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Recognition forces involved in mitochondrial binding to a low-affinity trimetazidine binding site related to anti-ischemic activity

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Abstract

A number of heterogeneous drugs previously shown to bind to trimetazidine (TMZ) binding sites on mitochondria and to inhibit mitochondrial swelling (Morin *et al.*, Br J Pharmacol 1998;123:1385–94) were investigated here for their physicochemical properties. The molecular parameters measured were the partition coefficients of the neutral and monocationic forms in the *n*-octanol/water and dichloroethane/water systems, their distribution coefficients at pH 7.4 in these two solvent systems, as well as their distribution coefficients at pH 7.4 in a phosphatidylcholine (PhC) liposomes/water system ($\log D_{lip}^{7.4}$). Most of these properties were not correlated with affinity to mitochondria or inhibition of mitochondrial swelling. In contrast, $\log D_{lip}^{7.4}$ showed a modest correlation with binding to the low-affinity site ($r^2 = 0.52$) and a better correlation with anti-swelling activity ($r^2 = 0.69$), itself well correlated with binding to the low-affinity sites ($r^2 = 0.83$). Thus, these sites have recognition properties much like those of membranes, as they depend on lipophilicity–hydrophobicity (core binding) and ionic bonds (surface interactions).

Keywords: Mitochondria; Permeability transition; Trimetazidine; Lipophilicity; Liposomes; Recognition forces

1. Introduction

Acquired mitochondrial dysfunctioning is associated with more than 100 different diseases, e.g. certain types of blindness, deafness, obesity, diabetes, some myo- and cardiomyopathologies, and cellular ageing [1,2]. This dysfunctioning can be related to two primary causes, namely mitochondrial DNA mutations or mitochondrial functional damage caused by cellular Ca^{2+} overload, excess of radical oxygen species, or alterations in ATP synthesis. But whatever the original cause, mitochondrial dysfunctioning may lead to a collapse of membrane potential. It induces the so-called mitochondrial permeability transition (MPT), which is characterised by a non-specific increase of the inner membrane permeability due to the opening of a ''giant pore'' allowing the diffusion of solutes of molecular weight up to 1500 [3]. Such alterations are associated with apoptosis and are observed in various pathological states caused by ischemia–reperfusion [4–6].

The MPT is a voltage-dependent, cyclosporin A-sensitive, dynamic multiprotein complex, which is probably located at the contact site between the inner and the outer mitochondrial membrane [4,7,8]. The MPT participates in the regulation of matrix Ca^{2+} concentrations, pH, transmembrane potential and volume; it functions as a Ca^{2+}-, voltage-, pH-, and redox-gated channel with several levels of conductance and little, if any, ion selectivity [4]. Although the exact composition of the MPT is elusive, proteins from the cytosol (hexokinase), the outer membrane (voltage-dependent anion channel), the inner membrane (the adenine nucleotide translocator), and the matrix (cyclophilin D) are probably involved [4]. Bernardi *et al.* [9] have defined three regulatory binding sites involved in MPT opening. Phospholipase A2 inhibitors such as trifluoperazine may act by interfering with Ca^{2+} binding to one of these regulatory sites [9].

The anti-ischemic drug TMZ may also act on a binding site. Indeed, Morin *et al.* have recently demonstrated the

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Abbreviations: MPT, mitochondrial permeability transition; PhC, phosphatidylcholine; TMZ, trimetazidine.

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existence of trimetazidine (1-(2,3,4-trimethoxybenzyl)piperazine) binding sites on mitochondrial membranes [10]. To explain the effects of TMZ, Morin *et al.* had selected a series of potential inhibitors of MPT opening for their ability to modify transporter activities and ionic fluxes across the mitochondrial membranes, and/or to interact with known mitochondrial sites. The affinity of these compounds for the mitochondrial membrane was assessed together with their effect on mitochondrial swelling. The existence of specific TMZ binding sites on rat liver and brain mitochondria was demonstrated. Their nature remains to be established, but they do not seem to be related to other previously described mitochondrial sites [10,11]. An endogenous substance present in the cytosol of hepatocytes was proven capable of displacing [^{3}H]-TMZ from its binding sites [11].

Two classes of specific binding sites, one of high-affinity and one of low-affinity, were distinguished. The high-affinity component represented only 4% of the total binding sites, and was located on the outer membrane, whereas the low-affinity binding site was restricted to the inner mitochondrial membrane [10]. These binding sites were neither identical to the inner membrane anion channel nor to the voltage-dependent anion channel. Furthermore, drugs binding to other mitochondrial targets, including respiratory chain complex inhibitors, an adenine nucleotide carrier ligand, a monoamine oxidase inhibitor or a Ca^{2+} uniporter blocker, were not able to displace [^{3}H]-TMZ [10].

A relationship between binding to the low-affinity site, inhibition of mitochondrial swelling and MPT opening was observed [10]. Recently, it has been reported that the inhibition of MPT opening by TMZ may result from displacement of Ca^{2+} [11].

The objective of this study was to gain a better insight into the physicochemical properties underlying the mitochondrial binding and anti-swelling activity of the heterogeneous series of compounds tested by Morin *et al.* [10] (Table 1). To this end, partition and distribution coefficients (log *P* and log *D*, respectively) were measured in biphasic lipid/water systems known to yield different information on intermolecular recognition forces [12–14]. Thus, log *P* and log *D* were measured in the isotropic *n*-octanol/water and 1,2-dichloroethane/water systems to obtain information on the hydrophobicity and hydrogen-bonding capacity of the investigated agents. Furthermore, liposomes/water distribution coefficients were measured since they yield information on hydrophobicity, H-bonding and ionic bonding capacity. When liposomes are prepared from phospholipids plus another component such as a protein, the solutes can interact by too many mechanisms (i.e. partition and binding in variable proportions) for the response to be interpretable in physicochemical terms. For this reason, ''simple'' phosphatidylcholine liposomes were used here.

Table 1
Inhibition of mitochondrial swelling and [^{3}H]-TMZ binding [10][a]

Compounds[b]	pIC_{50} Binding (μM)[c]		pIC_{50} Swelling (μM)
	pIC_{50H}	pIC_{50L}	
1, Trimetazidine	5.53	3.98	200
2, S-00226	6.25	3.85	320
3, Ref-64329	5.78	3.31	400
4, S-64329	7.70	4.47	54
5, Acebutolol	4.51	3.19	700
6, Alprenolol	6.27	4.47	33
7, Bupivacaine	3.97	3.97	223
8, Chlorpromazine	6.28	4.71	39
9, Deprenyl	5.54	3.80	140
10, Dibucaine	5.91	4.06	50
11, Flunarizine	6.46	4.41	20
12, Prenylamine	6.68	4.71	15
13, Prilocaine	4.99	3.11	900
14, Promethazine	6.19	4.65	49
15, Propafenone	5.34	5.34	13
16-R, *R*-(+)-Propranolol	6.28	4.79	33
16-S, *S*-(−)-Propranolol	5.95	4.32	52
17, Quinacrine	5.60	3.97	72
18, Quinidine	4.44	4.44	45
19, Quinine	3.74	3.74	130
20, Trifluoperazine	6.49	4.32	25

[a] Mean values; N = 3 or 4 for binding data; N = 3 for swelling data.
[b] See [10,11] for the structure of trimetazidine derivatives **2–4**.
[c] pIC_{50L} and pIC_{50H} are the negative logarithms of the binding affinity for the low- and high-affinity TMZ binding sites, respectively [10].

2. Material and methods

2.1. Compounds and reagents

Egg PhC, grade I, was purchased from Lipoid. Extruders of type T001 were purchased from Sirius Analytical Instruments. The polycarbonate filters with pores of 0.1 μm were obtained from Osmonics.

The phospholipids enzymatic colorimetric test MPR2 691844 was purchased from Boehringer Mannheim. Anhydrous disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Fluka and potassium chloride from Merck. Octanol and methanol were purchased from Fluka and 1,2-dichloroethane was purchased from SDS. All organic phases were of highest purity. Titrisol solutions of potassium hydroxide and hydrochloric acid were obtained from Merck.

Trimetazidine dihydrochloride and trimetazidine derivatives were kindly offered by Institut International de Recherche Servier. The other drugs were obtained from Aldrich, Fluka, ICN, RBI and Sigma Chemie.

2.2. Determination of ionisation constants

2.2.1. Potentiometry

Potentiometric titrations of compounds **1–20** (Table 1) were performed with a PCA101 or GLpKa apparatus [15] (Sirius Analytical Instruments) as previously described [16,17]. Solubility or availability problems prevented the

incorporation in this work of three compounds also previously investigated for their interaction with mitochondria [10], namely S-00240, flupenthixol and verapamil.

The low aqueous solubility of compounds **4**, **7**, **8**, **10–12**, **14**, **15** and **17–20** required pK_a measurements to be performed in the presence of MeOH or 1,4-dioxane as cosolvent. At least three separate semi-aqueous solutions with solute concentrations ranging from 0.39 to 1.43 mM, in 10.8–58.4% (v/v) MeOH (compounds **4**, **7**, **8**, **14**, **15** and **17–20**) or 10.2–40.1% (v/v) 1,4-dioxane (compounds **10** and **12**; exceptionally 39.2–50.8% (v/v) 1,4-dioxane in the case of flunarizine **11**) were initially acidified to pH 1.8. The solutions were then titrated with standardised KOH to pH 12.2. All titrations were carried out at $25.0 \pm 0.1^\circ$ and under a slow argon flow to avoid CO_2 absorption. The initial estimates of the p_sK_s values, which are the apparent ionisation constants in the mixed solvent, were obtained by the use of Bjerrum plots and refined by a weighed nonlinear least-squares procedure. The refined values were then extrapolated to 0% cosolvent following the Yasuda–Shedlovsky procedure ($r^2 \geq 0.97$).

2.2.2. UV spectroscopy

The pK_{a1} of dibucaine **10** was determined by potentiometry, but due to the presence of cosolvent no accurate determination of pK_{a2} was possible. The basic pK_{a2} was therefore, determined by UV spectroscopy using a Hewlett-Packard diode array spectrophotometer and Eq. (1):

$$pK_a = pH + \log\frac{A - A_N}{A_I - A} \quad (1)$$

where A is the monitored absorbance at a certain pH, and A_N and A_I are, respectively, the absorbance when 100% of the conjugated base or acid is present, all at the analysis wavelength.

The experiments were performed in triplicate at room temperature with 0.05 mM dibucaine solutions in a 10 mM citrate buffer of pH 5 (3.3 mM citric acid, 6.8 mM Na_2HPO_4) with 0.15 M KCl [18]. The wavelength of analysis was 232 nm. The absorbance at pH 0 (in 1 M HCl) and pH 5 were taken as A_I and A_N, respectively. Absorbance A was monitored while steadily decreasing the pH with ca. 0.6 pH units by dropwise addition of 0.1 M HCl and finally 5 M HCl to 20 mL 0.05 mM dibucaine.

2.3. Lipophilicity in n-octanol/water

2.3.1. Potentiometric experiments

At least three separate alkalimetric titrations with various volumes of octanol (volume ratio octanol/water ranged from 0.0052 to 1.52), were performed in the pH range 1.8–12.2 with the GLpKa instrument, under argon and at $25.0 \pm 0.1^\circ$. The concentration of the compounds ranged from 0.41 to 2.31 mM. The water phase was acidified to pH 1.8 before the titration was started. The log P values were estimated from difference Bjerrum plots [15] and refined by a linear least squares procedure [19] by including the earlier determined pK_a values as unrefined contributions. The $\log D_{oct}^{7.4}$ was determined by a simulation, using the pK_a(s) and the log P of the neutral and cationic form.

2.3.2. Shake-flask experiments

In order to determine the partition coefficients of the monocationic forms ($\log P_{oct}^{C}$) of compounds **10–12**, shake-flask experiments were performed at pH 5. Because of the low solubility of flunarizine and prenylamine, all three solutes were solubilised in *n*-octanol instead of buffer. If possible, drug concentrations were determined in both phases by HPLC analysis.

A citrate–phosphate buffer (3.27 mM citric acid, 0.102 M Na_2HPO_4) with pH 5.0 containing 0.15 M KCl was used [18]. Both *n*-octanol and buffer were saturated in one another by stirring 150 mL *n*-octanol and 600 mL buffer vigorously overnight. After phase separation in a separatory funnel, the two phases were collected and centrifuged at 2500 rpm for 15 min (MSE Mistral 2000° centrifuge) in order to remove also the last drops of the other phase.

Drug solutions of 1 mM in with buffer saturated *n*-octanol were prepared. To 1 mL drug solution 9 mL *n*-octanol saturated buffer was added and the mixture was shaken overnight at room temperature. Three Pyrex tubes were used for each compound. After 15 hr, the phases were separated by centrifugation at 4000 rpm for 15 min, followed by analysis of both phases and measurement of the pH of the buffer phase.

Table 2
The ionisation constants determined by potentiometry[a]

Compounds	pK_{a1}	pK_{a2}	pK_{a3}
1, Trimetazidine	9.15	4.52	
2, S-00226	8.49	4.10	
3, Ref-64329	10.12	7.87	4.05
4, S-64329	7.86[b]	3.64[b]	
5, Acebutolol	9.52		
6, Alprenolol	9.59		
7, Bupivacaine	8.35[b]		
8, Chlorpromazine	9.16[b]		
9, Deprenyl	7.41[d]		
10, Dibucaine	8.83[c]	1.9[e]	
11, Flunarizine	7.40[c]	1.8[f]	
12, Prenylamine	9.34[c]		
13, Prilocaine	7.96		
14, Promethazine	8.62[b]		
15, Propafenone	9.43[b]		
16, Propranolol	9.57		
17, Quinacrine	9.97[b]	7.74[b]	
18, Quinidine	8.52[b]	4.46[b]	
19, Quinine	8.52[b]	4.46[b]	
20, Trifluoperazine	7.78[b]	3.81[b]	

[a] The pK_a values are reported in decreasing order of basic strength.
[b] Methanol used as cosolvent, see Section 2.
[c] Dioxane used as cosolvent, see Section 2.
[d] Value used in the potentiometric determination of the partition coefficients in the *n*-octanol/water system and the liposomes/water system.
[e] Value determined by UV spectroscopy.
[f] Value taken in analogy with the pK_{a1} of hydroxyzine.

Table 3
Partition coefficients of neutral (log P^N) and monocationic (log P^C) forms in octanol/water and dichloroethane/water

Compounds	log P^N_{oct}	log P^C_{oct}	log P^N_{dce}	log P^C_{dce}	Δ log $P^N_{oct-dce}$
1, Trimetazidine	1.22[a]	−0.51[b]	1.04[c]	−3.78[d]	0.18
2, S-00226	1.69		2.07	–[f]	−0.38
3, Ref-64329	0.99		1.86	–[f]	−0.87
4, S-64329	3.00[a]	−0.72[e]	4.30	0.44	−1.30
5, Acebutolol	2.02	−0.50	0.81[c]	−2.22[g]	1.21
6, Alprenolol	3.10	0.25	3.26[c]	−1.84[g]	−0.16
7, Bupivacaine	3.72[a]	0.59[a]	4.25	–[f]	−0.53
8, Chlorpromazine	5.22[a]	1.58[a]	5.50	–[f]	−0.28
9, Deprenyl	2.90[a]	−0.35[a]	3.53	–[f]	−0.63
10, Dibucaine	4.19[a,l]	1.38[a,m]	4.67	0.44	−0.48
11, Flunarizine	4.83[a,l]	–[f]	4.62	–[f]	0.21
12, Prenylamine	4.83[a,l]	–[f]	5.46	–[f]	−0.63
13, Prilocaine	2.12[a]	−0.38[a]	2.62	–[f]	−0.50
14, Promethazine	4.14[a]	0.78[a]	4.88	0.63	−0.74
15, Propafenone	3.83[a]	1.00[a]	4.00	–[f]	−0.17
16, Propranolol	3.48	0.88	3.11[c]	−2.08[g]	0.37
17, Quinacrine	5.09[a]	1.16[e]	4.60	0.60	0.49
18, Quinidine	3.55[a]	1.19[a]	2.50[i]; 1.96[j]	−1.37[k]	1.59
19, Quinine	3.55[a]	1.19[a]	2.41[h]; 1.94[j]	–[f]	1.61
20, Trifluoperazine	4.71[e]	1.90[n]	5.45	1.96	−0.74

[a] Measured by potentiometry; N $\geq$ 3, SD $\leq$ 0.08.
[b] SD = 0.11.
[c] Measured by potentiometry; N = 4, SD < 0.05 [23].
[d] Measured by cyclic voltammetry; at least six measurements at different pH; SD = 0.16 [24].
[e] 0.21 $\leq$ SD $\leq$ 0.27.
[f] This value could not be determined.
[g] Measured by cyclic voltammetry; at least six measurements at different pH; SD < 0.30 [23].
[h] Measured by cyclic voltammetry.[1]
[i] Measured by centrifugal partition chromatography, SD = 0.04 [25].
[j] Measured by potentiometry; N = 2, mean deviation $\leq$ 0.02.
[k] Measured by cyclic voltammetry; at least six measurements at different pH; SD = 0.16 [25].
[l] Value is weighed mean: N = 2 or 3.
[m] Value obtained by shake-flask.
[n] SD = 0.46.

The two phases were analysed by HPLC using a LiChrosphere 60-RP-select B column (Merck), a Kontron UV detector and a Perkin-Elmer Nelson 1020 integrator. The mobile phase consisted of 60% (v) acetonitrile and 40% (v) 25 mM KH_2PO_4 pH 5. Both acetonitrile and the potassium dihydrogen phosphate solution had been filtered. The λ_{max} was determined for each compound. The drug concentrations at equilibrium were determined by the use of a calibration plot of the drug in the mobile phase. In contrast to the buffer phase, which was not diluted before injection, the octanol phase was diluted at least 30-fold before injection.

The log P^C_{oct} was determined using Eq. (2):

$$\log P^C_{oct} = \log \frac{[\text{drug}]_{oct,f}}{[\text{drug}]_{aq,f}} \quad (2)$$

in which $[\text{drug}]_{oct,f}$ and $[\text{drug}]_{aq,f}$ represent the drug concentration in the *n*-octanol and aqueous phase at equilibrium, respectively.

It is important to stress that the log P^C values so obtained are apparent partition coefficients of the cations, i.e. log *P* values depending on the *Galvani* potential between the organic and aqueous phase. The *Galvani* potential, in turn, depends on the buffer, the volumes of the phases and the nature and lipophilicity of the counterion(s) used [20,21].

2.4. Lipophilicity in 1,2-dichloroethane/water

The study of the partition behaviour of the compounds in the 1,2-dichloroethane/water system was carried out as in the *n*-octanol/water system (see above). At least three separate alkalimetric titrations with various volumes of dichloroethane (volume ratios dichloroethane/water ranged from 0.10 to 1.59), were performed in the pH range 1.8–12.2 with the GLpKa instrument, under argon and at 25.0 ± 1.0°. The solute concentrations ranged from 0.31 to 1.14 mM. Dichloroethane was handled with all necessary precautions to avoid inhalation and skin contact [22].

2.5. Lipophilicity in liposomes/water

Large unilamellar phosphatidylcholine liposomes for the equilibrium dialysis experiments were prepared by hydration of PhC with buffer pH 7.4 as described in

[1] Géraldine Bouchard. Personal communication, 2000.

[17]. The dialysis experiments were also performed as described [17], with the difference that they were only performed at pH 7.4. The molar [lipid]/[ligand] ratios varied from 6.83 (deprenyl **9**) to 144.48 (quinacrine **17**). The analytical wavelengths were 224, 258, 264, 268, 270, 272, 278, 280, 300, 306, 328, 332 and 332 nm for prilocaine **13**, deprenyl **9**, bupivacaine **7**, S-64329 **4**, trimetazidine **1**, S-00226 **2**, Ref-64329 **3**, quinacrine **17**, promethazine **14**, chlorpromazine **8**, dibucaine **10**, quinidine **18** and quinine **19**, respectively.

Experiments with hydrophilic compounds lasted longer (e.g. 300 min for trimetazidine **1**) than with lipophilic compounds (e.g. 100 min for chlorpromazine **8**). Mass balance experiments were performed to control that no loss of solute had occurred. All experiments were performed in triplicate and at room temperature.

3. Results and discussion

Ionisation constants are listed in Table 2. Most compounds examined are monobasic and hence showed only one pK_a value, whereas several are dibasic and Ref-64329 has three basic centres. In Table 2, pK_{a1} denotes the highest pK_a value. The partition coefficients of the neutral forms ($\log P^N$) and of the monocations ($\log P^C$) in the *n*-octanol/water and 1,2-dichloroethane/water are reported in Table 3. The distribution coefficients at pH 7.4 in the *n*-octanol/water, 1,2-dichloroethane/water and liposomes/water systems can be found in Table 4.

Due to solubility problems the liposomes/water distribution coefficients of flunarizine **11**, prenylamine **12**, propafenone **15** and trifluoperazine **20** could not be determined. The liposomes/water distribution coefficients reported for acebutolol **5**, alprenolol **6** and propranolol **16** were literature values [23].

The objective of this study was not to examine relations between structure and physicochemical parameters, but to unravel and interpret correlations between physicochemical parameters (Tables 3 and 4) and biological data (Table 1), with a view to obtain information on the high- and low-affinity binding sites and their role in anti-swelling activity.

No correlation can be observed between the affinity for the high-affinity binding site (pIC_{50H}) and $\log D^{7.4}_{oct}$, $\log D^{7.4}_{dce}$ or $\log D^{7.4}_{lip}$ as exemplified by the non-significant correlation coefficients reported in Table 5. A weak correlation between $\Delta \log P^N_{oct-dce}$ (which expresses the solute's capacity to form H-bonds [12–14]) and pIC_{50H} ($r^2 = 0.40$) may indicate that H-bonding plays a role in the binding to the TMZ high-affinity site.

Looking at correlations with low-affinity binding reveals a different picture. First, there is no correlation between pIC_{50L} and lipophilicity ($\log D^{7.4}_{oct}$ or $\log D^{7.4}_{dce}$) or H-bonding capacity ($\Delta \log P^N_{oct-dce}$) (Table 5). In contrast, there is a modest correlation between pIC_{50L} and $\log D^{7.4}_{lip}$ (Table 5) such that about 50% of the variance is explained. This reveals that binding to the low-affinity site is not structure-specific but depends on the recognition forces encoded in $\log D^{7.4}_{lip}$, namely lipophilicity plus ionic interactions. These are precisely the types of forces that must influence non-specific drug–membrane interactions.

Table 4
Distribution coefficients at pH 7.4 in the three partition systems

Compound	$\log D^{7.4}_{oct}$ [a]	$\log D^{7.4}_{dce}$ [b]	$\log D^{7.4}_{lip}$ [c]
1, Trimetazidine	−0.22	−0.72	0.80
2, S-00226	0.57	0.95	0.98[d]
3, Ref-64329	0.39	1.26	1.36
4, S-64329	2.41	3.71	2.20
5, Acebutolol	0.04	−1.31	1.15[e]
6, Alprenolol	1.00	1.07	2.50[e]
7, Bupivacaine	2.73	3.25	1.79
8, Chlorpromazine	3.46	3.73	3.65[f]
9, Deprenyl	2.59	3.22	2.16
10, Dibucaine	2.76	3.23	2.93
11, Flunarizine	4.52	4.32	–[g]
12, Prenylamine	2.89	3.52	–[g]
13, Prilocaine	1.46	1.95	1.34
14, Promethazine	2.89	3.64	3.35
15, Propafenone	1.86	1.97	–[g]
16, Propranolol	1.45	0.94	2.81
17, Quinacrine	2.03	1.54	2.70
18, Quinidine	2.41	0.81	2.39
19, Quinine	2.41	0.79	2.55
20, Trifluoperazine	4.18	4.92	–[g]

[a] $\log D^{7.4}$ in *n*-octanol/water system, calculated using the weighted log *P* contribution of the electrical forms present at pH 7.4.
[b] $\log D^{7.4}$ in 1,2-dichloroethane/water system, calculated using the weighted log *P* contributions of the electrical forms present at pH 7.4.
[c] $\log D^{7.4}$ in liposomes/water determined by dialysis; N = 3; SD ≤ 0.10.
[d] SD = 0.17.
[e] $\log D^{7.4}$ in liposomes/water determined by dialysis; N = 3; SD < 0.10.[2]
[f] $\log D^{6.0}$ in liposomes/water determined by dialysis.
[g] Measurement precluded by a low aqueous solubility.

Morin *et al.* [10] have shown that binding to the low-affinity site is correlated with the capacity to inhibit mitochondrial swelling. This relation is apparent in Table 5 ($r^2 = 0.83$) and is shown in Fig. 1A. Not unexpectedly, therefore, $\log D^{7.4}_{lip}$ is also correlated with pIC_{50sw} (Fig. 1B), and even much better so than with pIC_{50L} since about 70% of the variance are now explained (Table 5).

In conclusion, the present structure–activity relationship study confirms that the low-affinity TMZ sites on mitochondria are involved in the inhibition of mitochondrial swelling. In addition, the results reveal that these low-affinity sites have recognition properties much like those of membranes, as they depend on lipophilicity–hydrophobicity (core binding) and ionic bonds (surface interactions). Had a specific receptor been involved, geometric, steric and electrostatic complementarity (i.e. a stereoelectronic pharmacophore) would have been the predominant factor

[2] Catherine a Marca Martinet. Personal communication, 1999.

Table 5
Squared correlation matrix of pharmacological and physicochemical parameters[a]

	pIC_{50L}	pIC_{50sw}	$\log D^{7.4}_{oct}$	$\log D^{7.4}_{dce}$	$\log D^{7.4}_{lip}$	$\Delta \log P^{N}_{oct-dce}$
pIC_{50H}	0.34	0.38	0.05	0.22	0.06	0.40
pIC_{50L}	–	**0.83**	0.18	0.15	**0.52**	0.01
pIC_{50sw}	–	–	0.37	0.25	**0.69**	0.01
$\log D^{7.4}_{oct}$	–	–	–	0.80	0.72	0.04
$\log D^{7.4}_{dce}$	–	–	–	–	0.48	0.40
$\log D^{7.4}_{lip}$	–	–	–	–	–	0.00

[a] Squared correlation coefficients written in bold type are discussed in the text.

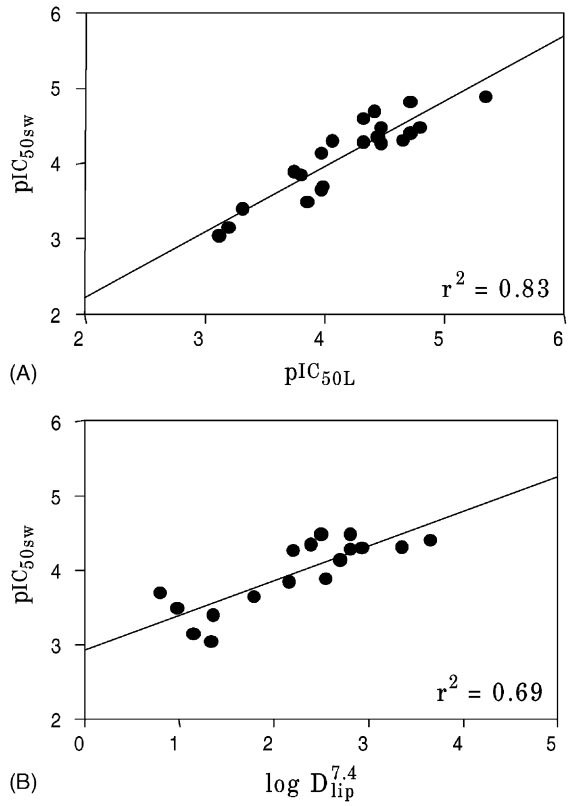

Fig. 1. Plots of (A) pIC_{50sw} vs. pIC_{50L} [10], and (B) pIC_{50sw} vs. $\log D^{7.4}_{lip}$.

controlling the mitochondrial activity of these drugs. This interpretation is compatible with the results of Gudz *et al.* [26] and Sokolove and Haley [27], who concluded that the effect of butylhydroxytoluene and related compounds on permeability of the inner mitochondrial membrane may be caused by interaction with the hydrophobic core of the membrane.

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