Biochemical Pharmacology, Vol. 37, No. 20, pp. 3953–3957, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

THE INFLUENCE OF LIPOPHILIC CHARACTER ON RECEPTOR BINDING AFFINITY OF A SERIES OF β-CARBOLINES

PIER ANDREA BOREA,*† MARIA CHIARA PIETROGRANDE‡ and GIAN LUIGI BIAGI§

* Istituto di Farmacologia, Universita' di Ferrara, Ferrara, ‡ Dipartimento di Chimica, Universita' di Ferrara, Ferrara, and § Istituto di Farmacologia, Universita' di Bologna, Bologna, Italy

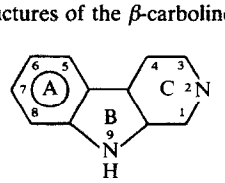
(*Received* 1 *October* 1987; *accepted* 11 *May* 1988)

Abstract—A quantitative study of the relationship between structure and receptor binding affinity of a series of 16β-carbolines showed the influence of lipophilic character and hydrogen bonding capability of substituents in position 3. Some data taken from the literature enabled us to add some evidence about the influence of planarity of ring C and bulky substituents in position 1 in determining the receptor binding affinity.

Recently several new drugs, among which the β-carboline derivatives, have been discovered which exert their pharmacological action by interacting with the benzodiazepine (BDZ) receptor in mammalian CNS [1]. These drugs have been classified according to their spectrum of biological activity as (a) agonists (which are, *inter alia*, anxiolytic), (b) inverse agonists (anxiogenic), or (c) antagonists (without any *per se* biological effect but preventing the interaction of agonists and inverse agonists with the receptor). The β-carboline derivatives, which are chemically unrelated to the benzodiazepines, interact with the BDZ receptors displaying the full spectrum of agonist, inverse agonist and antagonist properties according to their molecular substituents [2]. Qualitative studies have been devoted in very recent times to the structure–activity relationships in this class of drugs [3–8]. On the other side the literature is very poor in reports dealing with studies of the quantitative structure–activity relationships (QSAR) in the β-carboline series [9, 10]. In a previous paper a study with the Free and Wilson method allowed confirmation on a quantitative basis of the qualitative findings of the literature [10]. In particular the available data on the receptor binding of β-carbolines seem to point out that β-carbolines display high binding affinity for the BDZ-receptor only when all the following conditions are fulfilled: (a) presence of an esteric, sometimes amidic, function in position 3, capable of accepting hydrogen bonds from an hypothetic donor of the receptor; (b) full aromaticity of both the six membered rings, that is planarity of

† To whom correspondence should be addressed.

Table 1. Structures of the β-carboline derivatives



Compound	Name	R_1	R_3	R_4	R_5	R_6	R_7	Ring C
1	β-CC	H	COOH	H	H	H	H	Aromatic
2	Harman	CH_3	H	H	H	H	H	Aromatic
3	Harmine	CH_3	H	H	H	H	OCH_3	Aromatic
4	Harmol	CH_3	H	H	H	H	OH	Aromatic
5		H	CH_2OH	H	H	H	H	Aromatic
6	β-CCM	H	$COOCH_3$	H	H	H	H	Aromatic
7	β-CCE	H	$COOC_2H_5$	H	H	H	H	Aromatic
8	Pr-CC	H	$COOC_3H_7$	H	H	H	H	Aromatic
9	DMCM	H	$COOCH_3$	C_2H_5	H	OCH_3	OCH_3	Aromatic
10	ZK91296	H	$COOC_2H_5$	CH_2OCH_3	$OCH_2C_6H_5$	H	H	Aromatic
11	ZK93426	H	$COOC_2H_5$	CH_3	$O_iC_3H_7$	H	H	Aromatic
12		H	$COONHCH_3$	H	H	H	H	Aromatic
13	Harmaline	CH_3	H	H	H	H	OCH_3	Non-aromatic
14	Harmalol	CH_3	H	H	H	H	OH	Non-aromatic
15	ZK93423	H	$COOC_2H_5$	CH_2OCH_3	H	$OCH_2C_6H_5$	H	Aromatic
16	Nor-harman	H	H	H	H	H	H	Aromatic

the three-rings system [6]; (c) no substituents in position 1. However, the role played by physicochemical parameters, such as lipophilicity, has never been studied. The purpose of the present paper was a study of the quantitative relationship between structure and receptor binding affinity of a series of 16 β-carbolines (Table 1) by means of the Hansch approach [11]. In particular the role of the lipophilic character as expressed by the chromatographic R_m or log k' values, or by the log P values, was investigated.

MATERIALS AND METHODS

Chemicals. The β-carboline derivatives 6–11 and 16 were a generous gift from Dr R. Schmiechen, Schering AG, Berlin; the other derivatives were obtained from Sigma Chemicals (St Louis, MO). All other chemicals and solvents were of reagent or HPLC grade.

Determination of lipophilic character. The HPLC measurements were performed on a Spectra Physics Chromatograph consisting of an SP 87000 Solvent Delivery System and SP 8750 Organizer Module. A Varian Aerograph UV Detector operated at 254 nm. A 30 cm × 3.9 mm μBondapak C_{18} column from Waters Assoc. was used. The mobile phase was methanol in various mixtures (v/v%) with phosphate buffer (pH = 7.0; ionic strength = 0.05 M) at a flow rate of 1 ml/min. The carboline solution in methanol was injected into the column by a 10 μl loop.

The experiments were performed at room temperature. The retention time of potassium nitrate was taken as t_0. The capacity factor, k', was evaluated from the t_0 and the retention time of the solute, t_r, by the relationship $k' = (t_r - t_0)/t_0$.

For each compound the retention data were measured at different methanol concentrations (in the range 20–80%) in the mobile phase. Each measure was replicated at least three times. The HPTLC determinations were carried out on Whatman KC18F plates. A Camag Nanomat (Camag, Berlin) was used to spot compounds on the plates (about 100 nl of each carboline solution in methanol). The solutes were detected under UV light (254 nm). Solvent mixtures of methanol–phosphate buffer (pH 7.0) in the concentration range 45–80% were used as mobile phase. The R_m values were calculated by the relationship $R_m = \log (1/Rf - 1)$.

The log P values for three compounds (harman, harmine and norharman) were determined at pH 13.0 in octanol–water. The log P values of the other carboline derivatives were calculated from the experimental log P values of nor-harman, by taking advantage of the additive property of the Hansch π values [12].

Biological data. The receptor binding affinities of β-carbolines were assayed on the basis of their ability to displace ^{3}H-flunitrazepam from synaptosomal rat membranes. The results were expressed as the concentration of each test compound required to displace 50% of specific ^{3}H-flunitrazepam binding (IC_{50}). The IC_{50} (μM) values reported in Table 2 had been measured previously [10]. As ^{3}H-flunitrazepam is able to bind also to peripheral benzodiazepine binding sites present in brain, we tested whether the compounds used could interact with those sites.

Therefore for the compounds of Table 2 the receptor binding experiments were carried out also using ^{3}H-R_0 5-4864 as a radioligand [13]. All the test compounds displayed IC_{50} values $>10^{-5}$ M showing no or very poor affinity for peripheral benzodiazepine binding sites.

Statistical calculations. The data were treated by means of a multiple regression analysis [14]. The F test for equations 6–12 shows the significance of each of the independent variables added to equations 3–5.

Relationship between log P and R_m or log k' values. The chromatographic work showed that in both HPLC and HPTLC systems there is a linear relationship between log k' or R_m values and the methanol concentration in the mobile phase. The equations of the straight lines were used to calculate for each compound a theoretical log k' or R_m value at 0% methanol concentration in the mobile phase (Table

Table 2. Physicochemical parameters and biological activities of the β-carboline derivatives

Compound	log P	R_m	log k'	I_1	I_2	I_3	log $1/IC_{50}$ Obsd.	log $1/IC_{50}$ Calcd. (eq. 6)	log $1/IC_{50}$ Calcd. (eq. 12)
1	−0.19	0.63	0.76	1	0	1	−1.491	−0.966	0.735
2	3.50	3.35	2.54	0	1	1	−0.450	−1.134	−1.542
3	3.56	3.10	2.73	0	1	1	−1.980	−1.407	−1.529
4	3.06	2.82	2.10	0	1	1	−1.900	−1.712	−1.643
5	2.55	2.62	2.01	1	0	1	1.591	1.202	1.593
6	3.16	2.92	2.83	1	0	1	2.097	1.529	1.733
7	3.68	3.50	2.65	1	0	1	2.155	2.161	1.853
8	4.24	3.54	3.15	1	0	1	1.921	2.202	1.981
9	4.14	3.70	2.98	1	0	1	2.400	2.379	1.958
10	4.56	4.60	3.86	1	0	1	2.959	3.359	2.054
11	4.73	4.76	3.89	1	0	1	2.960	3.533	2.093
12	2.75	2.71	2.11	1	0	1	1.780	1.300	1.639
13	0.86	1.92	1.71	0	1	0	−2.813	−2.692	−4.235
14	0.21	2.04	1.10	0	1	0	−2.748	−2.561	−4.383
15	4.56	3.93	3.98	1	0	1	2.959	2.629	2.054
16	3.17	3.12	2.51	0	0	1	−1.000	−1.385	0.596

2), as an expression of the lipophilic character of the molecule. The acidic compound No. 1 with a pK_a value of about 1.9 [15] as well as the basic compounds No. 13 and 14 with pK_a values of about 11.0 [15] are to be considered as completely ionized at the pH 7.0 of both chromatographic systems. On the other side all other basic compounds with pK_a values of about 5.0 [12] are to be considered as completely unionized at that pH. Accordingly the log P values of compounds No. 1, 13 and 14 were calculated for the ionized forms. All this seems to be justified by the fact that the binding assays were carried out at pH 7.1. The R_m or log k' values proved to be very well correlated with the log P values.

$$R_m = 1.363\ (\pm 0.176) + 0.577\ (\pm 0.052)\ \log P$$
$$N = 16; r = 0.948, s = 0.336; F = 123.67; \quad (1)$$
$$P < 0.005$$

$$\log k' = 1.020\ (\pm 0.182) + 0.517\ (\pm 0.054)\ \log P$$
$$N = 16; r = 0.932; s = 0.348; F = 92.56; \quad (2)$$
$$P < 0.005$$

Equations 1 and 2, which are very similar, point out the closeness of the two chromatographic methods. On the other hand, both equations confirm the validity of the π system in calculating log P values as well as the usefulness of the chromatographic technique.

Structure–activity relationships. The physicochemical parameters and the biological activity data (log $1/IC_{50}$) of the β-carboline derivatives are reported in Table 2. The influence of the lipophilic character on the receptor binding affinity is described by equations 3–5.

$$\log 1/IC_{50} = -4.141\ (\pm 1.325) + 1.516\ (\pm 0.410)\ R_m$$
$$N = 16; r = 0.703; s = 1.618; F = 13.68; \quad (3)$$
$$P < 0.005$$

$$\log 1/IC_{50} = -4.007\ (\pm 1.136) + 1.774\ (\pm 0.419)\ \log k'$$
$$N = 16; r = 0.749; s = 1.507; F = 17.91; \quad (4)$$
$$P < 0.005$$

$$\log 1/IC_{50} = -2.211\ (\pm 0.847) + 0.922\ (\pm 0.250)\ \log P$$
$$N = 16; r = 0.701; s = 1.621; F = 13.56; \quad (5)$$
$$P < 0.005$$

These show that the lipophilic character explains, 53, 62 and 49% of the variability in log $1/IC_{50}$ data, respectively. On the other hand, the introduction of the quadratic term into equations 3–5 did not improve the correlation coefficient significantly.

As a further step in the analysis, the substituents in the position 3 were considered. An indicator variable, I_1, with a value of 1 was used for the compounds characterized in that position by substituents able to accept a hydrogen bond. In fact, it is well known that β-carbolines with such substituents display higher receptor binding affinity [3–7, 9, 10].

$$\log 1/IC_{50} = -4.784\ (\pm 0.376) + 1.089\ (\pm 0.120)\ R_m + 3.132\ (\pm 0.244) I_1$$
$$N = 16; r = 0.981; s = 0.455; F = 168.62; \quad (6)$$
$$P < 0.005$$

$$\log 1/IC_{50} = -4.300\ (\pm 0.444) + 1.175\ (\pm 0.177)\ \log k' + 2.917\ (\pm 0.328) I_1$$
$$N = 16; r = 0.969; s = 0.588; F = 98.33; \quad (7)$$
$$P < 0.005$$

$$\log 1/IC_{50} = -3.401\ (\pm 0.257) + 0.663\ (\pm 0.074)\ \log P + 3.136\ (\pm 0.247) I_1$$
$$N = 16; r = 0.981; s = 0.459; F = 165.52; \quad (8)$$
$$P < 0.005$$

The analysis of variance showed that the introduction of the I_1 term in equations 3, 4 and 5 yields a significant improvement in equations 6, 7 and 8 respectively. No collinearity was shown between the independent variables. The positive coefficient associated with the indicator variable shows that the substituents able to accept a hydrogen bond can provide a higher receptor binding affinity.

Finally the influence of the methyl substitution in position 1 was taken into consideration. An indicator variable, I_2, with a value of 1 was used for the compounds bearing a CH_3 group in that position.

$$\log 1/IC_{50} = -4.359\ (\pm 0.594) + 1.077\ (\pm 0.121)\ R_m + 2.749\ (\pm 0.480)\ I_1 - 0.468\ (\pm 0.504)\ I_2 \quad (9)$$
$$N = 16; r = 0.982; s = 0.457; F = 111.50;$$
$$P < 0.005$$

$$\log 1/IC_{50} = -3.911\ (\pm 0.756) + 1.160\ (\pm 0.182)\ \log k' + 2.571\ (\pm 0.633)\ I_1 - 0.428\ (\pm 0.664)\ I_2 \quad (10)$$
$$N = 16; r = 0.970; s = 0.601; F = 62.74;$$
$$P < 0.005$$

$$\log 1/IC_{50} = -3.077\ (\pm 0.526) + 0.655\ (\pm 0.076)\ \log P + 2.836\ (\pm 0.491) I_1 - 0.368\ (\pm 0.517)\ I_2 \quad (11)$$
$$N = 16; r = 0.982; s = 0.468; F = 106.31;$$
$$P < 0.005$$

The introduction of the I_2 term did not yield any significant improvement in equations 9, 10 and 11. However, the negative coefficient associated with the indicator variable seems to indicate that a methyl group in that position decreases receptor binding affinity. This would be in agreement with the hypothesis of a region of steric hindrance around the heterocyclic nitrogen in position 1 [4, 6, 7].

Since in the present series of 16 compounds only

Table 3. Structures, physicochemical parameters and log $1/IC_{50}$ values of 18 β-carbolines from literature data

Compound	R_1	R_3	R_4	R_5	R_6	R_7	Ring C	log P	I_1	I_2	I_3	log $1/IC_{50}$ Obsd.	log $1/IC_{50}$ Calcd. (eq. 12)
17	H	$COOC_6H_5$	H	H	H	H	Aromatic	4.73	1	0	1	3.000	2.093
18	CH_3	$COOC_2H_5$	H	H	H	H	Aromatic	4.24	1	1	1	−0.690	−0.233
19	H	$COOC_2H_5$	H	H	H	H	Non Aromatic	3.79	1	0	0	−0.690	−0.209
20	H	$COOCH_3$	H	H	H	H	Non Aromatic	2.60	1	0	0	−1.230	−0.482
21	H	COOH	H	H	H	H	Non Aromatic	−4.04	1	0	0	−1.924	−2.005
22	CH_3	$COOC_2H_5$	H	H	H	H	Non Aromatic	4.24	1	1	0	−2.602	−2.320
23	H	H	H	H	H	H	Non Aromatic	3.17	0	0	0	−2.694	−1.490
24	CH_3	H	H	H	H	H	Non Aromatic	3.50	0	1	0	−3.161	−3.629
25	C_2H_5	$COOCH_3$	H	H	OH	H	Aromatic	3.51	1	1	1	−0.859	−0.401
26	C_2H_5	$COOCH_3$	H	H	H	H	Aromatic	4.18	1	1	1	−0.974	−0.247
27	C_2H_5	H	H	H	H	H	Aromatic	4.19	0	1	1	−2.495	−1.384
28	H	$COOC_4H_9$	H	H	H	H	Non Aromatic	4.55	1	0	0	−0.681	−0.034
29	H	$COOC_4H_9$	H	H	H	H	Aromatic	4.44	1	0	1	1.538	2.027
30	H	CN	H	H	H	H	Aromatic	2.60	1	0	1	2.523	1.605
31	H	$COOCH_3$	H	H	OH	H	Aromatic	2.49	1	0	1	2.479	1.579
32	C_6H_5	$COOCH_3$	H	H	H	H	Non Aromatic	5.23	1	1	0	−2.337	−2.092
33	C_6H_5	$COOCH_3$	H	H	H	H	Aromatic	5.01	1	1	1	−0.687	−0.056
34	H	$COOCH_3$	H	H	OH	OCH_3	Aromatic	3.03	1	0	1	2.426	1.703

two of them do not show full aromaticity of the three-rings system (Compound Nos. 13 and 14), we did not take into consideration this parameter, which is indicated in the literature as an important one [5, 7, 10].

Structure–activity relationships with data from the literature. Since in the present series of 16 compounds it was not possible to show a significant role of both alkyl substituents in position 1 and aromaticity of ring C, we turned our attention to some data from the literature. In Table 3 are reported structures, physicochemical parameters and log $1/IC_{50}$ values of 18 β-carbolines [16, 17]. Equation 12 calculated with the data from both Tables 2 and 3 takes into account the effect of lipophilic character and hydrogen bonding capability of substituents in position 3 (I_1), as well as the influence of both bulky substituents in position 1 (I_2) and aromaticity of ring C (I_3). A statistical significance is shown for all four terms even if a somewhat high standard error of the estimate indicated that the regression does not fit the data very well.

$$\begin{aligned} \log 1/IC_{50} = &-2.218\,(\pm 0.499) + 0.229 \\ &(\pm 0.094)\log P + 1.140 \\ &(\pm 0.428)\,I_1 - 2.214\,(\pm 0.390) \\ &I_2 + 2.086\,(\pm 0.375)\,I_3 \\ &N = 34;\ r = 0.912;\ s = 0.934;\ F = 36.01; \\ &P < 0.005 \end{aligned} \qquad (12)$$

DISCUSSION

The hydrogen bonding capability of substituents in position 3 is playing a significant role both in our series of compounds and in the series including the data from the literature. The effect of both bulky substituents in position 1 and aromaticity of ring C (i.e. planarity of the three-rings system) came out to be significant only when we took into consideration the series of 34 compounds. Although all this points out the importance of the particular series which one is dealing with, the influence of bulky substituents and the importance of aromaticity of ring C seem to be confirmed. In a previous paper [6] based mainly on stereochemical and receptor binding affinity data, we suggest that the recognition site for β-carbolines can be a planar cleft where the main drug–receptor interaction is mediated by the carbonyl group of the esteric or amidic function in position 3. The carbonyl group is a typical hydrogen-bonding acceptor. Moreover the fact that 1-substitution prevents binding was taken as an indirect evidence that the main interaction region is located in the C=O, N_2 and N_9 line.

Equations 9–12 show the important role played by the indicator variables I_1, I_2 and I_3. In general, the use of indicator variables has to be considered with caution; in this case, however, they account for specific structural properties which cannot be explained by continuous linear free energy related parameters (or their combinations). In other words this is one of the cases where a rationalization of the activity data would be impossible without the introduction of discontinuous variables. Finally the present data show the significant role played by the lipophilic character in determining the receptor binding affinity of β-carbolines. Since in the literature we did not find any report about this point, the present result seems to be rather interesting. In an *in vitro* system, such as that used for a receptor binding assay, the lipophilic character might exert a role via an increase of the entropic term (caused by solvent disorganization) of the binding process itself and/or by an accumulation of lipophilic β-carbolines in the lipid membrane phase.

It would be very interesting to compare the present *in vitro* system with the problems faced in the whole animal where the lipophilic character should play a much more important role in determining the ran-

dom walk of the molecules through many biological membranes to the final target. In conclusion the present data seem to confirm on a quantitative basis previous qualitative findings. At the same time it seems to point out the importance of the lipophilic character of molecules.

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