ELSEVIER

Biochimica et Biophysica Acta 1558 (2002) 151–160

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Substituted aniline interaction with submitochondrial particles and quantitative structure–activity relationships

Emanuele Argese *, Cinzia Bettiol, Matteo Fasolo, Alfonso Zambon, Francesca Agnoli

Department of Environmental Sciences, Università Ca' Foscari di Venezia, Calle larga S. Marta 2137, 30123 Venice, Italy

Received 10 August 2001; accepted 26 September 2001

Abstract

The toxic effects of eighteen substituted anilines were determined by means of a short-term in vitro assay, using submitochondrial particles (SMP) as biosensors. The assay allows for the quantification of the effects of toxicants that act specifically on mitochondrial respiratory functions, like uncouplers and inhibitors, or non-specifically, by disturbing the structure and functioning of the inner mitochondrial membrane. The obtained EC_{50} values range from 72.5 to 1910 μmol/l. The type and position of the substituents are of fundamental importance in determining the toxic potency. In general, the presence of electron-withdrawing substituents produces higher toxic effects, whereas electron-donating groups seem to reduce the toxicity. Quantitative structure–activity relationships (QSAR) showed that toxicity values were correlated with the Hammett σ constant and with hydrogen bonding capacity descriptors, such as E_{LUMO}, E_{HOMO} and Q^+. The results indicate that toxicity increases with increasing the hydrogen bonding donor capacity of the NH_2 group and support the hypothesis of a mechanism of action based on hydrogen bonding formation between the amino group of anilines and polar groups at the membrane/water interface. Such an interaction would cause a derangement of the membrane structure and, as a consequence, a disturbance of its functioning.

Keywords: Submitochondrial particle; Substituted aniline; Toxicity; Polar narcosis; Quantitative structure–activity relationship

1. Introduction

Aniline and its substituted derivatives can be introduced into the environment through a number of sources. Chloro-, nitro- and dimethylanilines (xylidines) are widely used as intermediates for the synthesis of chemicals in the pharmaceutical, agrochemical and pigment industries and can be released into the environment through industrial discharges [1]. Halogenated anilines can also be formed as a result of the partial degradation of certain aniline based pesticides [1,2].

These compounds are known to cause a variety of toxic effects, whose magnitude and type depend on the nature of the substituent groups, on their number and, in addition, on their position in the aromatic ring. Various studies demonstrated that aniline and halogenated anilines can induce methaemoglobinaemia, as well as renal and hepatic toxicity both in vitro and in vivo [2–4]. Aminophenols, which are primary products of aniline metabolism, were also shown to induce nephrotoxicity [4]; in addition, evidence of recombinagenic activity is reported for these compounds, and they were shown to be more potent than aniline itself [5]. *para*-Aminophenol, in particu-

* Corresponding author. Fax: +39-41-257-8584.
E-mail address: argese@unive.it (E. Argese).

0005-2736/02/$ – see front matter
PII: S0005-2736(01)00424-2

lar, is a known nephrotoxin; this compound is a metabolite of aniline and also of acetaminophen, an analgesic and antipyretic drug [4].

In Quantitative structure–activity relationships (QSAR) studies anilines are commonly included in the class of polar narcotic chemicals, which are supposed to exert their toxic effects by disturbing the structure and functioning of biomembranes. Besides the important role played by the lipophilic character of the compounds, the mechanism underlying the toxicity of polar narcotics was shown to be related to their electronic properties and to their hydrogen bonding capability [6,7].

In a previous study we have investigated the toxic effects of a series of chlorinated anilines towards mitochondrial respiration, a process of primary importance for cellular functioning and viability in eucaryotic organisms [8]. To this purpose, we have utilized as biosensors submitochondrial particles (SMP), which are obtained by sonic disruption of beef heart mitochondria and consist of closed vesicles of inner mitochondrial membrane. Toxic effects were quantified by means of a short-term assay, based on the process of reverse electron transfer (RET), which is induced by ATP and succinate and involves three membrane-bound enzymatic complexes: succinate dehydrogenase, NADH dehydrogenase and ATP synthase [9,10].

As in the case of the physiological mitochondrial respiration, an essential requirement for a correct functioning of reverse electron transfer is the integrity of the inner membrane structure. As a consequence, besides specifically acting chemicals, like uncouplers and inhibitors, the assay is also sensitive to compounds that elicit their toxic action in a nonspecific manner, by interfering with the structure of the SMP membrane. The response of the assay, therefore, could also provide evidence of the effects of chemicals eliciting a disrupting action on functional membranes in general.

The findings obtained for chloroanilines were consistent with the polar narcosis mode of action and supported the hypothesis of an interaction between these toxicants and submitochondrial particles at membrane level [8]. In particular, this interaction was supposed to involve either the external hydrophilic region of the membrane or the internal hydrophobic one, or both. According to the assumed mechanism of action, the NH_2 group of anilines could form hydrogen bonds with polar groups at the membrane/water interface, such as phosphates, carbonyls, carboxyls and amino groups, whereas the hydrophobic moiety, i.e., the chlorine substituted benzene ring, could enter the hydrophobic region of the membrane. Such an action can lead to disorder and expansion of the membrane structure, resulting in a disturbance of its functioning. In the submitochondrial particle assay, this will result in a slowing down or a complete inhibition of reverse electron transfer.

The present study was aimed at acquiring further experimental evidence on the importance of the hydrogen bonding donor capacity of the NH_2 group in eliciting the effects of substituted anilines towards mitochondrial respiration. To this purpose, the study formerly carried out on a congeneric series of anilines, such as the chlorinated ones, was extended to a heterogeneous group of aniline derivatives, containing different substituents on the aromatic ring (Fig. 1). Specifically, we selected a set of anilines with substituents characterized by a wide difference in the electron donor-withdrawing capability, whereas hydrophobicity, which varied in a narrow range, was comparable for most compounds. The choice of such a set of anilines was aimed at emphasizing the contribution to the toxicity due to the electronic properties and the hydrogen bonding capacity, with respect to the hydrophobic and steric factors.

The toxic effects of these compounds were assessed by the submitochondrial particle assay, and the relationships between the toxicity and physicochemical and structural properties were investigated by correlating the obtained EC_{50} values with both classical molecular descriptors and quantum chemical parameters.

2. Materials and methods

2.1. Chemicals

ATP, antimycin A, NAD^+, sodium succinate, sucrose and $MgSO_4$ were purchased from Sigma–Aldrich (Milan, Italy). The substituted anilines were purchased from Aldrich (Milan, Italy). They were dissolved in analytical grade dimethyl sulfoxide

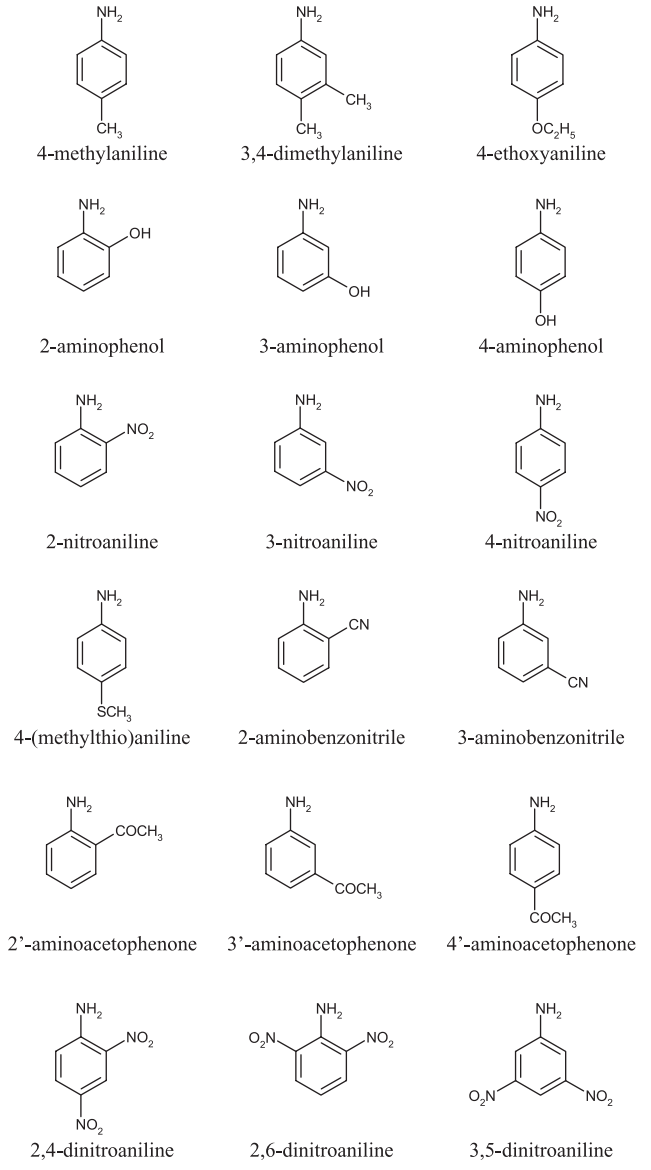

Fig. 1. Structures of the 18 substituted anilines.

(DMSO) or absolute ethanol. Antimycin A was dissolved in absolute ethanol. All the other chemicals were dissolved in ultrapure Milli-Q water (Millipore, Bedford, MA).

2.2. Submitochondrial particle assay

Beef heart mitochondria were prepared following the method of Löw and Vallin [11], with slight modifications. Submitochondrial particles were prepared according to the method of Hansen and Smith [12]. They were suspended in a medium containing 250 mM sucrose and 10 mM Tris–HCl (pH 7.4) at an approximate protein concentration of 25 mg/ml and were stored in a freezer at −20°C.

The test with phosphorylating SMPs was carried out by determining the effect of toxicants on the RET process, where exogenous NAD^+ is reduced to NADH, which absorbs strongly at 340 nm. The NADH production rate was measured using a Jasco V-530 spectrophotometer.

The assay medium contained sucrose (180 mM), Tris–HCl, pH 7.5 (50 mM), sodium succinate (100 mM), $MgSO_4$ (30 mM), NAD^+ (1 mM), antimycin A (0.7 μg/ml), ATP (2 mM) and submitochondrial particles (0.06 mg protein/ml). The assay was carried out at 30°C.

The rate of NADH production in the presence of subsequent cumulative toxicant doses was recorded and used to calculate the percent inhibition. The EC_{50} values were calculated from the dose–response curve, by extrapolating the concentration of toxicant at which the rate of NADH production was diminished by 50% [10]. The total amount of toxicant solution added to the medium did not exceed 0.5% (v/v). Equivalent volumes of organic solvent (ethanol or DMSO) exhibited no observable effects in control experiments.

Throughout the whole experiment the SMP stock suspension was maintained in ground ice, in order to minimize the activity loss. The repeatability of the SMP response during a 12-h experimental period was evaluated by executing eight replicates of the EC_{50} determination, using the same toxicant solution and the same SMP stock suspension. The repeatability was within a relative 95% confidence interval of 7%, as calculated by applying the Student's *t* distribution to the EC_{50} values.

2.3. Quantitative structure–activity relationships (QSAR)

The EC_{50} values obtained by the SMP assay were expressed as the logarithm of the inverse molar concentration (log $1/EC_{50}$). The hydrophobicity of substituted anilines was described by the octanol/water partition coefficient (log K_{ow}). The values of this parameter were experimentally measured and were retrieved from the on-line version of the LOGKOW program of the Syracuse Research Corporation

(http://esc-plaza.syrres.com/interkow/kowdemo.htm). For 4-methylthioaniline and 2,4-dinitroaniline, since the experimental data were not reported, log K_{ow} values estimated using the LOGKOW program were used. The Hammett σ constant was used to describe electronic effects. The σ values for the *meta* and *para* substituents were taken from Hansch et al. [13]. For strong electron withdrawing substituents ($COCH_3$, CN, NO_2), the nucleophilic σ_p^- was used, which takes into account the so-called 'through resonance' effect, present when these groups are conjugated with an electron-donating group, such as NH_2 in anilines [14]. As concerns the *ortho* substituents, which can exert proximity effects, σ constants are not univocally defined. In the present study, the σ_o values taken from the compilation of Charton and derived from the hydroxyl chemical shift of *ortho*-substituted phenols were used [15]. Regarding the disubstituted anilines, the summation of the single substituent constants was used.

Besides these classical descriptors, the following quantum chemical parameters were used to establish QSARs: E_{HOMO} (the energy of the highest occupied molecular orbital), E_{LUMO} (the energy of the lowest unoccupied molecular orbital), Q^- (the most negative partial charge on any non-hydrogen atom) and Q^+ (the most positive partial charge on any hydrogen atom). E_{HOMO} and Q^- are used as descriptors for hydrogen bonding acceptor capacity, which increases with increasing E_{HOMO} and decreasing Q^-. The hydrogen bonding donor capacity is described by E_{LUMO} and Q^+, and increases with a decrease in E_{LUMO} and an increase in Q^+ [16–18].

Molecular orbital energies were calculated using the Spartan program package [19] running on an Indy workstation. Geometry optimization was performed using the semiempirical PM3 procedure, and energies and partial charges (Mulliken) were recalculated at the ab initio 6–31 G level.

Moreover, molecular volumes (V_{mc}) were used to evaluate the influence of the steric properties of substituted anilines. These parameters were calculated using the HyperChem 6.01 program [20].

2.4. Statistical analysis

QSARs were developed by using linear regression analysis. The quality of the models was estimated by means of the coefficient of determination (R^2 and R^2_{adj}), the standard error of estimate (s) and the analysis of variance (ANOVA) ratio for regression (F). A cross-validation procedure (the leave-one-out method [21]) was used to evaluate the predictive power of the established QSAR models; the obtained R^2_{cv} values are reported in the results.

3. Results and discussion

3.1. EC_{50} determination

The EC_{50} values determined by means of the SMP assay are presented in Table 1, together with the parameters used to develop QSARs. It can be seen that the EC_{50} values for monosubstituted anilines vary by more than one order of magnitude, from a minimum of 72.5 μM for 4′-aminoacetophenone to 1910 μM for 2-aminophenol. These results point out the importance of substituent type in determining substituted aniline effects on reverse electron transfer. In general, anilines containing electron-donating groups show a lower toxicity, whereas the presence of electron withdrawing substituents produces increased toxic effects.

As can be argued from the data obtained for dinitroanilines, the addition of a second electron withdrawing substituent on the benzene ring significantly increases the toxicity; these compounds are, in fact, the most toxic in the whole examined series, with EC_{50} values in the range 25.7–60 μM. On the other hand, 3,4-dimethylaniline presents an EC_{50} value of 1140 μM, which is slightly higher than that of 4-methylaniline (921 μM). For electron donating groups, therefore, the addition of a further substituent has a lower influence and even seems to decrease the toxicity.

These results are in accordance with the findings achieved previously for chloroanilines and are consistent with the hypothesized mechanism of action [8]. In anilines, the lone pair associated with the nitrogen atom is conjugated with the electron cloud of the benzene ring. The result of the effect of electron withdrawing substituents will be a reduction in electron density at the N atom and, in turn, an attraction of the electron clouds associated with the NH_2 hydrogen atoms. Electron withdrawal thus leads to an

Table 1
Toxicity values obtained for substituted anilines by the SMP assay and molecular descriptors used to develop QSARs

Substituted aniline	Substituent	SMP EC_{50} (μM)	SMP log $1/EC_{50}$ (M)	log K_{ow}	σ	E_{HOMO} (Hartree)	E_{LUMO} (Hartree)	Q^+ (a.u.)	Q^- (a.u.)	V_{mc} (Å^3)
4-Methylaniline	CH_3	921	3.04	1.39	−0.17	−0.31097	0.14495	0.348123	−0.861574	421.07
3,4-Dimethylaniline	CH_3	1140	2.94	1.84	−0.24	−0.28031	0.14600	0.352739	−0.869141	462.33
4-Ethoxyaniline	OCH_2CH_3	701	3.15	1.24	−0.24	−0.29957	0.14103	0.347370	−0.861574	503.32
4-(Methylthio)aniline	SCH_3	876	3.06	1.68	0.00	−0.28905	0.13647	0.349868	−0.861087	474.66
2-Aminophenol	OH	1910	2.72	0.62	−0.41	−0.30506	0.13884	0.349293	−0.832184	389.02
3-Aminophenol	OH	604	3.22	0.21	0.12	−0.29478	0.14974	0.358335	−0.869817	387.91
4-Aminophenol	OH	1040	2.98	0.04	−0.37	−0.30301	0.13784	0.347546	−0.860794	390.67
2′-Aminoacetophenone	$COCH_3$	133	3.88	1.63	1.06	−0.33811	0.09300	0.358734	−0.871710	495.96
3′-Aminoacetophenone	$COCH_3$	230	3.64	0.83	0.38	−0.30508	0.09600	0.357638	−0.865102	468.19
4′-Aminoacetophenone	$COCH_3$	72.5	4.14	0.83	0.84	−0.33712	0.08984	0.354940	−0.860430	472.56
2-Aminobenzonitrile	CN	150	3.82	1.40	1.18	−0.34778	0.08932	0.361723	−0.849960	423.43
3-Aminobenzonitrile	CN	499	3.30	1.07	0.56	−0.31904	0.08643	0.362692	−0.866539	423.10
2-Nitroaniline	NO_2	223	3.65	1.85	1.20	−0.36015	0.06173	0.360479	−0.812037	427.31
3-Nitroaniline	NO_2	178	3.75	1.37	0.71	−0.32576	0.05485	0.365544	−0.864830	427.21
4-Nitroaniline	NO_2	106	3.98	1.39	1.27	−0.36710	0.05590	0.360454	−0.857954	431.47
2,4-Dinitroaniline	NO_2	35.5	4.45	1.84	2.47	−0.39730	0.02229	0.380462	−0.834819	485.94
2,6-Dinitroaniline	NO_2	60.0	4.22	1.79	2.40	−0.39624	0.02361	0.381547	−0.791581	480.84
3,5-Dinitroaniline	NO_2	25.7	4.60	1.89	1.42	−0.35534	0.02069	0.373031	−0.862420	489.95

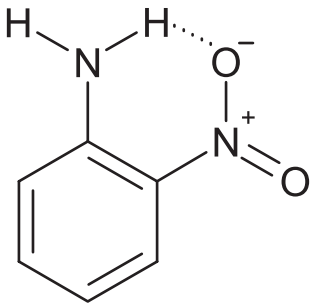

Fig. 2. Structure of 2-nitroaniline, showing the intramolecular hydrogen bond.

increased polarity of the N–H bond in the amino group and, as a consequence, in an increased strength of the N–H···A hydrogen bond, where A indicates, in general, an electronegative atom in the biomembrane.

The results in Table 1 also show that the position of the substituent has an influence on toxicity. In the case of electron withdrawing groups, such as $COCH_3$ and NO_2, the substitution at the *para* position seems to cause a higher toxicity, whereas for aminophenols the *meta* isomer was found to be the most toxic. In addition, for both mono and dinitroanilines, as well as for aminophenols, the substitution in the *ortho* position seems to be associated with a lower toxicity.

The higher toxicity of 4-nitroaniline and 4′-aminoacetophenone can be ascribed to the concomitant field/inductive (I) and mesomeric (M) effects exerted by the NO_2 and $COCH_3$ groups, respectively. When these groups are placed in the *para* position, they are conjugated with the mesomeric electron-donating NH_2 group of anilines; therefore, the overall magnitude of electron-withdrawal is greater than in the case of the *meta*-substituted isomers, where the resonance effect is negligible. This is also confirmed by the values of the Hammett σ_p of the NO_2 and $COCH_3$ groups, which are significantly higher than the corresponding σ_m.

As concerns aminophenols, OH is an electron-withdrawing group by induction, whereas it is a strong mesomeric electron-donating group. In 3-aminophenol, where the OH group is not conjugated with NH_2, the field/inductive electron withdrawing effect prevails, as shown by the positive value of the Hammett σ_m. This leads to a higher polarity of the N–H bond, which could account for the higher toxicity of this isomer. Conversely, in 4-aminophenol the +M and −I effects of the OH group are opposed, resulting in a lower electron withdrawing effect and, as a consequence, a lower toxicity.

The lower toxicity presented by the *ortho* substituted aminophenol and nitroanilines gives further support to the hypothesis of the involvement of hydrogen bonding in the toxic interaction between anilines and submitochondrial particles. In fact, in these compounds an intramolecular hydrogen bond is probably formed between the hydrogen of the NH_2 group and the oxygen atom of OH and NO_2, respectively [22] (Fig. 2); as a consequence, the formation of intermolecular hydrogen bonding between NH_2 and polar groups in the membrane is less favoured and, as a consequence, the disrupting action on the biomembrane would be reduced. However, 2′-aminoacetophenone, which could also form an intramolecular hydrogen bond, would seem to be an exception.

3.2. Quantitative structure–activity relationships

Quantitative structure–activity relationships were

Table 2
Results of regression analysis between EC_{50} values and QSAR parameters

Parameter	R^2	R^2_{adj}	R^2_{cv}	a	b	F	s	n
σ	0.81	0.80	0.76	3.20 ± 0.08	0.57 ± 0.07	69	0.25	18
σ (excluding outliers)	0.91	0.90	0.88	3.16 ± 0.05	0.53 ± 0.05	136	0.16	16
E_{HOMO}	0.71	0.69	0.65	−0.83 ± 0.71	−13.4 ± 2.1	39	0.31	18
E_{HOMO} (excluding outliers)	0.84	0.82	0.74	−0.30 ± 0.47	−11.7 ± 1.4	66	0.20	15
E_{LUMO}	0.82	0.81	0.78	4.62 ± 0.13	−11.0 ± 1.3	72	0.25	18
E_{LUMO} (excluding outliers)	0.86	0.85	0.83	4.58 ± 0.12	−11.0 ± 1.2	90	0.22	17
Q^+	0.67	0.65	0.59	−12.2 ± 2.8	44.0 ± 7.7	33	0.39	18
Q^+ (excluding outliers)	0.77	0.75	0.70	−13.1 ± 2.4	46.2 ± 6.5	50	0.28	17

n is the number of data; a and b are the regression coefficients for the equation: $\log(1/EC_{50}) = a + b\,X$, where X represents the molecular descriptor. The statistical parameters are described in Section 2. Outliers are indicated in the text.

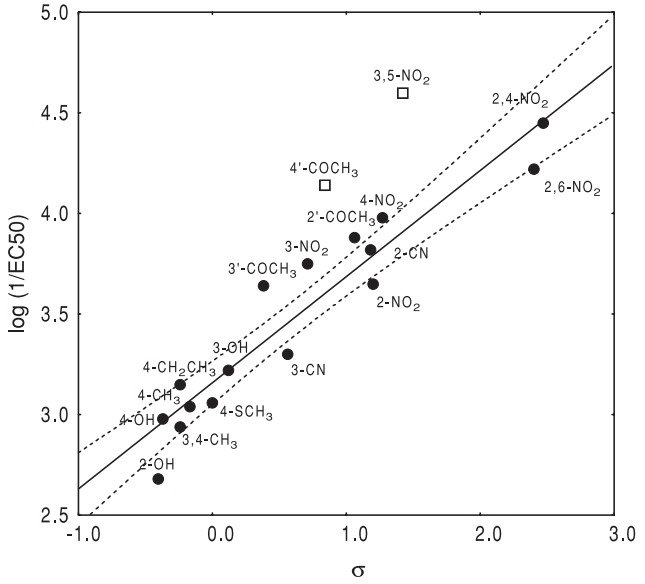

Fig. 3. Regression of log ($1/EC_{50}$) versus the Hammett σ. The statistical outliers are identified by the symbol □ and are not included in the regressions. The dotted lines represent 95% confidence limits.

developed in order to get a deeper insight into the dependence of the toxicity of substituted aniline on their structures and properties, and to confirm the hypothesized mechanism of toxic action.

As can be seen from the data in Table 1, substituted anilines with comparable log K_{ow} values exhibit very different toxic effects, and a clear relationship between this parameter and the toxic potency cannot be identified ($R^2 = 0.22$). A comparison with the results of other studies is not straightforward, since the data sets used and the involved toxicity endpoints often present significant differences. In various QSAR studies, log K_{ow}, though not always sufficient on its own as a descriptor or predictor of polar narcotic toxicity, is found to be one of the most important parameters [17,23–26]. Conversely, Kaiser [27] found a low correlation ($R^2 = 0.19$) between log P and the toxicity of 23 *para*-substituted anilines in the Microtox assay. Cronin and Schultz as well did not obtain a significant relationship when trying to model the toxicity of polar narcotic phenols to *Vibrio fisheri* using log P [28].

Our previous findings on chloroanilines showed that log K_{ow} was a significant descriptor of toxicity towards submitochondrial particles [8]; however, the compounds included in that study contained one to five chlorine substituents and log K_{ow} values ranged from about 2 to 5. In this study, instead, the investigation was focused mainly on monosubstituted compounds, except for the three dinitroanilines; log

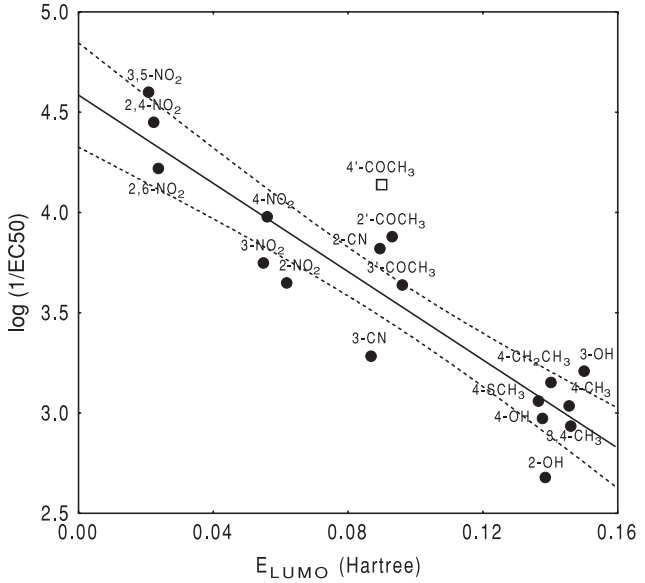

Fig. 4. Regression of log ($1/EC_{50}$) versus E_{LUMO}. The statistical outliers are identified by the symbol □ and are not included in the regressions. The dotted lines represent 95% confidence limits.

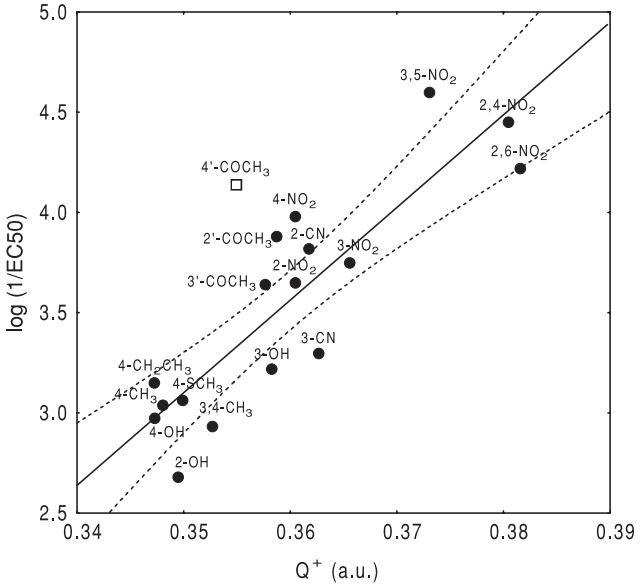

Fig. 5. Regression of log (1/EC50) versus Q^+. The statistical outliers are identified by the symbol □ and are not included in the regressions. The dotted lines represent 95% confidence limits.

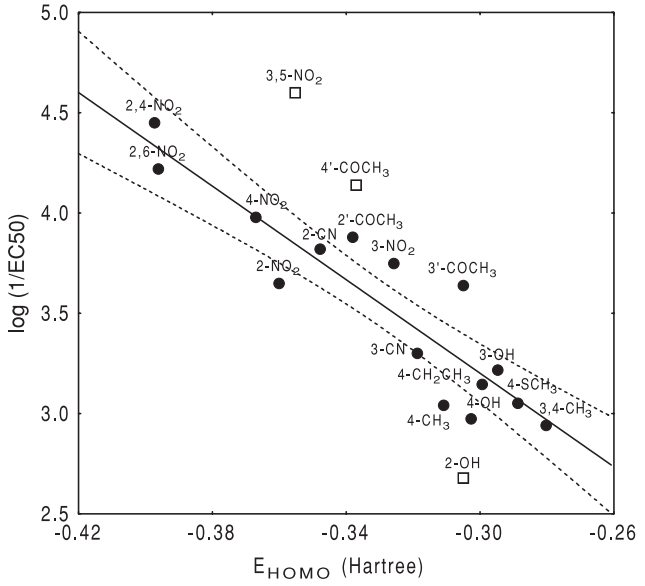

Fig. 6. Regression of log ($1/EC_{50}$) versus E_{HOMO}. The statistical outliers are identified by the symbol □ and are not included in the regressions. The dotted lines represent 95% confidence limits.

K_{ow} values, as already mentioned, varied in a narrow range and were always lower than 2. In addition, for chloroanilines it was difficult to distinguish the contribution to toxicity of interactions different from the hydrophobic ones, since most of the other QSAR descriptors were highly correlated with log K_{ow}.

The set of compounds examined in the present study, on the contrary, was designed with the purpose of pointing out the influence of other kinds of interactions, mainly the electronic ones.

As shown in Table 2 and Fig. 3, the descriptor that gives the highest quality QSAR is just the electronic parameter Hammett σ. The prevalent importance of σ as a descriptor of substituted aniline toxicity underlines the remarkable role played by electronic interactions. Specifically, the obtained QSAR equation confirms the trend towards increasing toxicity by increasing the electron-withdrawing effects of the substituents.

Two compounds, 3,5-dinitroaniline and 4′-aminoacetophenone, were found to be outliers and were excluded from the regression; their elimination significantly improved the quality of the model, as indicated by the statistical parameters in Table 2. One explanation of the excess toxicity exhibited by these compounds could be related to the possibility of the NO_2 and $COCH_3$ groups to form further hydrogen bonding by acting as acceptors.

The results in Table 2 also point out the influence of hydrogen bonding descriptors. In particular, significant correlations are found when using the descriptors for hydrogen bonding donor capacity, that is E_{LUMO} and Q^+ (Figs. 4 and 5). In both cases, the results are improved by excluding from the regressions the statistical outlier 4′-aminoacetophenone.

In the QSAR equations including E_{LUMO} and Q^+, the values of the slopes, negative and positive respectively, indicate that toxicity increases with an increase in the hydrogen bonding donor capacity, as expected on the basis of the supposed mechanism of toxic action of anilines in submitochondrial particles.

In addition, E_{HOMO} (Fig. 6) also results as a significant descriptor, though a more modest correlation is obtained. Three compounds were found to be statistical outliers, that is 3,5-dinitroaniline, 4′-aminoacetophenone and 2-aminophenol. The slope of the regression line has a negative value, indicating that toxicity increases when decreasing the hydrogen bonding acceptor capacity of the mole-

Table 3
Correlation matrix showing correlation coefficients among the different molecular descriptors

	log K_{ow}	σ	E_{HOMO}	E_{LUMO}	Q^+	Q^-	V_{mc}
log K_{ow}	1.00	0.56	−0.49	−0.54	0.49	0.31	0.63*
σ		1.00	−0.95*	−0.92*	0.92*	0.54	0.42
E_{HOMO}			1.00	0.90*	−0.83*	−0.64*	−0.29
E_{LUMO}				1.00	−0.90*	−0.46	−0.37
Q^+					1.00	0.49	0.33
Q^-						1.00	0.02
V_{mc}							1.00

The values indicated with an asterisk are significant at the level $P < 0.01$.

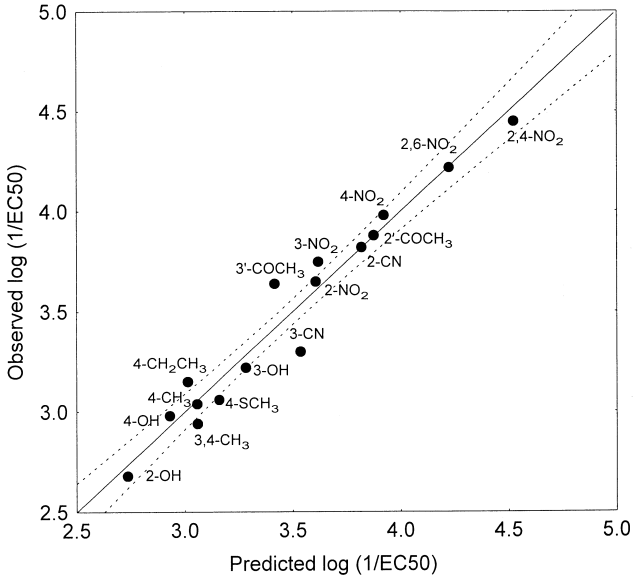

Fig. 7. Plot of observed versus predicted log ($1/EC_{50}$) values, on the basis of the multiple regression including the Hammett σ and Q^- as independent variables. The dotted lines represent 95% confidence limits.

cules. The correlation of toxicity with Q^-, instead, is not significant ($R^2 = 0.07$).

The molecular volume (V_{mc}) is also not a significant descriptor ($R^2 = 0.27$), showing that the differences in the steric hindrance are of minor importance in with respect to electronic properties in describing the toxic activity of the examined compounds.

In an effort to improve the quality of the obtained QSAR models, multiple regression analysis was performed. It must be noticed that a high degree of correlation exists between some parameters, as shown in the correlation matrix in Table 3, and this was taken into account when carrying out the multiple regression analysis.

The addition of the hydrophobicity descriptor, log K_{ow}, does not produce an improvement in any of the QSAR models. On the other hand, the addition of Q^- as a second independent variable in the regression that includes σ, significantly enhances the quality of the model. The following equation is obtained:

$$\log(1/EC_{50}) = 0.68(\pm 0.07)\sigma - 8.4(\pm 2.7)Q^- - 4.0(\pm 2.3)$$

with $R^2 = 0.89$, $R^2_{adj} = 0.87$, $s = 0.20$, $F = 59$, $n = 18$. The exclusion of the identified statistical outliers, which are the same as in the simple regression that includes σ (3,5-dinitroaniline and 4′-aminoacetophenone), further improves the regression quality and yields the equation:

$$\log(1/EC_{50}) = 0.61(\pm 0.04)\sigma - 5.9(\pm 1.7)Q^- - 1.9(\pm 1.4)$$

with $R^2 = 0.95$, $R^2_{adj} = 0.94$, $s = 0.12$, $F = 130$, $n = 16$. The observed versus predicted toxicity values are shown in Fig. 7. By comparing Figs. 3 and 7, it can be seen that the QSAR that includes both σ and Q^- seems to give a better estimate of the toxicity of some compounds, in particular the *ortho* substituted ones, for which σ alone would give an overestimate of toxicity.

As concerns the other descriptors, multiple regression analysis did not yield improved results.

4. Conclusion

The determination of EC_{50} values by means of the submitochondrial particle assay and the development of quantitative structure–activity relationships allowed for the achievement of a deeper insight into the mechanism of toxic action of substituted anilines. The inclusion in the study of a series of anilines containing substituents covering a wide range of electron donor-withdrawing capability gave the possibility to point out the importance of electronic interactions in determining aniline toxicity. In addition, the investigation on quantitative structure–activity relationships showed that the hydrogen bonding donor capacity of substituted anilines also plays an important role. Conversely, the narrow range of variation of log K_{ow} values did not allow us to point out the importance of this parameter as a descriptor of the toxicity of the examined compounds. In fact, a low degree of correlation was found between log K_{ow} and EC_{50} values.

These findings are consistent with the results obtained previously for chlorinated anilines, supporting the hypothesis of a mechanism of toxic action based on hydrogen bonding between the NH_2 group of substituted anilines and polar groups at the membrane/water interface. These interactions may lead to a disorder in the membrane structure and, as a consequence, to a disturbance of its functioning.

Submitochondrial particles have therefore proved

suitable biosensors for detecting and investigating the toxic effects of compounds that cause a derangement of the lipid bilayer of functional membranes, influencing negatively the processes that take place therein.

References

[1] L. Fishbein, The Handbook of Environmental Chemistry, Vol. 3, Part C – Anthropogenic Compounds, Springer, Berlin, 1984, pp 1–40.
[2] S.K. Hong, D.K. Anestis, T.T. Henderson, G.O. Rankin, Toxicol. Lett. 114 (2000) 125–133.
[3] L.D. Dial, D.K. Anestis, S.R. Kennedy, G.O. Rankin, Toxicology 131 (1988) 109–119.
[4] M.A. Valentovic, J.G. Ball, S.K. Hong, B.A. Rogers, M.K. Meadows, R.C. Harmon, G.O. Rankin, Toxicol. In Vitro 10 (1996) 713–720.
[5] R.J. Brennan, R.H. Schiestl, Mutagenesis 12 (1997) 215–220.
[6] G.D. Veith, S.J. Broderius, Environ. Health Perspect. 87 (1990) 207–211.
[7] A.P. Van Wezel, A. Opperhuizen, Crit. Rev. Toxicol. 5 (1995) 255–279.
[8] E. Argese, C. Bettiol, F. Agnoli, A. Zambon, M. Mazzola, A. Volpi Ghirardini, Environ. Toxicol. Chem. 20 (2001) 826–832.
[9] G.A. Blondin, L.M. Knobeloch, H.W. Read, J.M. Harkin, Bull. Environ. Contam. Toxicol. 38 (1987) 467–474.
[10] E. Argese, A. Marcomini, P. Miana, C. Bettiol, G. Perin, Environ. Toxicol. Chem. 13 (1994) 737–742.
[11] H. Löw, I. Vallin, Biochim. Biophys. Acta 69 (1963) 361–374.
[12] M. Hansen, A.L. Smith, Biochim. Biophys. Acta 81 (1964) 214–222.
[13] C. Hansch, A. Leo, R.W. Taft, Chem. Rev. 91 (1991) 165–195.
[14] C. Hansch, A. Leo, Substituents Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1999.
[15] M. Charton, J. Am. Chem. Soc. 91 (1969) 6649–6654.
[16] E. Urrestarazu Ramos, W.H.J. Vaes, P. Mayer, J.L.M. Hermens, Aquat. Toxicol. 46 (1999) 1–10.
[17] E. Urrestarazu Ramos, W.H.J. Vaes, H.J.M. Verhaar, J.L.M. Hermens, J. Chem. Inf. Comput. Sci. 38 (1988) 845–852.
[18] C.J. Cramer, G.R. Famini, A.H. Lowrey, Acc. Chem. Res. 26 (1993) 599–605.
[19] Spartan 4.0, Wave Function, Irvine, CA, 1995, USA.
[20] HyperChem 6.01, Hypercube, Gainesville, FL, 2000.
[21] D.C. Montgomery, E.A. Peak, Introduction to Linear Regression Analysis, Wiley, New York, 1992.
[22] M.E. Vaschetto, B.A. Retamal, A.P. Monkman, J. Mol. Struct. 468 (1999) 209–221.
[23] L.M. Arnold, D.T. Lin, T.W. Shultz, Chemosphere 21 (1990) 183–191.
[24] J. Hermens, P. Leeuwangh, A. Musch, Ecotoxicol. Environ. Saf. 8 (1984) 388–394.
[25] A. Harada, M. Hanzawa, J. Saito, K. Hashimoto, Environ. Toxicol. Chem. 11 (1993) 973–980.
[26] H.J.M. Verhaar, E. Urrestarazu Ramos, J.L.M. Hermens, J. Chemometr. 10 (1996) 149–162.
[27] K.L.E. Kaiser, QSAR in Environmental Toxicology – II, D. Reidel Publishing, Dordrecht, The Netherlands, 1997, pp. 169–187.
[28] M.T.D. Cronin, T.W. Schultz, Sci. Tot. Environ. 204 (1996) 75–88.