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Research Article

Synthesis and Cytotoxic Evaluation of 3-Dimethyl Carbamoyl Emodin

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Abstract

Emodin (6-methyl-1,3,8-trihydroxyanthraquinone) is a natural anthraquinone derivative with potential pharmacological such as cytotoxic effects. The structure modification could be performed to determine the functional groups that have the role of substance activities. In this study, we modified one hydroxy group in the emodin structure to become dimethyl carbamoyl moiety. Emodin was reacted with dimethyl carbamoyl chloride and potassium carbonate to create 3-dimethyl carbamoyl emodin. The structure of the product was elucidated using mass spectrophotometer (MS), Fourier transform infrared (FTIR), proton and carbon nuclear magnetic resonance (H-NMR and C-NMR). These substances were tested for cytotoxicity against HepG2 cell lines using the MTT assay. According to the evaluation, 3-dimethyl carbamoyl emodin is less cytotoxic than emodin. As a result, the hydroxy group at the C3 position of emodin has been identified as a functional component that contributes to its cytotoxic effect.

Keywords: carbamoyl, cytotoxic, emodin, HepG2

1. INTRODUCTION

Liver cancer is the third leading cause of cancer death worldwide, and it is one of the top five causes of cancer death in 90 countries. Despite data demonstrating that most occurrences of liver cancer are preventable, this remains the case. According to the most recent predictions, 905,700 persons were diagnosed with liver cancer worldwide in 2020, with 830,200 dying from the disease. If present incidence and mortality rates remain constant, may be predicted that 1.4 million people will be diagnosed with liver cancer and 1.3 million will die from the disease by 2040.

Nowadays, cancer management consists of a combination of drug uses, surgical for removing cancer tissue, radiation therapy, and palliative. However, there are still limitations on cancer management, so it needs further research to inhibit cancer growth, one of which is utilizing medicinal plants. Empirically, several medicinal plants have been widely used to treat a tumor or cancerous diseases. Antitumor drugs contain active compounds that are toxic to a certain phase of the tumor cell cycle but not toxic or interfere with normal cells.

Emodin (1,3,8-trihydroxy-6-methyl-9,10-anthraquinone) could be found as an active compound in the extract of *Cassia occidentalis* (Leguminosae)[1], *Rumex japonicus*[2], *Fallopia japonica* dan *Fallopia sachalinensis*[3], *Polygenum multiflorum*, *P. cuspidatum*, *Rumex patentia*, *Rhamnus catharticus*, *Rhamnus orbiculatus*, *Aloe vera*, *Acorus tatarinowii*, *Cassia obtusifolia*, *C. occidentalis*, *Rheum palmatum*, *R. officinale*, *Eriocaulon buergerianum*, *Dendrobium thyrsiflorum*, *Fibraurea tinctoria*, *Coptis chinensis*, *Scutellaria baicalensis*, *Isatis indigotica*, and *Rumex chalepensis* [4].

Emodin, in particular, demonstrates anti-neoplastic[5], anti-inflammatory[6], anti-angiogenesis[7], anti-atherogenic effect[8] and toxicological potential for pharmacology *in vitro* and *in vivo*. Emodin demonstrates cytotoxic effects (e.g., cell death) through the arrest of the cell cycle and the induction of apoptosis in cancer cells[9]. The overall molecular mechanisms of emodin include cell cycle arrest, apoptosis, and the promotion of the expression of hypoxia-inducible factor 1a, glutathione S-transferase P, N-acetyltransferase, and glutathione phase I and II detoxification

enzymes while inhibiting angiogenesis, invasion, migration, chemical-induced carcinogen-DNA adduct formation, HER2/neu, CKII kinase, and p34cdc2 kinase in human cancer cells[10].

As a part of our drug discovery program on anticancer agents, we carry out chemical transformations to improve the therapeutic application of a lead compound. The structure modification could be performed to determine the functional groups that have the role of substance activities. In this study, we modified one hydroxy group in the emodin structure to become dimethyl carbamoyl moiety. Both emodin and its derivative were performed cytotoxic by MTT assay.

2. RESEARCH METHODS

Material

Emodin (CAS 518-82-1) from Shaanxi Pioneer Biotech Co., Ltd China, dimethyl carbamoyl chloride from Sigma-Aldrich and HepG2 cell lines were obtained from Laptiab BRIN cell cultures collection.

Preparation of 3-dimethyl carbamoyl emodin (D'Souza1 and Kevill, 2015)

A stirred solution of emodin (100 mg, 1 mmol) in acetone (10 mL was added with 1 gram of K_2CO_3 at 10°C (ice bath). Dimethyl carbamoyl chloride (10.9 mmol) in 10 mL acetone was added slowly to the mixture. The reaction mixture was stirred for 2 hours. It was then filtered to separate carbonate and then the filtrate was evaporated under reduced pressure. The crude product was chromatographed on silica gel to afford the desired compound.

Cytotoxic evaluation

Cell viability was monitored by MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) colorimetric assay[12]. The cell cultures of HepG2 were treated with emodin and its derivatives for 24 hours. Subsequently, the culture medium was removed and changed to the fresh culture medium containing 0,5mg/mL of MTT. After a 4-hour incubation at 37 ^{o}C, SDS 10% was added to dissolve the MTT formazan. After incubation overnight at room temperature and dark condition, the absorbance value was measured at 570 nm using an ELISA plate reader. Cell viability inhibition (%) was calculated by (OD of blank cells-treated cells/OD of blank cells) x 100. Blank cells mean the cell culture without samples treated. The half-maximal inhibitory concentration (IC_{50}) was developed by constructing a dose-response curve and determining the concentration that inhibits 50% of the cells.

3. RESULTS AND DISCUSSION

At this stage, there has been a reaction to change the OH group at position C3 from emodin to ester group. As is known in the emodin structure, there are 3 hydroxy groups at the position of C1, C3, and C8. In this study, it was expected that only the hydroxy group at the C3 position would be converted into dimethyl carbamoyl. This may occur because the OH groups in C1 and C8 coexist with the ketone group, so it is possible that a hydrogen bond between H of the hydroxy group and O of the ketone group may occur. Thus, the OH group at positions C1 and C8 will be more difficult to replace.

Carbamoyl chloride is a functional group with the formula $R_2NC(O)Cl$. The master structure of carbamoyl chloride, H_2NCOCl is unstable, but many analogs are known. One example is dimethyl carbamoyl chloride (melting point of -90°C and boiling point of 93°C). Most of these compounds are sensitive to moisture, colorless, and soluble in nonpolar organic solvents[13]. Due to the influence of amino groups, these substances are less sensitive to the hydrolytic than ordinary acid chlorides[14]. The reactions of carbamoyl chloride with an alcohol or phenol led to stable carbamate esters (urethanes)[11].

The emodin reaction with dimethyl carbamoyl chloride produces 3-dimethyl carbamoyl emodin. The HCl also produced from the compound is neutralized by the addition of potassium carbonate.

The purity of products was confirmed by TLC and HPLC. The structure of the product was confirmed using IR, MS, ^{1}H, and ^{13}CNMR spectra. The product was a crystalline yellow solid that was very soluble in hot ethyl acetate and acetone, less soluble in hexane and chloroform, and insoluble in water. The melting point of the compound is 169 ^{o}C.

The 3-dimethylcarbamoyl emodin compound as the synthesis product was characterized from the TOF MS ES+ results, which appeared the peak at *m/z* 342.1606 as the peak ion $[M+H]^+$, *m/z* 343.1507 as the peak ion $[M+2H]^+$ and m/z 364.1297 as the peak ion $[M+Na]^+$ or a compound with Molecular weight (MW) 341.1606 with the molecular formula $C_{18}H_{15}NO_6$. The result of calculation with the Chembiooffice Program trial version (Perkin Elmer, UK) is known Exact mass calculation of [3-dimethylcarbamoyl emodin $+H]^+$ is 342.0972. Thus, the accuracy of the result of the measurement of the compound of the synthesis reached 99.98%.

The FTIR spectrum of the synthesis product shows the characteristic band of the carbonyl group at the wave number 1735 cm^{-1}. The tertiary amide group indicated the band at 1662 cm^{-}

[1] and the N-H bond at wave number 3147 cm^{-1}. The presence of an aromatic ring is indicated by the presence of a ribbon pair at 1598 and 1452 cm^{-1}. The methyl group is indicated by a peak at 1352 cm^{-1}. The band at 1244 is due to the stretching vibration of the C-O bonds.

The ^{1}H-NMR spectrum of the reaction product of showed there are 12 numbers of integral, which could be indicated as the number of protons in the compound. The protons consisted of 4 protons in the aromatic group with a chemical shift of 6.88 ppm to 7.90 ppm each having a proton. The appearance of two peaks of the proton singlet at a chemical shift of 2.34 ppm indicated there are 2 methyl groups attached to the amide nitrogen group. The two methyl groups from dimethyl carbamoyl appear in different chemical shifts because the dimethyl carbamoyl group has an unequal rotational rate due to the resonance interaction between unpaired electrons in N and the carbonyl group. Resonance requires molecules to adapt to become planar in a free rotational geometry. If the time to rotate freely is longer than the NMR transition, the NMR spectrum will display two different methyl groups, one from the same side of C=N and the other on the other side. So, this cluster differs its magnetic environment and raises the peak in different shifts. The protons of the two hydroxy groups are not visible on the spectrum.

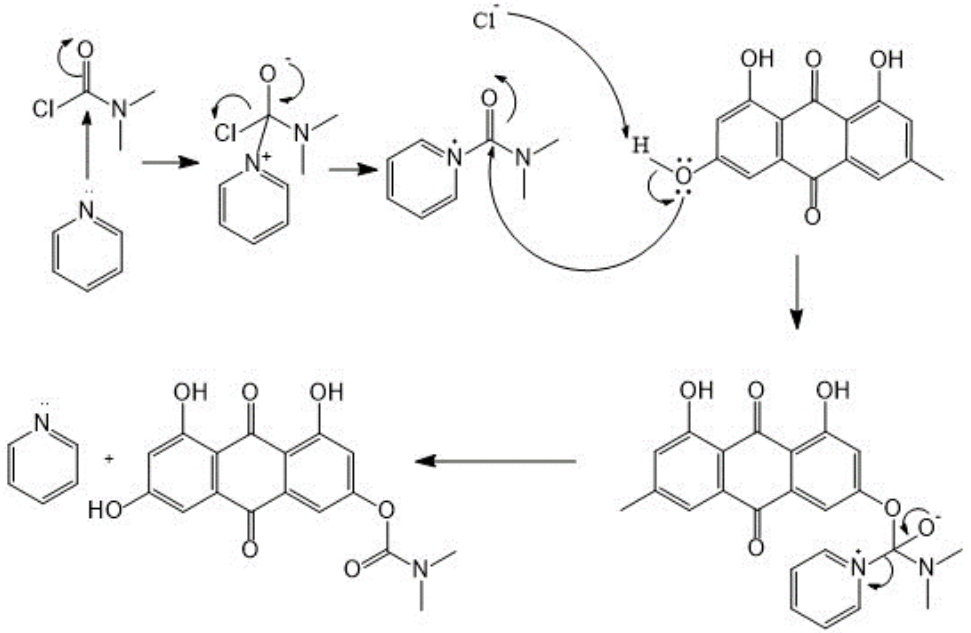

Figure 2. Reaction of emodin and dimethyl carbamoyl chloride

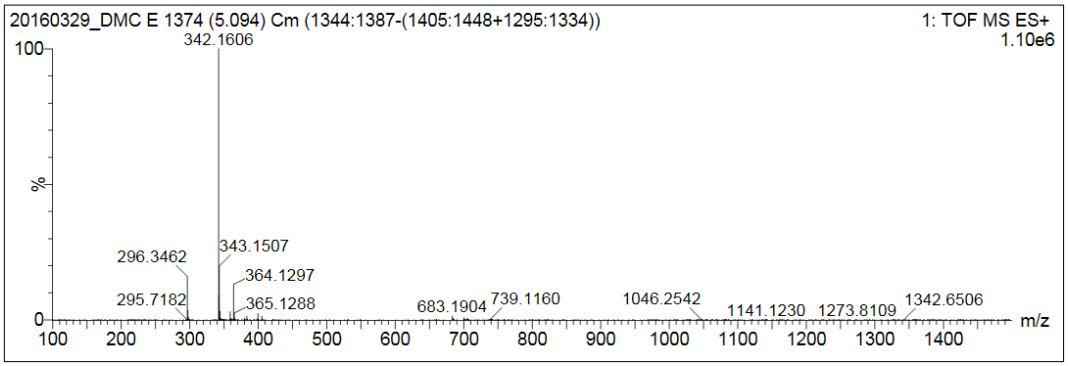

Figure 3. Mass Spectrum of 3-dimethyl carbamoyl emodin

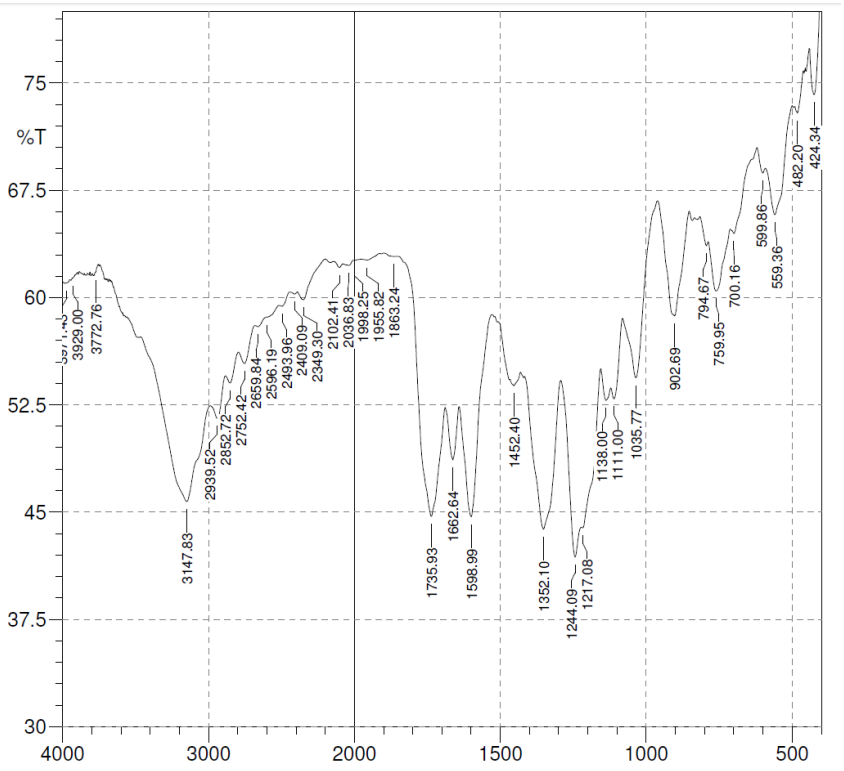

Figure 4. FTIR spectrum of 3-dimethyl carbamoyl

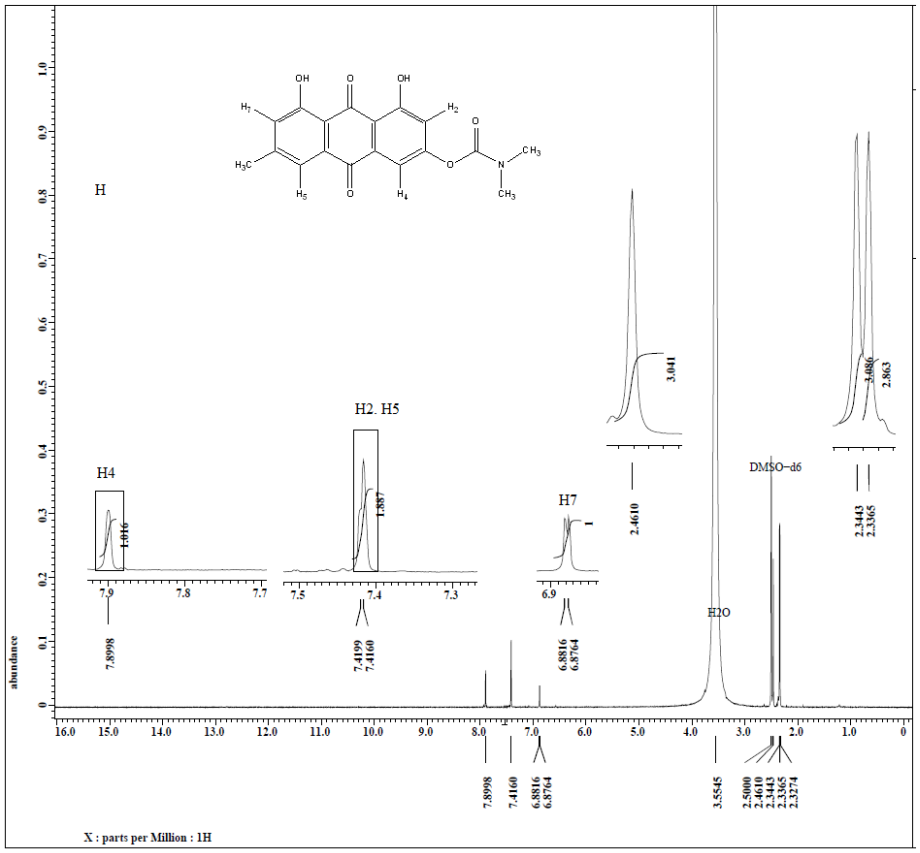

Figure 5. [1]H-NMR (500 MHz, CDCl3/TMS) of 3-dimethylcarbamoyl emodin

The [13]C-NMR spectrum showed 18 peaks which indicated there are 18 carbons moieties. The carbon atom of the methyl group appears at a chemical shift of 21.07; 21.09 and 21.18 ppm; The aromatic C-H atom appears in chemical shifts of 111.5 (C4); 117.1 (C2); 122.8 (C5); and 125.4 (C7). The Aromatic atom arises at a chemical shift of 131.1 (C9a); 133.9 (C10a); 135.7 (C10b); 146.3

(C5); 149.9 (C6); 152.5 (C1'). The carbon that binds to OH appears at a chemical shift of 163.7 (C1) and 169.2 (C8) and. The carbon of the carbonyl group appears in 169.4 (C1'), 179.2 (C10) and 182.0 (C9) ppm.

Based on the analysis, it can be concluded that emodin reaction with dimethyl carbamoyl chloride produces 3-dimethyl carbamoyl emodin. Emodin and 3-dimethyl carbamoyl emodin were evaluated for their cytotoxic activity against HepG2 cell lines that have been widely used to assess the toxic effects of a wide variety of chemicals and drugs[15,16]. The HepG2 cell line has also been used in genotoxicity testing, as these cells express metabolizing enzymes required for the activation of DNA-reactive carcinogens[15,17]).

The results of a cytotoxic evaluation against HepG2 cell lines showed that the IC_{50} value of emodin and dimethyl carbamoyl emodin as 0.54 and 25mM respectively.

Table 1. The IC_{50} value of emodin and dimethyl carbamoyl emodin

Compound	IC_{50} (mM)
Emodin	0.54
3-dimethyl carbamoyl emodin	25.08

It could be suggested that 3-dimethylcarbamyl emodin is non-toxic to cells relatively. Thus, it is known that the hydroxyl group in C3 is a pharmacophore, which is an important binding group that are required for activity. It is proven that the conversion of this group into ester form resulted in the decrease of the cytotoxic effects.

Hydroxy groups such as alcohol and phenol are functional groups which are common in many drugs and are often involved in hydrogen bonding. The oxygen can act as a hydrogen bond acceptor, and the hydrogen or proton can act as a hydrogen bond donor. One or all of these interactions may be important in binding emodin to the binding site in receptor which cause cytotoxic activity. So, if the proton is removed, the hydrogen bond will lose. Thus, the activity will decrease.[18]

Physicochemical properties such as hydrophobic, electronic, and steric parameters, are known have effect in biological response which is the result of the interaction of drug molecules with functional groups of receptor molecules.[18] This interaction can take place because of the strength of certain chemical bonds. Based on the simulation result using Chembiooffice program, some physicochemical properties of emodin and 3-dimethylcarbamoyl emodin were calculated as shown in Table 2.

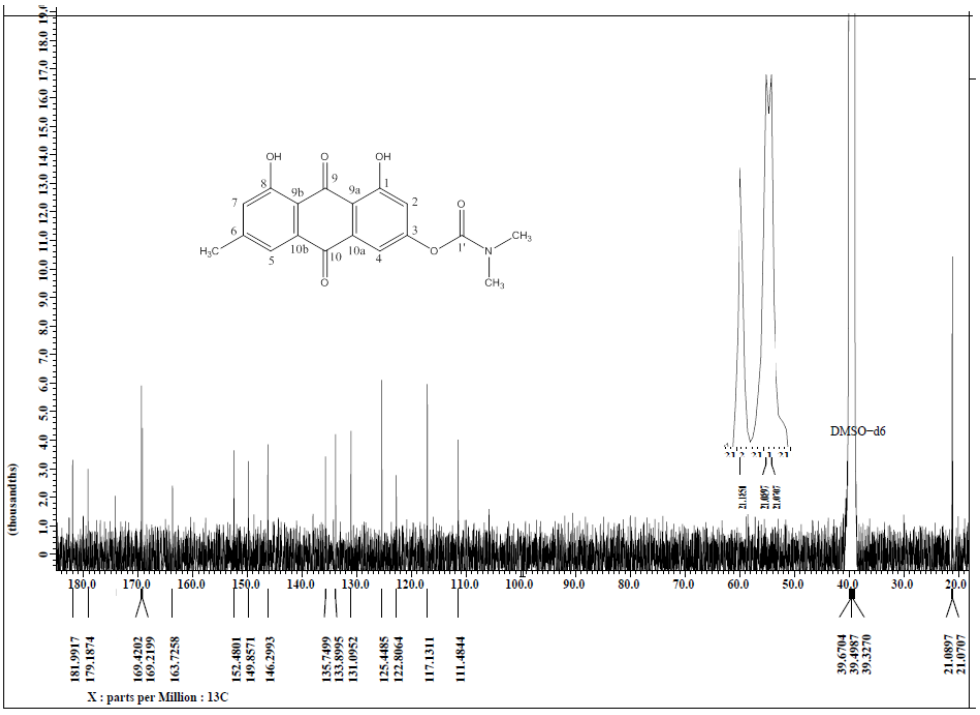

Figure 6. ^{1}C-NMR (500 MHz, CDCl3/TMS) of 3-dimethylcarbamoyl emodin

Table 2. Physicochemical properties of emodin and 3-dimethylcarbamoyl emodin

Compound	LogP	MR
Emodin	1.74	74.09
3-dimetylcarbamoyl emodin	1.86	92.4

Based on the physicochemical properties calculation and the cytotoxic evaluation, it indicated that decreasing of LogP (hydrophobic) and increasing of molar refractivity (steric) could decrease its activity. However, this suggestion remains to be proven by doing further research using more derived compounds to obtain suitable data for QSAR analysis.

4. CONCLUSIONS

The reaction between emodin and dimethyl carbamoyl chloride produces 3-dimethyl carbamoyl emodin. The MS, FTIR, H-NMR, and C-NMR spectra confirmed the structure of the product. Modifying emodin structure could affect its cytotoxic activity against HepG2 cell lines. The hydroxyl group in C3 is one functional group that affects its cytotoxic due to the conversion of this group into ester form resulting in the decrease of the cytotoxic effects.

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