A Novel and Useful Descriptor for Hydrophobicity, Partition Coefficient Micellar–Water, and Its Application to a QSAR Study of Antiplatelet Agents

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Herein we describe the introduction and application of a novel and useful descriptor, logarithm of micelle–water partition coefficients (log P_{mw}), for hydrophobicity of compounds. A QSAR study using log P_{mw} on antiplatelet activities *ex vivo* of 2-substituted phenyl and benzimidazolyl-5-methyl-4-(3-pyridyl)imidazoles showed its usefulness. Antiplatelet activities could be rationalized by means of only log P_{mw} with an excellent correlation ($r = 0.772$, $s = 0.257$, $F = 11.07$ **, $n = 18$), while use of other descriptors such as CLOGP values and retention factors (k') failed. P_{mw} values can easily be obtained using a normal HPLC system using a micelle aqueous solution as the mobile phase. HPLC techniques offer the advantage of tolerating lower sample purity, smaller sample size, and being dynamic range, compared with the flask-shaking method for measurement of logarithm of 1-octanol/water partition coefficients (log P), which is widely used in QSAR study. These indicated that log P_{mw} could be a useful descriptor for hydrophobicity of compounds.

Introduction

The hydrophobic character of a bioactive compound strongly affects its *in vivo* potency after oral administration since it is related to the distribution of the compound among body fluid compartments, lipid-rich phases, tissue proteins, etc.; thus, quantitation of hydrophobicity of compounds is of importance in drug design studies exemplified by the Hansch–Fujita quantitative structure–activity relationship (QSAR) method. The logarithm of partition coefficients in the biphasic solvent system of 1-octanol–water (log P) and hydrophobic substituent constant π values are widely used as an index for hydrophobicity.[1] However, the measurement of log P is still a tedious and sometimes troublesome task in spite of numerous efforts by many workers. The flask-shaking method may be the most popular method for measuring log P, but it has still several disadvantages such as requiring a pure and large sample size (100 mg to 1 g), being time-consuming, having limited dynamic range, and requiring special equipment. Application of π values is also limited since the parameter was originally defined for benzene derivatives, and its precision is not good for heterocyclic compounds and compounds having complicated and/or adjacent substituents.

Recently one of the authors has reported the superiority of using a liposome–water partition system in evaluating hydrophobic partition coefficients for QSAR analyses.[2,3] The liposome–water system is well-recognized as an excellent model of the membrane–water system *in vivo*, but this system is rather difficult to treat routinely in QSAR analyses. Therefore, a micelle/water partition system was selected in the present study. Micelle–water partition coefficients (P_{mw}) have been shown to be extracted by micelle chromatography (high-performance liquid chromatography (HPLC) using micelle aqueous solution as mobile phase).[4] For determination of P_{mw}, retention times are measured using a usual HPLC system at various concentrations of micelle in the aqueous mobile phase. HPLC techniques offer the advantages of needing lower sample purity, smaller sample size, and being dynamic range, compared with the flask-shaking method for log P measurement. The retention factor k' in the micelle–water system has been reported as a parameter for quantitative analysis of the biological activity of 4-substituted phenols, but not discussed with P_{mw}.[5]

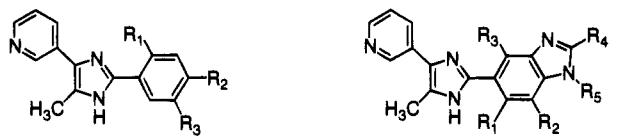

Figure 1. Structure of antiplatelet agents described in this paper. (a) 2-Substituted phenyl-5-methyl-4-(3-pyridyl)imidazoles. (b) 2-Substituted benzimidazolyl-5-methyl-4-(3-pyridyl)-imidazoles.

We recently reported the synthesis, antiplatelet activities with vasodilation, and structure–activity relationships of 2-substituted phenyl and benzimidazolyl-5-methyl-4-(3-pyridyl)imidazoles (Figure 1).[6] In continuous efforts to find useful antiplatelet agents, we have attempted some QSAR trials of those imidazoles using several indices such as retention time under usual conditions (eluents are water and acetonitrile). However, no good correlation with the antiplatelet activity could be offered.

We herein describe the application of the logarithm of P_{mw} to QSAR study of the imidazoles and demonstrate its effectiveness as a hydrophobic index for rationalizing antiplatelet activity.

Experimental Section

Apparatus. A Shimadzu gradient liquid chromatography system (LC-9A system) incorporating SPD-6A as detector and C-R5A chromatopac as calculator was used.

Reagents and Procedures. Polyoxyethylene(23) lauryl ether (Brij35, Katayama Chemical Co.) was used as micelle

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Table 1. Antiplatelet Activity and Physicochemical Data of 2-Aryl-5-methyl-4-(3-pyridyl)imidazoles

compd[a]	R_1	R_2	R_3	R_4	R_5	MW[b]	anti-platelet activity[c] %	anti-platelet activity[c] log(APA)[d]	log P_{mw}[e]	retention time (min)[f] 25	30	35	40	regression data[g] A	B	r	CLOGP[h]
2-Substituted Phenyl-5-methyl-4-(3-pyridyl)imidazoles																	
1	OCH_3	NHAc	Cl			357	65	1.4	2.04	7.95	7.01	6.32	5.78	4.12	3.21×10^{-2}	0.999	1.72
2	F	H	H			252	98	1.4	1.79	8.75	7.84	7.13	6.57	3.12	4.41×10^{-2}	1.000	3.04
3	NH_2	H	H			250	25	0.80	1.36	3.58	3.37	3.21	3.07	5.72	0.219	0.999	2.04
4	OCH_3	NHAc	NO_2			367	86	1.5	2.11	10.35	9.08	8.08	7.33	3.20	2.10×10^{-2}	1.000	1.29
5	CH_3	H	H			249	6	0.18	1.37	5.20	4.92	4.64	4.39	3.54	0.134	0.999	3.59
6	OCH_3	H	H			265	49	1.1	1.51	4.59	4.21	3.97	3.76	5.08	0.136	0.997	2.54
7	OEt	H	H			279	45	1.1	1.60	6.01	5.44	5.08	4.77	4.04	8.77×10^{-2}	0.997	3.07
8	OCH_2COOEt	H	H			337	12	0.60	2.40	9.21	7.12	6.57	6.18	4.32	1.46×10^{-2}	0.961	2.69
9	SCH_3	H	H			281	32	0.95	1.60	7.65	7.01	6.46	6.00	3.06	6.53×10^{-2}	1.000	3.76
10	$NHCONHCH_3$	H	H			307	46	1.1	1.62	6.34	5.78	5.35	5.00	3.88	7.94×10^{-2}	0.999	2.24
11	$NHCOOCH_3$	H	H			308	73	1.4	1.72	8.79	7.94	7.27	6.70	2.92	4.81×10^{-2}	1.000	2.84
12	Cl	H	H			270	9	0.38	1.68	8.63	7.83	7.17	6.64	2.88	5.19×10^{-2}	1.000	3.56
13	OCH_3	OCH_3	NHAc			352	24	0.93	1.35	2.75	2.60	2.50	2.41	8.20	0.325	0.998	1.22
2-Substituted Benzimidazolyl-5-methyl-4-(3-pyridyl)imidazoles																	
14	OCH_3	H	H	CH_3	H	319	21	0.83	1.34	2.23	2.16	2.06	1.99	11.3	0.455	0.997	2.55
15	OCH_3	Cl	H	CH_3	H	354	63	1.4	1.61	4.69	4.36	4.05	3.79	5.42	0.117	0.999	3.12
16	CH_3	H	H	CH_3	H	303	18	0.74	0.94	1.300	1.294	1.280	1.271	17.4	2.25	0.989	3.28
17	OCH_3	Cl	H	OH	H	356	17	0.79	0.81	1.320	1.301	1.296	1.289	14.4	2.23	0.953	2.39
18	Cl	H	H	CH_3	H	324	15	0.68	1.49	3.56	3.41	3.19	3.00	6.74	0.190	0.993	3.31
19	H	H	OCH_3	CH_3	H	319	16	0.72	1.40	2.79	2.70	2.56	2.43	8.44	0.297	0.991	2.55
20	H	Cl	OCH_3	CH_3	H	354	51	1.3	2.25	9.36	8.14	7.24	6.52	3.80	1.80×10^{-2}	1.000	3.28
21	H	Cl	OCH_3	A[i]	H	412	6	0.40	1.47	3.68	3.47	3.27	3.10	6.34	0.188	1.000	2.35
22	OCH_3	H	H	OH	CH_3	35	11	0.57	0.95	1.375	1.362	1.345	1.331	17.0	1.80	0.998	2.14

[a] See Figure 1. [b] MW: molecular weight. [c] Inhibitory activities on rat platelet aggregation induced by collagen *ex vivo* were measured after 1 h of oral administration of each compounds (10 mg/kg). [d] Antiplatelet activities (APA) were obtained by conversion of the above original antiplatelet activity data (%) to antiplatelet activity values at 10 μmol/kg. [e] log P_{mw} values were obtained according to the eq 3. [f] Retention time values were observed at 25, 30, 35, and 40 mM Brij35. [g] A and B values in the eq 2 for each compound were determined by regression analysis. (r: correlation coefficient). [h] The CLOGP (version 4.34) values were calculated using Corwin/Leo's software version 3.43. [i] A; CH_2CH_2COOH.

component. Brij35, which affords a neutral type of micelle in an aqueous solution, is suitable for determination of all kinds of compounds, since ionic types of micelle such as tetradecyltrimethylammonium bromide (cation type, $C_{14}TAB$) and sodium dodecyl sulfate (anion type, SDS) tightly bind with counterionic solutes, resulting in apparently very high partition coefficients. Stock solutions of Brij35 in phosphate buffer (PB, 10 mM, pH 4.01) and 10 mM PB (pH 4.01) were prepared in deionized water and were filtered through a 0.45 μm Cellulose ester membrane filter (HA type, Millipore Corp.). All compounds used in this paper were prepared in-house. The analytical column was a YMC-Pack C8 (A-201-10, S-10 μm, 120 A, 4.6 × 100 mm) from YMC Co., Ltd. All experiments were carried out at room temperature (22–25 °C). Retention time of $NaNO_3$ (0.949 min) was used as a dead retention time (t_0). Retention times of each compound were measured under 25, 30, 35, 40 mM of Brij35.

The relationship between retention factor k' and P_{mw} is represented by the following equation[7]

$$1/k' = [(P_{mw} - 1)V/(P_{sw}\Phi)]C_m + 1/(P_{sw}\Phi) \quad (1)$$

$$k' = (t - t_0)/t_0$$

where V is partial molar volume (V = 1.18 L/mol for Brij35), P_{sw} is partition coefficient between stationary phase and water, C_m is concentration of micelle in the mobile phase, and Φ is chromatographic phase ratio. Equation 1 could be written more simply as

$$1/k' = AC_m + B \quad (2)$$

where $A = (P_{mw} - 1)V/P_{sw}$ and $B = 1/(P_{sw}\Phi)$. The A and B values were determined by regression analysis of eq 2 using observed data (C_m and k'), where the calculation was done on the Cricket Graph program for correlation coefficient (r) and the MR2-8 in MVA package program[8] for F test and standard deviation (SD).

The P_{mw} values were estimated by

$$P_{mw} = A/(BV) + 1 \quad (3)$$

CLOGP values were calculated using Corwin/Leo's CLOGP software Version 4.34 by DAYLIGHT Chemical Information Systems, Inc.

All antiplatelet activities used in this paper were quoted from our previous papers.[6] However, these antiplatelet activities were observed as inhibition percentage (%) of control after 1 h of oral administration of 10 mg/kg (po).[9] In order to treat these biological data on a mole scale, we converted the inhibitory activities of compounds to those at 10 μmol/kg (po) doses (Table 1); therefore, we carried out QSAR studies about the relationship between log P_{mw} and logarithm of antiplatelet activity at a dose of 10 μmol/kg (po) [log(APA)].

Results and Discussion

Structure, antiplatelet activities (in rats), and log P_{mw} of imidazoles used in this study are shown in Table 1 with CLOGP values, observed HPLC data, and regression data for determination of logPmw.

Regression analyses between $1/k'$ and C_m were carried out to determine A and B values in eq 3 and showed good proportionality for all compounds with high correlation coefficients ($r > 0.95$) as shown in Table 1.

A plot between observed log P_{mw} values with log(APA) (Figure 2) showed that compounds **16**, **17**, and **22** are too hydrophilic (log $P_{mw} < 1.0$) to show *ex vivo* activity, and the relationship of other compounds and antiplatelet activities looks like a quadratic curve. A regression calculation except for the noted three compounds (**16**, **17**, and **22**) resulted in

$$\log(\text{APA}) = -2.06(\pm 1.73)(\log P_{mw})^2 + 8.03(\pm 6.35)(\log P_{mw}) - 6.51(\pm 5.64) \quad (4)$$

$$r = 0.632, s = 0.325, F = 5.32\ *, n = 19$$

The correlation coefficient (r = 0.632) is not very high but is encouraging for further treatment. In Figure 2, a compound having chlorine atom at the 2-position of

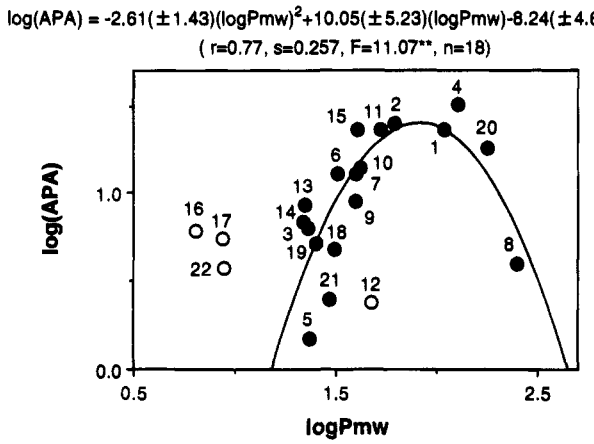

Figure 2. A plot of logarithm of antiplatelet activities *ex vivo* (log(APA) with logPmw values. Correlation coefficient value (0.772) is obtained except for compounds **12**, **16**, **17**, and **22** (see text).

phenyl ring (compound **12**) deviates from the regression curve, which indicated that the log P_{mw} value of compound **12** (1.68) is a little large compared with a presumed value (ca. 1.4). We consider that the reason for the hyper-estimated hydrophobicity of compound **12** is that the population of the ionized imidazole moiety, the soluble form in an aqueous solution, of the compound **12** is lower than that of the other compounds due to the electron-withdrawing property of the chlorine atom[10] and its large size, which caused the low hydrophilic property. Thus, we carried out another regression analysis omitting compound **12**, therefore studying 18 compounds, which resulted in

$$\log(\mathrm{APA}) = -2.61(\pm1.43)(\log P_{mw})^2 + 10.05(\pm5.23)(\log P_{mw}) - 8.24(\pm4.64)$$
$$= -2.6(\log P_{mw} - 1.9)^2 + 1.3 \quad (5)$$
$$r = 0.772,\ s = 0.257,\ F = 11.07\ **,\ n = 18$$

Omitting compound **12** gave a better regression (eq 5), while all constants were very similar to eq 4, as expected. Equation 5 indicated that a compound having a 1.9 log P_{mw} value can exert the maximum antiplatelet activity. This simulation result agrees with the experimental data; that is, compounds **4**, **1**, **2**, **11**, and **15** whose log P_{mw} value are 2.11, 2.04, 1.79, 1.72, and 1.61, respectively, are near to the 1.9 log P_{mw} value, and all these compounds exhibited the most potent antiplatelet activity while no compound whose log P_{mw} is far from the 1.9 log P_{mw} is potent. The good correlation coefficient ($r = 0.772$, $s = 0.257$, $F = 11.07$ **, $n = 18$) in eq 5 is amazing since it showed that the antiplatelet activity could be explained by only one parameter (log P_{mw}) while the antiplatelet activity value includes all kinds of factors such as *in vitro* potency, absorption, metabolism, distribution volume, and excretion after oral administration.

For comparison, we carried out a QSAR study using CLOGP and k' in 25 mM Brij35 as hydrophobic index. The CLOGP is widely used to estimate hydrophobicity of the compound in drug design because of its accuracy for simple compounds such as benzene derivatives and easiness to calculate by computer. A plot of antiplatelet activities with CLOGP and k' values showed that the

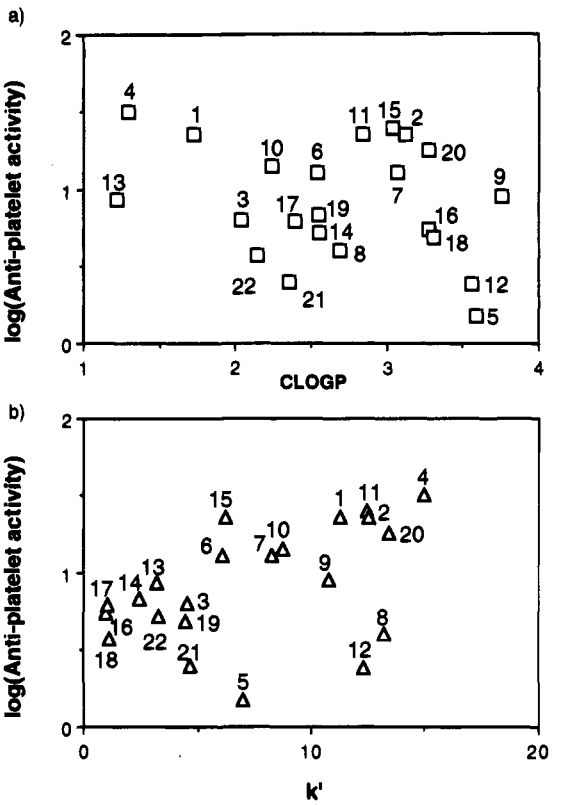

Figure 3. Plots of logarithm of antiplatelet activities (log(APA)) with (a) CLOGP values and (b) retention factors (k'). k' values were measured under the following HPLC conditions. Column: YMC-pack C8, 4.6 × 100 mm. Eluents: 25 mM Brij35 in 10 mM PB (pH 4.01), flow 1 mL/min. Detection: 254 nm.

antiplatelet activity has no relationship with them (Figure 3), while the k' was reported as a good descriptor for explanation of bioactivity of simple compounds such as 4-substituted phenols.[5]

Conclusion

We here introduced a novel and useful descriptor, log P_{mw}, for hydrophobicity of compounds. A QSAR study using log P_{mw} on antiplatelet activities *ex vivo* of 2-substituted phenyl and benzimidazolyl-5-methyl-4-(3-pyridyl)imidazoles demonstrated the usefulness; that is, the antiplatelet activities can be explained by means of only log P_{mw} with an excellent relationship ($r = 0.772$, $s = 0.257$, $F = 11.07$ **, $n = 18$), while other descriptors such as CLOGP values and retention factors (k') had no relationship. P_{mw} values can easily be extracted by a usual HPLC system using a micelle aqueous solution as the mobile phase. HPLC techniques offer several advantages of needing lower sample purity, smaller sample size, and being dynamic range, compared with the flask-shaking method for log P measurement. These advantages are very important for medicinal chemists since they generally synthesize a variety and small amount of compounds in a restricted short term to obtain useful drug as soon as possible. Thus log P_{mw} could be a useful and novel descriptor for hydrophobicity of compounds.

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(7) The original equation for correlation of k' and C_m is

$$1/k' = [(P_{mw} - 1)V/(P_{sw}\Phi)][C_m - \text{cmc}] + 1/(P_{sw}\Phi) \qquad (6)$$

where cmc is critical micellar concentration. In eq 6, the $[C_m - \text{cmc}]$ term stands for real concentration of micelle. The cmc is, however, only 0.1 mM for the Brij35; therefore, the $[C_m - \text{cmc}]$ can be replaced with C_m in this study.

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(9) Antiplatelet activity values were obtained according to the following procedure: Male Sprague–Dawley rats weighing about 250 g were used after overnight fasting. One hour after oral administration of the test compound (32 mg/kg or 10 mg/kg) or vehicle (control), blood was collected into a tube containing 0.1 volume of 3.8% sodium citrate. To 0.45 mL of blood, 0.05 mL of collagen (final concentration 5.0 (μg/mL) was added, and the mixture was incubated for 5 min at 37 °C under shaking. The reaction was terminated by addition of 1 mL of 10 mM phosphate-buffered saline (pH 7.4) containing 11.5 mM *N,N,N',N'*-tetrakis(carboxymethyl)-1,2-diaminoethane and 1% formalin. The reaction mixture was centrifuged at 70*g* for 5 min, and the platelet count of the upper phase was measured with a Platelet Analyzer 810 (Backer Instruments). Platelet aggregation was calculated according to the following formula:

$$\text{platelet aggregation (\%)} = (A - B)/A \times 100$$

A: platelet count after addition of vehicle

B: platelet count after addition of collagen

$$\text{inhibition (\%)} = (C - D)/C \times 100$$

C: platelet aggregation (%) of control

D: platelet aggregation (%) of test compound

(10) The similar effects of chlorine atom as ortho substituent was observed in effects on pK_a of 2-chlorophenol, that is, pK_a of 2-chlorophenol was 8.81[11] while that of 4-chlorophenol and phenol were 9.38[11] and 9.98,[12] respectively.

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