indication of IC₅₀ values substantially rather than the highest doses assayed.

from Sigma-Aldrich Co. and E. Merck.

General Procedures General procedures were followed for the reaction of primary amines with cantharidin. These compounds were prepared according to similar procedures, and the reaction took place in high-pressure tubes. Cantharidin was added to a tube containing 3 mL of dry ethanol and triethylamine, and the solution was stirred and heated to *ca.* 200°C. After being stirred for 2 h, the mixture was evaporated, and the residue mass was purified by column chromatography and recrystallized from methanol.

Antineoplastic Bioassays

Preparation of Test Solution and Assay Procedure for Cytotoxicity Media and sera for cell culture were purchased from Gibco/BRI (Grand Island, NY). Most chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). HL-60 (Human promyelocytic leukemia), MRC-5 (human hepatocellular H460 (non-small cell lung cancer tumor cell) and Hep G2 (human hepatocellular carcinoma cell line) obtained from American Type Culture Collection (ATCC) (Rockville, MD, U.S.A.). Hep G2 and MRC-5 cells were maintained as monolayers in Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, 100 µM nonessential amino acids, and 1 mM glutamine were prepared for cell culture.

MTT Assay for Cellular Viability Cells were seeded into 96-well plates and allowed to adhere for 24 h before drug were introduced. Then drugs and media were removed, and each well was treated with 100 µL of 500 µg/mL MTT in culture medium. The plates were centrifuged at 450×g for 10 min, and supernatants were removed and replaced with 100 µL of dimethyl sulfoxide (DMSO). Absorbance, as a measure of viable cell number, was read the following day in a model MA310 automated enzyme-linked immunosorbent assay (ELISA) plate reader at a wavelength of 550 nm. IC₅₀ values were obtained by a linear regression analysis of percent absorbance *versus* log drug concentration. It has been shown previously that viable cell numbers correlate with optical density as determined by the MTT assay.^{14,15)}

N-(Thiazolyl)cantharidinimine (**3a**): mp 173–175°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.28 (s, 6H, CH₃×2), 1.76–1.79 (m, 2H, CH₂), 1.85–1.89 (m, 2H, CH₂), 4.74 (t, 2H, *J*=2.4, 2.5 Hz, OCH×2), 7.32 (d, 1H, *J*=3.5 Hz, thiazolyl N-CH), 7.79 (d, 2H, *J*=3.4 Hz, thiazolyl S-CH); IR (KBr) 1721 (amide) cm⁻¹; high resolution (HR)-MS (EI, 80 eV) Calcd for C₁₃H₁₄N₂O₃S: 278.0721. Found: 278.0721.

N-[2-(5-Methylthiazolyl)]cantharidinimine (**3b**): mp 149–150°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.26 (d, 6H, *J*=5.6 Hz, CH₃×2), 1.73–1.76 (m, 2H, CH₂), 1.83–1.88 (m, 2H, CH₂), 2.48 (s, 3H, CH₃), 4.72 (t, 2H, *J*=2.6, 2.5 Hz, OCH×2), 7.42 (s, 1H, thiazolyl N-CH); IR (KBr): 1724 (carbonyl group) cm⁻¹; HR-MS (EI, 80 eV) Calcd for C₁₄H₁₆N₂O₃S: 292.0874. Found: 292.0874.

N-(2-Benzothiazolyl)cantharidinimine (**3c**): mp 158–160°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.31 (s, 6H, CH₃×2), 1.77–1.80 (m, 2H, CH₂), 1.86–1.91 (m, 2H, CH₂), 4.77 (t, 2H, *J*=2.6, 2.6 Hz, OCH×2), 7.42 (t, 1H, *J*=7.9, 8.0 Hz, H-5), 7.50 (t, 1H, *J*=8.2, 7.5 Hz, H-6), 7.89 (d, 1H, *J*=8.0 Hz, H-4), 8.12 (d, 1H, *J*=8.2 Hz, H-7); IR (KBr): 1723 (carbonyl group) cm⁻¹; HR-MS (EI, 80 eV) Calcd for C₁₄H₁₆N₂O₃S:

328.0882. Found: 328.0882.

N-[2-(1,3,4-Thiadiazolyl)]cantharidinimine (**3d**): mp 201–203°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.31 (s, 6H, CH₃×2), 1.78–1.79 (m, 2H, CH₂), 1.87–1.89 (m, 2H, CH₂), 4.76 (t, 2H, *J*=2.5, 2.4 Hz, OCH×2), 9.14 (s, 1H, thiadiazolyl H); IR (KBr): 1723 (carbonyl group) cm⁻¹, HR-MS (EI, 80 eV) Calcd for C₁₂H₁₃N₃O₃S: 279.0688. Found: 280.0942.

N-[5-(3-Phenyl-1,2,4-thiadiazolyl)]cantharidinimine (**3e**): mp 172–174 (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.25 (s, 6H, CH₃×2), 1.72–1.83 (m, 2H, CH₂), 1.88–1.91 (m, 2H, CH₂), 4.78 (t-like, 2H, OCH×2), 7.47 (t-like, 3H, overlap with H-4'), H-3', H-5', H-4'), 8.35 (dd, 2H, *J*=9.1, 1.2, 2.2 Hz, H-2', H-6'); IR (KBr) 1723 (amide) cm⁻¹, HR-MS (EI, 80 eV) Calcd for C₁₈H₁₇N₃O₃S: 355.1000. Found: 355.1000.

N-[3-(5-Phenylpyrazolyl)]cantharidinimine (**3f**): mp 197–198°C; ¹H-NMR (CDCl₃, 500 MHz): δ 1.26 (d, 6H, *J*=8.0 Hz, CH₃×2), 1.76–1.77 (m, 2H, CH₂), 1.85–1.87 (m, 2H, CH₂), 4.72 (t, 2H, *J*=2.6, 2.4 Hz, OCH×2), 6.98 (s, 1H, H-4), 7.37 (t-like, 1H, H-4'), 7.44 (dd, 2H, *J*=7.7, 7.3 Hz, H-3', H-5'), 7.73 (d, 2H, *J*=7.5 Hz, H-2', H-6'); IR (KBr): 1706 (carbonyl group), 3284 (secondary amine) cm⁻¹, HR-MS (EI, 80 eV) Calcd for C₁₉H₁₉N₃O₃: 337.1425. Found: 337.1426.

[4-(1,2,4-Triazolyl)]cantharidinimine (**3g**): mp 240–241°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.25 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), 1.80–1.90 (m, 4H, CH₂×2), 4.73 (t, 2H, *J*=2.6, 2.4 Hz, OCH×2), 8.31 (s, 2H, triazolyl H); IR (KBr): 1739 (carbonyl group) cm⁻¹; HR-MS (EI, 80 eV) Calcd for C₁₂H₁₄N₄O₃: 262.1072. Found: 262.1072.

N-Cantharidinimino-sulfamethazine (**6h**): mp 115–117°C (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.25 (s, 6H, CH₃×2), 1.74–1.77 (m, 2H, CH₂), 1.84–1.89 (m, 2H, CH₂), 2.35 (s, 6H, CH₃×2), 4.69 (t, 2H, *J*=2.6, 2.7 Hz, OCH×2), 6.64 (s, 1H, pyrimidinyl H), 7.52 (d, 2H, *J*=8.7 Hz, phenyl H), 8.26 (d, 2H, *J*=8.6 Hz, phenyl H); IR (KBr): 1710 (carbonyl group) cm⁻¹.

N-Cantharidinimino-sulfisomidine (**6i**): mp 99–101 (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.25 (d-like, 6H, CH₃×2), 1.72–1.75 (m, 2H, CH₂), 1.85–1.88 (m, 2H, CH₂), 2.56 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 4.69 (t-like, 2H, OCH×2), 6.96 (s, 1H, pyrimidinyl H), 7.56 (d, 2H, *J*=8.2 Hz, phenyl H), 8.06 (d, 2H, *J*=8.4 Hz, phenyl H); IR (KBr): 1710 cm⁻¹.

N-(2-Pyridyl)cantharidinimine (**8j**): mp 159–161 (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.27 (s, 6H, CH₃×2), 1.74–1.76 (m, 2H, CH₂), 1.85–1.89 (m, 2H, CH₂), 4.73 (d, 2H, *J*=1.8 Hz, OCH×2), 7.33–7.36 (dd, 2H, *J*=7.6, 5.14, 2.97 Hz, H-3, H-5), 7.83–7.86 (t, 1H, *J*=7.7, 7.7 Hz, H-4), 8.64–8.65 (d, 1H, *J*=3.9 Hz, H-6); IR (KBr): 1713 (C=O) cm⁻¹; MS *m/z* (rel., int.); 272[M]⁺ (5), 203 (100).

N-(5-Methyl-2-pyridyl)cantharidinimine (**8k**): mp 185–187 (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.26 (s, 6H, CH₃×2), 1.73–1.75 (m, 2H, CH₂), 1.84–1.87 (m, 2H, CH₂), 2.38 (s, 3H, CH₃-5), 4.72 (s, 2H, OCH×2), 7.21–7.23 (d, 1H, *J*=8.0 Hz, H-3), 7.64–7.66 (d, 1H, *J*=8.0 Hz, H-4), 8.45 (s, 1H, H-6); IR (KBr): 1709 (C=O) cm⁻¹; MS *m/z* (rel., int.) 286 [M]⁺ (5), 217 (100).

N-(3-Amino-2-pyridyl)cantharidinimine (**8l**): mp 281–283 (MeOH); ¹H-NMR (500 MHz, CDCl₃): δ (ppm) 1.29 (s, 6H, CH₃×2), 1.75–1.79 (m, 2H, CH₂), 1.88–1.92 (m, 2H, CH₂), 4.71 (s, 2H, OCH×2), 7.14–7.15 (d, 1H, *J*=8.0 Hz, H-4), 7.19–7.21 (m, 1H, H-5), 7.99 (s, 1H, H-6); IR (KBr): 1709 (C=O) cm⁻¹. MS *m/z* (rel., int.) 287 [M]⁺ (13), 217 (100).

N-(3-Amino-4-pyridyl)cantharidinimine (**8m**): mp 235–238 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.28 (s, 6H, $\text{CH}_3 \times 2$), 1.76–1.78 (m, 2H, CH_2), 1.87–1.91 (m, 2H, CH_2), 3.97 (bs, 2H, $\text{NH}_2 \times 3$), 4.69–4.70 (t, 2H, $J=2.5$, 2.3 Hz, $\text{OCH} \times 2$), 6.95 (s, 1H, H-5), 8.06–8.07 (d, 1H, $J=4.7$ Hz, H-6), 8.24 (s, 1H, H-2); IR (KBr): 1713 (C=O) cm^{-1} . MS m/z (rel., int.): 287 $[\text{M}]^+$ (13), 170 (100).

N-(4-Amino-3-pyridyl)cantharidinimine (**8n**): mp 273–276 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.29 (s, 6H, $\text{CH}_3 \times 2$), 1.76–1.78 (m, 2H, CH_2), 1.89–1.92 (m, 2H, CH_2), 4.51 (bs, 2H, $\text{NH}_2 \times 4$), 4.69 (s, 2H, $\text{OCH} \times 2$), 6.63–6.64 (d, 1H, $J=5.6$ Hz, H-5), 8.04 (s, 1H, H-2), 8.21–8.30 (d, 1H, $J=5.6$ Hz, H-6); IR (KBr): 1713 (C=O) cm^{-1} . MS m/z (rel., int.): 287 $[\text{M}]^+$ (100), 218 (75).

N-(5-Bromo-2-pyridyl)cantharidinimine (**8o**): mp 193–194 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.26 (s, 6H, $\text{CH}_3 \times 2$), 1.74–1.76 (m, 2H, CH_2), 1.84–1.87 (m, 2H, CH_2), 4.71–4.72 (d, 2H, $J=2.41$ Hz, $\text{OCH} \times 2$), 7.25–7.26 (t, 1H, H-3), 7.95–7.97 (q, 1H, $J=2.3$, 6.1, 2.4 Hz, H-4), 8.69 (d, 1H, $J=2.2$ Hz, H-6); IR (KBr): 1710 (C=O) cm^{-1} ; MS m/z (rel., int.): 350 $[\text{M}]^+$ (2), 281 (100), 283 (97).

N-(4-Pyridyl)cantharidinimine (**8p**): mp 139–140 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.26 (s, 6H, $\text{CH}_3 \times 2$), 1.75–1.77 (m, 2H, CH_2), 1.85–1.88 (m, 2H, CH_2), 4.69–4.70 (t, 2H, $J=2.7$, 2.61 Hz, $\text{OCH} \times 2$), 7.46–7.47 (d, 2H, $J=6.2$ Hz, H-3, H-5), 8.71–8.73 (d, 2H, $J=5.5$ Hz, H-2, H-6); IR (KBr): 1717 (C=O) cm^{-1} ; MS m/z (rel., int.): 272 $[\text{M}]^+$ (38), 203 (100), 204 (95).

N-(4,6-Dimethyl-2-pyridyl)cantharidinimine (**8q**): mp 185–186 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.26 (s, 6H, $\text{CH}_3 \times 2$), 1.73–1.75 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.35 (s, 3H, $\text{CH}_3 \times 6$), 2.53 (s, 3H, $\text{CH}_3 \times 4$), 4.71–4.72 (t, 2H, $J=2.9$, 2.6 Hz, $\text{OCH} \times 2$), 6.9 (s, 1H, H-5), 7.03 (s, 1H, H-3); IR (KBr): 1707 (carbonyl group) cm^{-1} ; MS m/z (rel., int.): 300 $[\text{M}]^+$ (12), 231 (100).

N-(5-Hydroxy-6-pyridyl)cantharidinimine (**8r**): mp 289–291 (MeOH); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 1.25 (s, 6H,

$\text{CH}_3 \times 2$), 1.71–1.73 (m, 2H, CH_2), 1.83–1.85 (m, 2H, CH_2), 4.69–4.70 (t, 2H, $J=2.7$, 2.7 Hz, $\text{OCH} \times 2$), 7.21–7.23 (t, 1H, $J=4.5$, 4.5 Hz, H-5), 7.35–7.37 (d, 1H, $J=6.9$ Hz, H-4), 8.10–8.12 (d, 1H, $J=3.2$ Hz, H-6); IR (KBr): 1712 (C=O) cm^{-1} ; MS m/z (rel., int.): 288 $[\text{M}]^+$ (18), 219 (100).

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