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Synthesis and cytotoxicity evaluation of naphthalenecarboxamide derivatives

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ABSTRACT

The core structure of ethyl 4-acetoxy-6,8-dimethoxynaphthalene-2-carboxylate was successfully synthesized via a two-step sequence including Stobbe condensation followed by cyclization from 2,4-dimethoxybenzaldehyde as the starting material. The ethyl ester moiety was then hydrolyzed to form the corresponding carboxylic acid which was subsequently in situ converted to carboxyl choride by reacting with thionyl chloride and then to carboxamides derivatives by treating with 4-methylbenzylamine and 3-morpholinopropylamine. The structures of the synthesized compounds were fully confirmed based on MS, ^{1}H-NMR, ^{13}C-NMR and DEPT spectroscopy data. Cytotoxicity evaluation showed that the two prepared napthalenecarboxamide derivatives had weak cytotoxicity against Lu cells and moderate cytotoxicity against Hep-G2 cell lines.

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1 INTRODUCTION

Naphthalene derivatives have been known to possess potential bioactivities such as anti-oxidant, anti-malaria, especially anti-cancer. Some naphthalene-containing drugs have been commercialized such as nafacillin, naftifine, tolnaftate, terbinafine. Due to this, a lot of effort has been made to isolate or synthesize new naphthalene-containing compounds and test for bioactivities for drug development in the world (Couladouros *et al.*, 2002; Kodihalli *et al*., 2008; Lowell *et al*., 2008). Subramaniam (2008) have isolated a novel compound named 1-hydroxy-5,7-dimethoxy-2-naphthalenecarboxaldehyde (Marmelin) from *Aegle marmelos*. This pure compound was capable of inducing apoptosis to a variety of cell lines. Reddy (2008) also reported the synthesis of 2-amino-1-aroylnaphthalene and 2-hydroxy-1-aroylnaphthalene derivatives as potent antitubulin agents. In previous paper, the reported synthesis of a new naphthalenebenzimidazole compound showed as good cytotoxocity against MCF-7 cell line (IC_{50} = 3.48 ± 0.52 µg/mL) as the positive control tamoxifen (4 µg/mL) (Huynh Thi Minh Hai *et al*., 2014). This paper continues to present the results on the synthesis and cytotoxicity against Hep G2 (hepatocellular carcinoma) and LU (human lung carcinoma) cell lines of two new naphthalenecarboxamide derivatives.

2 EXPERIMENTAL SECTION

2.1 Material

Reactions were monitored by thin-layer chromatography on 0.2 mm pre-coated silica-gel 60 F_{254} plates (Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advanced 500 MHz spectrometer. MS were recorded on a 1100 series LC-MSD-Trap-LS Agilent spectrometer by electrospray impact (ESI).

2.2 Synthesis

2.2.1 Synthesis of (E)-3-(ethoxycarbonyl)-4-(2,4-dimethoxyphenyl)but-3-enoic acid (2)

To a 100 mL round bottom flask containing *t*-BuOK (2.8 g – 0.025 mol) in *t*-BuOH (40 mL) was added diethyl succinate (3.48 g – 0.02 mol) and 2,4-dimethoxybenzaldehyde (**1**) (1.66 g – 0.01 mol). The resulting mixture was stirred at 40°C for 4 hours and acidified using acetic acid. The reaction mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water until neutral, and with brine, dried over Na_2SO_4, and solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography using PE:EtOAc = 5:1 as the eluent afforded a slight yellow crystalline solid (2.473 g – 84.1%, R_f = 0.51; PE:EtOAc = 1:2). ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 7.98 (s, 1H, -CH=C-); 7.27 (s, 1H, CH-Ar); 6.54 (d, *J* = 2.0 Hz, 1H, CH-Ar); 6.47 (d, *J* = 2.0 Hz, 1H, CH-Ar); 4.29 (q, *J* =7.0 Hz, 2H, CH_2-OCO-); 3.83 (s, 3H, -OCH_3); 3.75 (s, 3H, -OCH_3); 3.52 (s, 2H, -CH_2-COOH); 1.35 (t, *J* = 7.0 Hz, 1H, -CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$, δ ppm): 168.0 (-CO-); 162.1 (-CO-); 159.0 (C-Ar); 138.4 (C-Ar); 130.8 (CH-C=); 123.7 (=C=); 116.6 (C-Ar); 110.0 (C-Ar); 104.6 (C-Ar); 98.5 (C-Ar); 61.6 (-CH_2-); 56.5 (-OCH_3); 55.5 (-OCH_3); 34.2 (-CH_2-); 14.2 (-CH_3).

2.2.2 Synthesis of ethyl 4-acetoxy-6,8-dimethoxynaphthalene-2-carboxylate (3)

A mixture of compound **2** (0.294 g – 0.001 mol), AcONa (0.082 g – 0.001 mol) and Ac_2O (0.57 mL – 0.006 mol) in a 10 mL round bottom flask was stirred at 130°C for 3 hours. The resulting mixture was cooled down, and 15% NaOH aqueous solution was added. The mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water until neutral, and with brine, dried over Na_2SO_4, and solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography using the mixture Hex:EtOAc = 6:1 as the eluent provided a white crystalline solid (0.270 g – 85%, R_f = 0.6, PE:EtOAc = 1:1). ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 8.80 (d, *J* = 0.5 Hz, 1H, CH); 7.81 (d, *J* = 1.5 Hz, 1H, CH); 6.67 (d, *J* = 2.0 Hz, 1H, CH); 6.54 (d, *J* = 2.0 Hz, 1H, CH); 4.39-4.43 (q, *J* = 7.0 Hz, 2H, CH_2-); 4.02 (s, 3H, -OCH_3); 4.01 (s, 3H, -OCH_3); 1.41-1.45 (t, *J* = 7.0 Hz, 3H, CH_3-). ^{13}C-NMR (125 MHz, $CDCl_3$, δ ppm): 169.2 (-CO-); 166.3 (-CO-); 161.0 (>C<); 158.0 (>C<), 145.4 (>C<); 131.2 (>C<); 124.5 (>C<); 123.3 (CH), 122.3 (>C<); 119.2 (CH); 98.6 (CH); 91.7 (CH); 61.1 (-CH_2-); 55.8 (-OCH_3); 55.4 (-OCH_3); 21.0 (-CH_3); 14.4 (-CH_3).

2.2.3 Synthesis of 4-hydroxy-6,8-dimethoxynaphthalene-2-carboxylic acid (4)

A mixture of ethyl 4-acetoxy-6,8-dimethoxynaphthalene-2-carboxylate (**3**) (0.636 g – 1 mmol) and 3M KOH aqueous solution (0.8 mL) in ethanol 95% (0.8 mL) was stirred at 70°C for 5 hours. Acidification of the reaction mixture using 2M HCl solution. The resulting mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water until neutral, washed with brine, dried over Na_2SO_4 and solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography using Hex:EtOAc = 3:1 as the eluent gave a yellow crystalline solid (0.426 g – 86%, R_f = 0.55, Hex:EtOAc = 1:3). ^{1}H-NMR (500 MHz, $CDCl_3$ and MeOD, δ ppm): 8.47 (s, 1H, CH-naphthalene); 7.36 (d, *J* = 1.5 Hz, 1H, CH-naphthalene); 7.14 (d, *J* = 2.0 Hz, 1H, CH-naphthalene); 6.53 (d, *J* = 2.0 Hz, 1H, CH-naphthalene); 3.98 (s, 3H, CH_3-O-); 3.95 (s, 3H, CH_3-O-). ^{13}C-NMR (125 MHz, $CDCl_3$ and MeOD, δ ppm): 169.9 (-COOH); 159.8 (naphthalene-C-OCH_3); 157.5 (naphthalene-C-OCH_3); 151.8 (naphthalene-C-OH); 129.3 (C-naphthalene); 124.1 (naphthalene-C-COOH); 121.9 (C-naphthalene); 117.4 (CH-naphthalene); 108.3 (CH-naphthalene); 98.4 (CH-naphthalene); 92.7 (CH-naphthalene).

2.2.4 Synthesis of N-(4-methylbenzyl)-4-hydroxy-6,8-dimethoxy-2- naphthamide (5a)

To a 50 mL round bottom flask containing **4** (0.0248 g – 0.1 mmol) in acetonitrile (2 mL) was added slowly $SOCl_2$ (0.0476 g). The resulting mixture was stirred at room temperature for 3.5 hours, and solvent was evaporated under reduced pressure to obtain a brown solid which was then dissolved in acetonitrile (2 mL). 4-Methylbenzylamine (0.0363 g – 0.3 mmol) was then added, and the mixture was stirred at room temperature for 10 minutes. Water was added (10 mL), and the mixture was extracted with EtOAc (4 × 30 mL). The combined organic layers were washed with water (3 ×10 mL), brine (30 mL), dried over Na_2SO_4, and solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography using Hex:EtOAc = 2:1 as the eluent gave a slight yellow crystalline solid (0.0294 g – 84%); R_f = 0.5 (EtOAc:Hex=1:1); Mp = 135-137°C. MS (ESI) m/z $[M+H]^+$ = 352.1. $[M-H]^-$ = 350.2. ^{1}H-NMR (500 MHz, DMSO, δ ppm): 10.14 (s, 1H, O*H*); 8.99 (s, 1H, NH); 8.06 (d, *J* = 1.0 Hz, 1H, CH-naphthalene); 7.32 (d, *J* = 2.0 Hz, 1H,

CH-naphthalene); 7.22 (d, *J* = 8.0 Hz, 1H, CH-naphthalene); 7.13 (d, *J* = 8.0 Hz, 1H, CH-naphthalene); 7.04 (d, *J* = 2.0 Hz, 1H, CH-Ar); 6.64 (d, *J* = 2.0 Hz, 1H, CH-Ar); 4.42 (d, *J* = 6.0 Hz, 2H, -CH_2); 3.95 (s, 3H, -OCH_3); 3.87 (s, 3H, -OCH_3); 2.27 (s, 3H, -CH_3). ^{13}C-NMR (125 MHz, DMSO, δ ppm)*:* 166.7 (>*C*=O); 158.4 (>*C*<); 156.7 (>*C*<); 152.0 (>*C*<); 137.0 (>*C*<); 135.6 (>*C*<); 129.4 (>*C*<); 128.7 (*C*H); 127.5 (>*C*<); 127.2 (*C*H); 121.2 (>*C*<); 111.8 (*C*H); 107.9 (*C*H); 98.4 (*C*H); 92.6 (*C*H); 55.7 (O*C*H_3); 55.2 (O*C*H_3); 42.4 (*C*H_2); 20.6 (*C*H_3).

2.2.5 Synthesis of N-(3-morpholinopropyl)-4-hydroxy-6,8-dimethoxy-2-naphthamide (5b)

The synthetic method of **5b** is the same as described for the synthesis of **5a** but using 3-morpholinopropylamine as the starting material. Purification of the crude product by column chromatography using EtOAc:MeOH = 6:1 as the eluent gave a white crystalline solid (19.15 mg – 51.2%); R_f = 0.58 (EtOAc:MeOH = 3:1); Mp = 172-174°C. MS (ESI) m/z $[M+H]^+$ = 373.1, $[M-H]^-$ = 375.1. ^{1}H-NMR (500 MHz, DMSO, δ ppm): 8.51 (s, 1H, NH); 7.98 (s, 1H, CH); 7.30 (s, 1H, CH); 7.03 (d, *J* = 2.0 Hz, 1H, CH); 6.63 (d, *J* = 2.5 Hz, 1H, CH); 3.94 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 3.59 (t, *J*= 4.5 Hz, 4H, CH_2); 3.30 (q, *J*= 6.5 Hz, 2H, CH_2); 2.35 (t, *J* = 7.0 Hz, 6H, CH_2); 1.70 (m, 2H, CH_2). ^{13}C-NMR (125 MHz, DMSO, δ ppm*):* 166.8 (>C=O); 158.4 (>C<); 156,7 (>C<); 152.2 (>C<); 129.7 (>C<); 127.5 (>C<); 121.2 (>C<); 111.4 (CH); 108.0 (CH); 98.5 (CH); 92.7 (CH); 66.2 (CH_2); 56.5 (CH_2); 55.7 (CH_3); 55.2 (CH_3); 53.4 (CH_2); 38.2 (CH_2); 25.7 (CH_2).

2.3 Cytotoxicity assays

Human cancer cell lines consisting of Hep G2 and LU were cultured in suitable media supplemented with 10% fetal bovine serum and other necessary ingredients at standard conditions (5% CO_2, 37°C, 98% humidity, absolute sterility). The time between passaging cells depended on the characteristics of each cell line. Log-phase cells were used in cytotoxicity testing. 200 µL log phase cultures of test cell lines (Hep G2 and LU) at the concentration of 3×10^4 cells/mL were seeded to 96-well plates containing DMEM medium. Test samples were diluted to obtain different final concentrations including 128 µg/mL, 8 µg/mL, 2 µg/mL, and 0.5 µg/mL. The plates were then incubated at 37°C with 5% CO_2 for 3 days. The positive control containing only cell cultures and the negative control containing cell cultures with Ellipticine (Sigma) were also included. After 3 days of incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (0.2 mg/mL) was added; the plates were then incubated at 37°C in 4 hours. After removal of culture media in the wells, 100 µL DMSO was added into each well and mixed thoroughly. The plates were then measured at 540 nm by a Genios TECAN spectrophotometer.

Percentage of cell growth inhibition was calculated as follows:

$$IC\ (\%) = 100\ \frac{\text{Odtest sample - ODcontrol(+)}}{\text{ODcontrol(-) - ODcontrol(+)}}$$

IC_{50} value was calculated by Table Curve software using percentage growth inhibition.

3 RESULTS AND DISCUSSION

One of the most effective methods for the construction of naphthalene core substructure is the sequence Stobbe condensation between an aromatic aldehyde or ketone with dialkyl succinate under basic conditions followed by cyclization (Elias *et al*., 2002). Based on this method, a synthetic strategy was developed towards desired naphthalenecarboxamide derivatives as presented in Scheme 1. Starting from commercially available 2,4-dimethoxybenzaldehyde, the naphthalene core substructure **3** was constructed *via* a tandem Stobbe/cyclization sequence in 71% yield over two steps. The high yield observed in this case probably due to the presence of the two methoxy groups in the benzene ring which caused a high *E* alkene selectivity in the first step and activated the benzene ring in the subsequent cyclization sequence.

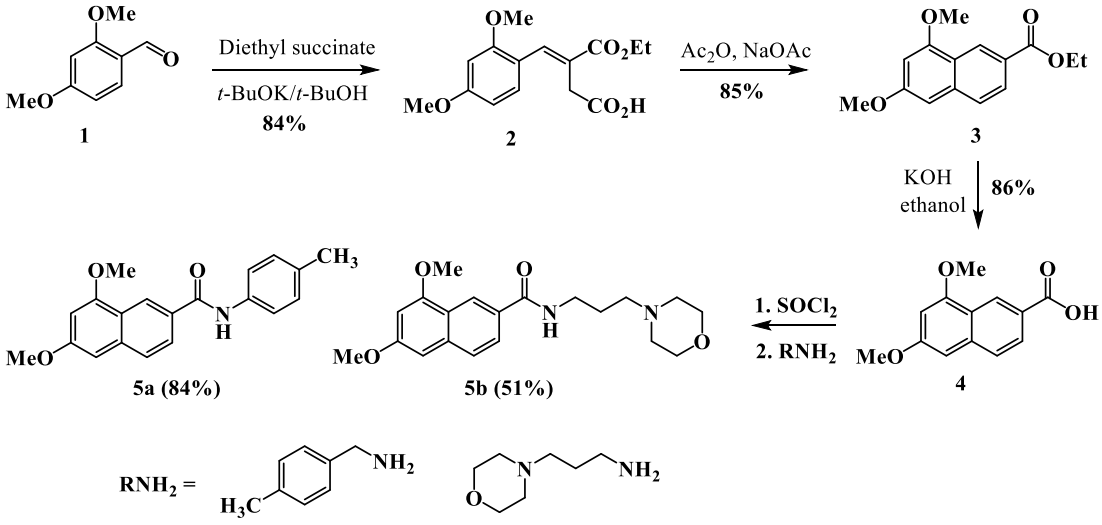

Fig. 1: Synthetic pathway towards naphthalenecarboxamide derivatives

For the formation of the desired naphthalenecarboxamide, the first effort was the direct aminolysis of the ester moiety using the corresponding amines. However, only the starting materials remained unchanged after the reaction probably due to steric hindrance of both ester **3** and the used amines and also due to the electron-releasing nature of the two methoxy groups which could deactivate the electrophilic character of the ester moiety towards nucleophilic attack. For that reason, the ester group must be activated prior reacting with amines. To that end, the ester moiety was then hydrolyzed to the carboxyl group by action of KOH solution in ethanol. Upon treating with thionyl chloride, the carboxyl group was converted into the corresponding acid chloride which was then reacted with amines to afford the desired naphthalenecarboxamide derivatives **5a** and **5b** in good to reasonable yields (84% and 51%, respectively).

The lower yield observed in the case of 5b compared to 5a could be explained by the presence of the tertiary amine of the morpholine moiety which could be partly protonated under acid conditions. As the results, this protonation step could either deactivate the nucleophilic property of the primary amine group or even make the morpholine moiety become a good leaving group and subsequently take part in a nucleophilic substitution or elimination under the reaction conditions, leading to the formation of different types of side products as observed in thin layer chromatography after the reaction.

The structures of the two naphthalenecarboxamide derivatives 5a and 5b were fully confirmed based on MS and NMR spectroscopic data.

Cytotoxicity assays against Hep G2 and LU cell lines were then carried out with the two synthesized naphthalenecarboxamide derivatives 5a and 5b. The results showed that compound 5a and 5b possessed moderate cytotoxicity against Hep G2 (IC_{50} of 11.74 and 12.25 μg/mL, respectively) compared to the control Ellipticine (IC_{50} = 0.51 μg/mL) and low cytotoxicity against LU cell line (IC_{50} of 59.53 and 58.32 μg/mL, respectively) compared to Ellipticine (IC_{50}= 0.52 μg/mL).

The research has been actively continuing in the lab in order to synthesize more naphthalene containing derivatives and screen for bioactivity especially cytotoxicity and antimicrobial activity.

4 CONCLUSIONS

Two naphthalenecarboxamide derivatives have been effectively prepared *via* a five-step sequence in rather good yield. The two prepared compounds showed moderate cytotoxicity against Hep G2 and low cytotoxicity against LU cell line compared to Ellipticine. This simple reaction pathway can easily applied to construct a potent naphthalene containing library for screening to find new leads for drug development.

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