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Structural features controlling the binding of β-carbolines to the benzodiazepine receptor

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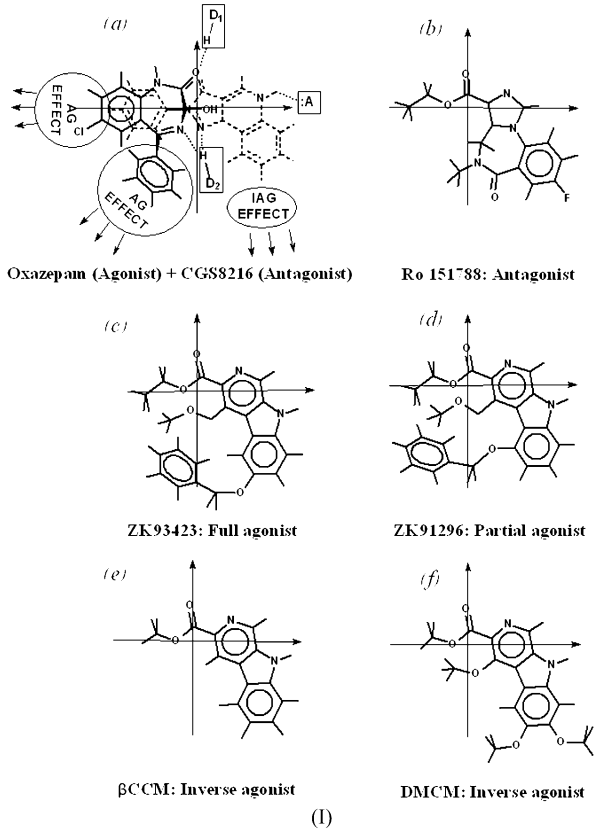
β-Carbolines are a class of drug which can interact with a high affinity with the benzodiazepine (BDZ) binding site of the $GABA_A$ receptor. The present paper, aimed at obtaining a deeper insight into the structure–properties relationships of this class of molecules, reports the crystal structures of four β-carbolines: ZK93423 (3-carboethoxy-4-methoxymethyl-6-benzyloxy-β-carboline), ZK91296 (3-carboethoxy-4-methoxymethyl-5-benzyloxy-β-carboline), FG7142 (*N*-methyl-3-carbamoyl-β-carboline) and the low-affinity ligand harmine hydrochloride (1-methyl-7-methoxy-β-carboline). This set of structural data is completed by the X-ray structures of other carbolines of known biological activity retrieved from the Cambridge Crystallographic Database and by the structures of β-CCE (3-carboethoxy-β-carboline), 6-PBC (3-carboethoxy-4-methoxymethyl-6-isopropoxy-β-carboline), PRCC (3-isopropoxy-β-carboline) and ZK93426 (3-carboethoxy-4-methyl-5-isopropoxy-β-carboline), which have been obtained by molecular-mechanics simulations. The structural features of all these molecules have been compared according to the stereochemical model we proposed in 1987. The structural comparison is integrated by the Free–Wilson analysis on 32 β-carbolines of known binding affinity data.

1. Introduction

β-Carbolines belong to a class of drugs, chemically unrelated to benzodiazepines (BDZs), which can interact with a high affinity with the BDZ binding site of the $GABA_A$ receptor. Here they are able to display different pharmacological properties by acting as full (*f*AG) or partial (*p*AG) agonists, inverse agonists (IAG) or antagonists (ANT; Braestrup *et al.*, 1983). The agonists are known to allosterically modulate the binding of GABA (γ-aminobutyric acid) to its receptor, increasing GABAergic transmission and producing anxiolytic, anticonvulsant, sedative–hypnotic and muscle-relaxant effects. Inverse agonists exert a negative cooperative effect on GABA binding, thereby producing proconvulsant and anxiogenic effects, while the antagonists have no intrinsic efficacy of their own but can inhibit either the positive effects of agonists or the negative effects of inverse agonists.

The $GABA_A$ receptor is a member of the family of ligand-gated ion channels (LGIC) and its detailed structure is presently unknown. Electron microscopy studies (Nayeem *et al.*, 1994) have shown that, in analogy with the other members of the LGIC family, it has a pentameric quaternary structure formed by homologous subunits assembled to form a central channel. Many present studies are devoted to identifying the amino acid residues involved in the BDZ drug–receptor interactions by means of affinity experiments on mutated

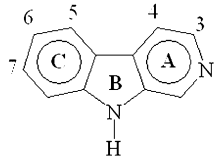
receptors (Buhr *et al.*, 1997; Schaerer *et al.*, 1998; Davies *et al.*, 1998; Sigel *et al.*, 1998; Dunn *et al.*, 1999; Renard *et al.*, 1999; Strakhova *et al.*, 2000; Newell *et al.*, 2000). These experiments might eventually confirm the previous structure–activity models which attempted to rationalize the stereochemical features controlling binding and the intrinsic-activity properties of the BDZ–receptor ligands in order to map the receptor binding site (Loew *et al.*, 1984; Codding & Muir, 1985; Borea *et al.*, 1987; Hollinshead *et al.*, 1990; Codding *et al.*, 1995; Zhang *et al.*, 1995; Cox *et al.*, 1998; Harris & Loew, 2000; Marder *et al.*, 2001). In this discussion we make particular reference to the



(I)

stereochemical model for the drug–receptor interaction we proposed in 1987 (Borea *et al.*, 1987), which intended to account for both the affinity and type of pharmacological activity of the BDZs, imidazo- and triazolo-BDZs, cyclopyrrolones, triazolopyridazines and β-carbolines. The model is based on the assumption that there is a rather diffuse unique recognition site for AGs, ANTs and IAGs where the different pharmacological profiles are accounted for by the different localizations of the ligands. It is essentially based on the principle that '*a generic ligand (. . .) can be considered as an effector able to modify the conformation of the receptor (. . .). Pushing leftwards and downwards on the left causes AG behaviour, downwards on the right IAG behaviour* [see (I*a*)]*; different balancing of the two forces produces any intermediate behaviour, pure ANTs representing only the point of perfect equilibrium of the two driving forces?*' (Borea *et al.*, 1987). A schematic stereochemical representation of the model is given in (I), where some BDZ–receptor ligands, oriented accordingly, are reported. In (*a*) the full agonist Oxazepam and the antagonist CGS8216 are superimposed, and the possible positions of the receptor residues involved in hydrogen-bond formation with the ligand carbonyl and N—H groups (rectangles) and arrows showing the 'pushing' directions and positions are also displayed. The antagonist Ro15-1788 and four carbolines, two agonists (ZK93423 and ZK91286) and two inverse agonists (DMCM and β-CCM), are sketched in (I*b–f*).

The present paper tries to obtain a deeper insight into the structure–properties relationships in β-carbolines by taking advantage of the relevant number of new experimental data available from the literature for this class of molecule and collecting new crystal data. To this aim, the paper reports the crystal structures of four β-carbolines, ZK93423 [3-carboethoxy-4-methoxymethyl-6-benzyloxy-β-carboline, *f*AG, (1)], ZK91296 [3-carboethoxy-4-methoxymethyl-5-benzyloxy-β-carboline, *p*AG, (2)], FG7142 [*N*-methyl-3-carbamoyl-β-carboline, IAG, (3)] and the low-affinity ligand harmine hydrochloride [1-methyl-7-methoxy-β-carboline (4)]. This set



Harmine.HCl [present work] R_1=CH_3; R_7= OCH_3

AG: **ZK91296** [present work] R_3=COOEt; R_4= CH_2OCH_3; R_5= OCH_2Ph

AG: **6-PBC** R_3=COOEt; R_4= CH_2OCH_3; R_6= O*n*Pr

IAG: **β-CCM** [CITRIG01,02,10] R_3=$COOCH_3$

IAG: **FG7142** [present work] R_3=$CONHCH_3$

ANT: **β-CC^{t}Bu** [JAFFIF] R_3=COO^tBu

ANT: **PRCC** : R_3=COO*n*Pr

AG: **ZK93423** [present work] R_3=COOEt; R_4= CH_2OCH_3; R_6= OCH_2Ph

AG: **Abecarnil** [YERRUI] R_3=COO*i*Pr; R_4= CH_2OCH_3; R_6= OCH_2Ph

IAG: **DMCM** [SEKPUT] R_3=$COOCH_3$; R_4= CH_2CH_3; R_6= OCH_3; R_7= OCH_3

IAG: **β-CCE** R_3=COOEt

IAG: **ZERHUZ** R_6= $NHCH_2Ph$

ANT: **ZK93426** R_3=COOEt; R_4= CH_3; R_5= O*i*P

ANT: **ZERHOT**: R_3=$COOCH_3$; R_6= $NHCH_2Ph$

(II)

of structural data is completed by the X-ray structures of other carbolines of known biological activity [DMCM (IAG: Bertolasi *et al.*, 1990); Abecarnil (*p*AG: Bock *et al.*, 1994); β-CCM (IAG: Bertolasi *et al.*, 1984); β-CC^{t}Bu (ANT: Codding *et al.*, 1988), ZERHOT and ZERHUZ (ANT and IAG, respectively: Codding *et al.*, 1995)] retrieved from the Cambridge Crystallographic Database (Allen, 2002) and by the structures of β-CCE (IAG), 6-PBC (*p*AG) PRCC and ZK93426 (both ANTs), which, not being available as crystal structures, have been obtained by molecular–mechanics simulations. The structural features of all these molecules, which are summarized in (II), have been compared by superposition techniques and the structural comparison study is integrated by the Free–Wilson analysis (Free & Wilson, 1964) on 32 β-carbolines of known binding affinities data.

Table 1
Experimental details.

	ZK93423	ZK91296	FG7142	Harmine
Crystal data				
Chemical formula	$C_{23}H_{22}N_2O_4$	$C_{23}H_{22}N_2O_4$	$C_{13}H_{11}N_3O$	$[C_{13}H_{13}N_2O]^+Cl^-\cdot 1/2H_2O$
M_r	390.43	390.43	225.25	257.71
Cell setting, space group	Monoclinic, $P2_1$	Orthorhombic, $P2_12_12_1$	Monoclinic, $C2/c$	Monoclinic, $C2/c$
a, b, c (Å)	5.0472 (2), 11.3381 (5), 16.8796 (7)	9.9033 (5), 11.5758 (4), 17.1805 (9)	14.3632 (5), 7.6804 (2), 19.6712 (7)	16.9891 (4), 12.5409 (3), 13.7967 (4)
β (°)	94.331 (3)	90.00	90.9340 (12)	121.8960 (11)
V (Å^3)	963.19 (7)	1969.58 (16)	2169.74 (12)	2495.66 (11)
Z	2	4	8	8
D_x (Mg m^{-3})	1.346	1.317	1.379	1.372
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
No. of reflections for cell parameters	4907	5595	5605	6879
θ range (°)	1.0–29.0	3.1–30.0	2.8–28.0	2.1–29.0
μ (mm^{-1})	0.09	0.09	0.09	0.30
Temperature (K)	100	293	293	150
Crystal form, colour	Prism, colourless	Plate, colourless	Prism, colourless	Prism, colourless
Crystal size (mm)	0.5 × 0.24 × 0.07	0.45 × 0.30 × 0.09	0.4 × 0.31 × 0.17	0.28 × 0.17 × 0.14
Data collection				
Diffractometer	Nonius Kappa CCD	Nonius Kappa CCD	Nonius Kappa CCD	Nonius Kappa CCD
Data collection method	φ and ω scans	φ and ω scans	φ and ω scans	φ and ω scans
Absorption correction	None	None	None	None
No. of measured, independent and observed reflections	4907, 2664, 2316	5595, 3201, 2330	5605, 2595, 1943	6879, 3317, 2448
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.034	0.033	0.026	0.023
θ_{max} (°)	29.0	30.0	28.0	29.0
Range of h, k, l	$-6 \Rightarrow h \Rightarrow 6$ $-15 \Rightarrow k \Rightarrow 15$ $-22 \Rightarrow l \Rightarrow 22$	$-13 \Rightarrow h \Rightarrow 13$ $-16 \Rightarrow k \Rightarrow 16$ $-23 \Rightarrow l \Rightarrow 23$	$-18 \Rightarrow h \Rightarrow 18$ $-10 \Rightarrow k \Rightarrow 10$ $-25 \Rightarrow l \Rightarrow 25$	$-23 \Rightarrow h \Rightarrow 23$ $-17 \Rightarrow k \Rightarrow 17$ $-18 \Rightarrow l \Rightarrow 18$
Refinement				
Refinement on	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.043, 0.103, 1.08	0.048, 0.111, 1.04	0.048, 0.148, 1.12	0.047, 0.129, 1.14
No. of reflections	2664	3201	2595	3317
No. of parameters	349	350	190	223
H-atom treatment	Refined independently	Refined independently	Mixture of independent and constrained refinement	Refined independently
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0457P)^2 + 0.304P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0406P)^2 + 0.4347P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0753P)^2 + 0.5664P]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0504P)^2 + 2.3654P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.004	0.073	0.054	0.002
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.38, −0.28	0.18, −0.18	0.18, −0.19	0.28, −0.36
Absolute structure	Flack (1983)	Flack (1983)	–	–
Flack parameter	1.4 (13)	−1.7 (15)	–	–

Computer programs used: *Kappa CCD server software* (Nonius, 1997), *DENZO-SMN* (Otwinowski & Minor, 1997), *SIR*97 (Altomare *et al.*, 1999), *SHELXL*97 (Sheldrick, 1997), *ORTEP*III (Burnett & Johnson, 1996), *PARST* (Nardelli, 1995), *PLATON* (Spek, 2001).

2. Experimental and computational

Crystals of the four compounds were obtained by slow evaporation from methanol/*iso*-amyl alcohol solutions at room temperature. Crystal data, data collection and refinement parameters are summarized in Table 1 and a selection of bond lengths and angles is reported in Table 2. X-ray diffraction data were collected on a Nonius Kappa CCD diffractometer using graphite-monochromated Mo $K\alpha$ radiation (λ = 0.71069 Å). Intensities were corrected for Lorentz and polarization. Structures were solved by direct methods with the *SIR*97 program (Altomare *et al.*, 1999) and refined by full-matrix least-squares with anisotropic non-H and isotropic H atoms using the *SHELXL*97 (Sheldrick, 1997) program. All other calculations were performed using the programs *PLATON* (Spek, 2001), *WinGX* (Farrugia, 1999) and *PARST* (Nardelli, 1995). *ORTEP*III (Burnett & Johnson, 1996) views of (1)–(4), oriented according to (I), are shown in Figs. 1–4. Hydrogen-bonding parameters for the four structures are reported in Table 3.

A total of 17 carboline structures have been retrieved from the Cambridge Structural Database (September 2003 version; Allen, 2002), from which seven compounds [CITRIG01,02,10 (β-CCM): Bertolasi *et al.*, 1984; Kubicki & Codding, 2001; Muir & Codding, 1985; CITROM10: Muir & Codding, 1984; JAFFIF (β-CC^{t}Bu): Codding *et al.*, 1988; SEKPUT (DMCM):

Bertolasi *et al.*, 1990; YERRUI, 01,02 (Abecarnil): Bock *et al.*, 1994; ZERHOT, ZERHUT, Codding *et al.*, 1995)], sketched in (II), display some activity with respect to the BDZ receptor.

The Free–Wilson method (Free & Wilson, 1964) is a multiple regression technique for the quantification of structure–activity relationships based on the simplifying assumption that a biological activity parameter of a homologous series of molecules can be expressed as the sum of a constant contribution, μ, because of the common molecular fragment (the pharmacophore) and the individual group contributions a_{ij} for each substituent i at the different positions j. This model has been applied to the analysis of the activity data of 32 β-carbolines (Hollinshead *et al.*, 1990; Cox *et al.*, 1998; Gessi *et al.*, 1999), all concerning binding inhibition constants of radiolabelled ligands on rat cerebella membranes. Calculations were performed with a modified version (Gilli, 2001) of the program originally proposed by Purcell *et al.* (1973). The affinity data of the 32 β-carbolines, variously substituted in positions 1, 3–7 and employed in the Free–Wilson analysis together with the observed and calculated values of the binding constant K_i, are available as deposited material.[1] The final results are displayed in Table 4.

The equilibrium structures of β-CCE (IAG), PRCC (ANT) and ZK93426 (ANT) have been obtained by molecular mechanics optimization (MM3 force field, *Alchemy*2000 system of programs; Tripos Inc., 1997). All the carbolines tested are characterized by an ester function in position 3. The conformation with the carbonyl *syn* with respect to N1 has been chosen because it is systematically more stable by 8–12 kJ mol^{-1} in agreement with the great majority of crystal structures so far determined (the only exception being DMCM: Bertolasi *et al.*, 1990) and because it is generally believed to be the active conformation in drug binding (Dorey *et al.*, 1989).

3. Results and discussion

3.1. Structural results

*ORTEP*III (Burnett & Johnson, 1996) views of the four structures, oriented according to (I), are shown in Figs. 1–4 and the packing schemes are shown in Figs. 5–8.

The bond distances and angles reported in Table 2 are quite similar and do not show significant discrepancies from the structural parameters of all other β-carbolines of known molecular structure. In (1) and (2) the methoxycarbonyl group is twisted with respect to the planar ring *A* by some 40° [ZK93423: N1—C1—C21—O4 141.1 (2)°; ZK91296: N1—C1—C21—O4 142.3 (3)°], because of the repulsion exerted by the 4-methoxymethyl substituent, in agreement with Abecarnil, the only other 4-substituted structure found in the CSD (Bock *et al.*, 1994), showing an out-of-plane rotation of some 30°. In all other 4-unsubstituted carbolines the ester group is coplanar with ring *A* to within 10°. Conversely, hydrogenation of this ring induces the molecule to adopt a 'butterfly' non-planar conformation (Borea & Ferretti, 1986; Codding, 1983) in which the ester substituent is nearly perpendicular to the mean plane of the ring, leading to the

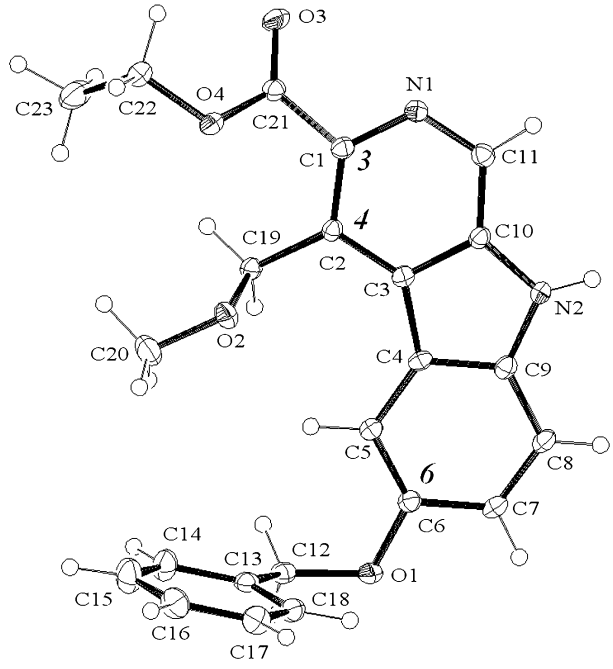


Figure 1
*ORTEP*III (Burnett & Johnson, 1996) view and atom numbering for ZK93423 (1). The displacment ellipsoids are drawn at 40% probability. The molecule is oriented according to (I). The standard position numbering is reported in bold.

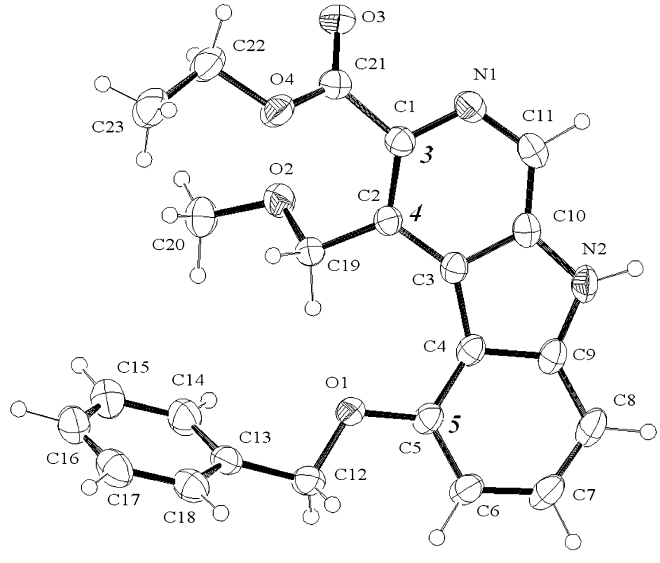


Figure 2
*ORTEP*III (Burnett & Johnson, 1996) view and atom numbering for ZK91296 (2). The displacement ellipsoids are drawn at 40% probability. The molecule is oriented according to (I). The standard position numbering is reported in bold italics.

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: NA5019). Services for accessing these data are described at the back of the journal.

Table 2
A selection of bond lengths (Å) and angles (°) for (1)–(4).

Molecule	(1)	(2)	(3)	(4)
N—C1	1.360 (3)	1.353 (3)	1.355 (2)	1.361 (3)
N1—C11	1.326 (4)	1.328 (4)	1.326 (2)	1.346 (2)
N2—C9	1.382 (3)	1.372 (3)	1.375 (2)	1.380 (2)
N2—C10	1.373 (4)	1.374 (3)	1.376 (2)	1.378 (2)
C1—C2	1.395 (4)	1.396 (3)	1.381 (2)	1.370 (2)
C2—C3	1.402 (5)	1.406 (3)	1.388 (2)	1.398 (2)
C3—C4	1.446 (4)	1.458 (3)	1.441 (2)	1.430 (2)
C3—C10	1.419 (4)	1.417 (3)	1.412 (2)	1.419 (2)
C4—C5	1.413 (3)	1.408 (3)	1.391 (2)	1.405 (2)
C4—C9	1.409 (4)	1.417 (3)	1.412 (2)	1.411 (2)
C5—C6	1.380 (4)	1.397 (3)	1.380 (3)	1.368 (2)
C6—C7	1.408 (4)	1.400 (4)	1.396 (3)	1.418 (3)
C7—C8	1.376 (4)	1.360 (4)	1.376 (3)	1.382 (3)
C8—C9	1.399 (4)	1.403 (4)	1.394 (3)	1.406 (2)
C1—N1—C11	118.8 (2)	118.3 (2)	118.6 (1)	124.8 (2)
C9—N2—C10	108.4 (2)	108.9 (2)	108.5 (1)	108.3 (2)
N1—C1—C2	124.6 (3)	125.0 (2)	124.0 (1)	120.8 (2)
C1—C2—C3	116.4 (3)	116.6 (2)	118.0 (1)	117.6 (2)
C2—C3—C4	134.7 (3)	137.0 (2)	135.9 (2)	134.2 (2)
C2—C3—C10	118.8 (3)	116.9 (2)	117.9 (1)	119.4 (2)
C4—C3—C10	106.4 (2)	105.9 (2)	106.2 (1)	106.4 (2)
C3—C4—C5	133.8 (3)	135.9 (2)	133.9 (2)	133.8 (2)
C3—C4—C9	106.0 (2)	105.9 (2)	106.4 (1)	106.6 (2)
C5—C4—C9	120.3 (2)	117.7 (2)	119.6 (2)	119.5 (2)
C4—C5—C6	117.4 (2)	119.2 (2)	119.2 (2)	118.3 (2)
C5—C6—C7	121.7 (3)	120.3 (2)	120.5 (2)	121.6 (2)
C6—C7—C8	121.5 (2)	122.5 (3)	121.7 (2)	121.8 (2)
C7—C8—C9	117.6 (2)	117.1 (3)	117.9 (2)	116.1 (2)
N2—C9—C4	109.9 (2)	109.6 (2)	109.4 (2)	109.4 (2)
N2—C9—C8	128.6 (3)	127.4 (2)	129.5 (2)	127.9 (2)
C4—C9—C8	121.5 (3)	122.9 (2)	121.1 (2)	122.7 (2)
N2—C10—C3	109.3 (2)	109.6 (2)	109.5 (1)	109.2 (2)
N2—C10—C11	130.7 (3)	128.6 (2)	130.3 (2)	129.0 (2)
C3—C10—C11	120.0 (2)	121.8 (2)	120.2 (2)	121.7 (2)
N1—C11—C10	121.4 (3)	120.8 (3)	121.3 (2)	115.8 (2)

conclusion that there is a significant π-conjugation between the pyridine *A* ring and the *R* function which can be moderately perturbed by bulky 4-substituents. ZK93423 and ZK91926 show the same packing scheme, displayed in Figs. 5 and 6, respectively. The molecules are linked by bifurcated N2—H···O1(N1) hydrogen bonds in chains running along the *b* direction and extended all along the crystal, whose parameters are reported in Table 3. Interestingly, this bifurcated bond is the most common in 3-substituted carboline crystals, as it occurs in the structures of Abecarnil, DMCM (Bertolasi *et al.*, 1990), β-CCM (Bertolasi *et al.*, 1984) and β-CC^{t}Bu (Codding *et al.*, 1988); consequently, it can be argued that a similar interaction could play a role in the ligand–receptor binding.

In (3) (FG7142) the 3-*R* moiety is replaced by a $CONHCH_3$ group which is almost perfectly coplanar with the pyridine ring [N1—C1—C12—N2 −1.9 (2)°]. The N1 atom is involved in a short N(amide)—H···N1 interaction [N···N 2.677 (2) Å; N—H···N 111 (1)°], which can be considered a weak intramolecular hydrogen bond, in agreement with the crystal structure of *N*-ethyl-3-carbamoyl-β-carboline (Muir & Codding, 1984) in which the N···N contact distance is 2.68 Å. The crystal packing (Fig. 7) is determined by chains running along the *a* direction and extending all along the crystal, where molecules are linked by N2—H···O1 bonds (see Table 4); the N···O distance of 2.759 (2) Å is typical of these heteronuclear hydrogen bonds, being in the range 2.71–2.75 Å (Gilli *et al.*, 2000).

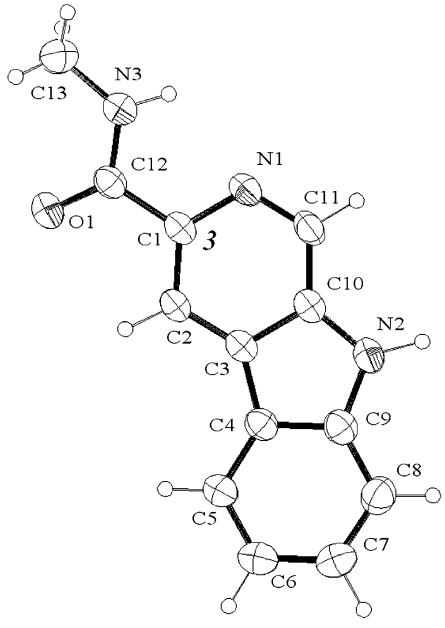


Figure 3
*ORTEP*III (Burnett & Johnson, 1996) view and atom numbering for FG7142 (3). The displacment ellipsoids are drawn at 40% probability. The molecule is oriented according to (I). The standard position numbering is reported in bold.

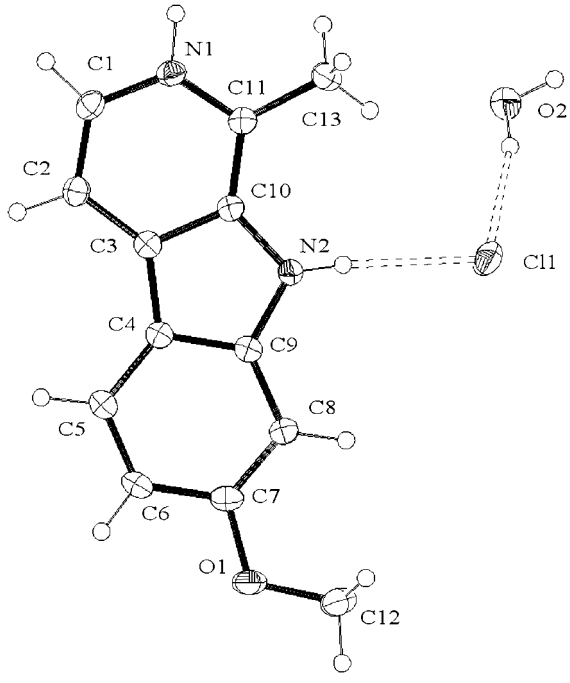


Figure 4
*ORTEP*III (Burnett & Johnson, 1996) view and atom numbering for harmine hydrochloride (4). The displacement ellipsoids are drawn at 40% probability. The molecule is oriented according to (I). The standard position numbering is reported in bold.

Table 3
Hydrogen-bonding parameters (Å, °).

Compound	Hydrogen bond	D—H	$D\cdots A$	H$\cdots A$	D—H$\cdots A$
(1), ZK93423	N2—H$\cdots$N1^{i}	0.85 (3)	3.092 (4)	2.33 (3)	149 (3)
	N2—H$\cdots$O3^{i}	0.85 (3)	2.986 (3)	2.31 (3)	137 (3)
(2), ZK91296	N2—H$\cdots$N1^{ii}	0.91 (3)	3.203 (3)	2.68 (3)	118 (2)
	N2—H$\cdots$O3^{ii}	0.91 (3)	2.970 (3)	2.07 (3)	168 (3)
(3), FG7142	N3—H$\cdots$N1	0.91 (2)	2.675 (2)	2.20 (2)	111 (2)
	N2—H$\cdots$O1^{iii}	0.99 (2)	2.759 (2)	1.84 (2)	153 (2)
(4), harmine	N2—H$\cdots$Cl	0.84 (2)	3.146 (2)	2.31 (2)	174 (2)
	O2—H1$\cdots$Cl	0.96 (9)	3.184 (4)	2.25 (9)	165 (8)
	N1—H$\cdots$Cl^{iv}	0.94 (2)	3.058 (1)	2.12 (2)	171 (2)
	O2—H2$\cdots$Cl^{v}	0.86 (7)	3.112 (4)	2.30 (7)	159 (6)

Symmetry codes used: (i) $2-x, y-\frac{1}{2}, 1-z$; (ii) $2-x, y+\frac{1}{2}, \frac{1}{2}-z$; (iii) $x-\frac{1}{2}, y+\frac{1}{2}, z$; (iv) $x+\frac{1}{2}, -y-\frac{1}{2}, z+\frac{1}{2}$; (v) $1-x, -y-1, 1$.

The asymmetric unit of (4) (harmine hydrochloride) is formed by a N1-protonated harmine molecule, a Cl^- ion and a half water molecule (disordered over two centrosymmetric positions). The packing scheme, reported in Fig. 8, is rather complex: each chloride ion bridges two harmine molecules *via* NH$\cdots$Cl hydrogen bonds forming zigzag ribbons. The N$\cdots$Cl distances (Table 3) lie in the range 2.91–3.52 Å, typical of this type of interaction, as reported by Jeffrey & Saenger (1991), and, according to the mostly electrostatic nature of the bond, the shortest distance [3.058 (1) Å] is the one involving the charged N1 atom. The ribbons are linked in a three-dimensional hydrogen-bonding network by the water molecules and bridge the anions at OH$\cdots$Cl distances of 2.25 (9) and 2.30 (7) Å.

3.2. Factors affecting affinity

The final results of the Free–Wilson analysis are given in Table 4 which reports, together with the statistical parameters of the regression, the individual group contributions, a_{ij}, of substituents i at the six positions j. Linearly dependent substituents chosen to assure the constraint condition $\Sigma_i a_{ij} = 0$ for any position j are marked by an asterisk.

In general, affinity is strongly affected by substitutions in positions 3 and 6 (Table 4), as evidenced by the large range of a_{ij} coefficients found for these positions (Δa_{ij} = 2.78 and 2.83, respectively). Positions 1, 4, 5 and 7 appear to be less important, although the presence of an ether group in position 4 seems to slightly enhance the binding affinity with respect to the unsubstituted molecule. As for position 3, high affinities are always associated with groups able to act as hydrogen-bond acceptors (ester carbonyl or CN). This strongly suggests that the C=O group is a primary site for β-carboline binding in analogy with what proposed for BDZs and the antagonist Ro151788 [see (I)]. Among $-R$ groups, the affinity appears to be related to both the length and linearity of the alkyl R chain, decreasing in the order $C_2H_5 > CH_2CH_2CH_3 > CH_3 > CH(CH_3)_2$) [COO*i*Pr (*aij* = 0.10), COO*n*Pr (*aij* = 0.61)]. Linear groups of moderate length are associated with the highest values. Substitution at position 3 by amide groups results in a considerable loss of affinity (*aij* values −1.36, −1.93) in spite of the presence of the required carbonyl group. This fact is to be ascribed to the wrong anti-conformation assumed by the −CONH*R* group because of the intramolecular N—H$\cdots$N short contact forming between N(amide)-H and β-carboline N1 atom, as clearly indicated by the structures of FG7142 (Fig. 2) and CITROM10 (Muir & Codding, 1984), in agreement with previous experimental evidence (Dorey *et al.*, 1989) that constrained β-carbolines having the carbonyl in an *anti* conformation bind much less than the normal compounds.

The most interesting substituents in position 6 from a pharmacological point of view are OCH_3 [able to enhance the binding affinity and present in DMCM; (1*f*) in (I)] and O—CH_2—Ph [present in ZK93423; (1*d*) in (I)] groups. Although

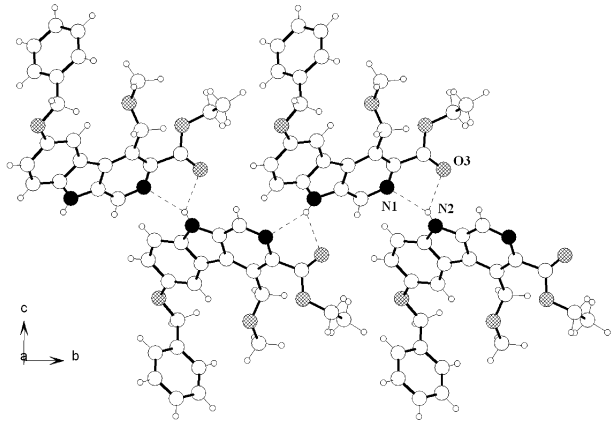


Figure 5
Packing scheme for ZK93423. Hydrogen bonds are drawn as dashed lines.

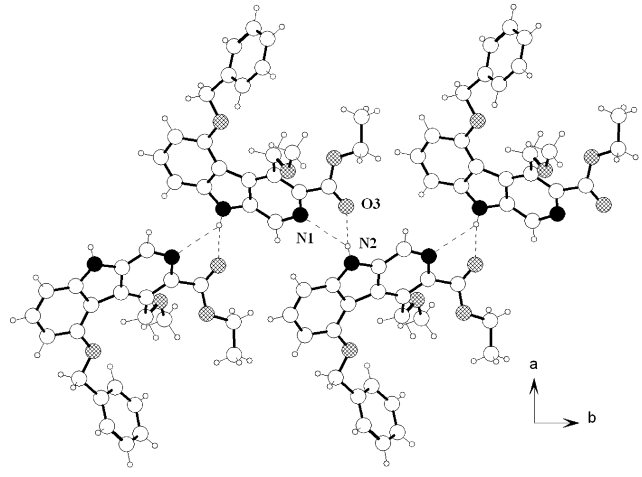


Figure 6
Packing scheme for ZK91296. Hydrogen bonds are drawn as dashed lines.

Table 4
Individual group contributions, a_{ij}, of substituent i present at positiom j as obtained from the Free–Wilson analysis.

$N = 32$, $r = 0.983F = 9.90$; explained variance = 87%; $\mu = -131$. Substituents marked by an asterisk are those chosen as linear dependent variables because of the constraint $\Sigma_i a_{ij} = 0$.

a_{i1}		a_{i3}		a_{i4}		a_{i5}		a_{i6}		$\forall_{i7}$	
CH_3*	0.09	$COOC_2H_5$	0.85	CH_2OCH_3	0.16	OCH_2Ph*	0.25	OCH_3	0.80	**H**	**0.01**
H	**−0.01**	CN	0.83	**H**	**−0.07**		**−0.01**	OH*	0.64	OCH_3*	−0.19
		COO(*n*Pr)	0.61	CH_2CH_3*	−0.38			**H**	**0.32**		
		$COOCH_3$	0.42					NO_2	0.32		
		COO(*i*Pr)	0.10					OCH_2Ph	−0.02		
		NCS	0.03					O(*n*Pr)	−0.14		
		O(*n*Pr)	−0.19					OCH_2-1-Napht	−0.57		
		$NHCSOCH_3$*	−0.77					$O(CH_2)_7CH_3$	−2.04		
		NH_2	−1.11								
		$CONHCH_3$	−1.36								
		$CONH_2$	−1.93								
		H	**−1.93**								
$\Delta a_{ij} = 0.10$		2.78		0.23		0.25		2.83		0.20	

bulky substituents appear to reduce the affinity capability, the $O-CH_2-Ph$ group is only slightly detrimental, most probably because of its large conformational freedom, thus allowing geometries not dissimilar from that shown in (1*d*) in (I).

3.3. Factors affecting intrinsic activity

To date, four carboline derivatives are known to display agonistic behaviour: ZK93423 (*f*AG), Abecarnil (*p*AG), ZK91296 (*p*AG) and 6-PCB (*p*AG), having, in their full extended conformation, strictly comparable molecular volumes in the range 313.1–366.7 Å^3 (calculated using the program *Alchemy*2000; Tripos Inc., 1997). Comparison of the four structures shows that they have some specific structural features in common, accounting for their agonistic behaviour:

(i) All molecules are substituted in positions 5 (ZK91296) or 6 (ZK93423, Abecarnil and 6-PCB) of ring *C* by a large hydrophobic group with much conformational freedom, evidenced by the comparison of their crystal structures. Abecarnil, in particular, crystallizes in three polymorphic forms (Bock *et al.*, 1994) in which the 6-benzyloxy group adopts different conformations, providing a good example of *conformational polymorphism* according to Bernstein's definition (Bernstein, 2002). According to the model of (I), this 6-bulky substituent can be easily oriented in the AG hydrophobic region, as it is shown by the superimposition of the full agonist ZK93423 and Oxazepam (Gilli *et al.*, 1978) of Fig. 9. In Fig. 9(*a*) the crystallographic structures are reported, while in Fig. 9(*b*) the two molecules are shown after a flexible alignment procedure (Molecular Operating Environment, 2003) that provides the best superimposition of the 6-benzyloxy substituent with the benzodiazepine-phenyl ring, using a carboline conformer whose energy is not higher than 8.36 kJ mol^{-1} with respect to the energy of the conformational absolute minimum. The volumes of superimposing rings are shown as Connoly dot-surfaces. Conversely, ZK91296, the 5-substituted positional isomer of the full agonist ZK93423, displays only a partial-agonist character, probably because the ether group in (4) limits the free rotation of the benzyloxy group. Moreover, comparison between ZK93423 and the partial agonist 6-PCB indicates that aromatic derivatives are to be preferred, suggesting a probable molecule–macromolecule interaction in this region *via* a π–π stacking.

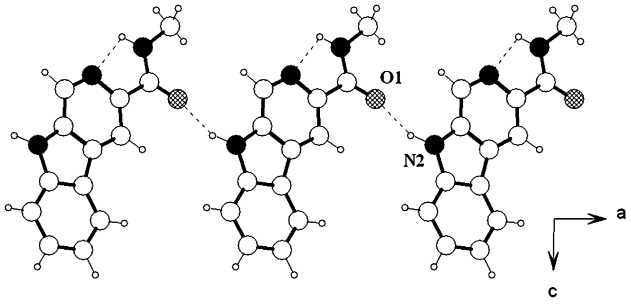


Figure 7
Packing scheme for FG7142. Hydrogen bonds are drawn as dashed lines.

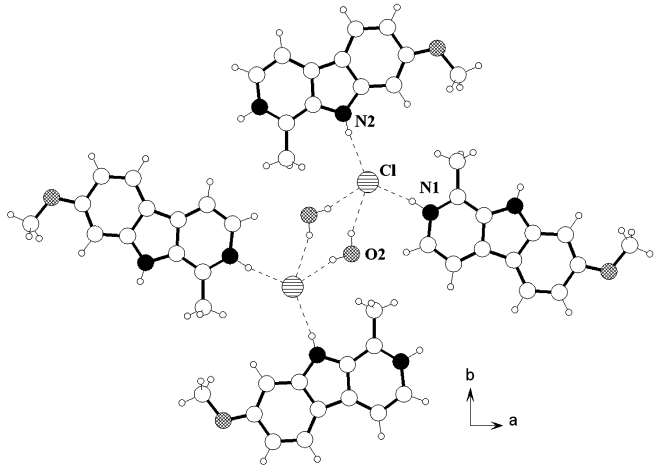


Figure 8
Packing scheme for harmine hydrochloride. Hydrogen bonds are drawn as dashed lines.

Table 5
Binding constants, GABA and PAL ratios for the molecules of (I).

GABA ratio = IC_{50} without GABA/IC_{50} with GABA; PAL ratio = IC_{50} in photoaffinity-labelled membranes/IC_{50} in non-photoaffinity-labelled membranes.

Compound	Intrinsic activity	K_i (nM)	GABA ratio	PAL ratio
Oxazepam	Agonist	8[a]	2.35[a]	97[a]
CGS9896	Partial agonist	0.3[a]	1.20[a]	1.2[a]
Ro151788	Antagonist	4.0[b]	1.05[b]	1.1[a]
ZK91296	Partial agonist	1.26[b]	1.18[b]	1.7[a]
ZK93423	Full agonist	1.19[b]	1.30[b]	1.2[c]
β-CCM	Inverse agonist	9.5[b]	0.72[b]	1.1[a]
DMCM	Inverse agonist	7.6[b]	0.60[b]	1.0[c]

† References: (*a*) McKernan & Whiting (1996); (*b*) Gessi *et al.* (1999); (*c*) P. A. Borea, unpublished results.

(ii) All molecules are substituted in position 4 of ring *C* with a methoxymethyl group. According to our model, it can be assumed that the oxygen is involved in a hydrogen bond [see (I*a*), D2], being located in the same zone as the N atom of the diazepinic ring. This hypothesis is confirmed by the fact that the ZERHOT molecule (Codding *et al.*, 1995), substituted in 6 by a $NH-CH_2-Ph$ group but with no substituent in position 4, is found to act as an antagonist.

(iii) All molecules are substituted in position 3 with ester groups carrying a linear and relatively short alkyl chain, since the replacement of COOEt with COO(*i*Pr) in Abecarnil is sufficient to make it a partial agonist. Moreover, as discussed above, all the ester groups show a noticeable out-of-plane *R* twisting.

Carbolines displaying inverse-agonist properties are DMCM, β-CCM, β-CCE and FG7142 [see (II)], out of which only DMCM is considered to be a full inverse agonist. It seems that the structural characteristics able to modulate the intrinsic activity are the shortening of the alkyl chain of the ester in the 3 position with respect to AGs and ANTs, and the presence of a methoxy group in position 7, which defines the specific interaction zone marked IAG in (I).

As for the antagonists they can be identified as those molecules whose substituents can partially occupy AG1, AG2 and/or IAG zones, a typical example being Ro151788 [see (I*b*)]. The ANT carbolines reported in (II) have long chains in the 3-position (PRCC and β-CCBu) which, according to the proposed model, are located in AG1 or an ether 5-substituent which is partially allocated in AG2.

The above considerations, based on structural as well as drug–receptor binding experimental data, seem to reasonably validate the stereochemical model presented in (I). Further support for the model comes from the analysis of the data in Table 5 which reports, for the molecules given in (I), together with the binding data and the GABA ratios, the PAL ratios (*i.e.* the ratio between IC_{50} measured in photoaffinity-labelled membranes and IC_{50} measured in non photoaffinity-labelled membranes). It is clear from the data of Table 5 that the labelling procedure strongly affects the binding of the benzodiazepine Oxazepam, while it has almost no effect on the binding of carbolines and Ro151788. This suggests that these molecules must interact with a different zone of the binding pocket. This is in agreement with the benzodiazepine/carboline relative orientation proposed by our model.

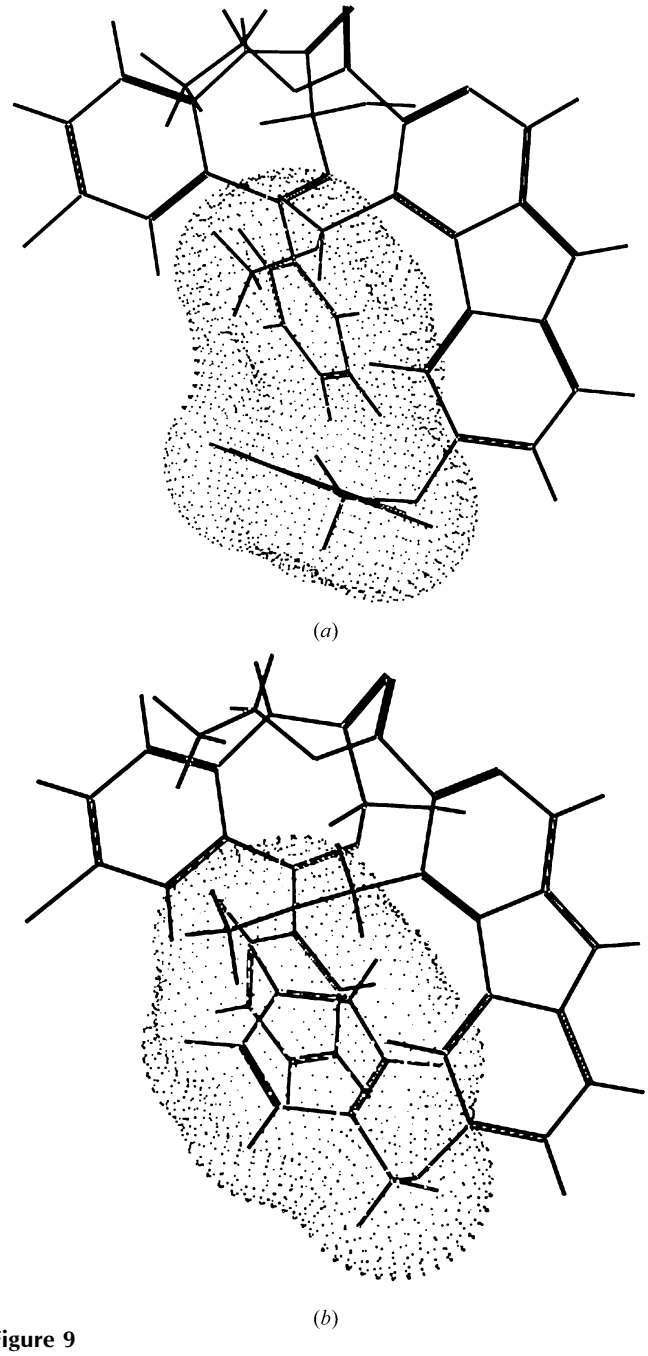


Figure 9
Superposition of the crystal structures of Oxazepam and ZK93423 (*a*) before and (*b*) after a flexible alignment procedure. The molecules are oriented according to (I). The Connoly surfaces of the aromatic substituents occupying the AG1 region are shown as dot-surfaces.

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