Retention Behavior of Neutral and Positively and Negatively Charged Solutes on an Immobilized-Artificial-Membrane (IAM) Stationary Phase

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The retention behavior of neutral, positively charged, and negatively charged solutes on the IAM.PC.DD2 stationary phase was investigated and compared. A set of monofunctional compounds and complex drugs (steroids, nonsteroidal anti-inflammatory drugs, and β-blockers) were selected for this study, *i.e.*, neutral solutes and solutes with acidic or basic functionalities which are positively charged or negatively charged at pH 7.0. The correlation between the retention factor $\log k_w$ at pH 7.0 on the IAM.PC.DD2 stationary phase and the partition coefficient $\log P_{oct}$ or the distribution coefficient $\log D_{7.0}$ showed that the retention mechanism depends on the charge state and structural characteristics of the compounds. The neutrals were least retained on the IAM.PC.DD2 stationary phase, and positively charged solutes were more retained than negatively charged ones. This implies that the retention of the charged solutes is controlled not only by lipophilicity but also by the electrostatic interaction with the phospholipid, with which positively charged solutes interact more strongly than negatively charged ones.

Introduction. – Successful drug development requires not only optimization of specific and potent pharmacological activity at the target site, but also efficient delivery to that site. Drug design and discovery must take pharmacokinetic behavior into account, in particular absorption and distribution. Numerous quantitative structure–permeability-relationship (QSPR) studies have clearly demonstrated that lipophilicity, as related to membrane partitioning and hence passive transcellular diffusion, is a key parameter in predicting and interpreting permeability [1][2]. Lipophilicity is generally expressed by the octan-1-ol/H_2O partition coefficient ($\log P_{oct}$, for a single chemical species) or distribution coefficient ($\log D_{oct}$, for a pH-dependent mixture of ionizable compounds). In some studies, a relationship has been established between $\log P_{oct}$ (or $\log D_{oct}$) and the absorption or permeability in intestinal models [3][4], blood-brain-barrier models [5], and cell-culture models [6–9], to name a few. However, in many other situations, $\log P_{oct}$ (or $\log D_{oct}$) cannot give a good estimate of a drug's absorption or permeation [10–14]. The lipophilicity parameters $\log P_{oct}$ or $\log D_{oct}$ fail to encode some important recognition forces, most notably ionic bonds, which are of particular importance when modeling the interaction of ionized compounds with biomembranes [15]. Because the majority of the drugs are ionizable [16], any prediction of their pharmacodynamic and pharmacokinetic properties should take their ionization into account.

[1]) The second author has the same contribution to this paper as the first author.

Thus the development of membrane-like systems such as immobilized-artificial-membrane chromatography has been of marked interest in the understanding of partitioning of ionized compounds [17][18]. Immobilized artificial membranes (IAMs) are solid-phase-membrane mimetics prepared by covalently bonding a monolayer of phospholipids to silica gel particles, thus mimicking the lipid environment of a fluid cell membrane on a solid matrix. Since IAMs provide the amphiphilic microenvironment of biological membranes, they should be able to take ionic bonds into account. In addition, IAM chromatography is a convenient process to measure partitioning of drugs because it involves the fast HPLC methodology.

The lipophilicity index from a IAM-HPLC stationary phase is derived from the capacity factor log k, which is calculated by *Eqn. 1*, where t_R and t_0 are the retention times of the solute and of an unretained compound, respectively. For lipophilic compounds, the retention times would be too long by using a purely aqueous mobile phase. Thus, log k values are determined at different concentrations of an organic modifier and extrapolated to pure aqueous mobile phase (log k_w) by *Eqn. 2*, where φ is the volume fraction of MeOH in the mobile phase, S the slope, and log k_w the intercept of the regression curve. For hydrophilic compounds, log k_w can be determined directly by using the aqueous mobile phase.

$$\log k = \log(t_R - t_0)/t_0 \quad (1)$$

$$\log k = -S\varphi + \log k_w \quad (2)$$

The interaction of drugs with phospholipids has been investigated by IAM-HPLC for different sets of neutral and ionized compounds such as β-blockers [19][20], nonsteroidal anti-inflammatory drugs [21], and dihydropyridine (DHP) calcium-channel blockers [22]. In these studies, the log k_w values obtained from IAM-HPLC were compared with the octan-1-ol/H_2O partitioning, and the occurrence of electrostatic interactions with phospholipids was found for ionized compounds.

To further understand the retention mechanism of solutes on a IAM stationary phase, we selected a set of monofunctional compounds and complex drugs (steroids, nonsteroidal anti-inflammatory drugs, and β-blockers). This set consists of neutral solutes and solutes with acidic or basic functionalities which are positively charged or negatively charged at pH 7.0, as shown in the *Table*. The retention behavior of different sets of solutes was investigated in terms of the influence of different functionalities, lipophilicity, and the charged state of the solutes.

Results and Discussion. – To obtain experimental conditions as close as possible to the physiologic pH and compatible with the stability of the stationary phase (highest pH limit is 7.5), log k values were determined at pH 7.0 on the IAM.PC.DD2 stationary phase. According to the pK_a values of the compounds shown in the *Table*, the monofunctional carboxylic acids **22**–**30** and the NSAIDs **31**–**36** are fully negatively charged at pH 7.0, whereas the (4-methylbenzyl)alkylamines **37**–**43** and β-blockers **44**–**49** are fully positively charged, and the very weak bases and acids **1**–**5**, **12**, and **13**) are fully neutral at this pH. The partition coefficient log P_{oct} and distribution coefficient

Table. *The Physicochemical Parameters of the Investigated Compounds* **1**–**49**. N = Neutral.

		log P_{oct} [a])	pK_a [a])	log $D_{7.0}$ [b])	Charge state	log k_w [c])
1	Acridine	3.40	5.58	3.40	N	2.42
2	$PhNH_2$	0.90	4.60	0.90	N	0.26
3	Ph_2NHEt	2.16	5.12	2.16	N	1.04
4	2-$ClC_6H_4NH_2$	1.91	2.64	1.91	N	1.14
5	2-$H_2NC_6H_4Ph$	2.84	3.82	2.84	N	2.02
6	$PhCH_2CN$	1.56	N	1.56	N	0.94
7	PhC(O)Me	1.58	N	1.58	N	0.86
8	$PhNO_2$	1.85	N	1.85	N	0.99
9	2-$ClC_6H_4NO_2$	2.24	N	2.24	N	1.58
10	$PhCH_2OH$	1.08	N	1.08	N	0.58
11	4-$ClC_6H_4CH_2OH$	1.96	N	1.96	N	1.21
12	3-ClC_6H_4OH	2.49	9.11	2.48	N	1.77
13	3-$O_2NC_6H_4OH$	2.00	8.40	1.96	N	1.38
14	Corticosterone	1.94	N	1.94	N	1.67
15	Dexamethasone	1.83	N	1.83	N	1.79
16	Estradiol	4.01	N	4.01	N	2.65
17	Estrone	3.13	N	3.13	N	1.92
18	Hydrocortisone	1.55	N	1.55	N	1,35
19	Hydrocortisone-21-acetate	2.19	N	2.19	N	1.78
20	Progestrone	3.87	N	3.87	N	3.01
21	Testosterone	3.29	N	3.29	N	2.51
22	$Ph(CH_2)_2COOH$	1.89	4.52	− 0.59	−	− 0.25
23	$Ph(CH_2)_3COOH$	2.42	4.72	0.14	−	0.06
24	$Ph(CH_2)_4COOH$	2.85	4.55	0.40	−	0.43
25	$Ph(CH_2)_7COOH$	4.09	5.03	2.12	−	2.02
26	PhCOOH	1.96	4.20	− 0.84	−	− 0.62
27	4-BrC_6H_4COOH	2.86	3.97	− 0.17	−	0.32
28	3-ClC_6H_4COOH	2.71	3.83	− 0.46	−	0.06
29	4-IC_6H_4COOH	3.13	3.96	0.09	−	0.51
30	1-Naphthoic acid	3.10	3.69	− 0.21	−	0.13
31	Aspirin	1.13	3.48	− 2.39	−	− 0.15
32	Flurbiprofen	3.81	3.91	0.72	−	1.78
33	Ketoprofen	2.77	4.29	0.06	−	1.26
34	Naproxen	3.06	4.15	0.21	−	1.35
35	Indomethacin	4.27	4.50	1.77	−	2.37
36	Mefenamic acid	5.12	4.33	2.45	−	2.35
37	4-$MeC_6H_4CH_2NHMe$	1.96	9.93	− 0.97	+	0.96
38	4-$MeC_6H_4CH_2NHEt$	2.38	10.04	− 0.66	+	1.02
39	4-$MeC_6H_4CH_2NHPr$	2.96	9.98	− 0.02	+	1.30
40	4-$MeC_6H_4CH_2NHBu$	3.49	9.98	0.51	+	1.87
41	4-$MeC_6H_4CH_2NH(CH_2)_4Me$	4.26	10.08	1.18	+	2.27
42	4-$MeC_6H_4CH_2NH(CH_2)_5Me$	4.96	10.17	1.79	+	2.77
43	4-$MeC_6H_4CH_2NH(CH_2)_6Me$	5.12	10.02	2.10	+	2.92
44	Metoprolol	1.95	9.63	− 0.68	+	1.45
45	Metipranolol	2.81	9.54	0.27	+	1.78
46	Oxprenolol	2.51	9.57	− 0.06	+	1.70
47	Penbutolol	4.62	9.92	1.70	+	3.70
48	Pindolol	1.75	9.54	− 0.79	+	1.31
49	Propranolol	3.48	9.53	0.95	+	2.48

[a]) Taken from [23–26]. [b]) Calculated according to $\log D = \log P_{oct} - \log(1 + 10^{pK_a - pH})$ for bases and $\log D = \log P_{oct} - \log(1 + 10^{pH - pK_a})$ for acids. [c]) $n = 3$, s.d. ≤ 0.05.

at pH 7.0, namely log $D_{7.0}$ calculated from pK_a and log P_{oct} values, are also summarized in the *Table*, together with the pK_a values and charge state of the compounds.

Relationship between log k *and* φ. The compounds **22**–**24** and **26**–**31** were eluted with a purely aqueous mobile phase. For the other solutes, four or five different MeOH concentrations in aqueous solutions were used as mobile phase for the extrapolation to log k_w values. Good linear relationships between log k and φ were found in the range of the eluent composition studied. The squared correlation coefficient was higher than 0.99, except for the log k_w of **3**, **4**, **10**, and **46** ($r^2 = 0.98$). The log k_w values are presented in the *Table* together with other physicochemical parameters.

Relationship between log k_w *and log* $P_{oct.}$ The correlation between log k_w and log P_{oct} is shown in *Fig. 1*. No correlation exists for the whole set of compounds. However, the correlations are good for neutral compounds or structurally related compounds. The corresponding correlation equations are shown as follows below (*Eqns. 3–7*), wherein 95% confidence limits are in parentheses, n is the number of compounds, r^2 the squared correlation coefficient, s the standard deviation, and F *Fisher*'s test.

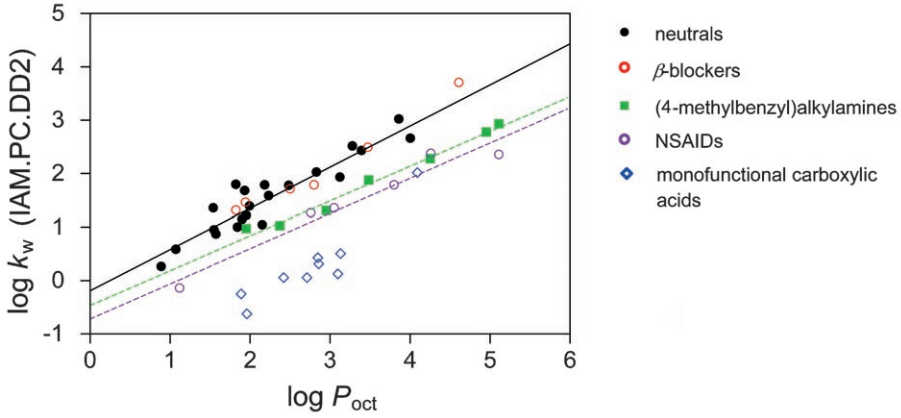

Fig. 1. *Correlation between log* k_w *values from the IAM.PC.DD2 stationary phase at pH 7 and log* P_{oct} *values for the compounds investigated*

For the neutral compounds **1**–**21**, *Eqn. 3* holds ($n = 21$, $r^2 = 0.87$, $s = 0.26$, and $F = 130$). The correlation coefficient for the 13 monofunctional solutes **1**–**13** became much more significant ($r^2 = 0.95$) if the steroids **14**–**21** (see *Table*) were excluded, implying that the correlation quality for neutral compounds is decreased by the increasing structural diversity of the complex drugs.

$$\log k_w = 0.77\ (\pm 0.13)\ \log P_{oct} - 0.19\ (\pm 0.33) \qquad (3)$$

For the β-blockers **44**–**49**, *Eqn. 4* holds ($n = 6$, $r^2 = 0.97$, $s = 0.18$, and $F = 115$). As shown in *Eqn. 4* and *Fig. 1*, the six β-blockers fit the correlation line for neutral compounds. This agrees with the study of *Barbato et al.* [19]. It means that the β-blockers under study can interact with phospholipids as strongly as neutral compounds with the same log P_{oct} values, although they are fully positively charged under the

experimental conditions. It suggests that the retention of the six β-blockers on the IAM.PC.DD2 stationary phase is governed not only by lipophilicity but also by extra interactions of which an electrostatic interaction between positively charged amines and negatively charged phosphates of the phospholipids plays a key role, as discussed by *Avdeef et al.* [27] and *Barbato et al.* [19][28] in their studies.

$$\log k_w = 0.84\ (\pm 0.16)\ \log P_{oct} - 0.34\ (\pm 0.48) \quad (4)$$

For the (4-methylbenzyl)alkylamines **37**–**43**, *Eqn. 5* holds ($n = 7$, $r^2 = 0.98$, $s = 0.11$, and $F = 320$). The retention of these seven positively charged (4-methylbenzyl)alkylamines on the IAM.PC.DD2 stationary phase is weaker than that of the β-blockers and neutral compounds with same log P_{oct} values, as shown by *Eqn. 5* and *Fig. 1*, implying that the strength of extra interactions between charged amines and the phospholipid membrane depends on the structural characteristics of the solutes.

$$\log k_w = 0.65\ (\pm 0.07)\ \log P_{oct} - 0.46\ (\pm 0.27) \quad (5)$$

For the NSAIDs **31**–**36**, *Eqn. 6* holds ($n = 6$, $r^2 = 0.95$, $s = 0.24$, and $F = 73$). The retention of the negatively charged NSAIDs and monofunctional carboxylic acids investigated is weaker than that of positively charged compounds. Contrary to the result from the study of *Barbato et al.* on the IAM.PC.MG stationary phase [21], where the correlation between log k_w and log P_{oct} results in a unique regression line for NSAIDs (with carboxylic function not directly linked to the aromatic ring) and neutral compounds, our study on the IAM.PC.DD2 stationary phase showed two separate regression lines for NSAIDs and neutral compounds, implying that the NSAIDs investigated are less retained than neutral compounds with same log P_{oct} values. It should be noted that the retention behavior of the negatively charged NSAIDs, especially of **32**–**35**, is very similar to that of the positively charged (4-methylbenzyl)-alkylamines.

$$\log k_w = 0.66\ (\pm 0.16)\ \log P_{oct} - 0.72\ (\pm 0.55) \quad (6)$$

For the monofunctional carboxylic acids **22**–**30**, *Eqn. 7* holds ($n = 9$, $r^2 = 0.86$, $s = 0.29$, and $F = 43$). These negatively charged carboxylic acids, except for **25**, are less retained than negatively charged NSAIDs, resulting in a different regression line between log k_w and log P_{oct}. The correlation coefficient of *Eqn. 7* is low, meaning that the retention behavior of this set of compounds cannot be well predicted by their log P_{oct} values.

$$\log k_w = 1.02\ (\pm 0.31)\ \log P_{oct} - 2.54\ (\pm 0.89) \quad (7)$$

Relationship between log k_w *on the IAM.PC.DD2 and log* $D_{7.0}$. The log P_{oct} and log $D_{7.0}$ values are highly interrelated for (4-methylbenzyl)alkylamines and NSAIDs investigated in this study; therefore, the relationship between log k_w and log $D_{7.0}$ values is not reported anymore here. The correlation between log k_w and log $D_{7.0}$ values for β-blockers is less significant than that between log k_w and log P_{oct}. However, for the

monofunctional carboxylic acids, a much more significant correlation equation is established between log k_w and log $D_{7.0}$ values (see *Eqn. 8*, $n = 9$, $r^2 = 0.94$, $s = 0.19$, and $F = 107$), as compared to *Eqn. 7*. It indicates that the retention can be much better predicted by the distribution coefficient log $D_{7.0}$ values for this set of compounds.

$$\log k_w = 0.82\ (\pm 0.16)\ \log D_{7.0} + 0.25\ (\pm 0.13) \tag{8}$$

The extra interaction between ionized solutes and the IAM.PC.DD2 stationary phase can be more clearly and logically shown in *Fig. 2*, the correlation between log k_w and the distribution coefficient log $D_{7.0}$ of the compounds investigated. From *Fig. 2*, it can be seen that the neutral compounds are the least retained on the IAM.PC.DD2 stationary phase. All the ionized solutes are more strongly retained than neutral ones with the same log $D_{7.0}$ values, to a different extent, depending on their charge and structural characteristics. The retention of positively charged solutes is stronger than that of negatively charged ones. Indeed, as discussed by *Avdeef et al.* in the liposomal membrane/water partitioning of ionized drugs [27], the charge distribution in the phospholipid membrane is anisotropic; as the ionized species moves in the direction of the aqueous exterior of the membrane, the first charges it experiences are those of the negatively charged phosphates. Further movement would bring the ionized drug substance in the vicinity of the positively charged trimethylammonium groups. Electrostatic pairing of charges would require a greater movement for weak acids, compared to weak bases. Therefore, the negatively charged solutes have lesser affinity for phosphatidylcholine-based membranes than positively charged solutes. The results from our study with the IAM.PC.DD2 stationary phase verified this point. Further, *Fig. 2* shows that the retention of the ionized solutes on the IAM.PC.DD2 phase also depends on their structural characteristics. For the positively charged amines investigated, β-blockers are slightly more retained than (4-methylbenzyl)alkylamines, which is also shown by *Taillardat–Bertschinger et al.* in their study [24]. For the negatively charged solutes, NSAIDs (except for mefenamic acid (**36**)) are more

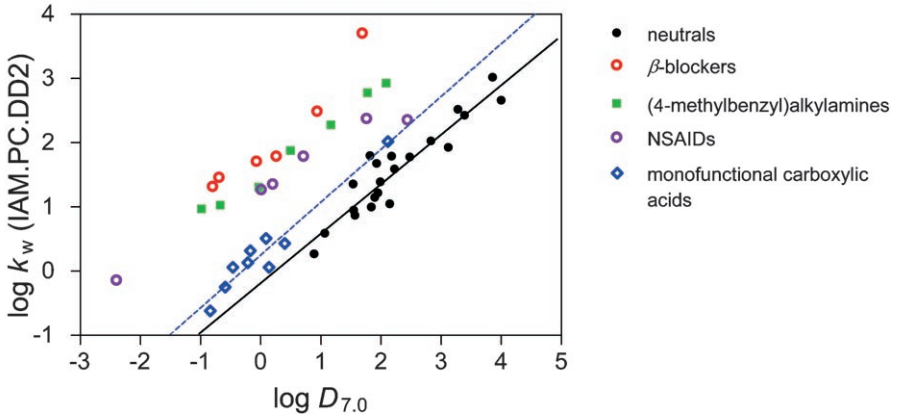

Fig. 2. *Correlation between log* k_w *values from the IAM.PC.DD2 stationary phase at pH 7 and log* $D_{7.0}$ *values for the compounds investigated*

retained than monofunctional carboxylic acids, confirming that the strength of the electrostatic interactions is influenced by different structural characteristics of the solutes.

Conclusion. – In this work, we compared the retention behavior of a set of neutral and positively or negatively charged solutes on the IAM.PC.DD2 stationary phase. Significant correlations were found between the retention factor $\log k_w$ on this stationary phase and $\log P_{oct}$ or $\log D_{7.0}$ for neutral or structurally related compounds, implying that the retention mechanisms are the same for neutral or structurally related compounds. The retention of the ionized compounds on the IAM.PC.DD2 is controlled not only by lipophilicity but also by extra interactions, mainly electrostatic interactions between charged solutes and phospholipids. For the solutes investigated in this study, positively charged compounds are more retained than negatively charged solutes. The ranking order of retention strength is: β-blockers > (4-methylbenzyl)alkylamines > NSAIDs > monofunctional carboxylic acids. This implies that the interaction between positively charged solutes and the phosphatidylcholine-based IAM stationary phase is larger than that between negatively charged solutes and the membrane, and that the electrostatic interaction depends on the structural characteristics of the solutes investigated.

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Experimental part

General. The (4-methylbenzyl)alkylamines **37**–**43** (*Table*) were synthesized by known procedures [29]. All other compounds were obtained from commercial sources (*Sigma–Aldrich*, Steinheim, Germany; *Carl Roth*, Karlsruhe, Germany; *VWR*, Leuven, Belgium) in the highest available purity. Distilled H_2O, HPLC-grade MeOH (*Alfa Aesar*, Karlsruhe, Germany) were used throughout.

Capacity Factors. The capacity factors were measured with a liquid chromatograph equipped with a HPLC pump *System-Gold-125* solvent module, a *System-Gold-507e* autosampler, and a *System-Gold-UV/VIS-168* detector (all from *Beckmann Coulter*, *Inc.,* Fuerton, CA, USA). The column was an IAM.PC.DD2 (100 mm × 4.6 mm i.d., 10 μm) from *Regis Technology* (Morton Grove, IL, USA). The mobile phases were either 0.02M phosphate buffer pH 7.0 or mixtures of 0.02M phosphate buffer pH 7.0 and MeOH in proportions varying from 70 to 10% (*v*/*v*) for all other compounds. The phosphate buffer was filtered under vacuum through a *HA-Millipore* filter (0.45 μm; *Millipore*, Milford, MA, USA) before being mixed with MeOH. The retention times were measured at r.t. by the UV/VIS detector at the λ_{max} of the analytes. The solns. to be injected (10^{-4} M to 10^{-3} M) were prepared by dissolving the solutes in the mobile phase; the injection volume was 10 μl. Citric acid was used as the unretained compound. The measurements were carried out at a flow rate of 1.0 ml/min for all compounds. For compounds **22**–**24** and **26**–**31**, the $\log k_w$ values were determined directly in the aq. mobile phase. For the other compounds, four or five different MeOH concentrations in aq. soln. were used as mobile phase for the extrapolation to $\log k_w$. The capacity factor $\log k$ was calculated by *Eqn. 1*. All $\log k$ values were the average of three measurements. The $\log k$ values were then extrapolated to 100% H_2O with *Eqn. 2* ($\rightarrow \log k_w$).

REFERENCES

[1] H. van de Waterbeemed, B. Testa, in 'Advances in Drug Research', Ed. B. Testa, Academic Press, London, 1987, Vol. 16, p. 87–227.
[2] G. Camenisch, G. Folkers, H. van de Waterbeemed, *Pharm. Acta Helv.* **1996**, *71*, 309.

[3] I. Komiya, J. Y. Park, A. Kamani, N. F. H. Ho, W. I. Higuchi, *Int. J. Pharm.* **1980**, *4*, 249.
[4] D. C. Taylor, R. Pownall, W. Burke, *J. Pharm. Pharmacol.* **1985**, *37*, 280.
[5] V. A. Levin, *J. Med. Chem.* **1980**, *23*, 682.
[6] P. Artursson, *J. Pharm. Sci.* **1990**, *79*, 476.
[7] A. Buur, L. Trier, C. Magnusson, P. Artursson, *Int. J. Pharm.* **1996**, *129*, 223.
[8] A. R. Hilgers, R. A. Conradi, P. S. Burton, *Pharm. Res.* **1990**, *7*, 902.
[9] L. Hovgaard, H. Brondsted, A. Buur, H. Bundgaard, *Pharm. Res.* **1995**, *12*, 387.
[10] M. S. Roberts, W. J. Pugh, J. Hadgraft, A. C. Watkinson, *Int. J. Pharm.* **1995**, *126*, 219.
[11] M. S. Roberts, W. J. Pugh, J. Hadgraft, *Int. J. Pharm.* **1996**, *132*, 23.
[12] W. J. Pugh, M. S. Roberts, J. Hadgraft, *Int. J. Pharm.* **1996**, *138*, 149.
[13] O. A. Raevsky, V. I. Fetisov, E. P. Trepalina, J. W. McFarland, K. J. Schaper, *Quant. Struct.-Act. Relat.* **2000**, *19*, 366.
[14] F. Wohnsland, B. Faller, *J. Med. Chem.* **2001**, *44*, 923
[15] B. Testa, P. A. Carrupt, P. Gaillard, R. S. Tsai, in 'Lipophilicity in Drug Action and Toxicology', Eds. V. Pliska, B. Testa, and H. van de Waterbeemed, VCH, Weinheim, Germany, 1996, pp. 275 – 304.
[16] J. Comer, K. Tam, in 'Pharmacokinetics Optimization in Drug Research: Biological, Physicochemical and Computational Strategies', Eds. B. Testa, H. van de Waterbeemed, G. Folkers, and R. H. Guy, Wiley-VHCA, Zürich, 2001, pp. 275 – 304.
[17] S. Ong, S. J. Cai, C. Barnal, D. Rhee, X. Qiu, C. Pidgeon, *Anal. Chem.* **1994**, *66*, 782.
[18] S. Ong, H. Liu, C. Pidgeon, *J. Chromatogr. A* **1996**, *728*, 113.
[19] F. Barbato, G. di Martino, L. Grumetto, M. I. La Rotonda, *Eur. J. Pharm. Sci.* **2005**, *25*, 379.
[20] R. Kaliszan, A. Nasal, A. Bucinski, *Eur. J. Med. Chem.* **1994**, *29*, 163.
[21] F. Barbato, M. I. La Rotonda, F. Quaglia, *J. Pharm. Sci.* **1997**, *86*, 225.
[22] F. Barbato, M. I. La Rotonda, F. Quaglia, *Eur. J. Med. Chem.* **1996**, *31*, 311.
[23] Bioloom version 1, Biobyte Corporation, Clarement, CA.
[24] A. Taillardat-Bertschinger, C. A. Marca Martinet, P. A. Carrupt, M. Reist, G. Caron, R. Fruttero, B. Testa, *Pharm. Res.* **2002**, *19*, 729.
[25] C. A. Marca Martinet, Ph.D Thesis, University of Lausanne, 2001.
[26] H. Tomida, T. Yotsuyanagi, K. Ikeda, *Chem. Pharm. Bull.* **1978**, *26*, 2832.
[27] A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, K. Y. Tam, *Pharm. Res.* **1998**, *15*, 209.
[28] F. Barbato, G. di Martino, L. Grumetto, M. I. La Rotonda, *Eur. J. Pharm. Sci.* **2004**, *22*, 261.
[29] W. R. Meindl, E. von Angerer, H. Schönenberger, G. Ruckdeschel, *J. Med. Chem.* **1984**, *27*, 1111.

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