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Synthesis and initial evaluation of novel, non-peptidic antagonists of the α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$

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ABSTRACT

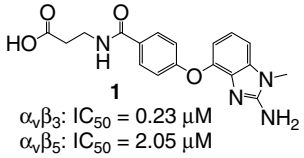
The discovery, synthesis and preliminary SAR of a novel class of non-peptidic antagonists of the α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ is described. High-throughput screening of an extensive series of ECLiPS™ compound libraries led to the identification of compound **1** as a dual inhibitor of the α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Optimization of compound **1** involving, in part, introduction of two novel constraints led to the discovery of compounds **15a** and **15b** with reduced PSA and much improved potency for both the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. Compounds **15a** and **15b** were shown to have promising activity in functional cellular assays and compound **15a** also exhibited a promising Caco-2 permeability profile.

Integrins belong to a superfamily of heterodimeric cell surface receptors with critical roles in regulating both physiological and pathological processes. They are involved in a variety of cell signaling pathways by mediating cell adhesion, migration and proliferation.[1] Many of the receptors in the integrin family recognize the tripeptidic RGD-sequence present in the respective ligands. Two integrins that have received considerable attention are α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Upregulation of these integrins has been associated with various pathological angiogenesis including ocular diseases, tumorigenesis and metastasis.[2] Recently a dual inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (SCH221153) has been reported to inhibit angiogenesis and tumor growth in vivo.[3] In addition, in clinical trials for recurrent glioblastoma multiforme, a highly invasive and vascular cancer, single-agent Cilengitide (i.e., EMD 121974, a peptidic dual inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins)[4] has demonstrated antitumor benefits and minimal toxicity.[5] As such there is significant interest in the identification and development of dual inhibitors of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins.

Many groups have reported small molecule integrin inhibitors designed as RGD (Arg-Gly-Asp) mimetics.[6] These RGD mimetics typically consist of: (i) a basic group as an arginine mimic, (ii) a subunit containing a carboxylic acid as an aspartic acid mimic, (iii) and a molecular core which can organize these two pharmacophores in the proper spatial orientation with respect to one another. The difficulty in obtaining $\alpha_v\beta_3$ inhibitors with good oral absorption has been recognized for many years as one of the major obstacles to overcome. One of the reasons for this is the zwiterionic character of the RGD mimetics. In the past few years a number of groups have reported $\alpha_v\beta_3$ inhibitors with good oral bioavailability.[6] The majority of these compounds incorporate basic groups with relatively low pK_a values (7–8) such as aminopyridyl variants.

High-throughput screening of an extensive series of ECLiPS™ libraries[7] employing a time resolved fluorescence (TRF) assay[8,9] resulted in the identification of compound **1** as an antagonist of αvβ3 (Fig. 1). Subsequent evaluation of the binding activity of **1** measured on the human $\alpha_v\beta_5$ receptor using a TRF assay with vitronectin as the ligand revealed this compound to be a dual inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$, with better potency against $\alpha_v\beta_3$.

Consideration of the physicochemical properties of compound **1** revealed it to be drug-like with respect to Lipinski's 'rule of 5'.[10] As mentioned earlier, most $\alpha_v\beta_3$ inhibitors with good oral bioavailability have basic groups with relatively low pK_a values (i.e., between 7 and 8). We have estimated the basic aminobenzimidazole moiety in our molecule to be in this range, having a calculated pK_a value of 6.9. However, the polar surface area (PSA) of compound **1** is potentially high as indicated by calculation of 'fast polar surface area'



1

$\alpha_v\beta_3$: IC_{50} = 0.23 μM
$\alpha_v\beta_5$: IC_{50} = 2.05 μM

Figure 1. Dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonist **1**.

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(FPSA).[11] The calculated FPSA of **1** is 120.3 Å^2, and we reasoned that this may potentially limit the prospect for good absorption.[12]

Since the high FPSA value for compound **1** is due in large part to the presence of four H-bond donors (COO**H**, amide N**H**, and N**H$_2$** of aminobenzimidazole), we examined the incorporation of constraints into the molecule as shown in Figure 2. Each of these constraints reduces polar surface area (PSA) by eliminating an H-bond donor (i.e., amide N**H** and N**H** of amino group) and has the potential to improve absorption while maintaining potency against α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$.

The compounds shown in Table 1 were synthesized via the general synthetic routes outlined in Schemes 1–3 and the synthesis of compounds **2a**, **2b**, **10** and **15a** has been previously described.[13] Compound **2a** was prepared as shown in Scheme 1. First, treatment of benzylbromide **4** with *tert*-butyl-3-aminopropionate in the presence of base with heating followed by BBr_3-mediated demethylation gave the phenol **5**. S_NAr_2 reaction of phenol **5** with 2,6-difluoronitrobenzene followed by a second fluoro displacement with methylamine and subsequent nitro-reduction gave aminoaniline **6**. Finally, treatment of intermediate **6** with cyanogen bromide to form the aminobenzimidazole followed by ester hydrolysis gave desired compound **2a**.

The synthesis of compounds **2b**, **2c** and **10** is shown in Scheme 2. Compounds **2b** and **2c** were prepared from the commercially available lactams **7a** and **7b**, respectively. First the phenol was protected as the benzyl ether by benzylation with benzyl bromide. Next the lactams were N-alkylated with ethyl 3-bromopropanoate to give intermediates **8a** and **8b**. After removal of the benzyl protecting group by hydrogenolysis, the conversion of **8a** and **8b** to **2b** and **2c** was carried out in a similar manner to that for the conversion of **5** to **2a**. The synthesis of compound **10** was similar but involved extra steps to prepare the tricyclic benzimidazole moiety via intermediate **9c**. Briefly, after removal of the benzyl protecting group of intermediate **8a**, S_NAr_2 reaction of the resultant phenol with 2,6-difluoronitrobenzene followed by a second fluoro displacement with 3-amino-1-propanol gave intermediate **9c**. After nitro-reduction of intermediate **9c**, followed by reaction of the resultant aminoaniline intermediate with cyanogen bromide to form the aminobenzimidazole, the hydroxyl group was converted to a chloride by treatment with thionyl chloride. The chloro intermediate then underwent cyclization upon heating to form the 6-membered ring constraint. Ester hydrolysis then gave compound **10**.

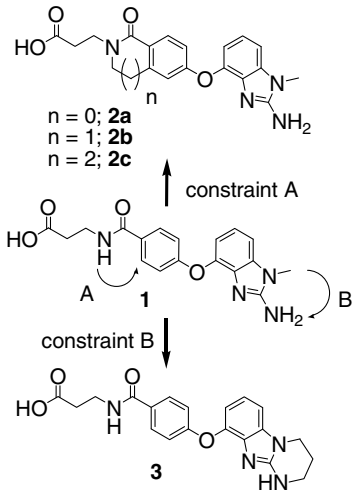
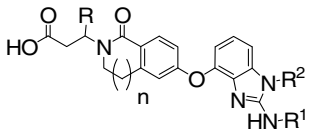


Figure 2. Two possible constraints of compound **1**.

Table 1
In vitro biological activity of compounds **2a**, **2b**, **2c**, **10**, **15a** and **15b**



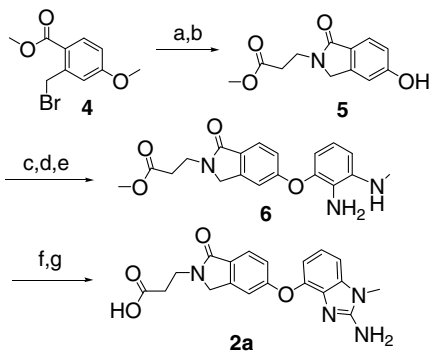
Compound	*n*	R	R^1	R^2	$\alpha_v\beta_3$ IC$_{50}$[a] (μM)	$\alpha_v\beta_5$ IC$_{50}$[a] (μM)	$\alpha II_b\beta_3$ IC$_{50}$[a] (μM)
2a	0	H	H	Me	0.18	4.8	>10
2b	1	H	H	Me	0.039	0.60	>10
2c	2	H	H	Me	0.37	4.3	>10
10	1	H	–(CH$_2$)$_3$–		0.007	0.31	6.3
15a	1	3,4-Benzdioxolyl	–(CH$_2$)$_3$–		0.002	0.014	0.167
15b	1	3-Pyridyl	–(CH$_2$)$_3$–		0.001	0.005	0.323

[a] Values are means of at least two experiments with standard deviation less than 20%.

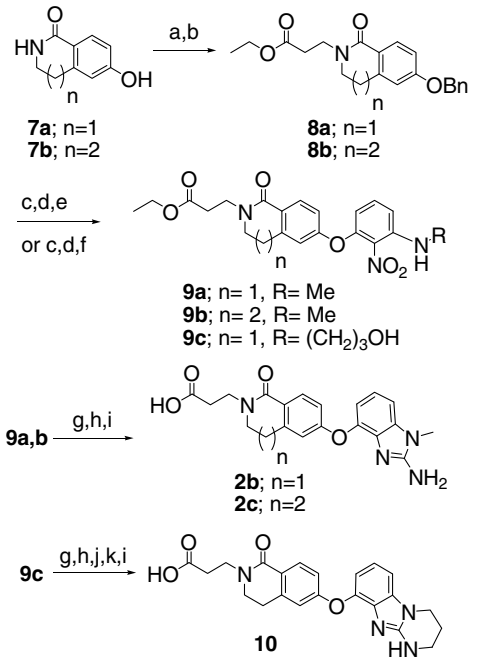
The preparation of compounds **15a** and **15b** is summarized in Scheme 3. First phenol **7a** was reacted with benzylbromide to protect the phenol as the benzyl ether. This benzyl ether intermediate was then converted into intermediates **12a** and **12b** using chemistry developed by Katritzky[14] involving lanthanide triflate catalyzed reaction of benzotriazole intermediates **11a** and **11b** with the commercially available silylketene acetal 1-(*tert*-butyldimethylsilyloxy)-1-methoxyethene. Conversion of intermediates **12a** and **12b** to compounds **15a** and **15b** was similar to that described for the conversion of intermediate **8a** to compound **10**.

The compounds synthesized in this study were initially evaluated using a TRF assay for their ability to antagonize integrin–ligand interactions for αvβ3 and $\alpha_v\beta_5$. The fibrinogen receptor ($\alpha_{IIb}\beta_3$) was also tested using the same assay for selectivity characterization.[9] As illustrated in Table 1, replacement of the benzamide spacer with bicyclic lactam scaffolds in which the ring size of the lactam ring was varied from 5 to 7 atoms (i.e., constraint A in Fig. 2) was investigated first. The 6-membered lactam analog **2b** was preferred and represented a 6-fold increase in potency at $\alpha_v\beta_3$ and a 3-fold increase in potency at $\alpha_v\beta_5$ compared to the lead compound **1**. The 5-membered lactam analog **2a** was about 2-fold more active at $\alpha_v\beta_3$ but 2-fold less active at $\alpha_v\beta_5$ compared to the library hit **1**. The 7-membered lactam analog **2c** showed decreased potency versus compound **1** at both $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Compounds **2a**, **2b** and **2c** were all virtually inactive (IC$_{50}$ > 10 μM) at the related integrin $\alpha_{IIb}\beta_3$.

To further increase the binding affinity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and reduce PSA (by deletion of an H-bond donor), our efforts focused on



Scheme 1. Reagents and conditions: (a) *tert*-butyl-3-aminopropionate∗HCl, Et_3N, MeOH, reflux; (b) BBr_3, CH_2Cl_2, −78 °C to 23 °C; MeOH; (c) 2,6-difluoronitrobenzene, $CsCO_3$, DMF, 80 °C; (d) $MeNH_2$ (2.0 M in MeOH), DMF, 23 °C; (e) H_2 (g) (1 atm), 10% Pd/C, EtOH; (f) BrCN, MeOH; (g) LiOH, MeOH/THF/H_2O (3:2:1).



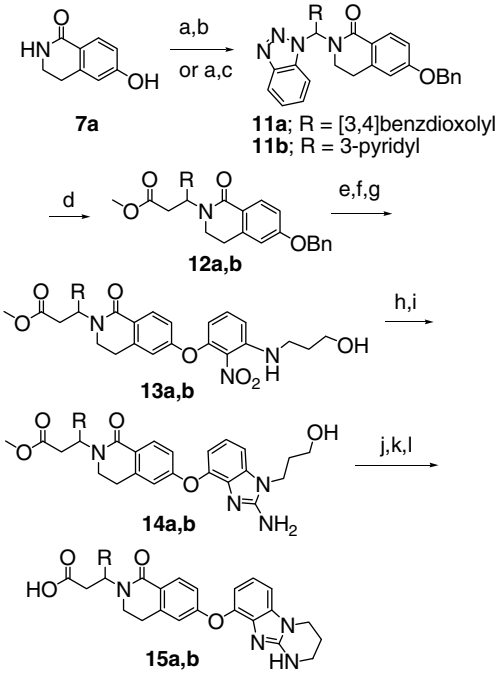
Scheme 2. Reagents and conditions: (a) benzylbromide, K_2CO_3, DMF; (b) NaH, ethyl 3-bromopropanoate, DMF; (c) H_2 (g) (1 atm), 10% Pd/C, EtOH; (d) 2,6-difluoronitrobenzene, $CsCO_3$, DMF, 80 °C; (e) $MeNH_2$ (2.0 M in THF), DMF, 23 °C; (f) 3-amino-1-propanol, EtOH, reflux; (g) H_2 (g) (1 atm), 10% Pd/C, EtOH; (h) BrCN, MeOH; (i) LiOH, MeOH/THF/H_2O (3:2:1); (j) $SOCl_2$, reflux; (k) K_2CO_3, TBAI (cat.), DMSO, 120 °C.

the aminobenzimidazole moiety next. Specifically, the exocyclic primary amine substituent contributes substantially to the overall high PSA of our molecules since it contains two H-bond donors. One way to eliminate one of the H-bond donors on the exocyclic primary amine substituent (and therefore decrease PSA) would be to simply introduce an alkyl group (e.g., methyl) onto the amino substituent. However, in earlier SAR studies around the aminobenzimidazole moiety, we demonstrated that introduction of a methyl substituent onto the exocyclic amino group (i.e., –NHMe vs $–NH_2$) resulted in a large decrease in binding affinity at both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (not shown). We reasoned that this loss in potency could be attributable to an unfavorable steric interaction between the methyl group on the exocyclic amine substituent and the methyl substituent of the imidazole nitrogen which would disrupt the correct orientation of the remaining H-bond donor (i.e., –N**H**Me) for binding to the integrin receptors.

To test this hypothesis, we prepared constrained analogs in which the alkyl group on the exocyclic nitrogen and the alkyl group on the imidazole nitrogen were joined together forming a 6-membered ring (see Fig. 2, constraint B). This constraint led to a substantial (5-fold) increase in binding affinity at $\alpha_v\beta_3$. The effect at $\alpha_v\beta_5$ was more moderate, resulting in a 2-fold increase in potency (compare analogs **10** and **2b**). Interestingly, the activity of compound **10** at the integrin $\alpha_{IIb}\beta_3$ was enhanced compared to analog **2b**, but compound **10** is still highly selective for both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ versus $\alpha_{IIb}\beta_3$. Binding affinities at both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ could be further enhanced by incorporating certain aryl and heteroaryl substituents at the 3-position of the propionic acid side-chain which have been utilized in potent $\alpha_v\beta_3$ antagonists described by Merck.[6g] For example, introduction of a 3,4-benzdioxolyl substituent to give compound **15a** resulted in a 3- to 4-fold increase in potency at $\alpha_v\beta_3$ and a 22-fold increase in potency at $\alpha_v\beta_5$ compared to the unsubstituted analog **10.** Analog **15b** containing a 3-pyridyl substituent was even more active at both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ with single digit nanamolar potency at both receptors. Introduction of the 3,4-benzdioxolyl or 3-pyridyl substituents also enhanced activity at $\alpha_{IIb}\beta_3$. Still, analog **15a** exhibits over 80-fold selectivity for $\alpha_v\beta_3$ versus $\alpha_{IIb}\beta_3$ and greater than 10-fold selectivity for $\alpha_v\beta_5$ versus $\alpha_{IIb}\beta_3$. Analog **15b** demonstrates an even better selectivity profile, with greater than 300-fold selectivity for $\alpha_v\beta_3$ versus $\alpha_{IIb}\beta_3$ and over 60-fold selectivity for $\alpha_v\beta_5$ versus $\alpha_{IIb}\beta_3$.

Because of their excellent potency at both $\alpha_v\beta_3$ and $\alpha_v\beta_5$, and their good selectivity profile versus integrin $\alpha_{IIb}\beta_3$, analogs **15a** and **15b** were further evaluated in functional cellular assays (see Table 2). To predict their potential for GI absorption, Caco-2 permeability was also determined. Both analogs demonstrated good potency in inhibiting FGF and VEGF-induced human umbilical vein endothelial cells (HUVEC) proliferation[15,16] and HUVEC migration.[17,18] In fact, both analogs were essentially equipotent in inhibiting proliferation and were approximately 10-fold more active in reducing cell migration compared to the known $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonist cycloRGDfV.[19] In early in vitro ADME profiling, compound **15a** displayed a promising Caco-2 permeability profile (P_{app} = 89.6 nm/s), but compound **15b** exhibited low permeability.

In summary, a novel, non-peptidic, dual inhibitor of the α_v-integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ was identified in a broad screen of Pharmacopeia's ECLiPS™ library collection. Introduction of two novel constraints, aimed in part at minimizing PSA, and incorporation of a 3,4-benzdioxolyl or 3-pyridyl substituent at the 3-position of the propionic acid side-chain resulted in the identification of compounds **15a** and **15b** which display nanomolar affinity for both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in binding assays. These analogs also display good selectivity over the related integrin $\alpha_{IIb}\beta_3$. In addition, both analogs show promising efficacy in functional cellular assays and compound **15a** displayed promising Caco-2 permeability. Therefore, compound **15a** was chosen as a lead structure for further SAR studies in pursuit of potent dual inhibitors for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ with potentially good oral bioavailability.



Scheme 3. Reagents and conditions: (a) benzylbromide, K_2CO_3, DMF; (b) piperonal, benzotriazole, TsOH (cat.), toluene, reflux, –H_2O (Dean–Stark); (c) nicotinaldehyde, benzotriazole, TsOH (cat.), toluene, reflux, –H_2O (Dean–Stark); d) 1-(*tert*-butyldimethylsilyloxy)-1-methoxyethene, $Yb(OTf)_2$, CH_2Cl_2; (e) H_2 (g) (1 atm), 10% Pd/C, MeOH; (f) 2,6-difluoronitrobenzene, $CsCO_3$, DMF, 80 °C; (g) 3-amino-1-propanol, EtOH, reflux; (h) H_2 (g) (1 atm), 10% Pd/C, MeOH; (i) BrCN, MeOH; (j) $SOCl_2$, reflux; (k) DIPEA, NMP, 120 °C; (l) 1 N NaOH (aq), MeOH, 60 °C; H+.

Table 2
Functional cell-based activity of compounds **15a**, **15b** and cycloRGDfV and Caco-2 permeability of compounds **15a** and **15b**

Compound	HUVEC proliferation/ FGF IC_{50}[a] (μM)	HUVEC proliferation/ VEGF IC_{50}[a] (μM)	HUVEC migration IC_{50},[a] (μM)	Caco-2 permeability P_{app} (nm/s)
15a	2.9 (±1)	1.1(±0.5)	0.040 (±0.020)	89.6
15b	1.1 (±1)	4.3(±2)	0.037 (±0.028)	0.0
CycloRGDfV	3.1 (±3)	4.5(±1.5)	0.53 (±0.22)	ND

[a] Values are means of two experiments, standard deviation is given in parentheses (ND, not determined).

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16. Assays for FGF or VEGF-induced HUVEC proliferation were performed as described in Ref. 15 with modifications. Briefly, HUVEC cells were seeded at 1000 cells per well in 200 μL of complete EBM-2 media in 96 well-format. The cells were cultured at 37 °C with 5% CO_2 for 24 h. The media was then decanted and 200 μL of proliferation media containing EBM-2 plus 2% FBS and 3 ng/mL FGF with the compound was added and the cells were incubated for an additional 48 h. Cells were then fixed with 2.5% glutaraldehyde for 30 min, washed and stained with 0.1% crystal violet. Absorbance at 595 nM was measured after being washed and destained using 10% acetic acid.
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18. HUVEC migration assay was performed essentially as described in Ref.17. Briefly, the polycarbonate filters from a 24-well Boyden chamber were coated with 2 μg/mL of vitronectin in coating buffer containing 50 mM Hepes, 100 mM NaCl, 1 mM of $MgCl_2$, $CaCl_2$ and $MgCl_2$ for 1 h at 37 °C, followed by blocking with 2% BSA in the coating buffer for 1 h at 37 °C. HUVEC cells were then seeded to the upper chamber at 20,000 per well in 100 μL of migration buffer in the presence or absence of the compound. Migration buffer (500 μL) was added to the bottom chamber followed by incubation at 37 °C for 4 h. The non-migrated cells were removed from the upper side of the filters with a cotton ball. The migrated cells were fixed with 2.5% glutaraldehyde followed by washing and staining using 0.1% crystal violet. The stained cells were photographed and analyzed using ScanImage.
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