Structure Activity Relationships of α_v Integrin Antagonists for Pulmonary Fibrosis By Variation in Aryl Substituents

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ABSTRACT: Antagonism of $\alpha_v\beta_6$ is emerging as a potential treatment of idiopathic pulmonary fibrosis based on strong target validation. Starting from an $\alpha_v\beta_3$ antagonist lead and through simple variation in the nature and position of aryl substituent, the discovery of compounds with improved $\alpha_v\beta_6$ activity is described. The compounds also have physicochemical properties commensurate with oral bioavailability and are high quality starting points for a drug discovery programme. Compounds **33S** and **43E1** are pan α_v antagonists having *ca* 100 nM potency against $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$ in cell adhesion assays. Detailed structure activity relationships with these integrins are described which also reveal substituents providing partial selectivity (defined as at least a 0.7 log difference in pIC50 values between the integrins in question) for $\alpha_v\beta_3$ and $\alpha_v\beta_5$.

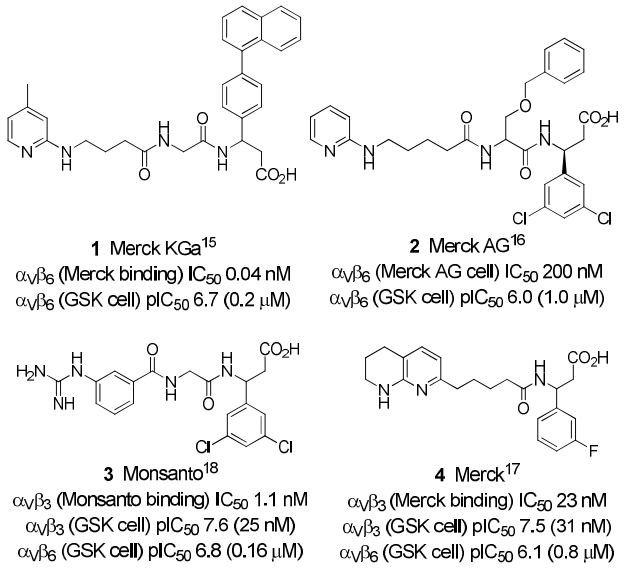
Once diagnosed, life expectancy for patients with idiopathic pulmonary fibrosis (IPF) is usually only a few years and the mortality rate exceeds that of many cancer indications.[1,2] A recent study in the UK also suggests that the incidence of IPF is rising with > 5,000 new cases diagnosed each year.[3] Although pirfenidone is now marketed for the treatment of IPF in some countries,[4] there remains an urgent need for effective new medicines. Recent research into IPF together with estimated peak sales for an effective treatment at around $2 billion per annum, have driven significant commercial activity around potential biological and small molecule fibrosis assets in the pharmaceutical sector.[5]

There is now reasonable evidence that the RGD (arginine-glycine-aspartic acid) integrin receptor $\alpha_v\beta_6$ may play an important role in the initiation and progression of IPF *inter alia*.[6,7] This receptor is predominantly expressed in injured lung tissue and inhibition of the receptor with $\alpha_v\beta_6$ antibodies or its absence in knock-out mice leads to substantial protection from the development of fibrosis in both bleomycin[8] and radiation[9] induced animal models. Selective $\alpha_v\beta_6$ antibodies are in development for fibrotic diseases.[5]

The $\alpha_v\beta_6$ receptor is a cell surface heterodimeric receptor composed of a non-covalently bound complex between the α_v and β_6 proteins. Three closely related integrins where only the β subunit is varied are $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$. Despite substantial research and drug discovery over the last decade on antagonists of the $\alpha_v\beta_3$ receptor for osteoporosis and other indications,[10-13] there is remarkably little literature on potent and selective small molecule antagonists of $\alpha_v\beta_6$. The selectivity profiles of the $\alpha_v\beta_3$ antagonists in the literature rarely include cross-screening data against $\alpha_v\beta_6$ or other α_v integrins with the exception of a Pfizer series of compounds for which a broad range of integrin data is reported (although the potencies are quite weak as $\alpha_v\beta_6$ antagonists).[14] Other exceptions are the Merck KGa,[15] Merck AG,[16] Merck,[17] Monsanto[18] compounds (**1**–**4**, Figure 1) and JNJ-26076713[19] (where the Arg guanidine and Asp acid mimetics are apparent at the either end of a chain) and very recently α_v integrin data in patents from Ruminski and Griggs at the University of St Louis.[20]

We describe here detailed structure activity relationship (SAR) studies of novel analogues of **4** describing for the first time their profiles against $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$ in cell adhesion assays. By varying the substituent pattern on the aryl ring, we have discovered pan α_v antagonists such as 33S and 43E1 ("pan" defined here as having antagonism against all the α_v integrins tested, namely $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$), antagonists with partial selectivity (defined as at least a 0.7 log difference in pIC50 values between the integrins in question) for $\alpha_v\beta_3$ (such as **27**) and partial selectivity for $\alpha_v\beta_5$ (such as **35**). The profiles of antagonists with partial dual selectivity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (such as the known compounds **4**[17] and **42**[17]) and are also described. Seemingly fairly subtle changes on just the aryl ring of the structures exert a profound effect on the overall selectivity profile, and in this series, it appears considerably more marked than in the Merck KGa series.[15] Measured lipophilicity values for compounds (chrom. logD)[21] are also provided. (When the sum of chrom. log D plus aromatic ring count is <6, there is an increased likelihood of a compound being more developable as an oral drug).[21]

Figure 1. Exemplar α_v integrin antagonists from the literature together with selected reported activities and activities in the assays described herein.

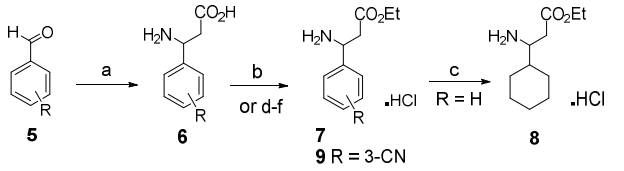


The combination of the $\alpha_v\beta_6$ activity of these compounds with their physicochemical properties, which are commensurate with potential oral bioavailability, identifies them as high quality starting points for a drug discovery programme and amongst the best currently known in the literature.

Derivatives of ethyl or methyl 3-amino-3-phenylpropanoate **7** were prepared in two steps from the appropriate aldehydes **5** by the Rodionov reaction[22] followed by esterification to **7**, or were purchased from commercial sources (Scheme 1). Ethyl 3-amino-3-(3-pyridyl)propanoate **10** was made in the same way from 3-formylpyridine. Indane-5-carboxaldehyde was prepared in 38% yield by treatment of indane with hexamethylenetetramine/TFA as described by Arora, *et al.*[23]

Hydrogenation of amino ester hydrochloride **7** (R = H) gave the cyclohexyl derivative **8**. The 4-cyano analogue **7** (R = 4-CN) was obtained in low yield after the esterification owing to a competing Pinner reaction. To circumvent this problem in the case of the 3-cyano analogue, the amino group was protected as the benzyl carbamate (CBZ), followed by esterification under mildly basic conditions and CBZ-group deprotection to give **9**.

Scheme 1. Synthesis of 3-aryl-3-aminopropanoic ester intermediates.



Reagents and conditions. a: malonic acid, ammonium acetate, MeOH or EtOH or ^{i}PrOH, reflux; b: $SOCl_2$, EtOH, −15 °C then reflux; c: H_2 (4 bar), 5% Rh/Al_2O_3, EtOH, 80 °C; d: *N*-(benzyloxycarbonyloxy)succinimide, $EtNPr^i_2$, CH_2Cl_2, room temp., 2 h; e: *N*,*N*'-carbonyldiimidazole, THF, room temp., then EtOH; f: H_2 (1 bar), 10% Pd/C, EtOH, room temp., 24 h. All compounds are racemic unless indicated in the text.

Amide coupling of 1,8-naphthyridin-2-yl)pentanoic acid **11**[17] to amino esters **7** (R = 2-OMe, 3-Me, 3-OCF_3 and 3,4-$(OMe)_2$, Scheme 2) gave the corresponding esters **12** in 31-67% yields. Selective hydrogenation of the naphthyridine ring gave the corresponding compounds **13** (R = 2-OMe, 3-Me, 3-OCF_3 and 3,4-$(OMe)_2$) (44-96%). For all the other compounds, the tetrahydronaphthyridine pentanoic acid **14**[17] could be coupled directly to the amino esters **7-9** to give compounds **13**. Hydrolysis of the esters **13** under basic conditions then afforded the target acids (see Table 1 for numbering).

For the preparation of the single enantiomers of **33**, commercially available assigned (*R)* and (*S)* enantiomers of the 3-CF_3 amino ester **7** were used. For the enantiomers of **37** and **43**, the precursor ethyl esters **13** (R = 3,5-Cl_2 and 3-CF_3,4-Cl respectively) were separated by preparative chiral HPLC and then hydrolysed to afford the target compounds.

The compounds were tested in $\alpha_v\beta_6$, $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$ cell adhesion assays as previously described.[24] Lipophilicity was determined using an HPLC chromatographic logD protocol.[21]

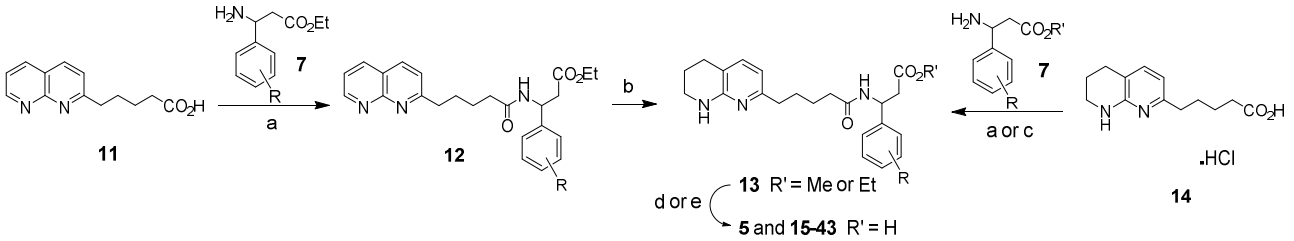
Preliminary unpublished work led to the selection of template **4** as suitable for exploring $\alpha_v\beta_6$ activity and in particular investigating what the impact of different aryl substituents might be. Although structure activity relationships (SAR) have been described by Merck for this template,[17] these predominantly relate to $\alpha_v\beta_3$ with little, if any, $\alpha_v\beta_6$ data published. Data from the present study are presented in Table 1. In order to establish preliminary α_v SAR rapidly, fluoro, chloro, methyl and methoxy analogues were prepared in the *ortho*, *meta* and *para* positions.

The parent phenyl compound **15** is a micromolar $\alpha_v\beta_6$ antagonist and substantially more potent against $\alpha_v\beta_3$ and $\alpha_v\beta_5$.[25,26] In every case, the fluoro, chloro, methyl and methoxy compounds are similarly more potent against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ than $\alpha_v\beta_6$ with activity against $\alpha_v\beta_8$ being similar to or less than the $\alpha_v\beta_6$ values. The $\alpha_v\beta_3$ and $\alpha_v\beta_5$ values are generally similar to each other. The superior antagonism against $\alpha_v\beta_3$ is perhaps unsurprising given the series emanates from one designed as $\alpha_v\beta_3$ antagonists.

The SARs are idiosyncratic although there are compounds with approximately 10-fold selectivity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ over $\alpha_v\beta_6$ and $\alpha_v\beta_8$ such as R = H (**15**), *m*-F (**4**), *m*-OMe (**22**) and *p*-OMe (**26**). More intriguingly perhaps, given the relative lack of such compounds in the literature, some show suggestions of selectivity for $\alpha_v\beta_5$ with the *o*-F (**16**), *m*-Cl (**20**) and the *m*-Me (**21**) having 0.5 log selectivity over $\alpha_v\beta_3$ and about a log selectivity over $\alpha_v\beta_6$ and $\alpha_v\beta_8$. Recent studies suggest there may be a role for $\alpha_v\beta_5$ antagonists in the treatment of sepsis.[27] Based on these data, the next iteration of analogues focussed on further mono-substituents in the *meta* and *para* positions as these showed more consistent $\alpha_v\beta_6$ activity and are more synthetically accessible compared to the *ortho* analogues.

Data from further mono-*para* substituents were explored (Table 1, **27**–**31**). Activities against $\alpha_v\beta_6$ are similar (pIC_{50} 6.1–6.4) despite varying size and electronic properties. In contrast, there is a ten-fold range in activity against $\alpha_v\beta_3$ (pIC_{50} 6.6–7.6) with a *p*-OMe **26** showing the most potent activity and a *p*–CF_3 **28** the least suggesting the electronic properties of the substituent may play a role.

Scheme 2. Synthesis of integrin antagonists.



Reagents and conditions. (a) *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide, *N*-methylmorpholine, 1-hydroxybenzotriazole hydrate, 20 °C; (b) H_2 (1 bar), 10% Pd/C, EtOH, 20 °C; (c) 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate, Et_3N, CH_2Cl_2, room temp.; (d) $NaOH_{(aq.)}$, EtOH, 20 °C; (e) LiOH, THF/H_2O, 20 °C. All compounds are racemic unless indicated in the text.

A similar pattern is seen with $\alpha_v\beta_5$ whereas antagonism of $\alpha_v\beta_8$ is flat and essentially similar to $\alpha_v\beta_6$. The *p*-CN analogue **27** suggests some selectivity for $\alpha_v\beta_3$. However these data do not indicate mono substitution in the *para* position is useful for increasing $\alpha_v\beta_6$ activity and so no further analogues were prepared.

In contrast, mono-substitution in the *meta*-position (Table 1, **32**–**34**) proved to be more influential on $\alpha_v\beta_6$ antagonism with around a tenfold range of activity (pIC_{50} 6.1–7.1). The size of the substituent is more influential (compare *m*-F **4** (pIC_{50} 6.1) with *m*-CF_3 **33** (pIC_{50} 7.0)) than electronic properties (compare *m*-MeO **22** and *m*-Me **21** with *m*-CN **32** and *m*-Cl **20** (pIC_{50} values of 6.5 and 6.4 *vs* 6.6 and 6.6 respectively)). The enantiomers of the most potent *m*-CF_3 analogue **33** (racemate pIC_{50} 7.0) were prepared from commercially available pure (*S*) and (*R*) enantiomers of **7** thus allowing **33S** and **33R** to be assigned as the (*S*) and (*R*) trifluoromethyl enantiomers respectively and with corresponding $\alpha_v\beta_6$ pIC_{50} values of 7.1 and 5.2. Given the high sequence homology between $\alpha_v\beta_3$ and $\alpha_v\beta_6$, this is consistent with the more potent *m*-fluoro analogue **4** against $\alpha_v\beta_3$ also having (*S*) stereochemistry as described elswhere.[28]

An approximately tenfold range in activity for *m*-substituents is also seen for $\alpha_v\beta_3$ (pIC_{50} 6.4–7.5) and $\alpha_v\beta_5$ (pIC_{50} 7.1–7.8) with antagonist activities generally being greater than those observed for $\alpha_v\beta_6$. The SAR is idiosyncratic suggesting that multiple low energy binding conformations may be possible. As seen with the *para*-substituents, the $\alpha_v\beta_8$ SAR essentially tracks the $\alpha_v\beta_6$ SAR.

The *m*-CN analogue **32** shows an approximate 0.7–1.4 log selectivity for $\alpha_v\beta_5$ (pIC_{50} 7.4) over the other α_v integrins. The *m*-CF_3 analogue **33S** is the most potent antagonist against $\alpha_v\beta_6$ (pIC_{50} 7.1) and equipotent against $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$ (pIC_{50} 7.0, 7.2 and 6.9). *Meta*-substitution is clearly highly influential on the selectivity profile and further work is ongoing.

Given the impact of mono aryl substitution on potency and selectivity, we decided to explore also the impact of disubstitution because if there are multiple binding pockets in this region of the active site, then synergistic effects might be observed. As the *meta* position had proved most sensitive we preserved substitution at this position whilst varying the position of the second substituent selecting analogues (**35**–**43**) which could be prepared from readily available starting materials.

A greater range in potency was seen with di-substituted analogues compared with mono substituted analogues. Preparation of substitution patterns of dichloro analogues gives an interesting range of selectivity profiles. The 2,3-dichloro **35** shows micromolar potency for $\alpha_v\beta_5$ and ten-fold selectivity over the other α_v integrins. The 3,4-dichloro **36** and the 3,5-dichloro **37** restore $\alpha_v\beta_6$ activity (pIC_{50} 6.7 and 6.8 respectively) with the 3,5 analogue **37** being a pan α_v antagonist. Separation of the 3,5-Cl_2 enantiomers[29] gave $\alpha_v\beta_6$ pIC_{50} activities of 6.8 (**37E2**) and 5.2 (**37E1**) with **37E2**remaining predominantly a pan antagonist.

As expected, the known 3,4-methylenedioxy analogue **42**[17] has approximately nanomolar potency and greater than ten-fold selectivity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ over $\alpha_v\beta_6$ and $\alpha_v\beta_8$; it also has increased potency by 0.6–0.7 log units over the 3,4-dimethoxy substituents **41** which is less selective for $\alpha_v\beta_3$ and $\alpha_v\beta_5$. The presence of the oxygens is important as both the corresponding indane **39** and 3,4-dimethyl substituents **38** are almost a log unit less potent against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and are also less selective. The same applies to the 3,4-dichloro analogue **36**. The 3-trifluoromethyl-4-chloro analogue **43** is also a pan α_v antagonist with the more active enantiomer **43E1**[29] having a similar profile.

Presented here are SAR studies of a series of integrin antagonists against $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$. Although **4** and **42** have previously been described as $\alpha_v\beta_3$ antagonists,[17] the studies described here show a more detailed picture of their profile with both compounds potent against $\alpha_v\beta_3$ but also being equipotent against $\alpha_v\beta_5$. The SARs presented here clearly show that by simple variation of the position and nature of the aryl substituent, the cell adhesion potency against $\alpha_v\beta_6$ can be increased and, comparatively, potency against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ reduced. Comparison of the lead compounds described here (e.g. **33S** and **43E1**) with the standards **1** and **3** from the literature (*cf.* Figure 1) show they have similar $\alpha_v\beta_6$ activity but with structural features perhaps more commensurate with oral bioavailability properties. Their lipophilicities are reasonable (chrom. logD values of 2.72 for **33S** and 3.28 for **43E1**) and they possess good permeability and solubility (data not shown). Indeed analogues of these compounds prepared by Merck have been shown to have good oral bioavailability in dog.[17,28]

Table 1. Activity of aryl substituted analogues in α_v integrin cell adhesion assays.[a]

Comp.	R	$\alpha_v\beta_6$ cell pIC_{50}	$\alpha_v\beta_3$ cell pIC_{50}	$\alpha_v\beta_5$ cell pIC_{50}	$\alpha_v\beta_8$ cell pIC_{50}	Chrom. logD[b]	Partial selectivity[c] for...
15	H	5.7	7.0	7.1	6.1	1.56	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
16	2-F	6.0	6.5	7.0	5.4	1.88	
17	2-Cl	<5.0	5.7	5.8	<5.0	1.92	
18	2-Me	6.4	7.3	7.7	5.9	1.85	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
19	2-OMe	<5.0	6.3	6.3	<5.0	1.83	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
4	3-F	6.1	7.5	7.8	5.8	1.92	$\alpha_v\beta_3$ and $\alpha_v\beta_5$[d]
20	3-Cl	6.6	7.1	7.6	6.3	2.30	
21	3-CH_3	6.4	6.8	7.3	6.2	1.95	
22	3-OMe	6.5	7.4	7.2	6.3	1.66	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
23	4-F	6.3	7.0	7.2	6.3	1.75	
24	4-Cl	6.4	6.9	6.9	6.2	2.14	
25	4-Me	6.1	7.4	7.0	6.0	1.97	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
26	4-OMe	6.3	7.6	7.3	6.3	1.64	$\alpha_v\beta_3$ and $\alpha_v\beta_5$
27	4-CN	6.4	7.1	6.4	6.1	1.47	$\alpha_v\beta_3$
28	4-CF_3	6.2	6.6	6.5	6.1	2.72	pan
29	4-OCF_3	6.2	6.8	6.3	6.1	2.85	
30	4-SO_2Me	6.2	6.9	6.4	6.1	1.08	
31	4-Ph	6.4	7	6.2	6.5	3.10	
32	3-CN	6.6	6.7	7.4	6.0	1.67	$\alpha_v\beta_5$
33	3-CF_3	7.0	6.7	7.4	6.8	2.73	
33S	(*S*)-3-CF_3	7.1	7.0	7.2	6.9	2.72	pan, $\alpha_v\beta_6$ lead
33R	(*R*)-3-CF_3	5.2	<5[d]	5.7[d]	<5[d]	2.61	
34	3-OCF_3	6.7	6.4	7.1	6.6	3.11	
35	2,3-Cl_2	5.1	5.4	6.3	5	2.73	$\alpha_v\beta_5$
36	3,4-Cl_2	6.7	7.3	7.0	6.7	2.62	pan
37	3,5-Cl_2	6.8	6.4	6.7	6.6	2.93	
37E1	3,5-Cl_2 (Ent. 1)	5.2[d]	<5[d]	6.0[d]	<5	2.97	
37E2	3,5-Cl_2 (Ent. 2)	6.8	6.9	6.6	7.0	2.80	pan
38	3,4-Me_2	6.7	7.2	7.2	6.3	2.29	
39	3,4-$CH_2CH_2CH_2$	6.8	6.9	7.1	6.4	2.52	
40	3,5-Me_2	6.3	6.4	6.4	6.2	2.43	
41	3,4-$(OMe)_2$	6.5	7	7.2	6.4	1.37	
42	3,4-OCH_2O-	6.6	7.8	7.9	6.1	1.47	$\alpha_v\beta_3$ and $\alpha_v\beta_5$[d]
43	3-CF_3-4-Cl	7.0	6.5	6.9	6.6	3.1	pan
43E1	3-CF_3-4-Cl (Ent. 1)	7.2	6.8	7.2	6.9	3.28	pan, $\alpha_v\beta_6$ lead
43E2	3-CF_3-4-Cl (Ent. 2)	<5	5.8[e]	6.2[e]	<5[e]	3.25	

[a] *All compounds racemic unless shown. All biological data are means from at least n = 2 and within ± 0.42 of the mean. pIC_{50} values are the negative log of the IC_{50}. The lower limit of the assays is around pIC50* [5]*;* [b] *Chromatographic logD – see reference 21;* [c] *Defined as at least a 0.7 log difference in pIC50 values between the integrins in question;* [d] *See reference 17;* [e] *Values are n=1.*

Although a crystal structure of $\alpha_v\beta_6$ is currently not available, a homology model is available[30] and the $\alpha_v\beta_3$ crystal structure has been described.[31] From the latter, it is known that the RGD motif (or ligand mimetic) binds at the interface between the α_v and the β_3 (or β_6) subunits with the arginine (or mimetic) binding to the α_v unit and the aspartic acid (or mimetic)

binding to the β_3 (or β_6) subunit. There is considerable sequence similarity between the β_3 and β_6 subunits so the gross topology is also likely to be similar. Based on this, an extended conformation for these ligands in $\alpha_v\beta_6$ is likely to be the conformation for antagonism and is also consistent with why small differences in aryl group substitution might have the profound impact on the selectivity between the different α_v integrins observed.

Further studies to improve $\alpha_v\beta_6$ potency are underway. Clearly sufficient potency is required to drive an antifibrotic response at a realistic clinical dose but a compromise may be required between the ideal potency and ideal selectivity. Although our focus here is IPF, a pan α_v antagonist has recently been reported[32] as being efficacious in a number of models of fibrotic diseases (see supporting information).

Described here are SAR relationships against $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$ with the aim of ultimately identifying an orally bioavailable selective and potent $\alpha_v\beta_6$ for treating IPF. The lead compounds **33S** and **43E1** have been derived from $\alpha_v\beta_3$ antagonists to pan α_v antagonists with antagonisms around pIC_{50} 7 (100 nM). This has been achieved whilst maintaining physicochemical properties commensurate with oral bioavailability. Compounds which are partially selective for $\alpha_v\beta_5$ have also been identified. Further studies will be reported in due course.

ASSOCIATED CONTENT

Supporting Information. Experimental details and spectroscopic data for the compounds described in this paper (37 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors have given approval to the final version of the manuscript.

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