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NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS BASED UPON A 4-SUBSTITUTED PIPERIDINE SCAFFOLD

Scott I Klein,* Bruce F Molino, Mark Czekaj, Jeffrey S Dener,
Robert J Leadley, Ralph Sabatino, Christopher T Dunwiddie, and Valeria Chu
Rhone-Poulenc Rorer, Departments of Medicinal Chemistry and Cardiovascular Biology, 500 Arcola Rd, Collegeville PA 19426

Abstract: Structure–activity relationships developed from work with peptide based fibrinogen receptor antagonists have been successfully applied to the development of simple and highly potent nonpeptide agents of the same class.

Introduction:

Thrombotic disorders such as unstable angina and myocardial infarction are to a large extent dependent upon the processes of platelet activation, adhesion and ultimately aggregation.[1] Platelet aggregation, in turn, depends upon the binding of the glycoprotein fibrinogen to its platelet membrane receptor glycoprotein IIb/IIIa (GpIIb/IIIa).[2] Expression of GpIIb/IIIa is the final common pathway of platelet aggregation regardless of the agonist involved and inhibition of the fibrinogen-GpIIb/IIIa interaction represents a well established intervention point in the treatment of thrombosis.[3] The binding of fibrinogen to GpIIb/IIIa is mediated by the tripeptide sequence Arg-Gly-Asp (RGD).[4] Small peptides containing this sequence are capable of blocking the fibrinogen-GPIIb/IIIa interaction and preventing subsequent platelet aggregation.[5] Among the more potent small peptides are the tetrapeptides RGDS and RGDF, both found in fibrinogen, and the tetrapeptide RGDV, found in vitronectin.

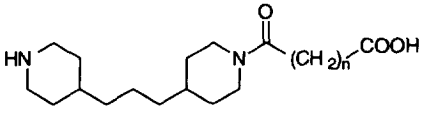
A wealth of information has become available in recent years describing the structure-activity relationships for fibrinogen receptor antagonists.[6] Whether one is discussing peptides, peptide mimetics, or nonpeptides, several key points are consistently realized. A basic functional group that mimics the side chain of the arginine residue in the RGDX tetrapeptides and a carboxylic acid to represent the aspartyl side chain are absolute requirements for activity. Some sort of lipophilic group proximal to the carboxylic acid, which can approximate the position of the side chain of the C-terminal amino acid of the tetrapeptides, generally enhances potency. The remainder of the molecule serves largely to hold these three functional groups in the proper spatial arrangement. The design of new fibrinogen receptor antagonists is therefore reduced to the problem of finding an appropriate scaffold to which a basic group, carboxylic acid, and lipophile can be appended in the appropriate orientation. This sort of approach, in which a central template has been used to properly orient the functional groups necessary for binding to the GPIIb/IIIa receptor, has been used successfully by others[7] to produce a variety of potent non-peptide fibrinogen receptor antagonists. In these approaches the central template has generally been of a highly constrained nature.

Results and Discussion:

All IC_{50} values reported are for the inhibition of fixed, activated human platelets.[8]

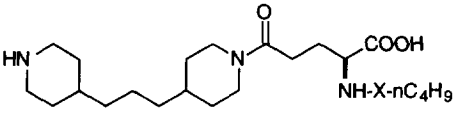
For this work, the commercially available trimethylene dipiperazine was chosen as a starting point. This molecule offers an advantage in that it contains both a basic functional group and a central template to which a carboxylic acid may be easily appended. Piperidine has been previously shown to be a reliable isostere for the guanidino group of arginine.[9] Additionally, the flexibility inherent in this system was anticipated to allow the proposed analogs to attain a conformation favorable for binding to the fibrinogen receptor.

A series of dicarboxylic acids was coupled with trimethylene dipiperazine to quickly give a group of compounds that displays the sensitivity to distance between the basic and acidic termini that is characteristic of fibrinogen receptor antagonists. The compound in which n = 3, the most potent of this group, was chosen as a basis for future analogs. The dicarboxylic acid used to produce this derivative, glutaric acid, corresponds to the amino acid glutamic acid. This allows for the ready addition, via glutamic acid derivatives, of alkyl groups that can mimic the side chains of the C-terminal amino acid found in the more potent RGD containing tetrapeptides (e.g., RGDV and RGDF).



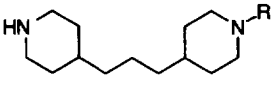
	n	$IC_{50}(\mu M)$
1	2	>25
2	3	3.0
3	4	6.7

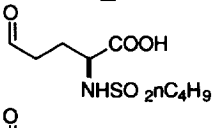
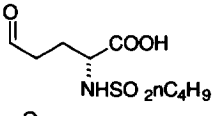
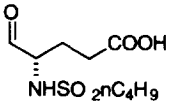
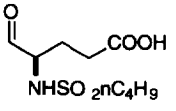
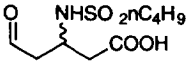
Planning to append these alkyl groups via the α-amino group of glutamic acid, the method of attachment was next explored. Sulfonamides are preferred to amides and both of these are much preferred to simple amines. Addition of an alkyl group to access the lipophilic binding site occupied by the C-terminal amino acid in the RGD tetrapeptides gives a 30-fold increase in potency.



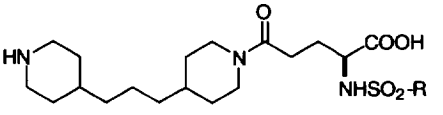
	X	IC_{50} (μM)
4	CH_2	8.4
5	CO	.25
6	SO_2	.11

Next the position and stereochemistry of the sulfonamide along the glutaric acid chain was examined. There is a clear preference for analogs prepared from L-glutamic acid (compare **7** and **8** or **9** and **10**), while position along the chain is of less importance (compare **7** and **9**).



	R	IC_{50} (μM)
7		.11
8		>25
9		.13
10		>25
11		.95

Finally, the nature of the alkyl group on the sulfonamide linkage was examined. Chain extension resulted in a small increase in potency, while conversion to an aryl sulfonamide gave a more significant enhancement. The para-*n*-propyl benzenesulfonamide (**18**) displayed an IC_{50} 10-fold greater than the parent *n*-butyl sulfonamide for inhibiting platelet aggregation.



	R	IC_{50} (μM)	IC_{50} for inhibition of ^{125}I Fg binding to activated human platelets (μM)[10]
12	n-C_4H_9	.11	.07
13	n-C_8H_{17}	.07	.13
14	Phenyl	.03	
15	1-Napthyl	.04	.01
16	2-Naphthyl	.04	.01
17	Benzyl	.02	.02
18	p-n-Propyl Phenyl	.01	.02
19	4-Biphenyl	.03	.03

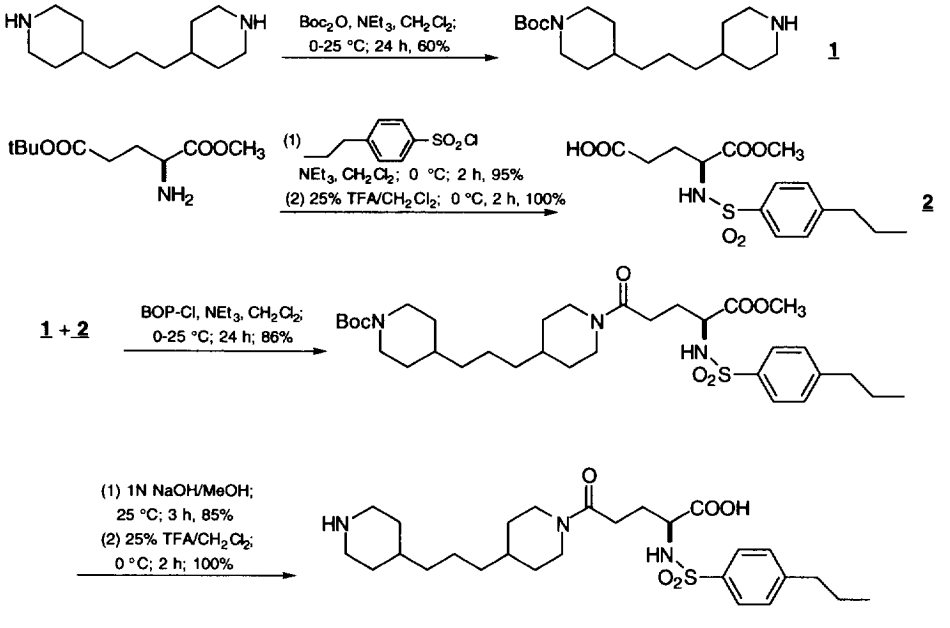
The *n*-butyl sulfonyl derivative (**12**) was tested in vivo in dogs in a canine model of coronary artery occlusion.[11] At a dose of 1 mg/kg (iv bolus) this compound completely abolished cyclic flow reductions, demonstrating the utility of these derivatives as antithrombotics.

In summary, a structurally simple, yet highly potent group of fibrinogen receptor antagonists have been prepared from readily available materials. This work illustrates the applicability of the structure–activity relationships that have been gleaned largely from work with peptides and peptide mimetics to the efficient design of novel nonpeptides.

Chemistry:

A representative example of the synthesis of one of the more potent compounds illustrated above is detailed below.

The synthesis is very straightforward and consists of coupling mono-Boc-protected trimethylene dipiperazine to a suitably protected glutamic acid residue via the side chain carboxyl group. Subsequent deprotection affords the desired product. Purification is accomplished via reverse phase HPLC using an acetonitrile/water gradient buffered with 0.1% TFA. Final products are obtained as fluffy solids after lyophilization.



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   Blood was obtained from human volunteers, all of whom had been free of any medications for at least 14 days prior to blood donation. In all cases the first 1–2 mL of blood obtained were discarded in order to avoid the traces of thrombin that have been shown to be generated during venipuncture. The remainder of each blood sample was mixed with 10% of its volume of a 3.8% sodium citrate solution. Gel filtered platelets were isolated following the procedures of Marguerie. For the preparation of fixed, activated platelets, washed platelets were activated with human α-thrombin (Enzyme Research Lab., South Bend, IN) at a final concentration of 1 U/mL for 2 min at room temperature, followed by the addition of the thrombin inhibitor I-2581 (Kabi, Pharmacia Harper, Franklin, OH) at a final concentration of 20 μM. To the activated platelets, paraformaldehyde (Sigma) was added to a final concentration of 0.5% and incubated for 30 min at room temperature. The fixed, activated platelets were then collected by centrifugation at 650 × *g* for 15 min. Platelet pellets were washed four times withTyrode's-HSA buffer and resuspended to 2×10^8 cells/mL in the same buffer.

   Platelet aggregation was performed using fixed, activated platelets according to the turbidometric method of Born. Various doses of a given compound were incubated with 0.4 mL of platelet suspension for 1 min and aggregation was initiated by the addition of fibrinogen (Calbiochem) to a final concentration of 250 μg/mL (0.72 μM). A platelet aggregation profiler model PAP-4 (Bio Data, Hatsboro, PA) was used to record platelet aggregation. The extent of inhibition of aggregation was expressed as the percent of the rate of aggregation observed in the absence of antagonists. The IC_{50}s were then calculated for each compound.
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    Fibrinogen (Kabi, Stockholm, Sweden) was purified according to Hawiger and Timmons and radioiodinated using a modification of the procedure of Fraker and Speck. Competitive binding assays were performed according to Hawiger and Timmons. Reactions were carried out in duplicate in Tyrode's buffer and 1×10^8 platelets/mL, 100 nM ^{125}I-fibrinogen and either 100 μM TRAP (SFLLRN-NH2, Peninsula Labs, Belmont, CA) or 10 μM ADP. When inhibitors of ^{125}I-fibrinogen binding were tested, both inhibitor and ^{125}I-fibrinogen were added prior to agonist addition. Following a 30 min incubation at room temperature, the reactions were layered onto a 20% sucrose cushion and centrifuged at $10{,}000 \times g$ for 3 min. The reaction tubes were frozen with liquid nitrogen and the tips of each tube clipped off and counted in a γ-counter.

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    Mongrel dogs (12–20 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg iv bolus, with supplements administered throughout the experiment, as needed), intubated and respired mechanically. A femoral artery catheter was inserted for monitoring blood pressure. A femoral vein catheter was inserted for administering intravenous saline (approximately 0.5 mL/min) and for administering drug and supplemental anesthesia.

    A left thoracotomy was performed at the level of the fifth intercostal space. The heart was suspended in a pericardial cradle and a 2 cm section of the left circumflex coronary artery was carefully isolated, tying off all branches in this section. An electromagnetic flow probe was placed around the vessel to monitor blood flow. A mechanical obstructer was placed around the vessel to create a site of critical stenosis. Endothelial cell and intimal smooth muscle cell injury were produced by compressing the vessel several times with vascular clamps. Under these conditions, platelets adhere to the damaged area of the vessel wall near the stenosis and aggregate until the vessel is completely occluded and blood flow through the vessel decreases to zero. The platelet rich thrombus is then dislodged by mechanical manipulation of the obstructer and blood flow is restored. A new wave of platelets adheres and the process is repeated, producing a characteristic, repetitive blood flow pattern referred to as cyclic flow reductions (CFRs). The number of CFRs per unit time is used to quantitate antithrombotic effects.

    Consistent CFRs were established for a control period of at least 20 min. The drug was then administered iv as a bolus injection of 1 μg/kg and the number of CFRs over the next three 20 min periods were recorded.

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