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Synthesis and cytotoxic activities of 1-benzylidine substituted β-carboline derivatives

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ABSTRACT

A series of new β-carboline derivatives, bearing a benzylidine substituent at position-1, has been prepared and evaluated in vitro against a panel of human cell lines. The N^2-benzylated β-carbolinium bromates represented the most interesting cytotoxic activities. In particular, compounds **19** were found to be the most potent compounds with IC_{50} values lower than 5 μM against 10 strains human tumor cell lines. These results confirmed that the N^2-benzyl substituent on the β-carboline ring played an important role in the modulation of the cytotoxic activities and suggested that further development of such compounds may be interest.

The β-carboline core is common to many natural and synthetic products associated with a broad spectrum of biochemical effects and pharmaceutical properties.[1] Previously, numerous investigations focused on the effects of β-carboline alkaloids on the central nervous system (CNS), such as their affinity with benzodiazepine (BZ),[2] 5-hydroxytryptamine (5-HT),[3] dopamine (DA),[4] and imidazoline[5] receptors. However, considerable recent interests in these alkaloids were stimulated by their potential antitumor activities. Ishida et al.[6] reported that harmine (Fig. 1), which naturally occurs in the medicinal plants of *Peganum harmala* and *Eurycoma longifolia*, and β-carboline analogues exhibited significant antitumor activities in vitro and α-(4-nitrobenzylidine)-harmine (Fig. 1) was found to be the most active compound with a broad cytotoxicity spectrum. β-Carbolines bearing a flexible alkylamine side chain at position-3 demonstrated potent DNA intercalating abilities resulting in remarkable antitumor activities.[7] The complex polycyclic ring system in manzamine A can be replaced with simpler amino substituents to provide active compounds.[8] In addition, β-carboline amino acid ester conjugates displayed potent cytotoxic activities and the Lys/Arg conjugates demonstrated the most significant antitumor activities in vitro.[9] Our previous reports described the syntheses of numerous β-carboline derivatives bearing various substituents at position-1, 2, 3 and 9 of β-carboline nucleus and evaluated their antitumor activities in vitro[10–13] and in vivo.[10,12] The structure–activity relationships (SARs) analysis provided evidence that (1) the molecular feature essential for the antitumor activity was the β-carboline moiety; (2) the introduction of appropriate substituents into position-1, 2, 3 and 9 of β-carboline ring remarkably enhanced the antitumor activities; (3) the methoxy group at position-7 of β-carboline nucleus played a very crucial role in determining their remarkable neurotoxic effects.[1] Taking advantage of previously developed SARs of β-carbolines as potential antitumor agents, in the present investigation we have designed and synthesized a number of new β-carboline derivatives bearing various 4-substituted benzylidine at position-1. The purpose of this study was to investigate effect of benzylidine substituent on the antitumor activity, with the ultimate aim of developing novel potent antitumor agents, together with lower side effects.

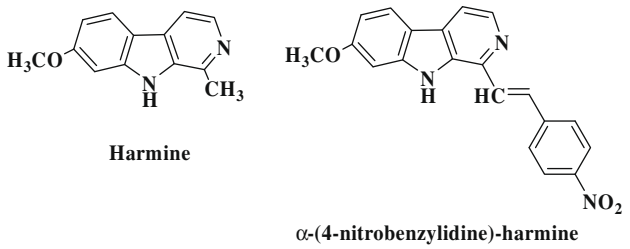

Figure 1. Chemical structure of harmine and α-(4-nitrobenzylidine)-harmine.

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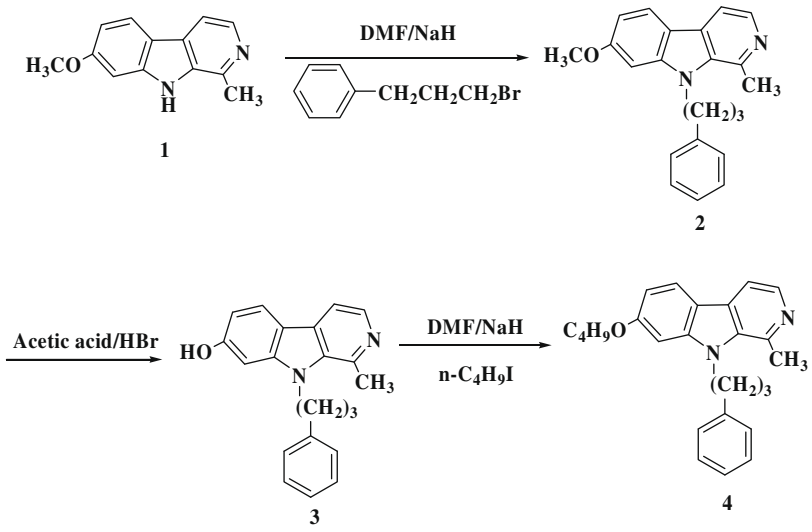
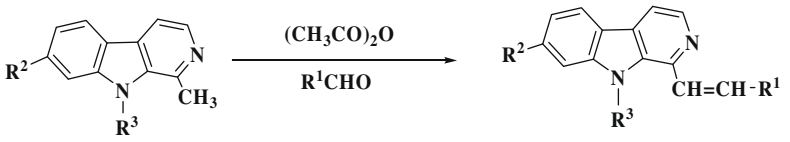
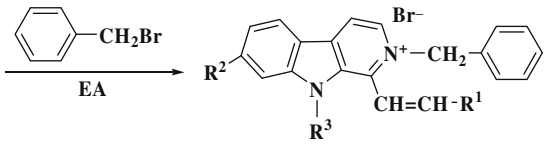


Scheme 1. Synthesis of 7-methoxy-9-(3-phenylpropyl)-1-methyl-β-carboline.



4 R^2=OC_4H_9 R^3=$(CH_2)_3C_6H_5$
5 R^2=H R^3=H
6 R^2=H R^3=n-C_4H_9
7 R^2=H R^3=$(CH_2)_3C_6H_5$

8 R^1=C_6H_5 R^2=H R^3=H
9 R^1=C_6H_4(p-OCH_3) R^2=H R^3=H
10 R^1=C_6H_2(3,4,5-trimethoxy) R^2=H R^3=H
11 R^1=C_6H_4(p-OCH_3) R^2=H R^3=n-C_4H_9
12 R^1=C_6H_4(p-OCH_3) R^2=H R^3=$(CH_2)_3C_6H_5$
13 R^1=C_6H_4(p-OCH_3) R^2=OC_4H_9 R^3=$(CH_2)_3C_6H_5$
14 R^1=C_6H_4(p-NO_2) R^2=H R^3=$COCH_3$
15 R^1=C_6H_4(p-NO_2) R^2=H R^3=$(CH_2)_3C_6H_5$
16 R^1=C_6H_4(p-NO_2) R^2=OC_4H_9 R^3=$(CH_2)_3C_6H_5$



17 R^1=C_6H_4(p-OCH_3) R^2=H R^3=H
18 R^1=C_6H_4(p-OCH_3) R^2=H R^3=n-C_4H_9
19 R^1=C_6H_4(p-OCH_3) R^2=H R^3=$(CH_2)_3C_6H_5$
20 R^1=C_6H_4(p-OCH_3) R^2=OC_4H_9 R^3=$(CH_2)_3C_6H_5$
21 R^1=C_6H_4(p-NO_2) R^2=H R^3=$COCH_3$
22 R^1=C_6H_4(p-NO_2) R^2=OC_4H_9 R^3=$(CH_2)_3C_6H_5$

Scheme 2. Synthesis of 1-benzylidene substituted β-carboline derivatives.

The synthesis of β-carbolines **2** and **5–7** has been described in our previous reports.[10,12] Compound **2** was demethylated in acetic acid and hydrobomic acid (1:2) to afford the expected product **3** (yield 80%). O^7-Butylated β-carboline **4** was prepared from compound **3** by the action of sodium hydride in dry DMF followed by addition of *n*-butyl iodide (Scheme 1).

Compounds **8–22** were prepared as shown in Scheme 2. 1-Benzylidine substituted β-carbolines **8–10**[14] were readily prepared by reaction of 1-methyl-β-carboline **5** with the corresponding aromatic aldehyde in refluxing acetic anhydride. The same synthetic procedure was used for the preparation of compounds **11–16**.[14] Unexpectedly, the reaction of 1-methyl-β-carboline **5** with 4-nitrobenzaldehyde in refluxing acetic anhydride gave N^9-acetylated β-carboline **14**. The N^2-benzylated β-carbolinium bromate derivatives **17–22**[15] were prepared from compounds **9**, **11–14**, and **16** by the addition of benzyl bromide in refluxing ethyl acetate. However, the same synthetic procedure was used for the preparation of N^2-benzylated **15** but failed to afford the expected β-carbolinium bromate.

The cytotoxic potential of all newly synthesized β-carboline derivatives was evaluated in vitro against a panel of human tumor cell lines according to procedures described in our previous reports.[10] The tumor cell line panel consisted of cervical carcinoma (HeLa), liver carcinoma (Bel-7402 and HepG2), gastric carcinoma (BGC-823), non-small cell lung carcinoma (A549), malignant melanoma (A375), renal carcinoma (786-0 and 769-P), colon carcinoma

Table 1
Cytotoxic activities of β-carboline derivatives in vitro[c] (IC_{50}, μM[a])

Compound	HeLa	Bel-7402	BGC-823	HepG2	A549	A375	786-0	HT-29	769-P	KB
5[b]	186	151	215	115	—	—	—	—	—	—
6[b]	102	145	264	145	—	—	—	—	—	—
7[b]	>1000	449	987	449	—	—	—	—	—	—
8	139	190	>200	171	36.7	71.4	37.5	62.9	7.8	>200
9	142	118	167	60.5	47.1	42.2	12.9	21.3	25.6	29.5
10	50.5	34.6	66.2	32.2	12.8	23.9	14.4	27.8	5.7	24.6
11	88.8	29.7	141	66.0	38.1	59.5	67.4	>200	25.2	30.1
12	>200	>200	>200	186	127	>200	92.3	>200	123	58.9
13	92.3	>200	>200	92.9	78.6	64.3	89.1	54.3	62.9	150
14	>200	>200	>200	197	>200	>200	>200	>200	>200	>200
15	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
16	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
17	32.1	22.5	22.9	9.6	10.8	28.2	10.0	9.4	4.9	17.9
18	6.7	7.7	21.8	6.7	9.1	8.4	11.2	7.8	5.1	3.9
19	4.5	2.9	0.46	2.1	3.9	0.68	3.2	4.3	4.6	0.93
20	6.8	1.6	7.1	8.9	3.4	7.3	4.6	3.2	8.2	2.7
21	14.4	17.9	14.8	5.2	7.6	25.3	28.6	10.5	19.8	15.4
22	2.9	1.5	4.1	1.3	2.8	4.5	2.7	2.5	3.1	2.4

[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.
[b] See Ref. 8.
[c] Data represent the mean values of three independent determinations.

(HT-29), and oral squamous carcinoma (KB). Compounds **8–16** were all prepared in the form of hydrochloride in order to enhance the solubility in aqueous solution by the usual methods before use. The results were summarized in Table 1.

As shown in Table 1, compounds **8–10** with no substituents at position-9 exhibited moderate cytotoxic activities, but compound **10**, bearing a 3,4,5-trimethoxybenzylidine substituent at position-1, was more active. In our previous investigation, we found that introducing an n-butyl or phenylpropyl substituent into position-9 of β-carboline nucleus facilitated antitumor activities in vitro [10–13] and in vivo.[10,12] Unfortunately, in the present investigation, compounds **12–13** and **15–16** were almost inactive to all tumor cell lines investigated at the concentration of 200 μM.

The N^2-benzylated β-carboline derivatives **17–22** represented the most interesting cytotoxic activities. As predicted, the IC_{50} values of these compounds were lower than 10 μM against most of human tumor cell lines investigated. Interestingly, compounds **19** was found to be the most active derivative with IC_{50} value of 0.46 μM, 0.68 μM, and 0.93 μM against BGC-823, A375, and KB cell lines, respectively. These results further confirmed that the position of the N^2-benzylated substituent on the β-carboline ring played a very vital role in the modulation of the cytotoxic activities.

Some important molecular mechanisms of action of this class of bioactive compounds have been recently reviewed.[1] However, by far, the underlying mechanism of action for the antitumor effects of β-carbolines has not been completely defined. Our previous investigations reported that the ability of β-carbolines to act as intercalating agents and Topo I inhibitors was related to the antitumor activity[16] and β-carboline derivatives can pass through cell membrane and penetrate into nucleus quickly resulting in intercalating into DNA in cells.[17] Very recent researches in our laboratory indicated that N^2-benzylated β-carbolinium ions can penetrate into plasma membrane and nuclear envelop more easily than those molecules without substituents at position-2. However, further studies will be needed to elucidate the underlying mechanism of action of these compounds completely.

Furthermore, in order to confirm the utility of the β-carbolinium ion derivatives as an interesting antitumor agent, compounds **18–20** and **22** are now selected and submitted to further acute toxicity and antitumor activity studies in animal models, and the relative possible results will be reported in due course.

In conclusion, a number of novel β-carboline derivatives described in this paper proved to be remarkably potent antitumor activities. In comparison with already published antitumor agents of similar chemical structure, N^2-benzylated β-carbolinium bromates **19, 20**, and **22** were found to be the most potent compounds with IC_{50} values lower than 10 μM against a panel of human tumor cell lines. Therefore, N^2-benzylated β-carbolinium bromates **19, 20**, and **22** can be considered promising leads for further structural modifications guided by the valuable information derivable from our detailed SARs.

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14. *General procedure for the preparation of 1-benzylidine β-carboline derivatives **8**–**16***. A mixture of **4**–**7** (10 mmol), acetic anhydride (50 ml) and aldehydes (50–100 mmol) was refluxed for 24–48 h. After completion of the reaction as indicated by TLC, the solution was poured into ice-water (200 ml) and made basic with sodium bicarbonate. The aqueous mixture was extracted with ethyl acetate, and the organic phase was washed with water and brine and then dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the residue was dissolved in ethanol and made acidic with concentrated hydrochloric acid. The solvent was evaporated in reduced pressure and the resulting oil was crystallized from acetone to give yellow solid. The solid was dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous sodium sulfate, concentrated under vacuum. The oil residue was crystallized to give **8**–**16** in 12–65% yields. Compound **12**: yield 53%, mp 136–137 °C. FAB-MS *m*/*e* (M+1) 419. IR (KBr) 3053, 3030, 2970, 2922, 2841, 1630, 1603, 1555, 1509, 1446, 1415, 1361, 1328, 1291, 1242, 1222, 1171, 1032, 966, 821 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 8.46–8.47 (1H, d, J = 5.0 Hz, H-3); 8.10–8.12 (1H, d, J = 7.5 Hz, H-4); 7.66–7.86 (3H, m, H-5, H-6, H-7); 7.53–7.55 (3H, m, H-8, Ph***H***); 7.15–7.33 (7H, m, Ph***H***); 6.94–6.95 (2H, d, J = 8.5 Hz, –C***H***═C***H***–); 4.58–4.61 (2H, m, NC***H***$_2$CH$_2$CH$_2$Ph); 3.86 (3H, s, OC***H***$_3$); 2.75–2.78 (2H, m, NCH$_2$CH$_2$C***H***$_2$Ph); 2.30–2.33 (2H, m, NCH$_2$C***H***$_2$CH$_2$Ph). Anal. Calcd for $C_{29}H_{26}N_2O$: C, 83.22; H, 6.26; N, 6.69. Found: C, 83.40; H, 6.29; N, 6.67.
15. *General procedure for the preparation of 1-benzylidine β-carbolinium bromates **17**–**22***. A mixture of **9**, **11–14** or **16** (2 mmol) and benzyl bromide (10–20 mmol) in ethyl acetate (50 ml) was refluxed for 5–10 h. After completion of the reaction as indicated by TLC, the solution was cooled and filtered and crystallized to afford **17–22** in 44–76% yields. Compound **19**: yield 60%, mp 207–209 °C. FAB-MS *m*/*e* 509. IR (KBr) 3412, 3029, 3002, 2936, 2837, 1622, 1606, 1515, 1457, 1339, 1256, 1179, 1033, 971, 820 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 8.98–8.99 (1H, d, J = 6.5 Hz, H-3); 8.89–.90 (1H, d, J = 6.5 Hz, H-4); 8.57–.59 (1H, d, J = 8.5 Hz, H-5); 7.88–7.93 (2H, m, H-6, H-7); 7.03–7.04 (15H, m, H-8, Ph***H***); 6.82–6.84 (2H, d, J = 8.5 Hz, –C***H***═C***H***–); 6.07 (2H, s, C***H***$_2$Ph); 4.59–.62 (2H, t, NC***H***$_2$CH$_2$CH$_2$Ph); 3.84 (3H, s, OC***H***$_3$); 2.49–.51 (2H, m, NCH$_2$CH$_2$C***H***$_2$Ph); 2.28–.30 (2H, m, NCH$_2$C***H***$_2$CH$_2$Ph). Anal. Calcd for $C_{36}H_{33}BrN_2O$: C, 73.34; H, 5.64; N, 4.75. Found: C, 73.20; H, 5.66; N, 4.78.
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