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QSAR Study of the Peptidic Fibrinogen Inhibitors FK633, FR158999 and Related Derivatives, Using a Novel and Useful Hydrophobic Descriptor (logPmw)

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Abstract: A QSAR study using the novel hydrophobic descriptor (logPmw), which is a descriptor for membrane affinity, of our fibrinogen inhibitors FK633 (**1**), FR158999 (**21**), and related derivatives was performed, and resulted in good correlation (n=19, s=0.268, F=6.38**, r=0.667). Based on these results, we constructed a hypothesis by which these potent inhibitors bind to the receptor via the biomembrane and the C-terminal moiety functions as an anchor moiety.

A number of different descriptors are used in quantitative structure-activity relationship (QSAR) studies to reflect the hydrophobic properties of compounds.[1] Physical properties such as hydrophobicity are generally very important for a molecule in order to exhibit potent activities, probably because they are directly related to uptake and transportation of drugs in biological systems. For example, the logarithm of the partition coefficient in the biphasic solvent system of 1-octanol/water, logP[1] and CLOGP[2], are widely used. The measurement of logP is, however, still a difficult task,[3] and its physiological significance is not clear because 1-octanol, which is usually used as the organic phase for the measurement of logP, has not been established to have structural similarity with bio-membranes. Therefore, we have introduced a novel hydrophobic descriptor, the logarithm of the partition coefficient micelle/water (logPmw), which is thought to represent the membrane affinity of a compound, since micelles are structurally similar to bio-membranes, and we have already demonstrated its usefulness in previous papers.[4,5] This novel hydrophobic descriptor can be easily extracted by micelle chromatography (high-performance liquid chromatography (HPLC) using a micelle aqueous solution as the mobile phase), and affords several practical advantages such as small sample size, no requirement for high purity, and lower limitations in the dynamic range, among others.

We have now applied logPmw to a QSAR study of our fibrinogen inhibitors,[5] since a limited range of hydrophobicity was found to be required for these compounds to possess potent activities. Many fibrinogen inhibitors have been studied and reported in this decade, since the binding of fibrinogen to activated glycoprotein IIb/IIIa (GPIIb/IIIa) on the surface of a platelet is the final step, and one of the most important, in the platelet aggregation cascade.[7] In two previous papers, we reported the design, synthesis, and evaluation of two types of novel fibrinogen inhibitors.[6,8] One was designed based on the results of computer simulations,[6] and resulted in a clinically studied compound (FK633, **1**), and derivatives (compounds **1**–**12**, Table 1) possessing a carboxyl group at the carboxy terminus (C-terminal). The other was developed as an orally active analog since the antiplatelet activities of FK633 are not sufficient after oral administration, and an orally active drug is necessary for certain indications, and resulted in 4-[[(4-amidinophenoxy)butanoyl]aspartyl]valyl-thiomorpholine 1,1-dioxide (FR158999, **21**) and its derivatives (compounds **13**–**21**, Table 1).[8] FR158999 derivatives have a carbamoyl group or hydrogen atom at their C-terminal position.

QSAR study of the antiplatelet activities of FK633 derivatives using logPmw, CLOGP and STERIMOL

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parameters showed that only logPmw has a good relationship with the inhibitory activities (pIC50) in a quadratic curve (n=12, s=0.368, F=14.1**, r=0.871), while the other parameters gave poor results.[5] We herein describe further QSAR results on our fibrinogen inhibitors, including FK633 and FR158999 derivatives (compounds **1**–**21**), and a hypothesis about the binding route from solution state to binding state, by which potent inhibitors bind to the receptor via the biomembrane and the C-terminal moiety functions as an anchor.

LogPmw Measurement

Partition coefficient micelle/water (Pmw) was determined by micelle chromatography as described below.[4] The relationship between retention factor k' and Pmw is represented by the following equation:

$$1/k' = [(Pmw-1)V/(Psw \times F)] \times Cm + 1/(Psw \times F) \quad (1)$$

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

where t is retention time, V is partial molar volume of micelle component (V=1.18 l/mol[4] for polyoxyethylene (23) lauryl ether (Brij35) which was used as micelle component in this study), Psw is partition coefficient between stationary phase and water, Cm is concentration of micelle in the mobile phase, and F is chromatographic phase ratio. Equation 1 can be written more simply in the following form:

$$1/k' = A \times Cm + B \quad (2)$$

where

$$A=[(Pmw-1)V/(Psw \times F)] \quad (3)$$

$$B=1/(Psw \times F) \quad (4)$$

A and B values were determined by regression analysis of equation 2 using the observed data (Cm and k'). The desired Pmw values were estimated from equation 3 and 4 by the following equation:

$$Pmw = A/(B \times V) + 1 \quad (5)$$

The retention time, A, B, and estimated logPmw values of the fibrinogen inhibitors studied in this work, along with their structure and anti-platelet activities are summarized in Table 1. All A and B values were obtained with high correlation coefficients, as shown in Table 1 (r>0.98).

The important and advantageous points about the use and measurement of logPmw are 1) measurement of logPmw can be carried out using a small amount of sample, and high purity is not needed because it is enough to measure retention time of the compounds by HPLC, whereas high purity and >100mg of compound is usually necessary for logP measurement, 2) logPmw values are independent of the HPLC conditions such as column type, Psw, F, etc., as shown in equation 5, and 3) logPmw values can be easily obtained by use of an auto-sampler system for HPLC.

Results and Discussion

The observed values of logPmw and antiplatelet activity (pIC_{50}) are plotted in Figure 1a for compounds **1**–**21**. Statistical analysis of these data, excluding compounds **20** and **21** gives:

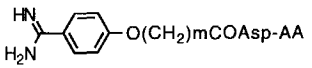
$$pIC50 = -2.36(\pm 1.45)(logPmw)^2 + 7.58(\pm 4.55)(logPmw) - 0.680(\pm 3.43) \quad (6)$$

$$= -2.36(logPmw - 1.61)^2 + 6.77 \quad (7)$$

(n=19, s=0.268, F=6.38**, r=0.667)

The values in parentheses in equation 6 indicate 95% confidence intervals. Equation 6 shows that the antiplatelet activities depend on their logPmw values, and equation 7 indicates that a compound having a logPmw value of 1.61 exerts the maximum activity (pIC_{50} = 6.77). This agrees with the experimental results: all compounds having suitable logPmw values, ranging from 1.3 to 1.7, showed potent antiplatelet activity (pIC_{50} > 6.2) and the most potent was compound **2**, whose logPmw value (1.58) is close to the presumptive optimum logPmw value (1.61). Interestingly, this result is consistent with our previous result,[4] in which compounds **1**–**12** only were considered: a compound having 1.57 of logPmw will exert the presumptive maximum activity

Table 1. Structures, Anti-platelet Activities, logPmw, and CLOGP values of Fibrinogen Inhibitors



	Structure		Inh.[a]	logPmw	Retention Time (min)[b]						Regression Result			CLOGP[d]
	m	AA	pIC_{50}		20	25	30	35	40	50 (mM)	A[c]	B[c]	r	
1 (FK633)	3	ValOH	6.96	1.29	5.90	-[e]	5.55	-	5.35	5.21	7.43	0.341	0.988	0.13
2	3	NleOH	7.12	1.58	11.11	-	10.21	-	9.49	8.94	4.63	0.107	0.999	0.79
3	3	IleOH	6.85	1.47	9.41	-	8.77	-	8.25	7.83	4.97	0.149	0.999	0.66
4	3	leuOH	6.87	1.47	9.70	-	9.04	-	8.49	8.06	4.79	0.144	0.999	0.66
5	3	γ-Me-LeuOH	6.64	1.59	14.11	-	12.96	-	12.06	11.19	3.51	0.0791	0.999	1.06
6	3	Tyr(Me)OH	6.89	1.96	14.85	-	13.01	-	11.82	10.91	4.62	0.0435	0.998	0.54
7	3	TyrOH	6.80	1.74	10.54	-	9.51	-	8.79	8.23	5.86	0.0920	0.999	-0.05
8	4	SerOH	4.92	0.904	3.32	-	3.27	-	3.24	3.20	10.6	1.28	0.995	-1.34
9	4	ValOH	6.64	1.32	7.55	-	7.12	-	6.83	6.52	5.43	0.23	0.999	0.66
10	4	TyrOH	6.19	1.82	12.79	-	11.47	-	10.57	9.72	4.93	0.0647	0.999	0.48
11	4	Tyr(Me)OH	6.57	2.00	17.87	-	15.67	-	14.24	12.91	3.82	0.0325	0.999	1.07
12	4	TyrOMe	5.35	2.09	20.18	-	17.31	-	15.56	14.28	3.54	0.0243	0.997	0.62
13	3	$NHCH(CH_3)CH(CH_3)_2$	5.90	1.20	-	5.44	5.12	4.86	4.63	-	11.2	0.643	0.999	0.54
14	3	$NHCH_2CH_2$(4-iPrOPh)	6.40	2.21	-	14.60	11.35	9.40	8.07	-	8.22	0.043	0.999	1.48
15	3	$NHCH_2CH_2$(4-EtOPh)	6.50	1.95	-	11.78	9.57	8.05	7.15	-	9.13	0.088	0.999	1.17
16	3	$NHCH_2CH_2$(3,4-MeOPh)	6.30	1.43	-	6.50	5.90	5.43	5.07	-	12.0	0.395	1.000	0.18
17	3	$ValNH_2$	6.20	0.891	-	3.70	3.63	3.56	3.50	-	14.9	1.86	0.999	-0.91
18	3	ValNH-chex	6.70	1.38	-	9.08	7.85	6.97	6.31	-	2.46	0.090	1.000	1.47
19	3	$ValNEt_2$	6.60	1.22	-	4.88	4.68	4.43	4.22	-	14.2	0.776	0.994	0.93
20	3	Val-(morpholine)	7.10	0.716	-	3.58	3.51	3.48	3.45	-	11.0	2.22	0.982	0.10
21 (FR158999)	3	Val-(dioxo-thiomorpholine)	7.50	0.804	-	3.51	3.46	3.41	3.37	-	14.5	2.29	0.999	-0.76

[a]Inhibitory activities were quoted from reference 8: $pIC_{50} = -\log(IC_{50})$. [b]Retention time values were measured under the conditions described in the Experimental Section. [c]See equation (2). [d]CLOGP values were calculated using Corwin/Leo's CLOGP software Version 4.34 by DAYLIGHT Chemical Information Systems, Inc. [e] -:not tested.

(pIC_{50} = 6.93) as shown in equation 8.

This consistency between our two QSAR studies strongly suggests that the antiplatelet activities of these peptidic inhibitors are dependent on logPmw.

$$pIC50 = -4.36(logPmw-1.57)^2 + 6.93 \quad (8)$$
$$(n=12, s=0.368, F=14.1^{**}, r=0.871)$$

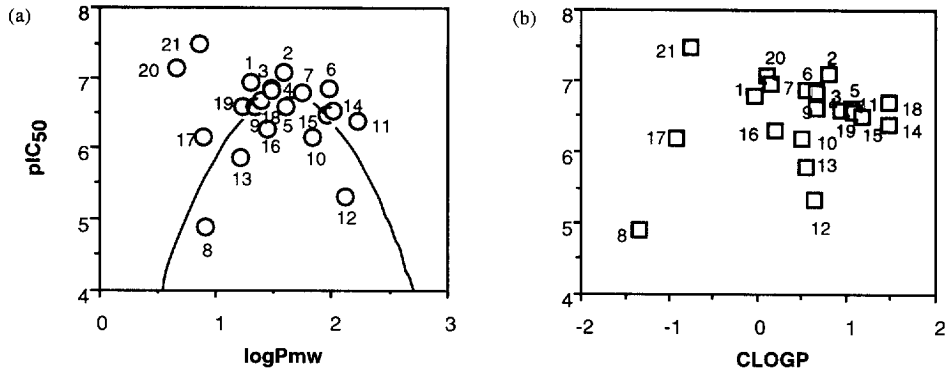


Figure 1. Plots of fibrinogen inhibitory activities (pIC50) versus logPmw (a) and CLOGP (b).

The compounds studied here are different only in the C terminal parts, and possess similar moieties in the remainder of the molecule. Therefore the role of the C terminal moiety can be discussed from the present results, and the following two possibilities can be deduced, 1) the C-terminal moiety plays an important role in interacting with the binding pocket of the GPIIb/IIIa receptor through hydrophobic interactions, and 2) potent inhibitors bind to the receptor via the biomembrane, as illustrated in route B of Figure 2, and the C-terminal moiety functions as an anchor by which the inhibitors can interact with the biomembrane through the hydrophobic nature of the C terminus.[9] In order to understand further, we next carried out QSAR studies using STERIMOL parameters[10] and CLOGP[11] values. QSAR studies on the C-terminal moiety using STERIMOL parameters were unsuccessful (data not shown), which is consistent with the previous study, indicating that the shape of the C-terminal moiety is not crucial for anti-platelet activity and is not recognized by the binding pocket of the receptor. A QSAR study using CLOGP values also resulted in a poor relationship (Figure 1b), in which some compounds having presumably suitable CLOGP values (0 – 1 values) showed weak antiplatelet activities (compounds **12** and **13**). This difference between QSAR results using logPmw and those using CLOGP is interesting to us since both are indicators of the same hydrophobic property of compounds. These results are thought to indicate that the important feature of the AA moiety in these derivatives is not their molecular shape (STERIMOL) or simple hydrophobicity (CLOGP), but membrane affinity (logPmw).

This importance of membrane affinity may give some insight into the importance of membrane processes in biological activity. Thus, the possibility is presented in this study that a potent inhibitor first interacts with biomembrane, and then binds to the receptor in the biomembrane, the membrane of platelets in this case (route B in figure 2), where the C-terminal moiety functions as an anchor.

According to this hypothesis, the reason for inactivity of compounds whose logPmw values are far from the maximum value (1.57) can be recognized as follows. A compound with low logPmw values, such as compound **8**, has to bind to the receptor directly from the solution state (route A in Figure 2) because it does not possess sufficient affinity for the membrane. In this case, compound **8** should have high binding affinity compared with others in order to have a good activity because it adapts an unfavorable binding route A in which there are two major demerits: 1) it is necessary to adapt an active conformation in solution for effective binding with the GPIIb/IIIa receptor, but this is unlikely,[12] and 2) probability for the inhibitor to meet the receptor via three

dimensional diffusion (route A) is much lower compared to that via two dimensional diffusion (route B).[13]

On the other hand, it is difficult for compounds whose logPmw is too high, such as compound **12**, to release itself from the membrane to bind to the binding pocket due to its very high membrane affinity, resulting in an overall low inhibitory activity.

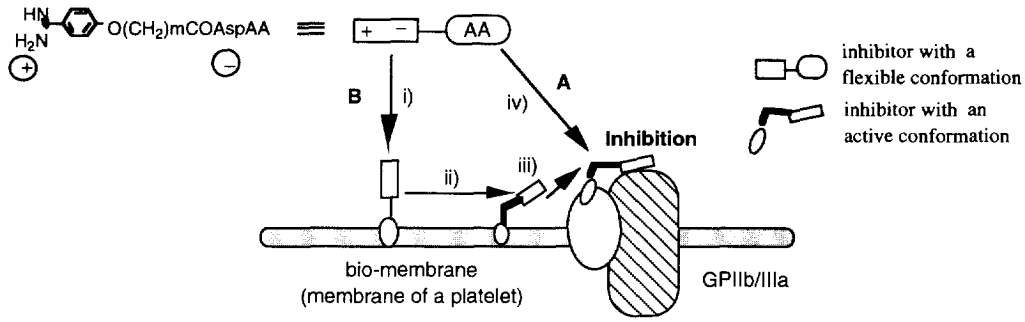


Figure 2. Two inhibitory pathways from the solution state to the binding state, and tentative route of compounds in this study(route **B**). i) Bind to the bio-membrane via hydrophobic interaction of the C terminal moiety, ii) construct a near active conformation under the bio-membrane conditions, iii) bind to the receptor, and iv) directly bind to the receptor only when the inhibitor adapts a near active conformation in solution. See text in detail.

Finally, comments are necessary about the two hydrophilic compounds **20** and **21**, which were excluded from the above discussion, but showed potent inhibitory activities, even though their logPmw values are very low. Because of poor membrane affinity, judged from the low logPmw values, they presumable bind to the receptor directly from aqueous solution via route A, like compound **8**. Therefore, the two compounds presumably have high binding affinity in order to recover the loss of adopting the disadvantageous binding route. This high binding affinity may derive from the functional groups of the C terminal moiety, such as an oxygen atom (**20**) or dioxosulfur atom (**21**), which are able to interact strongly with the receptor via hydrogen bonds. Thus, a 3D-QSAR analysis, in which electrostatic properties, molecular shape, and hydrophobicity are taken into account, must be applied for detailed discussion of the data of these compounds.

Conclusion

A QSAR study using a novel hydrophobic descriptor (logPmw), which is a descriptor for a compound's membrane affinity, of our two fibrinogen inhibitors, FK633, FR158999, and related derivatives was performed. Results indicated a good correlation (n=19, s=0.268, F=6.38**, r=0.667, excluding two compounds (**20**, **21**) in Figure 1a). On the other hand, QSAR studies using STERIMOL parameters, descriptors for molecular shape, and CLOGP, a common parameter for simple hydrophobicity, were unsuccessful.

Based on these QSAR results, we constructed a hypothesis by which potent inhibitors bind to the receptor via the biomembrane, and the C-terminal moiety thus functions as an anchor by which the inhibitors interact with the biomembrane (route B in Figure 2). These studies indicate that logPmw is a useful QSAR descriptor, and that a suitable membrane affinity for the fibrinogen inhibitors is vital for activity.

Experimental Section

All methods for the synthesis of the compounds and the measurement of anti-platelet activities used in this paper are the same as given in our previous papers.[6,8]

Measurement of logPmw.[5] A Shimazu gradient liquid chromatography system (LC-9A system) incorporating SPD-6A as a detector and C-R5A chromatopac as calculator, and a Nihhon-Bunko liquid chromatography system (Gulliver system) incorporating UV-975 as a detector and Bowin system for calculation

software, were used for compounds **1–12**, and for compounds **13–21**, respectively. Stock solutions of Brij35 in 0.1 % TFA aqueous solution (pH 2.37) were prepared in deionized water and were filtered through a 0.45 μm Cellulose ester membrane filter (HA type, Millipore Corporation). The analytical columns were TSK-gel ODS-80TM (5 μm, 120 A, 4.6 x 150 mm) from TOSOH Co., Ltd. for compounds **1–12** and YMC-Pack ODS-AM AM-301 (5 μm, 120 A, 4.6 x 100 mm) from YMC Co., Ltd. for compounds **13–21**, respectively. The flow rates were 0.7 ml/min (compounds **1–12**) and 1.0 ml/min (compounds **13–21**). All experiments were carried out at room temperature (22–25 ℃). Retention time of $NaNO_3$ (2.02 min for the Shimazu HPLC system, 2.53 min for the Nihhon-Bunko HPLC system) was used as a dead retention time (t_0). Retention times of compounds **1–12** were measured at 25, 30, 35, and 40 mM of Brij35 and for compounds **13–21** were observed at 20, 30, 40, and 50 mM of Brij35. Statistical calculations were performed using the MR2-8 in MVA package program.[14]

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References and Notes

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2. CLOGP is obtained by calculation to estimate logP, and is widely used in QSAR studies because it is readily obtained and accurate.
3. The shake-flask method may be the most conventional method for logP, but it still has several disadvantages such as requiring pure and large sample size (ca. 1 g), having limited dynamic range, being time-consuming, requiring special equipment, etc, which often interferes with its application to drug design work.
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11. CLOGP values were calculated using Corwin/Leo's CLOGP software Version 4.34 by DAYLIGHT Chemical Information Systems, Inc.
12. It is thought that a membrane existing in a bilayer state consists of a hydrophilic phase and a lipophilic phase. In the direct binding scheme from aqueous solution (route A in Figure 2), flexible agonists or antagonists of a membrane receptor have to be near to their active conformation. However, it is very rare to construct the near-active conformation in solution state. Recently, it has become widely accepted in medicinal chemistry to use aqueous lipid[12a] or micelle,[12b] conditions for determination of active conformations of bio-active flexible peptides by means of NMR. (a) Wakamatsu, K.; Okada, T.; Miyazawa, T.; *et al. Biochemistry* **1992**, *31* 5654. (b) Motta, A.; Pastore, A.; Goud, N. A. *et al. Biochemistry* **1991**, *30*, 10444.
13 The first step i) in route B is a little obstructive for compounds possessing suitable membrane affinity compared with step iv) in route A, because the area of the membrane is much larger than that of the receptor surface.
14 Takagi, T.; Tange, K.; Jikihara, T.; Onozawa, N.; Sakashita, K.; Fujiwara, H.; Sasaki, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 3606.