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Relative hydrophobicity and lipophilicity of drugs measured by aqueous two-phase partitioning, octanol-buffer partitioning and HPLC. A simple model for predicting blood–brain distribution

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Abstract

Relative hydrophobicity and lipophilicity of 63 compounds with known permeability through the blood–brain barrier (BBB) was examined by partitioning in aqueous dextran-poly(ethylene glycol) two-phase system and octanol-buffer system, and by gradient RP-HPLC at pH 7.4. Combination of the relative hydrophobicity estimates, $N(CH_2)$ obtained by aqueous two-phase partitioning and the lipophilicity ($\log D_{exp}$ or $\log D_{HPLC}$) values obtained by the shake-flask technique or HPLC technique allows one to differentiate between compounds capable of crossing the BBB and those that cannot. A simple model for predicting blood–brain distribution is proposed.
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Keywords: Hydrophobicity; Lipophilicity; Log P; Aqueous two-phase partitioning; Octanol-buffer partitioning; HPLC; Blood–brain barrier

1. Introduction

The ability of compounds to penetrate the blood–brain barrier (BBB) is of fundamental importance in drug discovery and design. High BBB permeability is needed for CNS-active drugs, while low BBB permeability may be desirable to minimise CNS-related side-effects of drugs with peripheral sites of action. The degree of BBB penetration is measured as the ratio of the steady-state concentrations of the drug in the brain and in the blood, expressed as $\log(C_{brain}/C_{blood})$ or log BB. Compounds with $\log BB > 0.3$ cross the BBB readily, while compounds with $\log BB < -1.0$ are only poorly distributed to the brain [1]. The determination of log BB is difficult, expensive, and time-consuming, requiring animal experiments. A rapid screening method for scoring BBB permeability for large sets of compounds is needed. Numerous attempts to correlate log BB of drugs with physicochemical parameters have been reported in Refs. [2–13]; however, none have succeeded in developing such a method.

Distribution of a compound in vivo may be viewed as a series of partitioning steps from one region to the next, in conjunction with diffusion through each region [14]. The process involves partitioning between aqueous media and biological membranes as well as between aqueous media having different solvent properties (e.g. interstitial fluid in brain and blood plasma). The affinity of a compound for biological membranes may be represented by its lipophilicity, log D, measured by the octanol-buffer partition technique [15]. The relative affinity of a compound for different aqueous media may be simulated by partitioning in aqueous two-phase systems [16,17].

Analysis of partitioning of a homologous series of monofunctional aliphatic compounds yields a linear relationship described by $\ln K = A + EN_C$, where N_C is the number of carbon atoms in the aliphatic alkyl chain of the partitioned solute molecule, and provides experi-

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mental values of an average ln K increment per CH_2 group (E) and of the total contribution of a polar moiety of the solute molecule (A) [14]. The coefficient E is related to the free energy of transfer of a CH_2 group between phases in a given two-phase system. The ratio expressed as

$$\ln K/E = N(CH_2) \quad (1)$$

has been defined as the equivalent quantity of methylene units and suggested [14,15,18–20] to be used as a measure of the relative hydrophobicity of a solute (or a moiety). A positive value of $N(CH_2)$ means that a given solute is hydrophobic and its relative hydrophobicity is equal to that of N units of methylene groups. A negative value of $N(CH_2)$ means that the solute is hydrophilic and its relative hydrophobicity is the reverse of that of N number of CH_2 units.

Relative hydrophobicity, $N(CH_2)$, has been used as a sole drug structure descriptor in QSAR analysis of drug permeability through porin channels of *E. coli*, and in combination with log D for QSAR analysis of tissue distribution of sulphonamides, and bitterness threshold of peptides (Zaslavsky et al., unpublished data). It seems that the combination of the two descriptors may be used for QSAR analysis when biological activity involves distribution of compounds throughout the body.

In this study we explored the possibility of using the combination of two descriptors representing the lipophilicity (as measured by octanol-buffer partitioning and RP-HPLC techniques) and relative hydrophobicity (as measured by aqueous two-phase partitioning) of organic compounds for analysis of drugs known to cross or not to cross the BBB.

2. Materials and methods

2.1. Materials

Dextran-67 (MW ~67 300, lot 119H1426), poly(ethylene glycol) (PEG-8000, MW ~8000, lot 85H0654), and commercially available pharmaceutical compounds were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. All organic solvents were of HPLC grade, and analytical reagent grade inorganic salts and reagents were used. Aqueous two-phase systems of final composition 6.05%wt. PEG-8000, 12.33%wt. Dex-67, and 0.15 M NaCl in 0.01 M universal buffer, pH 7.4 were prepared as described previously [18–21]. Octanol-buffer systems with volume ratio of 1:1 were prepared in 96 deep-well (1.2 mL) plates as described elsewhere [19].

2.2. Methods

Partitioning in aqueous two-phase systems was performed with Automated Signature Workstation (Analiza, Inc., Cleveland, OH) as described previously [18–21]. Compounds to be partitioned in the aqueous Dex-PEG systems were dissolved in water or DMSO at concentrations of ca. 0.5–5 mg mL^{-1}. Concentrations of partitioned compounds in both phases were measured by optical absorbance at the corresponding maximum wavelength using a Spectramax Plus^{384} UV–Vis spectrophotometer reader with pre-blank reading. The partition coefficient, K, is defined as the ratio of the sample concentration in the PEG-rich phase to the sample concentration in the dextran-rich phase. Each K-value was determined at 20 °C as the slope of the plot of the concentration in the upper phase as a function of the concentration in the bottom phase from five to six partition experiments. For the compounds introduced in the aqueous Dex-PEG two-phase system in DMSO the partition coefficients were re-calculated into those in the same system without DMSO using the linear relationship established previously [20].

The octanol-buffer partition experiments were performed with an automated shake-flask system (ADW, Analiza, Inc., Cleveland, OH) as described in detail elsewhere [21].

Liquid chromatograph 1050 (Agilent technologies) equipped with autosampler and DAD detector was used for performing HPLC measurements according to the modified gradient method developed by Donovan and Pescatore [22] and described in detail previously [19]. The standard deviation from the average log D_{HPLC} value does not exceed 5%.

3. Results and discussion

The results obtained in aqueous Dex-PEG systems for the compounds examined at pH 7.40 are presented as the corresponding relative hydrophobicity values, $N(CH_2)$, in Table 1. Out of 63 compounds examined here, only 2 compounds, estrone and ebastine, precipitated out in the aqueous Dex-PEG system and could not be partitioned. All the other compounds including those with high lipophilicity ($\log D^{7.40} > 3$), such as astemizole, perphenazine, thioridazine, etc. could be partitioned in the aqueous Dex-PEG two-phase system when dissolved in DMSO. The K-values determined for these compounds were transformed into those for the system without DMSO using the linear relationship described previously [20].

The results obtained in the octanol-buffer partition measurements are presented as log $D^{7.40}_{exp}$ values in Table 1 together with those obtained by HPLC (expressed as log $D^{7.40}_{HPLC}$). In attempting to correlate our experimental

Table 1
Relative hydrophobicity, $N(CH_2)^{7.4}$, lipophilicity, log $D^{7.4}_{exp}$, and chromatographic lipophilicity index, log $D^{7.4}_{HPLC}$ for the compounds examined

#	CNS	Compound	$N(CH_2)^{7.4}$	Log $D^{7.4}_{exp}$	Log $D^{7.4}_{HPLC}$
1	–	acyclovir	2.10±0.25	−1.75±0.10	−0.16
2	–	acetaminophen	11.60±0.20	0.40±0.10	0.91
3	–	albendazole	20.46±0.75	2.85±0.15	3.03
4	–	albuterol	3.51±0.20	−1.11±0.05	0.20
5	–	ampicillin	2.43±0.15	−1.61±0.04	0.91
6	–	antipyrine	7.25±0.16	0.28±0.05	1.21
7	–	astemizole 2HCl	28.88±0.60	3.88±0.20	4.19
8	–	atenolol	7.25±0.20	−1.03±0.05	0.49
9	–	cimetidine	6.92±0.25	0.25±0.05	1.35
10	– [a]	domperidone HCl	22.31±0.30	3.33±0.20	3.27
11	–	ebastine	precipitates	2.78±0.10	4.68
12	–	5-fluorouracil	1.59±0.10	−0.91±0.5	−0.25
13	–	ftorafur	3.53±0.15	−0.36±0.5	0.76
14	–	furosemide	13.45±0.41	−1.54±0.10	1.56
15	–	5-hydroxytryptophane	1.19±0.29	−1.70±0.20	
16	–	iproniazid	5.43±0.14	0.35±0.10	0.91
17	–	metoclopramide	8.54±0.26	0.32±0.26	
18	–	metoprolol	9.30±0.10	0.16±0.05	1.64
19	–	metronidazole	3.21±0.31	0.14±0.04	0.77
20	–	phenelzine	5.90±0.07	−0.33±0.10	
21	–	pirenzepine HCl	7.12±0.25	−0.39±0.06	1.29
22	– [a]	terfenadine HCl	29.16±0.68	n.d.	4.22
23	–	tiapride	5.59±0.06	−0.29±0.05	
24	+	amitriptiline HCl	15.71±0.10	2.95±0.20	3.82
25	+	chlorpromazine HCl	15.98±0.15	2.82±0.15	4.30
26	+	clomipramine	15.38±0.22	2.76±0.25	4.19
27	+	clonidine HCl	4.54±0.20	0.85±0.10	1.62
28	+	desipramine HCl	16.03±0.60	1.30±0.10	3.27
29	+	doxepin	12.08±0.16	2.10±0.20	3.49
30	+	doxylamine succinate	8.84±0.07	0.65±0.05	1.83
31	+	estrone	precipitates	n.d.	3.48
32	+	fluoxetine HCl	14.48±0.35	1.95±0.16	3.25
33	+	flupentixol 2HCl	15.63±0.30	2.81±0.20	3.68
34	+	fluphenazine	15.49±0.04	3.48±0.25	3.67
35	+	haloperidole HCl	14.15±0.25	3.16±0.20	3.68
36	+	homochlorcyclizine	10.75±0.18	2.20±0.20	3.78
37	+	hydroxyzine 2HCl	18.17±0.20	2.37±0.10	3.22
38	+	ibuprofen	5.08±0.30	0.81±0.05	1.91
39	+	imipramine HCl	18.97±0.25	2.20±0.10	3.76
40	+	indomethacin	10.29±0.70	0.77±0.04	2.36
41	+	lidocaine HCl	9.77±0.10	1.88±0.10	2.49
42	+ [a]	loperamide HCl	18.70±0.52	n.d.	3.51
43	+	maprotiline	13.37±0.09	1.44±0.15	3.08
44	+	mequitazine HCl	13.32±0.12	2.50±0.18	3.69
45	+	minaprine	11.05±0.27	1.58±0.12	2.35
46	+	mefexamide	6.74±0.16	0.48±0.10	1.98
47	+	naltrexone HCl	7.70±0.10	0.90±0.05	2.49
48	+	naloxone	7.66±0.09	1.28±0.14	2.58
49	+	nortriptyline	14.26±0.16	1.69±0.20	3.24
50	+	perphenazine 2HCl	18.18±0.31	3.51±0.20	3.72
51	+	physostigmine	8.90±0.25	0.97±0.06	1.89
52	+	progesterone	10.77±0.58	n.d.	3.47
53	+	promazine HCl	14.58±0.35	2.52±0.15	3.79
54	+	promethazine HCl	13.82±0.48	2.60±0.12	3.81
55	+	propranolol HCl	14.21±0.10	1.20±0.06	2.71
56	+	protriptyline	13.91±0.21	1.36±0.10	2.81
57	+	pyrilamine	11.41±0.21	1.31±0.10	2.69
58	+	thioridazine HCl	19.00±0.35	3.55±0.22	4.66
59	+	tranylcypromine	5.13±0.14	0.69±0.10	1.55
60	+	trazodone	8.19±0.21	2.64±0.23	3.35
61	+	trifluoperazine	14.86±0.20	3.14±0.25	3.95
62	+	triflupromazine	14.86±0.20	2.96±0.24	4.16

Table 1 (*Continued*)

#	CNS	Compound	$N(CH_2)^{7.4}$	Log $D^{7.4}_{exp}$	Log $D^{7.4}_{HPLC}$
63	+	trimipramine	15.09±0.22	1.98±0.17	4.08

n.d., Not determined; CNS, − denotes compounds not capable to cross the BBB; +, denotes compounds capable to penetrate the barrier.
[a] CNS+ or CNS− classification of these compounds varies in Refs. [8–10].

measurements of lipophilicity and relative hydrophobicity with blood–brain permeability data from the literature, we follow the examples of Seelig et al. [8,9] and Crivori et al. [12] by classifying the compounds under study into those with brain-penetrating ability (CNS+) and those with little or no ability to cross the BBB (CNS−). We did not use the experimental log BB values available in the literature, since they were measured using different experimental procedures with unknown comparability, and many of them have large inter-animal variations [23]. The experimental log BB values were judged by Feher et al. [13] to be highly heterogeneous and of fairly poor quality.

Three compounds domperidone, loperamide and terfenadine are classified as CNS− compounds according to [8,9] although they may also be considered as BBB permeable but transported back by P-glycoprotein [10]. Based on our data domperidone and terfenadine are located in CNS− region, whereas loperamide falls in the CNS+ region. Loperamide was also classified as a CNS+ compound by the model developed by Crivori et al. [12].

Of the three analytical techniques employed in this work, no single measurement correlated with blood–brain permeability behaviour. Indeed, it is the combination of two parameters, $N(CH_2)^{7.40}$ and log $D^{7.40}_{exp}$ (or log $D^{7.4}_{HPLC}$) which when plotted against each other allows discrimination between CNS+ and CNS− compounds as shown in Fig. 1a and b. Lack of correlation between lipophilicity, expressed in terms of log $D^{7.40}_{exp}$ or log $D^{7.4}_{HPLC}$ and relative hydrophobicity, $N(CH_2)^{7.40}$, for the compounds examined confirmed our earlier assumption [14] that these descriptors represent different aspects of molecular structure.

It should be mentioned that there is a rather poor correlation between the lipophilicity values obtained by the octanol-buffer partitioning (log $D^{7.40}_{exp}$) and the HPLC method (log $D^{7.4}_{HPLC}$) which may be described as:

$$\log D^{7.4}_{HPLC} = 1.43(\pm 0.10) + 0.82(\pm 0.05) \log D^{7.40}_{exp} \quad (2)$$

$$n = 55;\ r = 0.9181;\ s = 0.5239;\ F = 284.256$$

where 95% confidence limits are given in parentheses; n is the number of compounds with both lipophilicity estimates determined; r, correlation coefficient; s, standard deviation; F, Fisher's test. Nevertheless, both techniques result in highly predictive equations when combined with $N(CH_2)$ (see below).

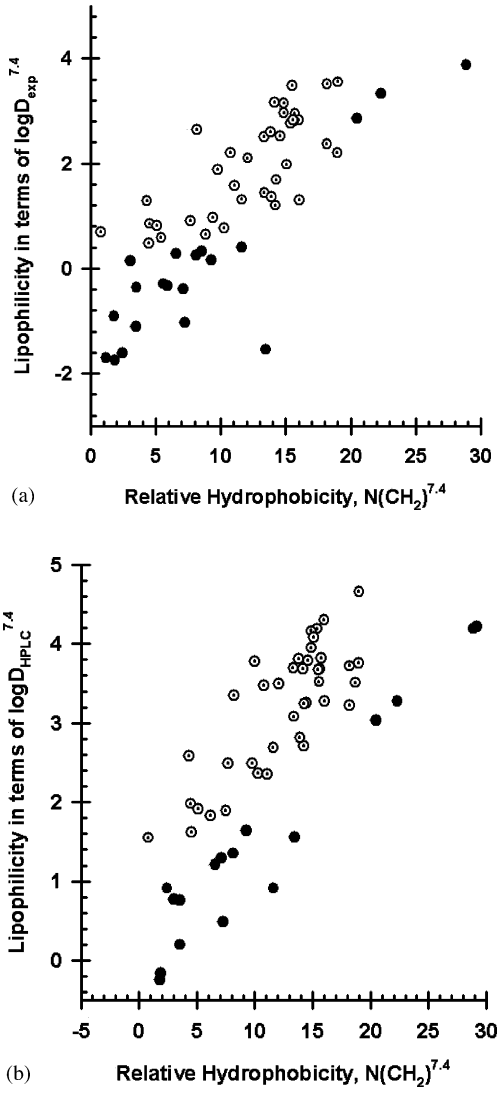

Fig. 1. (a) Lipophilicity expressed in terms of log $D^{7.40}_{exp}$ values plotted as a function of the relative hydrophobicity, $N(CH_2)$, at pH 7.4 for the compounds examined. (○) CNS+ compounds; (●) CNS− compounds. For assignment of compounds see Table 1. (b) Lipophilicity expressed in terms of chromatographic lipophilicity index, log $D^{7.40}_{HPLC}$ values plotted as a function of the relative hydrophobicity, $N(CH_2)$, at pH 7.4 for the compounds examined. (○) CNS+ compounds; (●) CNS− compounds. For assignment of compounds see Table 1.

To analyse the data for its ability to differentiate between BBB permeable (CNS = '+') and nonpermeable (CNS = '−') compounds, we used the following logistic regression model:

$$P(CNS = '+' \mid X) = \exp(b^T X)/(1 + \exp(b^T X))$$

or

$$\ln[\mathrm{P(CNS = `+' \mid } X)/(1 - \mathrm{P(CNS = `+' \mid } X))] = b^T X$$

where $b^T X = b_0 + b_1 X_1 + \ldots + b_k X_k$ is the linear combination of potential covariates $X_1, \ldots, X_k$ with coefficients $b_1, \ldots, b_k$, and b_0 is the intercept. The covariates included variables $N(CH_2)$, $\log(D_{exp})$, $\log D^{7.40}_{HPLC}$, and their interactions. Since the covariates were somewhat correlated, we considered the possibility that the final model could include only their subset, i.e., some of the regression coefficients could be specified as zeros. The potential subsets were fit using the penalised maximum likelihood approach to avoid the problem of complete separation in logistic regression [24]. The best subset of the covariates was found by comparing possible subsets with respect to the penalised likelihood ratio test (nested models) or the information criteria (non-nested models).

The prediction equation using $\log D_{exp}$ and its interaction with $N(CH_2)$ is as follows:

$$\ln[\mathrm{P(CNS = `+')}/(1 - \mathrm{P(CNS = `+')})] = -7.90 + 24.91 \log D_{exp} - 1.10 \log D_{exp} \times N(CH_2),$$

with concordance rate (the number of compounds where the predicted probabilities of CNS = '+' or CNS = '−' led to correct results) of 100% and $P < 0.0001$. Since the concordance rate was calculated for the same data that were used to fit the model there was a possibility of overfitting. To check this we used the cross-validation approach by refitting the model to all the compounds but one and predicting the results for the 1 compound that was left out. By repeating this procedure for each compound, we calculated the percentage of cases where the predicted value for CNS to be '+' or '−' corresponded to the actual value. The concordance rate dropped to 96.6%. An even better model included the $\log D^{7.40}_{HPLC}$ and its interaction with $N(CH_2)$. The prediction equation is as follows:

$$\ln[\mathrm{P(CNS = `+')}/(1 - \mathrm{P(CNS = `+')})] = -15.04 + 12.56 \log D_{HPLC} - 0.40 \log D_{HPLC} \times N(CH_2),$$

According to the model, for each fixed value of $N(CH_2) = c$, increasing the value of $\log D^{7.40}_{HPLC}$ by one unit increases the odds ratio, i.e., the probability to have CNS = '+' over the probability that CNS = '−', by $\exp(12.56 - 0.40c)$.

It seems that the $\log D^{7.40}_{HPLC}$-value represents the compound lipophilicity in regard to the BBB crossing better than the $\log D^{7.40}_{exp}$-value. This observation agrees with the suggestion [25,26] that the molecular mechanism and thermodynamics of reversed phase chromatographic partitioning mimic partitioning into a biological membrane better than octanol–water partitioning.

An explanation for the ability of the combination of the two descriptors, lipophilicity and relative hydrophobicity, to differentiate between CNS+ and CNS− compounds follows from the physicochemical bases of the descriptors. Lipophilicity, measured as $\log D_{exp}$ or $\log D_{HPLC}$, represents the relative affinity of different compounds for the nonaqueous environment, which is commonly viewed as simulating the environment in a biological membrane. The more lipophilic a given compound is, the greater its affinity for the membrane. Once distributed into the membrane, the compound may distribute further into one or the other of the two aqueous phases separated by the membrane, such as extracellular and intracellular media, for example, or interstitial fluid in brain and blood plasma. Distribution of a compound into one or the other aqueous phase from the biological membrane may depend upon its relative affinity for these two phases as well as on the presence of specific receptors in these phases. The relative hydrophobicity of a compound, $N(CH_2)^{7.4}$, represents the relative affinity of the compound for two aqueous media of different composition [18].

It seems that moderate levels of both relative hydrophobicity and lipophilicity values are required for compounds to be able to cross the BBB; more lipophilic compounds must be more sensitive toward their aqueous environment to cross the BBB. By integrating both types of partitioning data, we were able to discriminate this behaviour. We suggest that the combination of the relative affinity of a compound for a cellular membrane (represented by log D/log P-value) and that for the different aqueous media in blood and in interstitial fluid in brain (represented by $N(CH_2)$-value) may be predictive of the ability of a compound to permeate the BBB by passive diffusion.

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