Identification and *in vivo* efficacy of small-molecule antagonists of integrin $\alpha_v\beta_3$ (the vitronectin receptor)

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The integrin $\alpha_v\beta_3$ is thought to play a key role in the initiation and/or progression of several human diseases, including osteoporosis, restenosis following percutaneous transluminal coronary angioplasty (PTCA), rheumatoid arthritis, cancer and ocular diseases. Antagonism of integrin $\alpha_v\beta_3$ is therefore expected to provide an approach for the treatment and/or prevention of these diseases. A variety of potent, small-molecule $\alpha_v\beta_3$ antagonists have been identified, several of which are active in disease models, thereby demonstrating the therapeutic potential of $\alpha_v\beta_3$ antagonism. This review will focus on recent advances in the identification of small-molecule $\alpha_v\beta_3$ antagonists, with an emphasis on those studies where small-molecule $\alpha_v\beta_3$ antagonists have been used in proof-of-concept studies *in vivo*.

Integrins are a superfamily of heterodimeric transmembrane glycoproteins that function in cellular adhesion, migration and signal transduction[1–12]. These receptors consist of an α- and a β-subunit, which associate noncovalently in defined combinations. To-date, 17 α-subunits and eight β-subunits have been identified, which associate selectively to form at least 23 integrins.

The integrin $\alpha_v\beta_3$, also referred to as the vitronectin receptor, is expressed on a variety of cell types[5,9,11], including osteoclasts, vascular smooth muscle cells, endothelial cells and various tumor cells. In general, the level of expression of $\alpha_v\beta_3$ is low on most cell types and is greatly increased in remodeling or growing tissues. However, recent evidence suggests that it is expressed in normal lung tissue in rats[13]. Consistent with its expression profile, integrin $\alpha_v\beta_3$ mediates several biologically relevant processes, such as adhesion of osteoclasts to bone, vascular smooth muscle cell migration and angiogenesis. As a result, $\alpha_v\beta_3$ antagonists are expected to be useful for the treatment of osteoporosis, restenosis following percutaneous transluminal coronary angioplasty (PTCA), rheumatoid arthritis, cancer and ocular diseases.

Integrin $\alpha_v\beta_3$ binds to a variety of extracellular matrix proteins[5,9], including fibrinogen, fibronectin, osteopontin, thrombospondin, vitronectin and von Willebrand factor, largely through interaction with the Arg-Gly-Asp (RGD) tripeptide sequence. The related integrin $\alpha_{IIb}\beta_3$ also interacts through this mechanism and antagonists of $\alpha_{IIb}\beta_3$, particularly RGD peptides and small-molecule RGD peptidomimetics, have received considerable attention[14,15]. Research into $\alpha_v\beta_3$ has benefited from the discoveries made in $\alpha_{IIb}\beta_3$ research. Although the $\alpha_{IIb}\beta_3$ area is more mature, the identification of nonpeptide $\alpha_v\beta_3$ antagonists is the subject of vigorous research activity and significant

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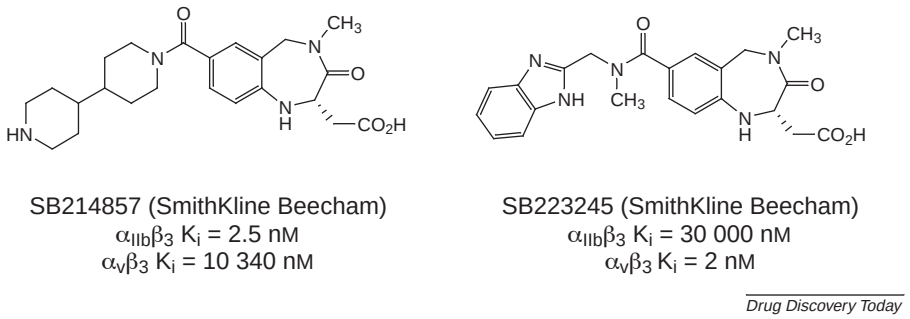

Figure 1. *Selectivity for $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ based on the guanidine mimetic.*

progress has been made. A survey of the recent patent literature indicates that no fewer than 80 patent applications claiming nonpeptide vitronectin receptor antagonists have been published and that several patents have been issued. Reports providing more detailed discussions of the advances made in this area have also begun to appear in the primary literature. This review will summarize the recent developments in the identification of nonpeptide $\alpha_v\beta_3$ antagonists, with an emphasis on those studies wherein small-molecule $\alpha_v\beta_3$ antagonists have been used for *in vivo* proof-of-concept. Generally, the review draws on reports published in the primary literature through the first quarter of 2000 but, where possible, information reported at recent conferences has also been included.

Requirements for selective interaction with integrin $\alpha_v\beta_3$ over integrin $\alpha_{IIb}\beta_3$

Initial investigations into $\alpha_v\beta_3$ antagonists focused on potency against the isolated receptor and selectivity over the closely related integrin $\alpha_{IIb}\beta_3$ (Refs 16–18). These early studies, using cyclic RGD peptides, suggest that $\alpha_v\beta_3$ recognizes a shorter overall separation between the key guanidine (Arg) and carboxylic acid (Asp) groups of the RGD tripeptide sequence. Presumably, the different length requirements are related to the preferential recognition of different conformations of the RGD sequence by the different integrins. The length requirements and conformational information obtained through these studies have served as the starting point for the design of small-molecule $\alpha_v\beta_3$ antagonists.

SmithKline Beecham (SB; King of Prussia, PA, USA) extended these initial observations to the small-molecule area with the identification of SB223245 (Fig. 1)[19], a potent $\alpha_v\beta_3$ antagonist (K_i = 2 nM in an isolated receptor binding assay) with good selectivity over $\alpha_{IIb}\beta_3$ (K_i = 30 000 nM). SB223245 is derived from a 1,4-benzodiazepine-based Gly–Asp template that had previously been used in SB214857, a potent $\alpha_{IIb}\beta_3$ antagonist (K_i = 2.5 nM) with good selectivity over $\alpha_v\beta_3$ (K_i = 10,340 nM)[20]. These studies support the observation that $\alpha_v\beta_3$ recognizes a shorter Arg-to-Asp distance than $\alpha_{IIb}\beta_3$, and demonstrate that a potent and selective $\alpha_{IIb}\beta_3$ antagonist can be converted to a potent and selective $\alpha_v\beta_3$ antagonist by changing the guanidine mimetic.

Another key finding of these early peptidomimetic studies is that a strongly basic guanidine mimetic is not an absolute requirement for high-affinity binding to $\alpha_v\beta_3$; SB223245 contains a neutral benzimidazole group (pKa = 6.2 as a guanidine mimetic), yet is a potent $\alpha_v\beta_3$ antagonist. In the $\alpha_{IIb}\beta_3$ area, the guanidine mimetics are generally strongly basic groups, such as guanidines, amidines and secondary amines. It seems that an amidine-like or guanidine-like disposition of nitrogens is more important than basicity for recognition by $\alpha_v\beta_3$. However, strongly basic guanidine mimetics are well-tolerated by $\alpha_v\beta_3$ as long as an amidine-like arrangement is maintained. For example, at both the *211th American Chemical Society National Meeting* (Duggan M.E. *et al.* Design and evaluation of potent nonpeptide ligands of $\alpha_v\beta_3$ as inhibitors of bone resorption. 24–28 March 1996, New Orleans, LA, USA, Abstract MEDI-234) and the *18th Annual Meeting of the American Society of Bone and Mineral Research*[21], Merck (West Point, PA, USA) described L748415 (Fig. 2), a cyclic-guanidine-containing compound that potently inhibits the binding of ^{125}I-echistatin to the membranes of $\alpha_v\beta_3$-expressing 293 cells (IC_{50} = 0.3 nM) and inhibits the adhesion of $\alpha_v\beta_3$-expressing 293 cells to osteopontin (IC_{50} = 3 nM) and vitronectin (IC_{50} = 0.8 nM). This compound is also a potent inhibitor of ADP-stimulated platelet aggregation (IC_{50} = 44 nM), suggesting high affinity for $\alpha_{IIb}\beta_3$.

Subsequent studies beginning with $\alpha_{IIb}\beta_3$ antagonist templates expanded on these early findings. Hoechst (Frankfurt, Germany) has described the identification of a series of $\alpha_v\beta_3$ antagonists, exemplified by compounds (a) and (b) (Fig. 2), that are derived from a hydantoin template

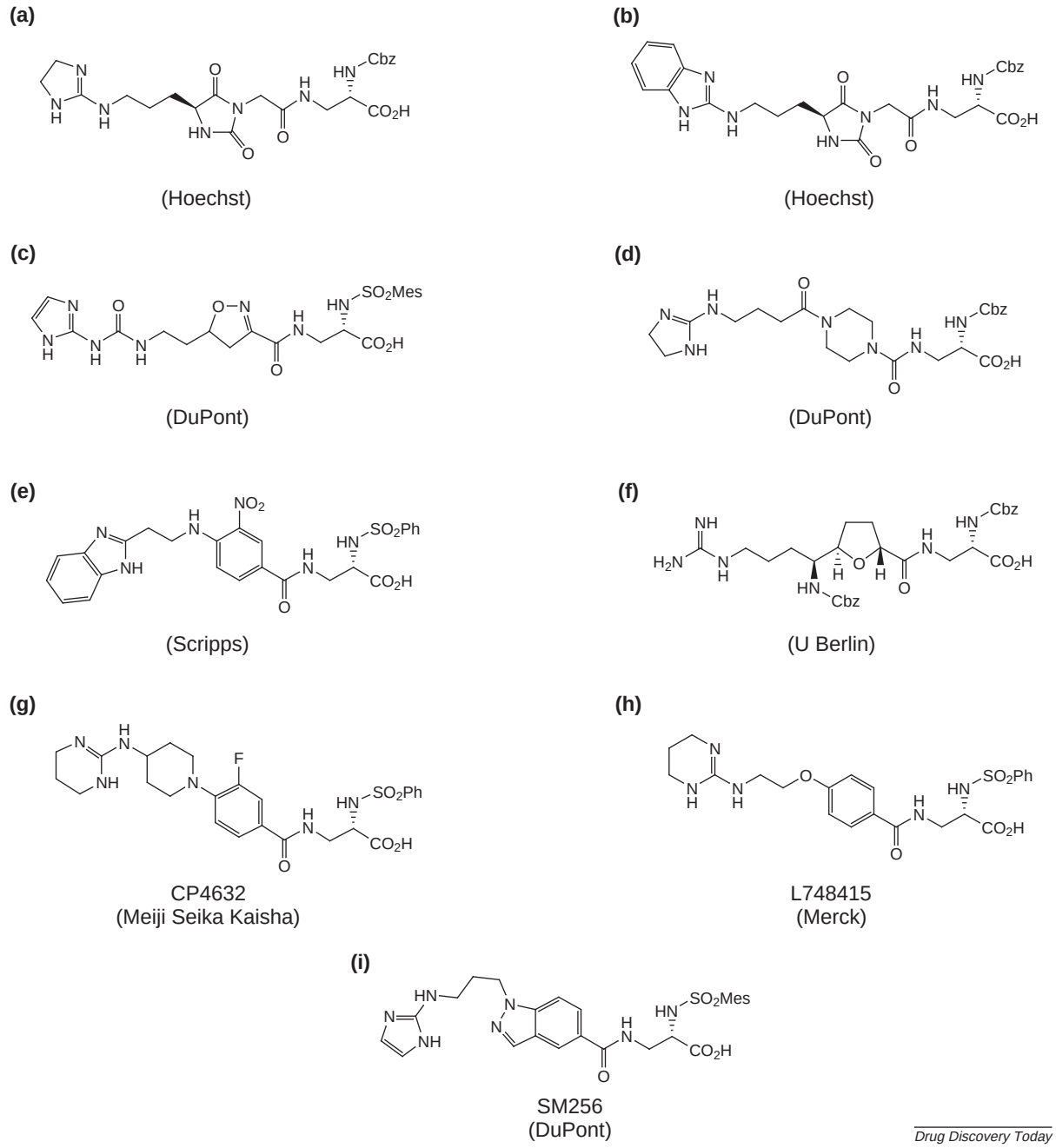

Figure 2. *Selected new $\alpha_v\beta_3$ antagonists. Mes = 2,4,6-trimethylphenyl; Cbz = benzyloxycarbonyl.*

originally used for selective $\alpha_{IIb}\beta_3$ antagonists[22]. Both compounds have good affinity for $\alpha_v\beta_3$ (IC_{50} = 20 and 40 nM, respectively, in inhibiting the binding of kistrin to $\alpha_v\beta_3$) and are selective relative to $\alpha_{IIb}\beta_3$ (IC_{50} >10 000 nM in inhibiting the binding of fibrinogen to $\alpha_{IIb}\beta_3$). In this series, the optimum Arg-to-Asp length for selective interaction with $\alpha_v\beta_3$ is shorter than for $\alpha_{IIb}\beta_3$ and cyclic guanidines are preferred over noncyclic guanidines. The Hoechst investigators speculated that binding to $\alpha_{IIb}\beta_3$ might occur through an 'end-on' interaction, so that noncyclic guanidines are preferred, while binding to $\alpha_v\beta_3$ occurs through a 'side-on' interaction, so that cyclic guanidines are tolerated.

DuPont (Wilmington, DE, USA) has described a series of $\alpha_v\beta_3$ antagonists derived from an isoxazoline template originally used for potent and selective $\alpha_{IIb}\beta_3$ antagonists. Initially using a solid-phase approach[23], followed by more

traditional lead optimization studies[24], the investigators identified potent and selective $\alpha_v\beta_3$ antagonists, exemplified by compound (c) (Fig. 2; IC_{50} = 34 nM in inhibiting adhesion of β3-transfected 293 cells to fibrinogen; IC_{50} = 32 000 nM in inhibiting $\alpha_{IIb}\beta_3$-mediated platelet aggregation in human gel-purified platelets). In this series, guanidine mimetics with a wide range of basicities are tolerated, although less basic mimetics (2-aminoimidazoles, acylated 2-aminoimidazoles and 2-aminopyridines) appear to impart greater selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$. In addition, the results with regioisomeric imidazole guanidine mimetics support the Hoechst findings that $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ might interact differently with guanidine-like groups. No conclusions could be drawn concerning the overall Arg-to-Asp length.

Both Hoechst and DuPont (as well as Merck) used derivatives of 2,3-diaminopropanoic acid (α-*N*-(benzyloxycarbonyl)-diaminopropanoic acid and α-*N*-sulfonyl-diaminopropanoic acid) as Asp mimetics, and the DuPont group has illustrated the importance of the α-*N* substituent in optimizing $\alpha_v\beta_3$ activity. The 2,3-diaminopropanoic acid subunit has found wide utility in both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ antagonists[25].

Requirements for selective interaction with integrin $\alpha_v\beta_3$ over integrins $\alpha_v\beta_5$ and $\alpha_5\beta_1$

Selectivity for $\alpha_v\beta_3$ over other integrins, such as $\alpha_v\beta_5$ and $\alpha_5\beta_1$, has also been achieved. DuPont has described a solid-phase synthesis of compound (d) (Fig. 2) that, in isolated receptor binding assays, has high affinity for $\alpha_v\beta_3$ (IC_{50} = 1.1 nM) and is highly selective over $\alpha_5\beta_1$ (IC_{50} = 660 nM)[26,27]. In addition, this compound has relatively modest activity in a cell-based $\alpha_v\beta_5$ adhesion assay (IC_{50} = 420 nM) and little activity in inhibiting ADP-induced platelet aggregation in platelet-rich plasma (IC_{50} = 20 000 nM). The selectivity for $\alpha_v\beta_3$ over $\alpha_v\beta_5$ is probably related to the α-*N*-(benzyloxycarbonyl)-diaminopropanoic acid subunit, which is reported to give excellent specificity for the β3 class of RGD-interactive integrins[26].

DuPont has also reported the identification[28] and detailed characterization[29] of SM256, an indazole-based RGD mimetic that is a potent inhibitor of both vitronectin binding to purified human $\alpha_v\beta_3$ (IC_{50} = 0.057 nM) and adhesion of β3-transfected 293 cells to fibrinogen (IC_{50} = 2.3 nM). Although SM256 has only modest selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ (IC_{50} = 21 nM in inhibiting aggregation of human gel-purified platelets), it has high selectivity over $\alpha_v\beta_5$ (IC_{50} = 920 nM in a cell adhesion assay) and $\alpha_5\beta_1$ (IC_{50} = 2300 nM in an isolated receptor binding assay). In addition, SM256 has comparable affinity and selectivity for $\alpha_v\beta_3$ from dog, mouse, rabbit and pig. The selectivity for $\alpha_v\beta_3$ over $\alpha_v\beta_5$ is presumably related to the diaminopropanoic acid subunit. In this indazole series, as in DuPont's isoxazoline series[24], less basic guanidine mimetics are preferred and the α-*N* substituent is important in optimizing $\alpha_v\beta_3$ activity.

Dual $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ antagonists

Dual $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ antagonists have the potential to inhibit events mediated by both $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ and therefore might have value in the treatment of restenosis following PTCA (Ref. 9). Antagonism of $\alpha_v\beta_3$ is expected to inhibit smooth muscle cell migration associated with vascular remodeling, while antagonism of $\alpha_{IIb}\beta_3$ might inhibit the formation of a platelet aggregate at the site of vascular injury. Studies with abciximab (ReoPro), a monoclonal antibody that interacts with $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, seem to support this hypothesis[30,31]. However, unambiguous validation of this hypothesis would require evaluating a selective $\alpha_v\beta_3$ antagonist in combination with a selective $\alpha_{IIb}\beta_3$ antagonist in a restenosis model, and comparing the results with those obtained with either agent alone.

The *218th American Chemical Society Meeting* (22–26 August 1999, New Orleans, LA, USA) received a report on work underway to identify a balanced $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist for the treatment of ischemic diseases (Ishikawa M. *et al.* Synthesis of potent, nonpeptide integrin $\alpha_v\beta_3$ antagonists. Abstract MEDI-063). The investigators have identified numerous potent antagonists but the preferred compound appears to be CP4632, a potent dual antagonist of $\alpha_v\beta_3$ (IC_{50} = 0.2 nM) and $\alpha_{IIb}\beta_3$ (IC_{50} = 0.17 nM) in solid-phase binding assays. Consistent with its dual antagonist profile, CP4632 is a potent inhibitor of vascular smooth muscle cell migration (IC_{50} = 37 nM) and inhibits platelet aggregation in human platelet-rich plasma (IC_{50} = 55 nM). Furthermore, CP4632 has good water solubility (1.5 mg ml^{-1}), a moderate intravenous (i.v.) half-life ($t_{1/2}$ = 34 min) and does not extend the bleeding time in dogs (bleeding time refers to the time required for bleeding to stop after a standardized lancet wound on the interior of the lip). Pharmacological studies are reported to be ongoing.

A report from Scripps (La Jolla, CA, USA) describes a series of derivatives closely related to the Merck compound L748415, wherein the central aromatic ring is substituted by a nitro group[32]. These compounds, which can be readily prepared through nucleophilic displacement of the fluoride group of *ortho*-nitro aryl fluorides, can generally be regarded as dual $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ antagonists. For example, compound (e) (Fig. 2), the most potent and selective $\alpha_v\beta_3$ antagonist identified, has high affinity for both $\alpha_v\beta_3$ (IC_{50} = 0.81 nM) and $\alpha_{IIb}\beta_3$ (IC_{50} = 24 nM) in isolated receptor binding assays. However, despite this level of affinity, this compound, and related potent $\alpha_v\beta_3$ antagonists, has

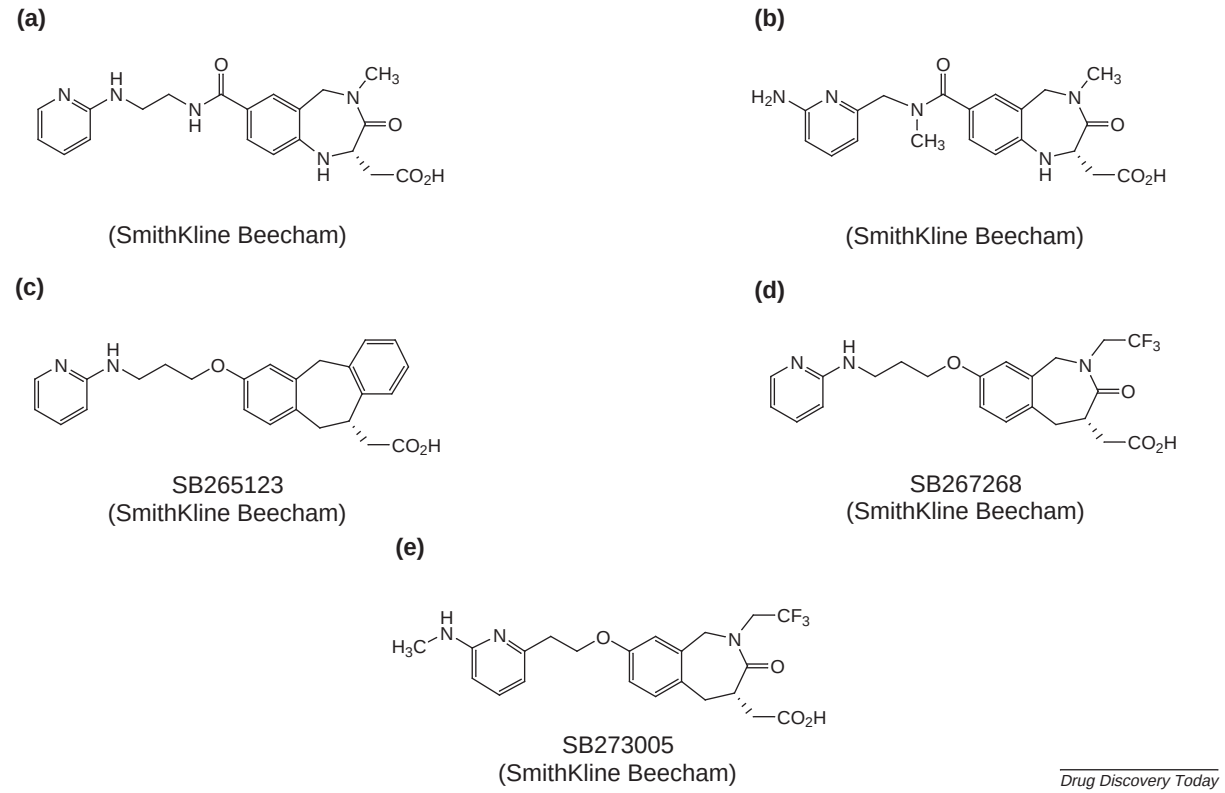

Figure 3. *Progress towards selective, orally bioavailable $\alpha_v\beta_3$ antagonists.*

relatively poor activity (IC_{50} >1 μM) in an $\alpha_v\beta_3$-mediated cell adhesion assay. None of the compounds prepared in this study have appreciable affinity for $\alpha_v\beta_5$ (IC_{50} >1 μM), presumably because of the presence of the α-*N*-sulfonyl-diaminopropanoic acid subunit[26].

Investigators at the Institut für Chemie der Humbolt-Universität zu Berlin (Berlin, Germany) in collaboration with Merck KGaA (Darmstadt, Germany) have described a series of *trans*- and *cis*-2,5-disubstituted tetrahydrofuran derivatives as integrin antagonists[33]. This study led to the identification of compound (f) (Fig. 2), which has good affinity for $\alpha_v\beta_3$ (IC_{50} = 52 nM) and $\alpha_{IIb}\beta_3$ (IC_{50} = 67 nM) and is selective over $\alpha_v\beta_5$ (IC_{50} >10,000 nM) in isolated receptor binding assays. The activity and selectivity in this series appear to be related to the linking group as well as to the relative and absolute configuration of the stereocenters about the tetrahydrofuran (THF) ring.

Identification of orally bioavailable $\alpha_v\beta_3$ antagonists

Historically, the discovery of $\alpha_{IIb}\beta_3$ antagonists with oral bioavailability greater than approximately 10% was difficult unless a prodrug strategy was employed to mask the ionic functionality[15]. However, considerable progress has been made in the identification of orally bioavailable small-molecule $\alpha_v\beta_3$ antagonists (Fig. 3).

The SB lead compound SB223245, while a potent $\alpha_v\beta_3$ antagonist, has poor pharmacokinetics and is therefore not suitable for *in vivo* proof-of-concept studies. Early follow-up studies, directed at improving the pharmacokinetics while maintaining or improving potency, concentrated on the 1,4-benzodiazepine series[34–36] and revealed that the benzimidazole guanidine mimetic could be replaced with nonbasic aminopyridine-based guanidine mimetics. In receptor binding assays, two representatives of this series – compounds (a) and (b) (Fig. 3) – have high affinity for $\alpha_v\beta_3$ (K_i = 3.5 and 35 nM, respectively) and high selectivity relative to $\alpha_{IIb}\beta_3$ (K_i = 28 000 and 32 000 nM, respectively).

Importantly, the aminopyridine-based guanidine mimetics appear to afford somewhat improved oral bioavailability, although the level is still rather low (approximately 10%). Subsequent studies combined these aminopyridine-based guanidine mimetics with modified Gly–Asp templates and identified several compounds with good oral bioavailability.

The dibenzocycloheptene derivative SB265123 (Fig. 3c) is a potent $\alpha_v\beta_3$ antagonist in both a receptor binding

assay (K_i = 4 nM) and in an $\alpha_v\beta_3$-mediated cell adhesion assay (IC_{50} = 60 nM)[37] and is selective for $\alpha_v\beta_3$ relative to the other RGD-binding integrins $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$. However, like SB223245, SB265123 has a high affinity for $\alpha_v\beta_5$ (K_i = 0.4 nM for SB223245; K_i = 1.3 nM for SB265123) in isolated receptor binding assays. Significantly, SB265123 has outstanding pharmacokinetics in rats, with a long half-life ($t_{1/2}$ = 3–6 h), low plasma clearance (Clp = 3 ml min^{-1} kg^{-1}) and very high oral bioavailability (approximately 100%). Although enterohepatic recirculation might contribute to this pharmacokinetic profile[38], the identification of SB265123 demonstrates that potent $\alpha_v\beta_3$ antagonists with high levels of oral bioavailability are achievable without having to resort to a prodrug strategy.

SB has also reported another class of potent, orally bioavailable small-molecule antagonists based on a 2-benzazepine template[39]. SB267268 (Fig. 3d) and SB273005 (Fig. 3e) are highly potent $\alpha_v\beta_3$ antagonists in both an isolated receptor binding assay (K_i = 0.9 and 1.2 nM, respectively) and an $\alpha_v\beta_3$-mediated cell adhesion assay (IC_{50} = 12 and 3 nM, respectively). SB267268 and SB273005 also have high affinity for $\alpha_v\beta_5$ (K_i = 0.6 and 0.3 nM, respectively) but are selective relative to $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$. Both compounds have good pharmacokinetics in rats, with moderate plasma clearance (Clp = 16 and 25 ml min^{-1} kg^{-1}, respectively) and high oral bioavailability (Fpo = 34 and 72%, respectively). These findings further demonstrate the feasibility of identifying potent $\alpha_v\beta_3$ antagonists with high oral bioavailability.

Other templates investigated for small-molecule $\alpha_v\beta_3$ antagonists

Other scaffolds have been investigated for the construction of nonpeptide $\alpha_v\beta_3$ antagonists, including carbohydrates[40], dianhydrohexitol[41] and biphenyls[42], but none of these templates has yet afforded potent $\alpha_v\beta_3$ antagonists. However, a series of cyclic peptides containing a carbohydrate linker[43] has shown low nanomolar affinity for $\alpha_v\beta_3$. Azapeptides and azapeptoids have been reported[44] but the compounds described have little affinity for $\alpha_v\beta_3$. In addition, a series of iminodiacetic acid derivatives have been prepared by liquid-phase parallel synthesis as potential integrin inhibitors, but no affinity data was reported[45].

Pharmacological evaluation

Osteoporosis

Osteoporosis is a debilitating bone disease characterized by a decrease in bone mass (osteopenia) leading to an increased risk of fracture[46]. The osteopenia associated with osteoporosis arises from an imbalance between bone resorption and formation, such that resorption exceeds formation. For bone resorption to occur, the bone-resorbing osteoclasts must first adhere to the bone matrix and this key adhesive event is mediated by integrin $\alpha_v\beta_3$ (Refs 9,47). Disruption of osteoclast adhesion inhibits bone resorption both *in vitro* and *in vivo*[48,49] and may provide a therapeutic approach to the treatment and/or prevention of osteoporosis.

Merck has reported the activity of L748415 in models of bone resorption. L748415 is a potent inhibitor of rat osteoclast-mediated bone resorption (IC_{50} = 40 nM) and also inhibits osteoclast formation in a murine osteoblast–bone marrow co-culture with an IC_{50} of 85 nM. In an *in vivo* thyroidectomized–parathyroidectomized (TPTx) rat model (which measures the ability of a compound to inhibit the parathyroid hormone (PTH)-induced calcemic response in hypocalcemic TPTx rats) an infusion of 2 mg kg^{-1} h^{-1} L748415 inhibits the calcemic response with an IC_{50} of 1 μM (circulating concentrations). This study provides evidence that small-molecule $\alpha_v\beta_3$ antagonists can inhibit bone resorption *in vivo*.

Monsanto/Searle (Chesterfield, MO, USA) has reported the identification and evaluation of SC56631 in models of bone resorption (Fig. 4)[50]. SC56631, identified through the evaluation of a library of synthetic RGD-mimetics, has high affinity for both $\alpha_v\beta_3$ (IC_{50} = 10 nM) and $\alpha_{IIb}\beta_3$ (IC_{50} = 9 nM) in solid-phase receptor binding assays but is relatively ineffective in blocking ADP-induced platelet aggregation in human (IC_{50} = 20 μM) or rat (IC_{50} >300 μM) platelet-rich plasma. SC56631 inhibits $\alpha_v\beta_3$-mediated adhesion of A3827 melanoma cells to fibrinogen, as well as attachment of $\alpha_v\beta_1$-expressing 293 cells (IC_{50} = 317 nM) and β5-transfected 293 cells (IC_{50} = 23 nM) but has little effect on $\alpha_5\beta_1$-mediated adhesion. In functional assays *in vitro*, SC56631 inhibits murine osteoclast-mediated bone resorption in a dose-dependent fashion with an IC_{50} of approximately 10 μM. The compound also inhibits rabbit osteoclast-mediated resorption of sperm whale dentin in a dose-dependent fashion, with an IC_{50} of approximately 5 μM.

In vivo, SC56631 is a potent inhibitor of bone resorption and estrogen-deficiency-induced osteopenia. In a TPTx rat model, continuous i.v. infusion of SC56631 inhibits the calcemic response in a dose-dependent fashion, providing approximately 40% inhibition at circulating blood levels of 3 μM. In an ovariectomized (Ovx) rat model of osteopenia, SC56631, on continuous i.v. infusion at rates of 0.1 and 0.5 mg kg^{-1} min^{-1} for six weeks, inhibits bone loss as determined by pQCT measurement of trabecular bone density. Histological evaluation of the bone from these animals confirmed the bone sparing effects. As SC56631 has a very short half-life ($t_{1/2}$<20 min), intravenous infusion was used to maintain circulating blood levels at approximately

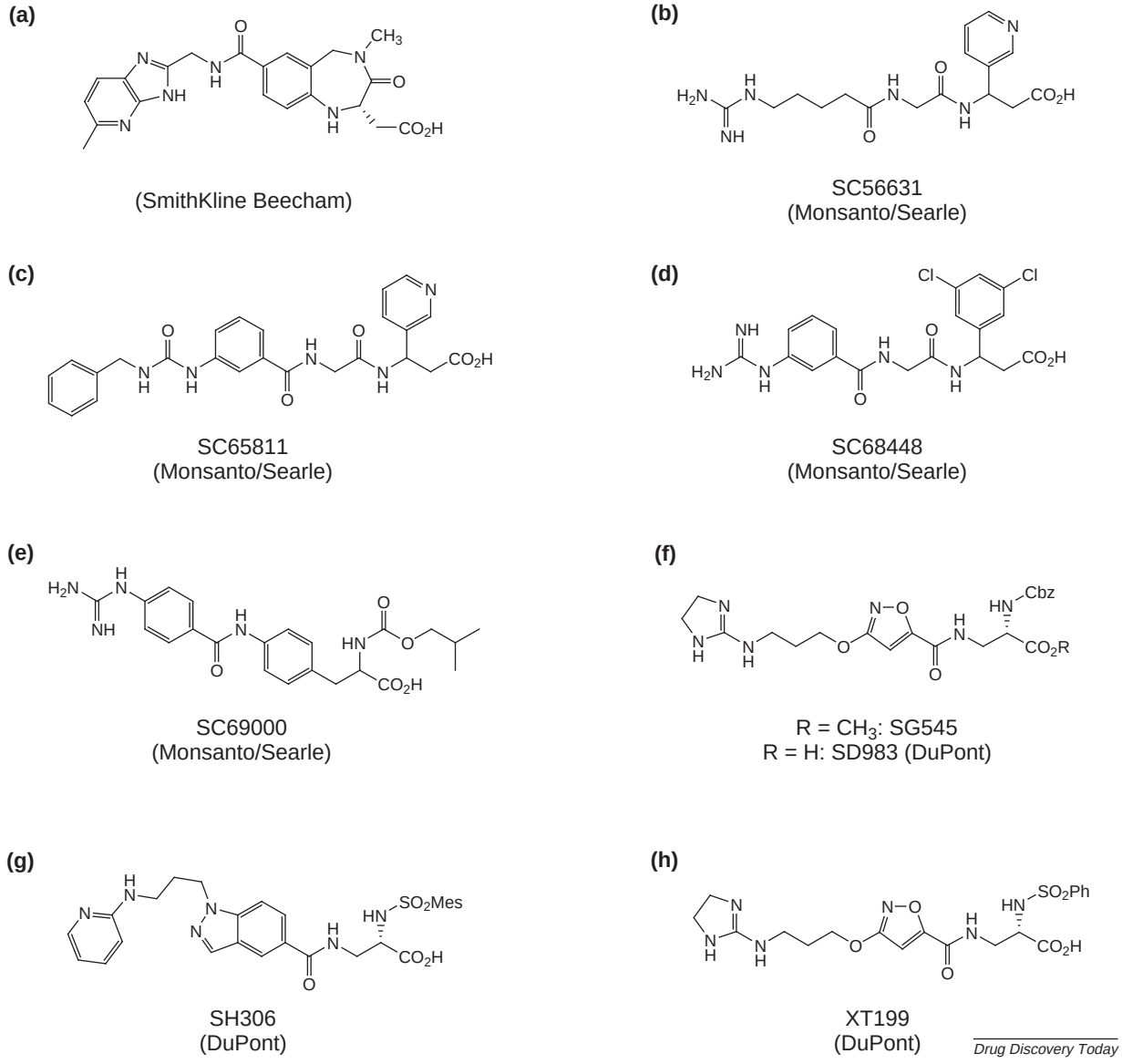

Figure 4. *Selected new $\alpha_v\beta_3$ antagonists. Mes = 2,4,6-trimethylphenyl; Cbz = benzyloxycarbonyl.*

1 and 10 μM for the low and high doses, respectively. Despite the difficult dosing protocol, this study was the first to demonstrate that small-molecule $\alpha_v\beta_3$ antagonists can be effective in this model of estrogen-deficiency-induced osteopenia.

Compound SB265123 (Fig. 3c) has been found to be active in models of bone resorption and bone loss[37,51]. An *in vitro* human osteoclast-mediated bone resorption assay found SB265123 to have an IC_{50} of 48 nM and, in an *in vivo* TPTx rat model of bone resorption, on continuous i.v. infusion at a rate of 2.5 mg kg^{-1} h^{-1}, SB265123 was found to give 85% inhibition of the calcemic response after 6 h. Most significantly, on twice-a-day oral dosing at 3, 10 and 30 mg kg^{-1} in an Ovx rat model, SB265123 has been found to inhibit bone loss in a dose-dependent fashion, as determined by bone mineral density (BMD) measurements. This was the first study to demonstrate oral efficacy with a small-molecule $\alpha_v\beta_3$ antagonist in any pharmacological model and suggests that $\alpha_v\beta_3$ antagonists have the potential to be orally administered agents for the treatment of human disease.

SB267268 and SB273005 (Fig. 3) have also been found to be active in models of bone resorption and osteopenia[39,52]. In an *in vitro* human osteoclast resorption assay, SB267268

has an IC_{50} of 29 nM and SB273005, an IC_{50} of 11 nM. In the TPTx rat model, SB267268 has an EC_{50} of 35 μM and SB273005, an EC_{50} of 20 μM. Furthermore, on twice-a-day oral dosing in the Ovx rat model, both SB267268 (at 5, 15 and 60 mg kg^{-1}) and SB273005 (at 3, 10 and 30 mg kg^{-1}) inhibit bone loss in a dose-dependent fashion, as determined by BMD measurements. Histomorphometric and biochemical marker studies with SB273005 indicate that this compound prevents bone loss by inhibiting bone resorption. These findings further support the assertion that $\alpha_v\beta_3$ antagonists have the potential to be orally administered drugs for the treatment of human disease.

Angiogenesis and cancer

Angiogenesis (the formation of new blood vessels) involves the $\alpha_v\beta_3$-mediated migration and proliferation of endothelial cells[5–7,9]. Significantly, $\alpha_v\beta_3$ is upregulated only in growing vessels and not in mature ones. Thus, $\alpha_v\beta_3$ antagonists should be useful in the treatment of diseases characterized by excessive or undesirable angiogenesis, such as cancer, rheumatoid arthritis, diabetic retinopathy and macular degeneration. In support of this hypothesis, $\alpha_v\beta_3$-selective antibodies and peptides have been shown to be effective inhibitors of angiogenesis[5–7,9].

Monsanto/Searle has reported that SC68448 (Fig. 4d) inhibits corneal neovascularization, Leydig cell tumor growth and the development of hypercalcemia of malignancy[53]. SC68448 is a potent $\alpha_v\beta_3$ antagonist (IC_{50} = 1.1 nM) with >100-fold selectivity relative to $\alpha_{IIb}\beta_3$ (IC_{50} = 152 nM). In functional studies *in vitro*, SC68448 dose-dependently inhibits the bFGF-stimulated proliferation of endothelial cells (IC_{50} = 1–10 μM), suggesting that the compound could be antiangiogenic. To test this hypothesis *in vivo*, the Monsanto/Searle group evaluated SC68448 in a rat model of corneal neovascularization. On intraperitoneal (i.p.) administration at 50 mg kg^{-1} twice-a-day for seven days, SC68448 inhibits bFGF-induced neovascularization by 56% relative to untreated controls. As expected, these effects are restricted to the neovasculature, as $\alpha_v\beta_3$ is expressed only on growing blood vessels. These results suggest that small-molecule $\alpha_v\beta_3$ antagonists could be useful as angiogenesis inhibitors for the treatment of ocular diseases.

As neovascularization is important to the growth and development of tumors, SC68448 was evaluated in a xenogenic severe combined immunodeficiency (SCID) mouse/rat Leydig cell tumor model. After ten days i.p. dosing at 50 mg kg^{-1} twice-a-day, SC68448 reduces the average tumor weight by 60% and tumor volume by 82%. Interestingly, SC68448 also inhibits the development of hypercalcemia associated with the Leydig tumor, which results from a stimulation of osteoclast-mediated bone resorption by humoral tumor-derived factors. Presumably, this effect results from the antiresorptive properties of $\alpha_v\beta_3$ antagonists. These data suggest that small-molecule $\alpha_v\beta_3$ antagonists might be useful as angiogenesis inhibitors for the treatment of cancer and also for managing humoral hypercalcemia of malignancy (HHM).

DuPont has evaluated several compounds in mouse models of angiogenesis and tumorigenesis[54]. In a mouse matrigel model of angiogenesis, subcutaneous (s.c.) administration of SM256 (Fig. 2; $\alpha_v\beta_3$ IC_{50} = 4 nM; $\alpha_v\beta_5$ IC_{50} = 1 μM in mouse endothelial cell adhesion assays) using seven-day osmotic minipumps decreases blood vessel formation with an ED_{50} of 0.055 μg kg^{-1} day^{-1}. Two other small-molecule antagonists from DuPont – SG545 (Fig. 4), which is a methyl ester prodrug of SD983 ($\alpha_v\beta_3$ IC_{50} = 2 nM; $\alpha_v\beta_5$ IC_{50} = 54 nM in mouse endothelial cell adhesion assays), and XT199 ($\alpha_v\beta_3$ IC_{50} = 40 nM; $\alpha_v\beta_5$ IC_{50} >1 μM) – are less active in the matrigel model (ED_{50} = 6 and 45 μg kg^{-1} day^{-1}, respectively). These compounds have also been evaluated in an *in vivo* mouse xenograft model using human colon carcinoma RKO cells that express $\alpha_v\beta_5$ (but not $\alpha_v\beta_3$). SM256 and XT199 are only moderately effective in inhibiting tumor growth, providing 20–21% inhibition (not statistically significant). However, SG545 produces a 57–59% reduction in tumor vascularity, which results in a 40% inhibition of tumor volume. The mouse xenograft results could suggest an important role for $\alpha_v\beta_5$ in tumor growth and development, although in this model, SG545 has significantly higher systemic exposure than the other two compounds. Interestingly, the apoptotic index increases significantly for all three small molecules relative to a vehicle-treated control, suggesting that increased cell death might contribute to decreased tumor volumes. Although the relationship between the *in vitro* α_v affinity and activity in the *in vivo* models is not entirely clear, these studies suggest that selective α_v antagonists with appropriate pharmacokinetic properties could be useful as inhibitors of angiogenesis and tumor growth.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a debilitating, systemic autoimmune disease in which there is massive bone and cartilage destruction within articulating joints[55]. Integrin $\alpha_v\beta_3$ is expressed on the vessels within the invasive pannus and could play a role in angiogenic vessel formation within the highly invasive hypertrophic synovium[56,57]. In addition, $\alpha_v\beta_3$ mediates the bone resorption process. As RA involves both angiogenic vessel formation and bone resorption, $\alpha_v\beta_3$ antagonists could prove useful in its treatment. In support of this hypothesis, a cyclic Arg-Gly-Asp peptide has been shown to be effective in a rabbit model of inflammatory arthritis[56].

At the *Fall 1999 American College of Rheumatology Meeting* (13–17 November 1999, Boston, MA, USA), SB described the activity of SB273005 (Fig. 3) in an inflammatory arthritis model[58]. SB273005 is a potent inhibitor of human endothelial cell migration *in vitro* (IC_{50} = 1.8 nM) and a potent inhibitor of bone resorption. It might therefore be effective in treating joint destruction in RA by preventing both bone resorption and vascularization of the synovial pannus. To test this hypothesis, the SB group evaluated SB273005 in an inflammatory arthritis model (adjuvant-induced arthritis) in the rat. In this model, on twice-a-day oral dosing at levels as low as 3 mg kg^{-1}, SB273005 inhibits joint swelling and the destruction of both bone and cartilage. These results suggest that small-molecule $\alpha_v\beta_3$ antagonists might be therapeutically beneficial in the treatment of inflammatory rheumatoid arthritis.

Restenosis

Restenosis refers to a significant, delayed loss of blood vessel lumen that generally occurs after PTCA. Vascular smooth muscle cell migration into the neointima is a necessary step in restenosis and $\alpha_v\beta_3$, which is expressed on smooth muscle cells, has been shown to mediate this migration[8]. In addition, vascular injury induced by PTCA causes a rapid, persistent and coordinated upregulation of $\alpha_v\beta_3$, $\alpha_v\beta_5$ and osteopontin during the period of neointimal development[59]. Studies have shown that blocking $\alpha_v\beta_3$ inhibits smooth muscle cell migration and that both peptide antagonists of $\alpha_v\beta_3$[60,61], as well as Vitaxin[62,63] (a humanized monoclonal antibody) are effective in reducing neointimal hyperplasia following arterial injury *in vivo*. Furthermore, Vitaxin treatment has also been associated with increased apoptosis of activated vascular smooth muscle cells. These findings suggest that $\alpha_v\beta_3$ antagonists could be useful for the treatment of restenosis following PTCA.

Compound (a) (Fig. 4), which contains an imidazopyridine group as a guanidine mimetic, has high affinity for $\alpha_v\beta_3$ (K_i = 45 nM) and inhibits the migration of rat vascular smooth muscle cells with an IC_{50} of 1.6 μM (Ref. 35). Although this compound has only modest potency, it has very low clearance (Clp = 1.8 ml min^{-1} kg^{-1}) and so was evaluated *in vivo* in a rat model of restenosis. On continuous i.v. infusion (10.8 mg kg^{-1} day^{-1} for seven days, then 3.6 mg kg^{-1} day^{-1} for the next seven days), this compound reduced the total neointimal lesion volume by 35% relative to vehicle control, thereby demonstrating the potential of small-molecule $\alpha_v\beta_3$ antagonists in the treatment of restenosis.

Monsanto/Searle has used SC69000 and SC65811 to study the effects of small-molecule $\alpha_v\beta_3$ antagonists on restenosis[64,65]. In isolated solid-phase receptor binding assays, SC69000 is a potent antagonist of both $\alpha_v\beta_3$ (IC_{50} = 0.56 nM) and $\alpha_{IIb}\beta_3$ (IC_{50} = 15.3 nM). SC69000 is also a potent inhibitor of cell attachment mediated by $\alpha_v\beta_5$ (IC_{50} = 0.091 nM) and $\alpha_v\beta_1$ (0.75 nM) and is >900-fold selective for $\alpha_v\beta_3$ versus $\alpha_5\beta_1$ (IC_{50} = 490 nM). Similarly, in isolated solid-phase receptor binding assays, SC65811 is a potent antagonist of $\alpha_v\beta_3$ (IC_{50} = 0.79 nM) but has little affinity for $\alpha_{IIb}\beta_3$ (IC_{50} = 3147 nM). In cell-based attachment assays, SC65811 is a potent inhibitor of $\alpha_v\beta_5$-mediated attachment (IC_{50} = 0.96 nM) but is relatively selective for $\alpha_v\beta_3$ over $\alpha_v\beta_1$ (IC_{50} = 79.2 nM) and $\alpha_5\beta_1$ (18 000 nM).

In functional studies, SC69000 and SC65811 inhibit the $\alpha_v\beta_3$-mediated binding of ^{125}I-kistrin to porcine smooth muscle cells (88% and 47% inhibition at 0.10 μM, respectively) and also inhibit insulin-like growth factor (IGF-I)-stimulated migration of porcine smooth muscle cells *in vitro* (88% and 82% inhibition at 0.10 μM, respectively). In an *in vivo* study in pigs, the compounds were delivered via osmotic minipumps directly to the site of vascular injury (Goldblatt clamps on the carotid and femoral arteries) at a rate of 5 μl h^{-1} for 14 days. Under these conditions, SC69000 provides a 51% decrease in neointimal area relative to vehicle (saline) control and SC65811 produces a 46% reduction. These results are consistent with those from a previous pig restenosis study (tantalum wire stents in coronary arteries), wherein an $\alpha_v\beta_3$-selective cyclic peptide provided a 42% reduction in lesion volume after a three-week infusion[61]. In the Monsanto/Searle study, the reduction in lesion size is attributed, at least in part, to inhibition of smooth muscle migration but an effect on apoptosis could not be ruled out.

At the *72nd American Heart Association Meeting* (7–10 November 1999, Atlanta, GA, USA) Genentech (San Francisco, CA, USA) reported that V0514 (structure unavailable) – a selective $\alpha_v\beta_3$ antagonist (no affinity data available) – inhibits neointima formation following balloon injury in porcine coronary arteries[66]. In this study, an i.v. bolus of V0514 (100 μg kg^{-1}) was administered at the time of PTCA, followed by an i.v. infusion at 10 μg kg^{-1} min^{-1} for 14 days. A 57% reduction in the neointima/media ratio was observed at day 28. A separate acute study in this model (6 h) indicates that the infusion of V0514 has no significant effect on platelet aggregation or thrombus formation.

Two studies from DuPont describe the effects of small-molecule $\alpha_v\beta_3$ antagonists in rabbit models of restenosis. In a rabbit cuff model of restenosis, SH306 not only reduces neointima formation but also has an effect on thrombus formation[67]. SH306 inhibits the adhesion of $\alpha_v\beta_3$-expressing 293 cells to fibrinogen with an IC_{50} of 48 nM, inhibits $\alpha_{IIb}\beta_3$-mediated platelet aggregation in human gel-purified platelets

with an IC_{50} of 220 nM (Ref. 28) and also inhibits the adhesion of fibrinogen to both human umbilical vein endothelial cells (HUVEC) and rabbit endothelial cells (IC_{50} = 20 nM and 500 nM, respectively). In the restenosis experiment, animals were dosed with an i.v. bolus of 5 mg kg^{-1} (30 min before surgery), followed by 10 mg kg^{-1} s.c. three times-a-day for three days. At day 21, neointima formation, as assessed by the intima/media ratio, is significantly decreased. Interestingly, SH306 also appears to afford protection from clotting, which might be because of an inhibition of platelet adhesion and/or aggregation. The relative role of $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ antagonism in mediating the apparent antithrombotic response of SH306 remains to be determined.

In a second study, reported at the *72nd American Heart Association Meeting*, Du Pont found XT199 to be effective in an atherosclerotic rabbit femoral artery model of restenosis[68]. In this experiment, animals were dosed with an initial i.v. bolus of 2.5 mg kg^{-1} followed by an i.v. infusion of 2.5 mg kg^{-1} day^{-1} for 14 days. At 28 days after balloon injury, histomorphometry shows an increase in the lumen size and a decrease in intimal area, and angiographic analysis shows a 30–40% reduction in restenosis. Notably, this study found that smooth muscle cell content in the lesion is not altered by treatment but that macrophage infiltration is reduced by 50%.

Taken together, these studies suggest that antagonism of integrin $\alpha_v\beta_3$ could provide a viable therapeutic approach to the treatment of restenosis. The inhibition of vascular smooth muscle cell migration, as well as anti-inflammatory actions, anti-angiogenesis[69] and induction of anoikis[70], might all contribute to the efficacy of $\alpha_v\beta_3$ antagonists.

Summary

A variety of potent, small-molecule antagonists of integrin $\alpha_v\beta_3$ have been identified. The majority of these compounds have high selectivity for $\alpha_v\beta_3$ relative to $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$, and several classes of compounds show selectivity for $\alpha_v\beta_3$ over $\alpha_v\beta_5$. In addition, compounds with high affinity for both $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ have been identified. Several small-molecule $\alpha_v\beta_3$ antagonists have been shown to be active in animal models of disease and orally active compounds have been discovered. The studies published to-date provide compelling evidence that small-molecule $\alpha_v\beta_3$ antagonists could be useful for the treatment and/or prevention of several human diseases, including osteoporosis, rheumatoid arthritis, restenosis, ocular neovascularization and cancer. In the near future, reports from clinical studies investigating the efficacy of small-molecule $\alpha_v\beta_3$ antagonists in man should begin to appear in the literature. These studies will provide the ultimate test of the value of integrin $\alpha_v\beta_3$ as a therapeutic target and will determine the direction of future developments within this exciting and promising field.

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