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DEVELOPMENT OF THE NEW POTENT NON-PEPTIDE GpIIb/IIIa ANTAGONIST NSL-95301 BY UTILIZING COMBINATORIAL TECHNIQUE

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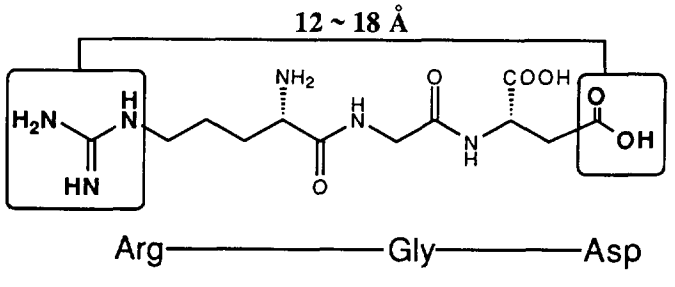
Abstract: The synthetic study of new non-peptide gpIIb/IIIa antagonist, NSL-95301, is presented. The combinatorial technique was engaged in the lead compound discovery process and optimization process to find (+)-NSL-95301. The IC_{50} value of collagen induced platelet aggregation inhibitory activity is 92 nM.

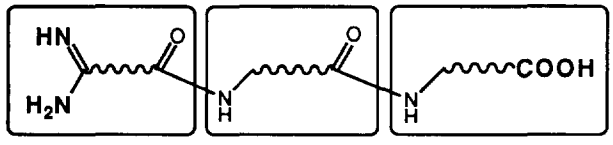
Since its discovery of Arg-Gly-Asp (RGD) sequence[1], common recognition sequence of integrins, much efforts have been made to replace the sequence with non-peptidic molecules to develop integrin antagonists[2].

Among such integrins, gpIIb/IIIa has been one of the major targets for the antagonist development. GpIIb/IIIa is the integrin that exists on the surface of the activated platelet, binding to fibrinogen to cause platelet aggregation[3]. The undesired platelet aggregation is suspected to play an important role in various vasoocculsive diseases[4] i.e., unstable angina, myocardial infarction, transient ischemic attacks and stroke. The effective drug to prevent such irregular platelet aggregation is in serious demand. Therefore gpIIb/IIIa antagonist should be an effective drug candidate to prevent such undesired platelet aggregation to cure related diseases. In this paper is presented the synthesis of RGD-based non-peptide gpIIb/IIIa antagonist utilizing combinatorial technique.



(Fig. 1)

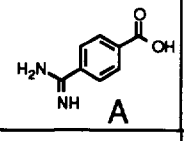
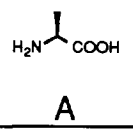
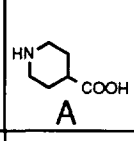
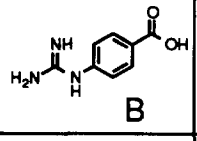
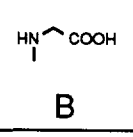
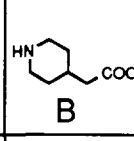
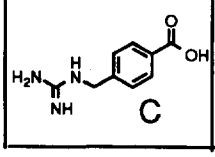
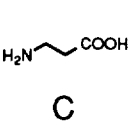
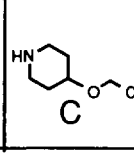
In general, the presence of guanidino group of Arg and the β carboxylic acid of Asp are essential to show its platelet aggregation inhibitory activity. Our previous studies[5] also support this result. According to the other works on the active conformation of RGD peptides[6], the distance between the guanidino group of Arg and the β carboxylic acid of Asp should be within the distance of 12 ~ 18 Å to show its platelet aggregation activity (Fig. 1). Those studies suggest that the major factor of RGD binding to the gpIIb/IIIa is based on ionic interaction. By adjusting N-terminus moiety and C-terminus moiety to the proper distance with an appropriate conformation, the molecule should function as a RGD receptor inhibitor. Three component strategy was engaged for combinatorial synthesis of target molecules. The candidate molecules are composed of 1: the N-terminal, 2: spacer, 3: C-terminal (Fig. 2) .



(Fig. 2)

To reduce the conformational flexibility, the ring structure was employed in both N-terminal unit and C-terminal unit as shown below (Fig. 3). Benzoic acid derivatives and piperidine derivatives are utilized as N-terminal unit and C-terminal unit respectively. The units were chosen to adjust distance between the functional groups and conformational flexibility. Each unit was connected in a combinatorial manner *via* peptide bond formation[7] and the IC_{50} values of collagen induced platelet aggregation inhibition for the final compounds were measured[8].

Compound Table

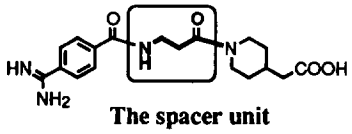
N-terminal	Spacer	C-terminal
 A	 A	 A
 B	 B	 B
 C	 C	 C

(Fig. 3)

Combination Table

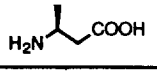
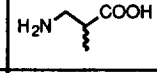
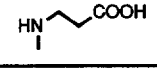
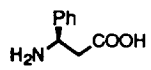
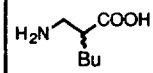
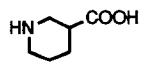
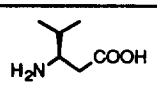
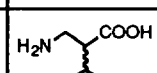
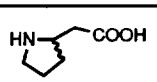
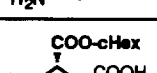
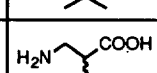
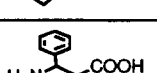
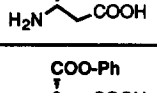
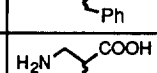
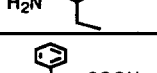
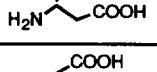
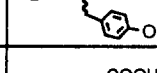
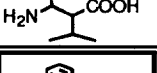
combination	IC 50 [μM]	combination	IC 50 [μM]	combination	IC 50 [μM]
AAA	110	BAA	>1000	CAA	710
AAB	30	BAB	740	CAB	92
AAC	-----	BAC	-----	CAC	-----
ABA	330	BBA	100	CBA	53
ABB	7.5	BBB	750	CBB	>1000
ABC	-----	BBC	-----	CBC	-----
ACA	33	BCA	750	CCA	>1000
ACB	2.5	BCB	200	CCB	-----
ACC	18	BCC	-----	CCC	-----

(Fig. 4)



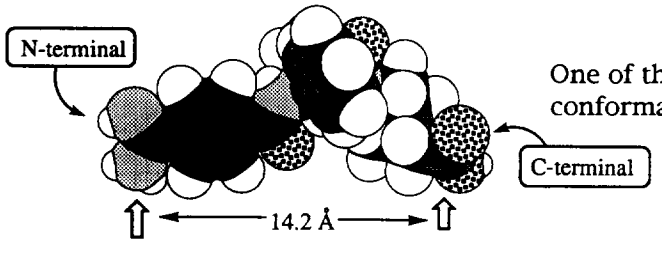
The spacer unit [ACB]

As the result, the combination of amidinobenzoic acid, 3-aminopropionic acid, and 4-piperidineacetic acid [ACB] showed the highest inhibitory activity. Thus [ACB] was chosen as the lead compound. Next, the optimization of the spacer unit was conducted. Various β and γ substituted 3-amino propionic acids were adopted for the spacer unit (Fig.5).

No	Structure	IC_{50} [μM]	No	Struncture	IC_{50} [μM]	No	Structure	IC_{50} [μM]
1		5.0	7		0.59	13		6.5
2		5.2	8		3.8	14		25
3		9.2	9		25	15		4.3
4		1.9	10		4.1	16		16
5		4.1	11		0.94	17		82
6		33	12		0.57	18		0.19

(Fig. 5)

According to the results (Fig. 5), the racemic NSL-95301(**18**)[9] whose spacer is 3-amino-3-phenyl-2,2-dimethyl-propionic acid, showed the best inhibitory activity. The enantiomers of NSL-95301(**18**) were separated from its racemic form by the chiral HPLC[10]. The (-)-NSL-95301 and (+)-NSL-95301 showed the IC_{50} value of 31 μM and 0.092 μM respectively. Also, the result of the conformational analysis[11] shows the distance between N-terminal and C-terminal of NSL-95301 of NSL-95301 was within 12-18Å.



One of the typical lower energy conformation of NSL-95301.

(Fig. 6)

In conclusion, by applying combinatorial technique to develop the fibrinogen receptor antagonist, highly active compound NSL-95301 was synthesized. Combinatorial technique is a powerful and efficient tool for lead compound discovery and optimization procedures when applied to the properly designed system. We are now conducting investigation of conformational study and derivatization of NSL-95301 for higher activity.

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7) All compounds were synthesized on ABI-431A peptide synthesizer based on Fast-Moc™ method using Fmoc-chemistry and purified over ODS reverse phase HPLC.

8) The platelet aggregation inhibitory activity was evaluated *in vitro* using human platelet-rich plasma (PRP) anti-coagulated with 0.38% trisodium citrate. Sample solutions at various concentrations were added to PRP and they were incubated for 1 min at 37°C and then platelet aggregation was induced by adding 5mg/ml collagen. The extent of platelet aggregation is determined by a change in light transmission through the PRP. The IC_{50} value is determined as the concentration of the sample required to achieve 50% inhibition.

9) PMR (270MHz, CD3OD, 60 C°): 1.29, ddd, J=2.9, 11.5, 24.1Hz, 1H: 1.42, ddd, J=3.1, 11.5, 24.1Hz, 1H: 1.52, s, 3H: 1.58, s, 3H: 1.95-2.07, m, 2H: 2.13-2.32, m, 1H: 2.36-2.43, m, 2H: 3.01-3.18, m, 2H: 4.58-4.70, m, 2H: 5.67, s, 1H: 7.46-7.55, m, 3H: 7.61-7.65, m, 2H: 8.08, dt, J=8.8, 1.7Hz, 2H: 8.17, dt, J=8.8, 1.7Hz, 2H $[\alpha]^{25}_D = +14.0°$ (*c* =1.0, MeOH) MS(EI) *m/z* 465.1 (M^+)

10) SUMICHIRAL OA-4700 (5 μm, 4.3 mmϕ x 250 mm) Hexane/THF/MeOH/TFA =70/20/10/0.2

11) The conformational analysis was conducted on MacroModel v.3.5X.

+Nippon Steel Chemical Co.

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