the chemotherapeutic activity of MTX, in particular because both compounds compete for cellular uptake [10] must be reconsidered in view of the low *in vivo* plasma concentrations of 7OH-MTX not bound to HSA.

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De novo analysis of receptor binding affinity data of β-carbolines

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β-Carbolines are a class of compounds, chemically unrelated to benzodiazepines (BDZs), able to interact at various affinity degrees with BDZ receptors in mammal Central Nervous System. Although several researches have been devoted to the study of quantitative structure–activity relationships (QSAR) for BDZs [1, 2], such an attempt has never been clearly carried out for the β-carbolines class of drugs. In this paper we report a QSAR study, making use of the Free and Wilson approach [3], on receptor binding affinity data for a series of 33 β-carbolines. The results of the analysis are briefly discussed in terms of the interactions of these compounds with the BDZ receptor.

Methods

(i) De novo *Model.* For QSAR studies the LFER (Linear Free Energy Relationships) approach [4] and the *de novo* model [3, 5, 6] are widely used. However, many of the independent substituents for the present series of compounds cannot be described by LFER continuous parameters. Therefore the *de novo* model, in its Free and Wilson version was preferred to the Hansch's approach. According to this model, the biological activities of molecules belonging to a homologous series can be expressed as the sum of a constant contribution, μ, and of as many individual contributions, α_{ij}, as there are substituents i in the different positions j. The group contributions were calculated by means of a modified version [7] of the Fortran program written by Purcell *et al.* [8].

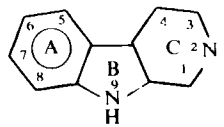
(ii) *Data set.* β-Carbolines' receptor binding affinities (RBAs) are expressed by their ability to displace ^{3}H-flunitrazepam from synaptosomal rat brain membranes as the concentrations of the test compounds required to displace 50% of specific ^{3}H-flunitrazepam binding (IC_{50}). IC_{50} (μM) values used here were taken from Braestrup and Nielsen [9] for compounds 1–6, 9, 11–18 of Table 1, from Cain *et al.* [10] for compounds 21–23, 27–30, 33, from Loew *et al.* [11] for compound 27, from Lippke *et al.* [12] for compounds 25 and 26, or measured in our laboratory for compounds 7, 8, 10–13, 24, 31 and 32 by the method given by Karobath and Supavilai [13]. Data are not completely homogeneous in the sense that they have been obtained by several researchers. However, our redeterminations for some reference compounds show that possible systematic differences due to small changes of experimental procedure are very small, in agreement with what is generally observed for binding experiments.

Results and discussion

Table 1 shows the 33 β-carbolines employed in the Free and Wilson analysis together with the observed and calculated values of -log IC_{50}. The atoms or groups selected as independent variables are shown in Table 2 together with the values of the individual group contributions, α_{ij}, obtained from the analysis.

The two independent variables, AR and NAR, take into account, respectively, the aromaticity or not of the C ring. Compounds 12 and 13 are 3,4-H while compounds 14–20, 25 and 29 are 1,2,3,4-H β-carbolines. The AR/NAR variable is conceived as an indicator both of the planarity of the three rings system and of the coplanarity of the esteric function with ring C. X-Ray diffraction studies show that the three rings system is almost perfectly planar when

Table 1. β-Carbolines taken into account in the Free and Wilson analysis and observed and calculated log $1/IC_{50}$ values in ^{3}H-flunitrazepam binding assay



Cpd	R_1	R_3	R_4	R_5	R_6	R_7	Ring C[a]	Log $\frac{1}{IC_{50}}$ (observed)[b]	Log $\frac{1}{IC_{50}}$ (calculated)
1	H	$COOC_6H_5$	H	H	H	H	AR	3.000	2.226
2 βCCE	H	$COOC_2H_5$	H	H	H	H	AR	2.155	1.331
3 βCCM	H	$COOCH_3$	H	H	H	H	AR	2.097	1.350
4 PrCC	H	$COOC_3H_7$	H	H	H	H	AR	1.921	1.921
5	H	$COOC_5H_{11}$	H	H	H	H	AR	1.347	1.347
6	CH_3	$COOC_2H_5$	H	H	H	H	AR	−0.690	−0.571
7 Harman	CH_3	$COOC_6H_5$	H	H	H	H	AR	−0.450	0.324
8 Norharman	H	H	H	H	H	H	AR	−1.000	−0.404
9 β-CC	H	COOH	H	H	H	H	AR	−1.491	−0.655
10 Harmol	CH_3	H	H	H	H	OH	AR	−1.900	−1.487
11 Harmine	CH_3	H	H	H	H	OCH_3	AR	−1.980	−1.560
12 Harmalol	CH_3	H	H	H	H	H	NAR	−2.748	−3.161
13 Harmaline	CH_3	H	H	H	H	OCH_3	NAR	−2.813	−3.233
14	H	$COOC_2H_5$	H	H	H	H	NAR	−0.690	−0.342
15	H	$COOCH_3$	H	H	H	H	NAR	−1.230	−0.324
16	H	COOH	H	H	H	H	NAR	−1.924	−2.328
17	CH_3	$COOC_2H_5$	H	H	H	H	NAR	−2.602	−2.245
18	CH_3	COOH	H	H	H	H	NAR	−3.800	−4.230
19 Tetrahydronorharman	H	H	H	H	H	H	NAR	−2.694	−2.078
20 Tetrahydroharman	CH_3	H	H	H	H	H	NAR	−3.161	−3.980
21	C_2H_5	$COOCH_3$	H	H	OH	H	AR	−0.859	−0.307
22	C_2H_5	$COOCH_3$	H	H	H	H	AR	−0.974	−1.133
23	C_2H_5	H	H	H	H	H	AR	−2.495	−2.888
24 ZK91296	H	$COOC_2H_5$	CH_2OCH_3	$OCH_2C_6H_5$	H	H	AR	2.959	2.959
25	H	$COOC_4H_9$	H	H	H	H	NAR	−0.681	−0.408
26	H	$COOC_4H_9$	H	H	H	H	AR	1.538	1.265
27	H	CN	H	H	H	H	AR	2.523	2.523
28	H	$COOCH_3$	H	H	OH	H	AR	2.479	2.176
29	C_6H_5	$COOCH_3$	H	H	H	H	NAR	−2.337	−2.348
30	C_6H_5	$COOCH_3$	H	H	H	H	AR	−0.687	−0.675
31 ZK93423	H	$COOC_2H_5$	CH_2OCH_3	H	$OCH_2C_6H_5$	H	AR	2.959	2.959
32 DMCM	H	$COOCH_3$	H	H	OCH_3	OCH_3	AR	2.400	2.400
33	H	$COOCH_3$	H	H	OH	OCH_3	AR	2.426	2.375

[a] AR = aromatic ring; NAR = non aromatic ring.

[b] IC_{50} values (μM) were taken from Braestrup and Nielsen [9], Cain *et al.* [10], Loew *et al.* [11], Lippke *et al.* [12] or determined in our laboratory by the procedure described by Karobath and Supavilai [13].

Table 2. Individual group contributions, α_{ij}, obtained from the Free and Wilson analysis. N = 33; r = 0.975; F = 13.11; explained variance = 88%; P < 0.01; regression constant, μ, = −0.285

Substitution on position															
1		3		4		5		6		7		Ring C	
Substituent	α_{ij}	Substituent	α_{ij}	Substituent	α_{ij}	Substituent	α_{ij}	Substituent	α_{ij}	Substituent	α_{ij}	Substituent	α_{ij}
H	0.87	CN	1.68	CH_2OCH_3	0.19	$OCH_2C_6H_5$	1.38	$OCH_2C_6H_5$	1.30	OH	0.70	Aromatic ring	0.56
												Non aromatic ring	−1.12
CH_3	−1.03	$COOC_6H_5$	1.39	H	−0.01	H	−0.04	OH	0.70	OCH_3	0.63		
C_6H_5	−1.16	$COOC_3H_7$	1.08			OCH_3	0.18	H	−0.12				
C_2H_5	−1.62	$COOCH_3$	0.51					H	−0.13				
		$COOC_5H_{11}$	0.51										
		$COOC_2H_5$	0.49										
		$COOC_4H_9$	0.42										
		H	−1.24										
		COOH	−1.49										
$\Delta\alpha_{ij}$ =	2.49		3.17		0.20		1.42		1.43		0.82		1.68

both A and C rings are aromatic, while hydrogenation of C ring produces a considerable "butterfly" bending of the molecule [14, 15]. The most important aspect concerns, however, the coplanarity of the esteric function with ring C when C is aromatic. This is produced by resonance and is observed in all β-carbolines structures [14, 15]. In conclusion AR can be considered the indicator of total molecular planarity, esteric function included; NAR an indicator of a completely different spatial arrangement while the three rings system in a "butterfly" conformation and the esteric function plane nearly perpendicular with the mean C ring plane, according to the minimization of intramolecular van der Waals repulsions. The following conclusions can be drawn from an analysis of the values of Table 2. (a) Generally speaking, RBAs are mainly determined by the nature of substituents at position 1 and 3 ($\Delta\alpha_{ij}$ = 2.49 and 3.17 respectively), the effect of substitution in positions 4 and 7 being of minor importance ($\Delta\alpha_{ij}$ = 0.20 and 0.82 respectively). Also positions 5 and 6 appear to be of some importance in affecting RBAs ($\Delta\alpha_{ij}$ = 1.42 and 1.43 respectively), the major contributions being given by highly hydrophobic $OCH_2C_6H_5$ groups (α_{ij} = 1.38 and 1.30 for positions 5 and 6 respectively). This is in agreement with the general observation that bulky lipophilic substituents generally increase the binding via an increase of the entropic term (caused by solvent disorganization) of the binding process. (b) Substituents other than hydrogen in position 1 are strongly detrimental for binding (α_{ij} CH_3 = −1.03, $\alpha_{ij}C_2H_5$ = −1.62, $\alpha_{ij}C_6H_5$ = −1.16, α_{ij}H = 0.87). (c) As to position 3 the presence of esteric groups is fundamental for an optimal drug–receptor interaction (α_{ij} in the range 0.42–1.39), the COOH group by itself being much less effective (α_{ij} = −1.49). However, even if based on a unique IC_{50} datum, the presence of a CN group appears to give the highest contribution to affinity (α_{ij} = 1.68). (d) Ring C aromaticity is also essential for high RBAs (α_{ij}AR = 0.56, α_{ij}NAR = −1.12).

Before discussing the present findings it is useful to recall that the crystal structure of β-CCM, one of the most efficient binders, has already been reported [14]; all the molecule has been found to be almost perfectly planar as far as both the three rings system and the carboxymethyl groups are concerned. The planarity of the $COOCH_3$ moiety was imputed to resonance of the conjugated double bond system going from N(9) to C=O through N(2).

On these grounds, the present results can be interpreted by assuming that the β-carbolines recognition site is a planar cleft.

Any change in the drug molecule able to destroy overall planarity hinders binding efficiency as indicated by the strong negative effect (α_{ij} = −1.12) in C ring hydrogenated compounds. In fact, 1–4 hydrogenation deletes completely the planarity of the ring C itself, as shown by the molecular structure of one of these compounds reported by Codding [15], and 3,4-hydrogenation causes the interruption of the conjugated system allowing the out-of-plane rotation of the carboxyalkyl group. Similar conclusions have been recently drawn out on β-carbolines by Loew *et al.* [11] on the basis of Quantum Mechanical Calculations.

The fact that the 3-carboxyalkyl-β-carbolines display high RBAs can be interpreted assuming that C=O group exerts a specific role in the binding process. As the carbonyl is a typical hydrogen bond acceptor it can be hypothesized that the main drug–receptor interaction is mediated by a hydrogen bond donated by the receptor. The highly negative α_{ij} value for COOH can be related to the hydrophilic character of this group, which would be particularly the case if it is at least partially ionized at the experimental pH value. It cannot be excluded that the carbonyl interaction is assisted by the two ring nitrogens and, on the other hand, the strong negative effect of any 1-substituent supports the idea that the closest drug–receptor contact happens in a region going from the C=O group to the N(9) atom.

The main feature of the esteric group is its ability to accept hydrogen bonds as its oxygen is a typical electron donor group. Probably any other functional group having the same hydrogen bonding accepting capability would display similar behaviour and in effect this could be the case of the 3CN group previously discussed.

It is worth mentioning the hypothesis, supported by crystallographic, quantum-mechanic and structure–activity studies, that BDZs also interact with their receptor mainly via hydrogen bonds and in an essentially planar molecular conformation [16, 17].

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Stabilizing action of L-carnitine on the energy-linked processes of mitochondria isolated from perfused rat liver

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L-Carnitine has recently been reported to stabilize rat liver mitochondria exposed to various stressing conditions as well as mitochondria isolated from the liver of L-carnitine treated animals [1]. L-Carnitine has also been found to prevent mitochondrial damage induced in leukaemia cells by the anticarcinogen methylglyoxal bis(guanilhydrazone) [2]. Retrospectively a protective action of L-carnitine on mitochondria might also explain the protection of mice against otherwise toxic doses of ammonium acetate [3], indeed the energy-linked phase of urea cycle takes place within liver mitochondria. In this paper we present evidence that addition of L-carnitine to the perfusion medium significantly improves the energy-linked processes of mitochondria, thereafter isolated from the liver, and their resistance against different damaging factors.

Materials and methods

Materials. Outdated human concentrated erythrocytes were kindly supplied by the Blood Bank of the Padova Hospital (Padova, Italy).

Ketalar® (2-(*O*-chlorophenyl)-2-methylaminocyclo-esanone) was purchased from Parke Davis S.p.A. as a 50 mg/ml solution.

Methods. Male Wistar rats (300–350 g) starved for 24 hr were anaesthetized by intraperitoneal administration of 0.35 ml (17.5 mg) of Ketalar®/100 g of body weight. In addition, 500 U.I. of heparine/100 g of body weight were injected.

The perfusion medium (modified Hanks' medium) contained 137 mM NaCl, 5.4 mM KCl, 0.44 mM KH_2PO_4, 0.33 mM Na_2HPO_4, 4.2 mM $NaHCO_3$, 10 mM Na–Hepes* (pH 7.4), 0.6 mM $MgSO_4$ and 1 μM Ca^{2+}.

Prior to being added to the perfusion medium the outdated human erythrocytes were washed twice in 10 vol. of 125 mM NaCl, 30 mM Tris HCl (pH 7.2), and the hematocrit of the resulting concentrated suspension (80–90%) was determined.

Reversed liver perfusion was achieved by cannulation of the caval vein (inlet) via the right atrium and the portal vein (outlet). A ligature was put around the inferior caval vein, just above the right renal vein, the hepatic vein and artery. During the perfusion, which was performed at a flow rate of 13 ml/min, the liver was left *in situ* and temperature (35°) and surface wetting were carefully controlled. The perfusion medium was constantly oxygenated with an oxygen:carbon dioxide (19:1) mixture, and the perfusate leaving the portal catether was discarded.

At the end of the perfusion the liver was excised, immediately immersed in ice-cold 0.25 M sucrose, 5 mM Na–Hepes (pH 7.4), washed thoroughly and then homogenized in 50 ml of 0.25 M sucrose, 5 mM Na–Hepes (pH 7.4) using a Potter homogenizer with Teflon pestle driven at 900 rev/min. Mitochondria were then isolated by differential centrifugation in the same buffered solution.

The protein content of mitochondrial suspensions was assayed according to Gornall *et al.* [4] using bovine serum albumin as the standard.

Mitochondrial oxygen consumption rates were measured at 20° with a Clark oxygen electrode (Yellow Spring Ind.) in

* Abbreviations used: Hepes: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.