Structure-Activity Relationships of RGD Mimetics as Fibrinogen-Receptor Antagonists

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Received April 5, 1999; Accepted June 30, 1999

The activities of a series of RGD mimetics, which contained a variety of cationic structures, for the inhibition of platelet aggregation and fibrinogen-receptor binding were measured. The stability of the coulombic ion-pairing complex of the model compounds with the acetate anion as a model for the receptor was calculated in terms of the ion interaction energy. The results suggest that stability is one of the significant factors which govern the inhibitory potency of fibrinogen-receptor binding. The distance between cationic and anionic groups might also affect the potency. A compound which contained an amidinophenyl structure as the cationic moiety showed exceptionally high inhibitory activity, suggesting that some other factors, in addition to coulombic interaction and the distance, affect the potency.

Key words: Arg-Gly-Asp; fibrinogen antagonist; anti-thrombotic; peptide mimetic; GPIIb/IIIa

Platelet aggregation represents a critical step in thrombus formation. Thrombus formation leads to the formation of pathologically acute vascular occlusions, which can result in myocardial infarction, unstable angina, transient ischemic attack and stroke. Platelet aggregation is mediated by the binding of fibrinogen to its receptor, platelet-membrane glycoprotein GPIIb/IIIa, which involves recognition of the Arg-Gly-Asp (RGD) sequence of fibrinogen. It is conceivable, therefore, that peptides which contain the RGD sequence might be capable of antagonizing the binding of fibrinogen to GPIIb/IIIa, resulting in the inhibition of platelet aggregation. Such peptides could serve as lead compounds for the development of potential therapeutic drugs for the treatment of thromboses which are related to cardiovascular and cerebrovascular diseases.

In the RGD sequence, a cationic unit, in this case, the guanidino group of the Arg side chain, and the β-carboxylic acid structure of Asp are required for inhibitory activity. In addition, the distance between these cationic and anionic functional groups is also an important factor for potency. The fibrinogen binding sites of GPIIb/IIIa have a number of Asp residues, and, because of this, the guanidino group of Arg in the case of RGD-type compounds is thought to be involved in ionic bonding with the carboxylate group of Asp in GPIIb/IIIa. To test this hypothesis, Zablocki et al. have prepared RGD mimetics having guanidino, amidinophenyl, aminomethylphenyl and imidazolonylphenyl groups as cationic groups, and measured their activity with respect to the inhibition of platelet aggregation, indicating that the variations in the potency of these compounds could be explained by the interaction energy between these cationic groups and the carboxylate of the receptor. However, since the number of compounds and the variety of their cationic groups were limited, the issue of whether the ionic interaction energy was the main factor governing the variation in activity is not absolutely clear.

To examine these suggestions, we synthesized a series of compounds which contained various cationic structures as surrogates of an Arg side chain. We quantitatively analyzed the inhibitory activity for fibrinogen binding according to the interaction energy of the compounds which was calculated by using their complex models. The effect of distance between cationic and anionic groups in the molecule was also investigated.

Materials and Methods

Chemistry. All syntheses were performed by the 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase method. Imidazo[1,2-b]pyridazine was synthesized by the procedures described in the literature. Other protected amino acids, reagents and the resin for the synthesis of peptide amides (Fmoc-4-methoxy-4’-(γ-carboxypropoxy)-benzhydrolamine linked to alanylaminomethyl resin with a substitution level of 0.5 meq/g; Kokusan Chemical Works) were obtained from commercial sources. 1H-NMR spectra were recorded by a Bruker ARX 500 spectrometer (500 MHz) or a Varian VXR-200 spectrometer (200 MHz). HR-FABMS were obtained with a JEOL JMS-HX110A spectrometer. The

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Abbreviations: Fmoc, 9-fluorenylmethoxycarbonyl; TFA, trifluoroacetic acid; DMF, N,N-dimethylformamide; DIPC, diisopropylcarbodiimide; HOBr, N-hydroxybenzotriazole; Asp(OtBu), β-t-buty-l-aspartryl; PRP, platelet-rich plasma; PPP, platelet-poor plasma; WP, washed human platelets
Structure-Activity Relationships of RGD Mimetics

A) Guanidination of an amino group.

**8-Guanidinooctanoyl-Asp-Trp-amide (1).** A solution of 3,5-dimethylpyrazole-1-carboxamidine nitrate (302 mg, 1.5 mmol) in DMF (5 ml) was added to 8-aminooctanoyl-Asp(OrBu)-Trp-resin (0.36 mmol), the pH value of the solution was adjusted to 9 with N,N-diisopropylethylamine, and the reaction mixture was stirred at room temperature for 24 h. 3,5-Dimethylpyrazole-1-carboxamidine nitrate (120 mg, 0.6 mmol) was then added to the reaction mixture. After stirring for 3 days, 3,5-dimethylpyrazole-1-carboxamidine nitrate (120 mg, 0.6 mmol) was again added to the mixture, and stirring was continued at room temperature for 3 additional days to give 8-guanidinoctanoyl-Asp-Trp-resin. After treating with a mixture (10 ml) of TFA, thioanisole, m-cresol, 1,2-ethanediol and distilled water (20:1:1:0.5:1, v/v) for 1.5 h at room temperature, the resin was removed by filtration through a sintered glass funnel. After evaporating the filtrate, the residue was washed with ice-cold anhydrous ether, and the crude material was purified by RP-HPLC before being lyophilized. Yield, 13%; 'H-NMR (DMSO-d$_6$) $\delta$: 1.23 (6H m, octanoyl 3CH$_2$), 1.43 (4H m, octanoyl 2CH$_2$), 2.04 (2H m, octanoyl CH$_2$), 2.43 and 2.65 (2H m, Asp $\beta$CH$_2$), 2.99 and 3.11 (2H m, Trp $\beta$CH$_2$), 3.07 (2H m, octanoyl CH$_2$), 4.39 (1H m, Trp $\alpha$CH), 4.53 (1H m, Asp $\alpha$CH), 6.96, 7.05, 7.09, 7.30, 7.32 and 7.55 (11H m, Trp 5CH and CONH$_2$, guanidino 2NH and NH$_2$), 7.71 (1H d, $J=7.8$, Trp NH), 8.08 (1H d, $J=7.6$, Asp NH), 10.81 (1H s, Trp ring NH), 12.06 (1H b, Asp COOH). HR-FABMS $m/z$: Calcd. for C$_{38}$H$_{58}$N$_{10}$O$_{8}$ 502.2778; Found, 502.2785.

B) Substitution with a tertiary amine.

**8-(1-Imidazo[1,2-$a$]pyridinio)octanoyl - Asp - Trp-amide (2).** A solution of imidazo[1,2-$a$]pyridine (110 µl, 1.08 mmol) in DMF (5 ml) was added to 8-bromooctanoyl-Asp(OrBu)-Trp-resin (0.36 mmol). The mixture was stirred for 3 days at 50°C to give 8-(1-imidazo[1,2-$a$]pyridinio)octanoyl-Asp-Trp-resin. The crude compound was cleaved from the resin and purified in a manner similar to that described for compound 1. Yield,
17%; 1H-NMR (DMSO-d$_6$) $\delta$: 1.24 (6H m, octanoyl 3CH$_2$), 1.43 (2H m, octanoyl CH$_2$), 1.80 (2H m, octanoyl CH$_2$), 2.03 (2H m, octanoyl CH$_2$), 2.44 and 2.66 (2H m, Asp $\beta$CH$_2$), 2.98 and 3.10 (2H m, Trp $\beta$CH$_2$), 4.41 (3H m, Trp $\alpha$CH, octanoyl CH$_2$), 4.53 (1H m, Asp $\alpha$CH), 6.94, 7.04, 7.09, 7.29 and 7.54 (7H m, Trp 5CH and CONH$_2$), 7.76 (1H d, J = 7.2, Trp NH), 8.13 (1H d, J = 8.0, Asp NH), 10.85 (1H s, Trp ring NH), 12.22 (1H b, Asp COOH). HR-FABMS m/z [M$^+$]: Calcd. for C$_{29}$H$_{37}$N$_2$O$_5$, 544.3499; Found, 544.3492.

C) Amide coupling. Isonisopicotyl-hexanoyl-Asp-Trp-amide (7). Fmoc-isonicotinic acid (315 mg, 0.9 mmol) was coupled with 6-aminohexanoyl-Asp(OrBu)-Trp-resin by using the DIPCI/HOBt method to give 6-(Fmoc-isonisopicotyl)-hexanoyl-Asp(OrBu)-Trp-resin. After the Fmoc group had been removed, the crude compound was cleaved from the resin and purified in a manner similar to that described for compound 1. Yield, 18%; 1H-NMR (DMSO-d$_6$) $\delta$: 1.20 (2H m, hexanoyl CH$_2$), 1.36 (2H m, hexanoyl CH$_2$), 1.43 (2H m, hexanoyl CH$_2$), 1.68-2.03 (8H m, isonicotinyl 4CH$_2$), 2.37 (1H m, isonicotinyl CH), 2.43 and 2.64 (2H m, Asp $\beta$CH$_2$), 2.88 (2H m, hexanoyl CH$_2$), 2.99 and 3.12 (2H m, Trp $\beta$CH$_2$), 3.00 (2H t, J = 5.7, hexanoyl CH$_2$), 3.49 (1H m, Trp $\alpha$CH), 4.53 (1H m, Asp $\alpha$CH), 6.96, 7.05, 7.09, 7.30 and 7.55 (7H m, Trp 5CH and CONH$_2$), 7.82 (1H d, J = 5.5, hexanoyl NH), 8.09 (1H d, J = 7.9, Asp NH), 8.44 (1H b, isonicotinyl NH), 10.80 (1H s, Trp ring NH), 12.13 (1H b, Asp COOH). HR-FABMS m/z [M + H$^+$]: Calcd. for C$_{27}$H$_{38}$N$_2$O$_5$, 543.2931; Found, 543.2939.

Compound 8 was synthesized by a procedure similar to that described for compound 7. NMR and MS data for compound 8 are as follows.

p-Amidinobenzoyl-$\beta$A A-Asp-Trp-amide (8). 1H-NMR (DMSO-d$_6$) $\delta$: 2.39 (2H m, $\beta$A A CH$_2$), 2.42 and 2.67 (2H m, Asp $\beta$CH$_2$), 3.00 and 3.15 (2H m, Trp $\beta$CH$_2$), 4.38 (1H m, Trp $\alpha$CH), 4.56 (1H m, Asp $\alpha$CH), 6.96, 7.04, 7.10, 7.30 and 7.56 (7H m, Trp 5CH and CONH$_2$), 7.85 (1H d, J = 7.5, Trp NH), 7.86 and 8.01 (4H Ar$_2$B$_2$, amidinobenzoyl 4CH$_2$), 8.27 (1H d, J = 7.7, Asp NH), 8.71 (1H t, J = 5.8, $\beta$A A NH), 8.89 and 9.37 (3H b, amidinobenzoyl NH and NH$_2$), 10.80 (1H s, Trp ring NH), 12.06 (1H b, Asp COOH). HR-FABMS m/z [M + H$^+$]: Calcd. for C$_{26}$H$_{32}$N$_2$O$_5$, 536.2257; Found, 536.2249.

Inhibition of human platelet aggregation. Inhibition of human platelet aggregation was measured by using the previously published method. Blood from human volunteers (50 ml), diluted with 130 mm trisodium citrate (5 ml), was centrifuged at 200 x g for 15 min at room temperature to give platelet-rich plasma (PRP) as the supernatant. After removing most of PRP (ca. 20 ml), the residual phase was centrifuged at 2000 x g for 10 min at room temperature to obtain platelet-poor plasma (PPP). PRP (200 $\mu$L) was incubated with each test compound (1-10 $\mu$L), which was dissolved in saline (154 mm NaCl at pH 6.4), for 2 min at 37°C. Platelet aggregation was induced by adding 10 $\mu$L of an aqueous solution of ADP (400 $\mu$m). Aggregation was measured by an ag-
Table 2. Inhibitory Activities for Platelet Aggregation and Fibrinogen Binding of the Test Compounds and Their Physicochemical Parameters

<table>
<thead>
<tr>
<th>No.</th>
<th>Aggregation [AG]</th>
<th>Binding [BD]</th>
<th>ΔEintert</th>
<th>ΔL</th>
<th>pIC50 (M)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.35(0.23)</td>
<td>5.30(0.01)</td>
<td>4.78</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>3.56(0.01)</td>
<td>4.01(0.02)</td>
<td>3.99</td>
<td>2.71</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>3.36(0.03)</td>
<td>3.40(0.10)</td>
<td>3.58</td>
<td>4.10</td>
<td>0.97</td>
</tr>
<tr>
<td>4</td>
<td>3.58(0.11)</td>
<td>3.92(0.02)</td>
<td>3.87</td>
<td>3.11</td>
<td>-0.94</td>
</tr>
<tr>
<td>5</td>
<td>3.78(0.17)</td>
<td>3.89(0.06)</td>
<td>3.88</td>
<td>3.08</td>
<td>-0.95</td>
</tr>
<tr>
<td>6</td>
<td>3.67(0.14)</td>
<td>3.89(0.08)</td>
<td>3.79</td>
<td>3.39</td>
<td>-0.94</td>
</tr>
<tr>
<td>7</td>
<td>4.08(0.17)</td>
<td>4.29(0.06)</td>
<td>4.83</td>
<td>-0.17</td>
<td>1.90</td>
</tr>
<tr>
<td>8</td>
<td>6.94(0.07)</td>
<td>7.18(0.03)</td>
<td>4.89</td>
<td>-0.37</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

a Values in parentheses are the standard deviation from at least 2 runs.
b The value of tetrapeptide RGDW is 4.58 from ref. 23.
c From Eq. 4.  
  d Interaction energy relative to that of compound 1. The raw value for compound 1 is 10.98 Å.

Iodination of human fibrinogen. Purified human fibrinogen (10 ml, 20 mg/ml; Green Cross) was radiolabeled by incubating with two IODO-BEADS (3.175 mm in dia; Pierce) and 37 MBq Na221 (580.9 MBq 221I/µg; Amersham) in a 100 mM phosphate buffer (pH 6.5) at room temperature for 10 min by using the previously published method.10, 11 221I-Fibrinogen was purified by gel filtration in a PD-10 column (Pharmacia) which had been equilibrated with a phosphate buffer (137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, and 1.5 mM KH2PO4 at pH 7.2) containing 0.1% BSA. The specific activity of radiolabeled fibrinogen ranged from 18 to 38 kBq/µg.

Inhibition of fibrinogen binding to washed human platelets. Washed human platelets (WP) were prepared according to the method of Morii et al.17 Blood samples from human volunteers (50 ml), diluted with 5 ml of a CCD buffer (100 mM trisodium citrate and 136 mM glucose at pH 6.5) were centrifuged at 180 × g for 15 min at room temperature to obtain PRP as the supernatant. PRP was diluted with the CCD buffer to 1.5 times its original volume and then centrifuged at 750 × g for 10 min at 4 °C to give a platelet pellet. This pellet was gently suspended in the same volume of a citrate buffer (13 mM trisodium citrate, 120 mM NaCl, and 30 mM glucose at pH 6.6) as that of original PRP and placed in an ice bath for 1 h. The mixture was then centrifuged at 750 × g for 10 min at 4°C to give a washed platelet pellet. This pellet was finally suspended in a modified Tyrode-HEPES buffer (137 mM NaCl, 2.7 mM KCl, 5.5 mM glucose, 3.8 mM NaH2PO4, 15 mM HEPES, and 0.1% BSA (w/v) at pH 7.4) and adjusted to 3 × 10⁶ cells/ml.

The Inhibition of fibrinogen binding to WP was measured by using the previously reported method.18 A mixture of the WP suspension (70 µl), 121I-fibrinogen in a phosphate buffer (pH 7.2, 10 µl, 90 µg/ml), and each test compound dissolved in saline (10 µl) was placed in a reaction tube. WP in the solution was activated by adding 10 µl of saline containing 100 µM ADP, 10 mM HEPES, and 10 mM CaCl2. The mixture was incubated at 37°C for 15 min. Fifty microliters of the resulting mixture were layered on 20% sucrose (100 µl) in saline containing 2% BSA in a polypropylene microtube and centrifuged at 7,500 × g for 5 min at room temperature. After the supernatant had been removed, the tip of each tube was cut off, and the radioactivity of the labeled fibrinogen, which was not only specifically but also nonspecifically bound to WP, in the tip was counted with a gamma-ray counter (ARC-600, Aloka). The 'non-inhibited' specific binding was evaluated as the difference in counts between the total binding, which was measured with the addition of saline only (10 µl), and the non-specific binding, which was measured in the presence of unlabeled fibrinogen (10 µl, 20 mg/ml). The total counts varied with the concentration of the test compound added, but counts for nonspecific binding were taken as being constant. The 50% inhibitory concentration (IC50[BD], M) for specific binding was estimated for each compound by a probit analysis. Each pIC50[BD] value which is the log of the reciprocal of IC50[BD], is listed in Table 2.

Molecular modeling. The model compounds were used for calculating the interaction energy and the distance which will be defined later. To simplify the calculation of the interaction energy, N-n-propyl compounds I–VI for compounds I–6, amide compounds VII and VIII for compounds 7 and 8 (Fig. 2.), and an acetate anion for the carboxylate group of the receptor were used. To calculate the distance, the Asp-Trp moiety, which is the common structure in compounds 1–8, was removed as representatively shown in Fig. 3 for compounds 1 and 5. All the model compounds were constructed by the SYBYL molecular modeling software package, version 6.4.19 and optimized by the MOPAC/PM3 semi-empirical molecular orbital method.20,21 To initially obtain model complexes, an acetate anion was placed at several positions in close proximity to the hydrogen atoms attached to the positively charged nitrogen atom of the model ligands. Each of the complexes was fully optimized by PM3. The most energetically stable complex was used for calculating the interaction energy.

Calculation of the interaction energy. The interaction energy of the cations of the model ligands with an acetate anion was calculated in a manner similar to that described in the literature.20 The energy was evaluated by using the ab initio GAUSSIAN98 program with a 6-31+G*/RHF basis set.22 During the ab initio calcula-
nitrogen atom and the carbonyl carbon atom. The $L$ value relative to that of compound 1, $\Delta L$, was used and each is listed in Table 2.

**Results and Discussion**

**Inhibitory activities for platelet aggregation and fibrinogen binding.** We synthesized peptide mimetics related to RGDW which has the highest activity in terms of the inhibition of platelet aggregation among the various RGDX' peptides ($X'=$Trp, Phe, Tyr, Val, Ser, Leu, Ile, and certain other unnatural amino acids).\(^{23}\) We adopted the ethylene backbone structure for the test compounds instead of the Arg-Gly amide bond (Fig. 1) in order to avoid their hydrolysis by peptidases and proteinases in blood. The C-terminal carboxylic acid was amidated, and the N-terminal amino group was removed to eliminate possible side effects resulting from their negative and positive charges, respectively, on the interaction with the receptor.

The inhibition of fibrinogen binding is due to the antagonism of peptide mimics against the receptor-binding of fibrinogen.\(^{6}\) Platelet aggregation may include processes other than fibrinogen binding. The fact that these two inhibitory activities are in good agreement with each other ($r=0.992$), however, strongly suggests that all of the compounds used here acted as fibrinogen antagonists. Compound 1 was six-fold more active than RGDW ($pIC_{50}[AG]=4.58$)\(^{25}\) for the inhibition of platelet aggregation (Table 2). Substitution of the guanidino group of compound 1 by a condensed-heterocyclic group (compounds 2 and 3) and by a cyclic or acyclic quaternary ammonium group (compounds 4-6) decreased both inhibitory activities. Compounds 7 and 8 contain piperidinyl and amidinophenyl groups, respectively, which are connected to the alkyl chain by an amide bond. Compound 7 was less active than compound 1 in the inhibition of platelet aggregation and fibrinogen binding by factors of 20 and 10, respectively. Of the compounds tested, compound 8 gave the highest

![Fig. 2. Structures of the Model Compounds Used for Calculating the Interaction Energy.](image)

**Calculation of the distance between the cationic group and Asp residue of the ligands.** The distance, $L$, was calculated with SYBYL for the model compounds, in which the Asp-Trp moiety of compounds 1-8 was substituted by an NH$_2$ group as representatively shown for compounds 1 and 5 in Fig. 3. For compounds 1 and 8, $L$ is defined as the distance between the center carbon atoms of the guanidino and amidino groups, respectively, and the carbonyl carbon atom. For compounds 2-7, $L$ is the distance between the positively charged

\[ \Delta E_{interact} = (E_{interact})_{cat} - (E_{interact})_{gua} \] (2)

where $(E_{interact})_{cat}$ and $(E_{interact})_{gua}$ are the interaction energy values for each model complex of compounds 2-8 and that of compound 1, respectively. To calculate $\Delta E_{interact}$, the value was scaled by 0.1 to make it nearly equiscalar with the $pIC_{50}[BD]$ value. Equations 1 and 2 show that the more negative the $\Delta E_{interact}$ Value, the more stable is the ionic complex. The $\Delta E_{interact}$ values are listed in Table 2.

![Model of Compound 1](image)

![Model of Compound 5](image)

**Fig. 3. Definition of Distance $L$.**
inhibitory activities in both assay systems and was approximately 40-fold and 70-fold more active than compound 1 in the inhibition of platelet aggregation and fibrinogen binding, respectively.

Relationship between the inhibitory activity for fibrinogen binding and the interaction energy and distance. We chose the inhibitory activity for fibrinogen binding, \( pIC_{50}[BD] \), for the subsequent structure-activity analysis, because this activity could be expected to more directly reflect the interaction of the compounds with the receptor than that for platelet aggregation. The inhibitory activity was quantitatively analyzed in relation to the interaction energy to give Eq. 3.

\[
pIC_{50}[BD] = -0.52(\pm 0.41) \Delta E_{\text{interact}} + 5.52(\pm 1.08) \quad (3)
\]

\( n=8, \ s=0.813, \ r=0.786, \ F_{1,6}=9.71 \)

In this and the following equations, \( n \) is the number of compounds included in the analysis, \( s \) is the standard deviation, \( r \) is the correlation coefficient, and \( F \) is the ratio of regression and residual variances. The figures in parentheses after each coefficient are the 95% confidence intervals of the regression coefficient. Equation 3 shows that the inhibitory activity tends to increase with a decrease in the interaction energy of the model complexes. It has been suggested that the guanidino group of RGD and its surrogates of the mimetics interact with the carboxylate anion of Asp or Glu in GP Ib/IIa.\(^9\) Zablocki et al. have shown that the stability of ionic interaction was one of the factors which governed the inhibitory potency of RGD mimetics.\(^10\) Equation 3 appears to verify their work.

A more detailed examination of the results, however, gave a much higher experimentally measured \( pIC_{50}[BD] \) value for compound 8 than the value calculated by Eq. 3. By excluding compound 8, Eq. 3 was much improved to give Eq. 4.

\[
pIC_{50}[BD] = -0.29(\pm 0.22) \Delta E_{\text{interact}} + 4.78(\pm 0.60) \quad (4)
\]

\( n=7, \ s=0.349, \ r=0.843, \ F_{1,5}=12.2 \)

The activity values calculated by Eq. 4 are listed in Table 2.

Various RGD mimetics that contain an amidinophenyl group like compound 8 have been reported to be highly potent inhibitors of platelet aggregation.\(^8,24-27\) The high potency of compounds with this group has been attributed to the stability of the ionic interaction of the group with the carboxylate moiety of the receptor.\(^10\) This type of interaction energy would, in part, account for the high activity of compound 8. Moreover, the amide moiety adjacent to the amidinophenyl group, as in compound 8, has been reported to be favorable for the activity.\(^25,28\) There seems to be hydrogen bond interaction between the amide moiety at this position and the receptor, increasing the activity. For amide compound 7, however, it is unlikely that the amide group adjacent to the piperidine group would contribute to increasing the activity. A plausible explanation is that the amide group is apart from the carbonyl group bound to the Asp-amino group by three more methylene units than the ethylene moiety at this position in compound 8. The fit of compound 7 with the receptor might become less favorable than that of compound 8. Interestingly, compound 7 was less active than compound 1, although the interaction energy calculated for compound 7 was lower than that for compound 1.

The distance between the cationic and anionic groups of RGD mimetics has been proposed to be a key determinant for inhibitory activity.\(^9\) The addition of the \( \Delta L \) and \( \Delta L^2 \) terms greatly improved Eq. 4 (results not shown; \( n=7, s=0.058, r=0.998 \)). The \( \Delta L \) value for compound 7 (1.90) is much larger than the optimum value (0.10), which resulted in the lower potency than compound 1. Since the number of compounds was not sufficient for this type of analysis,\(^29\) more compounds should be included to obtain more reliable information. Other factors such as the orientation and/or steric effects of the amidinophenyl or other relevant groups might also have affected the binding of RGD mimetics to GP Ib/IIa. To further analyze the role of these factors, three-dimensional quantitative structure-activity studies, using the comparative molecular field analysis (CoMFA) method, are now underway.

In summary, the platelet-aggregation inhibitory activity of a series of RGD mimetics is in good agreement with their inhibitory activity for fibrinogen binding. The latter activity, in turn, is correlated to some extent with the ionic interaction energy calculated with a model system for ligands and receptors. A distance factor evaluated in the molecule seems to have improved the correlation. The amidinophenyl group of the most potent compound among those tested in this study appears to have played an invaluable role in enhancing the activity. Although further studies are required, the present findings provide an important lead for the design of more potent drugs.

Acknowledgments

We appreciate the valuable suggestions of Emeritus Professor Toshio Fujita at Kyoto University. We are grateful to Drs. Youichiro Naito and Mitsuaki Imada of Yoshitomi Pharmaceutical Industries Ltd. for supplying human fibrinogen and for suggestions on the binding assay, and to Dr. Reiko Nakayama of Kyoto Women's University and Dr. Narito Morii in the Faculty of Medicine at Kyoto University for their helpful suggestions about the assay systems. Thanks are also given Dr. Naoshige Akimoto in the Faculty of Pharmaceutical Sciences at Kyoto University for the mass spectral measurements. The facilities of the Supercomputer Laboratory at the Institute for Chemical Research and the Radioisotope Research Center at Kyoto University were used for the calculations and radioisotope experiments, respectively. We also express our thanks to the human volunteers in our laboratory for providing blood samples.

References

12) Kobe, J., Stanovnik, B., and Tisler, M., Synthesis of pyridazine derivatives-XV: Some electrophilic substitutions on imidazo[1,2-
16) Markwell, M. A. K., A new solid-state reagent to iodinate prote-
17) Morii, N., Teru-uchi, T., Tominaga, T., Kumagai, N., Kozaki, S., Ushikubi, F., and Narumiya, S., A rho gene product in hu-
man blood platelet II. Effects of the ADP-ribosylation by buta-
20) Stewart, J. J. P., MOPAC Ver. 6, Quantum Chemistry Program Exchange, Program 455, Indiana University, Bloomington, IN, U.S.A.
23) Kamatsu, M., Uno, T., and Fujita, T., Application of a new hydrophobicity parameter of amino acid side chains to quantita-
25) Fisher, M. J., Gunn, B., Harms, C. S., Kline, A. D., Mullane,

J. T., Nunes, A., Scarborough, R. M., Artsten, A. E., Skelton, M. A., Um, S. L., Utterback, B. G., and Jakubowski, J. A., Non-peptide RGD surrogates which mimic a Gly-Asp β-turn: Po-
gen receptor antagonists which present an alternative pharma-
tide fibrinogen receptor antagonists. I. Synthesis and glycoprotein IIb-IIIa antagonistic activities of 1,3,4-trisubstitu-
ed 2-oxo piperdine derivatives incorporating side-chain func-
t aggregation based upon the Arg-Gly-Asp sequence of fibrino-
29) Topliss, J. G. and Costello, R., Chance correlation in struc-