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Original article

Design, synthesis and 3D-QSAR of β-carboline derivatives as potent antitumor agents

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ABSTRACT

In a continuing effort to develop novel β-carbolines endowed with better pharmacological profiles, a series of β-carboline derivatives were designed and synthesized based on the previously developed SARs. Cytotoxicities *in vitro* of these compounds against a panel of human tumor cell lines were also investigated. The results demonstrated that the N^2-benzylated β-carbolinium bromides **56**–**60** represented the most potent compounds with IC_{50} values lower than 10 μM. The application of 3D-QSAR to these compounds explored the structural basis for their biological activities. CoMFA ($q^2 = 0.513$, $r^2 = 0.862$) and CoMSIA ($q^2 = 0.503$, $r^2 = 0.831$) models were developed for a set of 47 β-carbolines. The results indicated that the antitumor pharmacophore of these molecules were marked at position-1, -2, -3, -7 and -9 of β-carboline ring.

1. Introduction

The β-carboline nucleus is common to many natural and synthetic products associated with a broad spectrum of biochemical effects and pharmaceutical properties. These compounds have been shown to intercalate into DNA [1–3], to inhibit CDK [4,5], topisomerase [1,3,6] and monoamine oxidase [7–9], and to interact with benzodiazepine receptors (BZ) [10–12], 5-hydroxy serotonin receptors (5-HT) [13,14], dopamine (DA) [15] and imidazoline receptors [16,17]. In addition, individual β-carboline derivative might selectively interact with specific targets so as to lead to a variety of pharmacological actions *in vitro* and *in vivo*. So far, β-carboline derivatives has been found to have various pharmaceutical functions including sedative, anxiolytic, hypnotic, anticonvulsant [18–20], antimicrobial [21,22], antiviral [23,24], parasiticidal [25,26] as well as antithrombotic activities [27,28].

Previous investigations focused on the effects of β-carbolines on the central nervous system (CNS). However, interests in these alkaloids were stimulated by their promising antitumor activities in the last decades. Recent reports [2, 29–34] our group investigations [35–40] on the synthesis of a variety of β-carboline derivatives and the evaluation of their antitumor activities unraveled that β-carbolines had potent antitumor activities and the activities was correlated to both the planarity of the molecule and the presence of the ring substituents. Preliminary structure–activity relationships (SARs) analysis suggested that the introduction of appropriate substituents into position-2, -3 and -9 played a vital role in determining their antitumor effects [37,38,41].

Despite these recent undoubted advances, β-carboline derivatives still present some limitations arising from the relative weak antitumor activities in animal models and the poorly understood action of mechanism [38]. Obviously, to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituents properties in β-carboline and antitumor activities, design and synthesis of more novel β-carboline derivatives with various substituents at different position of the β-carboline nucleus are needed.

In this context we began a systematic, long term study aiming at the synthesis of novel antitumor agents endowed with better pharmacological profiles. In the present report we described the design and synthesis, and the biological and 3D-QSAR studies of novel potent antitumor agents bearing various substituents in position-1, -2, -3, -7 and -9 of β-carboline ring. The aim purpose of this study was to elucidate the antitumor structure–activity relationships (SARs) of β-carbolines in finer detail, with the ultimate aim of developing a reliable 3D-QSAR model to probe the structural requirement at the 3D levels for highly potent antitumor activity.

2. Chemistry

1-(3,4,5-Trimethoxy)phenyl substituted β-carbolines **1**–**4** were synthesized according to already published methods [39]. The

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preparation of compounds **5**–**8**, **10**, **13**, **16**, **25**–**28**, **36**–**37** and **39** have been already described as antitumor agents in our previous reports [35,36,40]. Compound **9** was easily prepared in good yield according to the published method described by our group [35].

Previously, Xiao et al. [2] and our group [40] reported the synthesis of β-carbolines bearing an alkylamine side chain in position-3 in 18–27% yield. To improve yield and reaction conditions, in the present investigation, we have developed a straightforward cost-effective method involving microwave irradiation for the preparation of β-carbolines bearing an alkylamine side chain in position-3 in 82–90% yield. The crude mixture of ethyl 9-*n*-butyl-β-carboline-3-carboxylate **5** and ethylenediamine was subjected to microwave irradiation (150 °C, 15 min) to give the amidated derivatives **11** in 88% yield. The same synthetic protocol was used for the preparation of derivatives **12**, **14**–**15** and **17**–**18** from the starting materials compounds **6**, **7** and **8**, respectively (Scheme 1). Compared to the reaction time of 48 h under the conventional heating and refluxing, this synthetic protocol only took 15 min to finish the reaction under microwave irradiation.

The ester group in position-3 of compounds **6** and **9** was reduced to its corresponding alcohols by lithium borohydride ($LiBH_4$) in dry THF to provide compounds **21** and **22** [39], and further oxidized by MnO_2 in CH_3CN to afford 3-carboxaldehyde derivatives **23** and **24** [39] (Scheme 2).

The N^2-alkylated or benzylated β-carbolinium bromide derivatives **29**–**35** (Scheme 3) were prepared from the known compounds **25**–**28** by the addition of the corresponding alkylating or benzylating agents in refluxing ethyl acetate [38]. The same synthetic protocol was used for the preparation of the N^2-benzylated β-carbolinium bromide derivatives **56**–**60** (Scheme 4).

The N^9-alkylated harmine derivatives **36**–**37** and **39** were prepared according to the synthetic protocol described by our group [35]. The same synthetic procedure was used for the preparation of compound **38** from harmine and the commercially available isobutyl bromide. The preparation of compounds **40**–**43** followed a common synthetic scheme, characterized by demethylation of compound **36**–**39** using acetic acid and hydrobomic acid as reaction solvent (Scheme 4).

Compounds **44**–**55**, bearing alkoxy in postion-7 of β-carboline ring, were synthesized from compounds **40**–**43** by the action of sodium hydride in dry DMF followed by addition of the appropriate alkylating and arylating agents in 71–86% yield.

3. Results and discussion

3.1. Cytotoxicities in vitro

The cytotoxic potential of all synthesized β-carboline derivatives was evaluated *in vitro* against a panel of human tumor cell lines. Compound **3** was converted into its water-soluble sodium salts, and the other compounds (except **29**–**35** and **56**–**60**) examined were prepared in the form of hydrochloride by the usual methods before use. The results were summarized in Table 1.

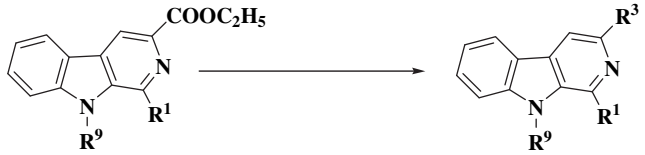
The first analysis of the structure–activity relationship concerned a general comparison of the cytotoxic activities among the four class of compounds described in this report. Compounds bearing a 3,4,5-trimethoxyphenyl substituent at position-1 of β-carboline ring gave the poorest cytotoxic activities whereas compounds bearing substituents at position-1, -2, -7 and 9 represented the most interesting class of antitumor agents with IC_{50} values lower than 10 μM against most of human tumor cell lines investigated.

Compounds **1**–**4** bearing a 3,4,5-trimethoxyphenyl substituent at position-1 exhibited weak to inactive cytotoxic activities, suggesting that the introduction of 3,4,5-trimethoxyphenyl substituent at position-1 had no positive effects on cytotoxic activities.

Of all 3, 9-disubstituted and 1,3,9-trisubstituted compounds **9**–**24**, compounds **11**–**12**, **14**–**15** and **17**–**18**, bearing a flexible aminoalkyl side chain at postion-3, displayed moderate to significant cytotoxic activities. Whereas compounds **13**, **16** and **19**–**20**, incorporating a hydroxyethyl substituent in position-3, only had marginal or no cytotoxic effect in any cell lines. These results indicated that hydroxyethyl side chain was unsuitable for the development of potent antitumor agents. Moreover, the substitution of the β-carboline ring with hydroxymethyl and carboxaldehyde determined a negative effect on the cytotoxic activities of derivatives **21**–**22** and **23**–**24**, suggesting that this class of substituents was unfavorable.

The N^2-alkylated and arylated derivatives **29**–**35**, as already reported in the literature [37,38], showed potent cytotoxic activities and the cytotoxic potency of derivatives followed the tendency of 33 (N^2-phenylpropyl) > **34** (N^2-benzyl) > **35** (N^2-*n*-butyl). A similar tendency also applied to derivatives **29** and **30** bearing a benzyl and phenylpropyl at position-2, respectively.

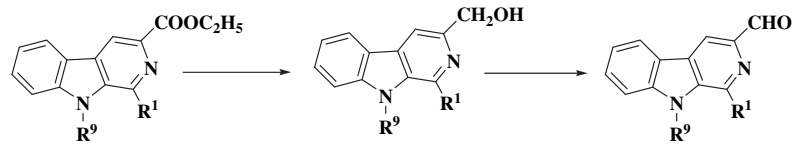
The cytotoxic potency of most 1,7,9-trisubstituted β-carboline derivatives **44**–**55** showed no distinct difference and the IC_{50}



5 R^1=H R^9=n-C_4H_9
6 R^1=CH_3 R^9=n-C_4H_9
7 R^1=H R^9=$CH_2C_6H_5$
8 R^1=CH_3 R^9=$CH_2C_6H_5$
9 R^1=H R^9=$CH_2C_6H_4$(p-F)
10 R^1=H R^9=$(CH_2)_3C_6H_5$

11 R^1=H R^9=n-C_4H_9 R^3=$CONH(CH_2)_2NH_2$
12 R^1=H R^9=n-C_4H_9 R^3=$CONH(CH_2)_6NH_2$
13 R^1=CH_3 R^9=n-C_4H_9 R^3=$CONH(CH_2)_2OH$
14 R^1=CH_3 R^9=n-C_4H_9 R^3=$CONH(CH_2)_2NH_2$
15 R^1=CH_3 R^9=$CH_2C_6H_5$ R^3=$CONH(CH_2)NH_2$
16 R^1=H R^9=$CH_2C_6H_5$ R^3=$CONH(CH_2)_2OH$
17 R^1=H R^9=$CH_2C_6H_5$ R^3=$CONH(CH_2)_2NH_2$
18 R^1=H R^9=$CH_2C_6H_5$ R^3=$CONH(CH_2)_6NH_2$
19 R^1=H R^9=$CH_2C_6H_4$(p-F) R^3=$CONH(CH_2)_2OH$
20 R^1=H R^9=$(CH_2)_3C_6H_5$ R^3=$CONH(CH_2)_2OH$

Scheme 1. Synthesis of 1,3,9-trisubstituted β-carbolines **11**–**20**.



6 R^1=CH_3 R^9=n-C_4H_9
9 R^1=H R^9=$CH_2C_6H_4$(p-F)

21 R^1=CH_3 R^9=n-C_4H_9
22 R^1=H R^9=$CH_2C_6H_4$(p-F)

23 R^1=CH_3 R^9=n-C_4H_9
24 R^1=H R^9=$CH_2C_6H_4$(p-F)

Scheme 2. Synthesis of 1,3,9-trisubstituted β-carbolines **21**–**24**.

values of this class of compounds were range from 10 to 100 μM. Exceptionally, compound **45** bearing a pentafluorobenzoxyl at position-7 displayed weak cytotoxic activity against human tumor cell lines, and the poor water-soluble property might be contributed to its weak activity.

The N^2-alkylated and arylated 1,7,9-trisubstituted β-carboline derivatives **56**–**60** 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. Interestingly, compound **57** was found to be the most active derivative with IC_{50} value of 0.93 μM against Hela cell line. These results further confirmed that the position of the N^2-arylated substituent on the β-carboline ring played a central role in the modulation of the cytotoxic activities.

3.2. 3D-QSAR study

All synthesized β-carboline derivatives were aligned to the common three-ring structure of β-carboline, and compound **57** with the highest pIC_{50} value was chosen as the template molecular. The aligned compounds were depicted in Fig. 1.

To further investigate the SARs of β-carbolines in more details, the cytotoxic data of HepG2 cell line were used to build the 3D-QSAR models. 40 compounds with determinate IC_{50} values were employed and their pIC_{50} values were calculated based on the original IC_{50} data.

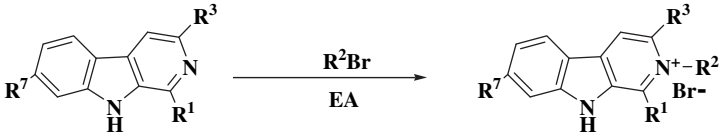
The PLS statistic parameters of CoMFA and CoMSIA models were summarized in Table 2. The cross-validation correlation coefficient q^2 which marked the predictive capacity and the conventional correlation coefficient r^2 which marked self-consistence were two key parameters to evaluate the qualities of PLS analysis. A q^2 value over 0.3 is considered significant for the chance of significant correlation being <95%. For CoMFA model, the q^2 and r^2 was 0.513 and 0.862, while for CoMSIA model was 0.503 and 0.831. The statistic results indicated the good predictive ability of CoMFA and CoMSIA models. The optimal number of components using to generate both CoMFA and CoMSIA model is 5, which were reasonable considering the number of compounds used to derive the models. The standard error of estimate of CoMFA and CoMSIA models were also reasonably low amounting to 0.223 and 0.247. *F*-test results were 42.583 and 33.517 for CoMFA and CoMSIA models, respectively.

To further test the validity of the 3D-QSAR models, as well as their predictive utility, an inactive test set made up of 8 compounds without determinate IC_{50} values ($IC_{50} > 200$ μM) were employed as external testing data. The predictive pIC_{50} values of the test set were obtained by using the predict property function of the QSAR module in SYBYL6.9. Considering the pIC_{50} data of the training set we selected to construct the 3D-QSAR models were range from 3.63 to 5.80, we considered a predictive pIC_{50} value lower than 4 here was reasonably inactive. The predictive results were summarized in Table 3. Both CoMFA and CoMSIA model shown fairly reliable predictive abilities as 7 out of 8 compounds exhibited predictive pIC_{50} values lower than 4 for CoMFA, while for CoMSIA all predictive pIC_{50} values were lower than 4.

Figs. 2 and 3 depicted the correlation between the actual pIC_{50} and the predictive pIC_{50} of CoMFA and CoMSIA model, respectively, demonstrating again the fair reliability of CoMFA and CoMSIA models. PLS Statistics of both CoMFA and CoMSIA models indicated that CoMFA model was somewhat better than CoMSIA model.

3D-QSAR contour maps of both CoMFA and CoMSIA models were depicted in Fig. 4, compound **49** with the highest pIC_{50} value was chosen to superimpose into the contour maps to better illustrate the SAR information. The steric contour maps are represented in green and yellow while the electrostatic contour maps are represented in red and blue. The green contours are indicative of favorable regions for sterically bulkier groups and the yellow contours are indicative of regions that are sterically less favorable. In a similar way the red contours represent regions that lead to the enhancement of activity with electron rich groups, and contrary to that the blue regions represent electron deficient regions and can lead to an increase in the activity of compounds.

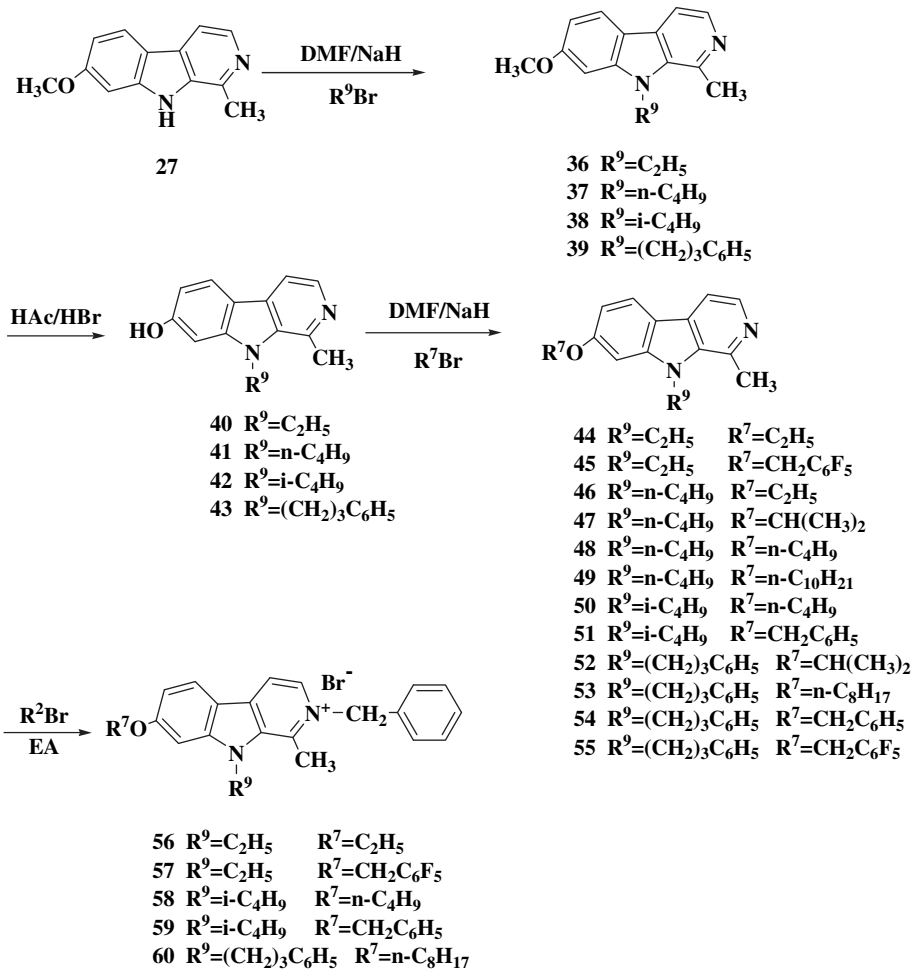
For CoMFA model, the steric term contributed 65.3% to the interacting energy, indicated the SAR of β-carboline were more



25 R^1=CH_3 R^3=R^7=H
26 R^1=CH_3 R^3=$CO_2C_2H_5$ R^7=H
27 R^1=CH_3 R^3=H R^7=OCH_3
28 R^1=R^3=R^7=H

29 R^1=CH_3 R^2=$CH_2C_6H_5$ R^3=R^7=H
30 R^1=CH_3 R^2=$(CH_2)_3C_6H_5$ R^3=R^7=H
31 R^1=CH_3 R^2=$CH_2C_6H_5$ R^3=$CO_2C_2H_5$ R^7=H
32 R^1=CH_3 R^2=$CH_2C_6H_5$ R^3=H R^7=OCH_3
33 R^1=R^3=R^7=H R^2=n-C_4H_9
34 R^1=R^3=R^7=H R^2=$CH_2C_6H_5$
35 R^1=R^3=R^7=H R^2=$(CH_2)_3C_6H_5$

Scheme 3. Synthesis of N^2-alkylated quaternary β-carbolines **29**–**35**.



Scheme 4. Synthesis of 1,7,9-trisubstituted and 1, 2, 7, 9-tetrasubsituted β-carbolines **40**–**60**.

influenced by steric effect than by electrostatic effect. The analysis of the contour signals represented in CoMFA map (Fig. 4A) leads to a conclusion that a high cytotoxic potency is associated to compounds placing hydrophobic bulky groups in two green regions in position-7 and -9 (i.e. compounds **48** and **52**); and electropositive atoms (nitrogen atoms) in the blue region located near position-2 (i.e. compounds **39** and **45**) and position-3 (i.e. compounds **11** and **21**); and electron rich groups such as benzyl or pentafluorobenzyl in position-2, -7 and -9 due to the electrostatic attractive effect with the red regions there (i.e. compounds **49** and **51**). Noticeably the blue region in position-3 was in good agreement with the electropositive DNA targeting substituent $-CONH(CH_2)_nNH_2$ that favored the antitumor activities.

Low activities compounds are experiencing steric repulsive contact with the yellow region in the far area of position-7 (i.e. compounds **33** and **47**) due to the long alkyl groups; and electrostatic repulsive interaction with the blue region in position-3 due to the electronegative groups (i.e. compounds **6** and **34**). Another electrostatic signal was visible in the far area of position-3, where a red region indicate the electropositive atoms here are unfavorable for the antitumor cytotoxicity (compounds **23** and **24**), this signal also indicated that the DNA targeting sequence $-CONH(CH_2)_nNH_2$ with a long carbon side chain ($n = 6$) were showing lower antitumor activities, this decreasing of cytotoxicity was considered to be constrained by the steric hindrance between two DNA strands as we described in our previous investigation.

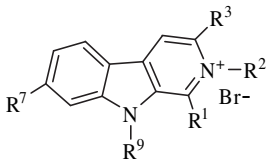
For CoMSIA model, the SAR signals representing in the resulting contour map (Fig. 4B) are similar and simpler than the CoMFA map, confirming the pharmacophore observations made in the CoMFA map analysis, particularly the contour signals placing in position-7 and -9 that hydrophobic bulky substituents, represented by the steric green regions and the electronegative red regions, favored the cytotoxicity; and at position-2 that presence of benzyl substituted, represented by the blue region in the electropositive nitrogen atom and red region in the electron rich benzene group, are associated with high cytotoxicity. Differ from CoMFA map, a yellow region can be seen near position-1 of CoMSIA map, explaining the decreasing cytotoxic activities of compounds with the bulkier substituent 3,4,5-trimethoxyphenyl in position-1 (i.e. compounds **1** and **2**), and also explaining the fairly higher activities of compounds with relative smaller methyl substituted in position-1 (i.e. compounds **20** and **41**).

In conclusion, the pharmacophore information of β-carbolines detected by the CoMFA and CoMSIA maps were in good agreement with most structural features of the SARs analysis elucidated before.

4. Conclusion

In summary, a number of novel β-carboline derivatives described in this paper proved to be potent antitumor agents. The N^2-benzylated β-carbolinium bromides **56**–**60** were found to be the most

Table 1
Chemical structures and cytotoxic activities of β-carboline derivatives *in vitro*.[c]



Compd	Substituents					IC_{50}(μM)[a]					
	R_1	R_2	R_3	R_7	R_9	HepG2[b]	Hela[b]	Bel-7402[b]	BGC-823[b]	MCF-7[b]	pIC_{50}[d]
1	3,4,5-trimethoxyphenyl	–	$CO_2C_2H_5$	H	H	167	>200	>200	>200	123	3.78
2	3,4,5-trimethoxyphenyl	–	$CO_2C_2H_5$	H	*n*-C_4H_9	>200	>200	>200	27.3	>200	–
3	3,4,5-trimethoxyphenyl	–	CO_2H	H	*n*-C_4H_9	208	166	187	36.2	115	3.68
4	3,4,5-trimethoxyphenyl	–	$CONH(CH_2)_2OH$	H	*n*-C_4H_9	87.4	>200	>200	37.2	105	4.06
9	H	–	$COOC_2H_5$	H	C_6H_4(p-F)	>200	>200	>200	>200	>200	–
11	H	–	$CONH(CH_2)_2NH_2$	H	*n*-C_4H_9	82.9	26.9	115.2	53.2	18.5	4.08
12	H	–	$CONH(CH_2)_6NH_2$	H	*n*-C_4H_9	95.8	53.0	58.5	110.3	42.8	4.02
13	CH_3	–	$CONH(CH_2)_2OH$	H	*n*-C_4H_9	140	80.4	90.6	45.0	97.3	3.85
14	CH_3	–	$CONH(CH_2)_2NH_2$	H	*n*-C_4H_9	21.9	13.3	56	17.3	7.5	4.66
15	CH_3	–	$CONH(CH_2)_2NH_2$	H	$CH_2C_6H_5$	34.8	11.0	53.6	20.3	4.7	4.46
16	H	–	$CONH(CH_2)_2OH$	H	$CH_2C_6H_5$	>200	>200	>200	>200	>200	–
17	H	–	$CONH(CH_2)_2NH_2$	H	$CH_2C_6H_5$	104	42.8	51.7	11.9	51.6	3.98
18	H	–	$CONH(CH_2)_6NH_2$	H	$CH_2C_6H_5$	58.7	24.2	56.8	34.8	54.1	4.23
19	H	–	$CONH(CH_2)_2OH$	H	C_6H_4(p-F)	>200	>200	>200	>200	>200	–
20	H	–	$CONH(CH_2)_2OH$	H	$(CH_2)_3C_6H_5$	>200	>200	>200	>200	>200	–
21	CH_3	–	CH_2OH	H	*n*-C_4H_9	128	100	122	127	107	3.89
22	H	–	CH_2OH	H	C_6H_4(p-F)	>200	>200	>200	>200	>200	–
23	CH_3	–	CHO	H	*n*-C_4H_9	143	169	123	>200	154	3.84
24	H	–	CHO	H	C_6H_4(p-F)	>200	>200	>200	>200	>200	–
29	CH_3	$CH_2C_6H_5$	H	H	H	69.5	69.2	74.0	77.4	89.2	4.16
30	CH_3	$(CH_2)_3C_6H_5$	H	H	H	33.0	49.8	20.5	3.5	23.8	4.48
31	CH_3	$CH_2C_6H_5$	$CO_2C_2H_5$	H	H	53.0	101	56.2	55.3	44.9	4.28
32	CH_3	$CH_2C_6H_5$	H	OCH_3	H	54.6	77.6	64.2	67.4	76.2	4.26
33	H	*n*-C_4H_9	H	H	H	94.0	130	302	326	338	4.03
34	H	$CH_2C_6H_5$	H	H	H	77.3	85.4	65.1	91.8	91.3	4.11
35	H	$(CH_2)_3C_6H_5$	H	H	H	44.6	44.3	24.6	7.1	5.7	4.35
40	CH_3	–	H	OH	C_2H_5	134	70.3	165	160	106	3.87
41	CH_3	–	H	OH	*n*-C_4H_9	81.5	89.8	69.5	90.2	46.9	4.09
42	CH_3	–	H	OH	i-C_4H_9	116	68.3	114	140	96.9	3.94
43	CH_3	–	H	OH	$(CH_2)_3C_6H_5$	28.3	45.3	35.1	29.9	34.5	4.55
44	CH_3	–	H	OC_2H_5	C_2H_5	68.9	30.7	27.4	57.1	14.4	4.16
45	CH_3	–	H	$OCH_2C_6F_5$	C_2H_5	236	258	241	238	103	3.63
46	CH_3	–	H	OC_2H_5	*n*-C_4H_9	43.3	32.1	36.1	37.2	12.0	4.36
47	CH_3	–	H	$OCH(CH_3)_2$	*n*-C_4H_9	30.2	21.2	26.8	19.1	19.5	4.52
48	CH_3	–	H	OC_4H_9	*n*-C_4H_9	15.5	17.4	69.9	15.8	17.3	4.81
49	CH_3	–	H	$OC_{10}H_{21}$	*n*-C_4H_9	127	106	18.8	28.4	62.7	3.90
50	CH_3	–	H	OC_4H_9	i-C_4H_9	12.1	20.8	42.5	12.1	7.2	4.92
51	CH_3	–	H	$OCH_2C_6H_5$	i-C_4H_9	22.2	50.5	26.8	36.0	54.1	4.65
52	CH_3	–	H	$OCH(CH_3)_2$	$(CH_2)_3C_6H_5$	14.6	48.6	36.2	16.3	45.3	4.84
53	CH_3	–	H	OC_8H_{17}	$(CH_2)_3C_6H_5$	105	69.6	21.8	17.4	45.9	3.98
54	CH_3	–	H	$OCH_2C_6H_5$	$(CH_2)_3C_6H_5$	15.7	72.6	29.4	19.5	63.3	4.80
55	CH_3	–	H	$OCH_2C_6F_5$	$(CH_2)_3C_6H_5$	147	73.5	18.6	7.88	38.7	3.83
56	CH_3	$CH_2C_6H_5$	H	OC_2H_5	C_2H_5	14.3	2.2	12.6	9.2	8.1	4.84
57	CH_3	$CH_2C_6H_5$	H	O $CH_2C_6F_5$	C_2H_5	1.6	0.93	4.0	4.6	5.7	5.80
58	CH_3	$CH_2C_6H_5$	H	OC_4H_9	i-C_4H_9	1.8	2.6	5.3	6.8	5.3	5.74
59	CH_3	$CH_2C_6H_5$	H	$OCH_2C_6H_5$	i-C_4H_9	1.9	12.4	5.0	5.2	3.9	5.72
60	CH_3	$CH_2C_6H_5$	H	OC_8H_{17}	$(CH_2)_3C_6H_5$	3.9	2.2	2.0	3.9	5.3	5.41

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound that causes a 50% growth inhibition to untreated cells using the MTT assay.
[b] Cell lines include liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (Hela), breast adenocarcinoma(MCF-7).
[c] Data represent the mean values of three independent determinations.
[d] pIC_{50} values of HepG2 human tumor cell lines.

interesting compounds with IC_{50} values lower than 10 μM against a panel of human tumor cell lines, and such compounds can be considered promising leads for further structural modifications guided by the valuable information derivable from our detailed SARs. In addition, 3D-QSAR models were developed to obtain detailed information on structure–activity relationships between β-carbolines and antitumor potencies, and the models provided effective tools to predict the antitumor potency of new compounds in silico.

Undoubtedly, to develop more refined and more reliable 3D-QSAR models for predicting the antitumor potency of new compounds, the synthesis of more larger and structurally more diverse chemical libraries is needed. Further biological evaluations on these and other unreported β-carboline derivatives in animal models are in progress in our laboratories. Moreover, the molecular mechanisms of these compounds are ongoing and the relative possible results will be reported in due course.

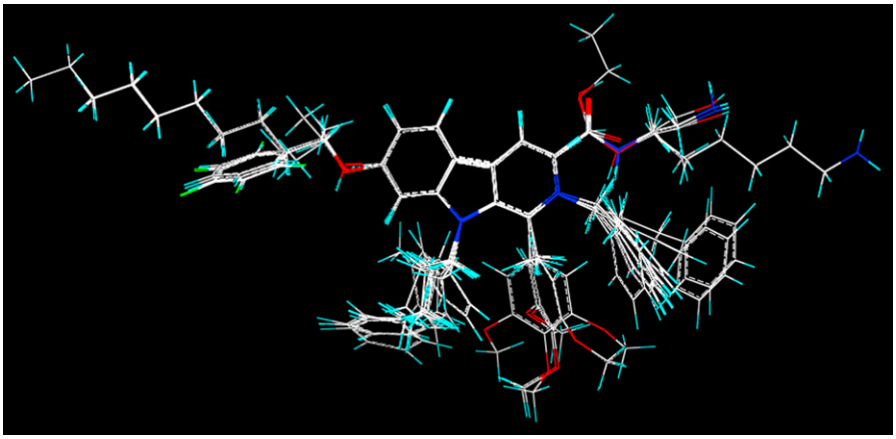


Fig. 1. 3D-QSAR structure alignment and superposition of 40 compounds using compound **57** as the template.

5. Experimental section

5.1. Reagents and general methods

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Harmine (7-methoxy-1-methyl-β-carboline **27**) was extracted from *Peganum multisectum Maxim*, a plant indigenous to western China, according to already published method [42]. The following compounds: ethyl 9-*n*-butyl-β-carboline-3-carboxylate **5** [35], ethyl 9-*n*-butyl-1-methyl-β-carboline-3-carboxylate **6** [36], ethyl 9-benzyl-β-carboline-3-carboxylate **7** [35], ethyl 9-benzyl-1-methyl-β-carboline-3-carboxylate **8** [36], ethyl 9-(3-phenylpropyl)-1-methyl-β-carboline-3-carboxylate **10** [36], *N*-(2-hydroxyethyl)-9-*n*-butyl-1-methyl-β-carboline-3-carboxamide **13** [40], *N*-(2-hydroxylethyl)-9-benzyl-β-carboline-3-carboxamide **16** [40], 1-methyl-β-carboline **25** [37], ethyl 1-methyl-β-carboline-3-carboxylate **26** [36], β-carboline **28** [35], 7-methoxy-9-ethyl-1-methyl-β-carboline **36** [35], 7-methoxy-9-*n*-butyl-1-methyl-β-carboline **37** [35], and 7-methoxy-9-(3-phenylpropyl)-1-methyl-β-carboline **39** [35], were prepared according to the described procedures.

Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. FAB-MS spectra were obtained from VG ZAB-HS spectrometer. FT-IR spectra were run as KBr pellets on a Bruker Equinox 55 Fourier Transformation Infrared Spectrometer. ^{1}H NMR spectra were recorded on a Varian INOVA 500NB spectrometer. Chemical shifts are reported in δ (ppm) downfield from an internal solvent peak and coupling constants, J in hertz. Elemental analyses (C, H and N) were carried out on an ElementarVario EL CHNS Elemental Analyzer. Silica gel F254 were used in analytical thin-layer chromatography (TLC) and silica gel were used in column chromatography respectively.

5.1.1. Preparation of ethyl 9-(4-fluorobenzyl)-β-carboline-3-carboxylate (**9**)

A mixture of ethyl β-carboline-3-carboxylate (24.0 g, 100 mmol) and anhydrous DMF (200 mL) was stirred at room temperature for 10 min, and then 60% NaH (8.0 g, 200 mmol) and 4-fluorobenzyl bromide (150 mmol) were added. The mixture was stirred at room temperature. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (500 mL), and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting oil was crystallized from ethyl ether to afford white crystal (30 g, 84%), mp 132–133 °C. FAB-MS m/z 349 (M + 1); IR (KBr, cm^{-1}): 3059, 3027, 2986, 2954, 2904, 1726, 1622, 1505, 1371, 1333, 1303, 1245, 1107, 1026, 746. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (1H, d, J = 4.5 Hz); 8.23 (1H, d, J = 8.0 Hz); 7.60–7.61 (1H, t, J = 4.5 Hz); 7.46 (1H, d, J = 8.0 Hz); 7.39 (1H, t, J = 7.5 Hz); 7.26 (1H, s); 6.95–7.14 (4H, m); 5.59 (2H, s); 4.51–4.55 (2H, m); 1.48 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.5, 160.6, 143.6, 137.4, 133.3, 131.9, 131.5, 129.8, 129.7, 124.3, 122.8, 121.1, 119.5, 116.4, 116.1, 112.4, 63.0, 46.9, 15.0. Anal. Calcd for $C_{21}H_{17}FN_2O_2$: C, 72.40; H, 4.92; N, 8.04. Found: C, 72.29; H, 4.96; N, 7.98.

5.2. General procedure for the preparation of 3-alkylamino substituted β-carbolines (**11**–**12**, **14**–**15** and **17**–**18**)

The mixture of the corresponding β-carboline-3-carboxy-lates (5 mmol) and alkylamines (15 mmol) was stirred under microwave irradiation at 150 °C for 15 min. The resulting solution was partitioned between water and methylene chloride. The aqueous phase

Table 2
PLS Statistics of CoMFA and CoMSIA Models.

	q^{2a}	N^b	r^{2c}	SE^d	F^e	Fraction[f]	
						Steric	Electrostatic
CoMFA	0.513	5	0.862	0.223	42.583	0.653	0.347
CoMSIA	0.503	5	0.831	0.247	33.517	0.518	0.482

[a] Cross-validated correlation coefficient.
[b] Optimum number of components obtained from cross-validated PLS analysis and same used in final non-cross-validated analysis.
[c] Non-cross-validated correlation coefficient.
[d] Standard error of estimate.
[e] F-test value.
[f] Field contributions.

Table 3
CoMFA and CoMSIA predictive pIC_{50} values of the inactive test set.

Compound	Predictive pIC_{50}	
	CoMFA	CoMSIA
7	3.50	3.44
8	3.58	3.35
9	3.95	3.75
13	3.64	3.31
14	3.45	3.26
18	3.93	3.66
36	3.56	3.40
53	4.10	3.90

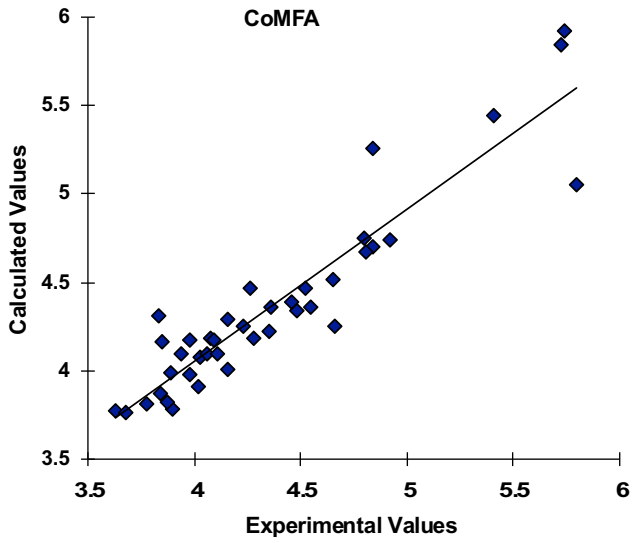


Fig. 2. Predictive vs. experimental pIC_{50} values derived from the CoMFA model.

was extracted with methylene chloride and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, evaporated. The residued crude oil was purified by a column chromatography, using a mixture of chloroform and methanol 20:1 as an eluent, to successfully afford the desirable β-carbolines bearing a flexible aminoalkyl side chain in good yield.

*5.2.1. N-(2-Aminoethyl)-9-n-butyl-β-carboline-3-carboxamide (**11**)*

Yellow oil was obtained (1.36 g, 88%). FAB-MS m/z (M + 1) 311. IR (KBr, cm^{-1}): 3366, 2957, 2930, 1655, 1623, 1528, 1496, 1462, 1333, 1257, 749. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (1H, s); 8.66 (1H, s); 8.49 (1H, t, J = 6.0 Hz); 8.10 (1H, d, J = 7.5 Hz); 7.54 (1H, t, J = 7.5 Hz); 7.37 (1H, d, J = 8.5 Hz); 7.25 (1H, t, J = 4.5 Hz); 4.26 (2H, t, J = 7.0 Hz); 3.69–3.73 (2H, m); 3.15 (2H, t, J = 6.0 Hz); 1.77–1.83 (2H, m); 1.29–1.36 (2H, m); 0.89 (3H, t, J = 7.5 Hz).

*5.2.2. N-(2-Aminohexyl)-9-n-butyl-β-carboline-3-carboxamide (**12**)*

Yellow oil was obtained (1.52 g, 83%). FAB-MS m/z (M + 1) 367. IR (KBr, cm^{-1}): 3382, 2931, 2859, 1658, 1624, 1588, 1530, 1497, 1464, 1334, 1258, 751. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (1H, s); 8.74 (1H, s); 8.21 (1H, t, J = 8.0 Hz); 8.15 (1H, d, J = 6.0 Hz); 7.61 (1H, t, J = 7.5 Hz); 7.47 (1H, d, J = 8.5 Hz); 7.32 (1H, t, J = 7.5 Hz); 4.39 (2H, t, J = 7.0 Hz); 3.50–3.54 (2H, m); 2.74–2.77 (2H, m); 1.86–1.91 (2H, m); 1.66–1.69 (2H, m); 1.54–1.55 (2H, m); 1.37–1.46 (6H, m); 0.95 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.3, 141.8, 140.4, 137.8, 131.6, 129.4, 128.4, 122.9, 121.3, 120.8, 114.3, 111.1, 43.3, 31.7, 31.5, 30.2, 27.1, 26.8, 20.5, 14.4.

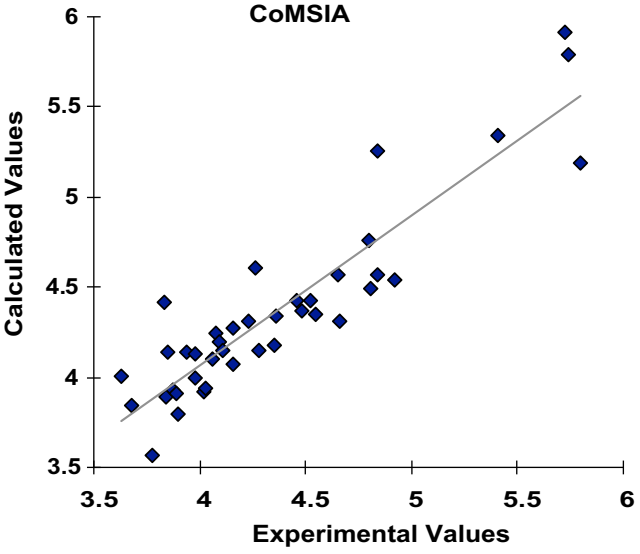


Fig. 3. Predictive vs. experimental pIC_{50} values derived from the CoMSIA model.

*5.2.3. N-(2-Aminoethyl)-9-n-butyl-1-methyl-β-carboline-3-carboxamide (**14**)*

White solid (1.37 g, 85%) was obtained, mp 157–158 °C (lit. [40] mp 144–145 °C). FAB-MS m/z (M + 1) 325. IR (KBr, cm^{-1}): 3377, 3056, 2961, 2865, 1463, 1521, 1457, 1352, 1249, 1110, 751. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90(1H, s); 8.76 (1H, s); 8.43 (1H, t, J = 6.0 Hz); 8.17 (1H, d, J = 8.0 Hz); 7.58 (1H, t, J = 7.5 Hz); 7.46 (1H, d, J = 8.0 Hz); 7.29 (1H, t, J = 7.5 Hz); 4.54 (2H, t, J = 7.0 Hz); 3.58–3.62 (2H, m); 3.04 (3H, s); 2.99 (2H, t, J = 6.0 Hz); 1.80–1.85 (2H, m); 1.41–1.49 (2H, m); 0.98 (3H, t, J = 7.5 Hz).

*5.2.4. N-(2-Aminoethyl)-9-benzyl-1-methyl-β-carboline-3-carboxamide (**15**)*

White solid (1.61 g, 90%) was obtained, mp 160–161 °C (lit. [40] mp 122–124 °C). FAB-MS m/z (M + 1) 359. IR (KBr, cm^{-1}): 3394, 3054, 2938, 1647, 1620, 1559, 1520, 1452, 1353, 1212, 733. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (1H, s); 8.22 (1H, d, J = 7.5 Hz); 7.53–7.56 (1H, m); 7.34–7.39 (2H, m); 7.25–7.27 (4H, m); 6.95 (2H, d, J = 7.5 Hz); 5.82 (2H, s); 3.57–3.61 (2H, m); 2.97–2.99 (2H, t, J = 6.5 Hz); 2.87 (3H, s).

*5.2.5. N-(2-Aminoethyl)-9-benzyl-β-carboline-3-carboxamide (**17**)*

White solid (1.51 g, 88%) was obtained, mp 193–195 °C. FAB-MS m/z (M + 1) 345. IR (KBr, cm^{-1}): 3395, 3052, 2928, 2867, 1660, 1625, 1590, 1554, 1525, 1464, 1334, 1269, 1206, 736. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (1H, s, H-1); 8.70 (1H, s); 8.36 (1H, d, J = 9.0 Hz); 8.25 (1H, d, J = 9.0 Hz); 7.59–7.62 (1H, m); 7.47 (1H, d, J = 10.5 Hz); 7.36 (1H, t, J = 7.5 Hz); 7.25–7.29 (3H, m); 7.14 (2H, d, J = 7.5 Hz); 5.61 (2H, s); 3.58–3.61 (2H, m); 2.97–3.00 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.4, 141.9, 140.8, 137.9, 137.7, 131.9, 129.6, 129.3, 128.8, 128.2, 127.5, 123.0, 121.5, 121.1, 114.5, 111.4, 46.9, 42.4, 41.8, Anal. Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27. Found: C, 72.28; H, 5.89; N, 16.30.

*5.2.6. N-(2-Aminohexyl)-9-benzyl-β-carboline-3-carboxamide (**18**)*

White solid (1.64 g, 82%) was obtained, mp 132–134 °C (lit. [40] mp 162–164 °C). FAB-MS m/z (M + 1) 401. IR (KBr, cm^{-1}): 3402, 3032, 2925, 2850, 1663, 1526, 1495, 1464, 1333, 1266, 725. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (1H, s); 8.68 (1H, s); 8.25 (1H, d, J = 9.0 Hz); 8.10 (1H, d, J = 9.0 Hz); 7.58–7.61 (1H, m); 7.47 (1H, d, J = 10.5 Hz); 7.36 (1H, t, J = 7.5 Hz); 7.26–7.30 (3H, m); 7.14 (2H, d, J = 7.5 Hz); 5.61 (2H, s); 3.50–3.54 (2H, m); 2.68–2.71 (2H, m); 1.38–1.49 (8H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.1, 141.9, 140.9, 137.9, 137.7, 131.8, 129.5, 129.3, 128.8, 128.2, 127.4, 123.0, 121.5, 121.1, 114.5, 111.4, 46.9, 41.8, 33.2, 30.3, 27.2, 27.0.

*5.2.7. Preparation of N-(2-hydroxylethyl)-9-(4-fluorobenzyl)-β-carboline-3-carboxamide (**19**)*

A solution of ethyl 9-(4-fluorobenzyl)-β-carboline-3-carboxylate **9** (3.48 g, 10 mmol) in ethanol (100 mL) containing ethanolamine (40 mmol) and 60% NaH (0.8 g, 20 mmol) was refluxed for 30 min. After completion of the reaction, as indicated by TLC, the solution was cooled and poured into ice-water (200 mL), and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil obtained was purified by silica

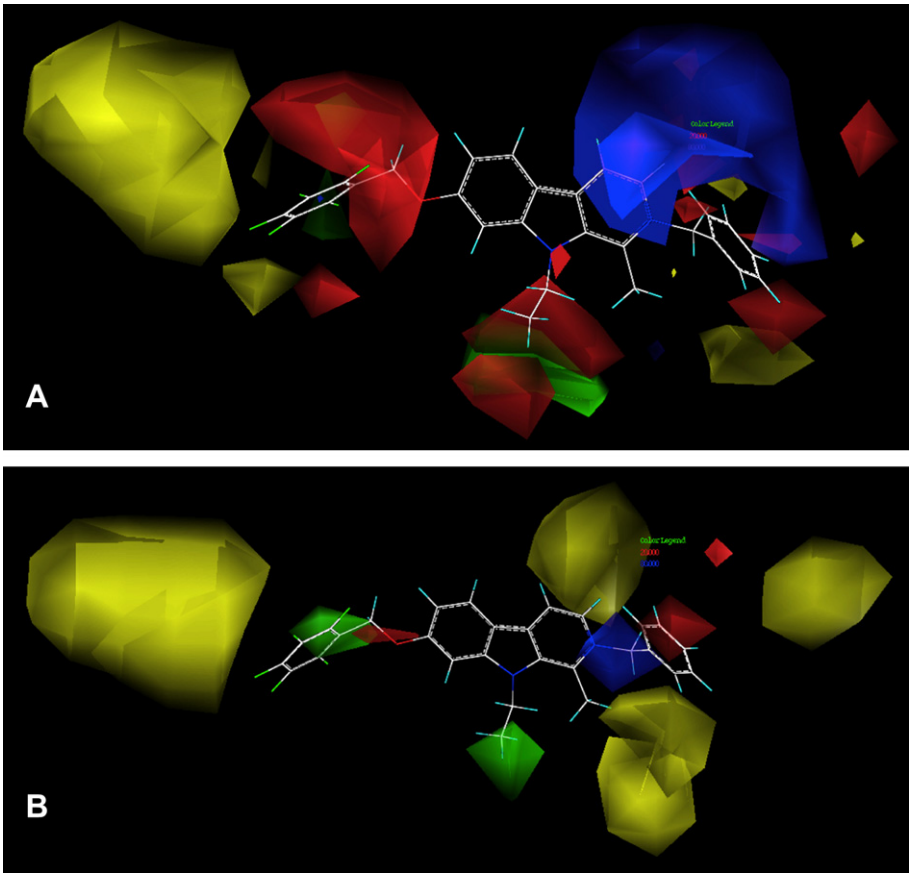


Fig. 4. Contour coefficient map of (A) CoMFA and (B) CoMSIA. Sterically favored regions are shown in green while certain sterically unfavorable regions are shown in yellow; the region favoring a positive charge are shown in red as do regions favoring a negative charge are shown in blue. The template compound **51** is shown embedded into the final field as a representative example. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

column chromatography with ethyl acetate as the eluent, and crystallized from ethyl acetate to give white crystals (2.94 g, 81%), mp 188–189 °C. FAB-MS *m*/*z* (M + 1) 364. IR (KBr, cm^{-1}): 3378, 3066, 2951, 2917, 2867, 1647, 1626, 1590, 1534, 1497, 1466, 1423, 1331, 1217, 1075, 825, 746. ^1H NMR (500 MHz, $CDCl_3$) δ 8.98 (1H, s); 8.66 (1H, s); 8.23 (1H, d, J = 8.0 Hz); 7.59–7.62 (1H, t, J = 8.5 Hz); 7.44 (1H, d, J = 8.5 Hz); 7.36 (1H, t, J = 8.5 Hz); 7.25 (1H, s); 6.95–7.12 (4H, m); 5.57 (2H, s); 3.88 (2H, t, J = 6.5 Hz); 3.68–3.71 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.8, 161.7, 160.5, 143.8, 137.0, 134.7, 133.2, 132.0, 129.8, 128.4, 123.7, 122.7, 120.9, 116.3, 116.1, 112.3, 60.1, 46.9, 43.2. Anal. Calcd for $C_{21}H_{18}FN_3O_2$: C, 69.41; H, 4.99; N, 11.56. Found: C, 69.28; H, 5.02; N, 11.59.

5.2.8. Preparation of N-(2-hydroxylethyl)-9-phenylpropyl-β-carboline-3-carboxamide (**20**)

Compound **20** was prepared by the same procedure as compound **19** from ethyl 9-phenylpropyl-β-carboline-3-carboxylate **10** (3.58 g, 10 mmol) to give white crystals (2.95 g, 79%), mp 126–127 °C. FAB-MS *m*/*z* (M + 1) 374. IR (KBr, cm^{-1}): 2884, 1659, 1624, 1517, 1474, 1389, 1235, 1131, 1037, 1011, 834, 748. ^1H NMR (500 MHz, $CDCl_3$) δ 8.90 (1H, s); 8.63 (1H, s); 8.19 (1H, d, J = 9.5 Hz); 7.58–7.62 (1H, m); 7.14–7.40 (8H, m); 4.40 (2H, t, J = 7.5 Hz); 3.88–3.91 (2H, m); 3.69–3.73 (2H, m); 2.70–2.74 (2H, m); 2.23–2.31 (2H, m). ^{13}C NMR (75 MHz, CDCl3) δ: 166.8, 141.5, 140.5, 139.8, 137.6, 130.0, 129.0, 128.9, 128.8, 128.4, 126.5, 122.4, 121.7, 120.6, 114.6, 109.8, 62.9, 43.1, 33.4, 30.5. Anal. Calcd for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25. Found: C, 73.90; H, 6.23; N, 11.30.

5.2.9. Preparation of 3-hydroxymethyl-9-n-butyl-1-methyl-β-carboline (**21**)

A fine suspension of ethyl 9-*n*-butyl-1-methyl-β-carboline-3-carboxylate **6** (3.1 g, 10 mmol) in dry THF (100 mL) was treated with $LiBH_4$ (30 mmol), and the mixture was stirred at room temperature for 9 h. The reaction was cooled, treated with 10% aq. HCl (20 mL), and stirred for 4 h. The reaction mixture was neutralized with 10% aq NaOH solution and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white crystals (2.1 g, 78%) were obtained. mp 115–116 °C. FAB-MS *m*/*z* (M + 1) 269. IR (KBr, cm^{-1}) 3153, 2967, 2918, 2838, 1619, 1557, 1477, 1362, 1278, 1245, 1208, 1075, 988, 871, 741. ^1H NMR (500 MHz, $CDCl_3$) δ 8.79–8.81 (1H, m); 8.52 (1H, s); 8.28–8.32 (1H, m); 8.14 (1H, d, J = 10.5 Hz); 7.96–8.00 (2H, m); 5.61 (2H, s); 5.17 (2H, t, J = 8.0 Hz); 3.77 (3H, s); 2.46–2.54 (2H, m); 2.08–2.18 (2H, m); 1.67 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 150.3, 142.1, 140.6, 134.0, 129.7, 128.6, 122.0, 121.3, 120.0, 110.9, 65.0, 44.6, 33.3, 23.7, 20.3, 14.5. Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.09; H, 7.51; N, 10.44. Found: C, 75.95; H, 7.54; N, 10.47.

5.2.10. Preparation of 3-hydroxylmethyl-9-(4-fluorobenzyl)-β-carboline (**22**)

Compound **22** was prepared by the same procedure as compound **21** from ethyl 9-(4-fluorobenzyl)-β-carboline-3-

carboxylate **9** (3.48 g, 10 mmol). White solid (1.8 g, 59%) was obtained, mp 177–178 °C. FAB-MS *m/z* (M + 1) 307. IR (KBr, cm^{-1}): 3307, 3068, 2934, 2867, 2682, 1625, 1602, 1555, 1506, 1465, 1366, 1333, 1261, 1221, 1153, 1097, 1021, 745. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (1H, s); 8.19 (1H, d, J = 8.5 Hz); 8.14 (1H, s); 7.65–7.67 (1H, m); 7.55 (1H, d, J = 9.5 Hz); 7.39–7.41 (1H, m); 6.93–7.27 (4H, m); 5.63 (2H, s); 5.02 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.6, 160.4, 151.8, 141.7, 135.8, 134.3, 132.1, 129.5, 129.0, 122.6, 121.4, 120.3, 116.2, 111.8, 110.9, 65.4, 46.0. Anal. Calcd for $C_{19}H_{15}FN_2O$: C, 74.50; H, 4.94; N, 9.14. Found: C, 74.68; H, 4.96; N, 9.18.

5.2.11. Preparation of 9-n-butyl-1-methyl-β-carboline-3-carboxaldehyde (**23**)

To a solution of compound **21** (5 mmol) in CH_3CN (300 mL) was added activated MnO_2 (20 mmol). The suspension was refluxed for 2 h and then cooled and filtered through Celite. The filtrate was passed through silica gel and washed with ethyl acetate, and the solvent was removed under reduced pressure. The residue was crystallized from acetone-petroleum ether to afford white crystals (0.95 g, 71%), mp 103–104 °C. FAB-MS *m/z* (M + 1) 267. IR (KBr, cm^{-1}): 3342, 3059, 3019, 2958, 2930, 2869, 1679, 1619, 1570, 1456, 1370, 1337, 1297, 1248, 1196, 1112, 736. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.45 (1H, s); 8.67 (1H, s); 8.21 (1H, d, J = 10.0 Hz); 7.40–7.73 (3H, m); 4.63 (2H, t, J = 8.0 Hz); 3.27 (3H, s); 1.87–1.93 (2H, m); 1.46–1.52 (2H, m); 1.01 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, CDCl3) δ: 193.0, 143.0, 142.2, 142.1, 137.1, 129.4, 128.6, 122.4, 121.5, 121.3, 113.8, 111.4, 44.9, 33.3, 23.9, 20.2, 14.4. Anal. Calcd for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.75; H, 6.84; N, 10.56.

5.2.12. Preparation of 9-(4-fluorobenzyl)-β-carboline-3-carboxaldehyde (**24**)

Compound **24** was prepared by the same procedure as compound **23** from compound **22** (3.06 g, 10 mmol). White solid (2.4 g, 79%) was obtained, mp 183–184 °C. FAB-MS *m/z* (M + 1) 305. IR (KBr, cm^{-1}): 3043, 2937, 2843, 2735, 1697, 1620, 1579, 1508, 1464, 1358, 1333, 1305, 1263, 1216, 1172, 848, 743. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.55 (1H, s); 9.11 (1H, s); 8.90 (1H, s); 8.33 (1H, d, J = 9.5 Hz); 7.76–7.80 (1H, m); 7.60 (1H, d, J = 9.5 Hz); 7.51–7.55 (1H, m); 7.03–7.16 (4H, m); 5.73 (2H, s). ^{13}C NMR (75 MHz, CDCl3) δ: 193.0, 144.6, 141.9, 138.7, 132.3, 131.6, 129.6, 128.4, 122.5, 122.0, 121.6, 116.4, 114.8, 110.4, 110.0, 46.9. Anal. Calcd for $C_{19}H_{13}FN_2O$: C, 74.99; H, 4.31; N, 9.21. Found: C, 75.10; H, 4.34; N, 9.25.

5.3. General procedure for the preparation of β-carbolinium derivatives (**29**–**35**)

A mixture of β-carboline derivatives **25**–**28** (2 mmol) and halogenated alkane (10 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 to afford yellow solid. The solid was crystallized from ethanol.

5.3.1. 2-Benzyl-1-methyl-β-carbolinium bromide (**29**)

Yellow crystals (0.46 g, 65%) were obtained, mp >270 °C. FAB-MS *m/z* 273. IR (KBr, cm^{-1}): 3420, 1750–3250, 1634, 1575, 1525, 1500, 1452, 1331, 1299, 1258, 1229, 1146, 740. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.94 (1H, s); 8.80 (1H, d, J = 7.0 Hz); 8.73 (1H, d, J = 6.5 Hz); 8.48 (1H, d, J = 7.0 Hz); 7.79–7.80 (2H, m); 7.25–7.47 (6H, m); 6.06 (2H, s); 3.06 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 144.2, 141.2, 135.8, 135.0, 132.5, 131.8, 129.8, 129.2, 127.7, 124.2, 122.3, 120.3, 116.6, 113.4, 60.2, 16.5. Anal. Calcd for $C_{19}H_{17}BrN_2$: C, 64.60; H, 4.85; N, 7.93. Found: C, 64.49; H, 4.88; N, 7.96.

5.3.2. 2-(3-Phenylpropyl)-1-methyl-β-carbolinium bromide (**30**)

Yellow crystals (0.58 g, 76%) were obtained, mp >270 °C. FAB-MS *m/z* 301. IR (KBr, cm^{-1}): 3250–3600, 2250–3250, 1634, 1574, 1522, 1499, 1453, 1330, 1229, 821,754. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (1H, s); 8.68 (1H, d, J = 6.5 Hz); 8.64 (1H, d, J = 6.5 Hz); 8.45 (1H, d, J = 8.0 Hz); 7.76–7.81 (2H, m); 7.42–7.46 (2H, m); 7.17–7.31 (5H, m); 4.73 (2H, t, J = 7.5 Hz); 3.10 (3H, s); 2.76 (2H, t, J = 7.5 Hz); 2.22–2.28 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 143.7, 141.1, 140.7, 135.3, 134.5, 132.1, 131.0, 129.0, 128.9, 126.7, 124.0, 122.1, 120.1, 116.3, 113.2, 56.9, 32.5, 32.3, 16.1. Anal. Calcd for $C_{21}H_{21}BrN_2$: C, 66.15; H, 5.55; N, 7.35. Found: C, 66.08; H, 5.58; N, 7.37.

5.3.3. 3-Ethoxycarbonyl-2-benzyl-1-methyl-β-carbolinium bromide (**31**)

Yellow crystals (0.42 g, 49%) were obtained, mp >270 °C. FAB-MS *m/z* 345. IR (KBr, cm^{-1}): 3421, 2250–3250, 1721, 1627, 1579, 1514, 1452, 1367, 1334, 1261, 1136, 1105, 1020, 735. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.37 (1H, s); 9.26 (1H, s); 8.61 (1H, d, J = 8.0 Hz); 7.87–7.88 (2H, m); 7.52–7.55 (1H, m); 7.33–7.39 (3H, m); 7.10–7.12 (2H, m); 6.21 (2H, s); 4.33–4.38 (2H, m); 3.14 (3H, s); 1.21 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.1, 144.8, 143.2, 142.3, 135.6, 135.2, 132.8, 131.9, 130.1, 129.4, 126.9, 124.3, 122.6, 120.6, 118.1, 113.6, 64.1, 57.6, 17.5, 14.4. Anal. Calcd for $C_{22}H_{21}BrN_2O_2$: C, 62.13; H, 4.98; N, 6.59. Found: C, 62.20; H, 5.01; N, 6.62.

5.3.4. 7-Methoxy-2-benzyl-1-methyl-β-carbolinium bromide (**32**)

Yellow crystals (0.64 g, 84%) were obtained, mp >270 °C. FAB-MS *m/z* 303. IR (KBr, cm^{-1}): 1750–3250, 1632, 1505, 1457, 1336, 1286, 1225, 1022, 830, 733; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.79 (1H, s); 8.73 (1H, d, J = 6.5 Hz); 8.57 (1H, d, J = 8.0 Hz); 8.32–8.37 (1H, m); 7.37–7.44 (3H, m); 7.22–7.24 (2H, m); 7.12–7.13 (1H, m); 7.06–7.09 (1H, m); 5.99 (2H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.5, 146.4, 139.4, 135.6, 135.1, 132.0, 129.8, 129.1, 127.7, 125.3, 115.2, 114.1, 113.5, 94.9, 59.8, 56.6, 16.3. Anal. Calcd for $C_{20}H_{19}BrN_2O$: C, 62.67; H, 5.00; N, 7.31. Found: C, 62.78; H, 5.02; N, 7.34.

5.3.5. 2-n-Butyl-β-carbolinium bromide (**33**)

Yellow crystals (0.46 g, 75%) were obtained, mp >270 °C. FAB-MS *m/z* 225. IR (KBr, cm^{-1}): 3404, 1750–3250, 1643, 1573, 1518, 1457, 1334, 1255, 1148, 1118, 869. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.86 (1H, s); 9.52 (1H, s); 8.85 (1H, d, J = 6.5 Hz); 8.77 (1H, d, J = 6.5 Hz); 8.51 (1H, d, J = 8.0 Hz); 7.79–7.84 (2H, m); 7.45–7.48 (1H, m); 4.78 (2H, t, J = 8.5 Hz); 1.97–2.03 (2H, m); 1.32–1.39 (2H, m); 0.94 (2H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 144.2, 135.1, 133.0, 132.6, 132.5, 129.9, 124.1, 122.1, 119.6, 118.5, 113.5, 60.9, 34.0, 19.7, 14.3. Anal. Calcd for $C_{15}H_{17}BrN_2$: C, 59.03; H, 5.61; N, 9.18. Found: C, 59.18; H, 5.63; N, 9.14.

5.3.6. 2-Benzyl-β-carbolinium bromide (**34**)

Yellow crystals (0.52 g, 77%) were obtained, mp >270 °C. FAB-MS *m/z* 259. IR (KBr, cm^{-1}): 3395, 1750–3250, 1642, 1520, 1498, 1337, 1258, 1209, 757, 732. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.86 (1H, s); 9.62 (1H, d, J = 7.0 Hz); 8.83–8.87 (2H, m); 8.50 (1H, d, J = 5.5 Hz); 7.79–7.84 (2H, m); 7.43–7.56 (6H, m); 6.00 (2H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 144.3, 135.9, 135.2, 133.1, 132.8, 132.6, 129.9, 129.7, 129.2, 124.1, 122.1, 119.5, 118.8, 113.4, 63.4. Anal. Calcd for $C_{18}H_{15}BrN_2$: C, 63.73; H, 4.46; N, 8.26. Found: C, 63.68; H, 4.48; N, 8.29.

5.3.7. 2-(3-Phenylpropyl)-β-carbolinium bromide (**35**)

Yellow crystals (0.61 g, 83%) were obtained, mp >270 °C. FAB-MS *m/z* 287. IR (KBr, cm^{-1}): 3499, 3431, 1750–3250, 1643, 1517, 1496, 1339, 1260, 1137, 826, 754. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.85 (1H, s); 9.51 (1H, s); 8.82 (1H, d, J = 6.5 Hz); 8.77 (1H, d, J = 6.5 Hz); 8.50 (1H, d, J = 8.0 Hz); 7.77–7.83 (2H, m); 7.43–7.46 (1H,

m); 7.12–7.26 (5H, m); 4.82 (2H, t, $J = 8.0$ Hz); 2.68 (2H, t, $J = 8.0$ Hz); 2.31–2.37 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 144.2, 141.0, 135.0, 133.0, 132.5, 132.4, 129.9, 128.9, 128.8, 126.5, 124.1, 122.0, 119.6, 118.4, 113.4, 60.1, 33.6, 32.4. Anal. Calcd for $C_{20}H_{19}BrN_2$: C, 65.40; H, 5.21; N, 7.63. Found: C, 65.35; H, 5.23; N, 7.66.

*5.3.8. Preparation of 7-methoxy-9-isobutyl-1-methyl-β-carboline (**38**)*

A mixture of 7-methoxy-1-methyl-β-carboline **29** (2.12 g, 10 mmol) and anhydrous DMF (50 mL) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and isobutyl bromide (6 mL, 50 mmol) were added. The mixture was continued to stir at RT. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (150 mL), and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil was crystallized from ethyl ether to afford white crystal (2.2 g, 82%), mp 112–113 °C. FAB-MS m/z (M + 1) 269. IR (KBr, cm^{-1}): 2961, 1621, 1564, 1496, 1446, 1403, 1338, 1254, 1207, 1139, 1044, 966, 815. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s); 7.95 (1H, d, $J = 8.5$ Hz); 7.73 (1H, d, $J = 5.0$ Hz); 6.85–6.88 (2H, m); 4.26 (2H, d, $J = 7.5$ Hz); 3.93 (3H, s); 3.01 (3H, s); 2.23–2.28 (1H, m); 0.92 (6H, d, $J = 5.5$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 160.9, 144.0, 141.1, 138.2, 135.4, 129.2, 122.9, 114.7, 112.8, 109.7, 95.0, 56.3, 51.4, 31.2, 23.9, 20.4. Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.09; H, 7.51; N, 10.44. Found: C, 76.27; H, 7.54; N, 10.39.

*5.4. General procedure for the preparation of 7-hydroxyl substitued β-carbolines **40**–**43***

A mixture of 7-methoxy substituted β-carboline derivatives (10 mmol), acetic acid (100 mL) and 40% hydrobromic acid (50 mL) was refluxed for 8–12 h. After completion of the reaction as indicated by TLC, the mixture was cooled and poured onto ice. The aqueous mixture, made basic with sodium hydroxide, yielded a precipitate that was filtered and dried. The solid was crystallized from anhydrous ethanol.

*5.4.1. 9-Ethyl-1-methyl-β-carboline7-ol (**40**)*

Afforded white crystals (1.8 g, 80%), mp 258–260 °C. FAB-MS m/z (M + 1) 227. IR (KBr, cm^{-1}): 3500–1750, 1627, 1568, 1451, 1410, 1347, 1315, 1260, 1215, 1092, 983, 821. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.74 (1H, s); 8.12 (1H, d, $J = 8.0$ Hz); 7.96 (1H, d, $J = 7.5$ Hz); 7.78 (1H, d, $J = 8.0$ Hz); 6.91–6.92 (1H, m); 6.73–6.75 (1H, m); 4.48–4.52 (2H, m); 2.92 (3H, s); 1.32 (3H, t, $J = 8.0$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.0, 146.2, 136.7, 133.6, 133.3, 129.5, 125.1, 114.3, 113.3, 112.7, 95.5, 18.5, 16.1. Anal. Calcd for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. Found: C, 74.38; H, 6.26; N, 12.43.

*5.4.2. 9-n-Butyl-1-methyl-β-carboline7-ol (**41**)*

Afforded white crystals (2.2 g, 87%), mp 205–206 °C. FAB-MS m/z (M + 1) 255. IR (KBr, cm^{-1}): 3500–1750, 1618, 1566, 1492, 1451, 1413, 1325, 1238, 1187, 1137, 1112, 980. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.72 (1H, s); 8.12 (1H, d, $J = 5.0$ Hz); 7.95 (1H, d, $J = 8.5$ Hz); 7.78 (1H, d, $J = 5.0$ Hz); 6.90–6.91 (1H, m); 6.72–6.75 (1H, m); 4.43 (2H, t, $J = 8.0$ Hz); 2.91 (3H, s); 1.67–1.74 (2H, m); 1.36–1.40 (2H, m); 0.93 (2H, t, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 159.3, 143.9, 140.5, 137.6, 135.0, 129.7, 123.2, 113.9, 112.7, 110.6, 95.9, 44.7, 33.1, 23.3, 20.3, 14.5. Anal. Calcd for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.69; H, 7.16; N, 10.97.

*5.4.3. 9-Isobutyl-1-methyl-β-carboline-7-ol (**42**)*

Afforded white crystals (2.0 g, 79%), mp 247–248 °C. FAB-MS m/z (M + 1) 255. IR (KBr, cm^{-1}): 3500–1750, 1626, 1568, 1447, 1392, 1203, 1136, 977, 820. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (1H, s); 8.13 (1H, d, $J = 5.0$ Hz); 7.95 (1H, d, $J = 8.5$ Hz); 7.79 (1H, d, $J = 5.0$ Hz); 6.93 (1H, s); 6.72 (1H, d, $J = 8.5$ Hz), 4.26 (2H, d, $J = 7.5$ Hz); 2.90 (3H, s); 2.11–2.17 (1H, m); 0.86 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 158.9, 144.2, 140.8, 138.2, 135.4, 129.5, 123.0, 113.8, 112.6, 110.4, 96.6, 51.6, 30.9, 23.9, 20.5. Anal. Calcd for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.38; H, 7.15; N, 11.04.

*5.4.4. 1-Methyl-9-(3-phenylpropyl)-β-carboline-7-ol (**43**)*

Afforded white crystals (2.5 g, 80%), mp 258–259 °C. FAB-MS m/z (M + 1) 317. IR (KBr, cm^{-1}): 3500–1750, 1613, 1567, 1492, 1452, 1412, 1352, 1246, 1194, 1160, 978, 823,746. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.74 (1H, s); 8.11 (1H, d, $J = 5.0$ Hz); 7.95 (1H, d, $J = 8.5$ Hz); 7.77 (1H, d, $J = 5.0$ Hz); 7.17–7.30 (5H, m); 6.87–6.88 (1H, m); 6.74–6.76 (1H, m); 4.45 (2H, t, $J = 7.0$ Hz); 2.77 (3H, s); 2.72 (2H, t, $J = 7.0$ Hz); 1.98–2.05 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 175.9, 159.1, 143.7, 141.6, 140.7, 138.0, 135.0, 129.5, 129.0, 126.6, 123.2, 113.9, 112.7, 110.5, 95.8, 44.4, 33.0, 32.5, 23.4. Anal. Calcd for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.68; H, 6.40; N, 8.87.

*5.5. General procedure for the preparation of 7-alkoxyl substituted β-carbolines **44**–**55***

A mixture of 7-hydroxyl-1-methyl-β-carboline derivatives (2 mmol) and anhydrous DMF (50 mL) was stirred at room temperature until clear, and then 60% NaH (3 mmol) and halogenated alkane (4 mmol) were added. The mixture was stirred at room temperature for 0.5–2 h. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (150 mL), and extracted with ethyl acetate. The organic phase was made acidic with concentrated hydrochloric acid. Upon removal of solvent, the residue was crystallized from acetone to afford yellow solid. The solid was dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil was crystallized from ethyl ether or ethyl ether–petroleum ether.

*5.5.1. 7-Ethoxy-9-ethyl-1-methyl-β-carboline (**44**)*

White crystals (0.39 g, 76%) were obtained, mp 117–118 °C. FAB-MS m/z (M + 1) 255. IR (KBr, cm^{-1}): 2978, 2931, 1620, 1560, 1446, 1407, 1342, 1208, 1139, 1048, 972, 817. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, $J = 5.5$ Hz); 7.96 (1H, d, $J = 8.5$ Hz); 7.74 (1H, d, $J = 5.5$ Hz); 6.87–6.90 (2H, m); 4.52–4.56 (2H, m); 4.16–4.20 (2H, m); 3.05 (3H, s); 1.43–1.51 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.3, 142.7, 140.6, 138.3, 135.2, 129.5, 122.5, 115.3, 112.4, 109.2, 93.9, 64.2, 39.7, 23.7, 15.9, 15.3. Anal. Calcd for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.54; H, 7.15; N, 11.06.

*5.5.2. 7-(Perfluorobenzyloxy)-9-ethyl-1-methyl-β-carboline (**45**)*

White crystals (0.63 g, 78%) were obtained, mp 205–206 °C. FAB-MS m/z (M + 1) 407. IR (KBr, cm^{-1}): 3063, 2977, 1620, 1565, 1507, 1442, 1408, 1350, 1208, 1139, 1060, 938, 815. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, d, $J = 5.0$ Hz); 8.01 (1H, d, $J = 8.5$ Hz); 7.55 (1H, d, $J = 5.0$ Hz); 6.93–6.99 (2H, m); 5.29 (2H, s); 4.55–4.59 (2H, m); 3.05 (3H, s); 1.46 (3H, t, $J = 7.5$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 153.7, 141.5, 139.5, 137.1, 135.5, 133.1, 131.3, 130.0, 123.8, 117.3, 111.0, 107.0, 104.8, 103.7, 89.5, 52.7, 34.3, 17.9, 10.1. Anal. Calcd for $C_{21}H_{15}F_5N_2O$: C, 62.07; H, 3.72; N, 6.89. Found: C, 61.98; H, 3.73; N, 6.91.

*5.5.3. 7-Ethoxy-9-n-butyl-1-methyl-β-carboline (**46**)*

White crystals (0.42 g, 75%) were obtained, mp 93–94 °C; FAB-MS m/z (M + 1) 283. IR (KBr, cm^{-1}): 3052, 2961, 2928, 2869, 1622, 1562, 1450, 1410,1360, 1255, 1193, 1139, 1048, 948, 809. ^{1}H NMR

(500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.0 Hz); 7.96 (1H, d, J = 8.5 Hz); 7.75 (1H, d, J = 5.0 Hz); 6.86–6.91 (2H, m); 4.45 (2H, t, J = 8.0 Hz); 4.16–4.20 (2H, m); 3.05 (3H, s); 1.78–1.84 (2H, m); 1.42–1.51 (5H, m); 0.98 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.2, 143.1, 140.6, 138.3, 135.4, 129.4, 122.4, 115.2, 112.4, 109.0, 94.2, 64.1, 44.9, 33.1, 23.8, 20.6, 15.3, 14.3. Anal. Calcd for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.70; H, 7.88; N, 9.90.

*5.5.4. 7-Isopropyloxy-9-n-butyl-1-methyl-β-carboline (**47**)*

Yellow oil (0.41 g, 72%) was obtained, FAB-MS m/z (M + 1) 297. IR (KBr, cm^{-1}): 3422, 2970, 2930, 1626, 1267, 1447, 1409, 1376, 1243, 1191, 1135, 1113, 986, 816. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.5 Hz); 7.95 (1H, d, J = 9.0 Hz); 7.73 (1H, d, J = 5.5 Hz); 6.87–6.88 (2H, m); 4.68–4.73 (1H, m); 4.44 (2H, t, J = 8.0 Hz); 3.04 (3H, s); 1.78–1.84 (2H, m); 1.37–1.47 (8H, m); 0.98 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.1, 143.3, 140.6, 138.2, 135.5, 129.5, 122.5, 115.4, 112.4, 110.1, 96.3, 70.8, 45.0, 33.1, 23.7, 22.5, 20.6, 14.3.

*5.5.5. 7-n-Butyloxy-9-n-butyl-1-methyl-β-carboline (**48**)*

White crystals (0.52 g, 84%) were obtained, mp 67–68 °C; FAB-MS m/z (M + 1) 311. IR (KBr, cm^{-1}): 3054, 2958, 2871, 1622, 1563, 1451, 1410, 1246, 198, 1141, 1044, 1004, 802. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.5 Hz); 7.96 (1H, d, J = 8.5 Hz); 7.76 (1H, d, J = 5.0 Hz); 6.86–6.91 (2H, m); 4.45 (2H, t, J = 7.5 Hz); 4.10 (2H, t, J = 8.0 Hz); 3.06 (3H, s, C***H***$_3$); 1.79–1.88 (4H, m); 1.52–1.60 (2H, m); 1.41–1.47 (2H, m); 0.97–1.03 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.5, 143.2, 140.7, 138.3, 135.5, 129.5, 122.4, 115.3, 112.4, 109.2, 94.4, 68.5, 45.0, 33.1, 31.8, 23.7, 20.6, 19.7, 14.3. Anal. Calcd for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.02. Found: C, 77.33; H, 8.46; N, 9.03.

*5.5.6. 7-Decyloxy-9-n-butyl-1-methyl-β-carboline (**49**)*

White crystals (0.65 g, 81%) were obtained, mp 68–69 °C. FAB-MS m/z (M + 1) 395. IR (KBr, cm^{-1}): 3427, 2925, 2852, 1620, 1462, 1338, 1231, 1194, 1138, 1039, 820. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.0 Hz); 7.97 (1H, d, J = 8.5 Hz); 7.78 (1H, d, J = 5.0 Hz); 6.86–6.92 (2H, m); 4.45–4.48 (2H, t, J = 8.0 Hz); 4.08–4.11 (2H, t, J = 8.0 Hz); 3.09 (3H, s); 1.78–1.89 (4H, m); 1.25–1.55 (16H, m); 0.98 (3H, t, J = 7.5 Hz); 0.83 (3H, t, J = 8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.4, 143.2, 140.6, 138.2, 135.4, 129.5, 122.4, 115.2, 112.4, 109.1, 94.2, 68.7, 44.9, 33.1, 32.3, 30.0, 29.9, 29.8, 26.5, 23.8, 23.1, 20.6, 14.6, 14.3. Anal. Calcd for $C_{26}H_{38}N_2O$: C, 79.14; H, 9.71; N, 7.10. Found: C, 79.24; H, 9.73; N, 7.08.

*5.5.7. 7-n-Butyloxy-9-isobutyl-1-methyl-β-carboline (**50**)*

Yellow oil (0.48 g, 78%) was obtained. FAB-MS m/z (M + 1) 311. IR (KBr, cm^{-1}): 2956, 2868, 2485, 1625, 1574, 1468, 1432, 1336, 1255, 1204, 1141, 1043, 804. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.5 Hz); 7.97 (1H, d, J = 9.5 Hz); 7.79 (1H, d, J = 5.5 Hz); 6.87–6.91 (2H, m); 4.28 (2H, t, J = 8.5 Hz); 4.10 (2H, d, J = 8.0 Hz); 3.07 (3H, s); 2.24–2.27 (1H, m); 1.82–1.88 (2H, m); 1.54–1.58 (2H, m); 1.01 (3H, t, J = 7.5 Hz); 0.93–0.95 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.3, 143.7, 140.7, 138.3, 135.7, 129.6, 122.3, 115.1, 112.3, 109.2, 95.1, 68.4, 52.1, 31.8, 30.9, 24.0, 20.6, 19.7, 14.3.

*5.5.8. 7-Benzyloxy-9-isobutyl-1-methyl-β-carboline (**51**)*

White crystals (0.59 g, 86%) were obtained, mp 110–112 °C. FAB-MS m/z (M + 1) 345. IR: 3432, 2959, 2928, 2871, 1618, 1568, 1448, 1254, 1196, 1136, 1004, 731. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.5 Hz); 7.96 (1H, d, J = 8.5 Hz); 7.74 (1H, d, J = 5.5 Hz); 7.30–7.49 (5H, m); 6.95–6.97 (1H, m); 6.92–6.95 (1H, m); 5.20 (2H, s); 4.23 (2H, d, J = 8.0 Hz); 3.01 (3H, s); 2.18–2.21 (1H, m), 0.87 (6H, d, J = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.3, 147.1, 137.3, 137.0, 133.9, 133.6, 129.3, 129.1, 128.6, 124.9, 114.9113.6, 113.4, 96.0, 70.8, 51.6, 31.2, 20.3, 18.4. Anal. Calcd for $C_{23}H_{24}N_2O$: C, 80.20; H, 7.02; N, 8.13. Found: C, 80.12; H, 7.04; N, 8.16.

*5.5.9. 7-Isopropyloxy-1-methyl-9-(3-phenylpropyl)-β-carboline (**52**)*

White crystals (0.54 g, 76%) were obtained, mp 121–122 °C. FAB-MS m/z (M + 1) 359. IR (KBr, cm^{-1}): 2975, 2927, 2858, 1621, 1563, 1494, 1448, 1409, 1374, 1326, 1240, 1158, 1111, 975, 754. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, J = 5.5 Hz); 7.92 (1H, d, J = 8.0 Hz); 7.71 (1H, d, J = 5.5 Hz); 7.19–7.32 (5H, m); 6.84–6.86 (1H, m); 6.68–6.69 (1H, m); 4.59–4.61 (1H, m); 4.43 (2H, t, J = 8.0 Hz); 2.89 (3H, s); 2.74–2.77 (2H, m); 2.12–2.18 (2H, m); 1.38–1.39 (6H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.1, 143.2, 140.9, 140.7, 138.4, 135.5, 129.6, 128.8, 128.6, 126.5, 122.5, 115.4, 112.4, 110.5, 95.9, 70.7, 44.4, 33.4, 32.1, 23.6, 22.5. Anal. Calcd for $C_{24}H_{26}N_2O$: C, 80.41; H, 7.31; N, 7.81. Found: C, 80.37; H, 7.33; N, 7.79.

*5.5.10. 7-Octyloxy-9-(3-phenylpropyl)-1-methyl-β-carboline (**53**)*

White crystals (0.61 g, 71%) were obtained, mp 79–81 °C. FAB-MS m/z (M + 1) 429. IR (KBr, cm^{-1}): 2947, 2920, 2852, 1622, 1564, 1496, 1448, 1412, 1244, 1161, 1039, 745. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, J = 5.5 Hz); 7.93 (1H, d, J = 8.5 Hz); 7.74 (1H, d, J = 5.0 Hz); 7.20–7.33 (5H, m); 6.86–6.89 (1H, m); 6.64–6.65 (1H, m); 4.44 (2H, t, J = 6.0 Hz); 3.97–3.99 (2H, t, J = 7.5 Hz); 2.92 (3H, s); 2.75–2.78 (2H, m); 2.13–2.19 (2H, m); 1.81–1.87 (2H, m); 1.30–1.54 (10H, m); 1.02 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.5, 143.1, 140.9, 140.7, 138.4, 135.4, 129.6, 128.8, 128.6, 126.5, 122.4, 115.2, 112.4, 109.6, 93.9, 68.6, 44.3, 33.3, 32.3, 32.1, 29.8, 29.7, 29.6, 26.6, 23.5, 23.1, 14.6. Anal. Calcd for $C_{29}H_{36}N_2O$: C, 81.27; H, 8.47; N, 6.54. Found: C, 81.30; H, 8.50; N, 6.52.

*5.5.11. 7-Benzyloxy-1-methyl-9-(3-phenylpropyl)-β-carboline (**54**)*

White crystals (0.68 g, 84%) were obtained, mp 139–140 °C. FAB-MS m/z (M + 1) 407. IR (KBr, cm^{-1}): 3060, 3026, 2938, 2863, 1620, 1563, 1495, 1448, 1412, 1385, 1224, 1160, 1001, 742. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, J = 5.5 Hz); 7.94 (1H, d, J = 8.5 Hz); 7.72 (1H, d, J = 5.0 Hz); 7.18–7.48 (10H, m); 6.94–6.96 (1H, m); 6.72–6.73 (1H, m); 5.09 (2H, s); 4.42 (2H, t, J = 6.5 Hz); 2.91(3H, s); 2.71–2.74 (2H, m); 2.09–2.15 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.0, 143.0, 140.9, 140.8, 138.4, 137.0, 135.4, 129.5, 128.9, 128.7, 128.3, 127.8, 126.6, 122.6, 115.5, 112.5, 109.8, 94.6, 70.7, 44.4, 33.3, 32.1, 23.6. Anal. Calcd for $C_{28}H_{26}N_2O$: C, 82.73; H, 6.45; N, 6.89. Found: C, 82.68; H, 6.47; N, 6.91.

*5.5.12. 7-Perfluorobenzyloxy-9-(3-phenylpropyl)-1-methyl-β-carboline (**55**)*

White crystals (0.72 g, 73%) were obtained, mp 149–150 °C. FAB-MS m/z (M + 1) 497. IR (KBr, cm^{-1}): 2934, 1623, 1565, 1505, 1448, 1205, 1162, 1135, 1058, 943, 744. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.0 Hz); 7.98 (1H, d, J = 9.0 Hz); 7.77 (1H, d, J = 5.0 Hz); 7.19–7.33 (5H, m); 6.91–6.93 (1H, m); 6.74–6.75 (1H, m); 5.14 (2H, s); 4.45 (2H, t, J = 8.0 Hz); 2.94 (3H, s); 2.76–2.79 (2H, m); 2.14–2.20 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.1, 147.6, 144.3, 142.8, 140.9, 140.8, 138.5, 135.5, 129.3, 128.8, 126.5, 122.8, 116.2, 112.6, 110.2, 109.5, 94.7, 58.1, 44.4, 33.2, 32.1, 23.5. Anal. Calcd for $C_{28}H_{21}F_5N_2O$: C, 67.74; H, 4.26; N, 5.64. Found: C, 67.82; H, 4.28; N, 5.67.

*5.6. General procedure for the preparation of β-carbolinium derivatives **56**–**60***

A mixture of 7-alkyloxy β-carboline derivatives (2 mmol) and benzyl bromide (10 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 to afford yellow solid. The solid was crystallized from ethanol.

*5.6.1. 7-Ethoxy-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (**56**)*

Yellow crystals (0.72 g, 85%) were obtained, mp 260–261 °C. FAB-MS *m*/*z* 345. IR (KBr, cm^{-1}): 3401, 2976, 2874, 1623, 1451, 1376, 1341, 1259, 1223, 1134, 1041, 826, 735. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (1H, d, *J* = 6.5 Hz, H-3); 8.60 (1H, d, *J* = 6.5 Hz, H-4); 8.37 (1H, d, *J* = 9.0 Hz); 7.36–7.43 (4H, m); 7.19 (2H, d, *J* = 7.0 Hz); 7.08–7.10 (1H, m); 6.04 (2H, s); 4.69–4.74 (2H, m); 4.26–4.30 (2H, m); 3.10 (3H, s); 1.37–1.45 (6H, m). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.1, 147.1, 139.4, 135.6, 134.9, 134.7, 133.2, 129.3, 128.5, 126.8, 124.8, 114.4, 113.5, 112.7, 94.1, 64.4, 59.9, 40.4, 16.2, 15.4, 14.6. Anal. Calcd for $C_{23}H_{25}BrN_2O$: C, 64.94; H, 5.92; N, 6.59. Found: C, 65.78; H, 5.93; N, 6.57.

*5.6.2. 7-(Perfluorobenzyloxy)-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (**57**)*

Yellow crystals (0.87 g, 76%) were obtained, mp 196–197 °C. FAB-MS *m*/*z* 497. IR (KBr, cm^{-1}): 3411, 2988, 1624, 1579, 1504, 1454, 1217, 1136, 1057, 973, 942, 828, 732. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (1H, d, *J* = 6.5 Hz); 8.66 (1H, d, *J* = 6.5 Hz); 8.43 (1H, d, *J* = 9.0 Hz); 7.63 (1H, m); 7.36–7.44 (3H, m); 7.14–7.22 (3H, m); 6.07 (2H, s); 5.48 (2H, s); 4.75–4.76 (2H, m); 3.13 (3H, s); 1.43 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.7, 146.5, 146.1, 144.2, 139.6, 138.0, 136.0, 134.7, 134.6, 132.8, 129.1, 128.3, 126.6, 124.8, 114.6, 113.3, 113.0, 109.7, 94.8, 59.8, 58.2, 40.4, 16.1, 15.2. Anal. Calcd for $C_{28}H_{22}BrF_5N_2O$: C, 58.25; H, 3.84; N, 4.85. Found: C, 58.18; H, 3.83; N, 4.87.

*5.6.3. 7-n-Butoxy-2-benzyl-9-isobutyl-1-methyl-β-carbolinium bromide (**58**)*

Yellow crystals (0.81 g, 84%) were obtained, mp 246–248 °C. FAB-MS *m*/*z* 401. IR (KBr, cm^{-1}): 3410, 2957, 2927, 2867, 1620, 1576, 1457, 1375, 1253, 1209, 1138, 1030, 828. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (1H, d, *J* = 6.5 Hz); 8.64 (1H, d, *J* = 6.5 Hz); 8.37(1H, d, *J* = 9.0 Hz); 7.35–7.44 (4H, m); 7.17 (2H, d, *J* = 7.0 Hz); 7.08–7.10 (1H, m); 6.04 (2H, s); 4.53 (2H, d, *J* = 7.5 Hz); 4.21 (2H, t, *J* = 7.5 Hz); 3.06 (3H, s); 2.04–2.09 (1H, m); 1.77–1.82 (2H, m); 1.47–1.55 (2H, m); 0.97 (3H, t, *J* = 7.5 Hz); 0.83 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.4, 148.7, 140.0, 136.3, 135.3, 135.2, 133.9, 129.7, 128.9, 127.1, 125.0, 114.8, 113.9, 112.8, 95.5, 68.7, 60.3, 51.9, 31.1, 30.8, 19.9, 19.2, 16.7, 14.2. Anal. Calcd for $C_{27}H_{33}BrN_2O$: C, 67.35; H, 6.91; N, 5.82. Found: C, 67.48; H, 6.93; N, 5.85.

*5.6.4. 7-Benzyloxy-2-benzyl-9-isobutyl-1-methyl-β-carbolinium bromide (**59**)*

Yellow crystals (0.86 g, 84%) were obtained, mp 218–220 °C. FAB-MS *m*/*z* 435. IR (KBr, cm^{-1}): 3408, 2959, 2921, 1622, 1581, 1454, 1351, 1253, 1222, 1137, 1029, 817, 743. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.5 Hz); 8.64 (1H, d, *J* = 6.5 Hz); 8.40 (1H, d, *J* = 8.5 Hz); 7.34–7.54 (9H, m); 7.17–7.19 (3H, m); 6.04 (2H, s); 5.35 (2H, s); 4.51 (2H, d, *J* = 7.5 Hz); 3.06 (3H, s); 2.02–2.05 (1H, m); 0.79 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.9, 148.5, 140.2, 137.0, 136.4, 135.4, 135.3, 134.0, 129.8, 129.2, 128.6, 127.2, 125.2, 115.1, 114.2, 113.2, 96.4, 70.8, 60.6, 52.2, 31.1, 20.2, 17.2. Anal. Calcd for $C_{30}H_{31}BrN_2O$: C, 69.90; H, 6.06; N, 5.43. Found: C, 69.82; H, 6.08; N, 5.46.

*5.6.5. 7-Octyloxy-9-(3-phenylpropyl)-2-benzyl-1-methyl-β-carbolinium bromide (**60**)*

Yellow crystals (0.9 g, 75%) were obtained, mp 185–186 °C. FAB-MS *m*/*z* 519. IR (KBr, cm^{-1}): 3421, 2993, 2926, 2856, 1620, 1579, 1453, 1371, 1248, 1155, 1134, 1031, 827, 727. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (1H, d, *J* = 6.5 Hz); 8.60 (1H, d, *J* = 6.5 Hz); 8.36 (1H, d, *J* = 9.0 Hz); 7.07–7.43 (12H, m); 6.04 (2H, s); 4.66 (2H, t, *J* = 8.0 Hz); 4.15 (2H, t, *J* = 8.0 Hz); 2.97 (3H, s); 2.68–2.71 (2H, m); 2.04–2.11 (2H, m); 1.78–1.84 (2H, m); 1.28–1.51 (10H, m); 0.86 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.5, 147.9, 141.2, 139.8, 136.1, 135.3, 135.2, 133.7, 129.7, 128.9, 128.8, 128.7, 127.1, 126.5, 125.1, 114.8, 113.9, 113.0, 94.6, 68.9, 60.2, 45.1, 32.3, 31.8, 31.7, 29.2, 29.1, 29.0, 26.0, 22.5, 16.5, 14.4. Anal. Calcd for $C_{36}H_{43}BrN_2O$: C, 72.11; H, 7.23; N, 4.67. Found: C, 72.18; H, 7.26; N, 4.68.

5.7. Cytotoxicity assays in vitro

Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described by our group [35]. Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Cultures were propagated at 37 °C in a humidified atmosphere containing 5% CO_2. Drug stock solutions were prepared in DMSO. The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentration without effect on cell replication. The tumor cell line panel consisted of liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (Hela), breast adenocarcinoma (MCF-7). In all of these experiments, three replicate wells were used to determine each point.

5.8. Data set

In this study, *in vitro* cytotoxic data against HepG2 cells were selected to construct the 3D-QSAR models, Table 1 list their structures and biological data. For CoMFA and CoMSIA analysis, 29 compounds with determinate IC_{50} values of HepG2 cell line were selected as the training set, while the rest compounds with indeterminate IC_{50} values (>200 μM) were assigned to an inactive test set to verify if models can correctly identify inactives. IC_{50} values were transformed to pIC_{50} values according to the following formula:

$$pIC_{50} = \log\frac{1}{IC_{50}}$$

5.9. Structure optimization and alignment

Construction and optimization of the compounds, CoMFA and CoMSIA modeling were all performed by using SYBYL6.9 molecular modeling package (TRIPOS Associates Inc.) installed on a Silicon Graphics Octane2 workstation 2400 with IRIX 6.5 operating system. Molecules were created by using the sketch module of SYBYL6.9. A molecular mechanics (MM) optimization was carried out, MMFF94 force field and MMFF94 charges were employed and the energy gradient was set to 0.001 kcal/mol. Subsequently, compounds were aligned to the common three-ring structure of β-carboline, and compound **57** with the highest pIC_{50} value was chosen as the template molecular.

5.10. CoMFA and CoMSIA studies

CoMFA computations were setup using default settings. The steric (Lennard–Jones) and electrostatic (Coulomb) interaction energies were generated using a sp^3-hybridized carbon steric probe atom with a positive charge of 1.00 as the electrostatic probe, grid spacing was set to 2 Å, a cutoff of 30 kcal/mol was adopted.

For CoMSIA computations, the steric and electrostatic fields were applied, the same grid spacing value and probe atom as CoMFA was used to evaluate the interaction energies. Different from the CoMFA, CoMSIA used a Gaussian type distance dependence of physicochemical properties. Thus no singularities occurred and no arbitrary cutoffs were required. The default value of 0.3 was used as the attenuation factor (*R*).

5.11. Partial least square (PLS) analysis

The partial least squares (PLS) linear regression method was used to modeling the 3D-QSAR results of CoMFA & CoMSIA, the CoMFA and CoMSIA descriptors were served as independent variables, while pIC_{50} values were served as dependent variables. SAMPLS leave-one-out (LOO) Cross-validated PLS was performed to determine the optimal number of components, corresponding to the highest cross-validated r^2 (q^2) of the PLS analyses. The q^2 was calculated using the following formula (eq (1)):

$$q^2 = 1.0 - \frac{\sum_Y \left(Y_{\text{pred}} - Y_{\text{actual}}\right)^2}{\sum_Y (Y_{\text{actual}} - Y_{\text{mean}})^2} \quad (1)$$

Where Y_{pred}, Y_{actual} and Y_{mean} are predicted, actual, and mean values of the target property (pIC_{50}), respectively.

Subsequently, the non-cross-validated calculation was employed using the optimal number of components to generate the final CoMFA and CoMSIA models. The predictive ability of the models is expressed by conventional correlation coefficient r^2 value, which is analogous to cross-validated r^2 (q^2). The standard error of estimate SE and the Fisher test F value were also derived to evaluate the predictive quality of the models.

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