Vietnam Journal of Science and Technology ***63*** *(4) (2025) 723-733*
doi:10.15625/2525-2518/19578

New coumarin-based mannich bases: synthesis and *in vitro* cytotoxic evaluation

Tran Duy Thanh, Vu Xuan Thach, Ho Duc Cuong, Dao Phuong Lan, Tran Khac Vu*

School of Chemistry and Life Sciences, Hanoi University of Science and Technology No 1, Dai Co Viet, Hai Ba Trung, Ha Noi, Viet Nam

*Emails: *vu.trankhac@hust.edu.vn*

Received: 5 December 2023; Accepted for publication: 2 July 2024

Abstract. This paper presents the synthesis of new Mannich bases **4a-h** derived from coumarin in good yields through a three-step procedure. Initially, the Knoevenagel reaction of 2,4-dihydroxybenzaldehyde (**1**) with Meldrum's acid in water under reflux for 10 h afforded coumarin acid (**2**) in 95 % yield. Compound **2** was next reacted with 3-methoxybenzylamine and 4-methoxybenzylamine in dimethylformamide (DMF) using 1-ethyl-3-carbodiimide hydrochloride (EDC.HCl) and 4-dimethylaminopyridine (DMAP) as a coupling system for 10-12 h to give compound **3a-b** in 76 - 83 % yield. Finally, the reaction of **3a-b** with the excess of paraformaldehyde and secondary amines in ethanol under reflux for 20 h gave new coumarin-based Mannich bases **4a-h** with yields ranging from 30 % to 69 %. The structures of the Mannich bases were characterized using NMR and MS spectra. Bioassay results revealed that some of the synthesized Mannich bases exhibited cytotoxic activity against SKLu-1 and MCF-7 cell lines, ranging from weak to moderate effect.

Keywords: Mannich, cytotoxicity, coumarin.

Classification numbers: 1.2.4.

1. INTRODUCTION

Cancer is a collection of diseases characterized by abnormal cell growth, with the potential to invade and spread to other parts of the body. In 2020, Globocan recorded a global total of 19.3 million cancer cases and 10 million deaths related to cancer [1]. Consequently, there has been a significant emphasis on cancer research to make substantial progress in diagnosing and treating this disease. Despite significant advancements in cancer research and treatment, the ongoing pursuit of innovative anti-cancer compounds is essential, mainly due to challenges related to selectivity, potency, and drug resistance. The exploration of small molecules that target specific components is crucial in uncovering new anti-cancer agents and is frequently underscored. One strategy for developing such agents involves introducing heterocyclic moieties into molecules, thereby creating compounds with distinct effects. Coumarin (2*H*-chromen-2-one) (Figure 1) and its derivatives are extensively found in nature, showcasing a diverse pharmacological profile.

The discussion around coumarin derivatives (CDs) is often prompted by their various biological properties. Recent literature has accumulated a wealth of information linking coumarin to numerous bioactivities, including anticancer [2], anticoagulant, estrogenic, dermal, photosensitizing, antimicrobial, vasodilator, molluscacidal, antihelminthic, sedative, hypnotic, analgesic, hypothermic [2], and free radical scavenging activities, particularly against superoxide anions generated by activated neutrophils [3]. Coumarin derivatives have garnered significant attention from organic and medicinal chemists, finding wide applications as fragrances, pharmaceuticals, and agrochemicals [2]. The anti-cancer potential of these compounds adds to their allure for further exploration in backbone derivatization and screening as novel therapeutic agents, highlighting crucial aspects of ongoing research in this field [4, 5]. Several reports underscore the efficacy of pure coumarins in inhibiting cell proliferation within cancerous cell lines [6]. Both experimental investigations and clinical/epidemiological findings have contributed evidence supporting the involvement of reactive oxygen metabolites or free radicals in the etiology of cancer [7].

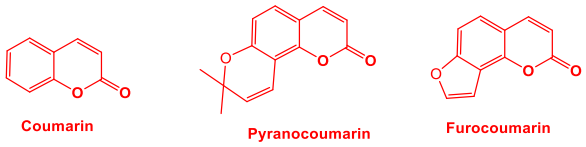


Figure 1. The structure of coumarin and its derivatives.

The Mannich reaction involves the interaction between aldehydes, amines, or heterocyclic acidic proton-containing compounds [8, 9]. Recognized as one of the fundamental C-C bond-forming reactions in organic chemistry, the Mannich reaction has undergone extensive study [10]. Its application extends to the synthesis of natural products and plays a pivotal role in medicinal chemistry, particularly in creating nitrogenous heterocyclic molecules [11 - 13]. Notably, the Mannich reaction has made significant strides in medicinal chemistry [14], offering a diverse array of biological activities such as anti-cancer [15], antibacterial [16], anti-inflammatory [17], anthelmintic [18], analgesic [19], and anticonvulsant effects [20]. In fact, many clinically approved drugs feature aminoalkyl chains, exemplified by compounds like ranitidine, amodiaquine, procyclidine.

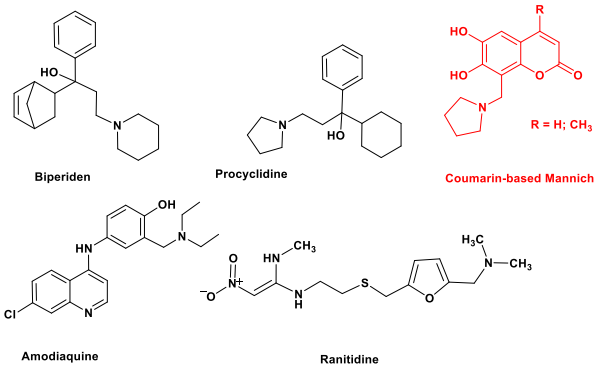


Figure 2. Several Mannich bases containing aminoalkyl chains and coumarin moiety.

Recently, some Mannich bases from coumarin have been synthesized and reported to show strong activity against some cancer cell lines (Figure 2) [21, 22, 2]. Considering the anti-cancer potential of the coumarin moiety and Mannich bases, it is anticipated that their synergistic action

will lead to enhanced anti-cancer efficacy. This study thus details the synthesis of novel Mannich bases incorporating the coumarin moiety and evaluate their cytotoxic activity against various cancer cell lines.

2. MATERIALS AND METHODS

2.1. Chemistry

Thin-layer chromatography (TLC) analysis was conducted on Whatman® 250 µm Silica Gel GF Uniplates, with visualization under UV light at 254 nm for all products. Melting points were determined using open capillaries on an Electrothermal IA 9200 Shimadzu apparatus and were reported without correction. Purification procedures included crystallization and open flash silica gel column chromatography, utilizing Merck silica gel 60 (240 to 400 mesh). Nuclear magnetic resonance spectra (^{1}H, ^{13}C NMR) were recorded on a Bruker 500 MHz spectrometer using tetramethyl silane (TMS) as an internal standard, with DMSO-d_6 as solvents. Chemical shifts are reported in parts per million (ppm) downfield from TMS, and coupling constants (*J*) are expressed in hertz (Hz). Mass spectra were obtained using FTICR MS Varian. Reagents and solvents were sourced from Aldrich, Fluka Chemical Corp, or Merck, unless otherwise specified. Solvents underwent distillation and drying before use.

2.2. Bioassay

All cell culture media, sera, and reagents employed in this study were sourced from GIBCO Co. Ltd. (Grand Island, New York, USA). Two human cancer cell lines, namely SKLU-1 (lung cancer) and MCF-7 (breast cancer), were supplied by the Institute of Biotechnology, Vietnam Academy of Science and Technology. The cytotoxicity assessment of the synthesized compounds followed the protocol outlined by the American National Cancer Institute (NCI), as detailed in the literature.

In brief, the cancer cell lines were cultivated as monolayers in a culture medium comprising 2 mM L-glutamine, 10 mM HEPES, 1.0 mM sodium pyruvate, and supplemented with 10 % fetal bovine serum (FBS) from GIBCO. The cells were cultured for 3 - 5 days post-transfer and maintained at 37 °C in a humidified atmosphere with 5 % CO_2. Assay samples, initially dissolved in DMSO, were serially diluted to appropriate concentrations with the culture medium immediately before the assay. Subsequently, the cells in each well, incubated for 24 h as described above, were exposed to 20 µL of samples at concentrations of 20 µg/mL, 0.8 µg/mL, and 0.16 µg/mL. The plates were further incubated for 48 h. Following removal of the medium, the cells were fixed using a 10 % trifluoroacetic acid solution. Staining was performed for 30 minutes using a staining solution (RSB method). The protein-bound dye was dissolved in a 10 mM tri-base solution, and optical densities (ODs) were measured at 510 nm using an ELISA reader. IC_{50} values were calculated using the Probits method. Ellipticine (Sigma) was used as a positive control, and the reported values for the compounds represent the average of three determinations.

Synthesis of 7-Hydroxy-2-oxo-2H-chromene-3-carboxylic acid (2)

A mixture of 2,4-dihidroxybenzaldehyde (**1**) (5.6 g; 40 mmol; 1 eq) and Meldrum's acid (7.3 g; 48 mmol; 1.2 eq) in H_2O (50 mL) was stirred and refluxed at 100 ^{o}C for 10 h. At the end of the reaction by checking thin layer chromatography, the insoluble precipitates were filtered

and washed several times on a Buckner funnel with water, dried in vacuum to obtain **2** (7.9 g, 95 %) as a solid in light brown powder.

Yield: 95 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 8.66 (s, 1H, H-4), 7.73 (d, *J* = 8.4 Hz, 1H, H-5), 6.83 (dd, 1H, *J* = 1.8 Hz, *J* = 8.4 Hz, H-6), 6.72 (d, *J* = 1.8 Hz, 1H, H-8). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 164.17 (COOH), 163.90 (C-2), 157.59 (C-7), 156.96 (C-9), 149.35 (C-4), 131.99 (C-5), 113.99 (C-6), 112.46 (C-3), 110.60 (C-10), 101.78 (C-8).

*Synthesis of compound **3a-b***

General procedure

A mixture of (0.5 g, 2.4 mmol; 1eq), 1-ethyl-3-carbodiimide hydrochloride (EDC.HCl) (0.56 g; 1.5 eq) and 4-dimethylaminopyridine (DMAP) (0.44 g, 1.5 eq) in dimethylformamide (DMF) (5 mL) was stirred at 0 ^{o}C for 30 minutes. Secondary amines including 3-methoxybenzylamine or 4-metoxybenzylamine (0.4 mL; 2.9 mmol; 1.2 eq) were added to the reaction. The reaction was stirred at 0 ^{o}C for 4 h, then at room temperature for 6 h and was monitored by TLC (*n*-hexane : EtOAc = 1:1, v:v). The reaction mixture was then diluted with H_2O (30 mL) and extracted with EtOAc (3 × 25 mL). The organic phase was separated and extracted with 1 M HCl and 1M $NaHCO_3$ solution, respectively. The organic phase was then separated and dried on anhydrous Na_2SO_4, evaporated under reduced pressure to obtain the residue that was washed with cold EtOAc and cold MeOH several times to obtain the corresponding amides **3a-b**.

7-Hydroxy-N-(3-methoxybenzyl)-2-oxo-2H-chromene-3-carboxamide (3a)

A yellow solid, yield: 76 %, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 9.03 (t, *J* = 6.0 Hz, 1H, NH), 8.79 (s, 1H, H-4), 7.81 (d, *J* = 8.4 Hz, 1H, H-5), 7.25 (t, *J* = 8.4 Hz, 1H), 6.90 (m, 2H), 6.88 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H, H-6), 6.82 (dd, *J* =1.8 Hz, *J* = 8.4 Hz, 1H), 6.8 (d, *J* = 2.4 Hz, 1H), 4.51 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 3.74 (s, 3H, OCH_3). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.75 (C-2), 161.73 (CONH), 161.02 (C-7), 159.36, 156.32, 148.14 (C-4), 140.64, 131.99 (C-5), 129.48, 119.49, 114.48 (C-6), 113.67 (C-3), 113.10, 112.29, 111.11, 101.81 (C-8), 54.98 (OCH_3), 42.61 (CH_2-NH).

7-Hydroxy-N-(4-methoxybenzyl)-2-oxo-2H-chromene-3-carboxamide (3b)

Yellow solid, yield: 83 %, ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.95 (t, *J* = 6.0 Hz, 1H, NH), 8.78 (s, 1H, H-4), 7.79 (d, *J* = 9.0 Hz, 1H, H-5), 7.26 (d, *J* = 8.4 Hz, 2H), 6.88 (m, 3H), 6.78 (d, *J* = 2.4 Hz, 1H, H-8), 4.44 (d, *J* = 5.4 Hz, 2H, CH_2-NH), 3.72 (s, 3H, OCH_3). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 163.68 (C-7), 161.52 (CONH), 161.04 (C-2), 158.35, 156.29, 148.12, 131.96 (C-5), 130.93, 128.84, 114.35 (C-6), 113.82, 113.65 (C-3), 111.12, 101.80 (C-8), 55.05 (OCH_3), 42.16 (CH_2-NH).

*Synthesis of Coumarin-based Mannich **4a-h***

General procedure

A mixture of secondary amines (1.5 eq) and paraformaldehyde (38 mg, 0.92 mmol, 3 eq) in ethanol (20 mL) was refluxed for 30 minutes. Compound **3a-b** (100 mg, 0.31 mmol, 1 eq) was added to the mixture. The reaction was stirred for 20 h and was monitored by TLC (DCM : MeOH = 20:1, v:v). The ethanol solvent was removed by vacuum evaporation to give the residue that was subjected to silica gel column chromatography using DCM: MeOH = 20:1 as a diluting system to afford the corresponding Mannich bases **4a-h**.

7-Hydroxy-N-(3-methoxybenzyl)-8-((4-methylpiperazin-1-yl)methyl)-2-oxo-2H-chromene-3-carboxamide (4a)

Light yellow solid, yield: 69 %. $C_{24}H_{27}N_3O_5$; ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 9.00 (t, *J* = 6.0 Hz, 1H, NH), 8.75 (s, 1H, H-4), 7.72 (d, *J* = 9.0 Hz, 1H, H-5), 7.24 (t, *J* = 8.4 Hz, 1H, H-5′), 6.90 (m, *J* = 7.2 Hz, 2H, H-4′, H-6′), 6.83 (dd, *J* =1.2 Hz, 5.4 Hz, 1H, H-2′), 6.79 (d, *J* = 8.4 Hz, 1H, H-6), 4.51 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 3.96 (s, 2H, CH_2-piperazine), 3.73 (s, 3H, OCH_3), 2.64 (brs, 4H, piperazine), 2.39 (brs, 4H, piperazine), 2.19 (s, 3H, CH_3-piperazine). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 165.72 (C-2), 161.83 (CONH), 160.88 (C-7), 159.31, 154.13 (C-9), 148.36 (C-4), 140.68, 130.72 (C-5), 129.40, 119.36, 114.86 (C-6), 112.95 (C-3), 112.22, 112.04, 110.24 (C-10), 107.38 (C-8), 54.93 (OCH_3), 54.07, 51.68, 51.52 (CH_2-piperazine), 45.31 (N-CH_3), 42.50 (CH_2-NH). ESI-MS: m/z 438.5 $[M+H]^+$.

7-Hydroxy-N-(3-methoxybenzyl)-2-oxo-8-(piperidin-1-ylmethyl)-2H-chromene-3-carboxamide (4b)

Light yellow solid, yield: 42 %. $C_{24}H_{26}N_2O_5$; ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 9.00 (t, *J* = 6.0 Hz, 1H, NH), 8.68 (s, 1H, H-4), 7.64 (d, *J* = 8.4 Hz, 1H, H-5), 7.24 (t, *J* = 8.4 Hz, 1H), 6.90 (m, 2H), 6.82 (dd, *J* = 1.8 Hz, 6.6 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H, H-6), 4.50 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 4.05 (s, 2H, CH_2-piperidine), 3.73 (s, 3H, OCH_3), 2.76 (brs, 4H), 1.61 (m, 4H), 1.48 (d, *J* = 4.80 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.55 (C-2), 162,19 (CONH), 161.18 (C-7), 159.31, 154.76 (C-9), 148.80 (C-4), 140.60, 130.94 (C-5), 129.40, 119.35, 116.28 (C-6), 112.95 (C-3), 112.20, 109.46, 108.76 (C-10), 105.62 (C-8), 54.93 (OCH_3), 52.47 (CH_2-piperidine), 42.44 (CH_2-NH), 24.40, 22.53. ESI-MS: m/z 423.5 $[M+H]^+$.

7-Hydroxy-N-(3-methoxybenzyl)-8-(morpholinomethyl)-2-oxo-2H-chromene-3-carboxamide (4c)

Light yellow solid, yield: 39 %. $C_{23}H_{24}N_2O_6$; ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 9.00 (t, *J* = 6.0 Hz, 1H, NH), 8.78 (s, 1H, H-4), 7.76 (d, J = 8.4 Hz, 1H, H-5), 7.24 (t, *J* = 8,4 Hz, 1H), 6.90 (d, *J* = 7.20 Hz, 2H), 6.87 (d, *J* = 8.40 Hz, 1H, H-6), 6.82 (dd, *J* = 1.80 Hz, 5.40 Hz, 1H), 4.51 (d, *J* = 6,0 Hz, 2H, CH_2-NH), 3.90 (s, 2H, CH_2-morpholine), 3.73 (s, 3H, OCH_3), 3.60 (t, J = 4.2 Hz, 4H), 2.57 (t, *J* = 4.2 Hz, 4H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 164.06 (C-2), 161.69 (CONH), 160.76 (C-7), 159.31, 154.20 (C-9), 148.41 (C-4), 140.62, 130.76 (C-5), 129.39, 119.36, 114.26 (C-6), 113.02 (C-3), 112.96, 112.22, 110.78 (C-10), 108.05 (C-8), 65.89, 54.93 (OCH_3), 52.46, 51.32 (CH_2-morpholine), 42.51 (CH_2-NH). ESI-MS: m/z 425.5 $[M+H]^+$.

7-Hydroxy-N-(3-methoxybenzyl)-2-oxo-8-(pyrrolidin-1ylmethyl)-2H-chromene-3-carboxamide (4d)

Light yellow solid, yield: 60 %, $C_{23}H_{24}N_2O_5$; ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 8.98 (t, *J* = 6.0 Hz, 1H, NH), 8.60 (s, 1H, H-4), 7.56 (d, *J* = 8.4 Hz, 1H, H-5), 7.24 (t, *J* = 8.4 Hz, 1H, H-5′), 6.88 (m, 1H, 2H, H-4′, H-6′)), 6.82 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H, H-6), 6.82 (d, *J* = 2.4 Hz, 1H, H-2′), 4.51 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 4.20 (s, 2H, CH_2-pyrrolidine), 3.74 (s, 3H, OCH_3), 3.02 (t, *J* = 6.0 Hz, 4H), 1.88 (t, *J* = 6.0 Hz, 4H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 172.76 (C-7), 162.65 (C-2), 161.55 (CONH), 159.32 (C-3′), 155.58, 147.83 (C-4), 140.96 (C-1′), 131.33 (C-5), 129.41 (C-5′), 119.33, 117.82 (C-8), 112.92 (C-6), 112.17 (C-2′), 107.46, 106.46 (C-3), 105.52, 54.98 (OCH_3), 52.88 ($2CH_2$, pyrrolidine), 49.15 (CH_2-pyrrolidine), 42.36 (CH_2-NH), 22.92 ($2CH_2$, pyrrolidine), ESI-MS: m/z 409.6 $[M+H]^+$.

7-Hydroxy-N-(4-methoxybenzyl)-8-((4-methylpiperazin-1-yl)methyl)-2-oxo-2H-chromene-3-carboxamide (4e)

Light yellow solid, yield: 69 %. $C_{23}H_{27}N_3O_5$ ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 9.94 (t, *J* = 6.0 Hz, 1H, NH), 8.75 (s, 1H, H-4), 7.73 (d, *J* = 9.0 Hz, 1H, H-5), 7.27 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 9.0 Hz, 1H), 4.46 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 3.96 (s, 2H, CH_2-piperazine), 3.73 (s, 3H, OCH_3), 2.63 (brs, 4H), 2.39 (m, 4H), 2.19 (s, 3H, CH_3-piperazine). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 165.70 (C-2), 161.83 (CONH), 161.64 (C-7), 160.89, 158.29, 154.11, 148.35, 130.97, 130.70, 128.69, 114.85, 113.76, 112.03, 110.25, 107.39, 55.01(OCH_3), 54.08, 51.69 (CH_2-piperazine), 51.52, 45.31, 42.04 (CH_2-NH). ESI-MS: m/z 438.7 $[M+H]^+$.

7-Hydroxy-N-(4-methoxybenzyl)-2-oxo-8-(piperidin-1-ylmethyl)-2H-chromene-3-carboxamide (4f)

Light yellow solid, yield: 42 %. $C_{24}H_{26}N_2O_5$; ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 8.93 (t, *J* = 6.0 Hz, 1H, NH), 8.68 (s, 1H, H-4), 7.65 (d, *J* = 9.0 Hz, 1H, H-5), 7.26 (d, *J* = 8.4 Hz, 2H), 6.90 (m, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 9.0 Hz, 1H), 4.46 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 4.06 (s, 2H, CH_2- piperidine), 3.73 (s, 3H, OCH_3), 2.50 (brs, 4H), 1.61 (br, 4H), 1.48 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.31 (C-2), 161.96 (CONH), 161.15 (C-7), 158.26, 154.67, 148.16, 131.08, 130.87, 128.67, 116.16, 113.74, 109.59, 108.83, 105.69, 54.99 ((OCH_3), 52.48 ((CH_2-piperidine), 41.97 (CH_2-NH), 24.43, 22.56. ESI-MS: m/z 423.6 $[M+H]^+$.

7-Hydroxy-N-(4-methoxybenzyl)-8-(morpholinomethyl)-2-oxo-2H-chromene-3-carboxamide (4g)

Light yellow solid, yield: 39 %. $C_{23}H_{24}N_2O_6$; ^{1}H-NMR (600 Hz, DMSO-d_6): δ (ppm) 8.99 (t, *J* = 6.0 Hz, 1H, NH), 8.82 (s, 1H, H-4), 7.80 (d, *J* = 9.0 Hz, 1H, H-5), 7.32 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 1H, H-6), 4.51 (d, *J* = 6.0 Hz, 2H, CH_2-NH), 3.93(s, 2H, CH_2-morpholin), 3.73 (s, 3H, OCH_3), 3.65 (t, *J* = 4.2 Hz, 4H), 2.61 (t, *J* = 4.2 Hz, 4H, 4H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 172.62, 166.92, 162.46, 161.54, 158.26, 155.57, 147.82, 131.69, 128.65, 117.76, 113.76, 107.20, 106.61, 105.59, 53.00 (OCH_3), 52.84, 51.32 (CH_2-morpholine), 49.12, 41.88. ESI-MS: m/z 425.6 $[M+H]^+$.

7-Hydroxy-N-(4-methoxybenzyl)-2-oxo-8-(pyrrolidin-1ylmethyl)-2H-chromene-3-carboxamide (4h)

Light yellow solid, yield: 30 %; $C_{23}H_{24}N_2O_5$; ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 8.98 (t, *J* = 6.0Hz, 1H, NH), 8.65 (s, 1H, H-4), 7.65 (d, *J* = 8.4 Hz, 1H, H-5), 7.32 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.82 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H, H-6), 4.50 (d, *J* = 6.0Hz, 2H, CH_2-NH), 4.20 (s, 2H, CH_2-pyrrolidine), 3.77 (s, 3H, OCH_3), 3.06 (brs, 4H), 1.92 (brs, 4H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 172.65 (C-7), 162,45 (C-2), 161.53 (CONH), 158.24, 155.52, 147.80 (C-4), 131.33 (C-5), 128.63, 117.75 (C-8), 113.75, 107.16 (C-6), 106.52 (C-3), 105.52, 55.00 (OCH_3), 52.84, 49.12 (CH_2-pyrrolidine), 41.88 (CH_2-NH), 22.91. ESI-MS: m/z 409.5 $[M+H]^+$.

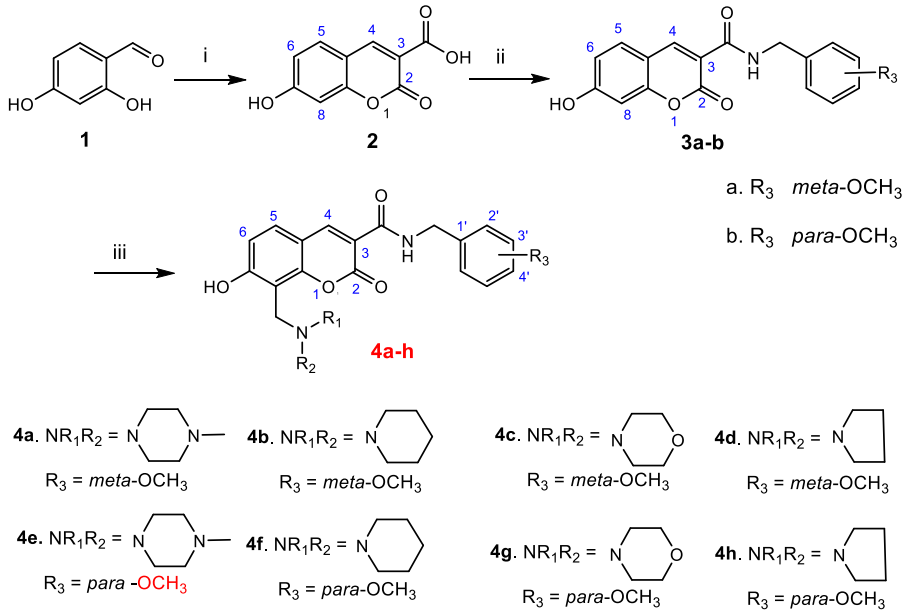
3. RESULTS AND DISCUSSION

3.1. Chemistry

Novel coumarin-based Mannich bases **4a-h** were synthesized in good yields *via* a three-step procedure (Scheme 1). The first step is the Knoevenagel reaction of 2,4-dihydroxybenzaldehyde (**1**) with Meldrum's acid in water under reflux for 10 h to afford coumarin acid (**2**) in 95 % yield. Compound **2** was next reacted with 3-methoxybenzylamine and

4-methoxybenzylamine in DMF using EDC and DMAP as a coupling system for 10 - 12 h to give compound **3a-b** in 76 - 83 % yields. The structure of compounds **2** and **3a-b** was confirmed based on NMR spectra and comparison with the previous reference [23].

Finally, the reaction of **3a-b** with the excess of paraformaldehyde and secondary amines in ethanol is quite specific to afford new coumarin-based Mannich bases **4a-h** with yields ranging from 30 % to 70 %.



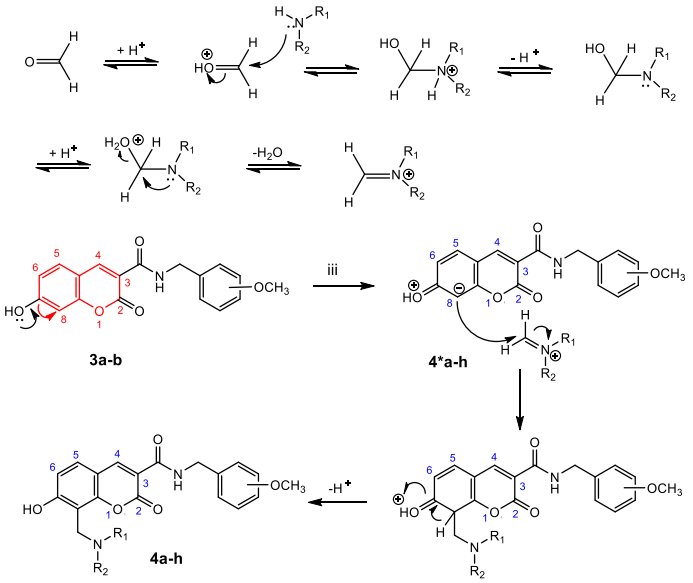
Scheme 1. Reagents and conditions: (i) Meldrum's acid, H_2O, 100 ^oC, 10 h, **2**, 95 %;
(ii) 3-methoxybenzylamine and 4-methoxybenzylamine, DMF, EDC, DMAP, r.t, 10 - 12 h, **3a-b**, 76 - 83 %;
(iii) secondary amines (1 - 3eq), ethanol, reflux, 20 h, **4a-h**, 39 - 69 %.

Theoretically, the Mannich reaction is an electrophilic substitution reaction on the aromatic ring of the coumarin moiety that can occur at positions 6 and 8. However, the reaction only produces a substitution product at position 8. This can be explained through the following mechanism (Scheme 2).

Initially, formaldehyde generated from paraformaldehyde reacts with secondary amines, undergoing deprotonation to remove water to form iminium ions as an electrophilic agent. The free electron pair of the 7-OH group of compound **3** pushes electrons to the C-8, making it more nucleophilic. This nucleophilic agent (**4*a-h**) participates in a nucleophilic addition reaction with iminium ion, then deprotonates to form Mannich bases **4a-h** [24]. The structure of **4a-h** was characterized by ^{1}H NMR, ^{13}C NMR, and MS spectra. Due to the structural similarity of target compounds, compound **4a** was used as an example to elucidate the structure.

The ^{1}H-NMR spectrum of compound **4a** shows the presence of 27 protons in the molecule. Noteworthy is the disappearance of the H-8 proton signal at δ_H 6.80. In addition, the signal of H-6 changes from a doublet of doublet (J = 2.4 Hz, 8.4 Hz) in the compound **3a** to a doublet (J = 8.4 Hz) indicating the absence of *meta*-interaction with H-8. The above evidence shows that the H-8 has been replaced. Besides, 4 new peaks appear in the high field at δ_H 3.96, 2.64, 2.39 and 2.19, indicating the presence of the newly substituted Mannich base groups. Compared with compound **3a**, the ^{13}C-NMR spectrum of **4a** recorded the appearance of 4 new carbon signals

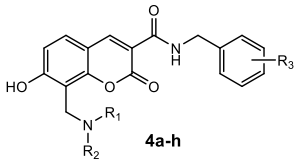
observed in the high-field region at δ_C 54.07, 51.68, 51.52 and 45.31, together with mass spectrum confirmed the structure of **4a**.



Scheme 2. Proposed mechanism of Mannich base formation **4a-h.**

3.2. Bioassay

Table 1. In vitro cytotoxic activity of **4a-h.**



No	Comps	NR_1R_2	R_3	IC_{50}, µM[a] / Cell lines [b]	
				SKLu-1	MCF-7
1	**4a**	Piperazine	*meta*-OCH_3	84.04 ± 9.72	95.21 ± 7,99
2	**4b**	Piperidine	*meta* -OCH_3	> 100	> 100
3	**4c**	Morpholine	*meta* -OCH_3	96.58 ± 5.13	> 100
4	**4d**	Pyrrolidine	*meta* -OCH_3	> 100	> 100
5	**4e**	Piperazine	*para* -OCH_3	> 100	> 100
6	**4f**	Piperidine	*para*-OCH_3	> 100	> 100
7	**4g**	Morpholine	*para*-OCH_3	> 100	> 100
8	**4h**	Pyrrolidine	*para* -OCH_3	> 100	> 100
Ellipticine				**1.66 ± 0.16**	**1.54 ± 0.20**

[a]Concentration (µg/mL) that produces a 50 % reduction in cell growth, the numbers represent the averaged results from triplicate experiments with deviation of less than 10 %. [b]Cell lines: SKLU-1, lung cancer; MCF-7, breast cancer.

The *in vitro* cytotoxicity of eight Mannich bases against SKLU-1 and MCF-7 was assessed using the SRB method [25]. Initial screening of all compounds was conducted at a constant concentration of 100 µg/mL. Active compounds underwent additional screening at reduced concentrations (e.g., 20 µg/mL, 4 µg/mL, 0.8 µg/mL, and 0.16 µg/mL) to determine IC_{50} values. Table 1 presents the results of the bio-assay.

Table 1 illustrates that three Mannich bases **4d-h** exhibited no activity against the two tested cancer cell lines. Meanwhile, Mannich bases **4a-c**, except compound **4b**, demonstrated cytotoxic effects on both human cancer cell lines. However, these compounds displayed weak cytotoxic activity against both cell lines. It was observed that the OCH_3 group at the *meta* position of the phenyl ring is more beneficial for cytotoxic activity than the *para* position. Although no Mannich bases synthesized can be compared with ellipticine in terms of cytotoxicity, the results of the study suggest that the presence of a mannich group in the coumarin group may have a beneficial effect on the cytotoxic activity of this type of derivative and can be a useful reference for future studies.

4. CONCLUSION

New coumarin-based Mannich bases have been synthesized and evaluated for their *in vitro* cytotoxicity against two human cancer cell lines, including SKLu-1 and MCF-7. The biological evaluation revealed that new Mannich bases **4a** and **4c** exhibited weak cytotoxic activity against both cell lines.

Acknowledgements. This work was financially supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under project number 03/2022/TN.

CRediT authorship contribution statement. Tran Duy Thanh, Vu Xuan Thach, Dao Phuong Lan carried out the main experimental work. Ho Duc Cuong collected and analysed experimental data. Tran Khac Vu designed and supervised the study and wrote the manuscript.

Declaration of competing interest. The authors have declared no conflict of interest.

REFERENCES

1. Siegel R. L., Miller K. D., Wagle N. S., Jemal A. - Cancer statistics, CA Cancer J. Clin. **73** (2023) 17-48. doi:10.3322/caac.21763.
2. La Quy Luong and Tran Khac Vu - Coumarin-Derived Mannich Bases: A Review of Biological Activities, Lett. Org. Chem. **21** (2024) 303-319. doi:10.2174/157017862 0666230622113356.
3. Paya M., Goodwin P. A., Heras B. D. L., Hoult R. S. - Superoxide scavenging activity in leukocytes and absence of cellular toxicity of a series of coumarins, Biochem. Pharmacol. **48** (1994) 445e51. doi.org/10.1016/0006-2952(94)90273-9.
4. Ojala T. - Screening of plant coumarins, PhD thesis, Helsinki: University of Helsinki, 2001, pp. 23e25.
5. Lacy A., O'Kennedy R. - Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer, Current Pharma. Des. **10** (2004) 3797e811. doi.org/10.2174/1381612043382693.
6. Budzisz E., Brzezinska E., Krajewska U., Rozalski M. - Cytotoxic effects, alkylating properties and molecular modelling of coumarin derivatives and their phosphonic

analogues, Eur. J. Med. Chem. **38** (2003) 597e603. doi.org/10.1016/S0223-5234(03)00086-2.

7. Singh R., Singh R. K., Mahdi A. A., Misra S., Rai S. P., Singh D., Cornelissen G. - Studies on circadian periodicity of urinary corticoids in carcinoma of the breast, In Vivo **12** (1998) 69e73.
8. Mannich C., Krösche W. Ueber ein Kondensationsprodukt aus Formaldehyd, Ammoniak und Antipyrin, Arch. Pharm. **250** (1912) 647.
9. Kobayashi S., Mori Y., Fossey J. S., Salter M. M. - Catalytic Enantioselective Formation of C−C Bonds by Addition to Imines and Hydrazones: A Ten-Year Update, Chem. Rev. **111** (2011) 2626. doi.org/10.1021/cr100204f.
10. Noble A., Anderson J. C. - Nitro-Mannich Reaction, Chem. Rev. **113** (2013) 2887-2939. doi.org/10.1021/cr300272t.
11. Iijima K., Harada M., Fukuhara G., Okada T. - Frozen Solution-Mediated Asymmetric Synthesis: Control of Enantiomeric Excess, J. Org. Chem. **85** (2020) 4525. doi.org/10.1021/acs.joc.9b03415.
12. Co´rdova A. - The Direct Catalytic Asymmetric Mannich Reaction, Acc. Chem. Res. **37** (2004) 102. doi.org/10.1021/ar030231l.
13. List B. - The Direct Catalytic Asymmetric Three-Component Mannich Reaction, J. Am. Chem. Soc. **122** (2000) 9336. doi.org/10.1021/ja001923x.
14. Overmann L., Ricca D. - Comprehensive Organic Synthesis, Vol. 2, Pergamon Press, Oxford, 1991, pp. 1007.
15. Xu T., Zheng Z., Guo Y., Bai L. P. - Semisynthesis of novel magnolol-based Mannich base derivatives that suppress cancer cells *via* inducing autophagy, Eur. J. Med. Chem. **205** (2020) 112663. doi.org/10.1016/j.ejmech.2020.112663.
16. Prakash C. R., Raja S. - Synthesis, characterization and *in vitro* antimicrobial activity of some novel 5-substituted Schiff and Mannich base of isatin derivatives, J. Saudi Chem. Soc. **17** (2013) 337. doi.org/10.1016/j.jscs.2011.10.022.
17. Sivakumar K. K., Rajasekaran A., Senthilkumar P., Wattamwar P. P. - Conventional and microwave assisted synthesis of pyrazolone Mannich bases possessing anti-inflammatory, analgesic, ulcerogenic effect and antimicrobial properties, Bioorg. Med. Chem. Lett. **24** (2014) 2940. doi.org/10.1016/j.bmcl.2014.04.067.
18. Bamnela R., Shrivastava S. P. - Synthesis and Characterizaton of Some N-Mannich Bases as Potential Antimicrobial, Anthelmintic and Insecticidal Agent, Chem. Sci. Trans. **1** (2012) 431. doi:10.7598/cst2012.184.
19. Koksal M., Gokhan N., Kupeli E., Yesilada E., Erdogan H. - Analgesic and antiinflammatory activities of some new mannich bases of 5-nitro-2-benzoxazolinones, Arch. Pharm. Res. **30** (2007) 419. doi.org/10.1007/BF02980214.
20. Czopek A., Byrtus H., Zagorska A., Siwek A., Kazek G., Bednarski M., Sapa J., Pawłowski M. - Design, synthesis, anticonvulsant, and antiarrhythmic properties of novel *N*-Mannich base and amide derivatives of β-tetralinohydantoin, Pharmacol Rep. **68** (2016) 886. doi.org/10.1016/j.pharep.2016.04.018.
21. Racane L., Kulenovic T. V., Fiser-Jakic L. - Synthesis of Bis-substituted Amidinobenzothiazoles as Potential Anti-HIV Agents, Heterocycles **55** (2001) 2085.

22. Kashiyama E., Hutchinson I., Chua M. S., Stinson S. F., Phillips L. R., Kaur G., Sausville E. A., Bradshaw T. D., Westwell A. D., Stevens M. F. - Antitumor Benzothiazoles, Synthesis, Metabolic Formation, and Biological Properties of the *C*- and *N*-Oxidation Products of Antitumor 2-(4-Aminophenyl)benzothiazoles, J. Med. Chem. **42** (1999) 4172. doi.org/10.1021/jm990104o.
23. Brahmachari G. - Room Temperature One-Pot Green Synthesis of Coumarin-3-carboxylic Acids in Water: A Practical Method for the Large-Scale Synthesis, ACS Sustainable Chemistry & Engineering **3** (2015) 2350-2358. doi.org/10.1021/acssuschemeng.5b00826.
24. Gao F., Tao D., Ju C., Yang B. B., Bao X, Q., Zhang D., Zhang T. T., Li L. - Regioselectivity of aminomethylation in 3-acetyl-7-hydroxycoumarins: Mannich bases and Betti bases, New Journal of Chemistry **45** (2021) 9864-9871). doi.org/10.1039/D1NJ01584B.
25. Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R. - New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening, J. Natl. Cancer Inst. **82** (1990) 1107. doi.org/10.1093/jnci/82.13.1107.