Solid-Phase Synthesis of a Nonpeptide RGD Mimetic Library: New Selective αv*β*3 Integrin Antagonists

Gábor A. G. Sulyok,[†] Christoph Gibson,[†] Simon L. Goodman,[‡] Günter Hölzemann,[§] Matthias Wiesner,[§] and Horst Kessler*[,†]

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching, Germany, Department of Immunology/Oncology, Merck KGaA, Frankfurter Strasse 250, D-64271 Darmstadt, Germany, and Department of Medicinal Chemistry, Merck KGaA, Frankfurter Strasse 250, D-64271 Darmstadt, Germany

Received November 21, 2000

The solid-phase synthesis of a low molecular weight RGD mimetic library is described. Activities of the compounds in inhibiting the interaction of ligands, vitronectin and fibrinogen, with isolated immobilized integrins αv*β*3 and αIIb*β*3 were determined in a screening assay. Highly active and selective nonpeptide αv*β*3 integrin antagonists with regard to orally bioavailability were developed, based on the aza-glycine containing lead compound **1**. An important variation is the substitution of the aspartic amide of **1** by an aromatic residue. Furthermore, different guanidine mimetics have been incorporated to improve the pharmacokinetic profile. Exchange of the *β*-amino acid NH by a methylene moiety in one set of RGD mimetics leads to the azacarba analogue compounds representing a novel peptidomimetic approach, which should increase the metabolic stability.

Introduction

Integrins are a family of heterodimeric transmembrane cell surface receptors that are involved in cell−cell and cell−matrix adhesion processes. Currently, more than 20 different integrin receptors have been identified consisting of various combinations of at least 17 α- and 9 *β*-subunits.[1] In the past decade the integrins of the *β*3-subfamily have been the focus of drug discovery research.[2−4] At the beginning most of the attention was directed toward the platelet integrin αIIb*β*3, also known as the fibrinogen receptor.[5] More recently, the vitronectin receptor αv*β*3, which is expressed on almost all cells originating from the mesenchyme, has received increasing attention.[6] This receptor represents an interesting therapeutic target because of its important role in pathologies as diverse as osteoporosis,[7,8] restenosis following percutaneous transluminal coronary angioplasty (PTCA),[9,10] acute renal failure,[6d,11] ocular diseases,[12,13] tumor-induced angiogenesis,[14,15] and metastasis formation.[16]

The tripeptide sequence RGD (Arg-Gly-Asp) is a common cell-recognition motif, which is part of integrin binding ligands, like fibronectin, fibrinogen, and vitronectin. It is responsible for the binding to the abovementioned integrin receptors.[6a,17] This sequence has been used as a lead for developing different integrin antagonists. First, the RGD sequence was incorporated into various linear and cyclic peptides.[18−25] One decade ago, we reported about the first selective small molecule antagonist of the αv*β*3 receptor, the cyclic pentapeptide c(−RGDfV−).[18,23,26,27] Systematic derivatization of this peptide resulted in the N-alkylated cyclopeptide c(−RGDf[NMe]V−),[28] which has entered clinical phase II studies as angiogenesis inhibitor (code EMD 121974, Merck KGaA). Furthermore, regression of primary tumors and eradication of micrometastases have been reported by combining a specific antiangiogenic therapy with our cyclopeptide and an immunotherapy with a tumor-specific antibody−interleukin 2 fusion protein.[29]

More recently, we[30,31] and other groups[32−46] focused on the development of selective nonpeptide αv*β*3 integrin antagonists. Because of enhanced metabolic stability, bioavailability, and biological absorption of aza-amino acid containing peptides,[47,48] we developed aza-glycine containing RGD mimetics. We have shown that glycine can be replaced by aza-glycine in RGD-containing cyclopeptides with preservation of the biological activity and selectivity.[49] A novel peptidomimetic approach is the substitution of the glycine or aza-glycine amide bond into the azacarba analogue, which is shown in Figure 1. This *azacarba* nomenclature should not be mixed with the *carbaza* expression for aza-peptides without one amide moiety, which was first coined by Limal et al.[50]

In the past decade combinatorial chemistry has received increasing attention as an important tool in drug discovery and development.[51] Here, we describe the solid-phase synthesis of a low molecular weight RGD mimetic library based on the aza-glycine containing compound **1**. The aza-RGD mimetic **1** was identified by an on-bead screening assay of a combinatorial library and shows already good affinity and selectivity toward the αv*β*3 integrin receptor (IC_{50}: 150 nM).[52,31] Regarding Pfizer's "Rule of 5" for orally available drugs (M < 500 g/mol, cLogP < 5 (cLogP is the logarithm of the partition coefficient for *n*-octanol/water), number of H-bond donors < 5, number of H-bond acceptors < 10, substrates of biological transporters are exceptions),[53] the resorption profile of the hydrophilic compound **1** had to be improved. To enhance the lipophilicity, we decided to furnish our RGD mimetics with a lipophilic residue.

* To whom correspondence should be addressed. Phone: ++49-89-2891 3301. Fax: ++49-89-2891 3210. E-mail: kessler@ch.tum.de.
[†] Institut für Organische Chemie and Biochemie.
[‡] Department of Immunology/Oncology.
[§] Department of Medicinal Chemistry.

10.1021/jm0004953 CCC: $20.00
Published on Web 05/05/2001

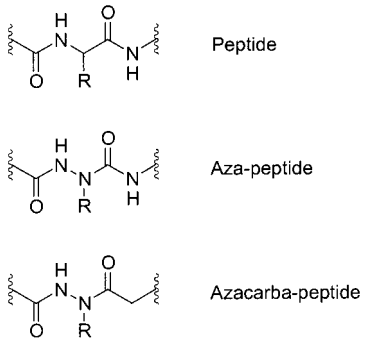


Figure 1. Comparison of peptide, aza-peptide, and azacarba peptide.

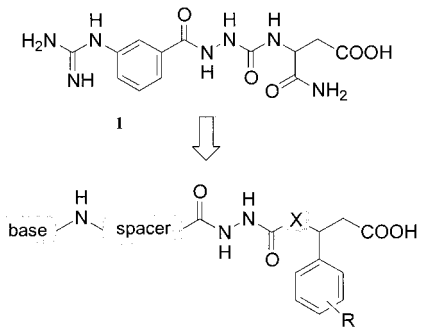


Figure 2. Aza-RGD mimetics with various aromatic β-amino acids (X = NH) or glutaric acids (X = CH_2) and different guanidine mimetics derived from compound **1**.

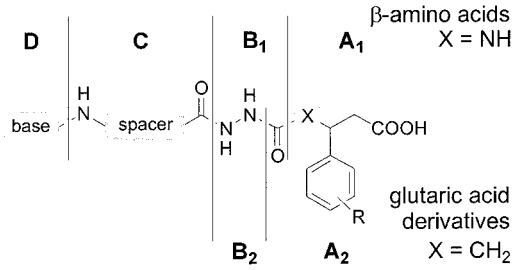


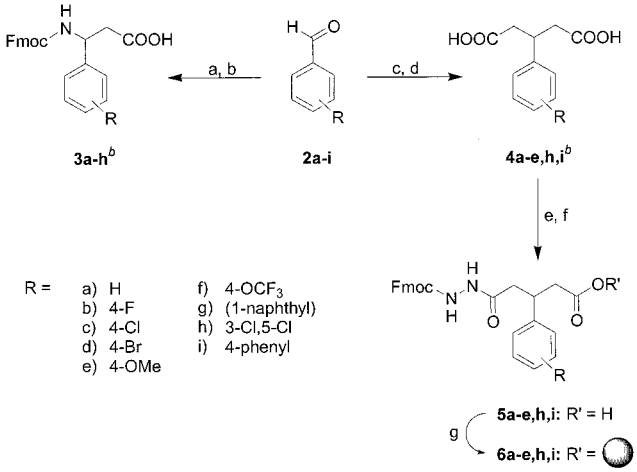
Figure 3. Retrosynthetic analysis of the RGD mimetic library obtaining four different building blocks: carboxylic acid $\mathbf{A_1}$ (β-amino acids) and $\mathbf{A_2}$ (glutaric acids), $\mathbf{B_1}$ (aza-glycine) and $\mathbf{B_2}$ (hydrazine), spacer **C**, and basic building block **D**.

The synthesized RGD mimetics include various β-amino acids (X = NH) or glutaric acids (X = CH_2) and different guanidine mimetics to improve the pharmacokinetic profile (Figure 2).

Chemistry

Previous results confirm that the essential pharmacophores of RGD mimetics for the binding to the above-mentioned integrins are the carboxylic acid and a guanidine-like functional group fixed at a certain distance.[6,54] We synthesized a library of RGD mimetics combining various carboxylic acid building blocks and different guanidine mimetics. Retrosynthetic analysis suggests a modular design of the two sets of RGD mimetics consisting of four building blocks **A**−**D** (Figure 3). For the carboxylic building block $\mathbf{A_1}$ of the first set (X = NH), different β-amino acids were used, which are known to mimic the aspartic acid in αvβ3 and αIIbβ3 antagonists.[4] The aza-glycine is then a good choise as building block $\mathbf{B_1}$. In the second set of RGD mimetics—the azacarba analogues—the NH of the β-amino acid is replaced by a methylene (X = CH_2). Thus, we decided to use glutaric acid derivatives as carboxylic building block $\mathbf{A_2}$ and hydrazine as building block $\mathbf{B_2}$ for the azacarba RGD mimetics.

Scheme 1[a]



[a] Reagents: (a) NH_4OAc, malonic acid, EtOH (74−88%); (b) Fmoc-Cl, $NaHCO_3$, dioxane (76−98%); (c) ethyl acetoacetate, piperidine (cat.) (42−85%); (d) 20 M KOH, 85 °C (68−99%); (e) acetic anhydride (62−89%); (f) *N*-Fmoc-hydrazine,[58] THF (100%); (g) TCP resin, DIEA, CH_2Cl_2. [b] Compounds **3d**, **3g**, and **4a** were purchased from commercially available sources.

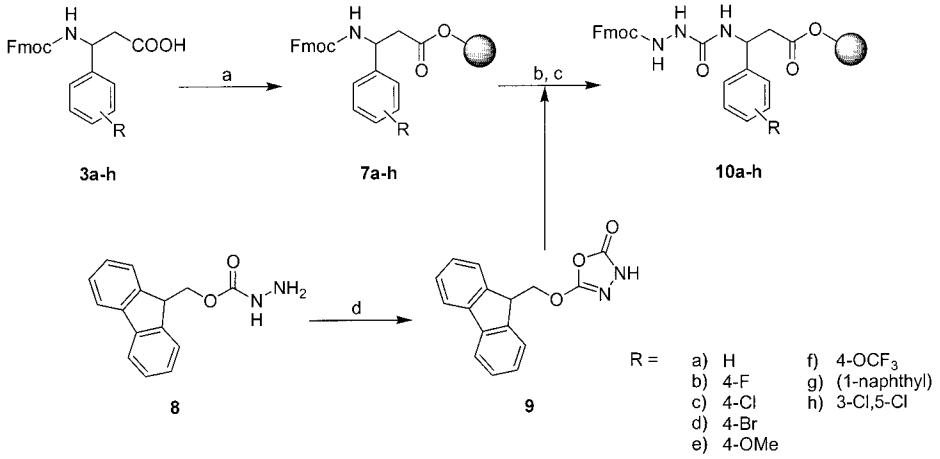
Building block **C** acts as an aromatic (3-aminobenzoic acid) or aliphatic spacer (5-aminopentanoic acid) for the guanidino building block **D**. Replacement of the guanidine by less basic functional groups is an alternative strategy to obtain more lipophilic compounds. Therefore, besides guanidine itself, methylamidine and 2-aminopyridine, which was shown to be a suitable guanidine mimetic,[39] were used as basic building block **D**. In the library, guanidine and methylamidine were incorporated in combination with an aromatic spacer, whereas aminopyridine was attached to an aliphatic spacer.

Synthesis of the RGD mimetics was performed on solid support by the Fmoc strategy, which is commonly used in solid-phase peptide synthesis.[55] First, we synthesized the different Fmoc-protected building blocks. Since benzaldehydes are commercially available in rich diversity, they are particularly suitable educts for the syntheses of the carboxylic building blocks **A**. Furthermore, they can be used for both the syntheses of β-amino acids ($\mathbf{A_1}$) and glutaric acids ($\mathbf{A_2}$) (Scheme 1).

Reaction of the benzaldehydes **2a**−**h** with ammonium acetate and malonic acid resulted in the corresponding racemic β-amino acids, which could be transformed into the Fmoc-protected derivatives **3a**−**h** in high yields. The 3-phenyl-substituted glutaric acids **4a**−**e,h,i** were synthesized by reaction of the benzaldehydes **2a**−**e,h,i** with ethyl acetoacetate and catalytic amounts of piperidine, followed by hydrolysis with aqueous KOH. The glutaric acids **4a**−**e,h,i** were converted into the cyclic anhydrides with acetic anhydride. Subsequently, ring opening with Fmoc-protected hydrazine afforded the 5-(*N*′-Fmoc-hydrazyl)-5-oxo-3-phenylpentanoic acids **5a**−**e,h,i** as a racemic mixture.

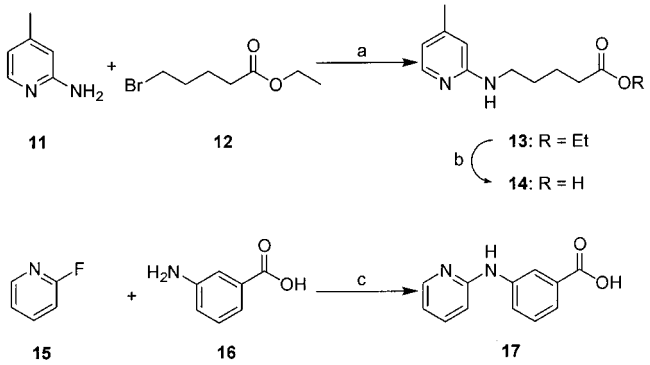
The Fmoc-protected carboxylic building blocks **3a**−**h** and **5a**−**e,h,i** were attached to trityl chloride polystyrol (TCP) resin using standard conditions. Different routes have been reported for the following incorporation of an

Scheme 2[a]



[a] Reagents: (a) TCP resin, DIEA, CH_2Cl_2; (b) 20% piperidine/DMF; (c) 5-(9*H*-fluoren-9-ylmethoxy)-1,3,4-oxadiazol-2(3*H*)-one (9);[30] (d) phosgene (1.9 M solution in toluene), sat. $NaHCO_3$, CH_2Cl_2 (85%).[30]

Scheme 3[a]



[a] Reagents: (a) 100 °C (23%); (b) 1 N NaOH (64%); (c) NaH, DMF, 80 °C (10%).

aza-glycine on solid support.[56,57] Recently, we reported an improved approach for the synthesis of activated Fmoc-protected aza-amino acids and their reaction with resin-bound amines.[30] *N*-Fmoc-hydrazine (**8**)[58] was carbonylated with an excess of a solution of phosgene in toluene to obtain the activated aza-glycine building block 5-(9*H*-Fluoren-9-ylmethoxy)-1,3,4-oxadiazol-2(3*H*)-one (**9**)[30] (Scheme 2). After Fmoc deprotection the resin-bound β-amino acids **7a**−**h** were treated with excess oxadiazolone (**9**) to yield the resin-bound Fmoc-protected aza-Gly-β-amino acid derivatives **10a**−**h**.

The following step of the solid-phase synthesis is the coupling of the spacer building block **C**, where *N*-Fmoc-protected 3-aminobenzoic acid and 5-aminopentanoic acid were used. In contrast to guanidine or amidine as building blocks **D**, which are coupled on solid support, 2-aminopyridine was first linked to the spacer molecule **C** in solution (Scheme 3). 2-Amino-4-methylpyridine (**11**) was treated with 5-bromopentanoic acid ethyl ester (**12**) at 100 °C to yield 5-(4-methylpyridine-2-yl)aminopentanoic acid ethyl ester (**13**). After hydrolysis with aqueous NaOH, building block **CD** 5-(4-methylpyridine-2-yl)aminopentanoic acid (**14**) was obtained by precipitation. The combination of aminopyridine and an aromatic spacer building block **C** was not used in library synthesis but in further synthesis of single compounds. Deprotonation of 3-aminobenzoic (**16**) acid with sodium hydride followed by addition of 2-fluoropyridine (**15**) afforded this building block combination **CD**, compound **17** in low yield. After deprotection of the resin-bound Fmoc-protected aza-compounds **6a**−**e,h,i** (X = CH_2) and **10a**−**h** (X = NH) with piperidine, the spacer building blocks **C** and **CD** were coupled using standard solid-phase coupling conditions (Scheme 4).

Solid-phase synthesis was completed by introduction of the basic building block **D**. Syntheses of the guanidine-containing mimetics were performed by treatment of the resin-bound amines **18a**−**e,h,i** (X = CH_2) and **19a**−**h** (X = NH) with *N,N′*-bis-Boc-1-guanylpyrazole[59] at 50 °C. After guanylation the resulting resin-bound aza-RGD mimetics were deprotected and cleaved from the resin in one step using trifluoroacetic acid. Treatment of the resin-bound amines **18a**−**e,h,i** (X = CH_2) with *S*-2-naphthylmethyl thioacetimidate hydrobromide[60] in the presence of the base DIEA and cleavage from the resin with acetic acid yielded the corresponding methylamidine containing aza-RGD mimetics **21a**−**e,h,i** (X = CH_2). By use of the same cleavage procedure, the aminopyridine-containing aza-RGD mimetics **23a**−**e,h,i** (X = CH_2) and **24a**−**h** (X = NH) were obtained.

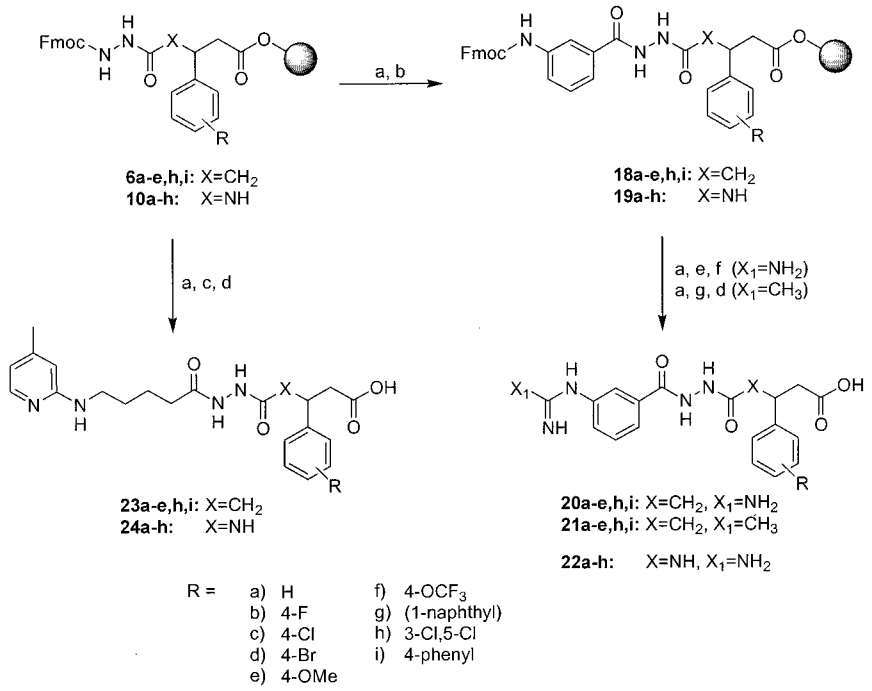
In addition, single compounds that are not included in the library were synthesized. Compounds **25** and **26** represent other combinations of the building blocks **C** and **D**, whereas compound **27** is an RGD mimetic without the aromatic residue of the carboxylic building block **A**.

Synthesis of compound **25** was performed on a solid support in analogy to compound **22a**. Resin-bound *N*-Fmoc-aza-Gly-β-phenylalanine (**10a**) was treated with 5-(*N*-Fmoc)aminopentanoic acid instead of 3-(*N*-Fmoc)-aminobenzoic acid as spacer building block **C**. After Fmoc deprotection, guanylation, and cleavage from the resin, compound **25** was achieved. Replacement of β-phenylalanine with β-alanine in the synthesis of compound **24a** resulted in compound **26**. Compound **27** was obtained by treating resin-bound 5-(*N*-Fmoc-hydrazino)-3-(3,5-dichlorophenyl)-5-oxopentanoic acid (**6h**) with the combined building block **CD**, 3-(pyridine-2-yl)aminobenzoic acid (**17**), and cleavage from the resin, in analogy to compound **23h**.

Results and Discussion

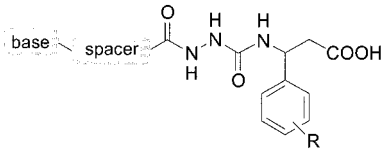
Biological Evaluation. Screening assays were performed by measuring the effect of RGD mimetics on the

Scheme 4[a]



[a] Reagents: (a) 20% piperidine/DMF; (b) 3-(*N*-Fmoc-amino)benzoic acid, HATU, collidine, DMF; (c) 5-(4-methylpyridine-2-yl)amino-pentanoic acid (14), HATU, collidine, DMF; (d) AcOH/TFE/CH_2Cl_2 (1:1:3); (e) *N,N′*-bis-Boc-1-guanylpyrazole,[59] $CHCl_3$, 50 °C; (f) 95% TFA/5% TIPS; (g) *S*-2-naphthylmethyl thioacetimidate hydrobromide,[60] DIEA, NMP.

Table 1. Effect of Substituent R and Basic Pharmacophore on IC_{50}[a] of Aza-RGD Mimetics



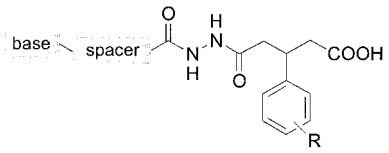
compd	R	base	spacer	cLogP	αvβ3 IC_{50} [nM]	αIIbβ3 IC_{50} [nM]
GRGDSPK[b]					1000	$>10^4$
c(RGDfV)[b]					2	850
1	(carboxamide)[c]	guanidine	*m*-C_6H_4−	−2.42	150	$>10^4$
25	none	guanidine	ω-C_4H_8	−0.26	40	1000
22a	none	guanidine	*m*-C_6H_4−	0.70	0.8	8500
22b	4-F	guanidine	*m*-C_6H_4−	0.86	6	1000
22c	4-Cl	guanidine	*m*-C_6H_4−	1.26	0.1	5500
22d	4-Br	guanidine	*m*-C_6H_4−	1.53	1	>1000
22e	4-OCH_3	guanidine	*m*-C_6H_4−	0.58	0.9	>1000
22f	4-OCF_3	guanidine	*m*-C_6H_4−	2.23	3	>1000
22g	(1-naphthyl)[c]	guanidine	*m*-C_6H_4−	1.70	27	$>10^4$
22h	3-Cl,5-Cl	guanidine	*m*-C_6H_4−	1.82	1	>1000
26	(H)[c]	2-NH_2-pyridine	ω-C_4H_8−	−0.07	1400	n.d.
24a	none	2-NH_2-pyridine	ω-C_4H_8−	1.64	37	$>10^4$
24b	4-F	2-NH_2-pyridine	ω-C_4H_8−	1.80	48	$>10^4$
24c	4-Cl	2-NH_2-pyridine	ω-C_4H_8−	2.20	0.3	7000
24d	4-Br	2-NH_2-pyridine	ω-C_4H_8−	2.47	7	n.d.
24e	4-OCH_3	2-NH_2-pyridine	ω-C_4H_8−	1.52	42	$>10^4$
24f	4-OCF_3	2-NH_2-pyridine	ω-C_4H_8−	3.17	130	n.d.
24g	(1-naphthyl)[c]	2-NH_2-pyridine	ω-C_4H_8−	2.64	140	n.d.
24h	3-Cl,5-Cl	2-NH_2-pyridine	ω-C_4H_8−	2.76	80	$>10^4$

[a] n.d. = not determined. [b] The peptides linear GRGDSPK and cyclo(RGDfV) were used as reference in the receptor binding assay. [c] Instead of substituted phenyl ring.

interaction between immobilized integrin receptors and biotinylated soluble ligands. The ability of RGD mimetics to inhibit the binding of vitronectin and fibrinogen to the isolated, immobilized αvβ3 and αIIbβ3 receptors was compared with that of the standard peptides GRGDSPK and c(RGDfV). The results of the screening assays are shown in Tables 1 and 2.

Chemistry. The synthesized library is derived from the structure of the aza-RGD mimetic **1**, which has been recognized by an on-bead screening assay.[31] An important variation of lead compound **1** was replacement of the aspartic amide with an aromatic residue of a β-amino acid (compounds **22a**−**h**). Different studies have pointed out aromatic β-amino acids to be suitable

Table 2. Effect of Substituent R and Basic Pharmacophore on IC_{50}[a] of Azacarba RGD Mimetics



compnd	R	base	spacer	cLogP	αvβ3 IC_{50} [nM]	αIIbβ3 IC_{50} [nM]
20a	none	guanidine	*m*-C_6H_4−	1.13	15	>10^4
20b	4-F	guanidine	*m*-C_6H_4−	1.28	4	>1000
20c	4-Cl	guanidine	*m*-C_6H_4−	1.68	2	>1000
20d	4-Br	guanidine	*m*-C_6H_4−	1.95	2	>1000
20e	4-OCH_3	guanidine	*m*-C_6H_4−	1.00	1	>1000
20h	3-Cl, 5-Cl	guanidine	*m*-C_6H_4−	2.24	2	1000
20i	4-phenyl	guanidine	*m*-C_6H_4−	2.80	0.7	1000
21a	none	methylamidine	*m*-C_6H_4−	1.44	23	>10^4
21b	4-F	methylamidine	*m*-C_6H_4−	1.60	160	>1000
21c	4-Cl	methylamidine	*m*-C_6H_4−	2.00	160	8000
21d	4-Br	methylamidine	*m*-C_6H_4−	2.27	40	>1000
21e	4-OCH_3	methylamidine	*m*-C_6H_4−	1.31	37	>1000
21h	3-Cl, 5-Cl	methylamidine	*m*-C_6H_4−	2.55	6	n.d.
21i	4-phenyl	methylamidine	*m*-C_6H_4−	3.11	26	>1000
23a	none	2-NH_2-pyridine	ω-C_4H_8−	2.07	7	>10^4
23b	4-F	2-NH_2-pyridine	ω-C_4H_8−	2.23	35	n.d.
23c	4-Cl	2-NH_2-pyridine	ω-C_4H_8−	2.63	15	n.d.
23d	4-Br	2-NH_2-pyridine	ω-C_4H_8−	2.90	6	9000
23e	4-OCH_3	2-NH_2-pyridine	ω-C_4H_8−	1.94	22	>10^4
23h	3-Cl,5-Cl	2-NH_2-pyridine	ω-C_4H_8−	3.19	9	>10^4
27	3-Cl,5-Cl	2-NH_2-pyridine	*m*-C_6H_4−	3.54	210	n.d.
23i	4-phenyl	2-NH_2-pyridine	ω-C_4H_8−	3.74	3	3000

[a] n.d. = not determined.

building blocks in potent RGD mimetics.[4] It is known that benzene interacts with amide bonds to form a complex that results in the so-called ASIS (aromatic solvent induced shift) effect.[61] Thus, we assume that aromatic residues substitute an amide bond in a favorable manner. The analogue compound **22a** containing β-phenylalanine shows a 200-fold increase in activity to the vitronectin receptor αvβ3 (IC_{50}, 0.7 nM) while preserving selectivity for the platelet receptor αIIbβ3. Different substitutions, especially at the para position of the phenyl ring, have been investigated (Table 1). All of the synthesized guanidine-containing aza-RGD mimetics **22a**−**h** are αvβ3 antagonists with excellent IC_{50} values in the nanomolar or subnanomolar range (compound **22c**). Compound **22c** with the highest affinity (IC_{50}, 0.1 nM) is about 2 orders of magnitude more active than the naphthyl derivative **22g**. Similar glycine-containing compounds synthesized by Searle/Monsanto show comparable affinity to the vitronectin receptor, but the specifitiy toward the fibrinogen receptor αIIbβ3 is about 1−2 log units smaller.[8] The importance of the meta-substituted aromatic spacer moiety could be shown by exchange with an alkyl chain, compound **25**. Because of higher flexibility of the alkyl spacer, a 50-fold decrease in αvβ3 receptor activity was observed.

As an indicator for lipophilicity of the synthesized compounds, we determined the calculated log *P* values (cLogP: estimation of the logarithm of the partition coefficient for *n*-octanol/water)[62] using a computer algorithm. Replacement of the lead compound amide with an aromatic ring resulted in a 3 order increase of cLogP. Despite enhancement of the lipophilicity by incorporation of the aromatic ring, the guanidine-containing compounds are still hydrophilic. In trying to improve the resorption profile, the polar guanidino moiety was replaced with a less basic aminopyridine moiety, combined with the alkyl spacer, in another set of aza-RGD mimetics (compounds **24a**−**h** and **26**). These mimetics are still very potent and highly specific antagonists of the αvβ3 integrin receptor with IC_{50} values in the nanomolar range. In comparison with the analogue guanidine-containing compounds, they show a slight (5- to 50-fold) decrease in the activity, while cLogP is increased by about 1 magnitude. The 4-chloro-substituted compound **24c** represents an exception with subnanomolar activity as well (IC_{50}, 0.3 nM). Since replacement of the basic pharmacophore does not influence the activity toward the platelet receptor αIIbβ3, further investigations of these selective RGD mimetics regarding the pharmacokinetic profile are promising. Both of the two basic pharmacophores, guanidine and aminopyridine, used in this set of RGD mimetics are suitable arginine mimetics when combined with the appropriate spacers.

Deletion of the aromatic residue of the β-amino acid, compound **26**, led to a 3 order decrease of activity to the αvβ3 receptor (IC_{50}, 1400 nM). This observed effect confirms the importance of the aromatic residue (or amide) in proximity to the carboxylic acid for a high binding affinity to the vitronectin receptor αvβ3.

Another series of compounds, the azacarba set, was synthesized to investigate the influence of the β-amino acid NH, which was replaced with a methylene moiety. We hope that this exchange of the amide bond will induce an improved metabolic stability of the RGD mimetics. The IC_{50} values of the obtained azacarba set of RGD mimetics (X = CH_2) are shown in Table 2. Analogue azacarba RGD mimetics have been synthesized with similar phenyl substitution patterns to allow comparison with the above-mentioned corresponding

aza-RGD mimetics. The guanidine-containing compounds **20a**−**e,h,i** have excellent affinities to the αvβ3 receptor in the nanomolar and subnanomolar ranges (compound **20i**) and high selectivities (low affinities) toward the platelet receptor αIIbβ3. Except for the unsubstituted compound **20a** showing a 200-fold decrease in activity, only small differences in IC_{50} values are observed in comparison with those of aza- and the analogous azacarba RGD mimetics (**22a**−**e,h** and **20a**−**e,h**), while cLogP values are slightly increased.

Reduction of basicity by replacement of guanidine with methylamidine, compounds **21a**−**e,h,i**, led to a further slight increase of cLogP while losing activity to the αvβ3 receptor (20- to 100-fold). These results suggest a binding contribution of the guanidine NH_2 moiety to the vitronectin receptor αvβ3. Only the high affinity of the 3,5-dichloro-substituted compound **21h** (IC_{50}, 6 nM) could be preserved. Exchange of the basic building block **D** by an aminopyridine, compound **27**, resulted in a 40- to 100-fold decrease in activity (IC_{50}, 210 nM) compared with the analogue 3,5-dichloro-substituted compounds **20h** (IC_{50}, 2 nM) and **21h** (IC_{50}, 6 nM). By analogy to the aza-RGD mimetics, aminopyridine has been used as a basic pharmacophore in combination with the pentanoic acid spacer. The obtained compounds **23a**−**e,h,i** also show high affinities to the αvβ3 receptor (IC_{50}, 4−35 nM) like the guanidine-containing azacarba RGD mimetics. The compounds are slightly more active than the corresponding aza-RGD mimetics **24a**−**h** (X = NH), except for the 4-chloro-substituted derivative **23c** (IC_{50}, 15 nM) with a 50-fold smaller activity compared to **24c**.

Although the aromatic spacer **C** is an important factor in guanidine-containing compounds, a decrease in activity resulted in the case of aminopyridine-containing RGD mimetics (compound **27**). The principle of additivity, which is often used in drug design, fails in this case. The reason might be the nonplanarity of the pyridine−benzene system, preventing the simple addition of individual effects. Therefore, the best combinations of building blocks **C** and **D** are arylguanidine and alkylaminopyridine.

When the two different sets of RGD mimetics (aza and azacarba) are compared, small differences in activity on the vitronectin receptor αvβ3 and in selectivity toward the platelet receptor αIIbβ3 can be observed. The amine of the β-amino acid appears to be less important for binding to the αvβ3 receptor. The aminopyridine-containing azacarba compounds show the highest cLogP values in the range 1.6−3.2 and are supposed to be compounds with enhanced metabolic stability, suitable for further resorption studies. Though nearly all compounds are very potent αvβ3 integrin antagonists (nanomolar IC_{50} values), the best results were achieved by 4-chloro substitution, compounds **22c** (IC_{50}, 0.1 nM) and **24c** (IC_{50}, 0.3 nM), as well as 4-phenyl substitution, compound **20i** (IC_{50}, 0.7 nM).

Since all compounds of the library were synthesized as racemic mixtures, the activities of the RGD mimetics can be further improved by synthesis of the pure *S*-enantiomers as shown by several groups.[4]

Conclusions

This work represents a further step in the development of highly specific, small nonpeptidic compounds with promising properties for oral bioavailability starting from the known linear RGD sequence. Our approach combines rational and combinatorial drug design to inhibit protein−protein interactions presented in Table 3. A peptidic anticancer drug (clinical studies phase II) was obtained by cyclization and optimization of the linear RGD sequence.[28] Further investigations led to aza-glycine-containing cyclic peptides and a library of aza-RGD mimetics. Optimization of the lead compound **1** with regard to an improved resorption profile is described in this work. The key step was the substitution of an amide bond by an aromatic residue, resulting in a 2 orders of magnitude increase in activity toward the vitronectin receptor αvβ3.

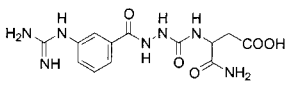
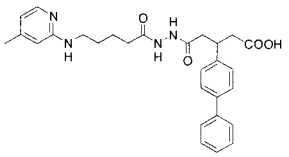
On the basis of the lead compound **1**, a library of RGD mimetics consisting of four building blocks **A**−**D** has been synthesized. Two sets of mimetics have been investigated. In the first set, β-amino acids have been used as carboxylic building block $\mathbf{A_1}$, whereas the second set—the azacarba analogues—represents a novel peptidomimetic approach with glutaric acids as carboxylic building block $\mathbf{A_2}$. These azacarba RGD mimetics with the amine of the β-amino acid substituted by a methylene moiety were designed to improve metabolic stability.

All of the guanidine-containing compounds show excellent affinities to the αvβ3 receptor in the nanomolar range. To increase lipophilicity of the RGD mimetics, the guanidino moiety has been substituted by methylamidine and aminopyridine as basic building block **D**. In particular, incorporation of the less basic aminopyridine led to RGD mimetics with promising properties for oral bioavailability while keeping nanomolar activity. Pharmacokinetic studies of these compounds are in progress. All compounds show little or no activities to the platelet receptor αIIbβ3 (IC_{50} > 10 000 nM), so we have obtained highly specific αvβ3 integrin antagonists (selectivities > 1000). Further investigations of activities and selectivities toward other integrin receptors, like αvβ5 and αvβ6, will be done. In summary, we have synthesized a library of highly active and selective RGD mimetics with promising properties for further pharmacokinetic investigations.

Experimental Section

General. Trityl chloride polystyrol (TCP) resin (0.94 mmol/g) was purchased from PepChem. Coupling reagents and amino acid derivatives were purchased from Advanced ChemTech, Perseptive Biosystems GmbH, Neosystem, and PurChem. All other reagents and solvents were purchased from Merck-Schuchardt, Lancaster, Aldrich, and Fluka and were used as received. The solvents ethyl acetate, ethanol, and methanol were distilled before use. Standard syringe techniques were applied for transferring dry solvents. Reactions on solid support were performed in filter columns (5 and 10 mL) from Abimed. Melting points (mp) were determined on a Büchi 510 apparatus and were uncorrected. ^{1}H spectra were obtained in $CDCl_3$ or DMSO-d_6 as solvent and internal reference on a Bruker AC250 or DMX500 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J* values) are given in hertz (Hz). Thin-layer chromatography was performed on silica gel 60 F_{254} plates from Merck. Flash chromatography was performed on silica gel 60 (230−400 mesh ASTM) from Merck. Reversed-phase HPLC analyses and separations were conducted on Amersham Pharmacia Biotech instruments using Omnicrom YMC columns (analytical, 4.6 mm × 25 mm, 5 μm C_{18}, 1 mL/

Table 3. Combination of Rational and Combinatorial Drug Design to Inhibit Protein−Protein Interactions

Step	Structure	IC_{50} ($\alpha v\beta 3$)	Sel.[a]	Feature	Ref.
Identification of binding sequence	linear GRGDSPK peptide ⇓	210 nM	8	active, nonselective	1
Cyclization and 'spatial screening'	c(RGDfV) ⇓	2 nM	415	superactive, $\alpha v\beta 3$ selective	18,23
Optimization	c(RGDf[NMe]V) ⇓	0.6 nM	1460	peptidic drug in clinical phase II	28
Peptidomimetics	c(RazaGDf[NMe]V) ⇓	6 nM	270	kinked structure, induced by aza-Gly	49
Linear aza-Gly RGD mimetics (combinatorial library)	 ⇓	150 nM	>70	active and selective, nonpeptidic, low molecular weight	31
Optimization (Pfizer's 'Rule of 5')		3 nM	1000	highly active and selective, improved pharmacokinetics	this work

[a] The selectivity designates the quotient of affinities to integrins $\alpha v\beta 3$ and $\alpha IIb\beta 3$.

min; preparative, 30 mm × 25 mm, 10 μm C_{18}, 25 mL/min) with a 30 min linear gradient from water (0.1% TFA) and CH_3CN (0.1% TFA) and detection at 220 nm. Mass spectra (ESI) were performed on a LCQ Finnigan instrument. Calculations of log *P* (cLogP) were performed with *ChemDraw Ultra* (version 6.0) using the *Crippen's* fragmentation algorithm.[62]

General Procedures. 3-[*N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-amino]-3-phenylpropionic Acids (3a−c,e,f,h). Benzaldehyde (30 mmol), malonic acid (3.06 g, 30 mmol), and ammonium acetate (4.63 g, 60 mmol) were refluxed in ethanol (30 mL) for 6 h. The amino acid precipitated from the reaction mixture as a white solid and was isolated by filtration. Fmoc-chloride (3.24 g, 12.5 mmol) in dioxane (20 mL) was slowly added to a stirred suspension of the 3-amino-3-phenylpropionic acid (12.0 mmol) in aqueous sodium carbonate (10%, 25 mL) and dioxane (12 mL) at 0 °C. After being additionally stirred for 1 h at 0 °C and 1 d at room temperature, the reaction mixture was poured into ice water (300 mL) and extracted with diethyl ether (2 × 100 mL). The pH of the aqueous layer was adjusted to 1 by addition of aqueous HCl (2 N). The precipitate was filtered off and dried under vacuo to yield product **3** as a white solid.

3-Phenylglutaric Acids (4b−e,h,i). A mixture of benzaldehyde (30 mmol) and ethyl acetoacetate (7.8 g, 60 mmol) was cooled to 0 °C, and piperidine (0.5 mL) was added dropwise. The mixture was left at room temperature for 3 days. The resulting solid was dissolved in ethanol (60 mL) and refluxed. After cooling to room temperature, the crystalline product was filtered off and dried under vacuo. The precipitate was added stepwise to an aqueous solution of KOH (20 M), and the mixture was stirred at 80 °C for 3 h. Ice (50 mL) and ethyl acetate (50 mL) were added. The aqueous phase was separated, and the pH was adjusted to 1 by cautious addition of concentrated aqueous HCl. The precipitate was filtered off, washed with water, and dried under vacuo to yield product **4** as a white solid.

5-[*N′*-(9*H*-Fluoren-9-ylmethoxycarbonyl)hydrazino]-5-oxo-3-phenylpentanoic Acids (5a−e,h,i). A suspension of 3-phenylglutaric acid **4** (10 mmol) in acetic anhydride (2.8 mL, 30 mmol) was refluxed at 130 °C until complete dissolution occurred. After the mixture was cooled to room temperature, diethyl ether (2 mL) was added dropwise. The precipitate was isolated by filtration, washed with cold ether, and dried under vacuo to yield the anhydride as a gray solid. A solution of the anhydride (2.0 mmol) and Fmoc-hydrazine (0.51 g, 2.0 mmol) in anhydrous THF (30 mL) was heated to reflux for 16 h. After evaporation of the solvent product **5** was obtained.

5-[*N*-(4-Methylpyridin-2-yl)-amino]pentanoic Acid Ethyl Ester (13). 5-Bromopentanoic acid ethyl ester (**12**) (12 mL, 100 mmol) and 2-amino-4-methylpyridine (**11**) (21.6 g, 200 mmol) were heated together at 100 °C for 12 h. After cooling to room temperature, the mixture was treated with saturated sodium hydrogen carbonate solution (100 mL) and extracted with ether (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield the crude ester **13** as yellow oil. After purification by flash chromatography (ethyl acetate/hexane 2:1) product **13** was obtained as a white solid (3.17 g, 23%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 7.78 (d, 2H, J = 7.0 Hz), 6.25−6.28 (m, 2H), 6.22 (s, 1H), 3.165 (m, 2H), 2.20 (m, 2H), 2.11 (s, 3H), 1.50 (m, 4H). MS(ESI): *m*/*z* 237.1 [M + H^+].

5-[*N*-(4-Methylpyridin-2-yl)amino]pentanoic Acid (14). Ester **13** (3.17 g, 13.4 mmol) was dissolved in methanol (5 mL) and treated with an aqueous NaOH solution (2 N, 10 mL) for 20 h at room temperature. The mixture was concentrated in vacuo and acidified with HCl (1 N) to pH 4.5. The precipitate was collected by filtration, washed with water, and dried under vacuo to yield the product **14** as a white solid (1.78 g, 64%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 7.79 (d, 1H, J = 5.2 Hz), 6.26−6.30 (m, 2H), 6.22 (s, 1H), 3.17 (m, 2H), 2.21 (t, 2H, J = 7.0 Hz), 2.11 (s, 3H), 1.41−1.58 (m, 4H). MS(ESI): *m*/*z* 209.1 [M + H^+].

3-[*N*-(Pyridin-2-yl)amino]benzoic Acid (17). Sodium hydride (0.88 g, 55% dispersion in mineral oil, 20.0 mmol, 2.0 equiv) was suspended in DMF (20 mL) and cooled to 0 °C. After slow addition of 3-aminobenzoic acid (**16**) (1.37 g, 10.0 mmol)

the suspension was stirred for 2 h at room temperature. 2-Fluoropyridine (**15**) (0.86 mL, 10.0 mmol) was added, and stirring was continued for 60 h at 80 °C. After addition of water (5 mL), the mixture was extracted with $NaHCO_3$ (30 mL) and ethyl acetate (2 × 30 mL). The aqueous layer was acidified with HCl (1 N) to pH 3 and extracted with ethyl acetate (2 × 30 mL). Evaporation of the solvent afforded the product **17** as a red solid (0.21 g, 10%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 9.65 (s, 1H), 8.12 (d, 1H, J = 6.5 Hz), 7.99 (s, 1H), 7.82 (d, 1H, J = 8.0 Hz), 7.72 (t, 1H, J = 7.5 Hz), 7.46 (d, 1H, J = 7.5 Hz), 7.41 (m, 1H), 6.95 (d, 1H, J = 8.5 Hz), 6.85 (t, 1H, J = 6.0 Hz). MS(ESI): m/z 215.2 [M + H$^+$].

General Procedures for Solid-Phase Synthesis. General Procedure Ia. Attachment of 3-(*N*-Fmoc)amino-3-phenylpropionic Acids 3a−h to TCP Resin. After swelling with CH_2Cl_2 (10 mL) for 20 min, TCP resin (2.00 g, theoretical 0.96 mmol/g, 1.92 mmol) was treated with a solution of Fmoc-protected β-amino acids (**3a**−**h**; 2.40 mmol) in 10 mL of CH_2Cl_2 and DIEA (514 μL, 3.00 mmol) at room temperature for 2 h. MeOH (1 mL) was added to cap the free sites, and the reaction mixture was shaken for 15 min. The resin was washed with CH_2Cl_2 (5 × 10 mL) and MeOH (3 × 10 mL) and dried under vacuo to give resin-bound 3-(*N*-Fmoc)amino-3-phenylpropionic acids (**7a**−**h**).

General Procedure Ib. Attachment of 5-(*N*′-Fmoc)-hydrazyl-5-oxo-3-phenylpentanoic Acids 5a-e,h,i to TCP Resin. After swelling with CH_2Cl_2 (10 mL) for 20 min, the TCP resin (2.00 g, theoretical 0.96 mmol/g, 1.92 mmol) was treated with a solution of Fmoc-protected pentanoic acids (**5a**−**e,h,i**; 2.40 mmol) in 10 mL of CH_2Cl_2 and DIEA (514 μL, 3.00 mmol) at room temperature for 2 h. MeOH (1 mL) was added to cap the free sites, and the reaction mixture was shaken for 15 min. The resin was washed with CH_2Cl_2 (5 × 10 mL) and MeOH (3 × 10 mL) and dried under vacuo to give resin-bound 5-(*N*′-Fmoc)hydrazyl-5-oxo-3-phenylpentanoic acids (**6a**−**e,h,i**).

General Procedure II. Coupling of Aza-glycine to Resin-Bound 3-(*N*-Fmoc)amino-3-phenylpropionic Acids 7a−h. Resin (0.20 g, 0.4−0.6 mmol/g, 0.08−0.12 mmol) was washed with CH_2Cl_2 (2 mL) and DMF (2 mL) and treated with a solution of 20% piperidine in DMF (2 × 2 mL) for 5 and 15 min. Resin was washed with DMF (6 × 2 mL) and CH_2Cl_2 (3 × 2 mL), and a solution of **9** (0.24−0.36 mmol) in CH_2Cl_2 (2 mL) was added. The reaction mixture was shaken at room temperature for 90 min and washed with CH_2Cl_2 (6 × 2 mL) and DMF (4 × 2 mL) to give resin-bound 3-[*N*-(*N*′-Fmoc)-hydrazinocarbonyl]-amino-3-phenylpropionic acids (**10a**−**e,h,i**).

General Procedure IIIa/b. Deprotection and Coupling with HATU. The Fmoc protecting group was removed using a solution of 20% piperidine in DMF (2 × 2 mL) for 5 and 15 min. The resin was washed with DMF (6 × 2 mL) and CH_2Cl_2 (3 × 2 mL), and a solution of Fmoc-3-aminobenzoic acid (**a**, 0.16−0.24 mmol) or 5-[*N*-(4-methyl)pyridin-2-yl]aminopentanoic acid **14** (**b**, 0.16−0.24 mmol), HATU (0.16−0.24 mmol), and collidine (1.60−2.40 mmol) in DMF (2 mL) was added to the resin. The reaction mixture was shaken at room temperature for 90 min and washed with DMF (6 × 2 mL).

General Procedure IVa. Guanylation of Resin-Bound Amines. The Fmoc protecting group was removed using a solution of 20% piperidine in DMF (2 × 2 mL). The resin was washed with DMF (6 × 2 mL) and $CHCl_3$ (3 × 2 mL), and a solution of *N*,*N*′-bis-Boc-1-guanylpyrazole[57] (0.48−0.72 mmol, 3 equiv) in $CHCl_3$ (1.5 mL) was added. The reaction mixture was shaken at 50 °C for 16 h and washed with CH_2Cl_2 (6 × 2 mL) to give the resin-bound Boc-protected guanidine.

General Procedure IVb. Amidation of Resin-Bound Amines. The Fmoc protecting group was removed with a solution of 20% piperidine in DMF (2 × 2 mL). The resin was washed with NMP (6 × 2 mL) and a solution of *S*-2-naphthylmethyl thioacetimidate hydrobromide[58] (0.16−0.24 mmol, 2 equiv), and DIEA (0.20−0.30 mmol) in NMP (3 mL) was added. The reaction mixture was shaken at room temperature for 16 h and washed with NMP (6 × 2 mL) and CH_2Cl_2 (3 × 2 mL) to give the resin-bound amidines.

General Procedure Va. Cleavage with Trifluoroacetic Acid (TFA). The resin was treated with a solution of 5% triisopropylsilane (TIPS) in TFA (2 mL) for 1.5 h and (2 × 2 mL) for 2 × 15 min. After removal of the resin by filtration, the filtrates were combined and solvent was removed in vacuo to yield the product as yellow oil. Purification was carried out by preparative reversed-phase chromatography (C_{18}, 20 → 45% ACN/H_2O).

General Procedure Vb. Cleavage with Acetic Acid. Resin was washed with CH_2Cl_2 (4 × 2 mL) and treated with a mixture of CH_2Cl_2, acetic acid, and trifluoroethanol (3:1:1; 2 mL) for 1.5 h and (2 × 2 mL) for 2 × 15 min. After removal of the resin by filtration, the filtrates were combined and solvent was removed in vacuo to yield the product as yellow oil. Purification was carried out by preparative reversed-phase chromatography (C_{18}, 20 → 45% ACN/H_2O).

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-phenyl-5-oxopentanoic Acid Trifluoroacetate (20a). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (6 mg, oil). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.98 (s, 1H), 10.33 (s, 1H), 9.95 (s, 1H), 9.78 (s, 1H), 7.76 (d, 1H, J = 7.8 Hz), 7.69 (s, 1H), 7.54 (t, 1H, J = 7.8 Hz), 7.49 (s, 4H), 7.41 (d, 1H, J = 7.8 Hz), 7.24−7.30 (m, 5H), 3.50 (m, 1H), 2.77 (dd, 1H, J = 5.5/16.0 Hz), 2.49−2.58 (m, 3H). FAB-HRMS: calcd, 384.1672; found, 384.1668 [M + H$^+$].

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-(4-fluorophenyl)-5-oxopentanoic Acid Trifluoroacetate (20b). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (17 mg, oil). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 10.32 (s, 1H), 9.97 (s, 1H), 9.95 (s, 1H), 7.76 (d, 1H, J = 7.8 Hz), 7.70 (s, 1H), 7.59 (s, 4H), 7.55 (t, 1H, J = 7.8 Hz), 7.41 (d, 1H, J = 7.8 Hz), 7.32 (m, 2H), 7.11 (m, 2H), 3.50 (m, 1H), 2.77 (dd, 1H, J = 5.5/16.0 Hz), 2.48−2.58 (m, 3H). MS-(ESI): m/z 402.2 [M + H$^+$].

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-(4-chlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (20c). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (9 mg, oil). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 10.31 (s, 1H), 9.96 (s, 1H), 9.93 (s, 1H), 7.75 (d, 1H, J = 7.8 Hz), 7.69 (s, 1H), 7.57 (s, 4H), 7.54 (t, 1H, J = 7.8 Hz), 7.41 (d, 1H, J = 7.8 Hz), 7.30−7.37 (m, 4H), 3.48 (m, 1H), 2.75 (dd, 1H, J = 5.7/16.0 Hz), 2.45−2.56 (m, 3H). FAB-HRMS: calcd, 418.1282; found, 418.1294 [M + H$^+$].

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-(4-bromophenyl)-5-oxopentanoic Acid Trifluoroacetate (20d). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (15 mg, white powder), mp 114−118 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 10.31 (s, 1H), 9.97 (s, 1H), 9.95 (s, 1H), 7.74 (d, 1H, J = 7.8 Hz), 7.69 (s, 1H), 7.59 (s, 4H), 7.53 (t, 1H, J = 7.8 Hz), 7.46 (d, 2H, J = 8.2 Hz), 7.40 (d, 1H, J = 7.8 Hz), 7.23 (d, 2H, J = 8.2 Hz), 3.49 (m, 1H), 2.76 (dd, 1H, J = 5.7/16.0 Hz), 2.47−2.58 (m, 3H). FAB-HRMS: calcd, 462.0777; found, 462.0757 [M + H$^+$].

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-(4-methoxyphenyl)-5-oxopentanoic Acid Trifluoroacetate (20e). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (9 mg, white powder), mp 107−111 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.99 (s, 1H), 10.33 (s, 1H), 9.94 (s, 1H), 9.95 (s, 1H), 7.76 (d, 1H, J = 8.0 Hz), 7.71 (s, 1H), 7.59 (s, 4H), 7.55 (t, 1H, J = 7.8 Hz), 7.41 (d, 1H, J = 7.8 Hz), 7.18 (d, 2H, J = 8.2 Hz), 6.85 (d, 2H, J = 8.2 Hz), 3.72 (s, 3H), 3.47 (m, 1H), 2.74 (dd, 1H, J = 5.5/15.4 Hz), 2.44−2.55 (m, 3H). FAB-HRMS: calcd, 414.1778; found, 414.1774 [M + H$^+$].

5-[*N*′-(3-Guanidinobenzoyl)hydrazino]-3-(3,5-dichlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (20h). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (12 mg, white powder), mp 104−107 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.15 (s, 1H), 10.32 (s, 1H), 9.95 (s, 1H), 9.91 (s, 1H), 7.74 (d, 1H, J = 7.8 Hz), 7.69 (s, 1H), 7.56 (s, 4H), 7.53 (t, 1H, J = 7.8 Hz), 7.41 (s, 1H), 7.40 (d, 1H, J = 7.8 Hz), 7.35 (s, 2H), 3.50 (m, 1H), 2.77 (dd, 1H, J = 5.9/16.4 Hz), 2.56−2.66 (m, 3H). FAB-HRMS: calcd, 452.0892; found, 452.0890 [M + H$^+$].

5-[*N′*-(3-Guanidinobenzoyl)hydrazino]-3-(4-biphenyl)-5-oxopentanoic Acid Trifluoroacetate (20i). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVa**, and **Va** (17 mg, white powder), mp 102−104 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 10.35 (s, 1H), 9.99 (s, 1H), 9.93 (s, 1H), 7.76 (d, 1H, J = 8.2 Hz), 7.70 (s, 1H), 7.64 (d, 2H, J = 7.8 Hz), 7.55−7.60 (m, 6H), 7.54 (t, 1H, J = 7.8 Hz), 7.45 (t, 2H, J = 7.5 Hz), 7.41 (d, 1H, J = 8.2 Hz), 7.36 (d, 2H, J = 7.8 Hz), 7.34 (d, 2H, J = 7.0 Hz), 3.57 (m, 1H), 2.80 (dd, 1H, J = 5.5/16.0 Hz), 2.55−2.63 (m, 3H). FAB-HRMS: calcd, 460.1986; found, 460.1968 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-phenyl-5-oxopentanoic Acid Trifluoroacetate (21a). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (15 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.10 (s, 1H), 10.26 (s, 1H), 9.92 (s, 1H), 7.82 (s, 3H), 7.43 (m, 2H), 7.36 (t, 1H, J = 8.0 Hz), 7.28−7.34 (m, 4H), 7.22 (m, 1H), 7.14 (d, 1H, J = 8.0 Hz), 3.55 (m, 1H), 2.82 (dd, 1H, J = 5.5/16.0 Hz), 2.47−2.62 (m, 6H). FAB-HRMS: calcd, 383.1719; found, 383.1735 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(4-fluorophenyl)-5-oxopentanoic Acid Trifluoroacetate (21b). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (8 mg, white powder), mp 81−85 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 11.25 (s, 1H), 10.37 (s, 1H), 9.98 (s, 1H), 9.52 (s, 1H), 8.63 (s, 1H), 7.89 (d, 1H, J = 7.8 Hz), 7.77 (s, 1H), 7.63 (t, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 8.0 Hz), 7.30 (m, 2H), 7.10 (m, 2H), 3.55 (m, 1H), 2.76 (dd, 1H, J = 5.5/16.0 Hz), 2.49−2.59 (m, 3H), 2.33 (s, 3H). FAB-HRMS: calcd, 401.1626; found, 401.1621 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(4-chlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (21c). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (9 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 11.24 (s, 1H), 10.37 (s, 1H), 9.98 (s, 1H), 9.51 (s, 1H), 8.63 (s, 1H), 7.88 (d, 1H, J = 7.8 Hz), 7.76 (s, 1H), 7.64 (t, 1H, J = 7.8 Hz), 7.48 (d, 1H, J = 7.8 Hz), 7.23−7.38 (m, 4H), 3.56 (m, 1H), 2.76 (dd, 1H, J = 5.5/16.0 Hz), 2.50−2.60 (m, 3H), 2.33 (s, 3H). FAB-HRMS: calcd, 417.1330; found, 417.1342 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(4-bromophenyl)-5-oxopentanoic Acid Trifluoroacetate (21d). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (5 mg, white powder), mp 98−102 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.08 (s, 1H), 11.23 (s, 1H), 10.34 (s, 1H), 9.98 (s, 1H), 9.51 (s, 1H), 8.63 (s, 1H), 7.89 (d, 1H, J = 8.0 Hz), 7.76 (s, 1H), 7.64 (t, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 8.0 Hz), 7.47 (d, 2H, J = 8.2 Hz), 7.23 (d, 2H, J = 8.2 Hz), 3.55 (m, 1H), 2.76 (dd, 1H, J = 5.5/16.0 Hz), 2.50−2.61 (m, 3H), 2.33 (s, 3H). FAB-HRMS: calcd, 461.0825; found, 461.0823 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(4-methoxyphenyl)-5-oxopentanoic Acid Trifluoroacetate (21e). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (5 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.03 (s, 1H), 11.25 (s, 1H), 10.37 (s, 1H), 9.99 (s, 1H), 9.53 (s, 1H), 8.63 (s, 1H), 7.89 (d, 1H, J = 7.8 Hz), 7.77 (s, 1H), 7.65 (t, 1H, J = 7.8 Hz), 7.47 (d, 1H, J = 7.8 Hz), 7.20 (d, 2H, J = 8.5 Hz), 6.85 (d, 2H, J = 8.2 Hz), 3.72 (s, 3H), 3.55 (m, 1H), 2.77 (dd, 1H, J = 5.5/16.0 Hz), 2.50−2.60 (m, 3H), 2.33 (s, 3H). MS(ESI): m/z 413.3 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(3,5-dichlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (21h). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (18 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.15 (s, 1H), 11.23 (s, 1H), 10.38 (s, 1H), 10.00 (s, 1H), 9.55 (s, 1H), 8.63 (s, 1H), 7.88 (d, 1H, J = 8.0 Hz), 7.76 (s, 1H), 7.63 (t, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 8.0 Hz), 7.42 (s, 1H), 7.35 (s, 1H), 3.50 (m, 1H), 2.77 (dd, 1H, J = 5.5/16.0 Hz), 2.53−2.69 (m, 3H), 2.33 (s, 3H). FAB-HRMS: calcd, 451.0941; found, 451.0930 [M + H$^+$].

5-[*N′*-(3-Acetimidoylaminobenzoyl)hydrazino]-3-(4-biphenyl)-5-oxopentanoic Acid Trifluoroacetate (21i). The procedure is similar to the general procedures for **Ib**, **IIIa**, **IVb**, and **Vb** (5 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.23 (s, 1H), 10.39 (s, 1H), 10.00 (s, 1H), 9.51 (s, 1H), 8.63 (s, 1H), 7.90 (d, 1H, J = 7.8 Hz), 7.77 (s, 1H), 7.61−7.68 (m, 3H), 7.58 (d, 2H, J = 8.5 Hz), 7.48 (d, 1H, J = 7.8 Hz), 7.45 (m, 2H), 7.31−7.39 (m, 3H), 3.56 (m, 1H), 2.79 (m, 1H), 2.51−2.63 (m, 3H), 2.32 (s, 3H). MS(ESI): m/z 459.1 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-phenylpropionic Acid Trifluoroacetate (22a). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (6 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.21 (s, 1H), 10.19 (s, 1H), 9.89 (s, 1H), 8.09 (s, 1H), 7.76 (d, 1H, J = 8.0 Hz), 7.70 (s, 1H), 7.48−7.57 (m, 5H), 7.40 (d, 1H, J = 8.0 Hz), 7.28−7.35 (m, 4H), 7.22 (t, 1H, J = 7.0 Hz), 7.01 (d, 1H, J = 8.5 Hz), 5.08 (m, 1H), 2.67−2.77 (m, 2H). FAB-HRMS: calcd, 385.1625; found, 385.1634 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-fluorophenyl)propionic Acid Trifluoroacetate (22b). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (7 mg, white powder), mp 116−120 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.26 (s, 1H), 10.19 (s, 1H), 9.95 (s, 1H), 8.12 (s, 1H), 7.76 (d, 1H, J = 7.8 Hz), 7.70 (s, 1H), 7.56 (s, 4H), 7.53 (t, 1H, J = 7.8 Hz), 7.33−7.42 (m, 3H), 7.13 (t, 2H, J = 8.8 Hz), 7.05 (d, 1H, J = 8.8 Hz), 5.06 (m, 1H), 2.65−2.77 (m, 2H). FAB-HRMS: calcd, 403.1530; found, 403.1537 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-chlorophenyl)propionic Acid Trifluoroacetate (22c). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (7 mg, white powder), mp 124−126 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.29 (s, 1H), 10.19 (s, 1H), 9.87 (s, 1H), 8.14 (s, 1H), 7.77 (d, 1H, J = 7.8 Hz), 7.70 (s, 1H), 7.48−7.57 (m, 5H), 7.40 (d, 1H, J = 8.4 Hz), 7.33−7.38 (m, 4H), 7.06 (d, 1H, J = 8.4 Hz), 5.06 (m, 1H), 2.66−2.78 (m, 2H). FAB-HRMS: calcd, 419.1235; found, 419.1230 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-bromophenyl)propionic Acid Trifluoroacetate (22d). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (5 mg, white powder), mp 147−152 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.18 (s, 1H), 10.19 (s, 1H), 9.83 (s, 1H), 8.13 (s, 1H), 7.76 (d, 1H, J = 7.8 Hz), 7.70 (s, 1H), 7.53 (t, 1H, J = 7.8 Hz), 7.50 (d, 2H, J = 8.5 Hz), 7.49 (s, 4H), 7.40 (d, 1H, J = 7.8 Hz), 7.30 (d, 2H, J = 8.5 Hz), 7.06 (d, 1H, J = 8.2 Hz), 5.03 (m, 1H), 2.71 (m, 2H). MS(ESI): m/z 463.1 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-methoxyphenyl)propionic Acid Trifluoroacetate (22e). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (5 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.89 (s, 1H), 10.20 (s, 1H), 9.78 (s, 1H), 8.06 (s, 1H), 7.78 (d, 1H, J = 7.8 Hz), 7.71 (s, 1H), 7.55 (t, 1H, J = 7.8 Hz), 7.48 (s, 4H), 7.41 (d, 1H, J = 7.8 Hz), 7.26 (d, 2H, J = 8.5 Hz), 6.93 (d, 1H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.5 Hz), 5.04 (m, 1H), 3.74 (s, 3H), 2.65−2.77 (m, 2H). FAB-HRMS: calcd, 415.1730; found, 415.1690 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-trifluoromethoxyphenyl)propionic Acid Trifluoroacetate (22f). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (8 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.29 (s, 1H), 10.19 (s, 1H), 9.80 (s, 1H), 8.15 (s, 1H), 7.77 (d, 1H, J = 7.8 Hz), 7.70 (s, 1H), 7.53 (t, 1H, J = 7.8 Hz), 7.46 (d, 2H, J = 8.2 Hz), 7.48 (s, 4H), 7.40 (d, 1H, J = 7.8 Hz), 7.31 (d, 2H, J = 8.2 Hz), 7.08 (d, 1H, J = 8.4 Hz), 5.10 (m, 1H), 2.75 (m, 2H). FAB-HRMS: calcd, 469.1447; found, 469.1435 [M + H$^+$].

3-{*N*-[*N′*-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(1-naphthyl)propionic Acid Trifluoroacetate (22g). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (5 mg, white powder), mp 151−154 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.28 (s, 1H), 10.21 (s, 1H), 9.94 (s, 1H), 8.14 (s, 1H), 8.09 (s, 1H), 7.94 (d, 1H, J = 8.0 Hz), 7.83 (d, 1H, J = 8.2 Hz), 7.76 (d, 1H, J = 7.5 Hz), 7.70 (s, 1H), 7.50−7.60 (m, 7H), 7.49 (t, 1H, J = 8.2 Hz), 7.39

(d, 1H, J = 7.5 Hz), 7.20 (d, 1H, J = 7.5 Hz), 5.91 (m, 1H), 2.74−2.91 (m, 2H). MS(ESI): m/z 435.2 [M + H$^+$].

3-{*N*-[*N*′-(3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(3,5-dichlorophenyl)propionic Acid Trifluoroacetate (22h). The procedure is similar to the general procedures for **Ia**, **II**, **IIIa**, **IVa**, and **Va** (5 mg, white powder), mp 143−147 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.05 (s, 1H), 10.21 (s, 1H), 9.71 (s, 1H), 8.24 (s, 1H), 7.78 (d, 1H, J = 7.8 Hz), 7.71 (s, 1H), 7.56 (s, 1H), 7.54 (t, 1H, J = 7.8 Hz), 7.43 (s, 4H), 7.41 (d, 1H, J = 7.8 Hz), 7.40 (s, 2H), 7.11 (d, 1H, J = 8.2 Hz), 5.04 (m, 1H), 2.76 (m, 2H). MS(ESI): m/z 453.1 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-phenyl-5-oxopentanoic Acid Trifluoroacetate (23a). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (5 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.91 (s, 1H), 11.98 (s, 1H), 9.71 (s, 1H), 9.65 (s, 1H), 8.37 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.20−7.28 (m, 4H), 7.17 (t, 1H, J = 6.2 Hz), 6.79 (s, 1H), 6.68 (d, 1H, J = 6.5 Hz), 3.46 (m, 1H), 3.26 (m, 2H), 2.77 (dd, 1H, J = 5.5/16.0 Hz), 2.39−2.48 (m, 3H), 2.31 (s, 3H), 2.13 (m, 2H), 1.57 (m, 4H). FAB-HRMS: calcd, 413.2190; found, 413.2187 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(4-fluorophenyl)-5-oxopentanoic Acid Trifluoroacetate (23b). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (9 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.07 (s, 1H), 12.07 (s, 1H), 9.71 (s, 1H), 9.65 (s, 1H), 8.59 (s, 1H), 7.80 (m, 1H), 7.38−7.47 (m, 1H), 7.25 (m, 1H), 7.01 (t, 1H, J = 8.5 Hz), 6.84 (s, 1H), 6.70 (d, 1H, J = 6.5 Hz), 3.46 (m, 1H), 3.27 (m, 2H), 2.91 (m, 1H), 2.70 (dd, 1H, J = 5.5/16.0 Hz), 2.37−2.52 (m, 2H), 2.32 (s, 3H), 2.13 (m, 2H), 1.57 (m, 4H). FAB-HRMS: calcd, 431.2095; found, 431.2110 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(4-chlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (23c). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (23 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.11 (s, 1H), 9.72 (s, 1H), 9.65 (s, 1H), 8.60 (s, 1H), 7.80 (s, 1H), 7.40 (m, 2H), 7.19−7.34 (m, 2H), 6.84 (s, 1H), 6.71 (d, 1H, J = 6.5 Hz), 3.45 (m, 1H), 3.28 (m, 2H), 3.00−3.18 (m, 1H), 2.88−2.98 (m, 1H), 2.70 (m, 1H), 2.39−2.53 (m, 1H), 2.31 (s, 3H), 2.13 (m, 2H), 1.52−1.70 (m, 4H). FAB-HRMS: calcd, 447.1800; found, 447.1807 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(4-bromophenyl)-5-oxopentanoic Acid Trifluoroacetate (23d). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (16 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.20 (s, 1H), 12.08 (s, 1H), 9.72 (s, 1H), 9.65 (s, 1H), 8.50 (s, 1H), 7.80 (d, 1H, J = 6.5 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.19 (d, 2H, J = 8.0 Hz), 6.81 (s, 1H), 6.68 (d, 1H, J = 6.5 Hz), 3.44 (m, 1H), 3.26 (m, 2H), 2.91 (m, 1H), 2.70 (dd, 1H, J = 5.5/16.0 Hz), 2.37−2.54 (m, 2H), 2.31 (s, 3H), 2.12 (m, 2H), 1.57 (m, 4H). FAB-HRMS: calcd, 491.1295; found, 491.1296 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(4-methoxyphenyl)-5-oxopentanoic Acid Trifluoroacetate (23e). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (5 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.14 (s, 1H), 9.69 (s, 1H), 9.65 (s, 1H), 8.61 (s, 1H), 7.80 (d, 1H, J = 6.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 6.84 (s, 1H), 6.81 (d, 2H, J = 8.5 Hz), 6.69 (d, 1H, J = 6.5 Hz), 3.70 (s, 3H), 3.41 (m, 1H), 3.24 (m, 2H), 2.67 (dd, 1H, J = 5.5/16.0 Hz), 2.35−2.47 (m, 2H), 2.32 (s, 3H), 2.13 (m, 2H), 1.54−1.66 (m, 4H). FAB-HRMS: calcd, 443.2294; found, 443.2279 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(3,5-dichlorophenyl)-5-oxopentanoic Acid Trifluoroacetate (23h). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (21 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.06 (s, 1H), 9.73 (s, 1H), 9.67 (s, 1H), 8.59 (s, 1H), 7.80 (m, 1H), 7.39 (s, 1H), 7.30 (s, 2H), 6.84 (m, 1H), 6.71 (d, 1H, J = 6.5 Hz), 3.45 (m, 1H), 3.27 (m, 2H), 3.06−3.20 (m, 1H), 2.95 (m, 1H), 2.71 (dd, 1H, J = 5.5/16.5 Hz), 2.58 (dd, 1H, J = 9.8/16.5 Hz), 2.32 (s, 3H), 2.28 (m, 1H), 2.12 (m, 1H), 1.54−1.66 (m, 4H). FAB-HRMS: calcd, 481.1410; found, 481.1402 [M + H$^+$].

5-{*N*′-[5-(4-Methylpyridin-2-ylamino)pentanoyl]hydrazino}-3-(4-biphenyl)-5-oxopentanoic Acid Trifluoroacetate (23i). The procedure is similar to the general procedures for **Ib**, **IIIb**, and **Vb** (18 mg, white powder), mp 176−177 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.00 (s, 1H), 9.73 (s, 1H), 9.65 (s, 1H), 7.79 (d, 1H, J = 6.5 Hz), 7.63 (d, 2H, J = 7.5 Hz), 7.56 (d, 2H, J = 8.2 Hz), 7.44 (m, 2H), 7.30−7.36 (m, 3H), 6.35 (m, 1H), 6.28 (d, 1H, J = 6.5 Hz), 6.25 (s, 1H), 3.52 (m, 1H), 3.17 (m, 2H), 2.75 (dd, 1H, J = 5.5/16.5 Hz), 2.55 (dd, 1H, J = 9.8/16.5 Hz), 2.44−2.51 (m, 2H), 2.31 (s, 3H), 2.09 (m, 2H), 1.46−1.58 (m, 4H). FAB-HRMS: calcd, 489.2503; found, 489.2522 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-phenylpropionic Acid Trifluoroacetate (24a). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (17 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.15 (s, 1H), 9.47 (s, 1H), 8.60 (s, 1H), 7.86 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.26−7.32 (m, 4H), 7.18−7.23 (m, 1H), 6.88 (d, 1H, J = 8.5 Hz), 6.83 (s, 1H), 6.70 (d, 1H, J = 6.2 Hz), 5.03 (m, 1H), 3.27 (m, 2H), 2.63−2.74 (m, 2H), 2.31 (s, 3H), 2.13 (t, 2H, J = 6.5 Hz), 1.54−1.62 (m, 4H). FAB-HRMS: calcd, 414.2141; found, 414.2145 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(4-fluorophenyl)propionic Acid Trifluoroacetate (24b). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (5 mg, white powder), mp 49−51 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.00 (s, 1H), 12.37 (s, 1H), 9.46 (s, 1H), 8.48 (s, 1H), 7.87 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.33 (m, 2H), 7.11 (m, 2H), 6.90 (d, 1H, J = 8.5 Hz), 6.80 (s, 1H), 6.68 (d, 1H, J = 6.5 Hz), 5.02 (m, 1H), 3.26 (m, 2H), 2.63−2.75 (m, 2H), 2.30 (s, 3H), 2.12 (t, 2H, J = 6.5 Hz), 1.54−1.61 (m, 4H). FAB-HRMS: calcd, 432.2047; found, 432.2041 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(4-chlorophenyl)propionic Acid Trifluoroacetate (24c). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (25 mg, white powder), mp 47−49 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.07 (s, 1H), 9.47 (s, 1H), 8.56 (s, 1H), 7.89 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.30−7.36 (m, 4H), 6.92 (d, 1H, J = 8.5 Hz), 6.82 (s, 1H), 6.69 (d, 1H, J = 6.5 Hz), 5.01 (m, 1H), 3.26 (m, 2H), 2.63−2.75 (m, 2H), 2.31 (s, 3H), 2.12 (t, 2H, J = 6.5 Hz), 1.54−1.61 (m, 4H). FAB-HRMS: calcd, 448.1752; found, 448.1769 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(4-bromophenyl)propionic Acid Trifluoroacetate (24d). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (16 mg, yellow powder), mp 58−62 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.03 (s, 1H), 9.47 (s, 1H), 8.50 (s, 1H), 7.90 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.48 (d, 2H, J = 8.0 Hz), 7.26 (d, 2H, J = 8.0 Hz), 6.93 (d, 1H, J = 8.5 Hz), 6.81 (s, 1H), 6.68 (d, 1H, J = 6.5 Hz), 5.00 (m, 1H), 3.26 (m, 2H), 2.62−2.77 (m, 2H), 2.31 (s, 3H), 2.12 (t, 2H, J = 6.5 Hz), 1.52−1.65 (m, 4H). FAB-HRMS: calcd, 492.1247; found, 492.1249 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(4-methoxyphenyl)propionic Acid Trifluoroacetate (24e). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (22 mg, white powder), mp 43−45 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.06 (s, 1H), 12.22 (s, 1H), 9.46 (s, 1H), 8.56 (s, 1H), 7.82 (s, 1H), 7.78 (d, 1H, J = 6.5 Hz), 7.21 (d, 2H, J = 8.7 Hz), 6.84 (d, 2H, J = 8.7 Hz), 6.82 (s, 1H), 6.79 (d, 1H, J = 8.5 Hz), 6.69 (d, 1H, J = 6.5 Hz), 4.97 (m, 1H), 3.71 (s, 3H), 3.26 (m, 2H), 2.60−2.72 (m, 2H), 2.31 (s, 3H), 2.12 (t, 2H, J = 6.5 Hz), 1.55−1.61 (m, 4H). FAB-HRMS: calcd, 444.2247; found, 444.2239 [M + H$^+$].

3-{*N*-[*N*′-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(4-trifluoromethoxyphenyl)-propionic Acid Trifluoroacetate (24f). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (24 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.89 (s, 1H), 9.46

(s, 1H), 8.43 (s, 1H), 7.89 (s, 1H), 7.77 (d, 1H, J = 6.5 Hz), 7.42 (d, 2H, J = 8.5 Hz), 7.28 (d, 2H, J = 8.5 Hz), 6.93 (d, 1H, J = 8.5 Hz), 6.80 (s, 1H), 6.68 (d, 1H, J = 6.5 Hz), 5.05 (m, 1H), 3.26 (m, 2H), 2.66−2.77 (m, 2H), 2.31 (s, 3H), 2.12 (m, 2H), 1.53−1.62 (m, 4H). FAB-HRMS: calcd, 498.1964; found, 498.1968 [M + H$^+$].

3-{*N*-[*N′*-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(1-naphthyl)propionic Acid Trifluoroacetate (24g). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (28 mg, white powder), mp 103−106 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.00 (s, 1H), 9.49 (s, 1H), 8.53 (s, 1H), 8.11 (d, 1H, J = 8.5 Hz), 7.94 (d, 1H, J = 7.5 Hz), 7.86 (s, 1H), 7.82 (d, 1H, J = 8.0 Hz), 7.76 (d, 1H, J = 6.5 Hz), 7.57 (t, 1H, J = 7.2 Hz), 7.50−7.55 (m, 2H), 7.46 (t, 1H, J = 7.5 Hz), 7.05 (d, 1H, J = 8.5 Hz), 6.81 (s, 1H), 6.66 (d, 1H, J = 7.5 Hz), 5.87 (m, 1H), 3.26 (m, 2H), 2.86 (dd, 1H, J = 6.5/16.2 Hz), 2.75 (dd, 1H, J = 7.5/16.2 Hz), 2.29 (s, 3H), 2.13 (m, 2H), 1.58 (m, 4H). FAB-HRMS: calcd, 464.2299; found, 464.2284 [M + H$^+$].

3-{*N*-[*N′*-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}amino-3-(3,5-dichlorophenyl)-propionic Acid Trifluoroacetate (24 h). The procedure is similar to the general procedures for **Ia**, **II**, **IIIb**, and **Vb** (26 mg, white powder), mp 150−152 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.05 (s, 1H), 9.50 (s, 1H), 7.95 (s, 1H), 7.76 (d, 1H, J = 5.5 Hz), 7.42 (m, 1H), 7.37 (m, 2H), 7.13 (s, 1H), 6.81 (s, 1H), 6.31 (m, 2H), 4.99 (m, 1H), 3.17 (m, 2H), 2.67−2.75 (m, 2H), 2.14 (s, 3H), 2.11 (m, 2H), 1.59 (m, 2H), 1.53 (m, 2H). FAB-HRMS: calcd, 482.1363; found, 482.1371 [M + H$^+$].

3-{*N*-[*N′*-(5-Guanidinopentanoyl)hydrazino]carbonyl}-amino-3-phenylpropionic Acid Trifluoroacetate (25). The procedure is similar to the general procedures for **Ia**, **II**, **III** (5-(*N*-Fmoc)-aminopentanoic acid), and **Vb** (22 mg, oil): ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.21 (s, 1H), 9.46 (s, 1H), 7.85 (s, 1H), 7.53 (m, 1H), 7.27−7.32 (m, 5H), 7.21 (m, 1H), 6.86 (d, 1H, J = 8.7 Hz), 5.03 (m, 1H), 3.90 (s, 4H), 3.08 (m, 2H), 2.69 (m, 2H), 2.10 (t, 2H, J = 6.5 Hz), 1.40−1.56 (m, 4H). FAB-HRMS: calcd, 365.1859; found, 365.1939 [M + H$^+$].

3-{*N*-[*N′*-(3-(*N*-Pyridin-2-yl)-aminobenzoyl)hydrazino]-carbonyl}amino-3-(3,5-dichlorophenyl)propionic Acid Trifluoroacetate (26). The procedure is similar to the general procedures for **Ia**, **II**, **III** (3-(*N*-pyridin-2-yl)-aminobenzoic acid **(17))**, and **Va** (16 mg, white powder), mp 110−114 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.03 (s, 1H), 10.22 (s, 1H), 9.89 (s, 1H), 9.65 (s, 1H), 8.12 (d, 1H, J = 6.5 Hz), 7.99 (s, 1H), 7.82 (d, 1H, J = 8.0 Hz), 7.72 (t, 1H, J = 7.5 Hz), 7.46 (d, 1H, J = 7.5 Hz), 7.41 (m, 1H), 7.39 (s, 1H), 7.35 (s, 2H), 6.95 (d, 1H, J = 8.5 Hz), 6.85 (t, 1H, J = 6.0 Hz), 3.51 (m, 1H), 2.78 (dd, 1H, J = 5.7/16.2 Hz), 2.63 (dd, 1H, J = 9.8/16.3 Hz), 2.57 (m, 2H). FAB-HRMS: calcd, 487.0941; found, 487.0945 [M + H$^+$].

3-{*N*-[*N′*-(5-(4-Methylpyridin-2-ylamino)pentanoyl)-hydrazino]carbonyl}aminopropionic Acid Trifluoroacetate (27). The procedure is similar to the general procedures for **Ia** (3-(*N*-Fmoc)-aminopropionic acid), **II**, **IIIb**, and **Vb** (27 mg, white powder), mp 107−110 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.17 (s, 1H), 9.43 (s, 1H), 8.62 (s, 1H), 7.80 (d, 1H, J = 6.8 Hz), 7.78 (s, 1H), 6.84 (s, 1H), 6.70 (d, 1H, J = 6.5 Hz), 6.33 (t, 1H, J = 5.8 Hz), 3.27 (m, 2H), 3.19 (m, 2H), 2.34 (t, 2H, J = 6.8 Hz), 2.32 (s, 3H), 2.12 (m, 2H), 1.52−1.62 (m, 4H). FAB-HRMS: calcd, 338.1828; found, 338.1823 [M + H$^+$].

Biological Assay. 1. Protein Purification. Human plasma vitronectin[63] and fibrinogen[64] were purified as previously described. The recombinant $\alpha v\beta 3$ receptor with binding properties identical to the native molecule from human placenta[65] was obtained from a baculovirus system using antibody affinity chromatography on an LM609 antibody column. The $\alpha IIb\beta 3$ receptor was purified by peptide affinity chromatography from outdated thrombocytes[66] with modifications,[67] using a linear GRGDSPK-conjugated CL-4B Sepharose column. Both integrins were >95% pure as judged by anti-integrin ELISA using α- and β-chain specific monoclonal antibodies and by SDS−PAGE.

2. Receptor Binding Assay. Inhibitory effects of aza- and azacarba RGD mimetics were quantified by measuring their effect on the interactions between immobilized integrin and biotinylated soluble ligands. Purified vitronectin or fibrinogen (1 mg/mL; pH 8.2) was biotinylated with *N*-hydroxysuccinimidobiotin (100 μg/mL; 1 h, 20 °C) before dialysis into PBS. Integrins diluted to 1 μg/mL (∼1:1000 dilution of stock) were adsorbed to 96-well nontissue culture treated microtiter plates. After free protein binding sites were blocked, the biotinylated ECM ligand at 1 μg/mL was added and incubation continued at 37 °C for 3 h. Unbound ligand was washed away, and bound biotin was detected with alkaline-phosphate-conjugated anti-biotin antibodies. For competition assays, compounds were serially diluted before addition to integrin-coated wells, followed by co-incubation with biotinylated ligand and detection as described above. Assays were performed in triplicate, the mean binding values were fitted to a sigmoid, and the IC_{50} was derived. Values shown in Tables 1−3 represent the mean of at least three separate determinations of IC_{50}. External standards of linear GRGDSPK and cyclo(RGDfV) were routinely included to monitor the dynamic range and the stability of the assay. Intra-assay variation, the relationship between IC_{50} values of standard inhibitors and the test substances, was typically less than ±5%, and inter-assay variation in absolute IC_{50} was typically a factor of less than ±2.

Acknowledgment. The authors gratefully acknowledge technical assistance from M. Kranawitter, B. Cordes, and A. Schröder. We thank D. Hahn (Merck KGaA) for performing the screening assays and Dr. J. Osterodt (Merck KGaA) for the FAB-HRMS analyses of the RGD mimetics. Financial support was provided by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Supporting Information Available: Additional synthesis procedures and table of analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0004953