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Original article

Design, synthesis, and biological evaluation of novel γ-carboline ketones as anticancer agents

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ABSTRACT

A series of novel γ-carboline ketones were designed, synthesized and evaluated for their cytotoxic activity *in vitro* against six human cancer cell lines (A549, SGC, HCT116, MCF-7, K562 and K562R). Biological evaluation revealed that almost all of the new compounds displayed moderate to potent cytotoxic activities against the tested cells. Among them, seven of the fourteen new compounds show more potent cytotoxic activities against K562R cell line than that of the positive control, taxol. Primary mechanism research on the most potent compound **6f** indicated that it was a potent tubulin polymerization inhibitor, with IC_{50} value of 4.3 μM, equivalent to that of CA-4, and arresting cell cycle in G_2/M phase.

1. Introduction

Microtubules, the key components of cytoskeleton of eukaryotic cells, are involved in numerous cellular functions, including cell motility, cell division and vesicle transport. Microtubules are in dynamic equilibrium with tubulin dimmers. Disruption of the dynamic equilibrium blocks the cell division machinery at mitosis and leads to cell death. Therefore, microtubule has attracted considerable attention as being a promising target of antitumor drugs [1]. Reported antitubulin agents can be divided into three groups, namely, the vinca-, the taxus-, and the colchicine-binding site inhibitors (CSIs) [2]. Although vinca alkaloids, taxanes are used for the treatment of cancer, their adverse effects, difficulty in synthesis and high cost limited their use [3]. So, the search for new antitubulin agents with better activity is still in progress. Recent years, CSIs have attracted more and more attention because of their simple structures and potent activities [4]. The common structural features of CSIs have been described [5,6]. It is reported that most CSIs bear two hydrophobic groups (ring A and B) and a *cis*-bridge between them. It is also reported that the existence of hydrogen-bond acceptor at ring A and hydrophobic groups or polar functional groups at ring B is beneficial for binding affinity.

Three series of γ-carboline derivatives with some displaying potent cytotoxicities have been reported in our previously studies on anticancer agents (**1**–**3**, Fig. 1.) [7]. We supposed that these compounds match well with the common structure of CSIs: the planar γ-carboline acts as the hydrophobic ring A of CSIs, and the N^2 position of carboline contributes as a hydrogen-bond acceptor; the aromatic ring connected to the linker is the hydrophobic ring B of CSIs. Further mechanism study showed they could inhibit tubulin polymerization (data haven't been revealed yet). Enlightened by these results and the fact that many CSIs bearing a carbonyl group, such as BPR0L075 (**4**) [8,9] and Phenstatin (**5**) [10], showed potent activity, we introduced a carbonyl group as the linker in designed compounds. We also introduced hydrophobic substituents, such as halogen and methoxy group, as well as polar functional group, such as amino group, on ring B. Here, we reported the synthesis and biological evaluation of this series of novel γ-carboline ketone compounds, with the aim to better understand the structure–activity relationships (SARs) of the γ-carboline series and to discover more potent antitubulin agents.

2. Chemistry

The synthetic routes of γ-carboline ketones **6a**–**n** are summarized in Scheme 1. γ-Carboline (**7a**) was prepared using our recently developed synthetic protocol [11]. Treatment of **7a** with various alkylating reagents (1.2 equiv.) and NaH in dry THF-DMF at room

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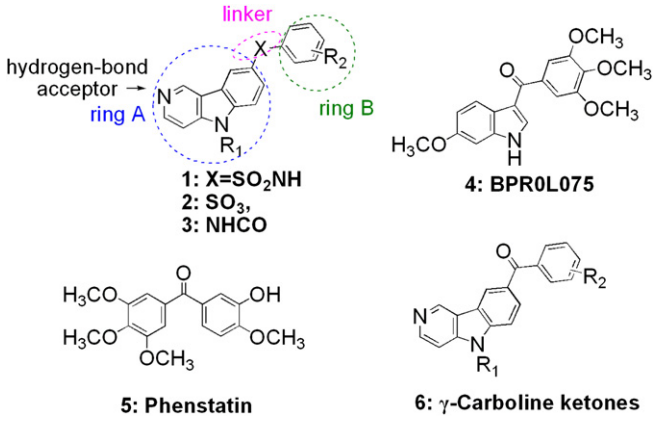


Fig. 1. Structure of BPR0L075 (**4**), Phenstatin (**5**), and γ-carboline derivatives (**1**–**3**, **6**).

temperature gave the corresponding compounds **7b**–**d** [12]. Increasing the amount of alkylating reagent (for example C_2H_5Br) to 2.4 equiv. led to the yield of quaternary γ-carboline **7e**. Then, compounds **7b**–**e** were treated with various benzoyl chlorides in dry CH_2Cl_2 in the presence of $AlCl_3$ at room temperature to afford target compounds **6a**–**k**, **6m** and **6n**. In addition, reduction of compound **6k** by Raney Ni in EtOH yielded the amino compound **6l** [13].

3. Biological studies and discussion

The synthesized γ-carboline ketones **6a**–**n** were tested for their cytotoxic activities *in vitro* against several human cancer cell lines including human non-small lung cancer cell A549, human gastric adenocarcinoma cell SGC, human colon cancer cell HCT116, human breast carcinoma cell MCF-7, human myeloid leukemia cell K562 and K562R (a multidrug resistant cell) by MTT assay. Taxol was employed as the positive control. The results are summarized in Table 1.

By a scrutiny of the MTT assay results, it showed that almost all of the new compounds displayed moderate to potent cytotoxic activities against the tested cells. Most of the γ-carboline ketones exhibited similar activities against MCF-7, K562, and K562R cell lines in comparison with taxol. It was interesting that seven of the fourteen new compounds showed more potent cytotoxic activities against K562R cell line than that of taxol, with IC_{50} values ranging from 0.66 to 3.68 μM. Comparing the cytotoxicities of **6a**–**c**

Table 1
In vitro cytotoxic activities of the synthesized compounds (**6a**–**n**) against six human cancer cell lines.

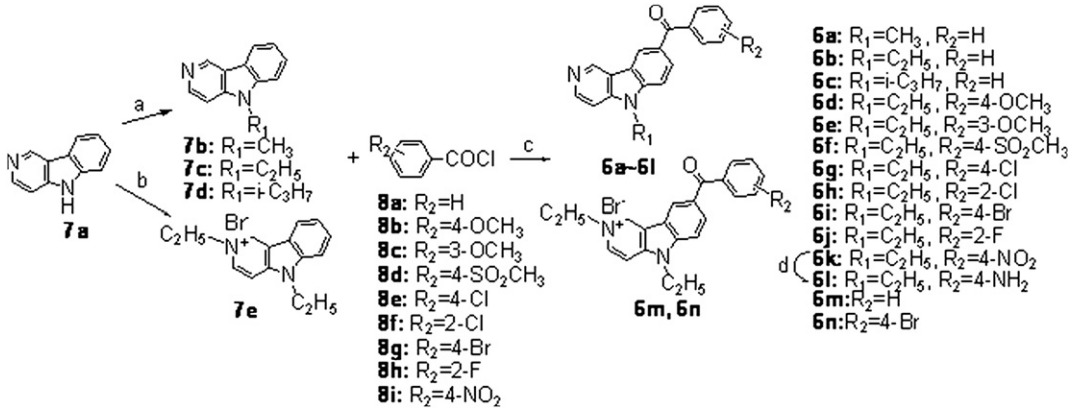
Compd.	Cytotoxicity (IC_{50}, μM)[a,b]					
	A549	SGC	HCT116	MCF-7	K562	K562R
6a	–[c]	–	–	–	4.82	–
6b	23.22	11.79	8.63	8.36	4.96	9.09
6c	–	–	–	–	6.46	–
6d	20.52	17.80	70.22	3.85	6.39	2.09
6e	21.37	18.66	34.30	9.99	6.42	1.57
6f	24.15	24.86	37.75	3.28	5.26	0.66
6g	27.18	62.49	62.58	30.62	4.69	1.58
6h	41.43	38.59	42.29	21.03	13.71	7.08
6i	35.15	33.96	24.26	>100	2.11	1.58
6j	46.81	35.59	35.94	4.74	12.19	1.45
6k	29.13	>100	59.22	3.21	16.79	3.68
6l	49.31	>100	40.65	5.04	13.41	23.34
6m	>100	41.43	>100	11.95	>100	>100
6n	>100	>100	>100	56.00	>100	>100
Taxol	2.46	3.34	4.37	2.11	1.16	5.63

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are means of three experiments.
[c] NT, not tested.

revealed that introducing methyl, ethyl, or isopropyl group at N^9 position of γ-carboline would do little effect on their cytotoxicities against K562 cell line. The influence of various substitutions on the B ring was also examined. Compound **6b** with an unsubstituted B ring exhibited the most potent activity against SGC and HCT116 cell lines, while compound **6f** bearing methylsulphonyl group at 4-position was the most potent one against MCF-7 and K562R cell lines. Besides, we also evaluated the effect of substitution on N^2 position. It was obviously that introduction of ethyl group at the N^2 position of **6b** and **6i** afforded the corresponding quaternary γ-carboline salts **6m** and **6n** with no activity. This was in agreement with the evaluated results of sulfonates **2** [7].

To investigate whether the cytotoxic activities of these compounds were related to an interaction with the microtubule system, compound **6f**, the most promising compound against MCF-7 and K562R cell lines, was evaluated for its tubulin polymerization inhibition. The IC_{50} value of compound **6f** was 4.3 μM, comparable with that of CA-4 (1.5 μM).

Then, flow cytometry experiments were carried out to determine whether compound treatment would lead to cell cycle arrest at G_2/M phase. A549 cells were treated with 2 μM **6f** for 48 h, and 1 μM CA-4 was used as a positive control. It was shown that compound **6f** caused a marked increase in the percentage of cells



Scheme 1. Reagents and conditions: (a) R_1X (1.2 equiv.), NaH, dry THF–DMF, r.t 0.5 h; (b) R_1X (2.4 equiv.), NaH, dry THF–DMF, r.t 0.5 h; (c) $AlCl_3$, dry CH_2Cl_2, N_2, reflux 3 h; (d) Raney Ni, EtOH, H_2, r.t 2 h.

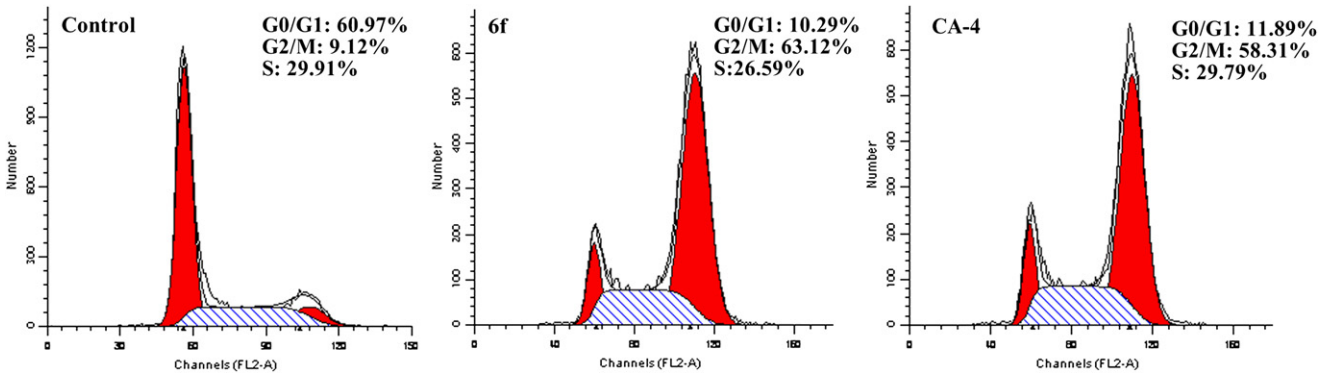


Fig. 2. Effect of compound **6f** (2 μM) and CA-4 (1 μM) on the cell cycle of A549 cells.

blocked in the G_2/M phase of the cell cycle, with a simultaneous decrease of cells in G_0/G_1 and S phase (Fig. 2). It was in the similar trend as CA-4.

4. Conclusion

In summary, a series of novel γ-carboline ketones were synthesized and tested for their cytotoxic activities *in vitro* against six human tumor cell lines. Most of the compounds showed moderate to potent cytotoxic activities against all the tested cells, especially for MCF-7, K562, and K562R cell lines. Primary mechanism research indicated that the synthesized compounds could inhibit tubulin polymerization. The IC_{50} value of compound **6f** was 4.3 μM, equivalent to that of CA-4. Moreover, it could also lead to cell cycle arrest at G_2/M phase. With all these results, further design and synthesis of potent antitumor agents are ongoing in our laboratory and the results will be reported in due course.

5. Experiment

Melting points were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brüker AM 400 instrument at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. Element analyses were performed on an Eager 300 instrument.

5.1. Synthesis

5.1.1. General procedure for synthesis of γ-carboline ketones *6a*–*n*

Benzoyl chloride (**8**, 2 mmol) and $AlCl_3$ (4.5 mmol) were added to a suspension of γ-carboline (**7**, 1 mmol) in dry CH_2Cl_2 (5 mL) under an inert atmosphere at room temperature. The mixture was stirred under reflux until completion of reaction (monitored by TLC). After adding ice water (10 mL), the mixture was neutralized with 10% NaOH, and extracted with AcOEt (3 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified over silica column chromatography using PE: EtOAc: EtOH (10:10:1, V/V/V) or PE: EtOAc: 95% EtOH (1:1:4, V/V/V) as eluent to afford **6a**–**n**.

*5.1.1.1. 8-Benzoyl-5-methyl-γ-carboline (**6a**).* Reagent: 5-methyl-γ-carboline (**7b**, 182 mg, 1 mmol), benzoyl chloride (280 mg, 2 mmol). White solid (23%), mp: 78–80 °C. ^{1}H NMR (δ, DMSO-d_6): 9.47 (s, 1H), 8.74 (s, 1H), 8.57 (d, 1H, J = 5.2 Hz), 7.98 (dd, 1H, J = 8.8, 1.2 Hz), 7.84 (d, 1H, J = 8.8 Hz), 7.80 (d, 2H, J = 7.2 Hz), 7.72 (m, 2H), 7.62 (t, 2H, J = 7.2 Hz), 3.97 (s, 3H, CH_3). ^{13}C NMR (δ, DMSO-d_6): 196.30, 144.85, 144.18, 143.72, 142.97, 136.74, 132.38, 131.66, 130.36, 129.98, 125.69, 121.46, 120.79, 120.25, 113.38, 109.92, 37.53. ESI-MS: m/z = 287 $[M+1]^+$. Anal. calcd for $C_{19}H_{14}N_2O$: C, 79.70; H, 4.93; N, 9.78. Found: C, 79.74; H, 5.01; N, 9.76.

*5.1.1.2. 8-Benzoyl-5-ethyl-γ-carboline (**6b**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), benzoyl chloride (280 mg, 2 mmol). White solid (51%), mp: 80–82 °C. ^{1}H NMR (δ, DMSO-d_6): 9.46 (s, 1H), 8.74 (s, 1H), 8.55 (d, 1H, J = 6.0 Hz), 7.96 (d, 1H, J = 8.8 Hz), 7.85 (d, 1H, J = 8.8 Hz), 7.79 (d, 2H, J = 7.2 Hz), 7.73 (m, 2H), 7.61 (t, 2H, J = 7.2 Hz), 4.54 (q, 2H, J = 6.8 Hz), 1.36 (t, 3H, J = 6.8 Hz). ^{13}C NMR (δ, DMSO-d_6): 195.60, 145.74, 144.74, 143.62, 142.29, 138.33, 132.34, 129.80, 129.54, 129.04, 128.71, 123.84, 120.78, 119.32, 109.80, 105.35, 37.77, 13.93. ESI-MS: m/z = 301 $[M+1]^+$. Anal. calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 80.14; H, 5.42; N, 9.16.

*5.1.1.3. 8-Benzoyl-5-isopropyl-γ-carboline (**6c**).* Reagent: 5-isopropyl-γ-carboline (**7d**, 210 mg, 1 mmol), benzoyl chloride (280 mg, 2 mmol). White solid (67%), mp: 134–136 °C. ^{1}H NMR (δ, DMSO-d_6): 9.48 (s, 1H), 8.75 (s, 1H), 8.53 (d, 1H, J = 4.4 Hz), 7.96 (d, 1H, J = 8.8 Hz), 7.93 (dd, 1H, J = 8.8, 0.8 Hz), 7.80 (m, 3H, J = 7.2 Hz), 7.71 (t, 1H, J = 7.6 Hz), 7.52 (t, 2H, J = 7.6 Hz), 5.24 (m, 1H), 1.67 (d, 6H, J = 7.2 Hz). ESI-MS: m/z = 315 $[M+1]^+$. Anal. calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.26; H, 5.84; N, 9.07.

*5.1.1.4. 5-Ethyl-8-(4-methoxybenzoyl)-γ-carboline (**6d**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 4-methoxybenzoyl chloride (340 mg, 2 mmol). White solid (29%), mp: 122–124 °C. ^{1}H NMR (δ, DMSO-d_6): 9.41 (s, 1H), 8.64 (s, 1H), 8.49 (d, 1H, J = 5.2 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.80 (d, 1H, J = 8.4 Hz), 7.76 (d, 2H, J = 8.4 Hz), 7.67 (d, 1H, J = 5.2 Hz), 7.07 (d, 2H, J = 8.4 Hz), 4.48 (q, 2H, J = 7.2 Hz), 3.82 (s, 3H), 1.31 (t, 3H, J = 7.2 Hz). ^{13}C NMR (δ, DMSO-d_6): 195.98, 158.31, 146.21, 145.23, 144.43, 143.17, 136.93, 126.74, 125.71, 123.66, 122.91, 115.23, 115.08, 112.81, 111.42, 110.84, 55.92, 40.27, 15.73. ESI-MS: m/z = 331 $[M+1]^+$. Anal. calcd for $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.26; H, 5.52; N, 8.72.

*5.1.1.5. 5-Ethyl-8-(3-methoxybenzoyl)-γ-carboline (**6e**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 3-methoxybenzoyl chloride (340 mg, 2 mmol). White solid (24%), mp: 96–98 °C. ^{1}H NMR (δ, DMSO-d_6): 9.49 (s, 1H), 8.76 (s, 1H), 8.56 (d, 1H, J = 6.0 Hz), 7.98 (d, 1H, J = 8.8 Hz), 7.87 (d, 1H), 7.75 (d, 1H, J = 6.0 Hz), 7.53 (t, 1H, J = 8.0 Hz), 7.34 (d, 1H, J = 8.0 Hz), 7.30 (s, 1H), 7.27 (d, 1H, J = 8.0 Hz), 4.56 (q, 2H, J = 7.2 Hz), 3.83 (s, 3H), 1.38 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 331 $[M+1]^+$. Anal. calcd for $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.52; H, 5.76; N, 8.26.

*5.1.1.6. 5-Ethyl-8-(4-methylsulfonylbenzoyl)-γ-carboline (**6f**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 4-methylsulfonylbenzoyl chloride (438 mg, 2 mmol). White solid (54%), mp: 161–162 °C. ^{1}H NMR (δ, DMSO-d_6): 9.50 (s, 1H), 8.78 (s, 1H), 8.57 (d, 1H, J = 4.8 Hz), 8.15 (d, 2H, J = 8.0 Hz), 8.08 (m, 3H), 7.90 (d, 1H, J = 8.0 Hz), 7.76 (d, 1H, J = 4.8 Hz), 4.55 (q, 2H, J = 7.2 Hz), 3.34 (s, 3H), 1.35 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 379 $[M+1]^+$. Anal. calcd for $C_{21}H_{18}N_2O_3S$: C, 66.65; H, 4.79; N, 7.40. Found: C, 66.76; H, 4.64; N, 7.47.

*5.1.1.7. 8-(4-Chlorobenzoyl)-5-ethyl-γ-carboline (**6g**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 4-chlorobenzoyl chloride (350 mg, 2 mmol). White solid (45%), mp: 115–117 °C. ^{1}H NMR (δ, DMSO-d_6): 9.48 (s, 1H), 8.74 (s, 1H), 8.56 (d, 1H, J = 5.6 Hz), 7.97 (d, 1H, J = 8.0 Hz), 7.87 (d, 1H, J = 8.0 Hz), 7.82 (d, 2H, J = 8.0 Hz), 7.74 (d, 1H, J = 5.6 Hz), 7.67 (d, 2H, J = 8.0 Hz), 4.56 (q, 2H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 335 $[M+1]^+$. Anal. calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37. Found: C, 71.73; H, 4.26; N, 8.27.

*5.1.1.8. 8-(2-Chlorobenzoyl)-5-ethyl-γ-carboline (**6h**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 2-chlorobenzoyl chloride (350 mg, 2 mmol). White solid (25%), mp: 98–100 °C. ^{1}H NMR (δ, DMSO-d_6): 9.47 (s, 1H), 8.72 (s, 1H), 8.55 (d, 1H, J = 4.4 Hz), 7.85 (d, 1H, J = 8.8 Hz), 7.74 (d, 1H, J = 8.8 Hz), 7.63 (m, 2H), 7.40 (t, 1H, J = 7.2 Hz), 7.29 (m, 2H), 4.51 (q, 2H, J = 6.8 Hz), 1.33 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 335 $[M+1]^+$. Anal. calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37. Found: C, 71.47; H, 4.75; N, 8.47.

*5.1.1.9. 8-(4-Bromobenzoyl)-5-ethyl-γ-carboline (**6i**).* Reagent: 5-ethyl-γ-carboline **(7c**, 196 mg, 1 mmol), 4-bromobenzoyl chloride (440 mg, 2 mmol). White solid (18%), mp: 139–141 °C. ^{1}H NMR (δ, DMSO-d_6): 9.43 (s, 1H), 8.68 (s, 1H), 8.50 (d, 1H, J = 6.0 Hz), 7.91 (d, 1H, J = 8.0 Hz), 7.82 (d, 1H, J = 8.0 Hz), 7.75 (d, 2H, J = 8.0 Hz), 7.68 (m, 3H), 4.48 (q, 2H, J = 7.2 Hz), 1.30 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 379 $[M+1]^+$. Anal. calcd for $C_{20}H_{15}BrN_2O$: C, 63.34; H, 3.99; N, 7.39. Found: C, 63.36; H, 4.04; N, 7.52.

*5.1.1.10. 5-Ethyl-8-(2-fluorobenzoyl)-γ-carboline (**6j**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 2-fluorobenzoyl chloride (318 mg, 2 mmol). White solid (39%), mp: 143–145 °C. ^{1}H NMR (δ, DMSO-d_6): 9.47 (s, 1H, H-4), 8.75 (s, 1H, H-5), 8.55 (d, 1H, J = 4.4 Hz), 7.94 (d, 1H, J = 8.8 Hz), 7.82 (d, 1H, J = 8.8 Hz), 7.71 (m, 2H), 7.64 (t, 1H, J = 7.2 Hz), 7.43 (m, 2H), 4.51 (q, 2H, J = 6.8 Hz), 1.34 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 319 $[M+1]^+$. Anal. calcd for $C_{20}H_{15}FN_2O$: C, 75.46; H, 4.75; N, 8.80. Found: C, 75.64; H, 4.84; N, 8.58.

*5.1.1.11. 5-Ethyl-8-(4-nitrobenzoyl)-γ-carboline (**6k**) and 5-ethyl-8-(4-aminobenzoyl)-γ-carboline (**6l**).* Reagent: 5-ethyl-γ-carboline (**7c**, 196 mg, 1 mmol), 4-nitrobenzoyl chloride (372 mg, 2 mmol). Yellow solid (67%), mp: 174–176 °C. ^{1}H NMR (δ, DMSO-d_6): 9.50 (s, 1H), 8.79 (s, 1H), 8.58 (d, 1H, J = 6.0 Hz), 8.44 (d, 2H, J = 8.4 Hz), 8.03 (m, 3H), 7.91 (d, 1H, J = 8.4 Hz), 7.77 (d, 1H, 1, J = 6.0 Hz), 4.56 (q, 2H, J = 6.8 Hz), 1.38 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 346 $[M+1]^+$. Anal. calcd for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17. Found: C, 69.63; H, 4.36; N, 12.26.

The nitro compound **6k** (345 mg) was dissolved in EtOH (5 mL) and Raney Ni (74 mg) was added. The reaction mixture was stirred at room temperature under H_2 for 2h. Then, the mixture was filtered over Celite, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (PE: EtOAc: EtOH, 3:3:1, V/V/V), yielded 5-ethyl-8-(4-aminobenzoyl)-γ-carboline (**6l**) as a yellow solid (69%), mp: 200–202 °C. ^{1}H NMR (δ, DMSO-d_6): 9.46 (s, 1H), 8.61 (s, 1H), 8.54 (d, 1H, J = 5.6 Hz), 7.86 (d, 1H, J = 8.4 Hz), 7.81 (d, 1H, J = 8.4 Hz), 7.71 (d, 1H, J = 5.6 Hz), 7.61 (d, 2H, J = 8.0 Hz), 6.66 (d, 2H, J = 8.0 Hz), 6.11 (s, 2H, 4′-NH_2), 4.54 (q, 2H, J = 7.2 Hz), 1.38 (t, 3H, J = 7.2 Hz). ESI-MS: m/z = 316 $[M+1]^+$. Anal. calcd for $C_{20}H_{17}N_3O$: C, 76.17; H, 5.43; N, 13.32. Found: C, 76.25; H, 5.62; N, 13.26.

*5.1.1.12. 8-Benzoyl-2, 5-diethyl-γ-carboline-3-ium bromide (**6m**).* Reagent: 2, 5-diethyl-γ-carboline-3-ium bromide (**7e**, 305 mg, 1 mmol), benzoyl chloride (280 mg, 2 mmol). White solid (45%), mp: 103–105 °C. ^{1}H NMR (δ, DMSO-d_6): 8.93 (s, 1H), 8.32 (d, 1H, J = 6.8 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.22 (m, 2H), 7.11 (t, 1H, J = 7.2 Hz), 6.85 (t, 2H, J = 7.2 Hz), 6.59 (d, 2H, J = 7.2 Hz), 4.38 (q, 2H, J = 6.8 Hz), 4.02 (q, 2H, J = 6.8 Hz), 1.46 (t, 3H, J = 6.8 Hz), 1.09 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 409 $[M+1]^+$. Anal. calcd for $C_{22}H_{21}BrN_2O$: C, 64.55; H, 5.17; N, 6.84. Found: C, 64.47; H, 4.94; N, 7.03.

*5.1.1.13. 8-(4-Bromobenzoyl)-2, 5-diethyl-γ-carboline-3-ium bromide (**6n**).* Reagent: 2, 5-diethyl-γ-carboline-3-ium bromide (**7e**, 305 mg, 1 mmol), 4-bromobenzoyl chloride (440 mg, 2 mmol). White solid (30%), mp: 121–122 °C. ^{1}H NMR (δ, DMSO-d_6): 9.23 (s, 1H), 8.50 (d, 1H, J = 6.8 Hz), 8.13 (s, 1H), 7.88 (d, 1H, J = 6.8 Hz), 7.63 (d, 1H, J = 8.8 Hz), 7.54 (d, 1H, J = 8.8 Hz), 7.15 (d, 2H, J = 8.4 Hz), 6.89 (d, 2H, J = 8.4 Hz), 4.55 (q, 2H, J = 6.8 Hz), 4.34 (q, 2H, J = 6.8 Hz), 1.59 (t, 3H, J = 6.8 Hz), 1.29 (t, 3H, J = 6.8 Hz). ESI-MS: m/z = 487 $[M+1]^+$. Anal. calcd for $C_{22}H_{20}Br_2N_2O$: C, 54.12; H, 4.13; N, 5.74. Found: C, 54.26; H, 3.97; N, 5.47.

5.2. Cytotoxic assay

The tumor cell lines (A549, SGC, HCT116, MCF-7, K562, K562R) were obtained from Shanghai Institute of Pharmaceutical Industry.

The cytotoxic activity *in vitro* was measured using the MTT assay [7]. MTT solution (10.0 μL/well) in RPMI-1640 (Sigma, St. Louis, MO) was added after cells were treated with drug for 48 h, and cells were incubated for a further 4 h at 37 °C. The purple formazan crystals were dissolved in 100.0 μL DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 nm. Assays were performed in triplicate in three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software 'Dose-Effect Analysis with Microcomputers'. The tumor cell line panel consisted of A549, SGC, HCT116, MCF-7, K562, and K562R. In all of these experiments, three replicate wells were used to determine each point.

5.3. Microtubule polymerization assay [14]

In vitro tubulin polymerization assays were conducted with reagents as described by the manufacturer (Cytoskeleton, Inc.). In brief, compound **6f** with series concentrations (3 μM, 0.75 μM, 0.1875 μM) was incubated with purified bovine tubulin and buffer containing 20% glycerol, 1 mM GTP, 80 mM PIPES (pH 6.9), 2.0 mM $MgCl_2$, and 0.5 mM EGTA at 37 °C and the effect of compound **6f** on tubulin polymerization was monitored kinetically using a fluorescent plate reader. The IC_{50} value is defined as the concentration of product which inhibits the rate of polymerization by 50%.

5.4. Flow cytometry analysis [15]

For flow cytometry analysis of DNA content. A549 cells in exponential growth were treated with **6f** (2 μM) for 48 h. Cells were washed twice with PBS and fixed in 75% ethanol. The cell pellet was resuspended in 100.0 μL of PBS containing 200.0 mg/mL RNase (Amersco, Solon, OH), then incubated at 37 °C for 0.5 h. After incubation, the cells were stained with 20.0 mL/L propidium iodide (PI, Sigma, St. Louis, MO) for 15 min. The fluorescence cell was measured with FACSCalibur (Becton–Dickinson, Lincoln Park, NJ).

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