Eur J Med Chem (1997) 32, 433–443 © Elsevier, Paris

Physicochemical parameters involved in the lethal toxicity of N,N-[(dimethylamino)ethyl]-4-substituted benzoate hydrochlorides: a QSAR study

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(Received 2 October 1996; accepted 21 November 1996)

Summary — A set of sixteen *para*-substituted *N*,*N*-[(dimethylamino)ethyl] benzoate hydrochlorides structurally related to procaine was synthesized. The apparent partition coefficients were determined by either shake-flask or HPLC methods and were taken as hydrophobic parameters. The IR stretching frequencies of the carbonyl group were determined in chloroform and taken as one of the electronic parameters. Additional physicochemical parameters were either taken from the literature: π , σ , \Im and \Re , MR₄, or calculated: log *P*. The lethal potency was determined in the mouse via the LD₅₀. In order to verify the nature and the relative contributions of the physicochemical parameters to lethal toxicity, QSAR equations were derived using regression analysis. A major contribution of hydrophobicity together with a smaller but still significant contribution of electronic or polar properties was found to describe the toxicity within this set of compounds.

physicochemical parameters / hydrophobicity / para-substituted N,N-[(dimethylamino)ethyl]benzoate hydrochlorides / lethal toxicity / QSAR

Introduction

Local anesthetics (LA) block the conduction in peripheral nerves by modifying the kinetics of opening and closing of the voltage-gated sodium channels [1-4]. However, local anesthetics do not only interfere with peripheral nerves. Any excitable membranes such as those of brain or heart cells can be altered by such drugs if they achieve sufficient tissue concentration, causing systemic effects. Although some of these effects have proven to be of therapeutic value, most of them are usually undesirable due to their toxic nature. In this respect the central nervous system (CNS) and cardiovascular system (CVS) are usually the most frequently affected. Therefore, the lethal toxicity of LA results from a conjunction of these untoward effects, and is consequently complex in terms of molecular mechanisms [4-8]. As both CNS and CVS toxicities apparently increase together

with LA potency, the assumption became widespread that the same structural features equally modulated both the anesthesiological and toxicological potencies of LA [4–8]. However, when this question is more carefully examined in a quantitative manner using QSAR, for example, the results do not strictly agree with this view.

Thus Recanatini et al [9], in a QSAR study involving a set of compounds structurally related to lidocaine found a differential contribution of lipophilicity to acute toxicity on the one hand and local anesthetic activity on the other. Moreover, the coefficients of log P_{app} and log P_{app}^2 were small, in the correlation equations, when either linear or non-linear models were employed to describe the biological activity. This led the authors to question the dependence of acute toxicity on log P in their set of compounds structurally related to lidocaine. In addition, it should be mentioned that in the set studied by Recanatini et al [9], a negative dependence on log P_{app} was found, describing the acute toxicity. This somewhat unexpected finding lcd us to examine the question further, using a different set of local anesthetics. These incompletely settled

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questions reflect the complexity of the molecular mechanisms underlying LA anesthetic and toxic effects [1-15].

In the case of acute toxicity, dealt with in the present work, an additional difficulty is that it is a whole body phenomenon and might therefore be more difficult to model, ie, the QSAR analysis may require parameters encoding information on metabolism, bioaccumulation, excretion, etc [10, 11, 14, 15]. However, it is still clearly worthwhile attempting to obtain a further understanding of this issue, and possibly disclosing structure–activity relationships that could in the long run contribute to obtain local anesthetics of lower toxicity [9, 10, 12, 13, 16].

The present work is thus aimed at obtaining a better understanding of the structural features and physicochemical parameters which are significantly involved in the lethal toxicity of this class of drugs. For this reason, a set of 16 *para*-substituted *N*,*N*-[(dimethylamino)ethyl]benzoate hydrochlorides, structurally related to procaine, has been synthesized and their lethal potencies evaluated. Each compound had its corresponding hydrophobic, electronic and polarizabilityrelated parameter either determined experimentally or taken from the data available in the literature [16–21]. Subsequently a QSAR analysis was performed to determine the nature and the relative contributions of the structural parameters that significantly modulate the lethal toxicity in the set of compounds studied.

Chemistry

Preparation of the compounds

In the design of the compounds subjected to the present QSAR study, Craig's criteria [22] were used to avoid significant intercorrelation of the physicochemical parameters used in the analysis. The compounds were obtained by an unambiguous method that requires only brief comment [23, 24]. They were prepared from the reaction of the corresponding benzoic acid with *N*,*N*-dimethylaminoethylchloride hydrochloride (*Method A*) or with *N*,*N*-dimethylaminoethanol hydrochloride (*Method B*). Good yields were achieved. The final products were purified by recrystallization from the appropriate solvent. All the compounds were characterized by their ¹H- and ¹³C-NMR spectra.

Lipophilicity parameters

The overall lipophilicity of each compound was assessed by its octanol/aqueous apparent partition coefficient, P_{app} , instead of the true partition coefficient, P [16, 25, 26, 38]. P_{app} values were obtained at

pH 7.40 by the shake-flask [21, 27] and at pH 7.24 by high-performance liquid chromatography (HPLC) methods, which were calculated from the reversed-phase HPLC capacity factors (log k') [27–30]. The true log P values were calculated by means of the ClogP program [17, 31], which takes into account only the unionized form of these N,N-[(dimethyl-amino)ethyl] benzoate hydrochlorides. Unless otherwise indicated, log P always refers to 1-octanol-water partition coefficients.

The Hansch–Fujita substituent constants, π , were taken from the literature [16, 19–21]. The π value for each substituent was also evaluated from the difference between the experimental log P_{app} values, determined respectively for the substituted and unsubstituted benzoates, and it was used in the analysis as the descriptor of the specific hydrophobic effect of the substituent.

Electronic parameters

The Hammett σ as well as the Swain–Lupton \Im and \Re substituent constants were used as electronic parameters, and were taken from the literature [16, 18–21].

The infrared carbonyl group stretching frequencies, (cm^{-1}) , $v_{C=0}$, of the compounds of the set (excepting 4-SO₂CH₃ and 4-CF₃ derivatives) were measured systematically in chloroform, and were also taken as an electronic parameter [24, 32]. For correlation purposes, the $v_{C=0}$ values were scaled by a factor of 0.01 in the QSAR analysis.

Polarizability-related parameter

The molar refractivity values of the substituent at the *para*-position of the aromatic ring, MR_4 , were taken from the literature. For correlation purposes, MR_4 values were scaled by a factor of 0.1 [16, 19–21].

Biology

The lethal potency of the compounds was assessed in the mouse. The median lethal doses (LD_{50}), expressed in mM/kg, and the 95% confidence limits were evaluated using probit analysis [33]. Log $1/LD_{50}$ expressed in M/kg, derived for correlation purposes, was taken as the biological parameter, indicating the lethal potency of the compounds.

QSAR analysis

Multiple regression analyses were performed using the BILIN program [16, 34] to determine the coefficients of the correlation equations. In addition to the *F*-test, the cross-validation test was applied to evaluate the significance of the correlations. In all the equations in this paper, the numbers in parentheses represent the 95% confidence interval of the coefficients; *n* is the number of points on which the equation is based; *r* is the correlation coefficient; *s* is the standard deviation; *F* is the Fisher significance test value; r_{cv}^2 and s_{PRESS} are the squared correlation coefficient and the standard deviation respectively associated with the cross-validation procedure [16, 34].

Results and discussion

The obtained results are displayed in tables I–VI and in equations (1-13). The chemical data, methods of preparation of the compounds and their yields, which were within 52.5–89.8%, are shown in table I. The proposed structures are in accordance with the spectral data of the compounds, which are presented in tables II and III.

The biological activity, ie, the median lethal doses (LD_{50}) of the compounds studied is displayed in table IV. The biological parameter, ie, the lethal potency, log 1/LD₅₀, together with the physicochemical parameters derived for correlation purposes are shown in table V. As can be seen, in this set of substituted N,N-[(dimethylamino)ethyl]benzoate hydrochlorides which are procaine analogs, the lethal potency varied over more than one logarithmic unit. In addition, as a matter of comparison, the LD₅₀ of a commercial procaine hydrochloride was also evaluated under the same experimental conditions. A value of 3.20 for its lethal potency was found, which is compatible to 3.14 obtained from a LD_{50} value of procaine found in the literature [7]. Thus comparatively, the most potent compound in the set, the $4-n-C_6H_{13}$ derivative with a potency of 3.16, as seen in table V, is about as potent as procaine in terms of lethal toxicity.

In table V, log P of the unionized form of the derivatives and π values are given for comparison. Throughout this study log P_{app} has been used as the measurement of the lipophilic interactions under the experimental condition of acute toxicity estimation. In the present work, the log P_{app} values were determined by both shake-flask and HPLC methods; see equation (1). This was performed to validate the HPLC measurements, which is an indirect method for log P determinations. It should be pointed out that for this set of compounds, ie, benzoates susceptible to hydrolysis, the HPLC method proved to be more useful because the time required for measurements was shorter

compared to the shake-flask method, which needed correction for hydrolysis regarding this set of benzoate derivatives. However, in spite of these limitations, both methods gave very similar results (table V) considering the experimental error and the slightly different pHs (7.40 and 7.24 respectively) at which the log P_{app} values were measured.

To sum up, the highly significant correlation in equation (1) found between the measurements obtained by HPLC and the shake-flask methods validates the use of the former as a lipophilic parameter in the QSAR analysis.

$$\log P_{app} (\text{HPLC}) = 1.02 (\pm 0.04) \log P_{app} (\text{SF}) -0.05 (\pm 0.08)$$
(1)

 $n = 16, r = 0.998, s = 0.086, F = 3319.6, r_{cv}^2 = 0.995, s_{PRESS} = 0.098$

As mentioned before, π values for the substituents in the set of benzoate derivatives were also evaluated from the experimentally measured log P_{app} values of the corresponding substituted and unsubstituted compound. The comparison of the tabulated π values [16, 19–21] with those obtained from experimental values were within the limits of experimental error. This finding shows that no specific hydrophobic effect of the substituent was observed in the set studied in this work.

As presented in table V and regarding the electronic and polar properties of the compounds in this set, $v_{(C=0)}$ were systematically [32] studied in this work, to obtain quantitative information on the nature and the effects of the substitution, on the electronic distribution of the carbonyl group, ie, to verify the nature of the electronic effects on the polarity of the carbonyl group [18, 20, 25, 32]. The $v_{(C=0)}$ values for 14 compounds were experimentally determined, and for the 4-SO₂CH₃ and the 4-CF₃ derivatives they were estimated from equation (2). The use of $v_{C=0}$ as a descriptor of the electronic effects transmitted by the substituents was validated by the application of the Hammett equation to the $v_{(C=0)}$ values, which led to significant correlations, as shown in equations (2) and (3).

$$v_{C=0} = 1725.0 (\pm 1.4) + 13.5 (\pm 3.8) \sigma$$
 (2)

$$n = 14, r = 0.912, s = 0.023, F = 58.955,$$

 $r_{cv}^2 = 0.780, s_{PRESS} = 0.027$

$$v_{C=0} = 1726.0 (\pm 1.3) + 6.71 (\pm 3.8) \Im + 18.9 (\pm 3.8) \Re$$
(3)

n = 14, r = 0.967, s = 0.015, F = 79.490, $r_{cv}^2 = 0.901, s_{PRESS} = 0.019$

Compound	R	Yield (%) (Method of preparation) ^a	Mp (°C) (Recrystallization solvent) ^b	Formula ^c (MW)					
1	Н	86.2 (A)	147-149 (D)	$\begin{array}{c} C_{11}H_{16}N_{1}O_{2}Cl_{1}\\(229.71)\end{array}$					
2	CH ₃	76.2 (A)	164–167 (D)	$\begin{array}{c} C_{12}H_{18}N_1O_2Cl_1\\ (243.73)\end{array}$					
3	C_2H_5	74.4 (A)	134–137 (D)	$\begin{array}{c} C_{13}H_{20}N_1O_2Cl_1\\ (257.76)\end{array}$					
4	<i>n</i> -C ₃ H ₇	88.7 (B)	103–105 (C)	$\begin{array}{c} C_{14}H_{22}N_1O_2Cl_1\\(271.78)\end{array}$					
5	n-C ₄ H ₉	86.0 (A)	100–103 (D)	$\begin{array}{c} C_{15}H_{24}N_1O_2Cl_1\\ (285.81)\end{array}$					
6	$t-C_4H_9$	80.1 (A)	175–178 (D)	$\begin{array}{c} C_{15}H_{24}N_{1}O_{2}CI_{1}\\ (285.81)\end{array}$					
7	OCH ₃	89.8 (A)	158–160 (D)	$\begin{array}{c} C_{12}H_{18}N_1O_3CI_1\\ (259.73)\end{array}$					
8	n-OC ₄ H ₉	52.5 (A)	122–124 (C)	$\begin{array}{c} C_{15}H_{24}N_1O_3Cl_1\\ (301.81)\end{array}$					
9	COCH ₃	56.8 (A)	173–174 (C)	$C_{13}H_{18}N_1O_3Cl_1$ (271.74)					
10	Cl	54.0 (A)	194–198 (E)	$\begin{array}{c} C_{11}H_{15}N_1O_2Cl_2\\ (264.15)\end{array}$					
11	NO ₂	68.3 (A)	181–185 (D)	$\begin{array}{c} C_{11}H_{15}N_2O_4Cl_1\\ (274.70)\end{array}$					
12	CN	68.6 (A)	205–206 (C)	$\begin{array}{c} C_{12}H_{15}N_2O_2Cl_1\\ (254.71)\end{array}$					
13	$n - C_6 H_{13}$	83.7 (B)	106–108 (C)	$\begin{array}{c} C_{17}H_{28}N_1O_2Cl_1\\ (313.87)\end{array}$					
14	$n-OC_{6}H_{13}$	60.0 (B)	128–130 (C)	$\begin{array}{c} C_{17}H_{28}N_{1}O_{3}Cl_{1}\\ (329.86)\end{array}$					
15	SO ₂ CH ₃	41,0 (B)	199–203 (D)	$\begin{array}{c} C_{12}H_{18}N_{1}O_{4}S_{1}Cl_{1}\\ (307.79)\end{array}$					
16	CF ₃	79.4 (B)	156–162 (D)	$\begin{array}{c} C_{12}H_{15}N_1O_2F_3Cl_1\\ (297.70) \end{array}$					

Table I. Chemical data and properties of a set of N,N-[(dimethylamino)ethyl]-4-substituted benzoate hydrochlorides.

^aSee experimental protocols for methods of preparation, A and B; ^brecrystallization solvent: (C) methanol/diethyl ether; (D) ethanol/diethyl ether; (E) ethanol; ^call of the compounds were analyzed for C, H, N and results agreed to within $\pm 0.4\%$ of calculated values.

436

Table II. ¹H-NMR chemical shifts^a for the *N*,*N*-[(dimethylamino)ethyl]-4-substituted benzoate hydrochlorides in CDCl₃^b.

Compound	$\delta(ppm)$
1	8.05–7.48 (m, 5H, Ar– <i>H</i>); 4.82 (t, 2H, OC <i>H</i> ₂); 3.64 (t, 2H, NC <i>H</i> ₂); 2.97 (s, 6H, N(C <i>H</i> ₃) ₂)
2	7.91–7.38 (dd, 4H, Ar–H); 4.84 (t, 2H, OCH ₂); 3.59 (t, 2H, OCH ₂); 2.99 (s, 6H, N(CH ₃) ₂); 2.44 (s, 3H, CH ₃)
3	7.97–7.38 (dd, 4H, Ar– <i>H</i>); 4.96 (t, 2H,OC <i>H</i> ₂); 3.98 (t, 2H, NC <i>H</i> ₂); 3.02 (s, 6H, N(C <i>H</i> ₃) ₂); 2.58 (qa, 2H, C <i>H</i> ₂ CH ₃); 1.37 (t, 3H, CH ₂ C <i>H</i> ₃)
4	13.03 (s, 1H, ⁺ N <i>H</i>); 7.97–7.25 (dd, 4H, Ar– <i>H</i>); 4.82 (t, 2H, OC <i>H</i> ₂); 3.50 (t, 2H, NC <i>H</i> ₂); 2.94 (s, 6H, N(C <i>H</i> ₃) ₂); 2.65 (t, 2H, C <i>H</i> ₂); 1.64 (m, 2H, C <i>H</i> ₂); 0.94 (t, 3H, C <i>H</i> ₃)
5	13.02 (s, 1H, ⁺ N <i>H</i>); 7.97–7.26 (dd, 4H, Ar– <i>H</i>); 4.84 (t, 2H, OC H_2); 3.49 (t, 2H, NC H_2); 2.95 (s, 6H, N(C H_3) ₂); 2.67 (t, 2H, C H_2); 1.61 (m, 2H, C H_2); 1.36 (m, 2H, C H_2); 0.93 (t, 3H, C H_3)
6	7.99–7.61 (dd, 4H, Ar–H); 4.95 (t, 2H, OCH ₂); 3.75 (t, 2H, NCH ₂); 3.15 (s, 6H, N(CH ₃) ₂); 1.59 (s, 9H, C(CH ₃) ₃)
7	12.6 (s, 1H, ⁺ N <i>H</i>); 8.00–6.80 (dd, 4H, Ar– <i>H</i>); 4.80 (t, 2H, OC <i>H</i> ₂); 3.80 (s, 3H, OC <i>H</i> ₃); 3.50 (t, 2H, NC <i>H</i> ₂); 2.90 (s, 6H, N(C <i>H</i> ₃) ₂)
8	12.91 (s, 1H, ⁺ N <i>H</i>); 8.02–6.90 (dd, 4H, Ar– <i>H</i>); 4.80 (t, 2H, OC <i>H</i> ₂); 4.02 (t, 2H, OC <i>H</i> ₂); 3.50 (t, 2H, NC <i>H</i> ₂); 2.96 (s, 6H, N(C <i>H</i> ₃) ₂); 1.79 (m, 2H, C <i>H</i> ₂); 1.52 (m, 2H, C <i>H</i> ₂); 0.98 (t, 3H, C <i>H</i> ₃)
9	12.94 (s, 1H, ⁺ N <i>H</i>); 8.21–8.01 (dd, 4H, Ar– <i>H</i>); 4.87 (t, 2H, OC <i>H</i> ₂); 3.54 (t, 2H, NC <i>H</i> ₂); 2.97 (s, 6H, N(C <i>H</i> ₃) ₂); 2.65 (s, 3H, COC <i>H</i> ₃)
10 ^c	8.03-7.40 (dd, 4H, Ar-H); 4.60 (t, 2H, OCH ₂); 3.60 (t, 2H, NCH ₂); 3.00 (s, 6H, N(CH ₃) ₂)
11 ^d	8.2-7.7 (dd, 4H, Ar-H); 4.8 (t, 2H, OCH ₂); 3.6 (t, 2H, NCH ₂); 3.0 (s, 6H, N(CH ₃) ₂)
12	13.23 (s, 1H, ⁺ NH); 8.28–7.77 (dd, 4H, Ar–H); 4.86 (t, 2H, OCH ₂); 3.48 (t, 2H , NCH ₂); 2.95 (s, 6H, $N(CH_3)_2$)
13	12.97 (s, 1H, ⁺ N <i>H</i>); 7.97–7.28 (dd, 4H, Ar– <i>H</i>); 4.84 (t, 2H, OC <i>H</i> ₂); 3.51 (t, 2H, NC <i>H</i> ₂); 2.94 (s, 6H, N(C <i>H</i> ₃) ₂); 2.66 (t, 2H, C <i>H</i> ₂); 1.62 (m, 2H, C <i>H</i> ₂); 1.30 (m, 6H, C <i>H</i> ₂); 0.88 (t, 3H, C <i>H</i> ₃)
14	12.94 (s, 1H, ⁺ N <i>H</i>); 8.02–6.91 (dd, 4H, Ar– <i>H</i>); 4.81 (t, 2H, OC <i>H</i> ₂); 4.01 (t, 2H, OC <i>H</i> ₂); 3.60 (t, 2H, NC <i>H</i> ₂); 2.94 (s, 6H, N(C <i>H</i> ₃) ₂); 1.79 (m, 2H, C <i>H</i> ₂); 1.33 (m, 6H, C <i>H</i> ₂); 0.91 (t, 3H, C <i>H</i> ₃)
15 ^e	11.33 (s, 1H, ⁺ N <i>H</i>); 8.69–8.38 (dd, 4H, Ar– <i>H</i>); 4.98 (t, 2H, OC <i>H</i> ₂); 3.69 (t, 2H, NC <i>H</i> ₂); 3.15 (s, 6H, N(C <i>H</i> ₃) ₂); 3.09 (s, 3H, C <i>H</i> ₃)
16 ^c	11.40 (s, 1H, ⁺ NH); 8.66–8.20 (dd, 4H, Ar–H); 4.98 (t, 2H, OCH ₂); 3.85 (t, 2H , NCH ₂); 3.16 (s, 6H, N(CH ₃) ₂)

^aIn ppm relative to TMS; ^ball spectra were recorded in CDCl₃ unless otherwise indicated; ^cD₂O used as a solvent; ^dacetone– d_6 used as solvent; ^eDMSO– d_6 used as a solvent.

The analysis of equation (2) indicates an electronic effect transmitted from the substituent to the carbonyl group describing both inductive and resonance effects. In equation (3), the electronic effect was analysed separately, using \Im and \Re as electronic descriptors, ie, the field and resonance effects respectively. The analysis of their ρ values (6.72 and 18.9 respectively), obtained in equation (3), indicates a major contribution of the resonance effect when compared with the field effect. This finding suggests a coplanarity between the substituent and the carbonyl group in this set of benzoates.

The inclusion of $v_{c=0}$ as an electronic parameter in addition to σ or \mathfrak{F} and \mathfrak{R} is further justified taking into account that the experimentally determined carbonyl frequency shifts constitute a descriptor of the

global electronic effects transmitted by the substituent to the carbonyl via inductive and/or resonance and/or field pathways, for these specific compounds. The MR_4 values, taken from the literature [16, 19–21], are also presented in table V. As discussed elsewhere [16, 25, 36], the molar refractivity may describe different properties related to the substituent such as electron polarizability, volume and lipophilicity. The degree of intercorrelation between the corresponding parameters dictates the nature of the property described by MR_4 in a certain set of compounds [15, 16, 36]. In the present work, the aim was that MR_4 values would mainly express the polarizability of the substituent.

In order to evaluate the nature and relative contributions of the physicochemical and structural parameters significantly involved in lethal potency in the set

Com- pound	C-1,5	C-2,4	C-3	С-6	C-7	C-8	C-9	C-10,11	C-12	C-13	R C-14	C-15	C-16	C-17
1 2 3 4 5 ^d 6 7 8 ^d 9 ^d	131.61 132.42 132.45 129.02 129.61 127.64 116.83 114.33 128.26	131.26 132.12 130.77 132.36 128.62 128.37 134.74 131.71 129.92	132.42 128.37 128.52 128.74 126.67 131.97 123.66 121.10 132.77	136.98 148.22 153.95 157.61 148.37 159.21 166.37 162.83 140.26	170.36 170.31 170.01 169.40 165.28 168.95 169.98 164.96 164.71	62.13 61.96 61.90 61.77 59.03 61.71 61.94 58.83 59.56	58.77 58.80 58.61 58.32 54.78 58.11 58.86 58.67 54.66	46.03 46.06 45.88 45.65 42.38 45.48 46.06 42.38 42.37	23.63 31.02 36.12 34.71 36.78 58.86 67.56	17.16 25.45 32.66 32.82 30.50 27.03	13.40 21.62 32.82 18.60	13.65 32.82 13.59		
10 11 12 ^d 13 ^d 14 ^d 15 ^e 16 ^{d.c}	128.20 129.65 133.69 132.63 129.63 114.33 127.04 125.87	123.52 133.67 137.07 130.29 128.56 131.71 130.85 126.63	126.20 126.52 133.12 126.62 121.09 133.44 130.74	142.19 153.26 115.59 148.63 162.82 142.81 133.53	164.97 168.97 168.38 164.17 165.30 164.96 165.51 164.55	62.14 62.90 59.75 59.03 58.82 59.59 59.92	54.00 58.43 58.68 54.59 54.82 54.82 54.83 55.52 54.91	45.77 46.08 42.32 42.42 42.39 42.86 42.63	118.01 35.04 67.86 42.29 125.72	30.98 28.41	30.48 25.03	28.18 30.89	21.97 21.98	13.86 13.83

Table III. ¹³C-NMR chemical shifts^a for carbon atoms^b for the set of N,N-[(dimethylamino)ethyl]-4-substituted benzoate hydrochlorides in D₂O^c.

^aIn ppm relative to TMS; ^bthe numbering of the carbon atoms is shown in the structure above. It does not follow the nomenclature, but it is used to simplify the assignment of chemical shifts; ^call spectra were recorded in D₂O unless otherwise indicated; ^dDMSO- d_6 used as solvent; ^cspectra recorded at 50 MHz in DMSO- d_6 used as solvent.

of studied compounds, a traditional QSAR analysis was carried out. The squared correlation matrix of the independent variables used in this analysis is displayed in table VI.

The Hansch approach [16, 21, 25, 37] applied to the lethal potency of all 16 *N*,*N*-[(dimethylamino)ethyl]-4-substituted benzoate hydrochlorides, using the linear model, and to the physicochemical parameters previously discussed, resulted in equations (4–8).

$$\log I/LD_{50} = 0.26 (\pm 0.06) \log P_{app} (HPLC) + 2.18 (\pm 0.12)$$
(4)

$$n = 16, r = 0.932, s = 0.133, F = 91.940,$$

 $r_{cv}^2 = 0.835, s_{\text{PRESS}} = 0.149$

$$\log I/LD_{50} = -0.67 (\pm 0.32) \sigma + 2.70 (\pm 0.13)$$
 (5)

 $n = 16, r = 0.765, s = 0.236, F = 19.806, r_{cv}^2 = 0.456, s_{PRESS} = 0.271$

$$\log 1/LD_{50} = -0.59 (\pm 0.58) \Im - 0.70 (\pm 0.56) \Re + 2.69 (\pm 0.20)$$
(6)

n = 16, r = 0.764, s = 0.246, F = 9.088, $r_{cv}^2 = 0.381, s_{PRESS} = 0.299$

$$\log 1/LD_{50} = -0.046(\pm 0.023) v_{(C=0)} + 81.62 (\pm 39.5)$$
(7)

n = 16, r = 0.753, s = 0.241, F = 18.357, $r_{cv}^2 = 0.433, s_{PRESS} = 0.276$

$$\log I/LD_{50} = 0.29 (\pm 0.16) MR_4 + 2.24 (\pm 0.25)$$
(8)

n = 16, r = 0.731, s = 0.251, F = 16.024, $r_{cv}^2 = 0.434, s_{PRESS} = 0.276$

In these equations, the regression statistics indicates that the lipophilic term explains more of the observed variation in the LD_{50} than the electronic and polariza-

438

Compound	R	<i>LD</i> ₅₀ ^a (95% <i>CL</i> ^b)
1	Н	4.33 (4.09–4.53)
2	CH ₃	2.87 (2.75-3.10)
3	C_2H_5	2.63 (2.56-2.71)
4	$n-C_3H_7$	1.66 (1.58–1.80)
5	$n-C_4H_9$	0.91 (0.82–1.05)
6	$t-C_4H_9$	1.16 (1.12–1.20)
7	OCH ₃	2.00 (1.92-2.05)
8	n-OC ₄ H ₉	0.84 (0.79–0.88)
9	COCH ₃	5.15 (4.87–5.40)
10	Cl	4.27 (4.10-4.49)
11	NO_2	3.69 (3.56-3.81)
12	CN	7.53 (7.31–7.80)
13	$n - C_6 H_{13}$	0.70 (0.62–0.74)
14	$n-OC_{6}H_{13}$	0.95 (0.84–1.04)
15	SO ₂ CH ₃	$\approx 8.4^{\circ}$
16	CF ₃	2.11 (1.88–2.25)

Table IV. Biological activity in a set of *N*,*N*-[(dimethyl-amino)ethyl]-4-substituted benzoate hydrochlorides.

 $^{a}LD_{50}$ values were calculated in mM/kg; $^{b}CL =$ confidence limits; $^{c}approximate$ value. Confidence limits could not be calculated; see text.

bility terms. However, in order to verify whether these two last terms could nonetheless contribute together with the lipophilic term to the lethal potency, a multiple regression analysis approach was subsequently applied.

The relative contributions to lethal toxicity of both lipophilic and electronic terms were evaluated in a subset of 15 compounds in which the hexyloxy-derivative was excluded. Thus, in this subset the collinearity between the analyzed terms became lower than that initially observed in the intercorrelation matrix presented in table VI. Therefore, the intercorrelation matrix r^2 values became the following: 0.350 (log *P* vs $v_{C=0}$); 0.469 (log *P* vs σ); 0.447 (log *P* vs \mathfrak{F}) and 0.223 (log *P* vs \mathfrak{R}). Whereas the contribution of σ

and \Im was not significant, a slight improvement in statistical significance is indicated by inclusion of either \Re or $v_{c=0}$ (equations (9, 10)).

$$log I/LD_{50} = 0.24 (\pm 0.06) log P_{app} (HPLC) - 0.32 (\pm 0.32) \Re + 2.19 (\pm 0.12)$$
(9)

$$n = 15, r = 0.952, s = 0.116, F = 57.518,$$

 $r_{cv}^2 = 0.859, s_{PRESS} = 0.142$

$$\log I/LD_{50} = 0.23 (\pm 0.07) \log P_{app} (HPLC) -0.017(\pm 0.015)v_{C=0} + 31.41 (\pm 25.2)$$
(10)

$$n = 15, r = 0.956, s = 0.111, F = 64.433, r_{cv}^2 = 0.869, s_{PRESS} = 0.137$$

As can be seen in these equations, the regression coefficients of the electronic terms are of borderline significance. Thus a sequencial (partial) *F*-test was further applied, and supported the inclusion of $v_{C=0}$ but not of \Re .

In equation [10], the better fit of the data observed with the inclusion of an electronic term, $v_{C=0}$, has two possible explanations. Either it describes electronic interactions affecting directly the lethal potency of the compounds. Or it accounts for electronic effects influencing indirectly the lethal potency via the degree of hydrolysis of the compounds thus affecting their effective concentrations on the biophase. It is interesting to recall that the compounds are benzoates and therefore susceptible to hydrolysis, which is directly influenced by substituent electronic effects. In the equations, the negative value of the coefficients is compatible with this last hypothesis. Actually, these different mechanisms could act either in an isolated or in a concerted manner, and to which extent each one explains the lethal potency cannot be evaluated at present.

The simultaneous use of log P_{app} plus MR₄ did not improve the statistical significance, even in a reduced data set (excluding the hexyl and hexyloxy derivatives), where the intercorrelation r^2 of log P vs MR₄ dropped from 0.578 (table VI) to 0.301.

Thus, to summarize, aside from a small contribution of the $v_{C=0}$ term, the log P_{app} parameter seemed to be the major descriptor of the lethal potency in the set of studied compounds. Moreover, a positive contribution of the log P_{app} term to acute toxicity was found, in contrast to a negative dependence found elsewhere in a different set of compounds [9].

It should be emphasized, however, that the regression coefficient of $\log P$ was always less than one in the equations, ie, it was apparently small [9, 14, 25]. This finding could be interpreted as indicative of the absence of a 'real' contribution of overall hydrophobi-

Compound	R	Obs	log 1/LD ₅₀ ª Calc ^b	121	π	$\log P_{calc}^{c}$	$\log P_{app}^{(SF)}$	^d log P _{app} ^{(HPI.}	^{.C)^e σ}	Ţ	ж	$V_{(C=0)}^{f}$	MR₄ ^g
1	Н	2.36	2.43	0.07	0.00	2.02	0.86	0.83	0.00	0.00	0.00	1726.5 ± 0.3	0.103
2	CH ₃	2.54	2.61	0.07	0.60	2.51	1.32	1.54	-0.17	-0.04	-0.13	1725.2 ± 0.1	0.565
3	C_2H_5	2.58	2.72	0.14	1.10	3.04	1.95	1.95	-0.15	0.00	-0.15	1724.1 ± 0.2	1.030
4	$n-C_3H_7$	2.78	2.84	0.06	1.60	3.57	2.43	2.45	-0.13	-0.06	-0.08	1723.7 ± 0.2	1.496
5	$n-C_4H_9$	3.04	2.94	0.10	2.10	4.10	2.86	2.89	-0.16	-0.06	-0.11	1723.6 ± 0.2	1.959
6	$t-C_4H_9$	2.94	2.86	0.08	1.88	3.84	2.74	2.63	-0.20	-0.07	-0.13	1724.8 ± 0.1	1.962
7	OCH ₃	2.70	2.63	0.07	-0.03	2.24	1.01	1.01	-0.27	0.26	-0.51	1717.1 ± 0.1	0.787
8	n-OC ₄ H ₉	3.07	2.99	0.08	1.47	3.82	2.71	2.63	-0.32	0.25	-0.55	1717.4 ± 0.3	2.166
9	COCH ₃	2.29	2.29	0.00	-0.39	1.73	0.57	0.48	0.50	0.32	0.20	1729.9 ± 0.2	1.118
10	Cl	2.37	2.56	0.19	0.73	2.81	1.63	1.63	0.23	0.41	-0.15	1729.5 ± 01	0.603
11	NO_2	2.43	2.27	0.16	0.22	1.92	0.70	0.73	0.78	0.67	0.16	1734.5 ± 0.1	0.736
12	CN	2.12	2.21	0.09	-0.33	1.63	0.43	0.36	0.66	0.51	0.19	1732.8 ± 0.2	0.633
13	$n - C_6 H_{13}$	3.16	3.20	0.04	3.10	5.16	4.0 ⁱ	4.0 ^j	-0.16	-0.06	-0.09	1723.4 ± 0.3	2.887
14	n-OC ₆ H ₁₃	3.02	3.26	0.24	2.47	4.88	3.7 ⁱ	3.8 ^j	-0.32	0.25	-0.55	1717.0 ± 0.2	3.090
15	SO_2CH_3	2.0^{h}	1.95	0.05	-1.20	0.55	-0.55	-0.7 ⁱ	0.72	0.54	0.22	1734.4 ^k	1.349
16	CF ₃	2.68	2.57	0.11	1.04	3.03	1.88	1.87	0.54	0.38	0.19	1732.0 ^k	0.502

Table V. Biological and chemical parameters used in deriving equations (1-13).

^aLD₅₀ in M/kg; ^bcalculated from equation (10); ^cfrom the ClogP program; ^dby the shake-flask method; ^eby the HPLC method; ^fin HCCl₃; ^gscaled by a factor of 0.1; ^happroximate value; ⁱestimated values from equation (13); ^jestimated values from equation (12); ^kestimated values from equation (2).

Table VI. Squared correlation matrix of the independent variables screened.

	π	log P _{calc}	$log P_{app}^{(SF)}$	$\log P_{app}^{(HPLC)}$	σ	T	R	$V_{C=O}$	MR_4
π	1.000	0.980	0.980	0.982	0.443	0.363	0.237	0.337	0.551
$\log P_{\rm calc}$		1.000	0.999	0.997	0.509	0.346	0.330	0.438	0.578
$\log P_{\rm app}^{\rm (SF)}$			1.000	0.996	0.511	0.353	0.327	0.436	0.586
$\log P_{app}^{(\text{HPLC})}$				1.000	0.522	0.364	0.330	0.439	0.560
σ					1.000	0.629	0.704	0.871	0.240
V						1.000	0.112	0.325	0.093
R							1.000	0.876	0.231
$V_{C=O}$								1.000	0.302
MR_4									1.000

city to the lethal toxicity, as suggested by others working with a different set of compounds [9]. Or possibly the contribution is only apparently small, perhaps encoding some hidden information [14, 15]. For example, these compounds, ie, benzoate derivatives, are susceptible to hydrolysis which could be present in every step involving hydrophobic interactions from the initial application of the drug till it reaches the final target(s). This could interfere with the real dependence of lethal toxicity on the lipophilic term.

Regarding theoretical models, neither the parabolic nor the bilinear [16, 21, 25, 38, 39] model fitted the data significantly. This could indicate an actual linear dependence of lethal toxicity on log P, or at least up to a log P_{app} value of 4.

Experimental protocols

Chemistry

Melting points were determined using a Kofler hot stage apparatus and are uncorrected. ¹H-NMR spectra were recorded using one of the following spectrometers: Bruker AC-200 (200 MHz), HX-90 E (90 MHz), Varian T-60 (60 MHz), in CDCl₃ as solvent unless otherwise indicated. ¹³C-NMR spectra were recorded on Bruker WM-250 (62.89 MHz) spectrometer, in D2O as solvent, unless otherwise indicated. The spectra of 4-SO₂CH₃ and 4-CF₃ derivatives were recorded on a Bruker AC-200 (50 MHz) spectrometer, in DMSO- d_6 as solvent. Chemical shifts were reported in δ (ppm) units relative to tetramethylsilane, TMS, or to the center of the DMSO- d_6 peak as reference standard. NMR spectra were in accordance with the given structure. Elemental analyses were performed by the analytical laboratory of the Chemistry Institute of the University of São Paulo, Brazil, and are within $\pm 0.4\%$ of the theoretical values of the indicated elements. The IR spectra for systematic carbonyl stretching frequencies shifts studies were recorded on a Perkin-Elmer-283 spectrophotometer in chloroform solutions. HPLC analyses for the log P value determinations were performed with a Waters instrument model 440 equipped with a Kontron 420 pump and a Beckman 160 UV detector with an analytical wavelength adjusted to 254 nm. In the shake-flask log P method, the concentrations were determined spectrophotometrically on a Carl Zeiss PM-QII instrument under thermostatically controlled conditions. Starting materials were obtained commercially, and redistilled or recrystallized before use. Solvents were used without purification except where indicated.

General procedures for the preparation of compounds 1-16

Method A

In a typical general procedure, 0.05 mol of the corresponding benzoic acid and anhydrous potassium bicarbonate (0.10 mol) were dissolved in anhydrous toluene (130 mL). The reaction mixture was kept under reflux with magnetic stirrer for about 6 h, and monitored by water formation in a Dean–Stark apparatus. When the reaction was completed, N,N-dimethylaminoethylchloride hydrochloride (0.05 mol) was added. Stirring and refluxing were continued for an additional 12–16 h, at which time the formation of water ceased. After cooling, the reaction mixture was treated with sodium hydroxide solution 10% (v/v), washed with water and dried over anhydrous magnesium sulfate. Anhydrous hydrogen chloride was bubbled into the toluene solution until salt formation was complete. The solvent was removed under reduced pressure and the residue was purified by recrystallization from the appropriate solvent, as indicated in table I.

Method B

In a typical general procedure, 0.02 mol of the corresponding benzoic acid and thionyl chloride (0.04 mol), dissolved in anhydrous toluene (100 mL), were kept under reflux with magnetic stirrer for ≈ 8 h. When the reaction was over, the excess of reagent and solvent were eliminated under reduced pressure, and N,N-dimethylaminoethanol hydrochloride (0.10 mol) was added dropwise to the corresponding benzoyl chloride, previously prepared or obtained commercially, when available. The reaction mixture was stirred for about 8-12 h, at which time no more formation of salt was observed. The reaction mixture was treated with potassium bicarbonate solution 10% (v/v) and further extracted with sulfuric ether. The organic phases were treated with sodium hydroxide solution 10% (v/v), washed with water and dried over anhydrous magnesium sulfate. Anhydrous hydrogen chloride was bubbled into the organic solution until salt formation was complete. The solvent was removed under reduced pressure and the residue was purified by recrystallization from the appropriate solvent, as indicated in table I.

In both methods A and B, good yields were achieved. All compounds were checked for their structures by 1 H- and 13 C-NMR spectra. Their physical constants, their methods of preparation and purification are presented in table I. Their spectral data are summarized in tables II and III.

Partition coefficient determinations

High-performance liquid chromatography (HPLC) method The log P_{app} (HPLC) values were determined by means of a reversed phase HPLC capacity factor procedure [28–30] using a 1-octanol coated ODS-Hypersil column (mean particle size 5 mm and 4.0 or 0.6 cm in length), as stationary phase. The mobile phase consisted of 1-octanol saturated phosphate buffer (1.47 mM KH₂PO₄; 8.06 mM K₂HPO₄ and 140 mM NaCl), pH = 7.24, adjusted to an ionic strength of 0.10 M. All the measurements were done at room temperature, controlled at 25 \pm 1 °C. The log *P* values for 13 of the 16 derivatives were calculated from the HPLC capacity factors (log k'), see equation (11). The values corresponding to 4-*n*-C₆H₁₃, 4-*n*-OC₆H₁₃ and 4-SO₂CH₃ derivatives could not be accurately measured, and were estimated from equation (12). The log P_{app} value of 4-SO₂CH₃ derivative was also determined and validated by the shake-flask method. No significant dependence of log *P* on compound concentration was observed. The log P_{app} values are presented in table V.

$$\log P_{\rm ann} \,({\rm HPLC}) = 1.04 \,(\pm 0.07) \,\log k' + 0.20 \,(\pm 0.14) \tag{11}$$

n = 13, r = 0.995, s = 0.096, F = 994.47, $r_{cv}^2 = 0.987, s_{PRESS} = 0.100$

 $\log P_{\rm app} \,({\rm HPLC}) = 1.02 \,(\pm 0.06) \log P_{\rm (calc)} - 1.22 \,(\pm 0.17) \quad (12)$

 $n = 13, r = 0.996, s = 0.080, F = 1423.338, r_{cy}^2 = 0.990, s_{\text{PRESS}} = 0.092$

Shake-flask method

The log P_{app} (SF) values were determined by means of the shake-flask method, as previously described [21, 24, 25, 27] using 1-octanol/Trizma buffer, pH 7.40, adjusted with KCl to an ionic strength of 0.20 M, under a thermostatically con-trolled temperature of 25 ± 1 °C. All the procedures were made in duplicate and in at least three different concentrations. The concentration range was $2-12 \times 10^{-5}$ M. There was no significant dependence of log P on compound concentration. The different determinations agreed to within ± 0.05 . Concentrations in the aqueous phase were determined spectrophotometrically. Following the same procedure, the log \hat{P} value for the 4-OCH₃ derivative was also determined in phosphate buffer at pH 7.40, adjusted with KCl to an ionic strength of 0.20 M, in order to verify the effect of the buffer composition on log P_{app} [35]. This compound, bearing an electron donor substituent, shows a lower susceptibility to hydrolysis and, as presented in table V, has a $\log P$ value of 1.01. These two properties improve the quality and accuracy of the $\log P$ experimental measurements. Thus, using two different buffers systems, phosphate and Trizma, at the same pH and ionic strength, non-significant effects of buffer composition on log P_{app} values, measured by the shake-flask method, were found. The values corresponding to $4-n-C_6H_{13}$ and $4-n-OC_6H_{13}$ derivatives could not be accurately measured, and were estimated from equation (13). The log P_{app} values are presented in table V.

 $\log P_{\text{app}} (\text{SF}) = 1.00 (\pm 0.03) \log P_{(\text{calc})} - 1.17 (\pm 0.09)$ (13) n = 14, r = 0.999, s = 0.052, F = 4987.440, $r_{\text{ev}}^2 = 0.996, s_{\text{PRESS}} = 0.066$

Infrared spectroscopy measurements

The infrared carbonyl absorption stretching band frequencies, cm⁻¹, $v_{(C=O)}$, were measured systematically [24, 32] in chloroform solution (0.040, 0.060 and 0.080 M), on a Perkin-Elmer model 283 grating spectrometer, using a 0.1 mm sodium chloride cell. The carbonyl region was scanned slowly and the spectra recorded in triplicate between 1800-1500 cm⁻¹. Calibration and paper alignment difficulties were minimized by recording the 1601.4 cm⁻¹ polystyrene band on each spectrum. The data presented in table V are averages of 15 readings on five runs. The carbonyl absorption band had a symmetric shape that simplified the determination of its position in the spectrum. The standard deviation, s, was calculated for each compound. Whenever the solubility permitted, three different concentrations of each compound were employed. The carbonyl stretching frequencies were not significantly dependent on the concentration. The $v_{(C=0)}$ values for the 4-SO₂CH₃ and 4-CF₃ derivatives were calculated from equation (2).

Biology

Median lethal doses (LD_{50})

The LD₅₀ was determined experimentally as follows: at least four different doses of each compound were injected ip in groups of 10–20 mice per dose. This took a minimum of 46 and a maximum of 100 animals per derivative. The percentages of death corresponding to each dose were calculated and transformed into empirical probits using a conversion table [33]. A linear regression of empirical probits on the logarithm of the corresponding doses yielded a provisional line, the ordinates of which provided expected probits. Working probits and weights corresponding to the expected probits were obtained from tables [33]. An iterative procedure using the weighted quantitics was performed till consecutive values of expected probits agreed to within 0.2. When a final regression line was obtained, the LD_{50} was estimated from this line by interpolation after checking for homogeneity by means of a goodness-of-fit χ^2 test [33]. The precision of the estimated LD_{50} was evaluated by calculating the 95% confidence interval [33]. The 4-SO₂CH₃ derivative was the only exception to this procedure, because probably due to its low potency, there was no clear doseresponse regression in its presence. Thus, it was only possible to obtain an approximate value of its LD_{50} . The time spent for the injection of all animals corresponding to each derivative was at most 1 h. This was thus the longest time any one compound remained in solution at room temperature, 24 ± 2 °C. Just prior to the injections, the compounds were dissolved in a buffered mammalian physiological solution of the following composition [mM]: NaCl [135]; KCl [5]; MgCl₂ [1]; CaCl₂ [2]; NaHCO₃ [15]; NaH₂PO₄ [1]; glucose [11], which was gassed continuously with a mixture of O_2 (95%) and CO_2 (5%). This kept the pH of the solution between 7.22-7.28. The solubilities of the $4 - n - C_4 H_9$, $4 - t - C_4 H_9$, $4 - n - OC_4 H_9$, $4 - NO_2$, $4 - n - C_6 H_{13}$ and 4-n-OC₆H₁₃ derivatives were increased by the addition of DMSO at a maximum concentration of 10%, v/v. In control experiments, DMSO dissolved in the physiological solution described above was injected into 25 animals. In no case did it induce lethality or any other obvious effect. In a further check, procaine hydrochloride was added to such a solution and the LD_{50} and confidence limits of the mixture were evaluated as described above. The values obtained, ie, 0.59 (0.54-0.65) mM/kg, did not differ significantly from those obtained for procaine in the absence of DMSO, 0.64 (0.58–0.69) mM/kg. So it is highly improbable that in this study DMSO induced any other effect aside from helping to solubilize some of the compounds,

Acknowledgments

The authors are most grateful to H Kubinyi, Drug Design Laboratory, BASF AG, Ludwigshafen, Germany, for his stimulating discussions, permanent constructive criticism and for kindly providing the BILIN-program; JK Seydel, Institut für Experimentelle Biologie und Medizin, Forschungsinstitut Borstel, Germany, for critical reading of the manuscript and for many helpful discussions; K Kim, Abbott Laboratories, IL, USA, and KJ Schaper, Institut für Experimentelle Biologie und Medizin, Forschungsinstitut Borstel, Germany, for their enlightening discussions and critical remarks; U Abraham for the $\log \tilde{P}$ calculations made with the ClogP program at BASF AG, Ludwigshafen, Germany; A Giesbrecht, J Nicolau, P Senise and MPB Ceneviva, University of São Paulo, Brazil, for their help and advice. This work was supported by grants from SV/DAAD (Project QF-15/Programa Brazil); FAPESP (Proc 89/3316-8; 90/2566-8; 92/2754-4; 94/2988-0); CNPq (301285/ 78); CAPES/DAAD; DFG and Pro-Reitoria de Pesquisa, University of São Paulo, Brazil.

References

- I Strichartz GR, Ritchie JM (1987) In: Local Anesthetics (Strichartz GR, ed) Springer, Berlin, 21-52
- 2 Butterworth JF, Strichartz GR (1990) Anesthesiology 72, 711-734

- 3 Ragsdale DS, McPhee JC, Scheuer T, Catterall WA (1994) Science 265, 1724–1728
- 4 Catterall WA, Mackie K (1996) In: *The Pharmacological Basis of Therapeutics* (Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds) McGraw-Hill, New York, 9th edn, 331–347
- 5 Luduena FP (1971) In: *Local Anesthetics* (Lechat P, ed) Pergamon, Oxford, 319-341
- 6 Covino BG (1987) In: Local Anesthetics (Strichartz GR, ed) Springer, Berlin, 187-212
- 7 de Jonge HR (1994) Local Anesthetics, Mosby, Saint Louis, 3rd edn
- 8 Feldman HS (1994) In: Anesthetic Toxicity (Rice SA, Fish KJ, eds) Raven Press, New York, 107–133
- 9 Recanatini M, Valenti P, Da Re P (1988) Quant Struct-Act Relat 7, 12-18
- 10 Gupta SP (1991) Chem Rev 91, 1109-1119
- 11 Cronin MT, Dearden JC (1995) Quant Struct-Act Relat 14, 117-120
- 12 Caliendo G, Di Carlo R, Greco G et al (1995) Eur J Med Chem 30, 603-608
- 13 Caliendo G, Greco G, Grieco P et al (1996) Eur J Med Chem 31, 99-110
- 14 Hansch C (1993) Drug Metab Rev 25, 1-48
- 15 Hansch C (1987) In: Molecular Structure and Energetics. VCH, Weinheim, 341–379
- 16 Kubinyi H (1993) In: Methods and Principles in Medicinal Chemistry, vol 1 (Mannhold R, Krosgaard-Larsen P, Timmerman H, eds) VCH, Weinheim
- 17 Leo A (1993) Chem Rev 93, 1281–1308
- 18 Hansch C, Leo A, Taft RW (1991) Chem Rev 91, 165-195
- 19 Hansch C, Leo AJ (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley, New York
- 20 Hansch C, Leo AJ, Hoekman D (1995) Exploring QSAR: Hydrophobic, Electronic and Steric Constants. ACS, Washington

- 21 Seydel JK, Schaper KJ (1979) Chemische Struktur und Biologische Aktivität von Wirkstoffen. Methoden der Quantitativen Struktur-Wirkung-Analyse. VCH, Weinheim
- 22 Craig PJ (1971) J Med Chem 71, 680-684
- 23 Rabjohn N, Fronabarger JW, Linstromberg WW (1955) J Org Chem 20, 271-273
- 24 Amaral AT do, Miyazaki Y, Stachissini S, Caprara L, Oliveira AC (1991) In: QSAR: Rational Approaches to the Design of Bioactive Compound (Silipo C, Vittoria A, eds) Elsevier, Amsterdam, 509–512
- 25 Hansch C, Leo AJ (1995) Exploring QSAR: Fundamentals and Applications in Chemistry and Biology. ACS, Washington
- 26 Kubinyi H (1979) Prog Drug Res 23, 97-198
- 27 Dearden JC, Bresnen GM (1988) Quant Struct-Act Relat 7, 133-144
- 28 Unger SH, Cook J, Hollenberg JS (1978) J Pharm Sci 67, 1364-1367
- 29 Unger SH, Chiang GH (1981) J Med Chem 24, 262-270
- 30 Terada H (1986) Quant Struct-Act Relat 5, 81-88
- 31 Daylight Chemical Information Systems Inc. ClogP Program. Calculations were performed at BASF-AG, Ludwigshafen, Germany (version 3.54)
- 32 Amaral AT do, Amaral L do (1976) J Org Chem 41, 1623-1627
- 33 Finney DJ (1980) Probit Analysis. Cambridge University Press, Cambridge, 3rd edn
- 34 BILIN-program, version 1994
- 35 Wang PH, Lien E (1980) J Pharm Sci 69, 662-668
- 36 Dearden JC, Bradburne SJA, Abraham MH (1991) In: QSAR: Rational Approaches to the Design of Bioactive Compound (Silipo C, Vittoria A, eds) Elsevier, Amsterdam, 143–150
- 37 Hansch C, Fujita T (1964) J Am Chem Soc 1616–1626
- 38 Hansch C (1969) Acc Chem Res 2, 232-239
- 39 Kubinyi H (1976) Arzneim-Forsch 26, 1991–1997