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2-Acylimino-3*H*-thiazoline Derivatives: A Novel Template for Platelet GPIIb/IIIa Receptor Antagonists

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Abstract—In the course of our research for the low-molecular weight RGD peptide mimics, we have found that a rigid 2-acylimino-3*H*-thiazoline structure is suitable for the peptide backbone mimics. Introduction of amidinophenyl and β-alanine moiety as arginine and aspartic acid side-chain surrogates to this backbone mimic resulted in a highly potent fibrinogen receptor antagonist 2-(4-amidinobenzoylimino)-3,4-dimethyl-*N*-(2-carboxyethyl)-3*H*-thiazoline-5-carboxamide (**7c**), namely PS-028 ($K_i = 46.5 \pm 5.8$ pM).

The aggregation of platelets is known to play a critical role in hemostatis and arterial thrombosis.[1] Thus, uncontrolled platelet aggregation and platelet adhesion to the subendothelium of damaged blood vessels may cause life-threatening diseases such as myocardial infarction, transient ischemic attack, and unstable angina pectoris.[2] The aggregation of platelet is invoked by various ways, such as stimulation with chemical inducers (ADP, collagen, TXA_2, etc.),[3] or sheer stress.[4] However, the crosslinking of the platelets by binding of dimeric fibrinogen to its receptor GPIIb/IIIa on the activated platelet membrane is the common pathway.[5] Therefore, inhibition of the binding of fibrinogen to GPIIb/IIIa has been regarded as an attractive strategy for a potent novel class of platelet aggregation inhibitors. In fact, a Fab fragment of GPIIb/IIIa monoclonal antibody Reo-Pro[6] can suppress the platelet aggregation invoked by various chemical inducers, and has now been launched as a therapeutic for the treatment of patients undergoing percutaneous transluminal coronary angioplasty (PTCA).

The minimum binding epitope of fibrinogen to its receptor, GPIIb/IIIa, is known to be the tripeptide sequence, Arg-Gly-Asp (RGD),[7] and, a number of RGD-including cyclic peptides,[8] peptide mimetics have been evaluated as GPIIb/IIIa antagonists. However, almost all of them are not systemically available when administered orally, and evaluated as parenteral agents for acute clinical settings. Therefore, development of orally active low-molecular weight GPIIb/IIIa antagonists are now desired for the treatment of chronic thrombotic disorders.

We now report the synthesis and pharmacological evaluations of a novel potent low molecular weight RGD mimic named PS-028. In the course of the investigation, we have utilized the benzamidino group as an arginine isostere[9] and β-alanine as an aspartate surrogate, and then evaluated the linkage structure of these two moieties. To avoid the difficulty in commercial large-scale synthesis, we have tended to evaluate achiral linkage systems.

Chemistry

The synthesis of target thiazole derivatives was accomplished as shown in Scheme 1. 2-Amino (**3a**) and methylamino (**3b**) thiazole derivatives were obtained from the corresponding 2-amino-4-methylthiazole-5-carboxylates in 3 steps. 3-Alkyl-4-methyl-2-imino-3*H*-thiazoline derivatives **5c**–**f** were synthesized from 4-cyanobenzoylamino intermediate (**4**) by selective alkylation in basic conditions (Scheme 2). Thus, treatment of **4** with sodium hydride in DMF and alkyl halides at 0 °C yielded 3-alkyl-3*H*-thiazoline **5c**–**f** as a single product. In an NMR study regarding **5c**,[10] the chemical shift of

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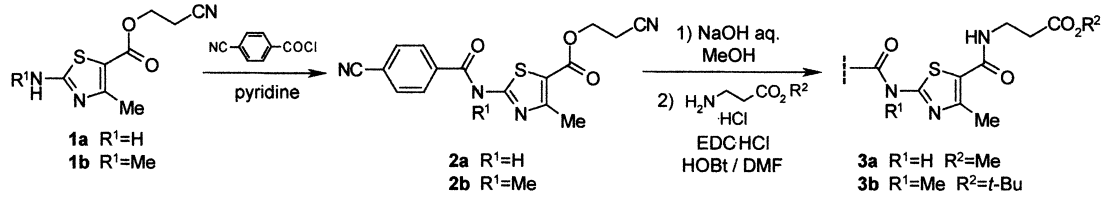
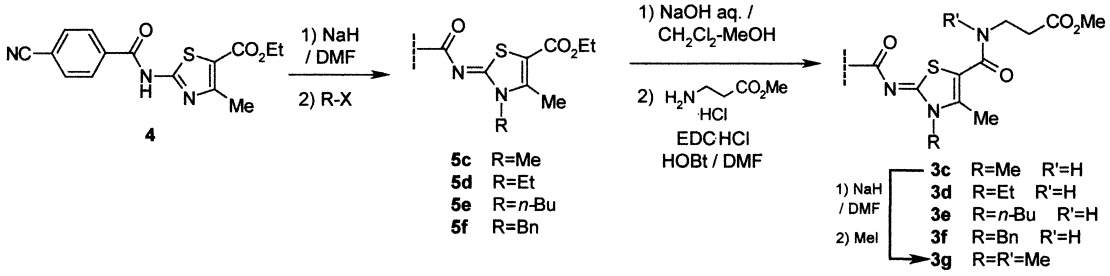
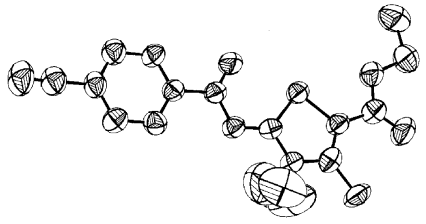


Scheme 1.



Scheme 2.

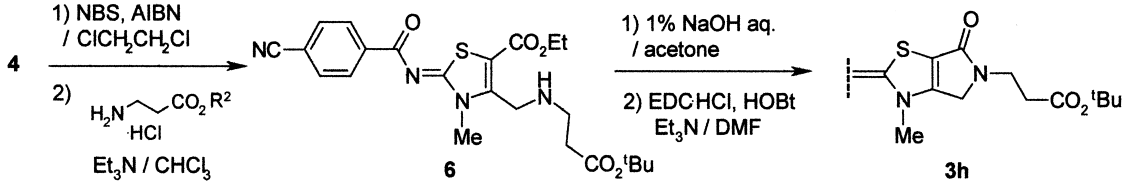
the 3-methyl proton was δ 3.83 (400 MHz, $CDCl_3$), and HMBC coupling was observed with the 4-position of the thiazoline carbon atom (δ 146.16), which was also observed coupling with the 4-methyl proton (δ 2.70). This indicates that the methylation of **4** proceeded at the 3-position of the thiazole ring. The X-ray crystallographic analysis[11] of crystalline intermediate (**5e**) revealed that the configuration of the acylimino moiety was *syn* (Scheme 3). Moreover, this indicates that the 2-benzoylimino-3-alkyl-3*H*-thiazoline template is an extremely constrained, planar conjugated system. Probably due to this conjugated structure, the acylimino moiety of these compounds is significantly stable for hydrolysis conditions, namely, they are tolerable even in refluxing 23% hydrobromic acid and 5% NaOH in aqueous methanol for hours. *N*-Methyl-β-alanine derivative **3g** was synthesized by methylation of **3c** in good yield (Scheme 2). Bicyclic thiazoline derivative **3h** was synthesized from **4** in 4 steps (Scheme 4). Thus, the 4-methyl group of the thiazoline ring in **4** was brominated by *N*-bromosuccinimide in the presence of a catalytic amount of 2,2′-azobisisobutyronitrile (AIBN), and subsequent introduction of the β-alanine side chain gave **6** in good yield. Hydrolysis of **6** with 1% aqueous NaOH and intramolecular cyclization using dehydrating reagent afforded the desired bicyclic derivative **3h**. The nitrile moiety in compounds **3a**–**h** was transformed into an amidine using conventional procedures[12] to afford desired compounds **7a**–**h** (Scheme 5).



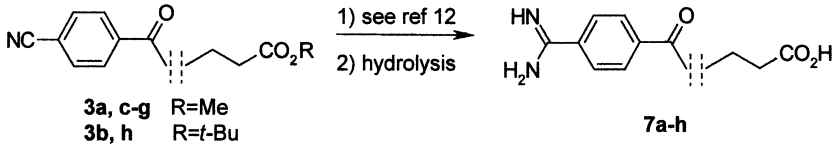
Scheme 3. ORTEP diagram of **5e**.

Results and Discussion

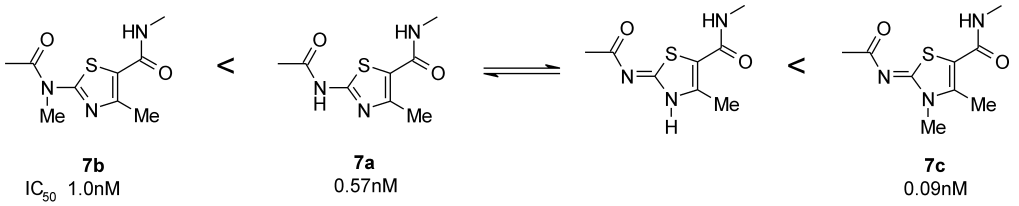
Evaluation of fibrinogen receptor binding ability of the compounds was performed using purified activated GPIIb/IIIa protein (ELISA)[13] and washed platelet (^{125}I-labelled fibrinogen).[14] First, according to the report by Alig et al., which indicated that the 1,3-disubstituted phenyl ring was suitable for this purpose,[15] we introduced a 2,5-disubstituted thiazole ring for a linkage of the terminal two moieties instead of phenyl ring. As shown in Table 1, the IC_{50} value of compound **7a** against Fbg-GPIIb/IIIa binding assay (0.57 nM) was almost equal to that of 1,3-disubstituted phenyl derivative **8** (0.55 nM), and about 2 times more potent in ^{125}I-labelled Fbg-WP binding assay (60.5 vs 105 nM). These data indicate that thiazole ring system is suitable for the linkage group for low-molecular weight GPIIb/IIIa antagonist. Introduction of methyl group on the nitrogen of the benzamide moiety reduced the activity by about half (**7b**: $IC_{50} = 1.0$ nM). This might be because of a loss of the ability for hydrogen bond donor to the binding site on the receptor, however, it could be due to the loss of rigidity of the linkage group. Thus, compound **7c** which possesses a methyl group at the nitrogen atom on the thiazoline ring and consequently lost



Scheme 4.

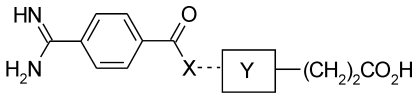


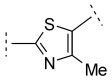
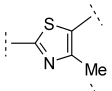
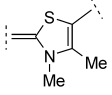
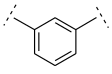
Scheme 5.



Scheme 6.

Table 1. Antiplatelet activity of compounds **7a**–**c**, **8** and Echistatin



Compds	X	Y	Fbg-GPIIb/IIIa ELISA[a] IC_{50} (nM)	^{125}I-Fg-WP[b] IC_{50} (nM)
7a	NH		0.57	60.5
7b	NMe		1.00	NT[c]
7c (PS-028)	N		0.090 ± 0.008	11.3
8[15]	NH		0.55	105
Echistatin			0.20	NT[c]

[a]Binding assay was performed with human fibrinogen (Fbg) versus purified activated GPIIb/IIIa[16] using ELISA method.
[b]Binding assay was performed with ^{125}I-labelled human Fbg versus washed platelet.
[c]NT = Not tested.

hydrogen bond donor at the benzamide moiety, still maintains the activity, and possesses 6-fold more potent (0.09 nM) activity than **7a**. Introduction of alkyl groups (**7d**,**e**) other than the methyl group on the 3-position of the thiazoline ring also increased activity, and even the introduction of the rather bulky benzyl group (**7f**) still maintained high potency (0.09 nM, Table 2). The substituents on the 3-position of the thiazole ring seemed to be widely tolerable, thus, it might act only to fix the *exo*-imino resonance form (Scheme 6).

Moreover, the rigid 2-acylimino thiazoline template of **7c** should fix two receptor-binding residues (an amidine and carboxyl group) to the suitable position to give a highly potent low-molecular weight GPIIb/IIIa antagonist. As in the case of a benzamide moiety at the 2-position of the thiazole ring, introduction of a methyl group on the nitrogen atom to the amide moiety at the 5-position of the thiazoline template of **7c** diminished the activity (**7g**, 0.89 nM). Substitution of the secondary amide group by a flexible tertiary amide group should result in the loss of the desired '*exo*' amide conformation. In fact, the '*endo*' amide derivative (**7h**), which had confirmed the amide moiety by ring-closure, showed dramatically reduced activity by about one-fifty magnitude (4.76 nM) as reported in the other templates.[17] We also investigated the relationship between IC_{50} value (ELISA) and concentration of fibrinogen. As shown in Figure 1, **7c** (PS-028) appeared to be a competitive inhibitor of fibrinogen-GPIIb/IIIa binding ($K_i = 46.5 \pm 5.8$ pM).[18] IC_{50} value of **7c** for ^{125}I-fibrinogen-washed human platelet binding was 11.3 nM which was almost the theoretical optimal value due to a large distribution of GPIIb/IIIa or the platelet membrane (up to 10^{-8} M in plasma),[19] and in the same concentration, **7c** strongly inhibits the ADP-induced platelet aggregation (IC_{50} for human PRP: 16.8 nM, Table 3).

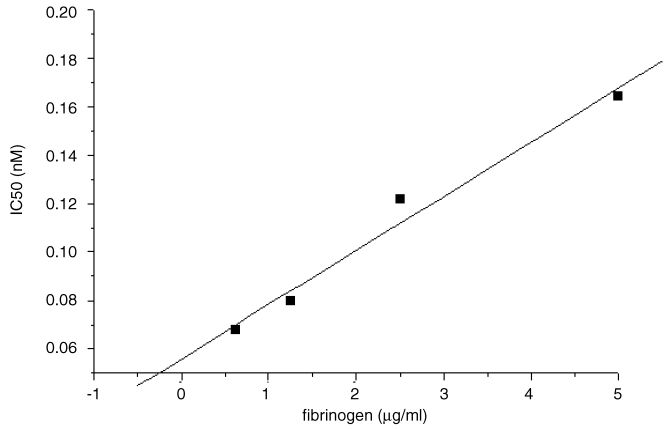
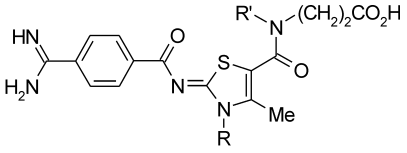
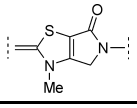


Figure 1. Relationship between IC_{50} value (ELISA, 0.4 μg/mL of GPIIb/IIIa) and concentration of fibrinogen (0.31–50.4 μg/mL).

Table 2. Antiplatelet activity of compounds **7c–h**



Compds	R	R′	Fbg-GPIIb/IIIa ELISA[a] IC_{50} (nM)	^{125}I-Fg-WP[bb] IC_{50} (nM)
7c	Me	H	0.09	11.3
7d	Et	H	0.12	NT[c]
7e	*n*-Bu	H	0.13	6.26
7f	Bn	H	0.09	NT[c]
7g	Me	Me	0.89	51.8
7h			4.76	67.9

[a–c]See Table 1.

Table 3. In vitro and ex vivo antiaggregation activities of compound **7c** (PS-028)

In vitro[a] (IC_{50}, nM)				Ex vivo[f] (iv, ED_{50}, mg/kg)
Human[b]	Mouse[c]	Dog[d]	Guinea pig[e]	
16.8	36.6	35.5	20.8	0.0063

[a]IC_{50} value for chemically induced aggregation of platelet-rich plasma monitored by Aggrecoder.[20]
[b]ADP (2 μM) was used as an inducer.
[c]ADP (5 μM) was used as an inducer.
[d]ADP (10 μM) and epinephrine (10 μM) were used as inducers.
[e]ADP (3 μM) was used as an inducer.
[f]ED_{50} value for ex vivo ADP (3 μM)-induced platelet aggregation of guinea-pig administered **7c** intravenously 30 min prior to the preparation of platelet-rich plasma.

Compound **7c** showed potent ex vivo antiaggregation activity in guinea pig (Table 3). Compound **7c** was found to be chemically and biologically stable (data not shown), and did not affect the RGD-mediated HUVEC adhesion[21] to extra cell matrix proteins (Table 4).

In conclusion, we have found a novel sterically constrained 2-imino-3*H*-thiazoline template for RGD peptide backbone mimics, and demonstrated a potent low molecular weight GPIIb/IIIa antagonist PS-028 (**7c**), which is suitable as a lead compound for orally active potent platelet aggregation inhibitors. Moreover, this unique template might be stable in chemical and biological conditions, it is of significance to use as the template for various kinds of pharmacological compounds.

Table 4. Selectivity for platelet fibrinogen receptor versus HUVEC receptor

Compds	IC_{50} for HUVEC adhesion IC_{50} (μM)[a] to plates coated with			
	Fibrinogen	Vitronectin	Laminin	Fibronectin
7c	>100	>100	>100	>100
echistatin	0.007	<0.001	0.01	0.1

[a]IC_{50} value for adhesion with immobilized HUVEC in the presence of each extra cell matrix.

Acknowledgements

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