The Emergence of Small Molecule Non-RGD-mimetic Inhibitors for RGD Integrins

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ABSTRACT

The RGD integrins are recognized therapeutic targets for thrombosis, fibrosis, and cancer, amongst others. Current inhibitors are designed to mimic the tripeptide sequence (arginine-glycine-aspartic acid) of the natural ligands; however, the RGD-mimetic antagonists for $\alpha_{IIb}\beta_3$ have been shown to cause partial agonism, leading to the opposite pharmacological effect. The challenge of obtaining oral activity and synthetic tractability with RGD-mimetic molecules, along with the issues relating to pharmacology, has left integrin-therapeutics in need of a new strategy. Recently, a new generation of inhibitor has emerged that lacks the RGD-mimetic. This

perspective will discuss the discovery of these non-RGD-mimetic inhibitors, and the progress that has been made in this promising new chemotype.

INTRODUCTION

Integrins are heterodimeric cell adhesion receptors that mediate the attachment of cells to the extracellular matrix (ECM) and are vital in cell to cell interactions.[1] These receptors were named "integrins" to represent the important role that they play in maintaining the integrity of the cytoskeletal-ECM linkage.[2] By recognizing binding motifs in ECM proteins and glycoproteins, integrins enable adhesion, migration, and proliferation of cells in their biological environment. At the time of this review, there are 24 known integrins in humans, each of which is composed of two non-covalently associated subunits: an α domain and a β domain (a representative schematic of the integrin structure is provided in Figure 1).[1] These domains exist solely as dimeric species at the cell surface; there is an excess of the β subunit found within the cell, and the availability of α subunit counterparts determines the number of receptors that move to the cell surface.[3] A study by Coller *et al*. hypothesized that patients with Glanzmann thrombasthenia (a rare bleeding disorder) may have a limited availability of β_3 subunits, which could account for the low levels of $\alpha_{IIb}\beta_3$ within the patients' platelets.[4]

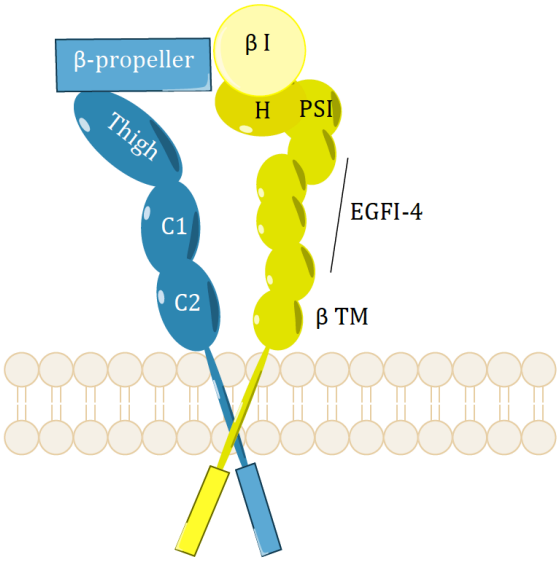


Figure 1. Illustrative representation of an arginine-glycine-aspartic acid (RGD) integrin: α domain (blue), β domain (yellow). Based on previously reported structural information.[5,6,7]

The α domain has a seven-bladed β-propeller at its head, which is supported by the leg structure; this is composed of a thigh, a calf-1 (C1), and a calf-2 domain (C2). Similarly, the β domain features a βI domain at its head, as well as a hybrid domain (H) and a plexin-sempahorin-integrin (PSI) domain. These are supported by a leg consisting of four cysteine-rich epidermal growth factor (EGF) repeats, with EGF1 residing in the head domain of some integrins.[1] The βI domain contains the cations found within the aptly named metal-ion-dependent adhesion site (MIDAS): it is at the MIDAS that ligand binding takes place.[4] The integrin receptors have flexible structures with a number of different conformations; the three main conformations are inactive, extended (with either a closed or open headpiece), and ligand occupied (Figure 2), which can be recognized by the conformation of the receptor.[5]

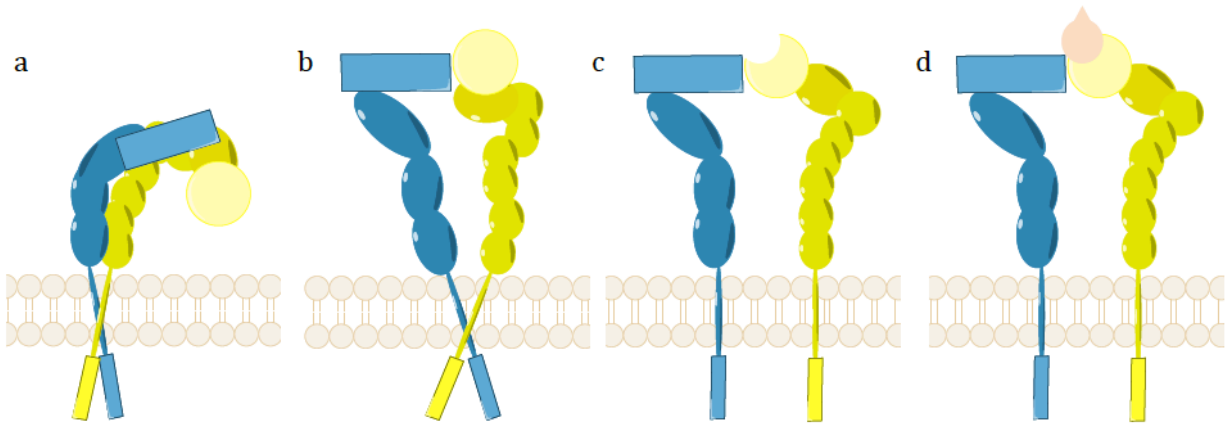


Figure 2. RGD integrin conformations: α domain (blue), β domain (yellow), and ligand (orange). (a) Bent, inactive; (b) Extended, closed headpiece conformation; (c) Extended, open headpiece conformation; (d) Ligand bound, open headpiece conformation. Based on previously reported structural information.[5, 6, 7]

In the inactive state the integrin is bent over towards the membrane and has a low affinity for ligands (Figure 2a); however, when the receptor extends out from the cell surface (Figure 2b), and the β H domain undergoes concomitant conformational change (Figure 2c), the receptor is primed and thus the integrin is activated. At the time of this review, the accepted paradigm is that the inactive integrin has a bent conformation, with the head region pointed towards the membrane; however, a report from Choi *et al*. suggested that in this inactive state the head of the receptor was orientated away from the lipid bilayer.[9] Further characteristic features of this inactive state are the crossing of the transmembrane helices and an cytoplasmic interchain salt bridge (Figure 2a).[10,11] The conformational changes can be caused by "inside-out" signaling, during which proteins within the cell, such as talin and kindlins, bind to the transmembrane base of the β domain and induce the change in conformation outside the cell by disrupting the salt bridge.[1,11-13] Alternatively, "outside-in" signaling can also result in conformational changes when

the integrin ectodomain interacts with a ligand, allowing the cell to sense and react to the extracellular environment.[12,16] It is in this activated state that the ligands can bind to the open headpiece; however, there is evidence that integrins can bind ligands during the bent, or partially bent, conformations.[14,15] A recent report has shown using biostructural studies of $\alpha_v\beta_3$ complexes with two forms of the physiologic ligand fibronectin, that key π-π interactions between Trp1496 and Tyr122 of the β_3 subdomain may be central to interacting with the inactive integrin without inducing any conformational changes in the receptor.[16]

The inhibition of integrins can be achieved by preventing the activation of the receptor or by blocking the binding site, with the latter being the more common approach.[17] Each integrin recognizes a specific type of ECM protein, thus the integrin family can be categorized into subsets based on the endogenous ligand; (i) leukocyte, (ii) collagen, (iii) RGD, or (iv) laminin (Figure 3). The RGD receptors are of particular therapeutic interest, with implications in thrombosis, fibrosis, and cancer, among other disease classes.[17] Accordingly, this class of receptor is, and has been, an active area for the development of novel medicines.

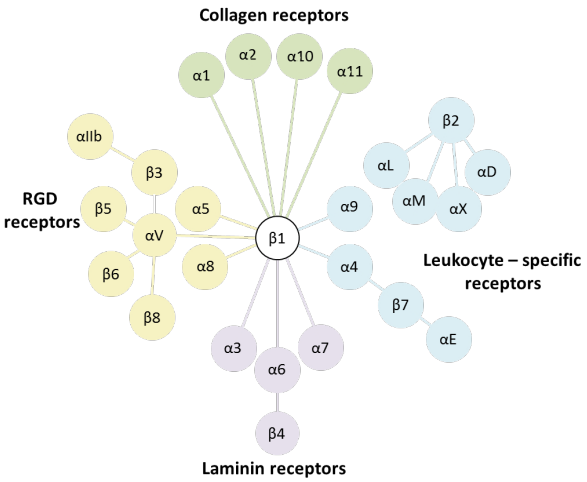
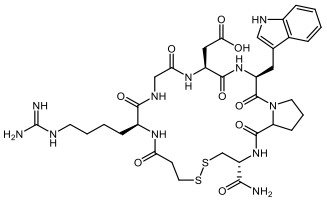
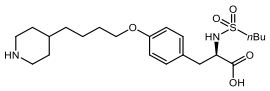
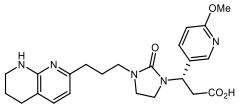
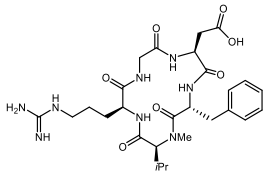
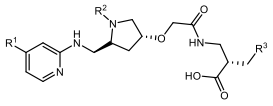


Figure 3. Integrin family, categorized by ligand type.

In the 1990s the first successful RGD integrin inhibitors were approved to reduce the risk of ischaemic events in patients undergoing percutaneous coronary intervention and those with acute coronary syndromes.[18] These pioneering inhibitors targeted the platelet $\alpha_{IIb}\beta_3$ integrin, also known as glycoprotein receptor (GP)-IIb/IIIa, an important target for the prevention of clot formation. This receptor is expressed uniquely on the surface of platelets and megakaryocytes, a type of platelet-producing cell in the bone marrow.[19] The three approved inhibitors are the antibody fragment abciximab,[20,21] and two small-molecule inhibitors eptifibatide (**1**),[22,23] and tirofiban (**2**)[24,25] (for a recent review see King *et al*.[26]). Although successful, these intravenously administered inhibitors are restricted to high-risk patients. Attempts to develop inhibitors for oral dosing were able to overcome the physicochemical challenges allied to zwitterionic compounds but there were issues related to pharmacology, with some orally-active antagonists of $\alpha_{IIb}\beta_3$ associated with a 30-35% increase in the risk of death.[27,28] Over eight million people have been treated using $\alpha_{IIb}\beta_3$ antagonists,[29] and to date $\alpha_{IIb}\beta_3$ remains to be the only RGD integrin for which pharmaceutical agents have been approved. The current clinical progress of selected RGD drugs is summarized in Table 1 (for a recent review of integrin-based therapeutics see Ley *et al*.[30]).

Table 1. Summary of select RGD integrin antagonists.

Name	Structure	Target Integrin(s)	Therapeutic Target (Stage)	Route of delivery	MW[a]	clogP[b]	PSA[c] (Å)
Abciximab[20,21] (ReoPro)	Antibody fragment	$\alpha_{IIb}\beta_3$	Thrombosis (Approved)	Intravenous	-	-	-
Eptifibatide[22,23] (Integrilin, **1**)		$\alpha_{IIb}\beta_3$	Thrombosis (Approved)	Intravenous	832	-5.06	328
Tirofiban[24,25] (Aggrastat, **2**)		$\alpha_{IIb}\beta_3$	Thrombosis (Approved)	Intravenous	441	0.598	112
Etaracizumab[31,32]	Monoclonal antibody	$\alpha_v\beta_3$	Melanoma (Phase II), solid tumors (Phase I)	Intravenous	-	-	-
MK0429[33-35] (L-000845704, **3**)		$\alpha_v\beta_3$ $\alpha_v\beta_5$	Prostate cancer (Phase I), Osteoporosis (Phase II)	Oral	440	-0.307	111
Cilengitide[36-38] (**4**)		$\alpha_v\beta_3$ $\alpha_v\beta_5$ $\alpha_5\beta_1$	Glioblastoma (Phase III)	Intravenous	589	-3.68	240
JSM6427‡[39] (**5**)		$\alpha_v\beta_5$	Age-related macular degeneration (Phase I)	Intravitreal	-	-	-
Volociximab[40,41]	Monoclonal antibody	$\alpha_5\beta_1$	Solid tumors (Phase I)	Intravenous	-	-	-

[a]Molecular weight (MW); [b]Calculated logP (clogP); [c]Topological polar surface area (PSA). Properties determined using JChem for Office (Excel).[42] ‡Structure not disclosed.

From consideration of the structures in Table 1, it can be noted that the non-peptide small-molecule inhibitors still retain RGD mimetics (or KGD) and thus resemble the native ligands of these integrins. It is understood that in the binding of the RGD sequence the acid coordinates with the Mg^{2+} and the arginine group resides in a narrow cleft on the β domain forming salt bridges with Asp150 and Asp218;[6] RGD-mimetic inhibitors, such as Cilengitide (**4**), are designed to make the same interactions as the native ligand (PDB 1L5G, Figure 4). Although these zwitterionic peptidomimetics provide potency, they often suffer from sub-optimal *in vivo* pharmacokinetic profiles due to their high molecular weight (MW), high PSA (topological polar surface area), low clogP, and, in some cases, high conformational flexibility.

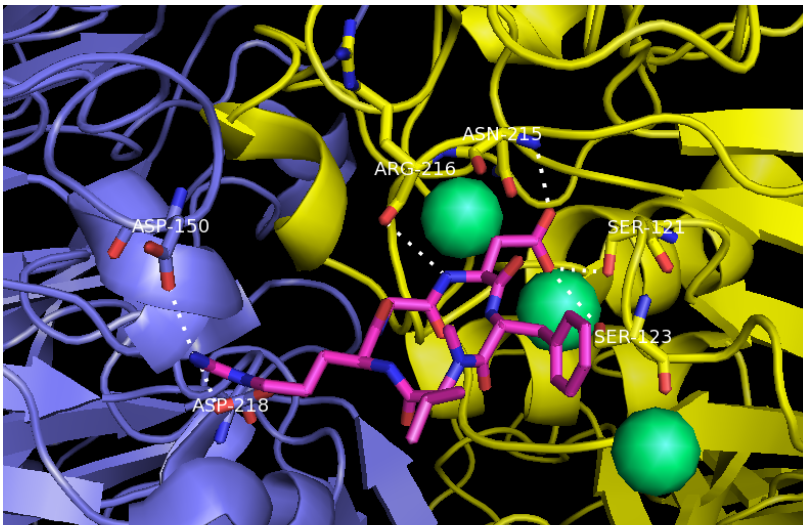
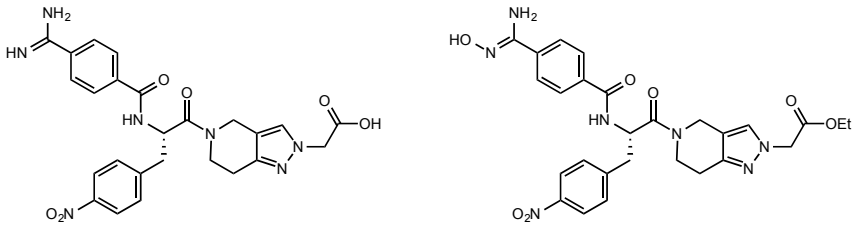


Figure 4. Cocrystal structure of **4** (pink) with $\alpha_v\beta_3$: α domain (blue), β domain (yellow), Mn^{2+} ions (green). PDB 1L5G, visualized using PyMOL.[43]

Furthermore, the use of oral RGD-mimetic $\alpha_{IIb}\beta_3$ inhibitors has led to a major issue in relation to pharmacology, as the compounds also have the potential to activate the receptor.[44] The approved $\alpha_{IIb}\beta_3$ inhibitors discussed previously have been reported to also cause partial agonism, resulting in the opposite pharmacological effect.[45,46] This reported partial agonism is a result of allosteric

changes upon binding, which prime the receptor. A preclinical study by Reynold *et al*. found that the $\alpha_v\beta_3$ antagonist cilengitide may promote tumor growth *in vitro*;[47] however, these results are highly debated, with many clinical trials of cilengitide indicating no safety signal.[48]

One RGD-mimetic small molecule antagonist of $\alpha_{IIb}\beta_3$ that does not induce receptor priming upon binding is UR-2922 (**6**), which is the active form of the prodrug UR-3216 (**7**) (Figure 5).[49,50] This antagonist (**6**), developed by Ube, has been shown to bind tightly to resting platelets, with a Ki of <1 nM. The excellent potency, along with a favorable pharmacokinetic profile, is advantageous for oral dosing of this antagonist. Although **6** has the RGD-mimetic of the typical RGD integrin antagonist, no partial agonism of the $\alpha_{IIb}\beta_3$ receptor has been reported as a result of it binding. Modelling studies carried out by Cox *et al*. suggested that this analogue does not interact with the MIDAS, and thus does not activate the receptor.[17] Instead of interacting with the cation, the authors propose that the carboxyl group of **6** forms an H-bond with Tyr166 and a salt bridge with Arg165. This proposed alternative binding of the acidic motif, along with additional π-π interactions with Phe160, would distinguish this RGD-mimetic inhibitor from the compounds described in Table 1; however, it is surprising that an RGD-mimetic inhibitor would bind via an alternative binding mode, and no biostructural evidence was reported to support the author's hypothesis.



6 (UR-2922)
IC_{50} = 31 ±3 nM
MW: 520; clogP: -0.69; PSA: 202 Å

7 (UR-3216)
MW: 564; clogP: 1.45; PSA: 195 Å

Figure 5. $\alpha_{IIb}\beta_3$ antagonist **6** and the prodrug form **7**. IC_{50} for inhibition of ADP-induced aggregation of platelets (human).[49,50]

A potential approach to avoid this unwanted activation of $\alpha_{IIb}\beta_3$ is to design inhibitors that block the binding site without forming the interactions made by the native RGD sequence. Thus the design of non-RGD-mimetic inhibitors could identify effective small-molecule antagonists that do not activate the integrin. This is a particularly promising strategy for integrin therapeutics targeting the RGD subfamily as the increase in mortality associated with the oral $\alpha_{IIb}\beta_3$ antagonists has been attributed to the ability of RGD-mimetic inhibitors to prime the receptor.[27,28] Furthermore, the development of non-RGD-mimetic inhibitors could lead to a more synthetically tractable series, that could provide easier explorations of SAR. By avoiding the RGD-mimetic, compounds may also have the potential to possess more favorable physicochemical properties, and therefore improved pharmacokinetic profiles.

$\alpha_{IIb}\beta_3$ NON-RGD-MIMETIC INHIBITORS

The first non-RGD-mimetic inhibitor of $\alpha_{IIb}\beta_3$ was identified in 2008 by Blue *et al*. using a medium throughput screen (MTS) of 33,264 small molecules.[51] This inhibitor, termed RUC-1 (**8**, IC_{50} = 9.7 ±1 μM),[51] was reported to be effective and selective at $\alpha_{IIb}\beta_3$, with no activity observed at the related integrin $\alpha_v\beta_3$, Figure 7. Through docking studies, **8** was determined to interact only

with the α_{IIb} domain and not with the β_3 subunit, thus explaining the specificity over $\alpha_v\beta_3$ displayed by this inhibitor. The authors also reported that this compound did not show any agonism of the integrin and hypothesized that the lack of interactions with the MIDAS metal ion may be the reason for this. Mutagenesis studies were carried out which supported the proposed binding mode.[52] Subsequently, the cocrystal structure of **8** with $\alpha_{IIb}\beta_3$ confirmed that the binding site was localized to the α_{IIb} domain and provided further insight into this small molecule inhibitor.[53] This crystal structure revealed that **8** binds to the closed conformation of the integrin headpiece and does not result in any priming of the receptor (PDB 3NIF). This is in contrast to RGD-mimetic antagonists, which have been shown to activate $\alpha_{IIb}\beta_3$ resulting in partial agonism.[44,45] The Coller group were able to develop **8** further, through structure-based design, and produce an analogue with approximately 100-fold higher affinity, termed RUC-2 (**9**, IC_{50} = 95 ±5 nM).[54] This more potent analogue was soaked into crystals of the $\alpha_{IIb}\beta_3$ headpiece, but no density for the metal ion at the MIDAS was observed (PDB 3T3M, Figure 6).[54] It was noted that **9** had the same binding interactions as **8** but with additional interactions in the β_3 βI domain. The primary amine of **9** was discovered to compete with the Mg^{2+} for interactions with the carboxyl oxygen of Glu220; thus, in high enough concentration, **9** displaced the metal ion of the MIDAS, explaining its absence from the crystal structure.

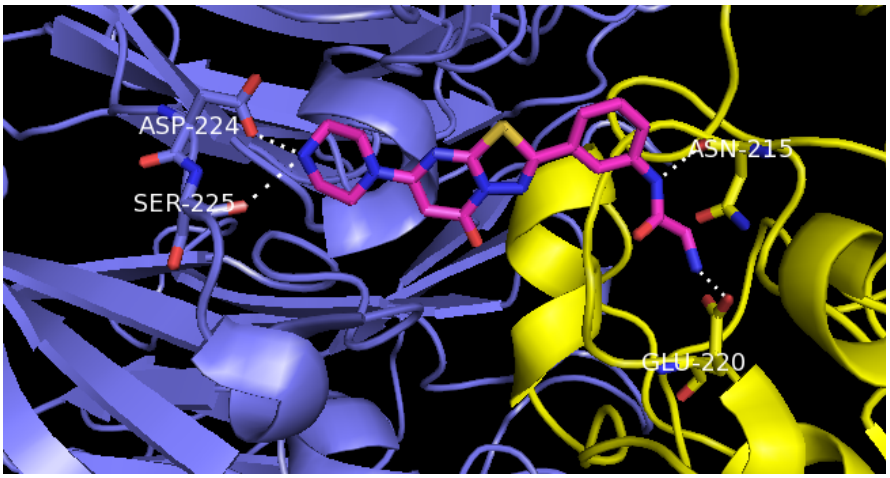


Figure 6. 9 (pink) bound within $\alpha_{IIb}\beta_3$: α domain (blue), β domain (yellow). PDB 3T3M, visualized using PyMOL.[43]

Furthermore, the authors report that **9** does not activate the integrin upon binding, thus preventing any unwanted signaling. This compound is the first in a novel class of integrin antagonists termed "ion displacement ligands" by the developers of **9**.[55] In 2014 the Coller group reported the SAR surrounding the optimization of **9**. Two analogues were disclosed: RUC-3 (**10**) and RUC-4 (**11**),[55] and the results of their docking studies compared with the binding of **9** suggested that additional water-mediated interactions of the nitrogen atoms with the receptor were responsible for the differences in the potencies observed, Figure 7. Unfortunately, **10** was found to be unstable in solution (DMSO or aqueous), and as a result was deprioritized. Through the ADMET profiling of **9** and **11**, it was found that **11** had a modest microsomal stability profile (23.2 μL/min/mg, human) whereas **9** was highly resistant (-1.16 μL/min/mg, human). Incorporation of a nitrogen into the aromatic ring at the core to give **11** increased the aqueous solubility (thermodynamic solubility at pH 7.4: **9** 28.0 μg/mL; **11** 239.5 μg/mL) and maintained the potency. This chemotype was recently patented,[56] and further research is underway for the

development of the more water soluble **11** towards a pre-hospital treatment of myocardial infraction.[57]

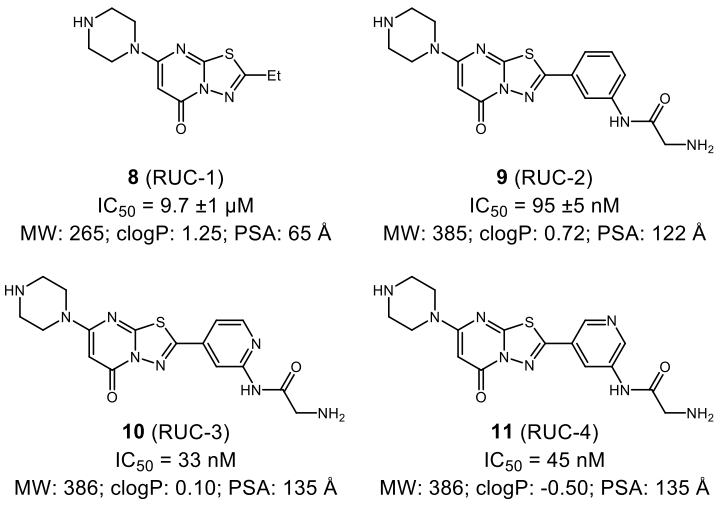
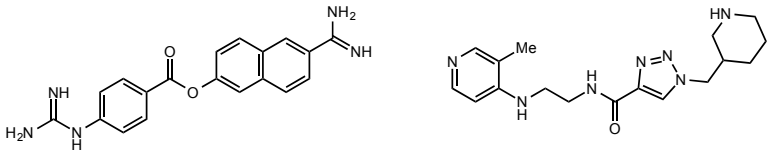


Figure 7. RUC-1 (**8**),[51] RUC-2 (**9**),[54] RUC-3 (**10**), and RUC-4 (**11**)[55] from the Coller group. IC_{50} for inhibition of ADP-induced aggregation of platelets (human).

Based on the unique binding mode of **8** and **9**, Negri *et al*. carried out a structure-based virtual screen with the aim of identifying new small molecule antagonists of $\alpha_{IIb}\beta_3$.[58] Over 2.5 million "lead-like" compounds of the ZINC database[59] were screened, and then five potential antagonists were selected from the top 500 scoring compounds. The ligands were selected based on the interactions observed during modelling, diversity of chemotype, and their commercial availability. Of the 5 compounds purchased (termed MSSM-1-5), four were successfully tested for biological activity against $\alpha_{IIb}\beta_3$, and two were found to show micromolar inhibition of $\alpha_{IIb}\beta_3$, including MSSM-1 (**12**),[58] Figure 8. These novel inhibitors were also found to show specificity for their desired integrin over $\alpha_v\beta_3$, and no undesired priming of the receptor was observed. In 2015 a second virtual screen of antagonists for $\alpha_{IIb}\beta_3$ was reported by Wang *et al*.[60] This work combined the structure-based *in silico* screen with a ligand-based pharmacophore screen of over

7.3 million "drug-like" compounds from the ZINC database. Their campaign identified 11 commercially available compounds, which were obtained and tested for their inhibitory effect against platelet aggregation. Three compounds were found to exhibit micromolar activity, with compound **13**[60] displaying the highest potency, Figure 8. Wang *et al*. also comment that this potential antagonist is predicted to have good solubility, permeability, ADMET properties, and low toxicity *in vivo*. This is perhaps surprising as the basic 4-aminopyridine motif will increase the polarity of **13**, thus lowering the permeability of this inhibitor; however, no measured permeability or pharmacokinetics were reported. With few non-RGD-mimetic inhibitors known in the literature, broad *in silico* screening appears to be a valuable tool for identifying novel scaffolds, but it is limited by the database(s) used.



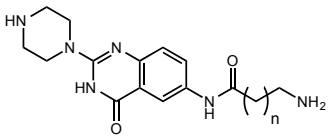
12 (MSSM-1)
IC_{50} = 12 ±1 μM
MW: 347; clogP: 2.52; PSA: 142 Å

13
IC_{50} = 20 ±4 μM
MW: 343; clogP: 0.03; PSA: 103 Å

Figure 8. $\alpha_{IIb}\beta_3$ inhibitors identified by virtual screening. IC_{50} for inhibition of ADP-induced aggregation of platelets (human).[58,60] Stereochemistry of **13** not reported.

A more recent report from Polishchuk *et al*. used quantitative structure–activity relationship (QSAR) and pharmacophore models to screen a number of databases for novel $\alpha_{IIb}\beta_3$ antagonists, but no hit compounds were identified.[61] The authors concluded that this was the result of the low number of zwitterionic compounds available in the commercial libraries used for their screen. Thus, the group used their models to design focused libraries of novel compounds, which were screened to identify ligands that could bind either the open or the closed form of the $\alpha_{IIb}\beta_3$

receptor. In order to model ligands for the open form, the RGD-mimetic inhibitor Tirofiban was used, whereas the closed form analogues were based on **9**. The virtual hit compounds were synthesized and through biological screening, one low nanomolar inhibitor for the closed receptor was successfully identified (compound **14**).[61] Docking studies carried out using compound **14** showed a similar binding mode to that of **9**, but this analogue showed superior levels of potency with an IC_{50} of 11 nM, Figure 9. A key feature of their model for a closed form binder was a distance of 15.8 Å between two positively charged centers. The authors later confirmed this hypothesis using a series of analogues in which this distance was varied.[62] This study showed that shortening the compound by one carbon (compound **15**) caused a greater than 100-fold decrease in potency, and lengthening by one carbon (compound **16**) was also unfavorable, resulting in micromolar inhibition, Figure 9.



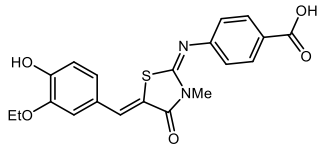
14, n = 2, IC_{50} = 11 ±1 nM; MW: 316; clogP: -0.95; PSA: 118 Å
15, n = 1, IC_{50} = 150 ±25 nM; MW: 302; clogP: -1.19; PSA: 118 Å
16, n = 3, IC_{50} = 1.4 ±0.17 μM; MW: 330; clogP: -0.91; PSA: 118 Å

Figure 9. $\alpha_{IIb}\beta_3$ non-RGD-mimetic inhibitors designed from **9**. IC_{50} for inhibition of ADP-induced aggregation of platelets (human).[61,62]

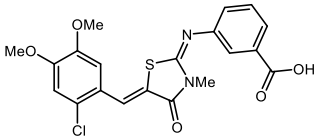
$\alpha_v\beta_3$ NON-RGD-MIMETIC INHIBITORS

To the best of our knowledge, the first example of a non-RGD-mimetic inhibitor of an RGD integrin was reported by Dayma *et al*. in 2006.[63] This work utilized common feature pharmacophore models, which were derived from known $\alpha_v\beta_3$ inhibitors. Two databases were screened (NCI2000 and Chemical Diversity) using the pharmacophore model, to give over 400

compounds. Hit compounds were then filtered by physicochemical properties, structural diversity, and commercially availability, to give 29 potential antagonists, which were obtained for further investigation. Through *in vitro* evaluation, four compounds were found to inhibit $\alpha_v\beta_3$, including the non-RGD-mimetic compound **17**,[63] Figure 10. The authors recognized the novelty of this inhibitor and carried out a small SAR study, which identified inhibitor **18**[63] with an 800-fold higher affinity than the initial hit **17**. This subnanomolar non-RGD-mimetic $\alpha_v\beta_3$ inhibitor was shown to have low cytotoxicity, and therefore the authors suggest this inhibitor has potential for the development of a noncytotoxic anticancer therapy. The 2-imino-rhodanine motifs at the core of **17** and **18** are often identified as screening hits, and thus are recognized pan-assay interference compounds (PAINS); therefore, these inhibitors may be unsuitable for further development.[64]



17
IC_{50} = 240 nM
MW: 398; clogP: 3.54; PSA: 102 Å

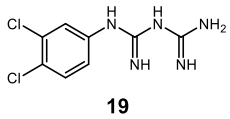


18
IC_{50} = 0.03 nM
MW: 433; clogP: 3.56; PSA: 91 Å

Figure 10. $\alpha_v\beta_3$ inhibitors identified by Dayam *et al*. IC_{50} for inhibition of $\alpha_v\beta_3$ receptor binding assay.[63]

This work was closely followed by a report from Zhou *et al*. in which a number of small molecule inhibitors of $\alpha_v\beta_3$ that lacked the aspartic acid mimetic were discovered.[65] By using a crystal structure of $\alpha_v\beta_3$ in complex with an RGD ligand (PDB 1L5G, Xiong *et al*.[6]), this group carried out a virtual screen of 88,695 commercially available organic compounds. A rigorous triage of docking results was used to successfully identify 50 potential inhibitors for biological testing. From the compounds that were selected for testing, seven were found to have inhibitory

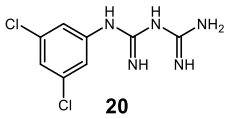
activity with an IC_{50} of <200 μM. The most potent of the hit compounds was inhibitor **19**[65] with an IC_{50} = 38.5 ±1.7 μM, which was progressed onto further testing *in vitro* and found to effectively inhibit cell migration and angiogenesis, Figure 11. Zhou *et al*. went on to explore this non-RGD-mimetic small molecule further through the synthesis of a series of analogues, which led to the identification of another inhibitor of similar potency, compound **20** (IC_{50} = 33.5 ±3.1 μM),[65] Figure 11. Docking studies of this compound identified that, in the most energetically favorable binding pose, the biguanide group can interact with a number of residues in the MIDAS through a series of H-bonds. This predicts that inhibitors of this chemotype will interact with the receptor through a significantly different binding mode than that of the more classic RGD-mimetic inhibitors, which is surprising as the biguanide might be expected to mimic the arginine. Unfortunately, no further structural information was obtained, and thus the alternative binding mode of these compounds remains unconfirmed. The authors do not report whether these non-RGD-mimetic compounds are both antagonists and/or partial agonists of the receptor; nevertheless, both inhibitor **19** and **20** having the low molecular weight of 246 Da makes the ligand efficiency of these compounds high.



19

IC_{50} = 38.5 ±1.7 μM

MW: 246; clogP: 1.93; PSA: 101 Å



20

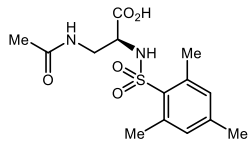
IC_{50} = 33.5 ±3.1 μM

MW: 246; clogP: 1.93; PSA: 101 Å

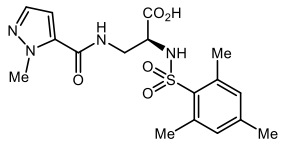
Figure 11. $\alpha_v\beta_3$ inhibitors identified by Zhou *et al*. IC_{50} for inhibition of $\alpha_v\beta_3$-mediated cell adhesion.[65]

Another series of small molecule non-RGD-mimetic $\alpha_v\beta_3$ inhibitors was reported by Elliot *et al*. in 2009.[66] This group had taken an alternative strategy and designed a series of novel inhibitors

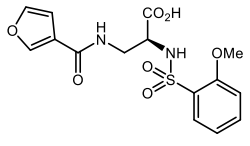
without the arginine motif. Their approach began with the screening of low molecular weight carboxylic acids and acid isosteres, from which they identified the hit compound **21**,[66] Figure 12. This initial hit had a promising IC_{50} of 800 nM, thus demonstrating that it is possible to achieve good affinity with $\alpha_v\beta_3$ using a non-RGD-mimetic chemotype, but the authors do not report any details of the assay used. Through exploration of the SAR, Elliot *et al*. were able to improve this potency with the development of lead compound **22** with an IC_{50} of 11 nM;[66] however, the compound had limited permeability (parallel artificial membrane permeability assay (PAMPA) = 0.021 x 10^6 cm s^{-1}) and the authors comment that there was only modest selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$. Based on analysis of previously reported crystal structures of these two integrins (PDB 1L5G vs. 1TY7) and modelling studies, the authors hypothesized that altering the *ortho* position of the sulfonamide could improve the selectivity. Through the synthesis of further *ortho* substituted analogues compound **23**[66] was discovered, with an IC_{50} of 700 nM and a >130-fold selectivity for their desired target $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$. This work shows that it is possible to design nanomolar inhibitors of $\alpha_v\beta_3$ without the use of an RGD-mimetic, although the PSA values of these inhibitors are high.



21
IC_{50} = 800 nM
MW: 328; clogP: 1.06; PSA: 115 Å



22
IC_{50} of 11 nM
MW: 394; clogP: 1.16; PSA: 133 Å

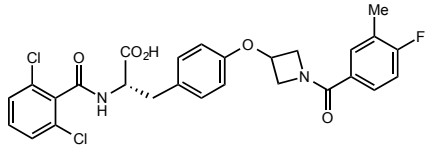


23
IC_{50} of 700 nM
MW: 368; clogP: 0.35; PSA: 138 Å

Figure 12. $\alpha_v\beta_3$ inhibitors identified by Elliot *et al*.[66] Assay protocol not reported.

$\alpha_5\beta_1$ NON-RGD-MIMETIC INHIBITORS

In 2007, a patent by AstraZeneca was reported claiming a series of small molecule inhibitors of the RGD integrin $\alpha_5\beta_1$.[67] The majority of compounds covered by the patent had the RGD-mimetics, but there were a small number of inhibitors claimed that did not fit this chemotype; for example, compound **24**[67] which lacks the arginine mimetic, Figure 13. This inhibitor was reported to have an IC_{50} of 4 μM in a cell adhesion assay and an IC_{50} of 466 nM using a second electrochemiluminescence ligand binding assay. Thus showing that this compound was able to inhibit $\alpha_5\beta_1$ *in vitro*, however, no *in vivo* data was reported. Although **24** is a non-RGD-mimetic inhibitor, this compound is quite large and lipophilic, with the high MW of 545 Da and high clogP of 5.44. Based on these physicochemical properties **24** might be predicted to suffer from poor ADMET; however, no measured pharmacokinetics were disclosed.



24

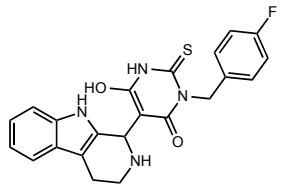
IC_{50} = 466 nM[a]; IC_{50} = 4 μM[b]

MW: 545; clogP: 5.44; PSA: 99 Å

Figure 13. $\alpha_5\beta_1$ inhibitor **24** reported by AstraZeneca.[67] [a]Electrochemiluminescence ligand binding assay. [b]Cell adhesion assay.

More recently, another non-RGD-mimetic inhibitor of $\alpha_5\beta_1$ was reported by Kang and Kim.[68] Termed IPS-05002, compound **25**[68] was identified by a screen of a phytochemical compound library using a ProteoChip-based protein-protein interaction assay for $\alpha_5\beta_1$ antagonists. This compound showed micromolar inhibition of $\alpha_5\beta_1$ in a cell proliferation assay and a cell migration assay, Figure 14. Furthermore, the authors report that this antagonist inhibits cell adhesion and

tubular network formation, and thus **25** may inhibit angiogenesis. This experimental evidence shows the potential for this novel small molecule to be developed into a potent antagonist; however, the complex structure may limit an SAR exploration of this scaffold if the synthetic route is not amenable to diversification.



25 (IPS-05002)
IC_{50} = 20.5 ±0.56 μM[a]; IC_{50} = 15.52 μM[b]
MW: 422; clogP: 2.58; PSA: 88 Å

Figure 14. $\alpha_5\beta_1$ inhibitor **25**.[68] [a]Cell proliferation assay. [b]Cell migration assay. Stereochemistry of **25** not reported.

In summary, the RGD integrins are recognized therapeutic targets. Numerous RGD inhibitors have been evaluated clinically for a range of therapeutic indications. For example $\alpha_v\beta_3/\alpha_v\beta_5$ inhibitor cilengitide alone has been studied in at least 35 clinical trials with issues appearing to be efficacy rather than safety. To date however, inhibitors have been approved for only one integrin from this class namely intravenous $\alpha_{IIb}\beta_3$. Safety issues were seen with oral $\alpha_{IIb}\beta_3$ inhibitors and ascribed to conformational changes in the receptor upon binding. The emergence of small molecule non-RGD-mimetic inhibitors has led to the discovery of new chemotypes with the promise of much increased synthetic tractability, which inhibit the RGD integrins, without - in the case of $\alpha_{IIb}\beta_3$ - the activation of the receptor, but the development of an efficacious, safe drug from this new generation of inhibitors is yet to be achieved. Initial compounds were identified using MTS and virtual screening; however, biostructural information on the non-RGD-mimetic inhibitors bound to the receptor, along with advancements in the understanding of the

unwanted integrin priming, has provided clues to the rational design of novel non-RGD-mimetic compounds. This new generation of RGD-integrin antagonists could lead to the development of a safe and efficacious oral dosing $\alpha_{IIb}\beta_3$ inhibitor, and help realize the potential of the RGD integrins as therapeutic targets.

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ABBREVIATIONS

ADMET, absorption, distribution, metabolism, elimination, and toxicity; ECM, extracellular matrix; EGF, epidermal growth factor; GP, glycoprotein receptor; MIDAS, metal-ion-dependent adhesion site; MTS, medium throughput screen; PAINS, pan-assay interference compounds; PSA, topological polar surface area; QSAR, quantitative structure–activity relationship RGD, arginine-glycine-aspartic acid; SAR, structure-activity relationship.

BIOGRAPHIES

Lisa M. Miller obtained her M.Chem degree at the University of Strathclyde in 2013. She began her Ph.D. studies with Dr. Allan J. B. Watson in 2013 where she is currently working on the development of new inhibitors for fibrosis drug discovery in collaboration with GSK.

John. M. Pritchard started with GSK (Glaxo) in 1984 as a medicinal chemist and has worked in the respiratory area for twenty years. As a team leader in the Fibrosis & Lung Injury Discovery Performance Unit in the Respiratory Therapeutic Area at GSK in Stevenage, U.K, integrin inhibitors have been the focus of his work for the last seven years.

Simon J. F. Macdonald has over 20 years' experience as a medicinal chemist in the pharmaceutical industry and has spent his entire career at GSK in its various incarnations. He is

currently a Director of Medicinal Chemistry in the Fibrosis & Lung Injury Discovery Performance Unit in the Respiratory Therapeutic Area at GSK in Stevenage, U.K., and is a Visiting Professor at the University of Nottingham.

Craig Jamieson obtained his B.Sc. (Hons) in Chemistry at the University of Glasgow in 1996 followed by Ph.D. studies with Professor R. Ramage at the University of Edinburgh (1999) and postdoctoral research with Professor S. V. Ley at the University of Cambridge. In 2001 he was appointed as a Principal Scientist in GSK's Discovery Medicinal Chemistry group working on a range of exploratory medicinal chemistry programs. In 2004, he joined Organon Laboratories (later Merck Research Labs) as a Group Leader in Medicinal Chemistry. He joined the University of Strathclyde in 2010 where his research interests are within synthetic and medicinal chemistry.

Allan J. B. Watson completed his M.Sci. at the University of Strathclyde in 2004 followed by Ph.D. research with Professor W. J. Kerr (2008) and postdoctoral work with Professor D. W. C. MacMillan at Princeton University, U.S. (2010). After an industrial postdoctoral research position at GSK, he returned to the University of Strathclyde in 2011 where his research interests are synthetic and medicinal chemistry.

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