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First total synthesis of isoquinolinone alkaloid marinamide and its methyl ester

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ABSTRACT

The first total synthesis of isoquinolinone alkaloid marinamide **1** and its methyl ester **2** was described. The key steps involved a regioselective Friedel–Crafts reaction of 1-benzyl-1*H*-pyrrole to form the intermediate **8**.

1. Introduction

Marinamide **1** and its methyl ester **2** were two novel isoquinolinone alkaloids, which were isolated from the metabolite of mixed fermentation of two mangrove endophytic fungi (strains Nos. 1924 and 3893) from the South China Sea. These two compounds were identified as 4-(2-pyrrolyl)-1-isoquinolone-3-carboxylic acid (**1**) and methyl 4-(2-pyrrolyl)-1-isoquinolone-3-carboxylate (**2**), respectively. Preliminary studies on the biological activity of these two compounds revealed that **1** and **2** both display significant antibacterial activity against *Escherichia coli* (diameter of bacteriostatics ring/cm: 1.4, **1**; 2.0, **2**), *Pseudomonas pyocyanea* (0.9, **1**; 1.7, **2**) and *Staphylococcus auereus* (1.0, **1**; 1.3, **2**) at the concentration of 1 mg/mL [1]. And they further demonstrated significant antitumor activity against HepG2 (IC_{50} = 2.52 μg/mL, **1** and 0.007 μg/mL, **2**), 95-D (IC_{50} = 1.54 μg/mL, **1** and 0.004 μg/mL, **2**), MGC832 (IC_{50} = 0.013 μg/mL, **1** and 0.091 μg/mL, **2**) and Hela cell lines (IC_{50} = 0.110 μg/mL, **1** and 0.529 μg/mL, **2**) [2]. Because of their promising biological activities, they can serve as ideal lead compounds for the development of drugs to combat cancer and other diseases. In order to provide an access to sufficient quantities of these materials for further pharmacological studies, we developed a facile and efficient approach to synthesize marinamide and its methyl ester.

2. Experimental

All reagents were purchased from commercial sources and used without further purification. Melting points were determined on a RY-1 hot stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 MHz instrument in $CDCl_3$ or DMSO-d_6, chemical shifts are given in part per million (ppm) relative to TMS as an internal standard. The HRMS spectra were obtained on a Thermo Finnigan spectrometer, model MAT 95XP.

2.1. Preparation of methyl 2-(1-benzyl-1H-pyrrole-2-carbonyl)benzoate (*8*)

2-(Methoxycarbonyl)benzoic acid (**4**, 12.6 g, 70 mmol) was dissolved in anhydrous dichloromethane (100 mL) and then thionyl chloride (12.5 g, 105 mmol) was added and the mixture was heated under reflux for 45 min. The solvent was evaporated under reduced pressure, toluene (50 mL) was added and the mixture was re-concentrated. The obtained residue was added into a mixture of 1-benzyl-1*H*-pyrrole (**7**, 7.5 g, 50 mmol), Zn powder (5.2 g, 80 mmol) and toluene (50 mL). The mixture was stirred at room temperature (Scheme 1). After the completion of the reaction as indicated by TLC analysis, the mixture was quenched with a saturated sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (2× 100 mL). Evaporation of the solvent followed by purification on silica gel afforded colorless liquid **8** (12 g, 78% yield). IR (cm^{-1}): ν 2950, 2921, 1721, 1630, 1457, 1397, 1323, 1331, 1263, 1122, 1078, 1030, 913, 871, 714. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.38 (s, 3H,), 5.70 (s, 2H), 6.18 (dd, 1H, *J* = 2.52, 4.05 Hz), 6.33 (dd, 1H, *J* = 1.71, 4.05 Hz), 7.23–7.46 (m, 7H), 7.60–7.66

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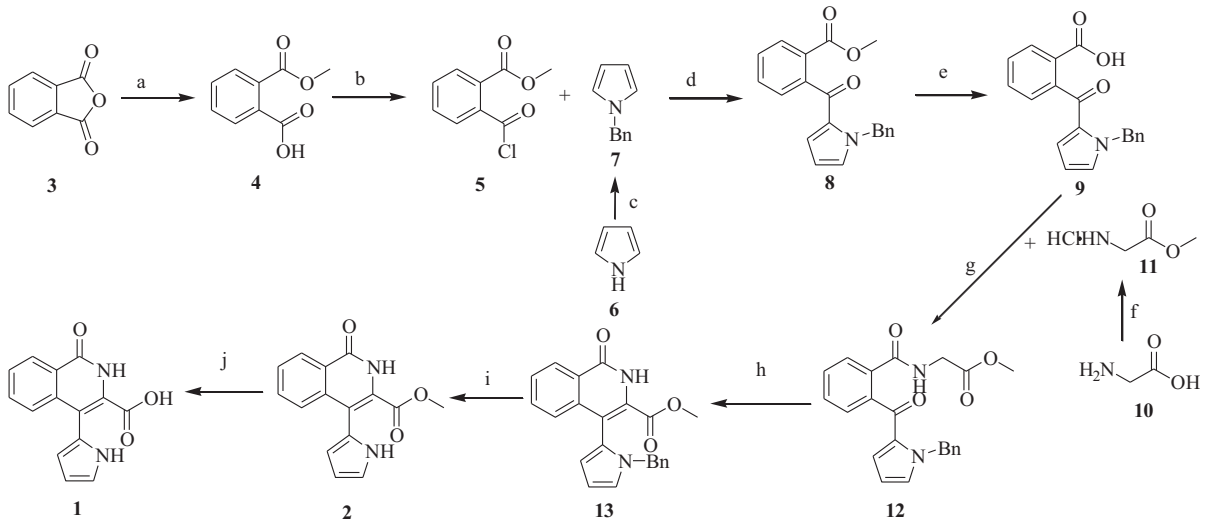

Scheme 1. The synthesis of marinamide (**1**) and its methyl ester (**2**). Reagents and conditions: (a) methanol, reflux; (b) $SOCl_2$, reflux; (c) KOH, DMSO, benzyl bromide, N_2; (d) Zn, toluene, r.t.; (e) 50% NaOH, methanol; (f) $SOCl_2$, methanol; (g) HOBT, EDCI, THF; (h) DBU, methanol/THF; (i) TFA/H_2SO_4, anisole; (j) 6 mol/L HCl, CH_3CN.

(m, 2H), 7.85–7.88 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 51.26, 51.98, 108.96, 122.58, 127.18, 128.13, 129.39, 129.39, 129.43, 129.43, 129.61, 129.67, 129.70, 130.64, 131.82, 132.01, 138.71, 141.51, 166.60, 185.05. HRMS calcd. for $C_{20}H_{18}NO_3$ [M+1]: 320.1287; found: 320.1289.

2.2. Preparation of 2-(1H-pyrrole-2-carbonyl)benzoic acid (**9**)

The compound **8** (12 g) was dissolved in methanol (100 mL) followed by the addition of a 50% NaOH solution (20 mL). The mixture was stirred at room temperature for 1 h and neutralized with hydrochloric acid. After methanol was evaporated under reduced pressure, the residue was extracted with ethyl acetate (3× 100 mL). After evaporation of the solvent, the solid was recrystallized from dichloromethane/methanol to give the colorless crystal **9** (9.6 g, 96% yield). Mp: 166–168 °C. IR (cm^{-1}): ν 3414, 3004, 2952, 1721, 1631, 1524, 1461, 1403, 1383, 1329, 1287, 1254, 1123, 1088, 1060, 961, 917, 886, 875, 777, 752. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.71 (s, 2H), 6.13 (m, 1H), 6.27 (m, 1H), 7.22–7.37 (m, 9H), 7.88 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.08, 108.90, 122.15, 126.95, 127.09, 127.09, 127.78, 127.78, 129.35, 129.48, 130.02, 130.02, 130.38, 131.47, 131.47, 138.87, 141.87, 167.39, and 185.79. HRMS calcd. for $C_{19}H_{16}NO_3$ [M+1]: 306.1131; found: 306.1130.

2.3. Preparation of [2-(1-benzyl-1H-pyrrole-2-carbonyl)benzoylamino]acetic acid methyl ester (**12**)

N-Hydroxybenzotrizole (HOBT) (1.6 g, 12 mmol) and compound **9** (3.5 g, 10 mmol) were dissolved in anhydrous THF (50 mL). Then, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDCI) (1.91 g, 10 mmol) was added, and the solution was stirred for 30 min. This solution was transferred to a solution of glycine methyl ester hydrochloride **11** (1.5 g, 12 mmol) and triethylamine (1.6 g, 16 mmol) in THF (60 mL) that had been prepared. The reaction was allowed to stir overnight at room temperature. Then the reaction was diluted with water (200 mL) and extracted with ethyl acetate (200 mL). The organic layer was washed with water (100 mL), a solution of saturated sodium bicarbonate (100 mL), 1 mol/L hydrochloric acid (100 mL), and finally water again (100 mL). After evaporation of the solvent, the crude product was crystallized from dichloromethane to give the colorless crystal **12** (3.4 g, 90% yield). Mp 187–189 °C; IR (cm^{-1}): ν 3428, 3294, 3100, 3056, 3028, 2950, 2927, 1762, 1660, 1624, 1591, 1544, 1525, 1457, 1409, 1396, 1348, 1316, 1256, 1209, 1175, 1153, 1084, 1036, 1011, 968, 920, 876, 758, 752, 688; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.60 (s, 3H), 3.86 (d, 2H, J = 6.6 Hz), 5.65 (s, 2H), 6.14 (m, 1H), 6.34 (m, 1H), 7.37–7.18 (m, 7H), 7.59 (m, 2H), 7.70 (m, 1H), 8.87 (d, 1H, J = 6.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.65, 52.23, 52.64, 109.27, 124.86, 125.81, 127.34, 127.53, 128.43, 128.56, 128.61, 129.71, 129.96, 130.48, 131.91, 133.09, 134.28, 138.05, 139.64, 167.94, 169.95, 186.73; HRMS calcd. for C_{22} $H_{21}N_2O_4$ [M+1]: 377.1501; found: 377.1504.

2.4. Preparation of 4-(1-benzyl-1H-pyrrol-2-yl)-1-oxo-1,2-dihydroisoquinoline-3-carboxylic acid methyl ester (**13**)

Compound **12** (0.38 g, 1 mmol) was dissolved in methanol (10 mL) and THF (5 mL), and then 1,8-diazabicyclo[5.4.0]-7-undecene (DBU, 0.3 g, 2 mmol) was added. The mixture was heated under reflux for 12 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between ethyl acetate and 1 mol/L hydrochloric acid, washed with aqueous sodium bicarbonate, and brine. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography to give **13** (0.3 g, 86% yield) as yellow powders. Mp: 182–183 °C; IR (cm^{-1}): ν 3433, 3169, 3109, 3049, 2948, 2917, 1737, 1655, 1602, 1495, 1463, 1431, 1336, 1294, 1249, 1230, 1154, 1124, 1067, 873, 751,724, 579. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.57 (s, 3H), 4.88 (m, 2H), 6.02 (m, 1H), 6.19 (m, 1H), 6.93–7.16 (m, 7H), 7.62 (m, 2H), 8.24 (d, 1H, J = 7.26 Hz), 11.43 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.64, 53.51, 112.83, 113.11, 117.69, 126.80, 127.81, 127.81, 128.06, 128.06, 128.30, 128.47, 128.76, 129.61, 129.61, 130.06, 130.06, 133.36, 134.53, 137.38, 160.94, 161.17. HRMS calcd. for $C_{22}H_{19}N_2O_3$ [M+1]: 359.1396; found: 359.1405.

2.5. Preparation of methyl ester of marinamide (**2**)

A solution of **13** (0.36 g, 1 mmol), trifluoroacetic acid (1.5 mL), methoxybenzene (0.5 mL) and concentrated sulfuric acid (0.1 mL) was heated at 90 °C for 30 min. The reaction mixture was dissolved in ice water and extracted with dichloromethane (3× 50 mL) and washed with saturated sodium bicarbonate (2× 30 mL). The organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a yellow solid

followed by purification on silica gel to afford pure product **2** (0.12 g, 46% yield). Mp >300 °C; IR (cm^{-1}): 3428, 3331, 1658. ^1H NMR (300 MHz, $CDCl_3$): δ 3.54 (s, 3H), 6.11 (m, 1H), 6.24 (m, 1H), 7.06 (m, 1H), 7.41–7.58 (m, 3H), 7.89 (m, 1H), 12.01 (s, 1H), 11.45 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.17, 108.54, 110.67, 112.48, 116.98, 122.23, 122.45, 123.61, 124.50, 124.63, 131.44, 140.07, 141.14, 167.45, 172.28. HRMS calcd. for $C_{15}H_{13}N_2O_3$ $[M+1]^+$: 269.0926; found: 269.0931.

2.6. Preparation of marinamide(*1*)

Compound **2** (0.1 g) was dissolved in acetonitrile (5 mL) followed by the addition of a 6 mol/L hydrochloric acid solution (5 mL). The mixture was stirred and refluxed for 10 h. After the methanol was evaporated under reduced pressure, the residue was extracted with ethyl acetate (3× 20 mL). The solvent was evaporated under reduced pressure; the obtained residue was recrystallized from dichloromethane to give marinamide **1** (0.06 g, 66% yield). Mp > 300 °C; IR (cm^{-1}): ν 3428, 3330, 1650. ^1H NMR (300 MHz, DMSO-d_6): δ 6.18 (m, 1H), 6.23 (m, 1H), 6.98 (m, 1H), 7.35–7.56 (m, 3H), 7.88 (m, 1H), 9.76 (s, 1H), 11.79 (s, 1H), 14.95 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 106.48, 109.17, 113.56, 120.18, 121.96, 122.54, 123.31, 123.48, 125.46, 134.34, 136.78, 146.72, 167.56, and 178.34. HRMS calcd. for $C_{14}H_{11}N_2O_3$ $[M+1]^+$: 255.0770; found: 255.0773.

3. Results and discussion

As illustrated in Scheme 1, the synthesis of intermediate **8** is the key step for the synthesis of marinamide and its methyl ester, which involved a regioselective Friedel–Crafts acylation of 1-benzyl-1*H*-pyrrole to form a 2-acylpyrrole derivative. The Friedel–Crafts acylation of pyrrole and its derivatives often produced a mixture of 2- and 3-acylated products because of their high reactivity in the electrophilic substitution reactions and general sensitivity to acid-catalyzed polymerizations [3]. Frequently, it was difficult to separate the mixtures. Therefore, it is crucial to only produce the 2-acylpyrrole derivative. Yadav once reported a general method for the selective 2-acylation of a range of pyrrole derivatives by exposing them to an acid chloride and zinc powder in toluene [6]. We attempted to adopt this method to synthesize the key intermediate **8**. The synthesis of **8** commenced with the synthesis of **4** from the monoesterification of phthalic anhydride **3** in methanol based on the procedures reported in the literature [4]. Then the intermediate **7** was synthesized by treating pyrrole **6** with benzyl bromide in anhydrous DMSO [5]. Based on what the literature described, reaction of compound **4** with $SOCl_2$ gave the acyl chloride **5**, which reacted with **7** through the catalysis of zinc powder in toluene at room temperature to successfully provide the key intermediate **8** in 68% yield [6].

Having synthesized the key intermediate **8**, the next step was to prepare intermediate **12**. This was achieved in three steps including hydrolysis of **8** to afford **9**, the synthesis of glycine methyl ester hydrochloride **11** and acylation of **9** with **11**. Thus, hydrolysis of ester **8** with 50% NaOH afforded the acid **9**, which reacted with **11** generated from the esterification of glycine in methanol with $SOCl_2$ to provide compound **12** in 90% yield [7].

Finally, the intermediate **13** was successfully synthesized by an intramolecular condensation of **12** promoted by DBU in methanol/THF (2/1) [8]. Then, deprotection of **13** with acid (TFA/H_2SO_4) and anisole at 90 °C successfully afforded the targeted methyl ester of marinamide **2** [9]. Hydrolysis of **2** with 6 mol/L hydrochloric acid afforded marinamide **1**.

The spectral data (IR, ^1H NMR, ^{13}C NMR, HRMS) of all new intermediates, marinamide, and its methyl ester were satisfactory. The spectral data of **1** and **2** were identical to those reported in the literature [1].

4. Conclusion

In conclusion, marinamide **1** and its methyl ester **2** have been successfully synthesized by employing a regioselectivity Friedel–Crafts acylation of a protected pyrrole as the key step. The syntheses described here will allow further investigations on their pharmacological properties and structure–activity relationship.

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