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Role of hydrophobicity on the monoamine receptor binding affinities of central nervous system drugs: a quantitative retention–activity relationships analysis using biopartitioning micellar chromatography

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Abstract

Biological action and activity reflect an aspect of the fundamental physicochemical properties of the bioactive compounds. As an alternative to classical QSAR studies, in this work different quantitative retention–activity relationships (QRAR) models are proposed, which are able to describe the role of hydrophobicity on the binding affinity to different brain monoamine receptors (H_1-histamine, α_1-noradrenergic and 5-HT_2-serotonergic) of different families of psychotherapeutic drugs. The retention of compounds is measured in a biopartitioning micellar chromatography (BMC) system using Brij-35 mobile phases. The adequacy of the QRAR models developed is due to the fact that both the retention of compounds in BMC and the drug–receptor interaction are described by the same hydrophobic, electronic and steric properties of compounds. The obtained results indicate that, for structurally related compounds that present the same molecular features as the basic pharmacophore, there is a retention range in which compounds present the highest affinity to all of monoamine receptors.

Keywords: Hydrophobicity; Quantitative retention–activity relationships; Biopartitioning micellar chromatography; Monoamine receptor

1. Introduction

A drug's mechanism of action is due to the interaction between the drug molecule and the molecules composing the biological target. The effects of biologically active compounds can be explained on basis of molecular interactions in terms of molecular structures or physicochemical properties of the molecules involved.

Ligand–receptor binding is usually reversible, and may be due to different types of forces between drug molecules and their receptors: electrostatic attraction, Van der Waals and hydrophobic forces. Among the interactions, the ion–ion type is the most important to many drugs at physiological pH where functional groups of the drug molecule may be ionized. The energy associated with this binding is about 17–33 kJ/mol. The importance of hydrophobic binding is due to the entropic changes observed when an interaction between the non-polar moieties of the molecules involved takes place. The energy associated to this binding is of 2.9 kJ/mol every $–CH_2–$ group and 8.4 kJ/mol every benzene ring. Summarizing, the drug–receptor binding energy has an enthalpy component that includes the electronic and Van der Waals forces and an entropy one, may be the most important, associated with hydrophobic interactions due to changes in the molecular degrees of freedom.

Steric effects are also important in the drug–receptor complex formation. The differences in biological activity between optical isomers are dependent on their ability to selectively react with a biological system, which is highly conditioned by the molecule geometry and configuration.

Compounds acting on the same receptor must be complementary to this receptor, which implies that they are structural and chemically related. However, there is still a certain degree of freedom for structural variation among compounds interacting with common receptors. For such drugs particular relationships may be found for structure and action [1].

These structure–activity relationships have been proposed by modern medicinal chemistry as an alternative to "in vivo" measurements. The usual physicochemical parameter employed in QSAR studies is the octanol–water

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partition coefficient (log *P*). In some cases, the steric factor and/or electronic effects are also important to describe the biological behavior of drugs.

The dynamic pharmacokinetic/pharmacodynamic processes of drug action are considered to have much in common with the processes on which chromatographic separations are based. The same molecular features (hydrophobicity, electrical charge and steric effects) affect not only transport processes and drug–biological target interactions, but also the compound retention in a chromatographic system under specific experimental conditions. The application of chromatographic parameters to quantitative structure–activity relationships has allowed the development of quantitative retention–activity relationships (QRAR) [2–5]. This approach that uses an unique parameter as independent variable may be an alternative to QSAR models in order to obtain an estimation or, at least, useful qualitative information about drug activity.

In this setting, our research group has demonstrated that the chromatographic system comprising a hydrophobic stationary phase and saline solutions of Brij-35 micelles as mobile phase can be used as a system to model drug biopartitioning [6–8]. We have named this methodology biopartitioning micellar chromatography (BMC). The success of QRAR models based on BMC could be attributed to the similarities between BMC systems and biological barriers and extracellular fluids [9–11]. This methodology has been applied for describing and predicting the biological activity of different pharmacological kinds of drugs [12], permeability across the intestinal barriers, blood–brain barrier and cornea [13].

From the beginning of psychopharmacology in 1950, when chlorpromazine was synthesized, the participation of biogenic amines and their corresponding CNS receptors in mental diseases was established. Receptor binding properties of drugs are determined by radioligand binding techniques using animal tissue (mainly rat brain) as the source of receptors. The therapeutic action of CNS drugs is related to their ability to modify the affinity of biological targets to their corresponding ligand (neurotransmitter). But CNS drugs also act at level of other different receptors, which is responsible for the adverse effects of drugs (although some of them can be used for therapeutically purposes) as well as their interactions with other drugs [14]. Taking into account these points it can be concluded that precise estimation of the binding constant of a ligand to one receptor allows one to obtain information about the relative potency of a particular compound and its possible undesirable consequences derived from it use. This fact is an important aspect of structure-based drug design.

In this paper, QRAR models for evaluating the role of hydrophobicity on binding affinities of CNS drugs to H_1-histaminic, α_1-adrenergic and 5-HT_2 serotonergic receptors are proposed. The drugs considered belong to one of the following groups: neuroleptics, tricyclic antidepressants and H_1-antihistamines. All of them are structurally related compounds that possess a special stereochemical and electronic feature: the aromatic ring and the nitrogen moieties, which are the primary binding groups to the receptors involved in this work. It is necessary to remark that the QRAR models developed comprise all those compounds with a proved therapeutic activity. Thus, in order to obtain meaningful estimates, the structural features of the new drug, including stereospecific ones (when required), must be represented in the original database from which the models were developed, i.e. interpolation within families of molecules is possible but extrapolation to a distant chemotype in not reliable.

2. Experimental

2.1. Instruments and measurements

The retention of the CNS drugs was measured using a Hewlett-Packard 1100 chromatograph comprised of an isocratic pump, a Rheodyne valve with a 20 μl loop; (Cotati, CA), thermostat, variable wavelength UV absorbance detector operated at 220 nm (tricyclic antidepressants and butyrophenones), 240 nm (antihistamines) or 254 nm (phenothiazines) and reversed-phase columns packed with 5 μm kromasil octadecyl silane, C_{18} (50 mm × 4.6 mm, i.d.). The mobile phase was pumped at a flow-rate of 1.5 μl/min (antihistamines) or 1 μl/min (rest of compounds). Data acquisition and processing were performed on an HP-Chemstation software (A0402, 1996).

All the assays were carried out at 36.5 °C. The retention factor, k, values were averages of the triplicate determinations. The relative standard deviations of log *k* values ranged between 0.1 and 0.9%.

2.2. Chemicals

The micellar mobile phases were prepared from polyoxy-ethylene-23 lauryl ether, Brij-35 (Acros Chimica, Geel, Belgium) at concentrations of 0.02, 0.04 and 0.06 M buffered at pH 7.4 with 0.05 M phosphate buffer (analytical reagent, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, 9.2 g/l NaCl (purissim, Panreac) was added to the mobile phases. They solutions were filtered through a nylon membrane filter (0.45 μm; Micron Separations, Westboro, MA) before use.

The CNS drugs were obtained from Spanish commercial pharmaceutical preparations (amitriptyline, *Tryptizol*, Merck Sharp and Dhome, Madrid; amoxapine, *Demolox*, Lederle, Madrid; clomipramine, *Anafranil*, Geigy, Barcelona; dothiepin, *Prothiaden*, Alter, Madrid; doxepin, *Sinequan*, Pfizer, Madrid; imipramine, *Tofranil*, Novartis, Barcelona; loxapine, *Desconex*, Alonga, Madrid; maprotiline, *Ludiomil*, Novartis; mianserin, *Lantanon*, Organon, Barcelona; trimipramine, *Surmontil*, Rhone-Poulenc Rorer, Madrid; chlorpromazine, *Largactil*, Rhone-Poulenc Rorer;

clozapine, *Leponex*, Sandoz-Pharma, Barcelona; thioproperazine, *Majeptil*, Rhone-Poulenc Rorer) or purchased from Sigma–Aldrich s.a. (Madrid, Spain) (chlorpheniramine, brompheniramine, doxylamine, chloropyramine, clemastine, antazoline, carbinoxamine, chlorcyclizine, cinnarizine, ketotifen, methapyrilene, orphenadrine, pyrilamine, promethazine, cyclizine, triprolidine) and Guinama (Valencia, Spain) (hydroxyzine, tripelennamine, cyproheptadine and diphenhydramine). Some of them were kindly donated by different laboratories: dimethindene (Novartis, Nyon, Switzerland) nortriptyline (Lilly, Madrid, Spain), perphenazine (Merck s.a., Barcelona, Spain) and the butyrophenones used (benperidol, bromperidol, droperidol, fluanisone, haloperidol, penfluridol, pipamperone) (Janssen-Pharma).

Stock solutions of tricyclic antidepressants and phenothiazines were prepared in micellar solutions of 0.04 M Brij-35. Stock solutions of butyrophenones and antihistamines were prepared using methanol as solvent. Working solutions were prepared by dilution of the stock solutions using mobile phase. The solutions were injected into the chromatographic system after filtering through 0.22 μm nylon membranes (Micron Separations, Westboro, MA). Barnstead E-pure deionized water (Sybron, Boston, MA) was used throughout.

2.3. Software, data processing and evaluation of the QRAR models predictive ability

Excel 7.0 from Microsoft Office software was used to perform the statistical analysis of the regressions. To evaluate the adequacy of the models, the fit error (root-mean-square error of calibration, RMSEC), the prediction error based on cross-validation (root-mean-square error of cross-validation, RMSECV), parameter that includes both interpolation and extrapolation information [15], and the RMSECVi [16] for measuring only interpolation information, were compared.

3. Results and discussion

The retention of the compounds included in Table 1, was measured using 0.02, 0.04 and 0.06 M Brij-35 mobile phases. The pH was adjusted to 7.4 to obtain experimental conditions as close as possible to physiological ones. All the CNS drugs studied (Fig. 1) are tertiary or secondary amines, include policyclic structures, and the molecular masses range between 225 g/mol (diphenhydramine) and 523.97 g/mol (penfluridol). These compounds are highly hydrophobic, and at physiological pH they most are positively charged (excepting of dimetindene, loxapine, mianserin and trimipramine which are mostly in their uncharged form).

As we commented previously, all of the compounds considered in this work have been demonstrated to show the binding affinities studied, which implies that all of them present the required structural and stereochemical features that allow them to bind a certain receptor. The molecular features hydrophobicity, total charge and steric properties affect not only the drug–receptor interaction, but also drug retention in the BMC system. Most of the compounds considered in this paper are positively charged, thus the differences observed between the compounds' retention can be explained in terms of hydrophobicity and, to a lesser extent, steric

Table 1
Logarithm of octanol–water partition coefficient and dissociation constants (pK_a) values of the psychotherapeutic drugs studied

CNS drug	log *P*	pK_a
Antidepressants		
Amitriptyline	4.64	9.42
Amoxapine	3.89	7.6
Clomipramine	5.19	9.38
Desipramine	3.97	10.44
Doxepine	3.88	9.0
Imipramine	4.53	9.5
Loxapine	4.75	6.6
Maprotiline	4.22	10.5
Mianserine	4.26	7.1
Nortriptyline	4.32	9.7
Trimipramine	4.73	6.77[a]
Phenothiazines		
Chlorpromazine	5.20	9.30
Chlorprothixene	5.30	7.60
Clozapine	4.27	8.0
Fluphenazine	5.90	3.9; 8.1
Perphenazine	5.57	7.8
Pimozide	6.30	7.3; 8.6
Prochlorperazine	6.15	3.78; 8.1
Trifluoperazine	6.48	8.1
Trimeprazine	4.59	9.0
Thioridazine	6.42	9.50
Thiothixene	4.80	7.67; 7.9
Antihistamines		
Antazoline	4.25	2.5; 10.1
Brompheniramine	2.88	9.79
Carbinoxamine	2.17	8.10
Chlorcyclizine	4.68	2.12; 8.15
Chlorpheniramine	2.73	9.16
Cyproheptadine	4.92	8.87
Diphenhidramine	3.36	9.00
Dimethindene	3.42	6.58[a]
Ketotifen	3.56	8.24
Methapyrilene	2.50	3.7; 8.90
Oxatomide	5.42	8.00
Phenindamine	3.74	8.30
Promethazine	4.65	9.10
Pyrilamine	2.77	4.02; 8.92
Tripelennamine	2.85	4.2; 8.71
Triprolidine	3.47	6.50; 9.5
Butyrophenones		
Benperidol	3.91	4.17
Bromperidol	3.95	8.65
Droperidol	3.5	7.6
Fluanisone	3.02	–
Haloperidol	3.52	8.3
Penfluridol	6.98	–
Pipamperone	1.84	8.28

[a] Measured potentiometrically in Brij35 medium.

contributions. [17]. Taking into account all these considerations, relationships between binding affinities and BMC retention can be mainly explained in terms of hydrophobicity.

Moreover, it is important to make a few points about the QRAR models developed. The micellar media used cannot distinguish between enantiomers and/or diastereoisomers; then, for a specific compound, all the corresponding optical isomers are going to present the same retention. Therefore, we are going to assign the retention data obtained to the active form of the compound.

Table 2 shows the BMC retention data of the available CNS drugs, log *k*, and their corresponding binding affinities

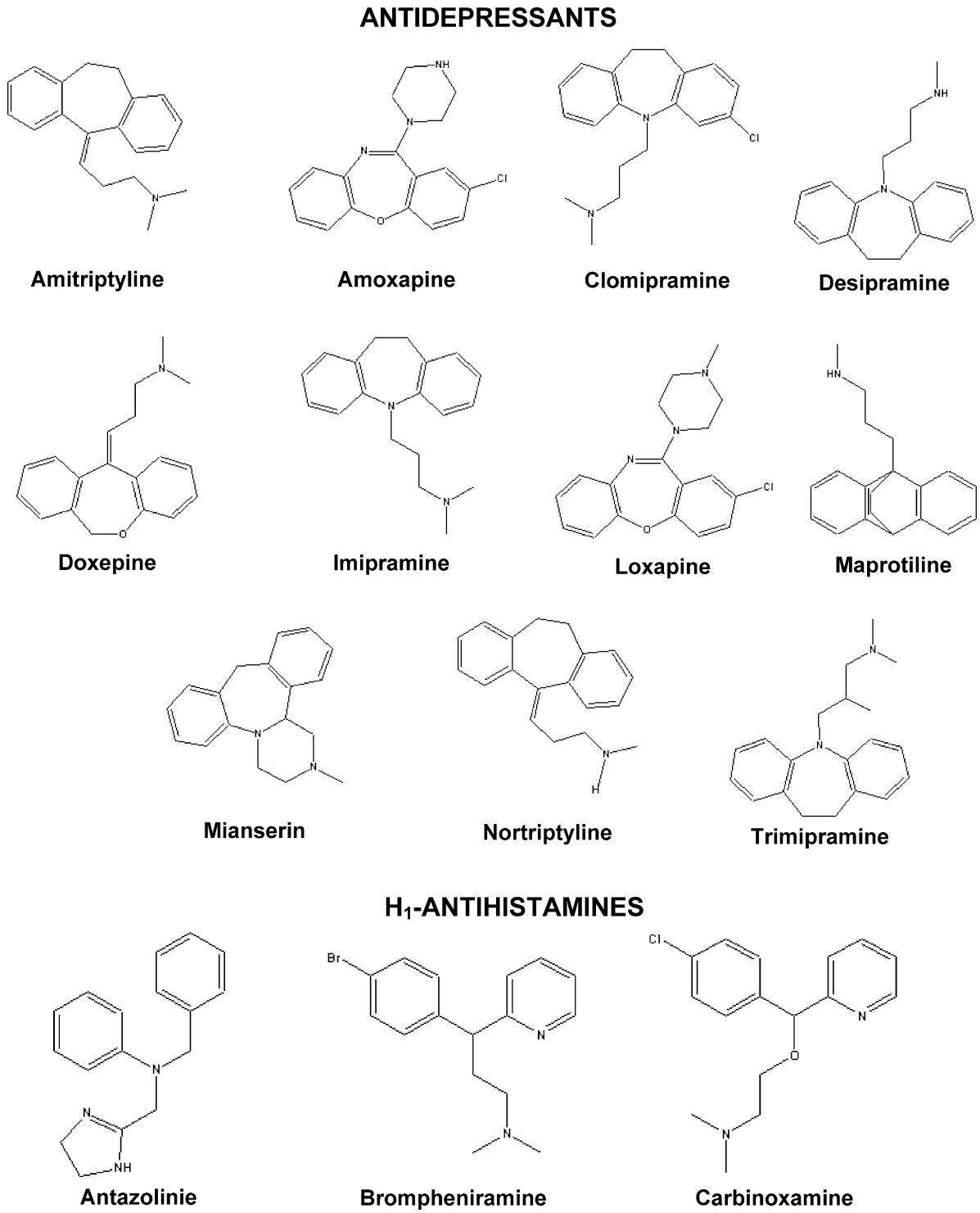
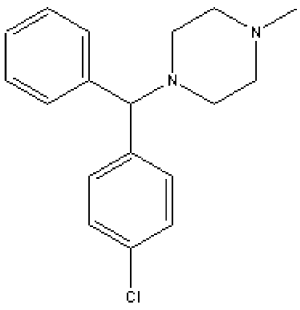
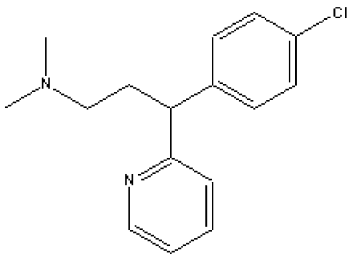


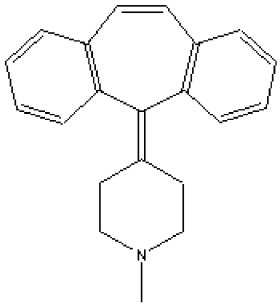
Fig. 1. Chemical structures of the psychotherapeutic drugs considered.



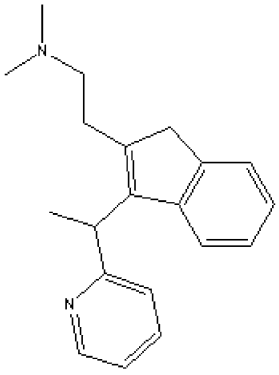
Chlorcyclizine



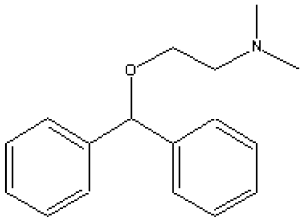
Chlorpheniramine



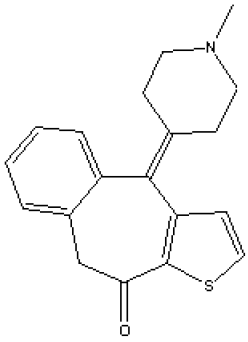
Cyproheptadine



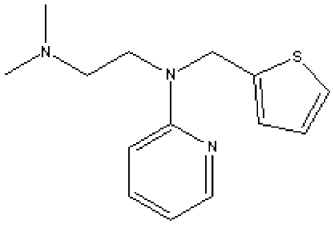
Dimetindene



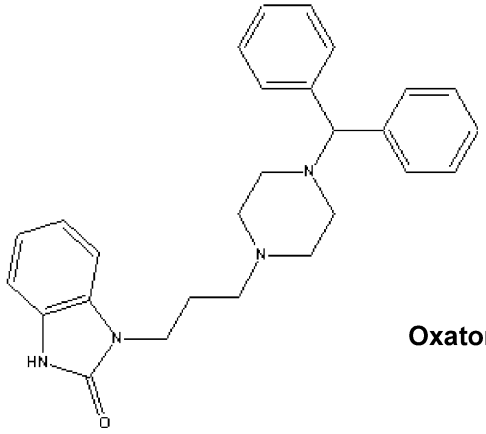
Diphenhydramine



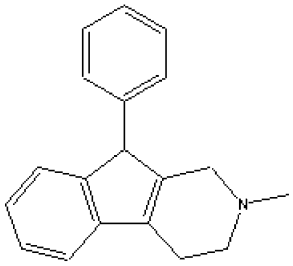
Ketotifen



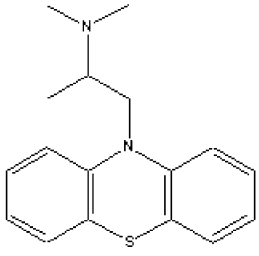
Methapyrilene



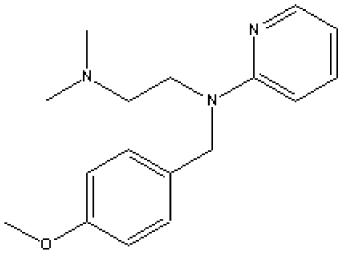
Oxatomide



Phenindamine

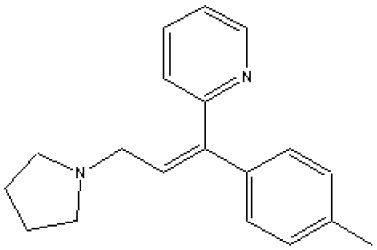


Promethazine

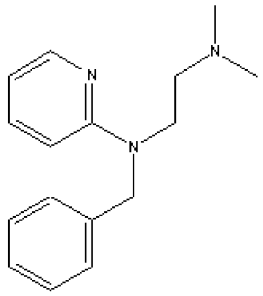


Pyrilamine

Fig. 1. (*Continued*).

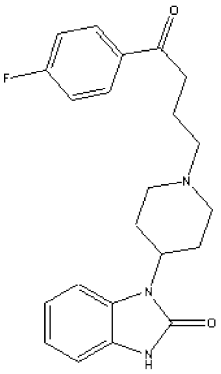


Tripelennamine

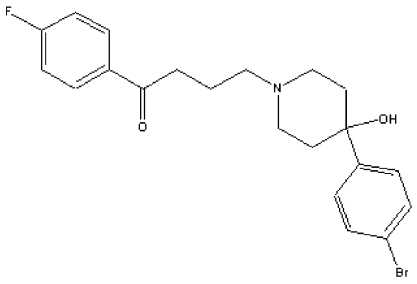


Triprolidine

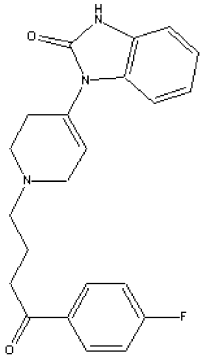
BUTYROPHENONES



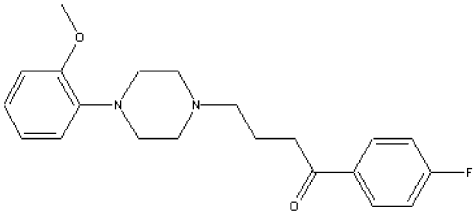
Benperidol



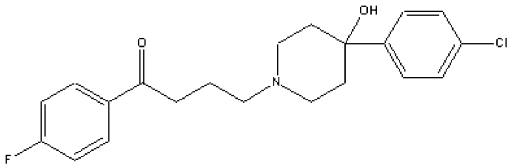
Bromperidol



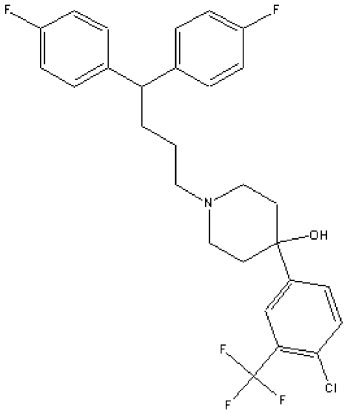
Droperidol



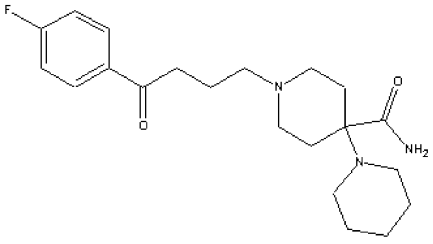
Fluanisone



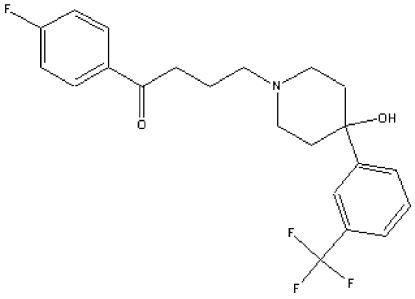
Haloperidol



Penfluridol



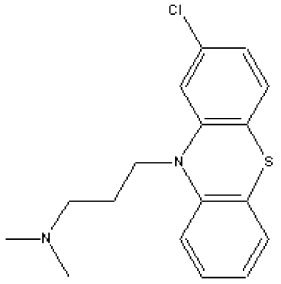
Pipamperone



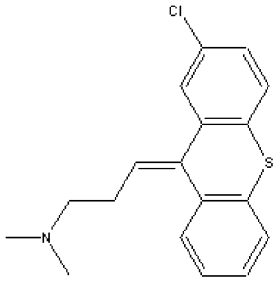
Trifluperidol

Fig. 1. (*Continued*).

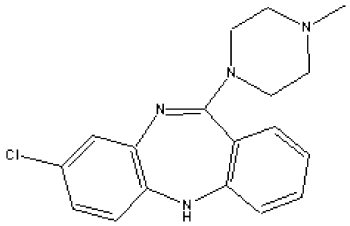
PHENOTHIAZINES



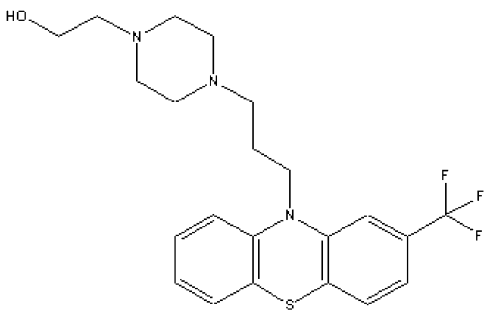
Chlorpromazine



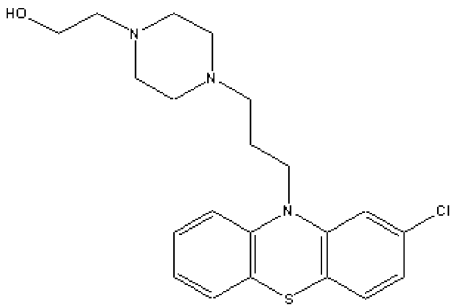
Chlorprothixene



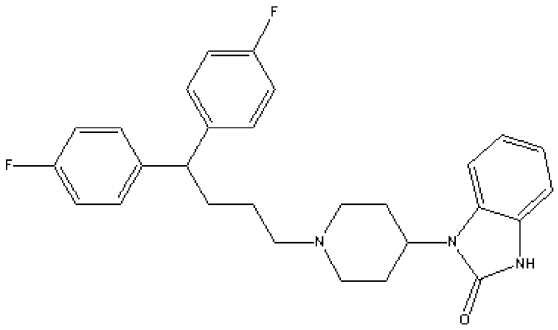
Clozapine



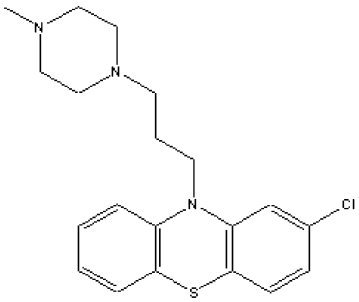
Fluphenazine



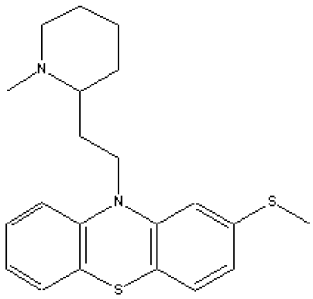
Perphenazine



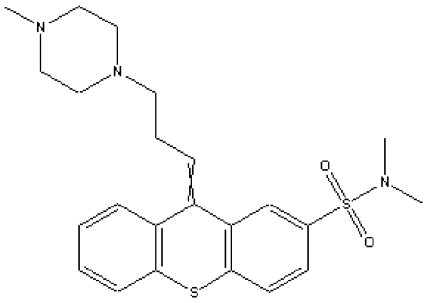
Pimozide



Prochlorperazine



Thioridazine



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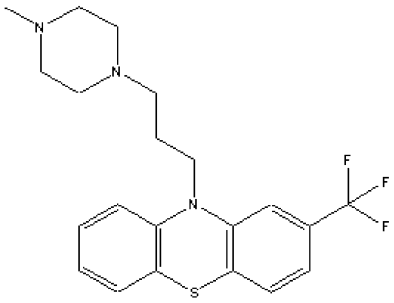
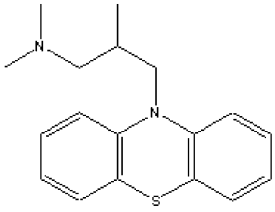


Trifluoperazine



Trimeprazine

Fig. 1. (*Continued*).

Table 2
Retention data in Brij-35 and monoamine receptor binding affinity values reported in literature of the psychotherapeutic drugs used in QRAR models development

CNS drug	Retention data (log *k*)			Binding constant (nM)			
	0.02 M	0.04 M	0.06 M	H_1	α_1	5-HT_2 (rat)	5-HT_2 (human)
Antidepressants							
Amitriptyline	2.25	1.84	1.76	4.1 [18]	24 [20], 21 [21]	4.2 [24], 6.2 [25]	29 [29]
Amoxapine	2.03	1.63	1.53	–	–	–	0.6 [29]
Clomipramine	2.31	1.89	1.79	33 [19]	–	–	27 [29]
Desipramine	1.80	1.42	1.35	250 [18]	148 [22]	78 [24]	280 [29]
Doxepine	2.10	1.69	1.55	0.7 [18]	23 [22]	–	25 [29]
Imipramine	2.22	1.80	1.67	26 [18]	58 [22], 51 [21]	37 [24]	80 [29]
Loxapine	2.26	1.86	1.75	–	–	2 [26]	1.7 [28]
Maprotiline	1.79	1.47	1.35	–	–	–	120 [29]
Mianserine	2.20	1.87	1.76	3 [19]	–	1.4 [24]	7 [29]
Nortriptyline	1.87	1.52	1.40	46 [18]	71 [22]	–	–
Trimipramine	2.26	1.86	1.75	–	–	19.4 [27]	32 [29]
Antihistamines							
Antazoline	1.35	1.04	0.94	610 [18]	–	–	–
Brompheniramine	2.19	1.88	1.86	4.7 [18]	–	–	–
Carbinoxamine	1.93	1.80	1.66	2.3 [18]	–	–	–
Chlorcyclizine	2.48	2.07	1.88	9 [18]	–	–	–
Chlorpheniramine	2.06	1.77	1.77	8 [18]	–	–	–
Cyproheptadine	2.47	2.08	1.88	3.1 [18]	–	0.44[24]	–
Diphenhidramine	1.95	1.73	1.61	17 [18]	–	–	–
Dimethindene	2.24	1.94	1.85	8 [18]	–	–	–
Ketotifen	1.97	1.59	1.50	–	–	17 [24]	–
Methapyrilene	1.86	1.69	1.48	4.5 [18]	–	–	–
Oxatomide	2.10	1.82	1.57	–	–	2.9 [25]	–
Phenindamine	2.33	1.95	1.79	20 [18]	–	–	–
Promethazine	2.25	1.88	1.75	2.9 [18]	55[21]	–	–
Pyrilamine	1.94	1.59	1.57	4.5 [18]	–	–	–
Tripelennamine	1.99	1.61	1.56	35 [18]	–	–	–
Triprolidine	2.30	2.05	2.03	5.6 [18]	–	–	–
Phenothiazines							
Chlorpromazine	2.42	2.18	1.92	28 [21], 36 [18]	4.3 [20], 6 [21], 5.2 [23]	3.3 [24], 2 [26]	1.4 [28]
Chlorprothixene	2.53	2.21	1.99	–	–	–	0.4 [28]
Clozapine	2.45	2.13	1.94	20 [20]	17 [20], 17 [23]	2.6 [24], 3.4 [25], 5 [26]	1.6 [28]
Fluphenazine	2.07	1.78	1.53	58 [20], 67 [18]	13 [20], 9.9 [23]	2.8 [25], 2.5 [26]	19 [28]
Perphenazine	1.98	1.67	1.40	–	7.4 [23]	5.6 [28]	
Pimozide	2.54	2.19	2.01	–	20 [20], 18 [23]	5.9 [24], 8 [26]	–
Prochlorperazine	2.51	2.31	2.00	–	–	6.3 [26]	15 [28]
Thioridazine	2.40	2.17	1.85	25 [20], 20 [18]	6.05 [20], 5.4 [23], 5.1 [21]	6.3 [26]	22 [28]
Thiothixene	1.97	1.76	1.42	37 [20], 27 [18]	11 [20]	–	–
Trifluoperazine	2.42	2.33	2.04	135 [20], 182 [18]	55.5 [20], 46 [23], 44 [21]	4 [26]	14 [28]
Trimeprazine	2.26	1.92	1.73	1.3 [18]	–	–	–
Butyrophenones							
Benperidol	1.84	1.64	1.49	–	–	–	–
Bromperidol	2.26	1.93	1.84	–	–	–	–
Droperidol	1.97	1.67	1.54	–	–	–	–
Fluanisone	1.49	1.50	1.39	150 [20]	–	–	–
Haloperidol	2.12	1.89	1.65	–	–	–	–
Penfluridol	2.55	2.30	2.12	–	–	–	–
Pipamperone	1.42	1.28	1.09	450 [20]	–	–	–

to H_1 (histaminic) α_1 (noradrenergic) and 5-HT_2 (serotonergic) receptors found in bibliography [18–29]. The binding affinities are expressed as the radioligand binding inhibition constant, K_i, and evaluated as the drug concentration that produces a certain degree of inhibition of the specific radioligand binding. In this case, all of the drugs studied present antagonist activity. When two or more data sources for the same compound were available, the median value to construct the corresponding QRAR model was used.

3.1. Retention-binding affinity on H_1-histamine receptor relationships

The antihistamine [3H]-mepyramine binds to H_1 receptors in mammalian brain membranes and it is used to evaluate the affinity to the H_1-receptor of the CNS drugs. This binding is saturable with a density of 10 pmol per gram of whole brain and has a dissociation constant of about 4 nM.

For many years, the most active histamine H_1-receptor antagonists have been represented by a general formula comprising two aromatic groups linked by a short chain of atoms to a secondary or tertiary amino group. The two aryl groups may be bridged to form tricyclic derivatives. A simpler description suggests that the most active antihistamines have an intramolecular distance of 5–6 Å between the side chain ammonium nitrogen and the center of one of the aromatic rings, but this is probably not exclusive [30]. It has been demonstrated that not only classical antihistamines, but also many other compounds of diverse chemical structures are potent histamine H_1-receptor antagonists. The most potent antagonists are certain tricyclic antidepressants (i.e. doxepin and amitriptyline [18]) and phenothiazine neuroleptics. Excepting fluanisone and pipamperone, which are relatively efficient in the displacement of [3H]-mepyramine from the histamine receptor [20], butyrophenones are extremely weak as H_1-receptor antagonists due to the lack of chemical resemblance to the described H_1-antagonist basic pharmacophore.

Quantitative structure–activity studies have been carried out to describe H_1 antagonist activity, these studies have been made in series of homologous compounds and the models obtained are expressed in terms of hydrophobic, steric and electronic parameters.

Fig. 2a shows the relationships between the retention data (k or $\log k$) obtained using a 0.04 M Brij-35 mobile phase and the binding affinities to H_1-receptor of different CNS drugs. Both plots provide the same information from a qualitative point of view: all the compounds with $\log k$ values ranged between 1.7 and 2.2 ($50 \leq k \leq 150$) present the lowest K_i values which implies that these compounds are going to form the most stable ligand–H_1-receptor complexes. For those compounds with $\log k$ values lower than 1.7 ($k \leq 50$) and over 2.2 ($k > 150$), K_i drastically increases indicating a loss of affinity to the histaminic receptor. Nevertheless, the reduction of the strength of binding seems to be less drastic

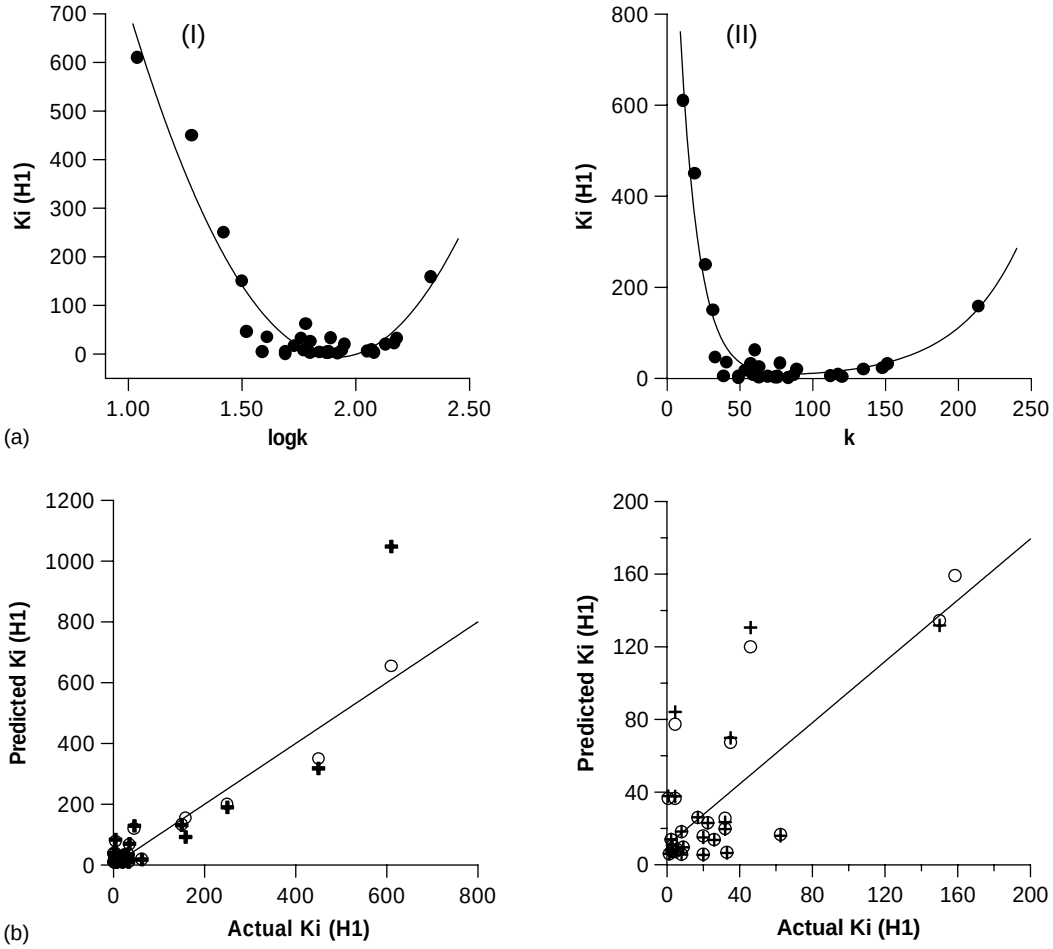


Fig. 2. (a) H_1-receptor binding affinity vs. $\log k$ (I) and k (II) relationships. (b) Validation plots for exponential QRAR model: predicted vs. actual values. Fitted (O) and cross-validated (+) results are shown.

Table 3
Statistical analysis and predictive features of the QRAR models for H_1-histamine receptor: $K_i(H_1)$ (nM) = $\exp(a + b^*k) + \exp(c^*k)$

[Brij-35] (M)	a ± asymptotic SE	b ± asymptotic SE	c ± asymptotic SE	R^2 (R_{adj})	SE	DW
0.02	7.27 ± 0.14	−0.040 ± 0.005	0.012 ± 0.002 0.90 (0.89)	45.9	2.0	
0.04	7.33 ± 0.10	−0.078 ± 0.006	0.024 ± 0.001	0.94 (0.94)	34.8	2.7
0.06	7.40 ± 0.12	−0.110 ± 0.010	0.040 ± 0.003	0.93 (0.92)	38.7	2.2

k: retention data of compounds obtained using Brij-35 mobile phases, asymptotic SE: asymptotic 95% confidence interval/2, $(R_{adj})^2 = R^2$ adjusted for degrees of freedom; SE: standard error of the estimate; DW: Durbin–Watson statistic.

for highly retained compounds. Similar graphical models were obtained from retention data using 0.02 and 0.06 M Brij-35 mobile phases but obviously with different intervals of $\log k$.

In order to obtain quantitative information, two different models were assayed. The K_i/$\log k$ relationship can be fitted to a second order polynomial model Eq. (1), whereas the K_i/k relationship can be fitted to a double exponential model Eq. (2):

$$K_i = a + b^*(\log k) + c^*(\log k)^2 \quad (1)$$

$$K_i = \exp(a + b^*k) + \exp(c^*k) \quad (2)$$

The first relationship agrees with the type of dependence that has been proved to be usual in previous QRAR studies for pharmacokinetics and biological responses of different groups of drugs [12]. The second model implies that compounds with retention within a certain range can show similar binding affinity to receptor. On the other hand, compounds with retention values outside of the described retention range, show low binding affinity to receptor, and these diminution of the binding affinity to the receptor is different for the lowest and highest retained compounds. As can be observed in Fig. 2a data were best fitted to the exponential model.

Table 3 summarizes the statistical analysis of the proposed models. In all cases statistically significant relationships between K_i and k values at the 95% confidence level were found, (the asymptotic 95% confidence interval does not contain the value 0). The R^2 statistic values indicate that the models, as fitted, explain between 90 and 94% of the variability in K_i data when different Brij-35 mobile phases are used. The Durbin–Watson statistic is greater than 1.4 implying a lack of autocorrelation in the residuals. The standard error of the estimate shows the standard deviation of the residuals to be between 35 (for 0.04 M Brij-35) and 46 (for 0.02 M Brij-35). These values can be used to construct the prediction limits for new observations.

The predictive ability of the model obtained using 0.04 M Brij-35 mobile phase was evaluated in term of cross-validated data: RMSEC = 33.0, RMSECV = 105.9 and RMSECVi = 39.0. As can be checked, the RMSECV is greater than RMSECVi value, indicating that some cautions must be taken with extrapolated data. Fig. 2b, shows the predicted (fitted and cross-validated) versus actual activity for the available data.

Table 4 contains the predicted binding affinity values for other CNS drugs with binding affinity data not found in literature but with proven therapeutic effect.

3.2. Retention–activity on α_1-noradrenergic receptor relationships

In order to evaluate the affinity to the α_1-adrenoreceptor class of CNS drugs, different binding assays have been reported in which the labeled α_1-antagonist WB-4101 (2-([2′,6′-dimethoxy]-phenoxyethylamino)-methylbenzodioxan) acts as radioligand. The binding is saturable, with K_D value of 0.48 nM [21].

Requirements for high α_1-adrenoreceptor affinity appear to be a positive charge and appropriate bulk/lipophilicity at opposite sides of this charge [31]. Then, the presence of the cyclic structure and the amino group, which provides the positive charge at physiological pH, is very important for the α_1-receptor activity. A wide variety of compounds present

Table 4
Predicted values of binding affinity to H_1-receptor in rat brain using 0.04 M Brij-35 mobile phase

CNS drug	$\log k$	K_i (nM) (95% confidence limits)
Tricyclic antidepressants		
Amoxapine	1.63	58 ± 19
Loxapine	1.86	11 ± 4
Melitracen	1.97	10 ± 2
Quinupramine	1.62	63 ± 20
Trimipramine	1.86	11 ± 4
Phenothiazines		
Pericyazine	1.60	72 ± 21
Methotrimeprazine	1.89	10 ± 3
Thioproperazine	1.71	32 ± 13
Chlorprothixene	2.21	46 ± 16
Ethopromazine	1.98	10 ± 3
Pimozide	2.19	38 ± 13
Prochlorperazine	2.31	123 ± 52
Antihistamines		
Doxylamine	1.39	229 ± 33
Hydroxyzine	1.60	72 ± 21
Orphenadrine	2.05	14 ± 3
Oxatomide	1.82	14 ± 6
Clemastine	2.22	50 ± 18
Cyclizine	1.96	10 ± 2
Cinnarizine	2.26	73 ± 28
Chloropyramine	1.86	11 ± 4
Ketotifen	1.55	77 ± 22

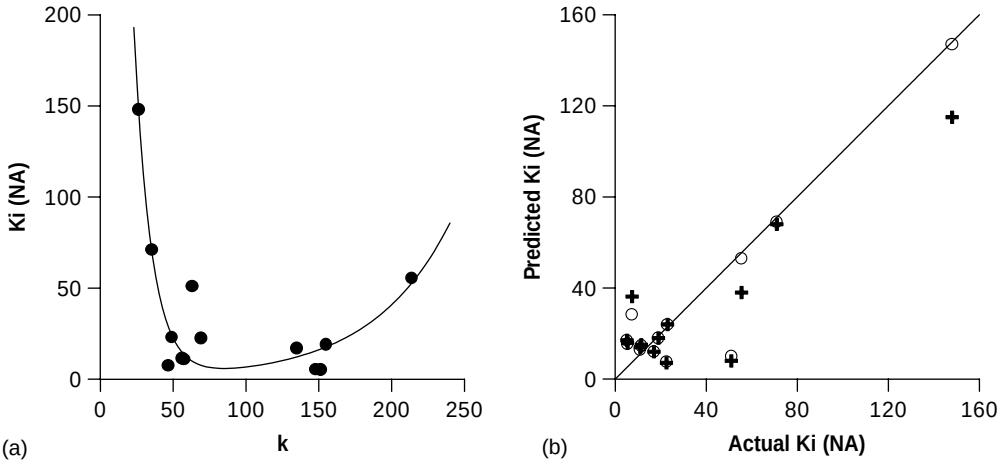


Fig. 3. (a) α_1-receptor binding affinity vs. and k relationship. (b) Validation plots for QRAR model: predicted vs. actual values. Fitted (O) and cross-validated (+) results are shown.

Table 5
Statistical analysis and predictive features of the QRAR models for α_1-noradrenergic receptor: $K_i(\alpha_1)\,(\text{nM}) = \exp(a + b^*k) + \exp(c^*k)$

[Brij-35] (M)	$a \pm$ asymptotic SE	$b \pm$ asymptotic SE	$c \pm$ asymptotic SE	R^2 (R_{adj})	SE	DW
0.02	10.0 ± 1.7	−0.08 ± 0.03	0.0101 ± 0.0015	0.75 (0.70)	22.1	1.5
0.04	7.2 ± 0.5	−0.084 ± 0.017	0.018 ± 0.001	0.86 (0.84)	16.3	2.2
0.06	8.65 ± 1.10	−0.20 ± 0.06	0.034 ± 0.004	0.72 (0.66)	23.4	1.6

See footnotes of Table 3.

these structural features and all of them are going to show antagonist activity on α_1-adrenergic receptors.

Among the drugs studied, the tricyclic antidepressants and the antipsychotic drugs display substantial potency in competing for the radioligand binding [22,23]. Regarding to antihistamines drugs, the α_1-adrenoreceptor-antagonist activity seems to be limited to the phenothiazine derivatives (i.e. promethazine) [32].

There are different QSAR studies reported in which binding affinity for α_1-adrenoreceptor is predicted. As could be expected, the resulting models provide a significant correlation of drugs electronic, steric and hydrophobic parameters with the biological affinities. These studies are based on computational chemistry and molecular modeling procedures [31,33,34].

We also studied the ability of the exponential QRAR model proposed (2) in explaining drug affinity at the noradrenergic receptor. As can be observed in Fig. 3a, the exponential model obtained using 0.04 M Brij-35 retention data was adequate. Compounds with k values ranging between 50 and 180 form the most stable antagonist–receptor complexes. Table 5 contains the results of the statistical analysis and the predictive features of the QRAR models proposed. The results indicate that there are statistically significant relationships at the 95% confidence level. Coefficients are also statistically significant at the same confidence level.

Better statistically model was obtained using 0.04 M Brij-35 retention data. The R^2 statistic value indicates that the exponential model for 0.04 M Brij-35 mobile phase explains 86% of the variability in α_1-receptor binding affinity data. The standard error of the estimate is 16.3. This value can be used to construct the prediction limits for new observations. In all cases, there is not any significant correlation in the residuals (DW > 1.4).

Regarding to the model's predictive ability, the calculated cross-validation statistics indicates that extrapolated data must be carefully considered (using Brij-35 0.04 M mobile phase, the cross-validation errors were: RMSEC = 14.3, RMSECV = 18.9 and RMSECVi = 17.3). Fig. 3b contains the predicted (fitted and cross-validated) versus actual activity plots. Models could not explain the binding affinity of promethazine ($k_i = 55 \pm 6$ nM) because retention of compound is in the zone of the greatest variability. Table 6 contains the predicted values of α_1-receptor binding affinity

Table 6
Predicted values of binding affinity to α_1-receptor in rat brain using 0.04 M Brij-35 mobile phase

CNS drug	log k	K_i (nM) (95% confidence limits)
Tricyclic antidepressants		
Amoxapine	1.63	39 ± 20
Dosulepine	1.74	16 ± 13
Maprotiline	1.47	113 ± 25
Quinupramine	1.62	42 ± 20
Phenothiazines		
Pericyazine	1.60	110 ± 24
Thioproperazine	1.71	21 ± 15
Chlorprothixene	2.21	20 ± 10
Prochlorperazine	2.31	44 ± 27

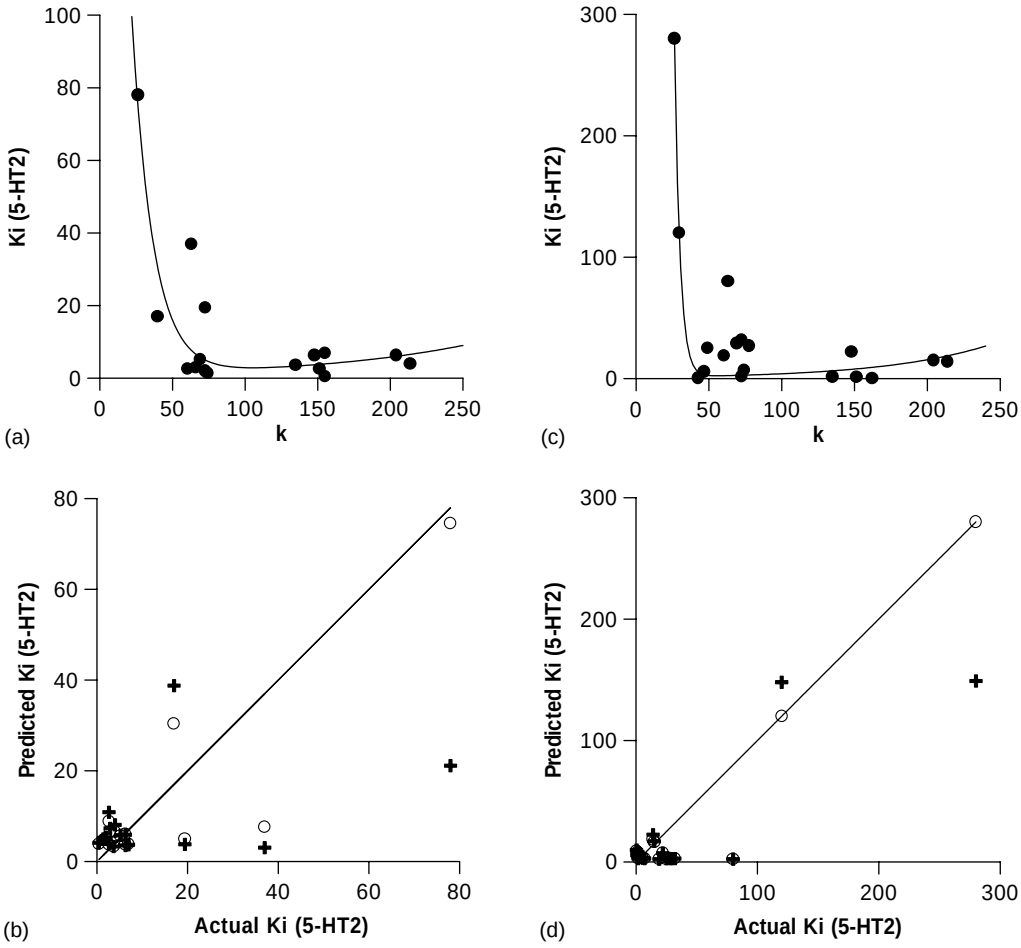


Fig. 4. 5-HT$_2$-receptor binding affinity vs. k relationships: (a) rat brain, (b) human brain. Fitted (O) and cross-validated (+) results are shown.

for different CNS drugs using exponential model from the experimental retention values in BMC.

3.3. Retention–activity on 5-HT$_2$ serotonergic receptor (S_2) relationships

The compound ketanserin is a quinazoline derivative with serotonin antagonist properties. Due to its marked selectivity and advantageous binding properties ($K_D = 0.42$ nM in Tris–HCl buffer), its corresponding [^{3}H] derivative is the most suitable of radioligands available so far for investigation of S_2 receptor binding sites [24].

A structure–activity relationships study of the S_2 receptor antagonist is a difficult task because of the abundance and structural diversity of such compounds although, among the families of compounds considered in this work few activity data were available. There are many published works about structure–activity of different S_2 antagonist compounds families [35–38]. One of the most important structural requirements to explain the activity of all the S_2 receptor antagonists seems to be one nitrogen atom located at 6.30 and 5.17 Å from two aromatic rings [39]. This structural requirement is also shared by the pharmacophore of H1 antihistamine and α1 noradrenergic receptors, which explain that the serotonin antagonists also interact with these receptor-binding sites.

Among the compounds considered in this work, phenothiazines, and the tricyclic derivatives (mainly those compounds with a carbon–carbon double bond connecting the middle ring with the side chain) such as antidepressants and the antihistamines ketotifen and cyproheptadine are moderate to potent inhibitors of [^{3}H]-ketanserin binding, whereas other antihistamines [32] and compounds of other chemical classes are inactive.

Fig. 4 shows the relationships between the compounds retention data (using 0.04 M Brij-35 mobile phase) and their binding affinities to 5-HT$_2$-receptor measured in rat (Fig. 4a) and human (Fig. 4b) brain. From a qualitative point of view, and according to the plots, those compounds with $k > 60$ show the highest affinity to both rat and human receptor. These results agree with the investigations reported [29].

Table 7a and 7b contains the corresponding statistical analysis for rat and human QRAR models respectively. In both cases, models and coefficients are statistically significant at the 95% confidence level. The R^2 statistic value indicates that the models, as fitted, explain between 72 and 88% of the variability in K_i data. The study of the models

Table 7
Statistical analysis and predictive features of the QRAR models for 5-HT_2 serotonergic receptor (S_2): $K_i(S_2)$ (nM) = $\exp(a + b^*k) + \exp(c^*k)$

[Brij-35] (M)	a ± asymptotic SE	b ± asymptotic SE	c ± asymptotic SE	R^2 (R_{adj})	SE	DW
(a) Rat brain						
0.02	7.7 ± 1.3	−0.05 ± 0.02	0.006 ± 0.002	0.75 (0.71)	10.7	2.0
0.04	6.1 ± 0.5	−0.069 ± 0.018	0.009 ± 0.005	0.77 (0.73)	10.3	2.7
0.06	6.5 ± 0.7	−0.12 ± 0.04	0.018 ± 0.007	0.75 (0.71)	10.7	2.3
(b) Human brain						
0.02	6.8 ± 0.6	−0.024 ± 0.009	0.008 ± 0.006	0.72 (0.68)	40.0	2.2
0.04	13 ± 2	−0.26 ± 0.08	0.014 ± 0.005	0.88 (0.86)	26.8	1.42
0.06	8.4 ± 1.4	−0.14 ± 0.06	0.026 ± 0.013	0.73 (0.69)	39.7	2.2

See footnotes of Table 3.

Table 8
Predicted values of binding affinity to S_2-receptor in rat and human brain from the exponential models obtained using 0.04 M Brij-35 mobile phase

CNS drug	log k	K_i (nM)	
		Rat brain (95% confidence limits)	Human brain (95% confidence limits)
Tricyclic antidepressants			
Amoxapine	1.63	25 ± 14	[a]
Melitracen	1.97	3 ± 3	4 ± 3
Quinupramine	1.62	27 ± 14	7.0 ± 10
Phenothiazines			
Pericyazine	1.60	30 ± 14	10 ± 15
Methotrimeprazine	1.89	4 ± 4	3 ± 2
Thioproperazine	1.71	15 ± 6	2 ± 2
Chlorprothixene	2.21	4 ± 7	9 ± 14
Ethopromazine	1.98	3 ± 2	4 ± 3

[a] Included in the model.

predictive ability concludes that extrapolated data must be prudently valuated (see Table 7a and 7b). Using 0.04 M Brij-35 mobile phase, the cross-validation errors were: RMSEC = 9.3, RMSECV = 17.4 and RMSECVi = 12.0 for the rat model and RMSEC = 22.8, RMSECV = 39.1 and RMSECVi = 25.3 for the human one. The use of 0.02 or 0.06 M Brij-35 mobile phases did not improve the results obtained using 0.04 M Brij-35 retention data (see Table 7a and 7b). Fig. 4c and d contains the predicted (fitted and cross-validated) versus actual activity plots. Table 8 shows the binding affinity predicted values using the QRAR exponential model for other CNS drugs.

4. Conclusions

The BMC methodology proposed is probably one of the most accessible, economical, robust and stable of the HPLC-based methodologies employed in QRAR analysis. The use of only one descriptor (the retention factor, k) is one of the most important advantages with regard to the classical QSAR studies.

The QRAR models obtained must be carefully used: the tested compounds, in the same way than the ones used in the model development, must present the same molecular and stereochemical features than the basic pharmacophores of the corresponding monoamine receptors. Taking into account these considerations, it has been shown that the BMC retention of a compound is able to describe the influence of hydrophobicity on the affinity of certain families of CNS drugs to monoamine receptors. This is presumably due to the fact that retention depends on the same interactions which condition drug–receptor binding. In this case, as all the compounds that comprise the set studied present similar electronic characteristics, differences observed in retention are explained by means of steric and, overall, hydrophobic contributions. This fact is going to allow us to relate drug–receptor binding to hydrophobicity.

The exponential models developed seems to be efficient to explain psychotherapeutic drugs binding affinity, overall for highly hydrophobic compounds. All those compounds with $50 < k < 150$ show the highest affinity for all of the monoamine receptors studied (H_1-histaminic, α_1-adrenergic and 5-HT_2 serotonergic). However small changes in the retention for the less hydrophobic compounds seem to affect more to drug–receptor complex stability than changes in the retention for the highly hydrophobic compounds. The estimation of the monoamine receptor binding affinity of other drugs is going to provide us useful qualitative information about the relative potency of these compounds and their possible side effects.

Similar QRAR models to the ones described in this work were assayed for dopamine receptor. Nevertheless, there were not enough available data in order to statistically validate these models.

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