Nanomolar Small Molecule Inhibitors for αv*β*6, αv*β*5, and αv*β*3 Integrins

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Integrin adhesion receptors frequently recognize a core amino acid sequence, Arg-Gly-Asp, in their target ligands. Inhibitors with the ability to inhibit one or a small subset of such RGD-dependent integrins have been invaluable in defining their biological function. Here, we have characterized low molecular weight inhibitors for their ability to specifically inhibit αv*β*6 integrin, a fibronectin/tenascin receptor. As of yet, no nonpeptidic inhibitor of αv*β*6 was known. New peptidomimetic and nonpeptidic compounds were examined in isolated integrin binding assays and in cell adhesion assays for their ability to block αv*β*6, αv*β*3, αv*β*5, and αIIb*β*3 integrins. The compounds are based on an aromatically substituted *β* amino acid or glutaric acid derivative as an acidic center and an aminopyridyl or guanidyl residue as a basic mimetic. We found several classes of inhibitors with different selectivities, especially mono- or biselectivity on the αv-integrins αv*β*6 and αv*β*3, and nanomolar activity. Furthermore, nearly all compounds are inactive on αIIb*β*3. Compound **11** is the first specific, peptidomimetic inhibitor of the αv*β*6 integrin receptor.

Introduction

Integrins are a family of heterodimeric cell surface receptors that regulate cell attachment and response to the extracellular matrix.[1] Integrins discriminate well between their target ligands, so it came as a surprise that for many integrins the recognition sequence in the matrix protein contains a core amino acid sequence, "Arg-Gly-Asp" (RGD). This has complicated the discovery of specific inhibitors. For example, at least the five αv-integrins (*β*1, *β*3, *β*5, *β*6, *β*8), as well as integrins αIIb*β*3, α5*β*1, and α8*β*1 bind their ligands via the RGD sequence. For some of these integrins, the specificity of interaction with the ligand involves a supplementary site distant from the RGD site.[2] It is, therefore, a major challenge to identify low molecular weight compounds that can discriminate between these integrins. Nevertheless, extremely selective nonpeptidic inhibitors have been discovered for several "RGD-dependent" integrins including αIIb*β*6,[3−6] αv*β*3,[7−14] α4*β*1,[15] and α5*β*1,[16] while peptidic inhibitors with high specificity and selectivity have been described for αIIb*β*3,[17,18] for αv*β*3,[19−25] for αv*β*6,[26] and for α5*β*1.[27]

We have attempted to find small molecule inhibitors that discriminate between subsets of the "RGD" integrins implicated in human pathologies, focusing on αv*β*6 for which no small molecule inhibitors have been described so far. αv*β*6 is a fibronectin/tenascin receptor specific to epithelia that is up-regulated during inflammatory events,[28] in tumor proliferation,[29−31] and during wound healing.[32,33] αv*β*6 has been linked with cell migration and proliferation,[34] gelatinase B expression,[35,36] and the activation of TGF-*β*1.[37] Other closely related integrins were also studied to demonstrate the specificity of the new compounds. We examined the following: αIIb*β*3, the platelet fibrinogen receptor, involved in thrombosis;[38] αv*β*3 a promiscuous receptor for vitronectin, fibronectin, fibrinogen, and other proteins, up-regulated on endothelia during tumor angiogenesis[39] and on smooth muscle cells during proliferation;[40] and αv*β*5 a vitronectin receptor involved in angiogenesis of retinopathy.[41]

Integrin inhibitors are of interest in three major areas. First, integrin inhibitors may lead to the development of drugs. Alterations of integrin activity and expression characterize numerous pathological conditions, whose consequences may be reversed by inhibitors of integrins. Specific inhibitors of individual integrins or of defined integrin groups have helped clarify the function of integrins in pathologies. Second, from the perspective of defining the basic biological role of the integrins, specific inhibitors can be used to investigate the role of individual integrins at the cellular level. Finally, from the perspective of structural analysis, the molecular basis of ligand recognition by integrins is for the most part mysterious, and novel structural probes are valuable for this recognition process. However, there are still gaps in our knowledge due to the lack of specific inhibitors.

Here, we describe the characterization of nanomolar αv*β*6 receptor inhibitors with mixed profiles that inhibit αv*β*6 and αv*β*3; or αv*β*6, αv*β*3, and αv*β*5; in each case, with little effect on αIIb*β*3. These inhibitors with similar structural features allow the investigation of structure−activity relationships for the different αv-integrin subtypes. Together, these inhibitors are very useful to study the combined function of αv-series integrins and, in particular, provide for the first time highly active small molecule inhibitors of αv*β*6.

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Experimental Section

Chemistry. The synthesis of the cyclic peptides has already been described: *N*-methylated peptides **1** and **2**[25] and aza-glycine containing peptides **3**−**5**.[42,43] The aza-RGD mimetics **6**−**8** and **13** were synthesized by solid-phase synthesis on TCP resin using the oxadiazolone route.[9] Details of the syntheses have been published elsewhere.[13] Non aza compounds **9**−**12** and **14**−**15** were synthesized in solution using conventional peptide coupling techniques.[44] The aromatic β amino acid was obtained from a reaction of the corresponding benzaldehyde with ammonium acetate and malonic acid.[13] After esterification, Boc-Gly-OH was coupled N-terminally. The Boc group of the dipeptide was cleaved and the resulting compound coupled with a readily prepared pyridin-2-ylamino alkylcarboxylic acid. The final product was obtained by saponification and purification by preparative HPLC (for details, see Supporting Information).

Biology. (a) Isolated Integrin-Ligand Binding Assays. The production of recombinant human $\alpha v\beta 3$ and $\alpha v\beta 6$ has been described elsewhere.[26,45] Recombinant human $\alpha v\beta 5$ was produced by similar technologies.[46] Human platelet $\alpha IIb\beta 3$ was isolated as described.[47] The inhibitory activity of the substances described here was tested in ligand inhibition assays,[25] using immobilized integrin as the target, and biotinylated human serum vitronectin ($\alpha v\beta 3$ and $\alpha v\beta 5$), fibronectin ($\alpha v\beta 6$), and fibrinogen ($\alpha IIb\beta 3$) as ligands. In brief, 96-well ELISA plates were coated by adsorption from neutral aqueous buffers of 1 μg mL^{-1} integrin. After blocking residual sites on the plate with BSA, biotinylated ligands (1 μg mL^{-1}) were added in the presence or absence of serial dilutions of inhibitor, and after incubation and washing, bound biotin was detected with peroxidase-coupled anti-biotin antibody and TMB substrate. IC_{50}, the concentration of inhibitor needed to inhibit ligand binding in the absence of inhibitor by 50%, was established by curve fitting, and the values presented are usually the mean of three or more such independent determinations. These ligand binding assays distinguish reproducibly between compounds with minimal activity (IC_{50} > 10 μM), and those with activity here defined as low (10 μM < IC_{50} < 1 μM), moderate (1 μM < IC_{50} < 10 nM), and high (IC_{50} < 10 nM). Standard compound **2** gave mean IC_{50} on $\alpha v\beta 3$ of 3.4 nM, a value within the experimental variability of our previously reported data.[25] Standard deviations were typically of the same order as the mean values.

(b) Cell Attachment Assay. The role of various αv-integrins in initial cell attachment to extracellular matrix was measured in a quantitative assay using lysosomal hexosaminidase to determine cell number.[47] M21 human melanoma cells attach to vitronectin using integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, and the $\alpha v\beta 3$ component can be examined by performing the assay in the presence of the $\alpha v\beta 5$ blocking antibody P1F6.[47] UCLAP-3 human lung adenocarcinoma cells attach to vitronectin exclusively via $\alpha v\beta 5$ integrin, and to fibronectin exclusively via $\alpha v\beta 6$, as confirmed by blocking with an RTDLXXL peptide as described for HT-29 cells.[26] In brief, the effect of inhibitors on cell attachment to surfaces coated with vitronectin or fibronectin was measured 1 h after the freshly harvested cells had been plated into a serial dilution of the inhibitors. IC_{50}, the concentration of inhibitor needed to inhibit cell attachment in the absence of inhibitor by 50%, was established by curve fitting, and the values presented here are the mean of two such independent determinations. Standard deviations were typically of the same order as the mean values.

Results

Chemistry. The structures of the compounds described in this paper are shown in Figure 1. A large number of different cyclic pentapeptides containing the RGD sequence have been synthesized in our group.[21,25,48] For example, we used *N*-methylated amino acids[25,49] and exchanged glycine for aza-glycine in some peptides.[42,43]

To obtain peptidomimetics, we synthesized compounds incorporating features that are present in the RGD peptide sequence. These mimetics possess, with the exception of **13**, an aminopyridine moiety as the basic part of the molecule. In compound **13**, the guanidino group itself served this role. The acidic, C-terminal component of the molecules is formed either by an aromatic β amino acid **(8**−**15)** or a substituted glutaric acid group (**6**, **7**). The glycine spacer is retained in **9**−**12** and **14**. Aza-glycine is used in **6**−**8** and **13**, and it is completely omitted in **15**.

Biology. We tested the activities of the novel synthetic compounds in inhibiting the interaction of ligands with isolated immobilized integrins. The results are shown in Table 1 and visualized in Figure 2. We have previously recognized that constraints in cyclic RGD-peptide stereoisomers can be used to tune the integrin inhibition profiles,[19,48] as shown by comparing compounds **1**−**5**, some of which have been previously described by ourselves. Compound **1** is a specific $\alpha v\beta 3$ inhibitor (IC_{50} 24 nM) with moderate activity on $\alpha v\beta 5$ and low activity on $\alpha v\beta 6$ and $\alpha IIb\beta 3$. The substitution of valine for alanine to furnish **2**, cyclic (-Arg-Gly-Asp-D-Phe-(NMe)Val-), EMD 121974, increased inhibitory activity on each integrin.[25] Interestingly, the aza-Gly derivative **3**, cyclic (-Arg-azaGly-Asp-D-Phe-(NMe)Val-),[42,43] also exhibits high activities and a similar activity profile (Table 1): compound **2** [**3**] has an IC_{50} value of 3 [10] nM on $\alpha v\beta 3$, 37 [40] nM on $\alpha v\beta 5$, and 590 [2800] nM on $\alpha IIb\beta 3$. Compounds **2** and **3** also have similar activities on $\alpha v\beta 6$, 470 nM vs 900 nM. Deletion of the *N*-methyl group in **4** resulted in a loss of activity on $\alpha v\beta 5$. Compound **4** is highly specific for integrin $\alpha v\beta 3$ with a selectivity of 2 orders over $\alpha v\beta 5$ and 3 orders over $\alpha v\beta 6$. Activity for $\alpha IIb\beta 3$ was strongly increased in compound **5** by exchanging the D,L-stereochemistry of the valine and phenylalanine groups, while the activity on $\alpha v\beta 3$ is still in the low nanomolar range. These variations in cyclic peptides confirmed that subtle alterations in stereochemistry could produce drastic alterations in both activity and selectivity of the integrin inhibitors.

Nonpeptidic azacarba-derivatives, compounds **6** and **7**, exhibit high activity for $\alpha v\beta 3$ and high selectivity toward $\alpha v\beta 5$. These compounds show only modest activity on $\alpha v\beta 6$ and are inactive on both $\alpha v\beta 5$ and $\alpha IIb\beta 3$. Switching first the methylene for an NH group, compound **8**, and then the NH of aza-glycine for a methylene, obtaining glycine containing compound **9**, an increase of activity to $\alpha v\beta 6$ and a decrease of $\alpha v\beta 3$/$\alpha v\beta 5$ selectivity is observed. With these variations, it is possible to switch $\alpha v\beta 3$/$\alpha v\beta 6$ selectivity in a modest way, as shown in the selectivity diagram (Figure 2). Scientists of Monsanto/Searle first described $\alpha v\beta 3$ integrin antagonists with the 3,5-dichloro substitution pattern (compounds **7**, **8**, and **9**) in 1998.[50]

Modification of the phenyl ring also produced drastic alterations in integrin inhibitory activity. The replacement of the 3,5-dichloro groups with a 3-trifluoromethoxy function, compound **10**, resulted in slight loss of activity on $\alpha v\beta 3$, and a gain of almost 2 orders of magnitude on $\alpha v\beta 5$, while remaining the activities for $\alpha IIb\beta 3$ (inactive) and $\alpha v\beta 6$ (subnanomolar). Derivatization of the phenyl ring at the 4-position with naphthyl or phenyl rings, compounds **11** and **12**, resulted in a 2 orders of magnitude decrease of inhibitory activity to

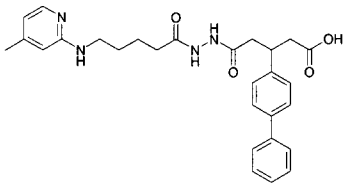
Compound **1** cyclo(RGDf(NMe)A)

Compound **2** cyclo(RGDf(NMe)V)

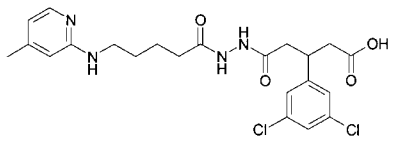
Compound **3** cyclo(R*aza*GDf(NMe)V)

Compound **4** cyclo(R*aza*GDfV)

Compound **5** cyclo(R*aza*GDFv)



Compound **6**



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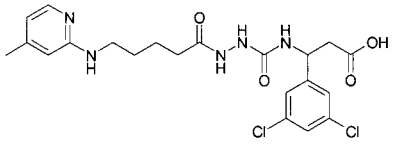

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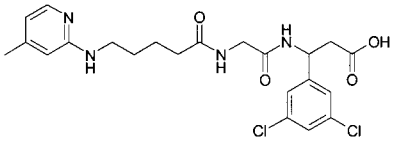
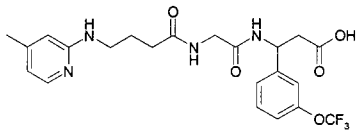
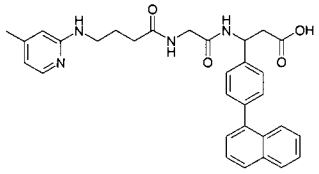


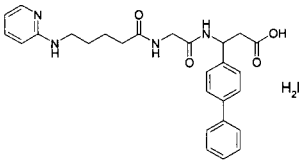
Compound **9**

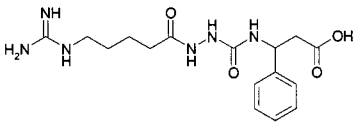


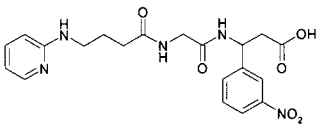
Compound **10**

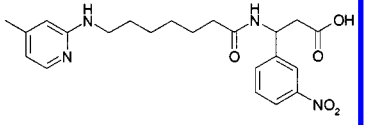


Compound **11**









Compound **12** Compound **13** Compound **14** Compound **15**

Figure 1. Structural formulas of compounds described in text. Lower case letters represent D-amino acids. NMe = *N*-methyl.

Table 1. Potent and Selective αvβ6 Inhibitors in Isolated Receptor Binding Assay[a]

	IC$_{50}$ (nM) on integrin				
compd	αvβ6 (st. dev.)	αvβ5 (st. dev.)	αvβ3 (st. dev.)	αIIbβ3 (st. dev.)	n^b
1	2400 (700)	662 (570)	24 (16)	6000 (4000)	3
2	470 (310)	37 (23)	3 (2)	590 (490)	6
3	900 (500)	40 (48)	10 (5)	2800 (1800)	2
4	6000 (2100)	540 (98)	3 (0.7)	3200 (3300)	2
5	530 (220)	>10000 −	3 (0.8)	45 (58)	2
6	53 (25)	8700 (1900)	4 (3.3)	3000 (640)	2
7	44 (12)	5700 (200)	6 (4)	>10000 −	2
8	1.7 (1.4)	2500 (3500)	64 (41)	>10000 −	4
9	0.4 (0.4)	350 (300)	53 (63)	>10000 −	3
10	0.15 (0.3)	39 (40)	14 (7)	>10000 −	3
11	0.04 (0.07)	5170 (2200)	8 (5)	4230 (3080)	6
12	0.6 (0.5)	2670 (3600)	0.45 (0.57)	4050 (3300)	3
13	460 (110)	26 (23)	40 (25)	1000 (1500)	2
14	4 (4)	42 (43)	13 (15)	7300 (1290)	3
15	61 (23)	1600 (1350)	53 (41)	580 (725)	3

[a] The concentrations (nM) of compounds, numbered as in text, necessary to inhibit ligand binding to 50% of control, the IC$_{50}$, on the isolated recombinant human integrins αvβ3 and αvβ5 (ligand vitronectin), αvβ6 (ligand fibronectin), and platelet αIIbβ3 (ligand fibrinogen) are shown. [b] n = number of experiments.

αvβ5, while leaving the activity against αvβ6 (high) and αIIbβ3 (low) essentially unchanged. Compound **12** is to our knowledge the first αvβ3/αvβ6 mixed inhibitor with subnanomolar activity. As suggested by the loss of αvβ5 activity between compounds **10** and **11**, the activity was apparently influenced by the bulk of the function, derivatizing the phenyl ring (compounds **6**, **11**, **12**). Although the αvβ6 inhibitory activity was relatively insensitive to modifications of the phenyl ring, it was sensitive to alterations at the basic end of the molecule. Replacement of the pyridin-2-ylamino by a guanyl function, compound **13**, resulted in a loss of activity on αvβ6, IC$_{50}$ 460 nM, and a αvβ3/αvβ5 mixed inhibitor with modest selectivity on αvβ6 was obtained. Activity on αIIbβ3 was slightly enhanced. The 3-nitro-phenyl derivative, compound **14**, in comparison to compound **10**, had similar activity on αvβ5 and αvβ3 and lost 1 order of magnitude activity on αvβ6, while the αIIbβ3 activity remained unchanged. As shown in Figure 2, compound **14** was thus a strong αvβ3, αvβ5, and αvβ6 mixed inhibitor. The necessity of the glycine amide function was demonstrated by replacing this unit by an alkyl chain, compound **15**, which had lost all activity for αvβ5 and little activity for αvβ6 but had gained more than 1 order of magnitude activity for αIIbβ3. The

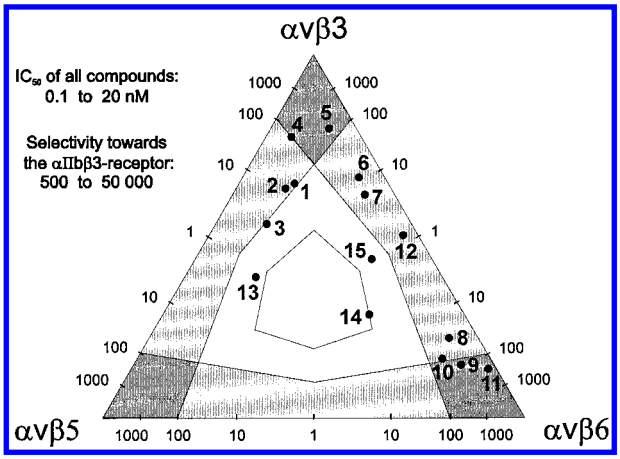


Figure 2. Inhibitor selectivity diagram. The selectivity of the highly active and selective RGD-mimetics described in the text is shown (black dots). Selectivity varies from compounds with high selectivity (dark gray areas), to those with biselectivity (gray/slightly gray areas), and to nonselective compounds with selectivity lower than 10 (white area). The scale shows the nanomolar IC_{50} for isolated receptor inhibition (for calculation of the dot-position, see Appendix).

methyl group of the aminopyridin has no significant influence on αvβ5 activity, as shown by comparison with compound **10**.

To visualize the different selectivities of the inhibitors described here for the αv-integrin receptor subtypes, we have summarized the data in a graphical representation (Figure 2). In this ternary selectivity diagram, each corner—in analogy to a phase diagram—represents one of the αv-integrin subtypes αvβ3, αvβ5, or αvβ6. The selectivities are the ratios of the activities of one compound for two integrin receptor subtypes, thus, each compound has three selectivity values, which are plotted into the diagram. Consequently, every compound is represented by one spot in the triangle. Compounds near the triangular corners (dark gray areas) are monoselective (compounds **4**, **5**, **9**−**11**), those near the edges (gray areas) are biselective (compounds **1**−**3**, **6**−**8**, and **12**), and the compounds in the middle of the triangle (slightly gray or white area) do not differentiate between αvβ3, αvβ5, and αvβ6 (compounds **13**−**15**). The selectivities of nearly all compounds toward the platelet receptor, αIIbβ3, are >500 over αvβ6, αvβ5, and αvβ3 (exceptions are compounds **5** and **15**).

To examine the relationship between activity at the receptor level with that in a cellular environment, we also tested the efficacy of the compounds for their ability to perturb initial cell attachment mediated by αvβ3, αvβ5, or αvβ6 integrins. The results are summarized in Table 2. In general, compounds found active at the receptor level on αvβ6 also inhibited αvβ6-dependent cell attachment of UCLAP-3 carcinoma cells to fibronectin, and compounds more active at the cellular level tended to rank more active at the receptor level, while compounds inactive at the receptor level tended to be weak inhibitors of cell attachment. For example, compound **10** also inhibited attachment over these integrins at the cellular level with a similar activity profile (IC_{50}: αvβ6, 30 nM; αvβ5, 27 μM; αvβ3, 24 μM). The specific αvβ6 receptor blocker compound **11** also had only weak activity on αvβ5 on cells (IC_{50}: αvβ3, 3 μM; αvβ6, 200 nM; αvβ5, 20 μM). However, as previously noted, the inhibitory activities at the cellular level are some 2−3 orders of magnitude lower than at the level of the receptor for αvβ3 inhibitors,[8] and similar loss of activity was seen for the αvβ5 and αvβ6 receptor inhibitors at the cell level. The compounds **6**, **7**, **13**, and **15** showed anomalous behavior at the cellular level, having a low but measurable activity, while at the receptor level they were inactive at the level of detection. The reason for this is not clear, but may be related to nonlinearities in ELISA-based receptor assays as used in this study.[51]

Table 2. Potent and Selective αvβ6 Inhibitors in Cell Adhesion Assays[a]

	IC_{50} (μM) for inhibition of cell attachment			
compd	UCLAP-3/FN αvβ6 (st. dev.)	UCLAP-3/VN αvβ5 (st. dev.)	M21/VN+P1F6 αvβ3 (st. dev.)	n[b]
1	37 (7.1)	1.3 (0.6)	3.9 (2.5)	2
2	10 (9)	0.4 (0.6)	0.4 (0.3)	4
3	n.d.[c] −	n.d.[c] −	n.d.[c] −	2
4	8.4 (7.9)	2.1 (0.4)	1.6 (0.1)	2
5	2.0 (0.1)	31 (9.9)	15 (7.1)	2
6	3.8 (0.4)	24 (14)	0.97 (0.5)	2
7	1.6 (1.1)	18 (6.4)	20 (7.8)	4
8	0.3 (0.13)	5 (2.3)	12 (3.3)	2
9	0.3 (0.03)	2 (0.2)	16 (7.8)	2
10	0.03 (0.02)	27 (30)	24 (1.4)	2
11	0.2 (0.1)	20 (0.7)	3 (0.57)	2
12	0.1 (0.1)	26 (2.1)	9.5 (5.0)	2
13	9.6 (10)	43 (1.4)	>50 −	2
14	0.09 (0.06)	1.2 (0.4)	6.9 (0.07)	2
15	2.1 (0.6)	21 (1.4)	>50 −	2

[a] The concentration of compounds (PM), numbered as in text, necessary to inhibit cell attachment to extracellular matrix coated substrates to 50% of control, the IC_{50}. The sensitivity of the αvβ6 was measured by inhibiting UCLAP-3 cells on fibronectin (FN), that of the αvβ5 integrin by inhibiting UCLAP-3 cells on vitronectin (VN), and that of the αvβ3 integrin by inhibiting M21 cells on vitronectin (VN), in the presence of monoclonal antibody P1F6. [b] n = number of experiments. [c] n.d. = not determined.

Discussion

Integrins convey a variety of signals from the extracellular matrix to the cytoplasm. Specific subgroups of the integrin family are differentially expressed or differentially activated in pathological over normal conditions. Integrins represent therapeutically interesting target molecules, such as αvβ3 and αIIbβ3. αvβ5 and αvβ6 are also differentially expressed but their precise functions and possible therapeutic relevance are as yet unknown. These four integrins have a partially overlapping ligand specificity, and a conserved amino acid sequence, RGD, within the target ligands is at the core of their recognition sites. Nevertheless, non-RGD peptides have been discovered with high specificity and inhibitory activity for αIIbβ3 and αvβ6.[17,26] Peptidic inhibitors have several disadvantages as low molecular weight inhibitors including their instability to serum degradation, which can be circumvented by use of small cyclic peptides,[52] and poor pharmacokinetics. In this study, we describe a series of peptidomimetic inhibitors with high affinity on αvβ6 integrin. Furthermore, we report about the first specific, low molecular weight αvβ6 integrin receptor antagonist, compound **11**. Together with certain inhibitors that have adjunct activity on the integrins αvβ3 or αvβ5, we show that a set of integrin specific inhibitors can be designed around a conserved (aza)-glycine phenyl-β-amino acid backbone.

Of the RGD-dependent integrins used here, αvβ3 is usually considered the most promiscuous in its sub-

strate specificity, closely followed by αIIbβ3. αvβ5 is a relatively specific vitronectin receptor, while αvβ6 binds primarily to fibronectin with some binding activity reported for tenascin-C. We predicted, therefore, that it should be possible to make a series of inhibitors that recognize one or more of the different αv-integrin receptor subtypes. The inhibitors we found fall into this class. We found one peptidomimetic inhibitor, phenylnaphthalene containing compound **11**, favoring integrin αvβ6 with subnanomolar activity. The activity of this compound was nearly 2 orders of magnitude higher on αvβ6 over αvβ3, with almost 5 orders of magnitude smaller activity on both αvβ5 and αIIbβ3. This compound is to our knowledge the first specific, low molecular weight αvβ6 inhibitor that is described in the literature. The selectivity is similar to the monospecific peptidic inhibitors of αvβ6 with the general structure RT/GDLXXLX.[26] The selectivity toward the αvβ5 integrin receptor can be obtained by incorporation of a bulky function at the phenyl ring, compounds **6**, **11**, **12**, and improved with the peptidomimetic azacarba moiety, compounds **6** and **7**.

On the other hand, we found bispecific compounds with interesting profiles. A similar profile to compound **11**, with 1 order of magnitude less activity both on αvβ6 and αvβ3, was seen in the aza-dichlorophenyl compound **8** and its peptidic-linked analogue compound **9**. An even more active biselective αvβ6/αvβ3 inhibitor was found in compound **12**, with similarly low activity on αvβ5 and αIIbβ3. Furthermore, compound **14** represents a mixed, triselective αvβ6/αvβ3/αvβ5 inhibitor with nanomolar activity for these integrin receptors.

At the cellular level, compared with the isolated receptor assays, the specificity of the compounds was usually retained, but with a loss of activity as measured by IC_{50}. We hypothesize that the loss of activity is due to the need to inhibit a large fraction of the receptors on a cell before attachment is lost. Cooperativity and clustering of integrins at the cell surface can also increase apparent local receptor affinity. The isolated receptor assay, by contrast, involves the inhibition of what is more nearly a simple bimolecular interaction, and nonlinear effects can enhance apparent IC_{50} in such assays.[51]

αvβ6, αvβ3, and αvβ5 integrins activate different intracellular signaling pathways, so the ability to specifically target desired combinations of these receptors may be therapeutically relevant. αvβ6 is expressed in proliferating keratinocytes[33] and in the invasive front of certain carcinomas,[30] and it may stimulate release of MMP9[35,36] or help activate latent TGF-β1,[37] while αvβ3 is up-regulated on neoangiogenic blood vessels,[53] where it may promote cell migration[54] and induce apoptosis.[55] Clearly, identifying the precise role of the integrin in such diseases awaits the availability of highly active, specific, and well-defined inhibitors, inhibitors such as are described here.

In conclusion, we describe here for the first time highly active small molecule inhibitors of integrin αvβ6. These compounds have nanomolar IC_{50} on αvβ6 at the receptor level combined with nanomolar activity on αvβ3 and/or on αvβ5, while having an IC_{50} over 10 μM on αIIbβ3. With compound **11**, we have obtained a specific inhibitor of αvβ6 with subnanomolar activity on this integrin receptor (IC_{50} 0.04 nM). As well as providing a starting point for further research on structure−activity relationships, these molecules provide useful structural information for the design of therapies targeting αv-series integrins.

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Appendix

For the calculation of the position $\vec{X} = (x_1, x_2)$ of the compounds (black dots) in the selectivity diagram, we used the following equations

$$E = \alpha(\vec{x} - \vec{A})^2 + \beta(\vec{x} - \vec{B})^2 + \gamma(\vec{x} - \vec{C})^2$$

$$\frac{\partial E}{\partial x_1} = 2\alpha(x_1 - a_1) + 2\beta(x_1 - b_1) + 2\gamma(x_1 - c_1) \overset{!}{=} 0$$

$$\frac{\partial E}{\partial x_2} = 2\alpha(x_2 - a_2) + 2\beta(x_2 - b_2) + 2\gamma(x_2 - c_2) \overset{!}{=} 0$$

$$\Rightarrow x_1 = \frac{\alpha \cdot a_1 + \beta \cdot b_1 + \gamma \cdot c_1}{\alpha + \beta + \gamma} \quad \text{and}$$

$$x_2 = \frac{\alpha \cdot a_2 + \beta \cdot b_2 + \gamma \cdot c_2}{\alpha + \beta + \gamma}$$

with the coordinates for the integrin receptors $\vec{A}$, $\vec{B}$, $\vec{C}$ (cornerpoints) and the activity factors α, β, γ:

$$\vec{A} = \left(\frac{\sqrt{3}}{2}, \frac{3}{2}\right), \quad \alpha = \sqrt[3]{\frac{1}{IC_{50}}} \qquad (\alpha v\beta 3\text{-integrin})$$

$$\vec{B} = (0, 0), \quad \beta = \sqrt[3]{\frac{1}{IC_{50}}} \qquad (\alpha v\beta 5\text{-integrin})$$

$$\vec{C} = (\sqrt{3}, 0), \quad \gamma = \sqrt[3]{\frac{1}{IC_{50}}} \qquad (\alpha v\beta 6\text{-integrin})$$

Supporting Information Available: Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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