Development of Predictive Retention−Activity Relationship Models of Tricyclic Antidepressants by Micellar Liquid Chromatography

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The distribution of tricyclic antidepressants from plasma to brain, where these drugs exert their main clinical action, and other organs is related to transport events across the cell membranes of the different tissues. It could be expected that all the molecular features that condition the transport processes (mainly hydrophobicity and molar total charge) also control the pharmacokinetic and biochemical behavior. Micellar liquid chromatography (MLC) has been proposed to emulate in vitro the partitioning process in the biomembranes. The use of micellar solutions of Brij35 as mobile phases in reversed-phase liquid chromatography has proven to be valid to predict the biological activities of local anesthetics, barbiturates, catecholamines, and benzodiazepines. In this paper, the relationships between the capacity factor in MLC and some pharmacokinetic parameters and biological responses of tricyclic antidepressants are studied. Predictive regression models for the estimation of these parameter values, using the logarithm of the retention data (log k) as independent variable, are also proposed.

Introduction

The tricyclic antidepressant drugs have achieved widespread clinical use in the treatment of depression. The tricyclic antidepressant action consists, in part, in presynaptic receptor inhibition, specific to each kind of biogenic amine such as serotonin (5-HT) and noradrenaline (NA) which permits the blockade of the reuptake of the amines into the neuron at the level of the cell membrane, and also in their action on other biogenic amine systems in the brain like a histamine-sensitive adenylate cyclase.[1]

To directly affect central nervous system (CNS) cells, tricyclic antidepressant drugs must appear in the fluid environment of these cells. The distribution of many drugs to the brain is a more selective process than the distribution to other organs. This fact is a consequence of the blood−brain barrier action which permits a restricted distribution from blood to brain.[2] Molecular exchange between blood and brain must take place through the cells. The most crucial step in this transcellular movement is the freedom with which a drug can escape from plasma and cross through the cell membrane. As this one has a lipid nature, hydrophobic compounds tend to cross through the membrane easily, but electronic and steric properties are also of great importance.

In computational quantitative structure−activity relationship (QSAR) studies, physicochemical characteristics of compounds are used as parameters to correlate biological activity with structures using different chemometric approaches. Classical QSAR based on multiple linear regression (MLR) cannot be established in many cases for a pharmacological family of drugs due to the fact that the number of biological activity data available is not enough with respect to the number of molecular descriptors needed to obtain interpretative and predictive equations. In addition sometimes the relationships between variables are not linear. As an alternative to QSAR models, investigations have been made to obtain single parameters which provide adequate predictive and interpretative models to describe the biological behavior of drugs.

Chromatography is a powerful technique for the measurement of physicochemical parameters. The application of chromatographic parameters in SARS gives rise to a new field: quantitative retention−activity relationships, QRAR.[3−5] To emulate the biological barriers, different reversed stationary phases have been developed, all of them based on the inclusion of polar groups in the chromatographic surfaces.[6−10]

A simpler and reproducible approach consists of the use of micellar liquid chromatography (MLC). It is a type of reversed-phase liquid chromatography, which uses a surfactant solution above the critical micellar concentration (cmc) as mobile phase.[11−12] When Brij35 (nonionic surfactant) is used to prepare micellar mobile phases, there are a number of similarities between the mobile phase/modified stationary phase in MLC and the membrane/water interface. The stationary phase modified by the adsorption of surfactant monomers resembles structurally the ordered array of the membranous hydrocarbon chains. In addition, the hydrophilic/hydrophobic character of surfactant adsorbed could be expected to resemble the polar membrane regions. As a consequence, the stationary phase provides both hydrophobic and electrostatic sites of interaction.[13,14] The use of retention data in MLC instead of molecular descriptors may solve the QSAR−MLR drawbacks for short data series. Successful MLC applications to QRARs have been reported to describe substituted phenol bioactivity,[15] anesthetic potency of local anesthetics,[16] hypnotic activity of barbiturates,[17] α- and β-adrenergic activity of catecholamines,[18] and toxicity and activity of benzodiazepines[19] and phenothiazines.[20] Extensive studies are

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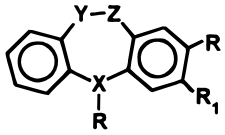
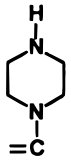
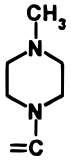
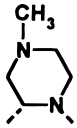
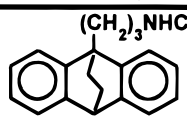
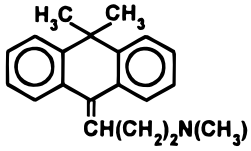
Table 1. Structure, pK_a,[a] log P[b] (for nonionic forms), and log D[c] Values of the Tricyclic Antidepressants Studied

GENERAL STRUCTURE



COMPOUND	X	Y	Z	R	R_1	R_2	pK_a	logP	logD
Amineptine	CH	CH_2	CH_2	$NH(CH_2)_6COOH$	H	H	5.24* 7.69*	2.44	2.26
Amitriptyline	C	CH_2	CH_2	$=CH(CH_2)_2N(CH_3)_2$	H	H	9.42	4.64	2.62
Amoxapine	O	N		-	H	Cl	7.6	3.89	3.48
Clomipramine	N	CH_2	CH_2	$(CH_2)_3N(CH_3)_2$	Cl	H	9.38	5.19	3.32
Desipramine	N	CH_2	CH_2	$(CH_2)_3NHCH_3$	H	H	10.44	3.97	1.05
Dothiepin	C	CH_2	S	$=CH(CH_2)_2N(CH_3)_2$	H	H	9.25	4.52	2.66
Doxepin	C	O	CH_2	$=CH(CH_2)_2N(CH_3)_2$	H	H	9.0	3.88	2.27
Imipramine	N	CH_2	CH_2	$(CH_2)_3N(CH_3)_2$	H	H	9.5	4.53	2.31
Loxapine	O	N		-	H	Cl	6.6	4.75	4.69
Mianserin	CH_2			-	H	H	7.1	4.26	4.08
Nortriptyline	C	CH_2	CH_2	$=CH(CH_2)_2NHCH_3$	H	H	9.7	4.32	2.02
Quinupramine	N	CH_2	CH_2		H	H	7.85*	4.94	1.36
Trimipramine	N	CH_2	CH_2	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H	H	6.77*	4.73	4.64
Maprotiline[d]							10.5	4.22	1.12
Melitracen[d]							7.38*	5.12	4.83

[a] pK_a = logarithm of the protonation constant. [b] log P = logarithm of the partition coefficient in the biphasic octanol−water solvent system (values taken from ref 16). [c] log D = lop P values at pH 7.4. [d] Not corresponding to the general structure indicated above. *log K measured potentiometrically in Brij35 medium.

needed to establish the conditions and the adequacy of this technique that allow mimicking of the biopartitioning of compounds into membranes.

In this paper, quantitative relationships between the MLC retention data of the tricyclic antidepressants and their pharmacokinetic parameters and biological responses are studied. Predictive models corresponding to each RAR have been proposed.

Experimental Section

Instruments and Measurements. A Hewlett-Packard 1100 chromatograph with an isocratic pump, a UV−visible detector, and a HP Vectra computer was used (Palo Alto, CA). Data acquisition and processing were performed on a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP−Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA), with a 20-μL loop. A Kromasil octadecylsilane C_{18} column (5 μm, 50- × 4.6-mm i.d.) and a guard column of similar characteristics (35 × 4.0 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1 mL min^{-1}. The detection was performed in UV at 220 nm. All the assays were carried out at room temperature.

Reagents and Standards. Mobile phases were prepared by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35, Acros Chimica, Geel, Belgium). Micellar eluent pH was adjusted to 7.4 with 0.05 M phosphate buffer, which was

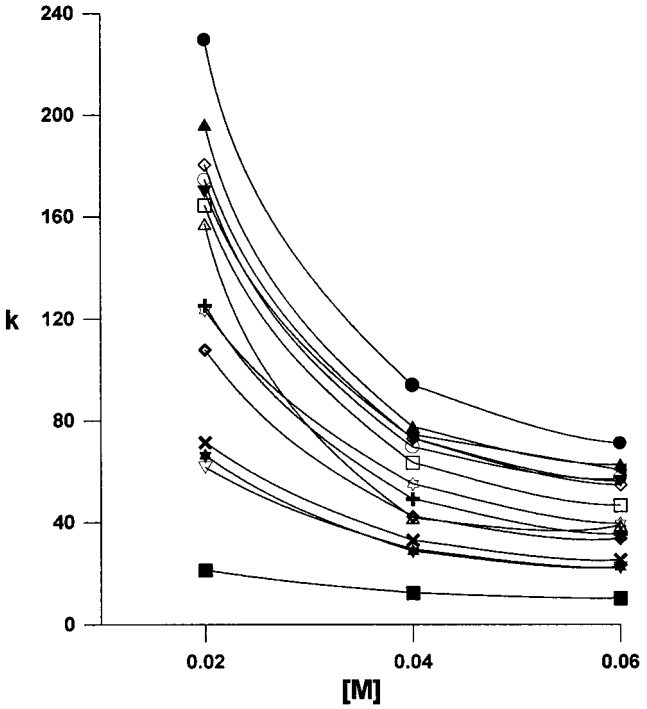


Figure 1. Effect of Brij35 concentration in the mobile phase on the retention of tricyclic antidepressants: (■) amineptine, (○) amitriptyline, (◇) amoxapine, (▲) clomipramine, (✱) desipramine, (✡) dothiepin, (+) doxepin, (□) imipramine, (◇) loxapine, (▽) maprotiline, (●) melitracen, (▲ pointing to the left) mianserin, (×) nortriptyline, (△) quinupramine, and (▼) trimipramine. Mobile phase pH was adjusted to 7.4 with 0.05 M phosphate buffer. To reproduce the osmotic pressure of biological fluids, NaCl (9.20 g/L) was also added to the micellar eluent.

prepared with disodium hydrogen phosphate and sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain). To reproduce the osmotic pressure of biological fluids, NaCl (9.20 g/L, purissim, Panreac) was added to the micellar mobile phase.

Some tricyclic antidepressants were kindly donated by several pharmaceutical laboratories: amineptine (Servier, Madrid, Spain); melitracen (Lundbeck, Copenhagen-Valby, Denmark); nortriptyline (Lilly, Madrid, Spain); quinupramine (Rhone-Poulenc Rorer, Vitry Sur Seine, France). Other ones were obtained from pharmaceutical preparations: mianserin (Lantanon, Organon, Barcelona, Spain); imipramine (Tofranil) and maprotiline (Ludiomil) (Novartis, Barcelona, Spain); clomipramine (Anafranil, Geigy, Barcelona, Spain); amitriptyline (Tryptizol, Merck Sharp and Dhome, Madrid, Spain); doxepin (Sinequan, Pfizer, Madrid, Spain); amoxapine (Demolox, Lederle, Madrid, Spain); dothiepin (Prothiaden, Alter, Madrid, Spain); loxapine (Desconex, Alonga, Madrid, Spain); trimipramine (Surmontil, Rhone-Poulenc Rorer, Madrid, Spain). Desipramine was bought as a hydrocloride derivate (Sigma, Barcelona, Spain).

Stock standard solutions of tricyclic antidepressants at 1000 mg/L were prepared using 0.04 M Brij35 (pH 7.4) as solvent. Working solutions were prepared by dilution of the stock standard solutions using 0.04 M Brij35 (pH 7.4) too. Solutions were stored at 4 °C. All retention factor values were averages of at least triplicate determinations.

Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45- and 0.22-μm nylon membranes, respectively (Micron Separations, Westborc, MA).

Software and Data Processing. Log *P* values for tricyclic antidepressants (nonionic forms) were taken from the literature.[21] Excel 7.0 Microsoft Office software was used to perform the statistical analysis of the MLR.

Evaluation of the QRAR Model Predictive Ability. To evaluate the predictive ability of the models in terms of cross-validated data, but pointing out the difference between interpolated and extrapolated data, the comparison between the fit error (i.e., root-mean-square error of calibration, RMSEC) and the prediction error based on cross-validation (i.e., root-mean-square error of cross-validation, RMSECV) was used.[19] RMSEC value informs us about the average deviation of the model from the data:

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n} (\bar{y}_i - y_i)^2}{n}} \qquad (1)$$

where $\bar{y}_i$ is the predicted activity when all the *n* molecules are

Table 2. Tricyclic Antidepressant Retention Data Obtained Using a 0.04 M Brij35 Mobile Phase (pharmacokinetic parameters and biological response values reported in the literature)

		pharmacokinetic parameters[a]				biological responses						
antidepressant	log *k*	$T_{1/2}$[b] (h)	V_d[c] (L/kg)	CL_M[d] (L/h)	T.P.L.[e] (ng/mL)	IC_{50}(NA)[f] (10^{-8} M)	IC_{50}(5-HT)[f] (10^{-8} M)	$IC_{50}(\alpha_1)$[f] (nM)	$IC_{50}(H_1)$[f] (nM)	K_i(Hipp)[g] (μM)	K_i(Neoc)[g] (μM)	$IC_{50}(H_2)$[g] (μM)
amineptine	1.09	–	–	–	–	–	–	–	–	–	–	–
amitriptyline	1.84	27.5	15	72	180	4.1	4.4	0.02	0.02	0.053	0.060	0.66
amoxapine	1.63	11.4	–	57.6	350	–		–	–	–	–	–
clomipramine	1.89	35	13.5	72.5	90	4.6	0.5	0.04	0.2	0.055	0.043	0.72
desipramine	1.46	37.5	22	123	212.5	0.2	35	0.25	0.8	0.35	0.32	3.8
dothiepin	1.74	19	44.5	–	–	–	–	–	–	–	–	–
doxepin	1.69	16.5	–	51	–	6.5	20	0.01	0.003	0.17	0.19	1.6
imipramine	1.80	18	30	67	275	4.6	4.4	–	0.06	0.16	0.16	1.9
loxapine	1.86	–	–	–	–	–	–	–	–	–	–	–
maprotiline	1.47	40	21.5	–	250	–	–	0.14	0.03	–	–	–
melitracen	1.97	–	–	–	–	–	–	–	–	–	–	–
mianserin	1.87	25	16	–	–	–	>500	0.07	0.006	0.065	0.071	0.88
nortriptyline	1.52	35.5	35	79	–	0.9	17	0.04	0.05	0.45	0.28	7.0
quinupramine	1.62	–	–	–	–	–	–	–	–	–	–	–
trimipramine	1.86	28	20	72.5	180	–	–	–	–	–	–	–

[a] Pharmacokinetic parameter values indicated correspond to the average of the range reported in the literature. [b] $T_{1/2}$ = half-life time; data taken from ref 26 ($T_{1/2}$ values of amitriptyline and amoxapine taken from ref 28). [c] V_d = volume of distribution; data taken from ref 26 (V_d values of amitriptyline and desipramine taken from ref 28). [d] CL_M = plasma clearance; data taken from ref 28. [e] T.P.L. = therapeutic plasma level; data taken from ref 27. [f] IC_{50} = drug concentration for 50% inhibition (NA = noradrenaline receptor, 5-HT = serotonin receptor, α_1 = adrenergic receptor, H_1 = histaminic receptor); data taken from ref 29. [g] K_i = brain adenylate cyclase inhibition constant (Hipp = hippocampus, Neoc = neocortex), IC_{50} = drug concentration for 50% inhibition (H_2 = brain adenylate cyclase histaminic receptor); data taken from ref 1.

included in the model construction. In contrast, the RMSECV value is a measure of the model's ability to predict pharmacokinetics and biological parameters of new compounds. RMSECV is defined as RMSEC in eq 1 except that now y_i are predictions for other antidepressants not included in the model formulation (e.g., each one of the calibration molecules is used as a test in turn for the model chosen on the remaining molecules, performing the procedure $n - 1$ times, which is referred to as the leave-one-out cross-validation). Since in the RMSECV parameter both interpolation and extrapolation information are mixed, we propose an additional parameter for measuring only the interpolation information (e.g., excluding the two extreme data, after ordering them by their log k values):

$$\mathrm{RMSECVi} = \sqrt{\frac{\sum_{i=2}^{n-1} (\bar{y}_i - y_i)^2}{n-2}} \qquad (2)$$

From a qualitative point of view, the more differences between RMSEC and RMSECV or RMSECVi exist, the lower the QRAR model's obtained robustness is and then more cautions must be taken in future predictions.

Results and Discussion

Retention Behavior of Tricyclic Antidepressants. Table 1 shows the structure, the logarithm of the protonation constants (pk_a), and the log P values for the nonionic form of the tricyclic antidepressants studied. The basic structure common to these drugs is a tricyclic system formed by two benzene rings and a seven-membered central ring (except maprotiline and melitracen which have a six-membered central ring), a chain of two or three carbon atoms, and one terminal secondary or tertiary amino group.[22] These structural features give tricyclic antidepressants a high hydrophobicity, which is reflected in their respective log P values. At physiological pH 7.4, all compounds are positively charged, but the molar total charge (calculated by the method proposed by Escuder et al.[23]) varies from −0.34 for amineptine to practically +1 for maprotiline and desipramine.

Figure 1 shows the effect of the Brij35 concentration (0.02, 0.04, 0.06 M) in the mobile phase on the retention of tricyclic antidepressants. As can be expected, for the highly hydrophobic compounds large changes in the retention were obtained upon increasing the surfactant concentration in the mobile phase, while for the slightly hydrophobic ones, the retention was scarcely modified.

The tricyclic antidepressant retention depends not only on the hydrophobic interactions but also on the molar total charge and steric properties of the compounds. In fact, the best log k−log P relationships were obtained when the molar total charge of compounds was included into the model (i.e., $R^2 = 0.91$ and 0.71 considering and without considering it for 0.04 M Brij35, respectively).

Retention−Activity Relationships. As the molecular features of drugs determine the interaction of drug−receptor and consequently their biological behavior, and also the retention in MLC, it could be expected that retention−activity relationships exist. Table 2 shows the retention data in 0.04 M Brij35 used in the model construction, the pharmacokinetic parameters, and some biological responses of tricyclic antidepressants reported in the literature. Similar models were

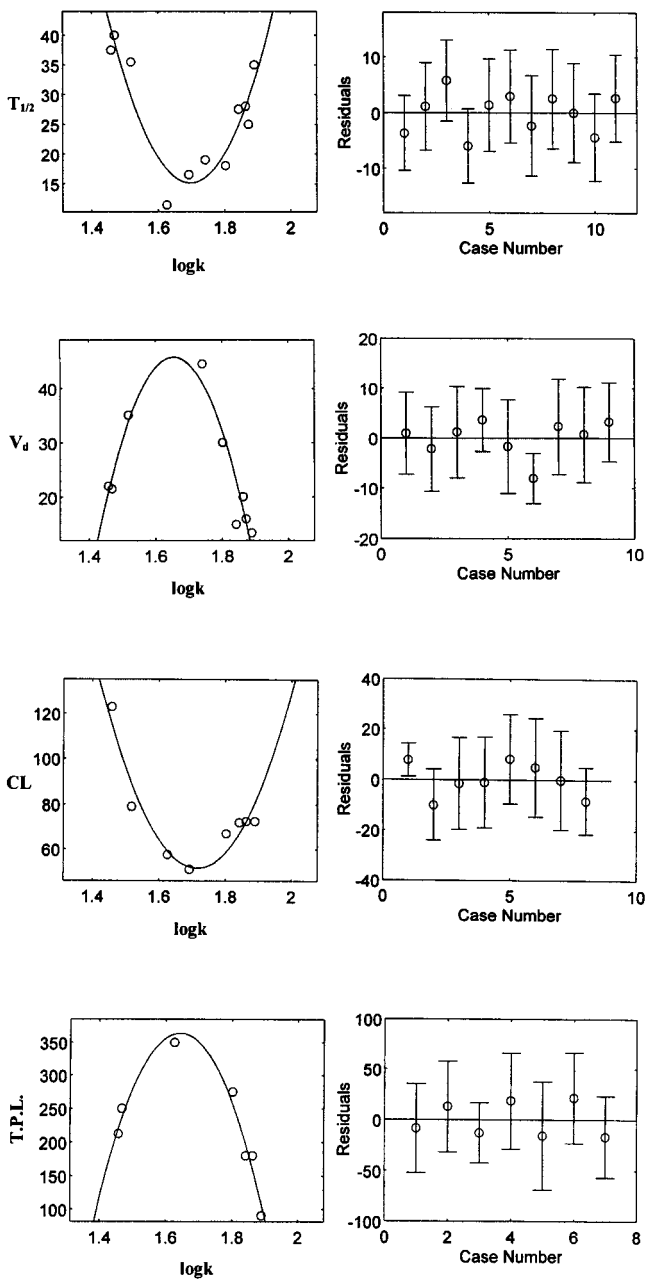


Figure 2. Log k−pharmacokinetic parameter (left) relationships for different tricyclic antidepressants at 0.04 M Brij35 concentration in the mobile phase. Residual plots (right) for these QRAR models are also included.

obtained using the retention data for 0.02 and 0.06 M Brij35 mobile phases. We checked that the relationships between the biological activities studied and the log P and the molar total charge values were not adequate or were statistically not so good as the relationships obtained from the QRAR models shown below (R^2 values vary from 0.05 to 0.71).

1. Pharmacokinetic Parameters. Tricyclic antidepressants are drugs well-absorbed through the gastrointestinal tract. The therapeutic plasma levels vary from 50 to 500 ng/mL, and drug concentration over 500−1000 ng/mL has toxic effects.[24] The determination of pharmacokinetic parameters of the tricyclic antidepressants is very difficult due to a high and variable hepatic clearance of these drugs, which results in a relative low and variable bioavailability.[25] Tricyclic antidepressants

Table 3.

(a) Statistical Analysis and Predictive Features of the QRAR Models[a]

pharmacokinetic parameter (n)	$a \pm L_a$ (p value)	$b \pm L_b$ (p value)	$c \pm L_c$ (p value)	R^2 $(R_{adj})^2$	SE	F (p value)	RMSEC	RMSECV1	RMSECV1i
$T_{1/2}$ (11) (h)	1300 ± 500 (0.0002)	−1600 ± 600 (0.0002)	460 ± 170 (0.0002)	0.86 (0.82)	4.1	23.7 (0.0004)	3.47	4.86	4.71
V_d (9) (L/kg)	−1700 ± 700 (0.0010)	2100 ± 800 (0.0008)	−600 ± 200 (0.0008)	0.88 (0.84)	4.2	21.7 (0.0018)	3.40	4.96	5.20
CL_M (8) (L/h)	2900 ± 1200 (0.0018)	−3300 ± 1500 (0.0022)	1000 ± 400 (0.0024)	0.90 (0.86)	8.0	22.8 (0.0031)	6.36	13.48	7.89
T.P.L. (7) (ng/mL)	−11000 ± 4000 (0.0015)	14000 ± 5000 (0.0013)	−4200 ± 1400 (0.0012)	0.96 (0.94)	20.9	45.0 (0.0018)	15.78	29.20	30.34

(b) Predicted Values for Other Tricyclic Antidepressants Not Included in Model Building

antidepressant	$T_{1/2}$ (h)	V_d (L/kg)	CL_M (L/h)	T.P.L. (ng/mL)
amoxapine	(included in model)	32−58	(included in model)	(included in model)
doxepin	(included in model)	31−58	(included in model)	277−430
loxapine	18−38	7−29	49−96	94−225
melitracen	35−65	0−19	78−150	0−88
quinupramine	7−28	32−58	36−84	286−437

[a] Pharmacokinetic parameter $= a + b(\log k) + c(\log k)^2$, corresponding to the retention data obtained using a 0.04 M Brij35 mobile phase. n = number of available activities, L = 95% confidence interval for coefficient estimates, $(R_{adj})^2$ = R-squared adjusted for degrees of freedom, SE = standard error of the estimate, F = F ratio, RMSEC = root-mean-square error of calibration, RMSECV1 = root-mean-square error of cross-validation (leave-one-out), RMSECV1i = root-mean-square error of cross-validation (leave-one-out) for interpolated data.

Table 4.

(a) Statistical Analysis and Predictive Features of the QRAR Models[a]

biological response (n)	$a \pm L_a$ (p value)	$b \pm L_b$ (p value)	$c \pm L_c$ (p value)	R^2 $(R_{adj})^2$	SE	F (p value)	RMSEC	RMSECV1	RMSECV1i
IC_{50}(NA) (6) (10^{-8} M)	−50 ± 30 (0.0085)	60 ± 30 (0.0147)	−16 ± 10 (0.0169)	0.97 (0.95)	0.1	49.1 (0.0051)	0.09	0.22	0.31
IC_{50}(5-HT) (6) (10^{-8} M)	−40 ± 60 (0.1142)	50 ± 70 (0.1407)	−15 ± 20 (0.1234)	0.89 (0.82)	0.3	12.5 (0.0350)	0.20	0.46	0.30

(b) IC_{50} Predicted Values for Other Tricyclic Antidepressants Not Included in Model Building[b]

antidepressant	IC_{50}(NA) (10^{-8} M)
amoxapine	3.4 (0.99−11)
loxapine	4.4 (1.4−14)
melitracen	1.3 (0.18−8.9)
quinupramine	3.2 (0.94−11)

[a] $\log IC_{50} = a + b(\log k) + c(\log k)^2$, corresponding to the retention data obtained using a 0.04 M Brij35 mobile phase. For abbreviations, see Table 3. [b] The numbers in parentheses represent the prediction limits at the 95% confidence level.

show a large apparent volume of distribution which results in an extensive and high tissue binding. Plasma protein binding range varies from 80% to 95%. As a consequence of their exceptionally large volume of distribution and high clearance, tricyclic antidepressants also show half-life time values with an extremely wide reported range.

The possibility to establish relationships between tricyclic antidepressant retention data and their half-life time ($T_{1/2}$),[26,28] therapeutical plasma level (T.P.L.),[27] plasma clearance (CL_M),[28] and volume of distribution (V_d)[26,28] values has been studied. Figure 2 shows the relationships between the pharmacokinetic parameter values of some tricyclic antidepressants and their retention data and the corresponding residual plots. In all cases there is a random distribution of the residuals, and practically they all were statistically equal to zero which suggests, from a qualitative point of view, the adequacy of a polynomial model to the data.

Table 3a shows the statistical analysis and the predictive features of the QRAR models obtained by adjusting the corresponding pharmacokinetic parameter values and the logarithm of the retention data. For all models, the p values were less than 0.01. It means that the relationships between the pharmacokinetic parameter values and the log k were statistically significant at the 99% confidence level. In all cases, coefficients were also significant ($p < 0.01$) at the same confidence level. The standard error of estimation shows the standard deviation of the residuals to be 4.1, 4.2, 8.0, and 20.9 for $T_{1/2}$, V_d, CL_M, and T.P.L., respectively. This value can be used to construct prediction limits for new observations. Based on these results, it will be possible to estimate the corresponding pharmacokinetic parameter values of those tricyclic antidepressants with non-reported data. Table 3b shows the predicted values for these compounds.

2. Biological Responses. Action on biogenic amine reuptake: Tricyclic antidepressants promote the actions of noradrenaline (NA) and of sympathetic nerve stimulation by preventing inactivation of the NA released into the synaptic cleft. That is a result of blockade of the reuptake of the amine into the neuron by inhibition of the amine-concentrating mechanism located at the level of the cell membrane of the adrenergic neuron.[22]

The biological action of tricyclic antidepressants is also related to the blockade of the reuptake of serotonin

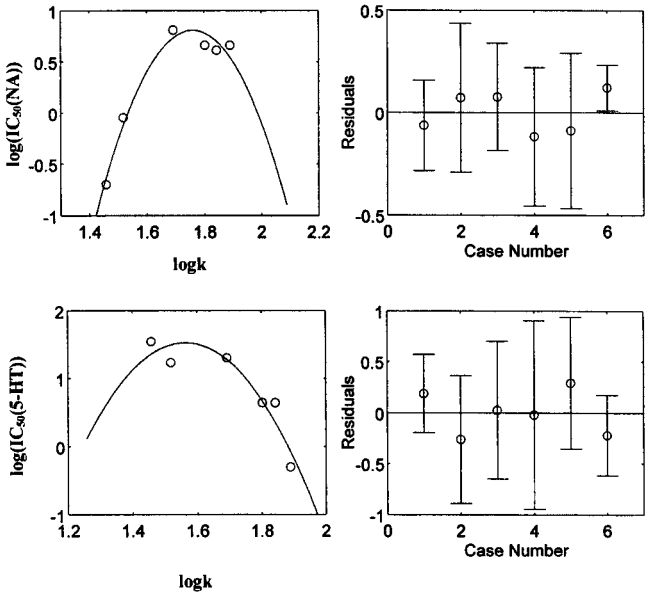


Figure 3. Log k−log IC_{50} relationships for the blockade of the NA and 5-HT reuptake (left) and residual plots (right) corresponding to these QRAR models.

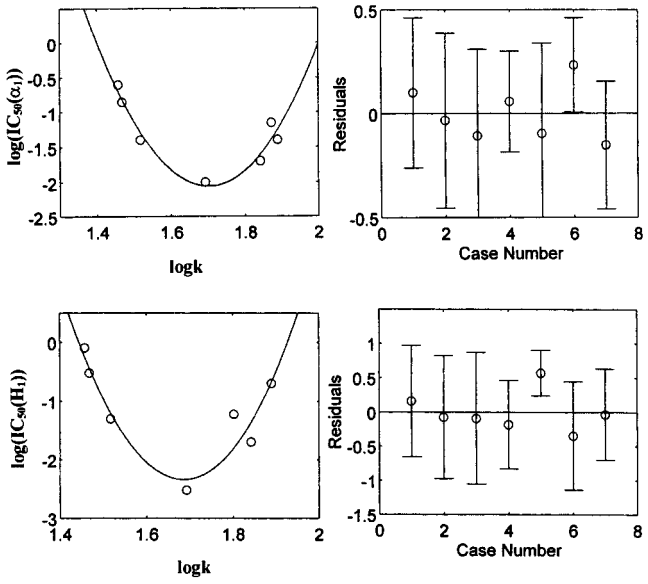


Figure 4. Log k−log IC_{50} relationships for the antagonist effect on α_1- and H_1-receptors (left) and residual plots (right) corresponding to these QRAR models.

(5-HT), but in this case, big differences between the reuptake inhibition degree of the drugs can be observed.[29,30] Relationships between the logarithm of the IC_{50} values (concentration for 50% inhibition measured in vitro) of tricyclic antidepressants for NA and 5-HT reuptake in rat brain[29] and the logarithm of retention data have been studied. Figure 3 shows the relationships between the log IC_{50} values of drugs and their retention data and the corresponding residual plots. In both cases the polynomial model was adequate.

Table 4a shows the statistical analysis and the predictive features of the corresponding QRAR models obtained. The coefficients in the QRAR model for the blockade of NA reuptake were statistically significant, but the corresponding values to the blockade of 5-HT reuptake were statistically nonsignificant. For both models, p values were less than 0.05 indicating that the relationships between log IC_{50} values for NA and 5-HT reuptake and log k values were statistically significant at the 95% confidence level. Table 4b shows the IC_{50} predicted values for NA reuptake of other tricyclic antidepressants. The prediction of IC_{50} values for 5-HT reuptake based on the proposed model was not considered.

Action on α_1-adrenergic and H_1-histaminic receptor sites: Tricyclic antidepressants present an accentuated antagonist effect on α_1-adrenergic and H_1-histaminic receptor sites.[29] Relationships between the corresponding log IC_{50} values for both receptor sites in rat brain and the log k values were obtained. As can be observed in Figure 4 there is an adequacy of a polynomial model to the data.

Table 5a shows the statistical analysis and the predictive features of the QRAR models obtained. For both models statistically significant relationships between log IC_{50} and log k values at the 95% confidence level exist (p values were 0.0068 and 0.0176 for the α_1- and H_1-receptor site inhibition models, respectively). The coefficient values were also significant at this confidence level. Table 5b shows the IC_{50} predicted values for α_1- and H_1-receptor site inhibition of other tricyclic antidepressants not included in the model building.

Effects on a histamine-sensitive brain adenylate cyclase: The activation by histamine of adenylate cyclase, which is coupled to the H_2-receptor in the brain, is one of the responsible processes of the histamine effect on the CNS.[1] Tricyclic antidepressant drugs are all

Table 5.

(a) Statistical Analysis and Predictive Features of the QRAR Models[a]

biological response (n)	$a \pm L_a$ (p value)	$b \pm L_b$ (p value)	$c \pm L_c$ (p value)	R^2 $(R_{adj})^2$	SE	F (p value)	RMSEC	RMSECV1	RMSECV1i
$IC_{50}(\alpha_1)$ (7) (nM)	60 ± 30 (0.0086)	−80 ± 40 (0.0049)	23 ± 11 (0.0052)	0.92 (0.88)	0.2	22.2 (0.0068)	0.13	0.22	0.22
$IC_{50}(H_1)$ (7) (nM)	110 ± 70 (0.0127)	−140 ± 80 (0.0096)	40 ± 20 (0.0100)	0.87 (0.81)	0.4	13.1 (0.0176)	0.27	0.42	0.48

(b) IC_{50} Predicted Values for Other Tricyclic Antidepressants Not Included in Model Building[b]

antidepressant	$IC_{50}(\alpha_1)$ (nM)	$IC_{50}(H_1)$ (nM)
amoxapine	0.012 (0.0029−0.048)	0.0063 (0.00034−0.12)
loxapine	0.035 (0.01−0.12)	0.084 (0.0055−1.3)
melitracen	0.4 (0.054−2.9)	1.5 (0.08−860)
quinupramine	0.012 (0.003−0.05)	0.0068 (0.00037−0.13)

[a] log $IC_{50} = a + b(\log k) + c(\log k)^2$, corresponding to the retention data obtained using a 0.04 M Brij35 mobile phase. For abbreviations, see Table 3. [b] The numbers in parentheses represent the prediction limits at the 95% confidence level.

Table 6.

(a) Statistical Analysis and Predictive Features of the QRAR Models[a]

biological response (n)	$a \pm L_a$ (p value)	$b \pm L_b$ (p value)	R^2 $(R_{adj})^2$	SE	F (p value)	RMSEC	RMSECV1	RMSECV1i
K_i(Hipp) (7) (μM)	2.6 ± 1.6 (0.0027)	−2.0 ± 0.9 (0.0021)	0.87 (0.85)	0.15	33.9 (0.0021)	0.1270	0.1834	0.1747
K_i(Neoc) (7) (μM)	2.2 ± 1.6 (0.0018)	−1.8 ± 0.9 (0.0044)	0.83 (0.80)	0.16	24.3 (0.0044)	0.1316	0.1736	0.1605
$IC_{50}(H_2)$ (7) (μM)	3.7 ± 1.8 (0.0222)	−2.0 ± 1.1 (0.0047)	0.83 (0.80)	0.18	23.6 (0.0047)	0.1493	0.2315	0.2180

(b) K_i (Hippocampus and Neocortex) and IC_{50} Predicted Values for Other Tricyclic Antidepressants Not Included in Model Building[b]

antidepressant	K_i(Hipp) (μM)	K_i(Neoc) (μM)	log $IC_{50}(H_2)$ (μM)
amoxapine	0.21 (0.08−0.57)	0.19 (0.07−0.52)	2.61 (0.83−8.18)
loxapine	0.07(0.03−0.19)	0.07 (0.03−0.2)	0.87 (0.27−2.81)
melitracen	0.04(0.02−0.13)	0.05 (0.02−0.14)	0.53 (0.15−1.90)
quinupramine	0.22 (0.08−0.59)	0.19 (0.07−0.53)	2.68 (0.85−8.44)

[a] log activity = $a + b$(log k), corresponding to the retention data obtained using a 0.04 M Brij35 mobile phase. For abbreviations, see Table 3. [b] The numbers in parentheses represent the prediction limits at the 95% confidence level.

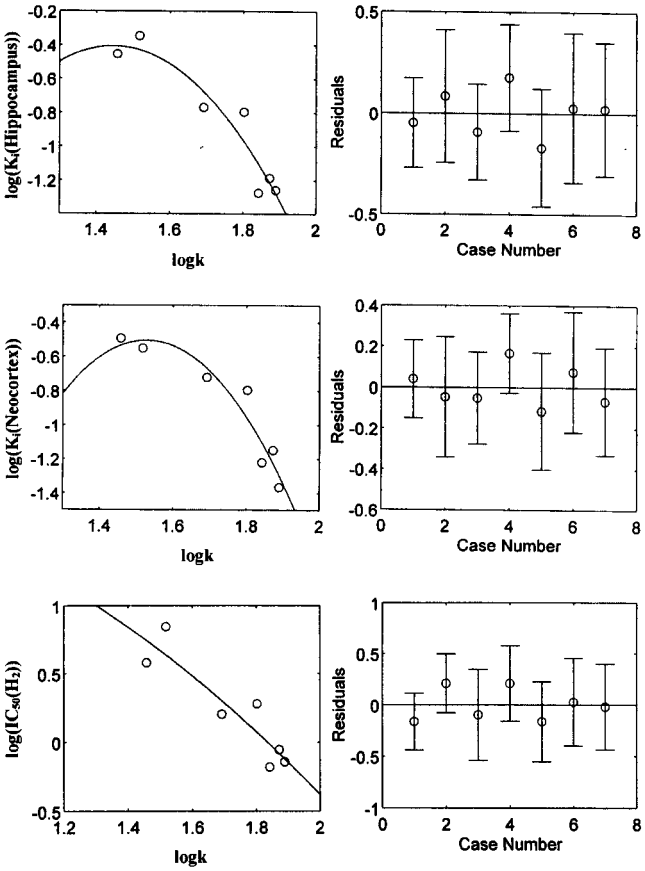


Figure 5. Log k−log K_i relationships for brain adenylate cyclase inhibition in hippocampus and neocortex of guinea pig, log k−log IC_{50} relationships for brain adenylate cyclase H_2-receptor (left), and residual plots (right) corresponding to these QRAR models.

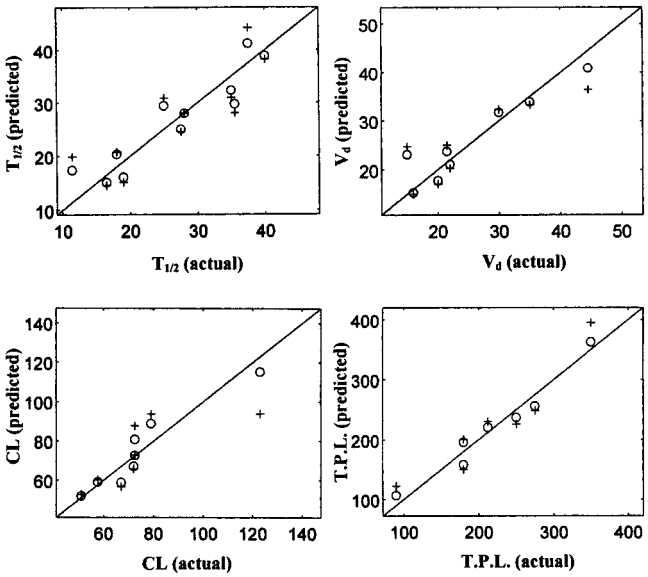


Figure 6. Validation plots for pharmacokinetic QRAR models: predicted pharmacokinetic parameters versus actual values. Fitted (○) and cross-validated (+) results are shown.

potent antagonists of the activation of brain adenylate cyclase by histamine.

Relationships between the logarithm of the inhibition constants of antidepressants for the adenylate cyclase activity (K_i, μM, estimated as the concentration of antidepressant required to produce maximal inhibition of the enzyme activity in the presence of 100 μM histamine), measured in homogenates of guinea pig hippocampus and neocortex, and the retention data were studied. It was possible, too, to establish a relationship between log IC_{50} (expressed as the concentration of antidepressant required to give 50% inhibition of the histamine effect on adenylate cyclase activity) and log k values. In Figure 5, the relationships between the biological parameters and log k values and the corresponding residual plots are shown. As can be observed, the polynomial models obtained for the hippocampus and neocortex inhibition constants and the IC_{50} were adequate to describe these activities (R^2 = 0.92, 0.96, and 0.83, respectively) and statistically significant at the 95% confidence level (p values were 0.0081, 0.0067, and 0.0043, respectively). However the coefficients were statistically nonsignificant at the 95% probability level.

To obtain models with predictive ability, linear models were assayed. Table 6a shows the statistical analysis and the predictive features of the lineal QRAR models obtained. In all cases, the corresponding p values to the models and their coefficients were less than 0.01. Table 6b shows the K_i (hippocampus and neocortex) and IC_{50} predicted values for some tricyclic antidepressants with no available data. For amineptine, which has a low retention, the application of linear models provided excessively large values (K_i(hippocampus) = 2.6 μM, K_i(neocortex) = 1.7 μM, and $IC_{50}(H_2)$ = 30.3 μM), with

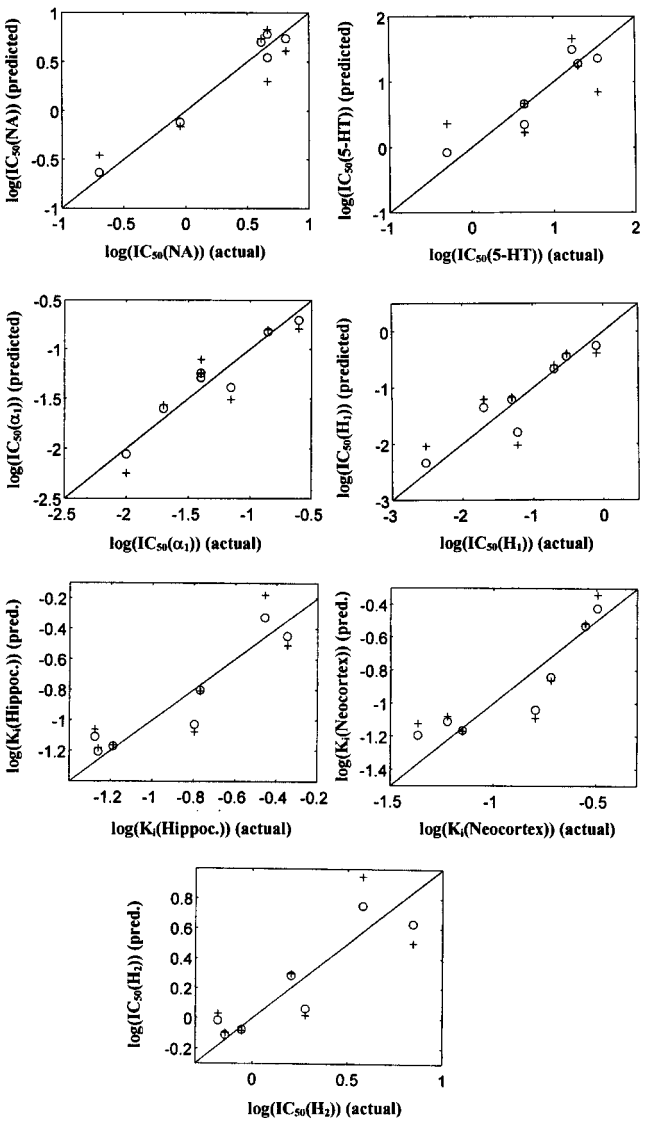


Figure 7. Validation plots for biological response QRAR models: predicted activities versus actual values. Fitted (○) and cross-validated (+) results are shown.

regard to the values reported in the literature from other tricyclic antidepressants. However, despite polynomial models not being used as predictive models, the estimated values from these models, included amineptine, were similar enough to those reported in the literature for other tricyclic antidepressants (K_i(hippocampus) = 0.11, 0.28, 0.066, 0.024, and 0.29 μM and K_i(neocortex) = 0.023, 0.27, 0.065, 0.020, and 0.28 μM for amineptine, amoxapine, loxapine, melitracen, and quinupramine, respectively). This fact supports the hypothesis that polynomial models are closer to the real behavior of tricyclic antidepressants and linear models could be adequate only for a narrow range of log *k* values.

Predictive Ability of QRAR Models. To compare the predictive ability of the models in terms of cross-validated data, but pointing out the difference between interpolated and extrapolated data, the RMSEC, RMSECV, and RMSECVi values for the QRAR models were obtained (see Tables 3−6). As can be observed for all models, except for that corresponding to CL, the RMSECV and RMSECVi values were similar. This suggests that both interpolations and extrapolations of pharmacokinetic and biological parameters based on the current QRAR models should be reasonably adequate. In contrast, for the CL model, the RMSECV value was notoriously larger than the RMSECVi one, which indicates that some cautions must be taken with extrapolated parameter data. Nevertheless, in this case the information obtainable may be useful from a practical point of view.

Figures 6 and 7 show, respectively, the predicted (fitted and cross-validated) versus actual activities for the available data. As can be observed in general, the ability of log *k* values in describing and predicting pharmacokinetic and biological responses is adequate.

Conclusions

The need to get a tool for pharmacokinetic and biological parameter estimation of new compounds (e.g., a new synthesized drug from a generic molecular structure) for clinical applications supports the postulation of predictive models as an alternative to conventional clinical assays.

Chromatographic surfaces modified by absorption of surfactants (MLC) resemble the lipid bilayers of biological barriers. In addition, the retention of compounds in MLC, which depends on hydrophobic, electronic, and steric features of compounds, is obtained in flow conditions in a similar way as the phenomena of absorption, transport, metabolism, and excretion of drugs occur in the body. Consequently, a single MLC retention parameter, log *k*, would be able to describe these processes. In addition, log *k* values are empirical variables easy to measure and are reproducible.

From a statistical point of view, the main problem to construct models with predictive ability of biological responses is the small number of available activity data of compounds because they have not been studied and/or reported. This approach involving quantitative retention−activity relationships (QRAR) may be a preferable alternative, and sometimes a unique option, to QSAR models in order to obtain estimation or at least useful qualitative information about drug activity.

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