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Note

Lipophilicity measurement of benzodiazepine-receptor ligands by reversed-phase liquid chromatography

Comparison between high-performance liquid and thin-layer chromatography

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Benzodiazepines (BDZs) are a class of drugs endowed with a wide spectrum of anxiolytic, sedative–hypnotic, anticonvulsant and muscle relaxant properties. A few years ago, specific receptors for these drugs were discovered in the mammalian CNS[1,2]. More recently, several new drugs, chemically unrelated to BDZs, have been found that are able to interact with BDZ receptors. They show a spectrum of biological activities ranging from compounds having full BDZ-like properties (agonists), to those with completely opposite actions (inverse agonists), and finally to the antagonists able to bind to the receptor without producing any definite pharmacological effect. These compounds belong to several chemical classes, namely β-carbolines, cyclopyrrolones, pyrazoloquinolines, imidazobenzodiazepines and triazolopyridazines[3].

Many studies have been devoted to the structure–affinity relationships of the compounds interacting with BDZ receptors[4–12]; in particular, BDZs and β-carbolines have been extensively studied.

It has been suggested[12] that BDZ receptor ligands exert their action by interacting with an unique, diffuse and substantially planar recognition site; the main drug–receptor interactions should be mediated by carbonyl or imine groups via hydrogen bonds. The observed differences in pharmacological profiles should be accounted for by the various localizations of the different ligands inside this unique binding site. The lipophilic character seems to play a significant role in determining receptor binding affinities of BDZs and β-carbolines[5,13]. On the other hand, many physico-chemical factors, *e.g.*, capability of forming hydrogen bonds, planarity of the molecules, steric hindrance of substituents and electronic factors[6–13], are involved in the receptor binding affinity of all BDZ receptor ligands. Therefore, it appears very intriguing to single out in a quantitative way the role played by the hydrophobicity of compounds. In this work, by means of reversed-phase thin-layer (TLC) and

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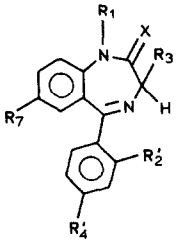
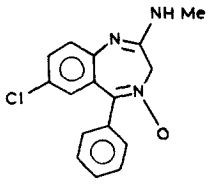
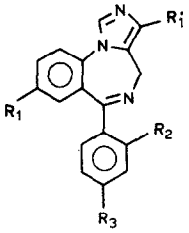
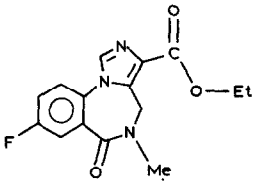
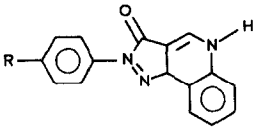
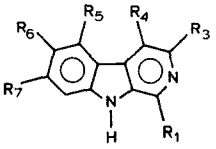
TABLE I

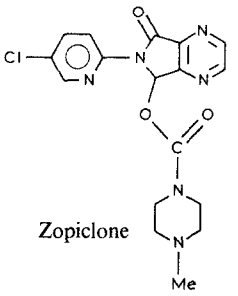
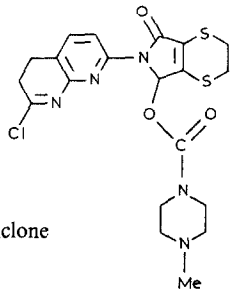
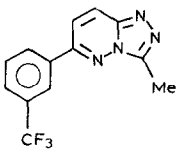
STRUCTURES OF BENZODIAZEPINE-RECEPTOR LIGANDS

Me = methyl; Et = ethyl; Pr = propyl; Ph = phenyl.

Chemical class	*Structure*
Benzo-diazepines	  Chlordiazepoxide Diazepam : R_1 = Me; R_7 = Cl; X = O Oxazepam : R_3 = OH; R_7 = Cl; X = O Prazepam : R_1 = CH_2-cC_3H_5; R_7 = Cl; X = O Medazepam : R_1 = Me, R_7 = Cl; X = H_2 Flunitrazepam : R_1 = Me; R_7 = NO_2; R'_2 = F; X = O
Imidazobenzo-diazepines	  RO 15-1624: R'_1 = $COOC_2H_5$; R_1 = R_2 = R_3 = H RO 15-8670: R_1 = Cl; R'_1 = $COOC_2H_5$; R_2 = R_3 = H RO 22-9735: R_3 = Cl; R'_1 = $COOC_2H_5$; R_1 = R_2 = H RO 21-8384: R_1 = R_2 = Cl; R'_1 = $CONH_2$; R_3 = H
Pyrazolo-quinolines	 CGS 9896: R = Cl CGS 8216: R = H
β-Carbolines	 βCCM : R_3 = COOMe; R_1 = R_4 = R_5 = R_6 = R_7 = H βCCE : R_3 = COOEt; R_1 = R_4 = R_5 = R_6 = R_7 = H PrCC : R_3 = COOnPr; R_1 = R_4 = R_5 = R_6 = R_7 = H Harman : R_1 = CH_3; R_3 = R_4 = R_5 = R_6 = R_7 = H ZK93423 : R_3 = COOEt; R_4 = CH_2OCH_3; R_6 = OCH_2Ph; R_1 = R_5 = R_7 = H

(*Continued on p. 406*)

TABLE I (*continued*)

Chemical class	*Structure*
Cyclopyrrolones	 Zopiclone
	 Suriclone
Triazolo-pyridazines	 Cl 218872

high-performance liquid chromatographic (HPLC) systems, we have measured the lipophilic character of some representative compounds belonging to the benzodiazepines, β-carbolines, cyclopyrrolones, triazolopyridazines, pyrazoloquinolines, phenylquinolines and imidazobenzodiazepines (Table I). The purpose was to determine the range of lipophilicity of compounds interacting with BDZ receptors and to compare the chromatographic systems.

EXPERIMENTAL

Materials

Imidazobenzodiazepines were a gift from H. Möhler (Hoffman-La Roche, Basle, Switzerland); β-CCM, β-CCE, PrCC and ZK 93423 were a gift from R. Schmiechen (Schering, Berlin, F.R.G.); Zopiclone and Suriclone were donated by Rhone-Poulenc (Vitry-sur-Seine, France); CGS 8216 and CGS 9896 were donated by Ciba-Geigy (Basle, Switzerland); and CL 218-872 was provided by American Cyanamide (Pearl River, U.S.A.). All other drugs and chemicals (analytical-reagent grade) were obtained from commercial sources.

Chromatography

The HPLC measurements were performed on a Spectra-Physics chromatograph consisting of an SP 87000 solvent delivery system and an SP organizer module. A Varian Aerograph UV detector operated at 254 nm was used. A 30 mm × 3.9 mm I.D. μBondapak C_{18} column from Waters Assoc. was used. The mobile phase was acetonitrile in various mixtures with phosphate buffer (pH = 7.0; ionic strength = 0.05 *M*) in the concentration range 30–70%. The TLC determinations were carried out on Whatman KC18F plates. A Camag Nanomat (Camag, Berlin, F.R.G.) was

used to spot 100 nl of the solute in methanol on the plates. The solutes were detected under UV light (254 nm). Mixtures of acetonitrile–phosphate buffer in the concentration range 30–65% and methanol–phosphate buffer in the 55–90% range were used as mobile phases. The R_M values for compounds 17–21 in the methanol systems had been determined previously[13]. Each HPLC and TLC measurement was replicated at least three times.

RESULTS AND DISCUSSION

In the HPLC system the linear relationship between log k' and acetonitrile concentration in the mobile phase allowed the calculation of theoretical log k' values at 0% acetonitrile (Table II). Similarly, theoretical R_M values were calculated in both TLC systems (Table II).

The log k' and R_M values in Table II were used in order to evaluate eqns. 1–3.

$$R_{M(CH_3CN)} = 0.177\ (\pm 0.177) + 0.885\ (\pm 0.080) \log k'_{(CH_3CN)} \quad (1)$$

$(n = 21; r = 0.931; s = 0.142; F = 123.87; P < 0.005)$

$$R_{M(CH_3OH)} = 0.033\ (\pm 0.297) + 1.431\ (\pm 0.138)\ R_{M(CH_3CN)} \quad (2)$$

$(n = 21; r = 0.922; s = 0.234; F = 107.41; P < 0.005)$

$$R_{M(CH_3OH)} = -0.022\ (\pm 0.227) + 1.406\ (\pm 0.102) \log k'_{(CH_3CN)} \quad (3)$$

$(n = 21; r = 0.954; s = 0.182; F = 190.74; P < 0.005)$

The R_M and log k' values were fairly similar when the mobile phase in both TLC and HPLC systems was based on acetonitrile. In fact, eqn. 1 has an intercept and slope close to 0 and 1, respectively, with a good correlation coefficient. On the other hand, eqn. 2 shows a similar correlation coefficient between the R_M values determined with methanol or acetonitrile in the mobile phase of the TLC system. However, the slope of eqn. 2 is greater than 1, which means that the extrapolated R_M values in the two systems are different. In fact, in the system with methanol in the mobile phase the R_M values range from 1.78 to 4.53, whereas with acetonitrile the R_M values range from 1.34 to 3.17. In other words, in the meethanol system there is a much wider range of R_M values. As a consequence, while describing a good correlation between the TLC and HPLC systems, eqn. 3 indicates again the wider range of R_M values in the TLC system with methanol in the mobile phase.

In our previous studies it was possible to show that the extrapolated R_M values were very similar when the mobile phase contained either methanol or acetone. Therefore, those data were considered as supporting the hypothesis that the extrapolated R_M values were really an expression of the partitioning between water and silicone oil. This does not seem to be so with acetonitrile in the mobile phase. This

TABLE II

LIPOPHILIC CHARACTER OF BENZODIAZEPINE-RECEPTOR LIGANDS

No.	*Compound*	$R_{M(CH_3OH)}$	$R_{M(CH_3CN)}$	*Log* $k'_{(CH_3CN)}$
1	CGS 9896	3.47	2.19	2.30
2	CGS 8216	2.41	1.88	1.96
3	Zopiclone	2.29	1.88	1.80
4	Suriclone	3.23	2.25	2.27
5	CL 218872	3.07	2.38	2.14
6	RO 15 1788	1.78	1.34	1.19
7	RO 15 1624	3.28	1.98	2.22
8	RO 15 8670	3.38	2.35	2.34
9	RO 22 9735	3.58	2.48	2.42
10	RO 21 8384	2.87	1.83	2.16
11	Diazepam	2.91	2.12	2.17
12	Flunitrazepam	2.71	1.95	1.97
13	Chlordiazepoxide	2.82	1.94	2.21
14	Oxazepam	2.30	1.45	1.65
15	Prazepam	3.45	2.42	2.47
16	Medazepam	4.53	3.17	3.26
17	β-CCM	2.92	2.02	1.93
18	β-CCE	3.50	2.12	2.36
19	PrCC	3.54	2.45	2.64
20	ZK 93423	2.93	2.02	2.28
21	Harman	3.35	2.15	2.33

could be due to some kind of interaction between acetonitrile and the stationary phase. However, as eqn. 1 shows that the R_M and log k' values are very similar, such an interaction should act in the same way in both TLC and HPLC. In fact, Braumann *et al.*[14], in describing the relationship between the log k' values of phenylurea herbicides with methanol in the mobile phase and those with acetonitrile, found an equation very similar to our eqn. 2 with a correlation of 0.939.

Obviously this study should be expanded to other series of chemicals in order to find out if there is a more general meaning.

The compounds in Table I belong to six classes. In Table II it can be seen that the examined compounds show quite different R_M values in the methanol system. However, when compounds 16 and 6, with the highest and lowest R_M values, respectively, are excluded the R_M values in the methanol system range from 2.29 to 3.58. This means that the compounds able to interact with the BDZ receptors are characterized by a narrow range of lipophilic character. This could suggest that lipophilic character plays only a secondary role in providing higher binding affinities to BDZ receptors.

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