Quantitative Structure−Activity Relationship Study of Fibrinogen Inhibitors, [[4-(4-Amidinophenoxy)butanoyl]aspartyl]valine (FK633) Derivatives, Using a Novel Hydrophobic Descriptor

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We recently reported a novel hydrophobic descriptor for quantitative structure−activity relationship (QSAR) studies, the logarithm of the partition coefficient micelle/water (log P_{mw}), which is easily determined by a HPLC system and is thought to be a descriptor for a compound's affinity to a biomembrane. We carried out QSAR studies using log P_{mw} on the antiplatelet activities of novel fibrinogen inhibitors, [[4-(4-amidinophenoxy)butanoyl]aspartyl]valine (FK633) derivatives, which resulted in a quadratic curve with a good correlation coefficient ($n = 12$, $s = 0.368$, $F = 14.1$**, $r = 0.871$), indicating that a suitable membrane affinity of the fibrinogen inhibitors is vital for their inhibitory activities. QSAR studies using STERIMOL parameters and/or CLOGP values were unsuccessful.

Introduction

Hydrophobic properties of bioactive compounds are generally thought to be important for their activity. The logarithm of the partition coefficient in the biphasic solvent system of 1-octanol/water (log *P*) may be the most common descriptor for a compound's hydrophobic property in a quantitative structure−activity relationship (QSAR) study.[1] The measurement of log *P* is, however, still a difficult task in spite of numerous efforts.[2] Recently, we reported a novel hydrophobic descriptor for QSAR studies, the logarithm of the partition coefficient micelle/water (log P_{mw}).[3] For measurement of log P_{mw}, the dependence of the retention time of the compound on micelle concentration in a micelle chromatography system (HPLC using micelle aqueous solution as mobile phase) is used; this method has several advantages such as small sample size, samples of high purity not required, no limited dynamic range, etc. Studies based on the structural similarity of 1-octanol to a biomembrane have been attempted because one important factor of a compound's hydrophobicity is thought to arise from that compound's affinity to a biomembrane. These studies, however, have not yet succeeded. On the other hand, the micelle/water system is recognized as a model of the biomembrane/water interface (Figure 1). Thus, log P_{mw} is thought to be able to describe a compound's affinity to a biomembrane and to be a useful hydrophobic descriptor in QSAR studies.

We thought to apply log P_{mw} to the QSAR study on our fibrinogen inhibitors, [[4-(4-amidinophenoxy)butanoyl]aspartyl]valine (FK633; Figure 2) derivatives,[4] since suitable hydrophobicity of these compounds seems to be important for possessing potent fibrinogen inhibitory activities. Uncontrolled platelet aggregation and platelet adhesion to the subendothelium of damaged blood vessels causes life-threatening diseases such as myocardial infarction,[5] transient ischemic attack,[5] and unstable angina.[6] Binding of a certain tripeptide in fibrinogen, ArgGlyAsp (RGD), to activated glycoprotein IIb/IIIa (GPIIb/IIIa) on the surface of a platelet is the final step in the platelet aggregation cascade and thought to be the most important step for platelet aggregation.[7] Thus, many fibrinogen inhibitors, RGD mimics, have been studied and reported to be clinically useful antiplatelet agents.[8] In particular, many structural studies on the RGD moiety of compounds by NMR and X-ray crystallography methods have been performed to understand the active conformation of the RGD moiety for rational drug design.[8,9] We have also designed and synthesized the FK633 derivatives from computer simulations based on our hypothesis in which the active conformation of the RGD moiety is a type II′ β-turn.[4]

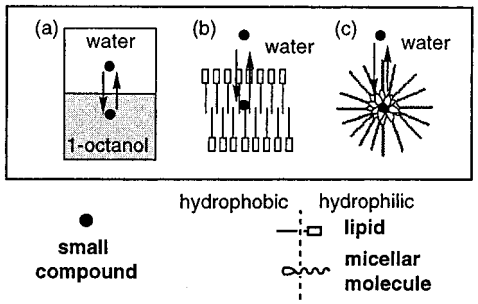

Figure 1. Partition of small compounds in (a) 1-octanol/water (log *P*), (b) membrane/water (*in vivo*), and (c) micelle/water (log P_{mw}) systems.

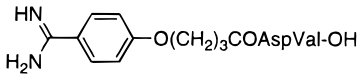

Figure 2. Structure of FK633 (**9**), a clinically studied fibrinogen inhibitor.

On the other hand, few studies have been reported on the relationship between the physical properties of these fibrinogen inhibitors and their antiplatelet activity although both structural and physical properties of bioactive compounds are generally important for possessing potent inhibitory activity. Thus, we carried out QSAR studies using log P_{mw}, CLOGP,[10] and STERIMOL

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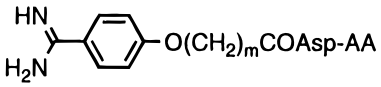
Table 1. Structures, Antiplatelet Activities, and log P_{mw} and CLOGP Values of Fibrinogen Inhibitors Studied in This Work



	structure		inhibitory		retention time (min)[b]				regression result			
	m	AA	activity[a] pIC_{50}	log P_{mw}	25 mM	30 mM	35 mM	40 mM	A^c	B^c	*r*	CLOGP[d]
9 (FK633)	3	ValOH	6.96	1.29	5.898	5.545	5.347	5.207	7.43	0.341	0.988	0.13
10	3	NleOH	7.12	1.58	11.11	10.21	9.491	8.943	4.63	0.107	0.999	0.79
11	3	IleOH	6.85	1.47	9.413	8.773	8.246	7.826	4.97	0.149	0.999	0.66
12	3	LeuOH	6.87	1.47	9.704	9.038	8.487	8.055	4.79	0.144	0.999	0.66
13	3	γ-Me-LeuOH	6.64	1.59	14.11	12.96	12.06	11.19	3.51	0.0791	0.999	1.06
14	3	Tyr(Me)OH	6.89	1.96	14.85	13.01	11.82	10.91	4.62	0.0435	0.998	0.54
15	3	TyrOH	6.80	1.74	10.54	9.51	8.79	8.23	5.86	0.0920	0.999	−0.05
16	4	SerOH	4.92	0.904	3.324	3.269	3.238	3.199	10.6	1.28	0.995	−1.34
17	4	ValOH	6.64	1.32	7.547	7.118	6.832	6.524	5.43	0.23	0.999	0.66
18	4	TyrOH	6.19	1.82	12.79	11.47	10.57	9.716	4.93	0.0647	0.999	0.48
19	4	Tyr(Me)OH	6.57	2.00	17.87	15.67	14.24	12.91	3.82	0.0325	0.999	1.07
20	4	TyrOMe	5.35	2.09	20.18	17.31	15.56	14.28	3.54	0.0243	0.997	0.62

[a] Inhibitory activities were quoted from ref 8: $pIC_{50} = -\log(IC_{50})$. [b] Retention time values were measured under the conditions described in the Experimetnal Section. [c] See eq 2. [d] CLOGP values were calculated using Corwin/Leo's CLOGP software version 4.34 by DAYLIGHT Chemical Information Systems, Inc.

parameters[11] on the antiplatelet activities of the FK633 derivatives to understand the relationship between their antiplatelet activity and physical properties. We herein describe measurement of log P_{mw}, results of QSAR studies on antiplatelet activity of the FK633 derivatives, and comparison of log P_{mw} and CLOGP.

log P_{mw} Measurement

Partition coefficient micelle/water (P_{mw}) can easily be determined by micelle chromatography (HPLC using micelle aqueous solution as mobile phase) as described below.[3]

Poly(oxyethylene)(23) lauryl ether (Brij35) was used as the micelle component. Brij35, which affords a neutral type of micelle in an aqueous solution, is suitable for determination of all kinds of compounds in several kinds of micelle components, but ionic types of micelle such as tetradecyltrimethylammonium bromide (C_{14}TAB, cation type) and sodium dodecyl sulfate (SDS, anion type) tightly bind with counterionic solutes, resulting in apparently very high partition coefficients.

The relationship between retention factor k' and P_{mw} is represented by the following equation:[12]

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

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

where t is retention time, V is partial molar volume of micelle component (V = 1.18 L/mol for Brij35[3]), P_{sw} is partition coefficient between stationary phase and water, C_m is concentration of micelle in the mobile phase, and F is chromatographic phase ratio. Equation 1 could be written more simply as the following equation:

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

where

$$A = [(P_{mw} - 1)V/(P_{sw}F)] \quad (3)$$

$$B = 1/(P_{sw}F) \quad (4)$$

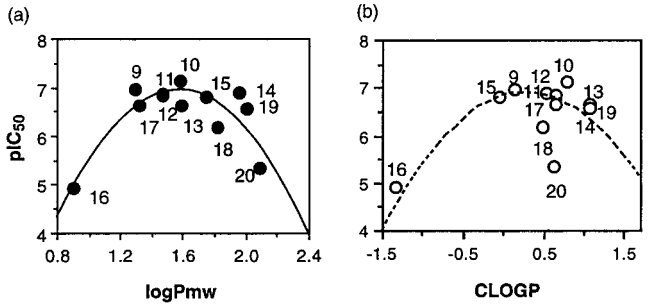


Figure 3. Plots of fibrinogen inhibitory activities (pIC_{50}) of compounds studied in this work with (a) log P_{mw} and (b) CLOGP. The dotted line in b stands for a presumable quadratic curve which was obtained from a statistical calculation on 10 compounds (excluding compounds **18** and **20**).

The A and B values were determined by regression analysis of eq 2 using the observed data (C_m and k').

The desired P_{mw} values were estimated from eqs 3 and 4 by the following equation:

$$P_{mw} = A(BV) + 1 \quad (5)$$

The retention time, A, B, and estimated log P_{mw} values in eq 1 of the fibrinogen inhibitors studied in this work with their structure and antiplatelet activities are summarized in Table 1. All A and B values are obtained with high correlation coefficients (r > 0.988, Table 1).

Results and Discussion

A plot of observed log P_{mw} values and antiplatelet activity (pIC_{50}) studied in this work is shown in Figure 3a, showing that this relationship looks like a quadratic curve. A statistical calculation on Figure 3a was carried out and resulted in:

$$pIC_{50} = -4.36(\pm 1.88)(\log P_{mw})^2 + 13.7(\pm 5.85)(\log P_{mw}) - 3.84(\pm 4.42) \quad (6)$$

$$= -4.36(\log P_{mw} - 1.57)^2 + 6.93 \quad (7)$$

$$n = 12,\ s = 0.368,\ F = 14.1^{**},\ r = 0.871$$

The values in parentheses in eq 6 indicate 95% confidence intervals. The good correlation coefficient (s =

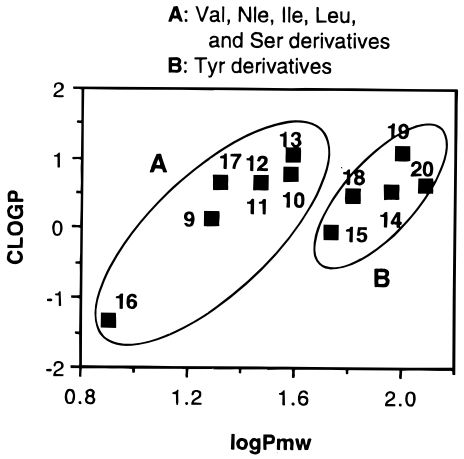


Figure 4. Plot of log P_{mw} and CLOGP values of compounds studied in this work.

0.368, $F = 14.1$**, $r = 0.871$) in eq 6 showed that the antiplatelet activities of the fibrinogen inhibitors (pIC_{50}) depend on their log P_{mw} values. Equation 7 indicates that a compound having a 1.57 log P_{mw} value exerts the maximum antiplatelet activity, which agrees with the experimental results; that is, the most potent compound **10**, whose log P_{mw} value is 1.58, is the one nearest to the 1.57 log P_{mw} value, and all compounds having suitable log P_{mw} values (1.3−1.7) such as compounds **9**−**13**, **15**, and **17** showed potent antiplatelet activity, while compounds whose log P_{mw} is far from the 1.57 log P_{mw} value are not potent (compounds **16** and **20**).

We carried out other QSAR studies on the antiplatelet activities using STERIMOL parameters and CLOGP values. QSAR studies using STERIMOL parameters, descriptors for molecular shape of compounds, were unsuccessful (data not shown), which indicates that the shape of the AA moiety is not the most important property for antiplatelet activity. Next, we performed a QSAR study using CLOGP values which is widely used as a parameter for hydrophobicity because of its accuracy and easiness to calculate by computer.[13] A plot of the antiplatelet activities and CLOGP, however, resulted in a poor relationship (Figure 3b). In Figure 3b, compounds **18** and **20** are far from a presumable quadratic curve (dotted line in Figure 3b), and these hydrophobicities appear to be underestimated. These QSAR results can be thought to indicate than an important factor of the AA moiety for their inhibitory activity is not just their molecular shape (STERIMOL) or simple hydrophobicity (CLOGP) but also their membrane affinity (log P_{mw}). Further structure−activity relationships and QSAR results of other FK633 derivatives will be reported in the future.

Finally, we compared the two hydrophobic descriptors log P_{mw} and CLOGP. A plot of log P_{mw} and CLOGP resulted in two groups as shown in Figure 4; that is, one involves compounds having only aliphatic side chains at their C-terminal such as Val, Leu, and Ser derivatives, and another consists of only Tyr derivatives having aromatic side chains. In both groups the log P_{mw} values appear to be directly proportional to CLOGP values, but the log P_{mw} values of aromatic derivatives are larger than those of CLOGP, which indicates that CLOGP tends to underestimate the hydrophobicity of aromatic moieties compared with those log P_{mw} values. This difference resulted in the underestimation of hydrophobicity of compounds **18** and **20** (Tyr derivatives) and a poor correlation coefficient in Figure 3b.

Conclusion

A QSAR study was successfully applied using a novel hydrophobic descriptor, the logarithm of the partition coefficient micelle/water (log P_{mw}), on novel fibrinogen inhibitors, [[4-(4-amidinophenoxy)butanoyl]aspartyl]-valine (FK633) derivatives, resulting in a good relationship ($n = 12$, $s = 0.368$, $F = 14.1$**, $r = 0.871$, Figure 3a). On the other hand, QSAR studies using STERIMOL parameters, descriptors for molecular shape, and CLOGP, a common parameter for a compound's hydrophobicity, were unsuccessful. The difference between the parameters for hydrophobicity, log P_{mw} and CLOGP, arose from estimation of the hydrophobicity of the aromatic moieties; that is, CLOGP tends to underestimate hydrophobicity of aromatic moieties compared to log P_{mw} in this work (Figure 4). This underestimation of the aromatic moiety by CLOGP resulted in a poor correlation coefficient in Figure 3b, while a QSAR study using log P_{mw} gave a good relationship. This indicates that log P_{mw} is a useful candidate as a QSAR descriptor and that a suitable membrane affinity of the fibrinogen inhibitors is vital for activity.

Experimental Section

All antiplatelet activities used this paper are quoted from our previous paper.[4] The synthesis of these compounds was also described in the previous paper.[4]

Measurement of log P_{mw}. A Shimazu gradient liquid chromatography system (LC-9A system) incorporating SPD-6A as a detector and C-R5A chromatopac as calculator were used. Stock solutions of Brij35 in 0.1% TFA aqueous solution (pH 2.37) were prepared in deionized water and filtered through a 0.45 μm cellulose ester membrane filter (HA type, Millipore Corp.). The analytical column was TSK-gel ODS-80TM (5 μm, 120 A, 4.6 × 150 mm) from TOSOH Co., Ltd. The flow rate was 0.7 mL/min. All experiments were carried out at room temperature (22−25 °C). Retention time of $NaNO_3$ (2.016 min) was used as a dead retention time (t_0). Retention time of each compound was measured under 25, 30, 35, and 40 mM Brij35. Statistical calculations were done using MR2-8 in the MVA package program.[14]

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(12) (a) The original equation[12b] for correlation of k' and C_m is $1/k' = [(P_{mw} - 1)V/(P_{sw}\Phi)][C_m - \text{cmc}] + 1/(P_{sw}\Phi)$, where cmc is critical micellar concentration. In the above equation, the $[C_m - \text{cmc}]$ term stands for real concentration of micelle. The cmc is, however, only 0.1 mM for Brij35;[3] therefore the $[C_m - \text{cmc}]$ can be replaced with C_m. (b) Armstrong, D. W. Micelles in Separation: A Practical and Theoretical Review. *Separat. Purif. Methods* **1985**, *14*, 213−304.
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