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DEVELOPMENT OF NEW NON-PEPTIDE GPIIb/IIIa ANTAGONISTS, NSL-95315 AND NSL-95317, ISOSTERES OF NSL-95300

Tohru Asari, Shigetaka Ishikawa, Toshiki Sasaki, Jun Katada, Yoshio Hayashi,*
Takeo Harada, Mako Yano, Emiko Yasuda, Isao Uno, and Iwao Ojima[†]

Life Science Research Center, Advanced Technology Research Laboratories, Nippon Steel Corporation, 3-35-1 Ida, Nakahara-ku, Kawasaki 211, Japan.
[†]Department of Chemistry, State University of New York at Stony Brook, NY 11794-3400, U.S.A.

Abstract: The synthetic and structure-activity relationship (SAR) studies of new non-peptide GPIIb/IIIa antagonists (**1a-f** and **3**) were conducted by replacing one amide bond of NSL-95300 (**2**) with an (*E*)-double bond or an enone group. NSL-95315 (**1a**) and NSL-95317 (**3**) showed the inhibitory activity for collagen-induced human platelet aggregation with IC_{50} values of 0.25 μM and 0.21 μM, respectively.
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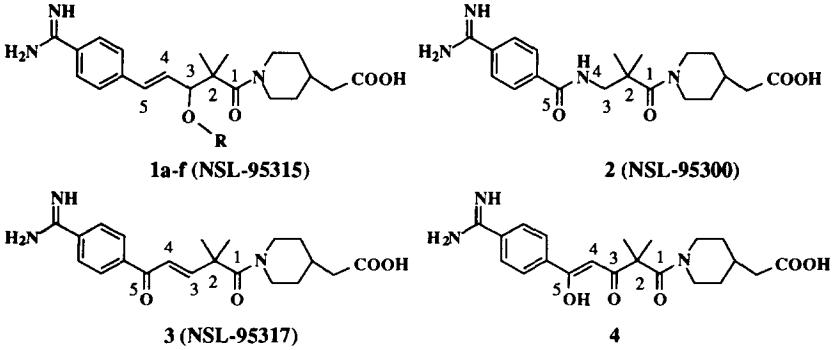
Glycoprotein (GP) IIb/IIIa is an integrin that exists on the surface of activated platelets, and binds to fibrinogen to cause platelet aggregation.[1] The development of highly selective and potent GPIIb/IIIa antagonists has been one of the major focuses of potential antithrombotic therapy.[2] Along this line, we have recently developed NSL-95300 (**2**) as one of the potent GPIIb/IIIa antagonists by utilizing a combinatorial technique.[3] There has been increasing interest in the modification of the amide bond-based backbone in biologically active peptides to increase stability and bioavailability,[4] e.g., an (*E*)-double bond in a peptide mimetics is found to closely resemble the three-dimensional structure of the parent peptide bond.[5]

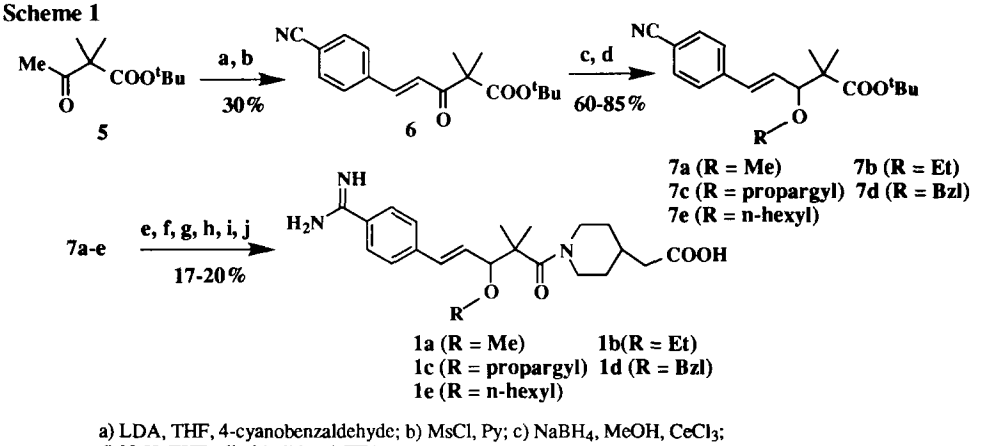
We report here a series of new GPIIb/IIIa antagonists, NSL-95315 (**1a-f**) and NSL-95317 (**3**), in which an amide bond of **2** is replaced with an (*E*)-double bond or an enone group.



(Fig.1)

Target molecules were designed as shown in Fig. 1. In compounds **1a-f**, an amide bond at the N4-C5 position of **2** was substituted by an (*E*)-double bond. In addition, an alkoxyl or hydroxyl group at the C3 position of **1a-f** was introduced to simplify the synthetic pathway and reduce the conformational flexibility. The enone or diketone system at the C3-5 position of **3** or **4** was utilized to keep both the phenyl and C3-C5 moieties on the same plane. 3-Alkoxy-2,2-dimethyl-4-pentenoic acid analogs (**1a-e**) were prepared as illustrated in Scheme 1. Readily available *tert*-butyl 2,2-dimethylacetoacetate (**5**)[6] was coupled with 4-cyanobenzaldehyde via aldol condensation, and the resulting δ-hydroxy ester was dehydrated to **6** with methanesulfonyl chloride under basic conditions. The 1,2-reduction of the enone system in **6** was accomplished by treatment with $NaBH_4$ and cerium(III) trichloride.[7] The resulting hydroxyl group was converted to sodium alkoxide with NaH, and then the addition of the appropriate alkyl iodide to the reaction mixture gave the corresponding 3-alkoxy-2,2-dimethyl-4-pentenoic acid *tert*-butyl ester (**7a-e**). The *tert*-butyl esters **7a-e** were deprotected by TFA, and the resulting free acid was coupled with readily available methyl piperidine-4-acetate[8] in the presence of BOP, i.e., benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate. The conversion of the cyano group of **7a-e** to the amidino group in 3 steps, followed by saponification of the methyl ester, gave the target compounds (**1a-e**).

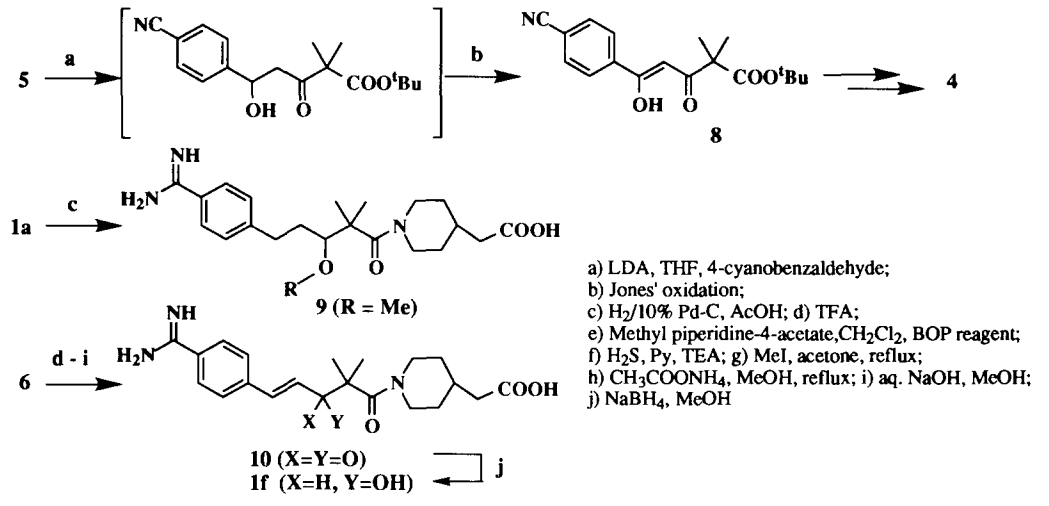
Scheme 1



a) LDA, THF, 4-cyanobenzaldehyde; b) MsCl, Py; c) $NaBH_4$, MeOH, $CeCl_3$;
d) NaH, THF, alkyl iodide; e) TFA;
f) Methyl piperidine-4-acetate, CH_2Cl_2, BOP reagent; g) H_2S, Py, TEA;
h) MeI, acetone, reflux; i) CH_3COONH_4, MeOH, reflux; j) aq. NaOH, MeOH

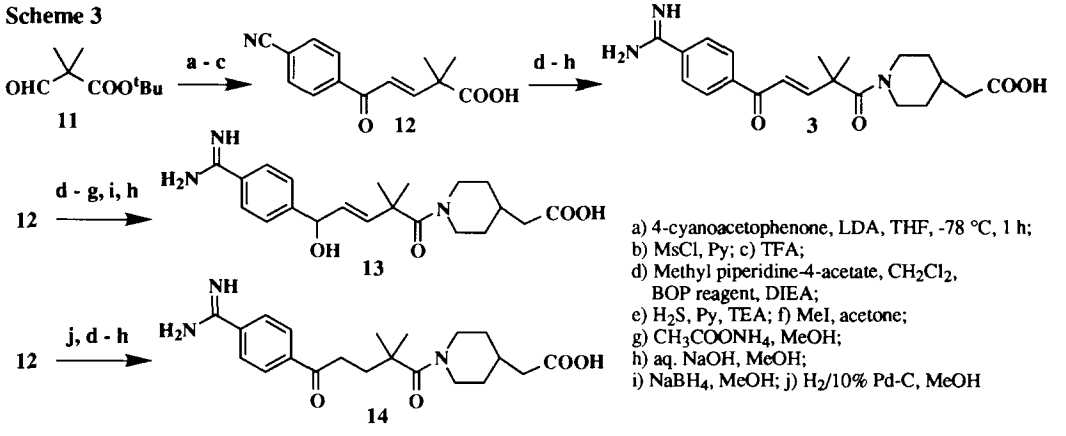
Related compounds **4** and **9** were also synthesized as shown in Scheme 2. After the coupling of **5** with 4-cyanobenzaldehyde, the resulting δ-hydroxy ester was immediately converted to **8** by Jones' oxidation. The conversion of **8** to **4** was carried out in the same manner as that illustrated in Scheme 1 for the synthesis of **1a-e**.

Scheme 2



Compound **9** was synthesized from **1a** through hydrogenation over 10% palladium on carbon. Compound **10** bearing a reversed enone system of **3** was obtained from **6** via a six-step procedure (deprotection of *tert*-butyl ester, coupling with methyl piperidine-4-acetate, amidination of nitrile, and hydrolysis of methyl ester) (Scheme 2).

Scheme 3



5-Oxopentanoic acid derivatives and related compounds (**3**, **13** and **14**) were synthesized as shown in Scheme 3. Aldehyde **11**[9] was converted to the key intermediate **12** by aldol condensation with 4-cyanoacetophenone followed by treatment with TFA. The coupling of **12** with methyl piperidine-4-acetate yielded the corresponding methyl ester. The synthesis of **3** was completed by amidination of the cyano group followed by hydrolysis of the methyl ester. Compound **13** was synthesized by treating the methyl ester of **3**

with $NaBH_4$ followed by saponification. Compound **14** was synthesized by the hydrogenation of **12** over 10% palladium on carbon followed by the same procedure as shown in Scheme 1 for the synthesis of **1**.

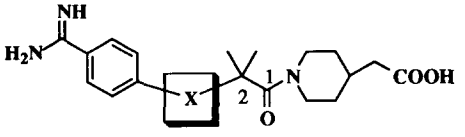


Table 1.

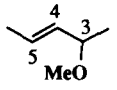
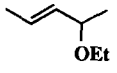
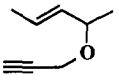
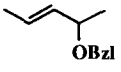
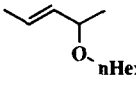
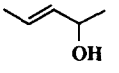
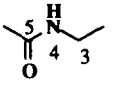
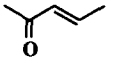
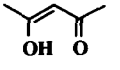
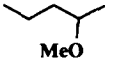
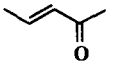
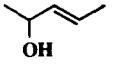
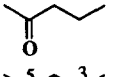
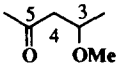
Compd.	X	IC_{50} (μM)
1a		0.25
1b		0.26
1c		0.27
1d		0.73
1e		1.6
1f		0.60

Table 2.

Compd.	X	IC_{50} (μM)
2		0.57
3		0.21
4		600
9		0.48
10		3.5
13		7.6
14		0.63
15		---

These new compounds were assayed for their activity against collagen-induced human platelet aggregation.[10] These results (IC_{50}) are summarized in Tables 1 and 2. NSL-95300 (**2**) (IC_{50} 0.57 μM) was used as the standard. These results indicate two important structural features for strong anti-platelet activity. One is an alkoxyl group at the C3 position, and the other is a carbonyl group at the C5 position. In the case of **1a-f**, where the amide bond is replaced by the (*E*)-double bond at the C4-C5 position, the alkoxyl group at the C3 position is varied to examine its effect on activity. As Table 1 shows, smaller groups such as methoxy, ethoxy and propargyloxy groups increase the activity (**1a-c**), but the compounds that have larger groups such as a benzyloxy or a *n*-hexyloxy group (**1d-e**) are less active. These results indicate the level of steric tolerance at this position upon binding to the GPIIb/IIIa receptor. As clearly shown for **10**, a carbonyl group at the

C3 position is not favorable.

A comparison of **2**, **3** and **14** with **10** and **13** indicates that the carbonyl function at the C5 position appears to be more important than the double bond. The good activity exhibited by **14** indicates that the amide bond at the N4-C5 position of **2** can be replaced by a ketomethylene group without decreasing activity. In the case of compound **4** that loses activity by three orders of magnitude, the characteristic of carbonyl functionality at the C5 position seems to be completely lost by the keto-enol tautomerism to the 99% enol form.[11]

On the other hand, the activities of compound **9** and **14** are slightly lower as compared with those of **1a** and **3**, which indicates that the conformational restriction at the C3-C5 position is also important for better activity.

In conclusion, we found two important structural features for strong anti-platelet activity in the NSL-95315 and NSL-95317 series. These are (i) an alkoxy group at the C3 position, and (ii) a carbonyl group at the C5 position. The importance of increased conformational rigidity by the introduction of the (*E*)-double bond is also recognized. Further studies on the structural modifications of NSL-95315 and NSL-95317 as well as their SAR are actively underway.

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6) *tert*-Butyl 2,2-dimethylacetoacetate was synthesized from isobutyryl chloride in 3 steps (esterification with *tert*-butyl alcohol, aldol condensation with acetaldehyde, and Jones' oxidation).

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8) Hydrogenation of pyridine-4-acetic acid with PtO_2 under acidic conditions followed by methylation with $SOCl_2$ in methanol gave methyl piperidine-4-acetate.

9) Aldehyde (**11**) was prepared from isobutyryl chloride in 2 steps (esterification with *tert*-butyl alcohol and aldol condensation with ethyl formate).

10) The platelet aggregation inhibitory activity was evaluated *in vitro* using human platelet-rich plasma (PRP) anti-coagulated with 0.38% trisodium citrate. Compound 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 5 μg/ml collagen. The extent of platelet aggregation is determined by a change in light transmission through the PRP. The IC_{50} is determined as the concentration of compound required to achieve 50% inhibition.

11) The diketone form was not observed in the ^{1}H-NMR spectrum.

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