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ARTICLE TYPE

Synthesis and biological evaluation of novel bivalent β-carbolines as potential antitumor agents

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A series of novel bivalent β-carbolines with a spacer of three to ten methylene units between the 3-carboxyl oxygen was synthesized and evaluated as antitumor agents. The results demonstrated that most compounds displayed good and selective cytotoxic activities against 769-P and KB cell lines. Acute toxicities and antitumor efficacies of the selected compounds in mice were also evaluated. Compound 22 exhibited potent antitumor activity against mice bearing Lewis lung cancer with tumor inhibition rate of 64.2%. Preliminary structure-activity relationships analysis indicated that (1) the length of the spacer affected cytotoxic activities in vitro and six methylene units were more favorable; (2) the introduction of substituent into position-1 of β-carboline ring might be detrimental to antitumor potency in vivo models.

Introduction

The β-carboline nucleus is common to many natural and synthetic products associated with a wide spectrum of biochemical and pharmaceutical functions. [1] Recently, there have been intense research efforts in the design and development of β-carbolines as a new class of antitumor agents.[2-9] These compounds were found to exert antitumor effects through multiple mechanisms of action including intercalating into DNA[10] inhibiting Topo I and II (topoisomerase I and II), [11] CDK (cyclin-dependent kinase), [12] MK-2 (mitogen activated protein kinase-activated protein kinase 2), [13] kinesin-like protein Eg5, [14] IKK (I-Kappa-B kinase)[15] and PLK (polo-like kinase). [16]

Our group previously reported the synthesis and biological evaluation of a large series of β-carbolines as a new class of antitumor agents.[17-27] Structure-activity relationship analysis of these compounds indicated that (i) the introduction of appropriate substituents into position-1 and 9 of β-carboline nucleus played a vital role in determining their antitumor effects; (ii) the butyl and 3-phenylpropyl substituents in position 9 of β-carboline nucleus were the optimal pharmacophoric group giving rise to significant antitumor agents.

Previous investigations[28] demonstrated that dimerization of various intercalating agents by an appropriate spacer could lead to a dramatic increase in the DNA binding affinity. Therefore, bivalent β-carbolines were expected to exhibit more potent antitumor efficacies than monomers. Recently, several bivalent β-carbolines were synthesized and evaluated as anti-Alzheimer's[29] and antitumor[30] agents (**Figure 1**) and bivalent β-carbolines were proved to exhibit more potent anti-Alzheimer potencies than monomers. However, to the best of our knowledge, no large, systematic study has been undertaken to examine the structure-activity relationships of bivalent β-carbolines as antitumor agents. We recently began such a study and have reported the synthesis and antitumor evaluation of bivalent β-carbolines with a spacer of three to ten methylene units between the indole nitrogen[31] (**Figure 1**). We report now the synthesis, in vitro evaluation, in vivo efficacies and preliminary structure-activity relationships for the new bivalent β-carbolines with a spacer of three to ten methylene units between the 3-carboxyl oxygen.

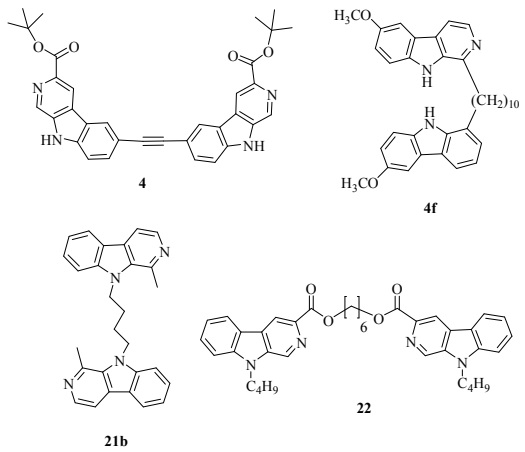
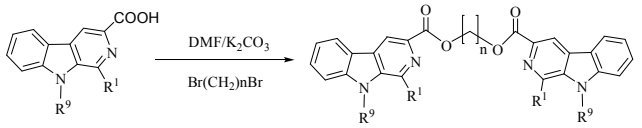


Figure 1 The chemical structure of the representative reported and newly synthesized bivalent β-carbolines

Chemistry

The synthetic route of the newly designed bivalent β-carbolines **14-44** was outlined in **Scheme 1**. Monovalent β-carboline-3-carboxylic acids **1-13** were synthesized according to our previously published methods. [17, 18, 21] The symmetrical bivalent β-carbolines **14-44** were prepared by reaction of the corresponding dibromoalkane with monovalent β-carboline-3-carboxylic acids **1-13** in anhydrous DMF in 55-94% yield.[32] The chemical structures of all the newly synthesized compounds were characterized by MS, HRMS, ^{1}H NMR and ^{13}C NMR.



1 R^1=CH_3 R^9=CH_3
2 R^1=CH_3 R^9=C_2H_5
3 R^1=H R^9=n-C_4H_9
4 R^1=CH_3 R^9=n-C_4H_9
5 R^1=H R^9=CH_2Ph
6 R^1=CH_3 R^9=CH_2Ph
7 R^1=H R^9=CH_2Ph(m-Cl)
8 R^1=H R^9=$(CH_2)_3Ph$
9 R^1=CH_3 R^9=$(CH_2)_3Ph$
10 R^1=Ph(p-OCH_3) R^9=C_2H_5
11 R^1=Ph(p-OCH_3) R^9=$(CH_2)_3Ph$
12 R^1=Ph(3,4,5-OCH_3) R^9=C_2H_5
13 R^1=Ph(3,4,5-OCH_3) R^9=$(CH_2)_3Ph$

Scheme 1 Synthesis of the bivalent β-carbolines 14-44.

Experimental section

Cytotoxicity *in vitro*

Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described by Cao et al. [23] Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μM penicillin and 100 μM streptomycin. Cultures were propagated at 37℃ in a humidified atmosphere containing 5% CO_2. The cells were exposed to different concentrations of drug, and kept for incubation for 48 h. DMSO was used as the solution for drugs. Final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentration without effect on cell replication. In all of these experiments, three replicate wells were used to determine each point.

Assay of acute toxicities

Acute toxicity assay was performed according to the method described by Cao *et al*. [23] Briefly, healthy C57BL/6 mice (9-12 weeks) weighing 18-22g were housed in rooms where the temperature was approximately 24±2□, with a relative humidity 60-70%, and in 12h light-dark cycle. The sterile food and water were provided according to institutional guidelines. All animals were provided by Xinjiang Medicine University Medical Laboratory Animal Center. All animal procedures were approved by the Animal Ethical Committee of the Xinjiang Medicine University. Prior to each experiment, mice were fasted overnight and allowed free access to water. Various doses of the bivalent β-carbolines ranging from 10-1500 μmol kg^{-1} dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution were given via intraperitoneal (i.p.) to different groups of healthy C57BL/6 mice, and each group contained 10 mice (5 males and 5 females). After the administration of the compounds, mice were observed continuously for the first 2 h for any gross behavioral changes and deaths, then intermittently for the next 24 h and occasionally thereafter for 14 days, and for the onset of any delayed effects. All animals were sacrificed on the 14th day after drug administration and checked macroscopically for possible damage to the heart, liver and kidneys. Mice dying immediately following drug administration were also examined for any possible organ damage. LD_{50} values were calculated graphically as described. [33]

Assay of antitumor activity

Antitumor activity against mice bearing CT-26 colon cancer and Lewis lung cancer was evaluated as described by Cao *et al*[23] with a slight modification. Briefly, CT-26 colon cancer and Lewis lung cancer cell lines were provided by Shanghai Institute of Pharmaceutical Industry. Tumor cells of CT-26 colon cancer and Lewis lung cancer were inoculated into mice. After 7 days, tumors were taken out and cells were harvested. Viable tumor cells (2×10^6 cells/mouse) were inoculated into to the armpit of mice by subcutaneous injection. Each compound was injected by intraperitoneally (i.p.) to different group mice (each group containing 10 female mice) 24 h after the inoculation at a dosage of about one fifth of LD_{50} value once a day for 7 consecutive days. Cyclophosphamide (CTX) at 114.9 μmol kg^{-1} was used as a positive control and vehicle as negative control. The weights of animals were recorded every 3 days. All animals were sacrificed on the 21st day after tumor inoculation and the tumors were excised and weighed. The inhibition rate was calculated as follows:

$$(C-T)/C\times100$$

T: average tumor weight of treated group; C: average tumor weight of negative control group.

Result and discussion

Cytotoxicity *in vitro*

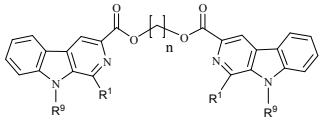
The cytotoxic potencies of all the newly synthesized bivalent β-carbolines against a panel of human tumor cell lines were investigated and compared with the monovalent β-carbolines **3** and **8** and the reference drug cisplatin. Our previous investigation indicated that BGC823, A375, 769-P, KB and SK-OV-3 tumor cells were more sensitive to β-carbolines than other tumor cells, so these tumor cells were selected and evaluated in the present investigation. The results were summarized in **Table 1**.

As shown in **Table 1**, compound **14** displayed a broad spectrum of cytotoxic activities with IC_{50} value of lower than 20μM against four tumor cell lines, while compounds **17**, **19**, **22**, **23**, **29**, **30**, **37** and **43** show significant and selective cytotoxicities with IC_{50} value of lower than 20μM against one or two tumor cell lines. Interestingly, most compounds showed selective cytotoxic activities against renal carcinoma (769-P) and epidermoid carcinoma of the nasopharynx (KB).

We examined the influence of the spacer length of bivalent β-carbolines on cytotoxic activities. The data collected in Table 1 showed that compound **22** with a spacer of six methylene units exhibited moderate to strong cytotoxic activities with IC_{50} value of 43.2, 18.2 and 15.6 μM against BGC823, A375 and KB cell lines, while compounds **23-25** with a spacer of eight to ten methylene units displayed weaker cytotoxic effect against tumor cell lines tested. Similarly, compounds **15**, **17**, **26**, **30**, **33**, **34**, **37** and **39** with a spacer of six methylene units showed good and selective cytotoxic effects against tumor cell lines, while other compounds had weaker cytotoxic activities. These results suggested that the length of the spacer might affect cytotoxic activities and six methylene units might be more favorable.

Next, we examined the influence of the substituents in position-1 and 9 of β-carboline ring on cytotoxic potencies. **Table 1** showed that compounds **22** displayed significant and selective cytotoxic

Table 1 Cytotoxic activity of bivalent β-carbolines *in vitro*



Compds	R^1	R^9	n	IC_{50} (μM) ±SD [a]				
				BGC823[b]	A375	769-P	KB	SK-OV-3
14	CH_3	CH_3	3	19.5±2.23	16.6±3.36	16.3±2.08	18.4±4.12	21.0±4.85
15	CH_3	CH_3	6	>200	82.6±12.4	28.3±6.32	165±30.2	>200
16	CH_3	CH_3	10	>200	125±16.8	42.9±5.42	198±32.1	>200
17	CH_3	C_2H_5	6	>200	32.9±4.25	43.1±6.64	16.5±2.32	>200
18	CH_3	C_2H_5	8	>200	86.3±14.2	21.7±3.41	>200	86.4±15.6
19	CH_3	C_2H_5	9	>200	>200	10.3±0.84	56.9±11.3	>200
20	H	C_4H_9	4	116±18.8	>200	42.6±10.6	89.0±18.9	123±18.7
21	H	C_4H_9	5	>200	>200	32.9±5.42	53.9±12.5	>200
22	H	C_4H_9	6	43.2±6.42	18.2±3.28	112±14.2	15.6±1.36	>200
23	H	C_4H_9	8	68.9±10.8	146±22.6	210±36.4	19.8±2.48	124±20.4
24	H	C_4H_9	9	>200	89.6±12.6	115±16.8	26.4±2.87	>200
25	H	C_4H_9	10	168±28.6	>200	89.2±14.6	45.2±5.86	>200
26	CH_3	C_4H_9	6	>200	>200	60.5±7.15	54.6±8.24	>200
27	CH_3	C_4H_9	7	>200	>200	95.9±12.5	>200	71.5±9.82
28	CH_3	C_4H_9	10	>200	42.0±4.86	114±12.7	>200	>200
29	H	$PhCH_2$	4	43.6±3.64	7.90±0.56	45.4±5.27	>200	>200
30	H	$PhCH_2$	6	164±26.5	>200	46.8±5.42	15.7±1.45	27.8±3.66
31	H	$PhCH_2$	8	>200	>200	68.6±8.61	162±30.2	>200
32	CH_3	$PhCH_2$	5	>200	125±20.6	72.4±10.6	42.4±6.27	>200
33	CH_3	$PhCH_2$	6	>200	>200	64.7±9.53	112±16.8	>200
34	H	(3-Cl)$PhCH_2$	6	>200	>200	21.9±2.87	46.9±6.54	19.0±2.32
35	H	(3-Cl)$PhCH_2$	9	>200	>200	145±20.8	71.2±8.21	>200
36	H	$Ph(CH_2)_3$	3	>200	>200	95.6±16.3	72.4±8.46	>200
37	H	$Ph(CH_2)_3$	6	>200	14.4±3.18	29.4±4.54	56.4±6.68	8.50±0.64
38	H	$Ph(CH_2)_3$	7	>200	143±20.1	43.5±6.54	78.9±10.2	>200
39	CH_3	$Ph(CH_2)_3$	6	>200	>200	21.8±4.21	>200	73.4±9.82
40	CH_3	$Ph(CH_2)_3$	9	>200	>200	108±16.8	50.7±8.24	>200
41	(4-OCH_3)-Ph	C_2H_5	6	>200	>200	65.9±8.64	89.2±10.2	>200
42	(4-OCH_3)-Ph	$Ph(CH_2)_3$	6	140±16.8	132±18.6	95.5±8.94	218±30.4	>200
43	3,4,5-tri(OCH_3)-Ph	C_2H_5	6	159±25.6	40.4±4.83	10.9±0.98	20.7±1.64	47.4±6.42
44	3,4,5-tri(OCH_3)-Ph	$Ph(CH_2)_3$	6	168±21.4	134±18.7	118±20.5	>200	>200
	3			125±14.0	79.6±8.52	42.7±4.87	67.7±8.20	>200
	8			36.7±4.21	67.6±7.64	56.8±6.12	86.4±7.86	>200
	Cisplatin			20.8±1.64	8.90±1.32	33.6±4.03	32.0±2.87	4.20±0.36

[a]Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay. The data represents mean values±SD of at least three independent experiments. Values > 100 μM indicate less than 50% growth inhibition at > 100 μM.

[b]Cell lines include gastric carcinoma (BGC823), malignant melanoma (A375), renal carcinoma (769-P), epidermoid carcinoma of the nasopharynx (KB) and ovarian carcinoma (SK-OV-3).

effects against most tumor cell lines. Introduction of a methyl group into position-1 of compound **22** led to compound **26**, which exhibited weaker cytotoxic activities. Similarly, compounds **33** and **39** having a methyl group in position-1 exhibited weaker cytotoxic potencies than compounds **30** and **37**. In comparison with compounds **37**, compounds **42** and **44** bearing an additional 4-methoxyphenyl and 3,4,5-trimethoxyphenl group in position-1 of β-carboline ring, respectively, were almost inactive against all tumor cell lines at the concentration of 100μM. In addition, the compounds **22**, **30**, **34** and **37** bearing a n-butyl (**22**), benzyl (**30**), 3-chlorobenzyl (**34**), 3-phenylpropyl (**37**) and having the same spacer of six methylene units showed good cytotoxic activities with the tendency of 3-phenylpropyl >3-chlorobenzyl >benzyl > n-butyl group. These results indicated that (1) the introduction substituent into position-1 of β-carboline ring might be detrimental to cytotoxic effects of this class of compounds; (2) the 3-phenylpropyl substituent in position-9 of β-carboline nucleus was the suitable group giving rise to potent cytotoxic agents.

Preliminary assessment of acute toxicity

The LD_{50} values of the selected bivalent β-carbolines in mice after administration by i. p. route were summarized in **Table 2**. All the tested bivalent β-carbolines resulted in acute toxic manifestations. Animals showed a decrease in locomotive activity after the administration of various bivalent β-carbolines. Death occurred mostly in the high dosage group within 4-8 hours after injection. All surviving animals returned to normal in the next day. Autopsy of the animals that died in the course of experiment and the necropsy findings in surviving animals at the end of the experimental period (14 days) revealed no obvious changes in any organs.

Of all investigated bivalent β-carbolines, compound **42** with a 4-methoxyphenyl group appended to position-1 displayed remarkable acute toxicity with LD_{50} value of 55.8 μmol kg^{-1}, while compound **37** having no substituent in position-1 of β-carboline ring demonstrated weaker acute toxicities with LD_{50} value of 134.7 μmol kg^{-1}. Replacement of the 3-phenylpropyl group in postion-9 of compound **37** with a butyl substituent gave compound **22** which also exhibited lower acute toxicity with LD_{50} value of 161.8 μmol kg^{-1}. In addition, monovalent β-carboline-3-carboxylic acids **3** and **8** exhibited weaker acute toxicity with LD_{50} value of 1119.4 and 1090.9 μmol kg^{-1}, respectively. These results suggested that the introduction of substituent into position-1 of β-carboline nucleus might play an important role in determining acute toxicity.

Table 2 **Acute toxic effects of bivalent β-carbolines in mice and antitumor activities of these compounds against mice bearing CT-26 colon cancer and Lewis lung cancer.**

Compds	LD_{50} (μmol kg^{-1})	Dosage (μmol kg^{-1})	Tumor inhibition rate (%)	
			CT-26	Lewis lung cancer
14	384.6	76.9	23.6	28.1
22	161.8	32.3	33.8	64.2
37	134.7	26.9	28.3	53.5
42	55.8	11.1	15.1	24.6
3	1119.4	223.8	18.7	45.4
8	1090.9	212.1	20.3	40.3
CTX		114.9	87.6	88.1

Evaluation of antitumor activity

Four bivalent β-carbolines were selected for evaluation in vivo against mice bearing CT-26 colon cancer and Lewis lung cancer and compared with monovalent β-carboline-3-carboxylic acids **3** and **8** and the reference drug cyclophosphamide (CTX). Our previous investigation demonstrated that mice bearing CT-26 colon cancer and Lewis lung cancer were more susceptible to β-carbolines than other animal models, therefore these animal models were selected and evaluated in the present investigation. The tumor inhibition rates of all investigated bivalent β-carbolines were summarized in **Table 2**. As shown in **Table 2**, Lewis lung cancer was more susceptible to all tested compounds than CT-26 colon cancer. Compounds **22** and **37** exhibited more potent antitumor activities against mice bearing Lewis lung cancer than their monovalent β-carboline-3-carboxylic acids **3** and **8**. Particularly, compound **22** was found to be the most potent antitumor agent with the tumor inhibition rate of 64.2% against mice bearing Lewis lung cancer. Compound **37** having a 3-phenylpropyl group in position-9 of β-carboline ring displayed good antitumor effects with the tumor inhibition rate of 53.5 % against mice bearing Lewis lung cancer at dose 26.9 μmol kg^{-1}, while compound **42** with an additional 4-methoxyphenyl group appended to position-1 displayed lower antitumor effects. Although compound **14** had significant cytotoxic activities against tumor cell lines, it displayed weaker antitumor potencies with tumor inhibition rates of 23.6 and 28.1% against mice bearing CT-26 colon cancer and Lewis lung cancer, respectively. These results suggested that the introduction of substituent into position-1 of β-carboline ring might be detrimental to antitumor potency.

Conclusions

In conclusion, we have synthesized a series of novel bivalent β-carbolines with a spacer of three to ten methylene units between the 3-carboxyl oxygen. Most compounds exhibited good and selective cytotoxic activities against 769-P and KB cell lines. Antitumor evaluation of the selected bivalent β-carbolines in animal models indicated that Lewis lung cancer was more susceptible to all tested compounds than CT-26 colon cancer, and compounds **22** and **37** bearing a butyl and 3-phenylpropyl group in position-9 of β-carboline nucleus, respectively, exhibited potent antitumor potencies with the tumor inhibition rate of over 50% against mice bearing Lewis lung cancer. Preliminary structure-activity relationships information revealed that (1) the length of the spacer affected cytotoxic activities in vitro and six methylene units were more favorable; (2) the introduction of substituents into position-1 of β-carboline ring might be detrimental to antitumor potency in vivo models. Further investigations to elucidate the pharmacological mechanisms of this class of compounds are underway in our laboratory, and the data will be published elsewhere.

Acknowledgments

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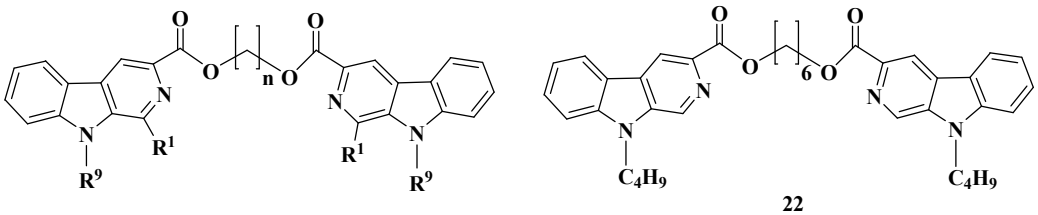
† Electronic Supplementary Information (ESI) available.

Graphic abstract



22

A series of bivalent β-carbolines with a spacer between the 3-carboxyl oxygen was synthesized and their cytotoxic activities in vitro and antitumor efficacies in vivo were evaluated. Compound **22** exhibited potent antitumor activity against mice bearing Lewis lung cancer with tumor inhibition rate of 64.2%.