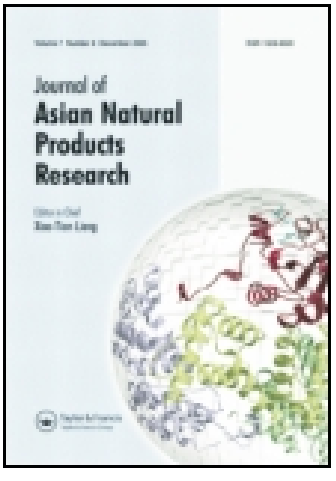

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An efficient synthesis method targeted to marine alkaloids marinacarbolines A-D and their antitumor activities

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An efficient synthesis method targeted to marine alkaloids marinacarbolines A–D and their antitumor activities

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Marinacarbolines A–D are a series of marine β-carboline alkaloids isolated from actinomycete *Marinactinospora thermotolerans* of the deep South China Sea with antiplasmodial activities. In inhibition assays of *in vitro* growth of *Plasmodium falciparum*, marinacarbolines exhibited antiplasmodial activity against drug-sensitive line 3D7 and drug-resistant line Dd2 of *P. falciparum*. However, approaches for the synthesis of such useful compounds are very limited. In this work, we reported a simple, efficient, and versatile process to synthesize marinacarbolines A–D (**1**–**4**). On the basis of that, the antitumor activities of marinacarbolines in a structure-dependent manner were allowed to be unveiled.

Keywords: marinacarbolines; marine β-carboline alkaloid; synthesis; antitumor activities

1. Introduction

Indole alkaloids are considered to be one of the most important structural units having great significance in medicinal chemistry. The β-carboline alkaloids, containing a tricyclic pyrido[3,4-b]indole ring [1], displayed a wide range of biological properties, such as antitumor, anti-HIV, antimalarial [2], and neuroprotecting activities [3]. Because of their practical importance, the β-carboline alkaloids have drawn tremendous interest from synthetic and medicinal chemists [1].

Marinacarbolines A–D (Figure 1, **1**–**4**), four new marine β-carboline alkaloids, have been isolated from *Marinactinospora thermotolerans* SCSIO 00652 obtained from the deep South China Sea, and exhibited antiplasmodial activities against *Plasmodium falciparum* of both the drug-sensitive (3D7) and drug-resistant (Dd2) strains [4]. In 2013, Ju et al. [3] successfully synthesized marinacarbolines A–D through an enzymatic approach. Very recently, Hibino and co-workers have reported the first chemical synthesis of marinacarbolines A–D from methyl 1-chloro-β-carboline-3-carboxylate in four steps [5]. However, the required compound methyl 1-chloro-β-carboline-3-carboxylate was prepared in another six-step sequence which is not an atom economical process [5]. Our previous studies on the structure–activity relationship indicated that the electron-withdrawing substitution at C-3 position of the β-carboline alkaloids confers antitumor activities [6]. Because of the limited amount of natural occurring compounds, the potential antitumor activities of marinacarbolines A–D remain unknown.

Given our longstanding interest in the β-carboline alkaloids [7] and the intriguing biological profile of marinacarbolines A–D, we undertook the development of a scalable total synthesis of marinacarbolines A–D to provide sufficient quantities for additional biological evaluation.

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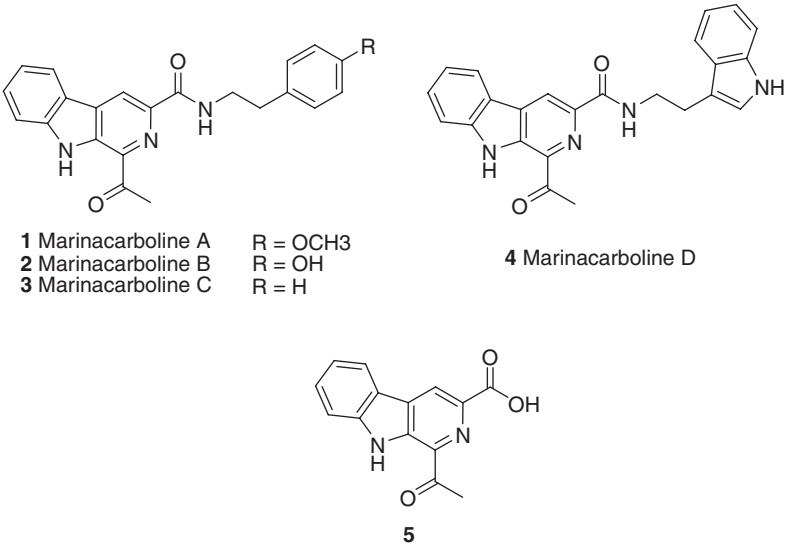


Figure 1. The structures of β-carbolines derivatives (**1–5**).

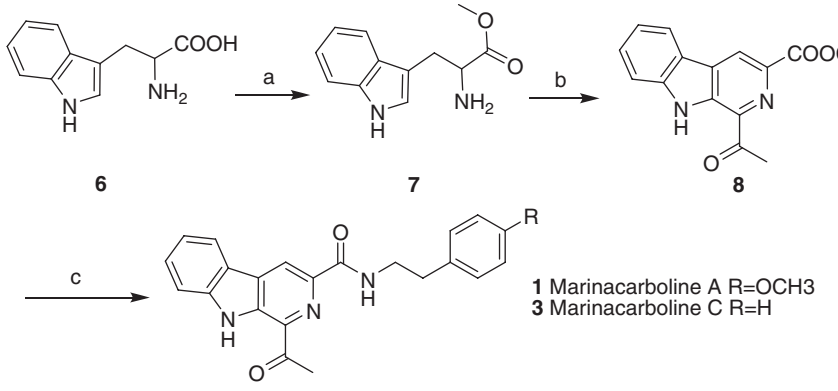
We herein report the total chemical synthesis of marinacarbolins A–D through the Pictet–Splengler reaction from L-tryptophan. In addition, antitumor activities of marinacarbolines A–D and compound **5** against a human colon cancer cell line (HCT116) and two human lung cancer cell lines (H1299 and A549) were investigated.

2. Results and discussion

2.1. Chemistry

The traditional methods for the synthesis of β-carboline alkaloids are mainly focused on the two-step methods of Pictet–Spengler and Bischler–Napieralski condensations [8–11], which is the cyclization of L-tryptophan with different appropriate aldehyde to provide a corresponding diastereoisomeric mixture of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid as the first step, followed by the aromatization of dehydrogenation to yield β-carboline. Very recently, Ahmed et al. [12] reported unexplored multi-coupled domino reactions for the total synthesis of marinacarboline and analogs. However, highly toxic iodine was employed in the reaction process. The modification method is to use *p*-toluenesulfonic acid as the acid catalyst to make the cyclization and aromatization in one step to form β-carboline directly with the reaction between L-tryptophan and different appropriate aldehydes [6]. This strategy for the construction of the β-carboline derivatives is efficient and straightforward, and based on the principle, we previously accomplished the total syntheses of marine alkaloid pityriacitry [6] and eudistomins Y_1–Y_7 [7].

The amide-substituted group at 3-position of β-carboline is unique to marine alkaloid marinacarbolines A–D. Therefore, the β-carboline-3-carboxylic acid is the key intermediate to facilitate the chemical synthesis of marinacarbolines A–D. Based on the established method, β-carboline-3-carboxylic acid was formed in lower yield as a by-product because the carboxyl group at 3-position was easily removed at the aromatization step [6]. Not surprisingly, our synthetic strategy for the key intermediate of β-carboline-3-carboxylic acid was envisioned to exploit



Scheme 1. Synthesis of marinacarbolines A (**1**) and C (**3**). Reagents and conditions: (a) HCl/CH_3OH, rt, 89%; (b) methyl–glyoxal/p-TSA/CH_3OH, 64°C, 120 min, 90%; (c) triethylamine/4-methoxyphenethylamine/$CHCl_3$, 50°C, for **1**, 85%; triethylamine/2-phenylethylamine/$CHCl_3$, 50°C, for **3**, 82%.

the lessons learned in our earlier synthesis, with important modifications to ensure efficient access to the natural product (Scheme 1).

To obtain 1-acetyl-3-carboxy-β-carboline, L-tryptophan methyl ester (**7**) was used to react with methyl-glyoxal in methanol, and *p*-toluenesulfonic acid was used as the catalyst for 2 h at 64°C. By using this condition, 1-acetyl-3-(methoxycarbonyl)-β-carboline (**8**) was formed in one-pot in a yield of 90%.

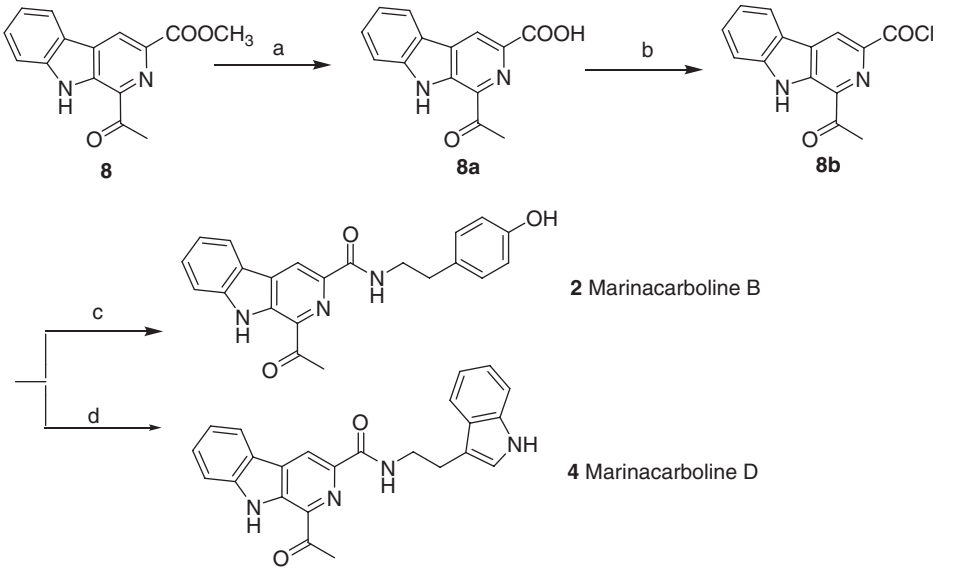
Subsequently, marinacarbolines A and C (**1** and **3**) were synthesized by the reaction of 1-acetyl-3-(methoxycarbonyl)-β-carboline (**8**) with different appropriate amines, 2-phenylethylamine and 4-methoxyphenethylamine, in $CHCl_3$ at 50°C, respectively. The reaction routes are outlined in Scheme 1.

However, marinacarbolines B and D (**2** and **4**) could not be obtained from 1-acetyl-3-(methoxycarbonyl)-β-carboline (**8**), because tyramine and tryptamin in the ester exchange reaction of amine were easy to form by-product, and the reaction activity was lower when heated up to 50°C. To increase the reaction yield, the target compounds, marinacarbolines B and D, were synthesized through the reaction of 1-acetyl-3-chlorocarbonyl-β-carboline with the corresponding amine (Scheme 2).

At 60°C, 1-acetyl-3-(methoxycarbonyl)-β-carboline (**8**) was treated with 18% aqueous NaOH to remove the methyl group and to form 1-acetyl-3-carboxy-β-carboline (**8a**) in a yield of 92%. **8a** was then treated with oxalyl chloride in dichloromethane to obtain 1-acetyl-3-chlorocarbonyl-β-carboline (**8b**). Finally, **8b** was used to react with tyramine or tryptamine in the presence of pyridine and CH_2Cl_2 as solvent to afford a target compound marinacarbolines B or D (**2** or **4**). The reaction routes are outlined in Scheme 2.

2.2. *Biological activities*

We measured the percentage of viable cancer cells after 48 h exposure to the β-carbolines derivatives, marinacarbolines A–D (**1–4**), and the key intermediate **5** at a concentration of 50 μM as compared with the compound-free control that is 100% viability (Figure 2) in the three cancer cell lines including two lung cancer (A549 and H1299) and one colon cancer (HCT116) cells. Compounds **4** and **5** showed considerable cytotoxicities in the three cancer cell lines tested.



Scheme 2. Synthesis of marinacarbolines B (**2**) and D (**4**). Reagents and conditions: (a) NaOH/H_2O/CH_3OH, 60°C, 92%; (b) $C_2Cl_2O_2$/ CH_2Cl_2; (c) tyramine/pyridine/CH_2Cl_2; 68%; (d) tryptamine/pyridine/DMF/CH_2Cl_2; 71%.

Subsequently, the IC_{50} in the three cancer lines was explored further using doxorubicin as a positive control (Table 1). All the β-carbolines derivatives showed moderate cytotoxicities in each tested cell line. Interestingly, the IC_{50} value for each compound was not proportional to the data shown in Figure 1, this indicated that the concentration-dependent cytotoxicity curve was unique to each compound. The data in Table 1 showed that compound **5** with carbonyl group and compound **4** with amide of tryptamine at the 3-position of β-carbolines were more toxic than other compounds in H1299 and HCT116 cells with IC_{50} which ranged from 11 to 18 μM and less toxic than the positive control of doxorubicin.

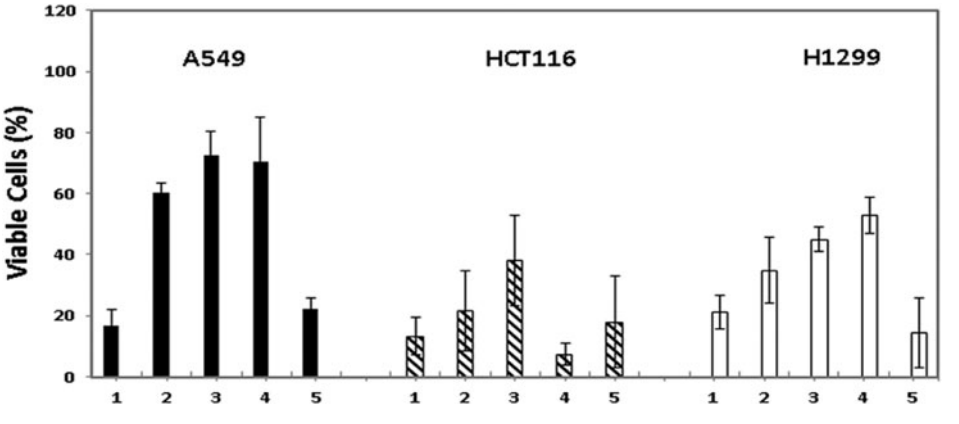


Figure 2. The percentage of viable cancer cells after 48 h exposure to compounds **1–5** at a concentration of 50 μM compared with the compound-free control (100% viability). The compounds were formulated initially in DMSO and then diluted in complete growth media (details are in experimental protocols) (■, A549; ⬔, HCT116; □, H1299).

Table 1. IC_{50} values of compounds **1–5** in cancer cells A549, H1299, and HCT116 after 48 h exposure to the compounds.

	$IC_{50}(\mu M)$		
Compound	A549	H1299	HCT116
Marinacarboline A (**1**)	33.9 ± 7.95	26.9 ± 4.75	24.4 ± 2.00
Marinacarboline B (**2**)	57.6 ± 1.50	63.7 ± 3.13	37.9 ± 0.86
Marinacarboline C (**3**)	47.5 ± 1.50	47.5 ± 3.14	37.4 ± 2.23
Marinacarboline D (**4**)	29.9 ± 6.13	15.0 ± 0.15	23.9 ± 3.81
intermediate (**5**)	32.9 ± 2.60	14.7 ± 0.25	18.7 ± 2.17
Doxorubicin	0.32 ± 0.06	6.70 ± 0.59	0.20 ± 0.09

3. Experimental

3.1. Materials and methods

Melting points were recorded on a micro melting point apparatus MP-500D and are uncorrected (Shanghai Instrument Physical Optics Instrument Co. Ltd., Shanghai, China). NMR spectra were recorded on a Jeol JNM-ECP spectrometer at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR with TMS as the internal standard (JEOL Ltd., Tokyo, Japan). Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), brs (broad singlet), and so on. Mass spectra were recorded using a Q-TOF Ultima™ Global by chemical ionization (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China). The starting materials and reagents purchased from commercial suppliers were used without further purification. All reactions were monitored by a thin-layer chromatography (TLC), on aluminum sheets (Silica gel 60-F_{254}, E. Merck, Darmstadt, Germany). Compounds were visualized by UV light. All reaction solvents were dried prior to use, according to the standard procedures. All primary reagents were commercially available. Silica gel chromatography solvents were of analytical grade.

3.2. General procedure for 1-acetyl-3-(methoxycarbonyl)-β-carboline synthesis (8)

To a solution of L-tryptophan methyl ester (0.18 g, 0.67 mmol) in CH_3OH (40 ml), *p*-toluenesulfonic acid monohydrate (0.14 g, 0.67 mmol) and 40% methyl–glyoxal (1.2 ml, 6.67 mmol) were added. The solution was stirred at 64°C for 2 h and the completion of the reaction was monitored by TLC. The resulting suspended solution was poured into 100 ml of ice water and the solid materials were collected by filtration and dried in vacuum to afford a yellow solid. The crude products were purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:2) as the mobile phase to provide compound **8**.

1-Acetyl-3-(methoxycarbonyl)-β-carboline (**8**): yellow solid; yield 90%; Mp 224–227°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 10.51 (s,1H), 9.02 (s, 1H), 8.20 (d, $J = 7.7$ Hz, 1H), 7.58–7.65 (m, 2H), 7.40 (t, $J = 7.7$ Hz, 1H), 4.09 (s, 3H), 2.97 (s, 3H). ESI-MS: (*m/z*) 269 $[M + H]^+$, 291 $[M + Na]^+$.

3.3. General procedure for marinacarboline A and marinacarboline C

To the solution of 1-acetyl-3-(methoxycarbonyl)-β-carboline (1 g, 3.7 mmol) in $CHCl_3$ (30 ml), triethylamine (1.5 g,

3.7 mmol) and 2-phenylethylamine (1.88 ml, 6.4 mmol) were added dropwise. The reaction mixture was heated at 50°C for 6 h and the completion of the reaction was monitored by TLC. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was poured into water (100 ml) and extracted with ethyl acetate (20 ml × 3). The combined organic phase was washed with 5% aqueous $NaHCO_3$ (20 ml × 3), H_2O (20 ml × 3), and brine (20 ml × 3), dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure to afford a yellow crude product. The residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:15) as the mobile phase to provide compound **1**.

Marinacarboline A (**1**): Yellow solid; yield 85%; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 10.39 (s, 1H), 9.09 (s, 1H), 8.22 (d, $J = 7.7$ Hz, 1H), 8.07 (t, $J = 5.5$ Hz, 1H), 7.59–7.64 (m, 2H), 7.38 (t, $J = 7.7$ Hz, 1H), 7.23–7.26 (m, 2H), 6.90 (d, $J = 8.8$ Hz, 2H), 3.79–3.83 (m, 5H), 2.96 (t, $J = 6.6$ Hz, 2H), 2.78 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 202.3, 164.6, 158.5, 141.6, 139.3, 136.3, 133.5, 132.7, 131.1, 130.0, 129.8, 122.4, 121.6, 121.1, 118.4, 114.3, 112.2, 55.4, 40.8, 35.1, 25.8.

Marinacarboline C (**3**): Yellow solid; yield 82%; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 9.08 (s, 1H), 8.21 (d, $J = 7.7$ Hz, 1H), 8.07 (t, $J = 5.5$ Hz, 1H), 7.58–7.66 (m, 2H), 7.32–7.40 (m, 5H), 7.26–7.29 (m, 1H), 3.86 (t, $J = 7.2$ Hz, 2H), 3.02 (t, $J = 7.1$ Hz, 2H), 2.75 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 202.4, 164.7, 141.7, 139.2, 133.6, 136.4, 132.7, 131.0, 129.8, 129.1, 129.0, 128.9, 128.8, 126.7, 122.4, 122.4, 121.2, 118.4, 112.3, 40.6, 36.0, 25.8.

3.4. General procedure for marinacarboline D and marinacarboline B

To the solution of 1-acetyl-3-(methoxycarbonyl)-β-carboline (**8**, 0.2 g, 0.75 mmol) in CH_3OH (10 ml), 18% aqueous NaOH (5.5 ml) was slowly added. After the addition, stirring was continued for 2 h at 60°C. The completion of the reaction was monitored by TLC. Subsequently, concentrated hydrochloric acid was slowly added to the reaction solution to adjust the pH to 1, and the resulting reaction mixture was filtered and dried *in vacuum* to afford a white crude product. The crude product was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/methanol/formic acid (50:2:1) as the mobile phase to provide the 1-acetyl-3-carboxy-β-carboline (**8a**) as a white solid in a yield of 92%.

To a solution of **8a** (1.0 g, 3.7 mmol) in anhydrous CH_2Cl_2 was added $(COCl)_2$ (1.1 ml, 11.1 mmol). The above mixture was stirred at room temperature for about 1 h and the completion of the reaction was monitored by TLC. The resulting solution was evaporated under reduced pressure. The residue was solved in anhydrous CH_2Cl_2 and tryptamine (62 mg, 0.39 mmol) and pyridine (94 μl, 1.17 mmol) were added. In addition, stirring was continued overnight at room temperature. The mixture was poured into 10 ml of ice water and extracted with ethyl acetate (30 ml × 2). The combined organic phase was washed with 2 N aqueous hydrochloric acid (20 ml × 3), H_2O (20 ml × 3), and brine (20 ml × 3), dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure to afford a yellow crude product. The crude product was purified by flash column chromatography using silica gel as the stationary phase and ethyl acetate/hexane (1:10) as the mobile phase to provide the title compound **4**.

Marinacarboline D (**4**): Yellow solid; yield 68%; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.17 (s, 1H), 10.90 (s, 1H), 9.11 (s, 1H), 8.78 (t, $J = 6.1$ Hz, 1H), 8.44 (d, $J = 8.3$ Hz, 1H), 7.83 (d, $J = 6.1$ Hz, 1H), 7.67 (d, $J = 6.1$ Hz, 1H), 7.62 (t, $J = 7.7$ Hz, 1H), 7.36 (d, $J = 8.1$ Hz, 1H), 7.35 (t, $J = 7.5$ Hz, 1H), 7.27

(s, 1H), 7.08 (t, $J = 7.7$ Hz, 1H), 6.98–7.01 (m, 1H), 3.72 (q, $J = 6.6$ Hz, 2H), 3.06 (t, $J = 7.7$ Hz, 2H), 2.83 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 201.1, 164.1, 142.4, 138.8, 136.5, 134.9, 134.0, 132.0, 129.3, 127.4, 122.9, 122.3, 121.1, 120.9, 120.4, 118.6, 118.4, 118.0, 113.4, 111.8, 111.5, 39.6, 26.1, 25.4.

Marinacarboline B (**2**): Yellow solid; yield 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ:12.17 (s, 1H) 9.23 (s, 1H), 9.08 (s, 1H), 8.68 (t, $J = 5.9$ Hz, 1H), 8.45 (d, $J = 8.1$ Hz, 1H), 7.82 (d, $J = 8.2$ Hz, 1H), 7.61 (t, $J = 8.2$ Hz, 1H), 7.32–7.35 (m, 2H), 7.11 (d, $J = 8.3$ Hz, 2H), 6.63 (d, $J = 8.3$ Hz, 2H), 3.58 (q, $J = 6.6$ Hz, 2H), 2.87 (s, 3H), 2.80 (t, $J = 7.4$ Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 203.3, 166.1, 141.4, 137.1, 136.5, 136.5, 135.3, 131.9, 129.8, 129.7, 122.1, 122.6, 121.7, 121.6, 121.3, 121.2, 120.9, 120.9, 112.4, 52.8, 29.9, 25.7.

3.5. *Biological activities*

A549 (2000 cells/well), H1299 (2000 cells/well), and HCT116 (2000 cells/well) cells were seeded in 96-well plates. After 24 h, cells were treated with serial concentrations of β-carbolines derivatives in 200 μl of serum complete media. After 48 h of treatment, 20 μl of resazurin (2 mg/ml dissolved in water, Sigma, St Louis, MO, USA) cells was added to the media. After 16 h incubation at 37°C, the fluorescent signal was monitored using 544 nm excitation wavelength and 595 nm emission wavelength by a Spectramax M5 plate reader (Molecular Devices, Sunnyvail, CA, USA). The relative fluorescence unit generated from the assay was proportional to the number of living cells in each well.

4. Conclusions

In summary, an efficient synthetic method for marinacarbolines A–D was developed. It was expected that this methodology could not only be used in the synthesis of other β-carbolines possessing an amino acid side chain, but also be effective in the synthesis of other complex natural products. The chemically synthesized marinacarbolines A–D and other analogs made wider bioassay studies possible.

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Note

1. These authors contributed equally to this work.

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