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Substituted Benzocycloheptenes as Potent and Selective α_v Integrin Antagonists

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Abstract—A novel series of potent and specific α_v integrin antagonists has been obtained by aminoalkyl substitutions on benzocycloheptene acetic acids as a rigid GD bioisostere. The preferred compounds **1**–**2**, **1**–**3** and **1**–**8**, showed nano- to subnanomolar IC_{50} values on $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, with favorable pharmacokinetics.

Integrins belong to a large class of ubiquitous heterodimeric cell surface glycoproteins involved in cell–cell and cell–matrix interactions that are for the most part mediated by the recognition of a tripeptidic RGD-sequence present in the extracellular ligands.[1]

Upregulation of specific α_v-integrins such as $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (vitronectin receptors) has been associated with various pathologies including arthritis[2] and metastatic cancer.[3] Therefore regulation of these integrin receptors has been acknowledged as a valuable therapeutic approach.

Although numerous publications and patents have been issued in this field,[4] few integrin antagonists, among them the cyclopeptide **Cilengitide**[5] for cancer and the peptidomimetic **SB273005**[6] for osteoporosis, are in clinical development.

Because some integrins are also implicated in essential phenomena, such as blood coagulation via the $\alpha_{IIb}\beta_3$ receptor,[7] selectivity of α_v integrin antagonists is essential for their development as drugs. For this purpose, several cyclic and acyclic peptidomimetic derivatives have been investigated.[8]

Crystal structures of the truncated $\alpha_v\beta_3$ integrin[9] and the conformational analyses of cyclopeptide antagonists point to constrained orientation of the glycine moiety, may be by intramolecular hydrogen bond, forming a γ-turn **a** (Fig. 1).[10] This twisted conformation of the RGD recognition sequence is an attractive hypothesis for the design of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonists selective versus the $\alpha_{IIb}\beta_3$ integrin (Fig. 1). Compounds **1** are proposed as constrained RGD bioisosteres, potentially specific for α_v integrins. The benzocycloheptene acetic acid replaces the GD γ-turn **a**, ensuring conformational rigidity, while various aminoalkyl side chains replace the arginine (Fig. 1).

Chemistry

A retrosynthetic analysis correlates **1** to the GD biomimetic **2**, using classical coupling reactions. This amine **2** can derive from aldehyde **3**, produced via one carbon homologation of ketone **4**. The racemic form of compound **4** was generated from mono Michael addition on the symmetrical benzocycloheptadienone **5**, readily obtained by a described procedure starting from dibromo-*o*-xylene (Fig. 2).[11]

The monoalkylation of ketone **5**, was controlled by combination with a TBS-ketene acetal, at low temperature, in the presence of catalytic mercury iodide, to give the corresponding TBS-enol ether **6**.[12] This intermediate was then carefully hydrolysed with etheral hydrochloric acid, to give ketone **4** in high yield. The one carbon homologation of ketone **4** was achieved by 1,2-addition

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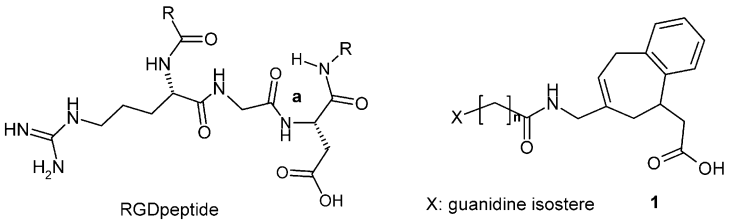


Figure 1. Integrin ligand and γ-turn mimic **1**.

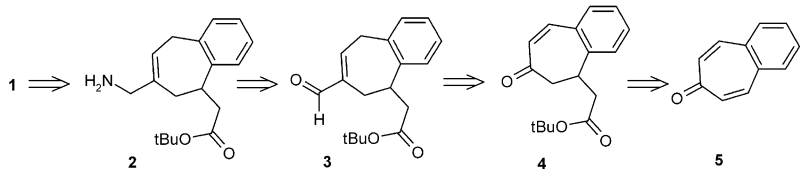


Figure 2. Retrosynthesis.

of chloromethyl-TMS anion to form an epoxide intermediate **7**, which was quantitatively isomerized into aldehyde **3**, on silica gel.[13] Various conditions of reductive aminations of aldehyde **3** led to partially isomerized conjugated amine.[14] Thus the stepwise procedure, comprising reduction of **3** in allylic alcohol,[15] bromination,[16] substitution with potassium phthalimide followed by hydrazinolysis produced the amine **2**,[17] in good overall yield, without double bond isomerisation (Scheme 1).

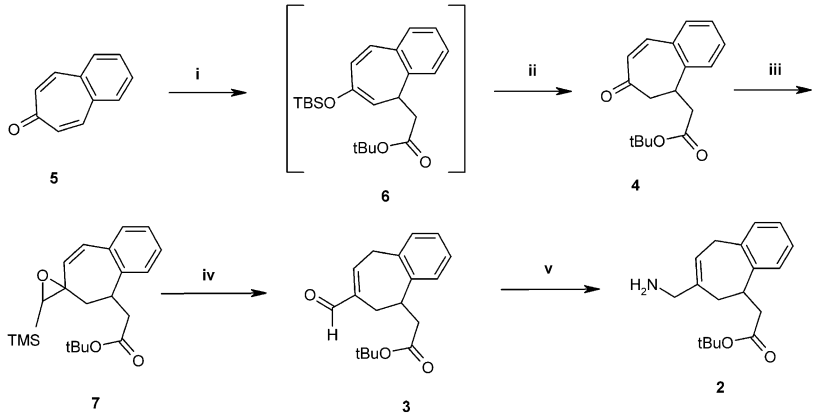
In the design of bioavailable vitronectin receptors antagonists, it has been shown that the guanidine moiety could be replaced advantageously by much less basic amino heterocycle surrogates.[18]

In order to validate our conformational hypothesis, we envisaged to couple synthon **2** with ω-aminoalkylcarboxylic acid containing these various guanidinomimetics. Pyridylaminoalkyl side chains were readily obtained from 2-chloropyridine-*N*-oxide and appropriate commercial β, γ or δ-aminoalkylcarboxylic acids to give **8**–**1** to **8**–**3**[19] ($n = 2$–4, respectively) (Scheme 2). To avoid any intramolecular lactam formation in the non-substituted pyridylaminoalkyl acid species, the corresponding triBOC derivatives **8**–**1** to **8**–**3** were used and coupling with **2** could then be performed without catalysis.[20]

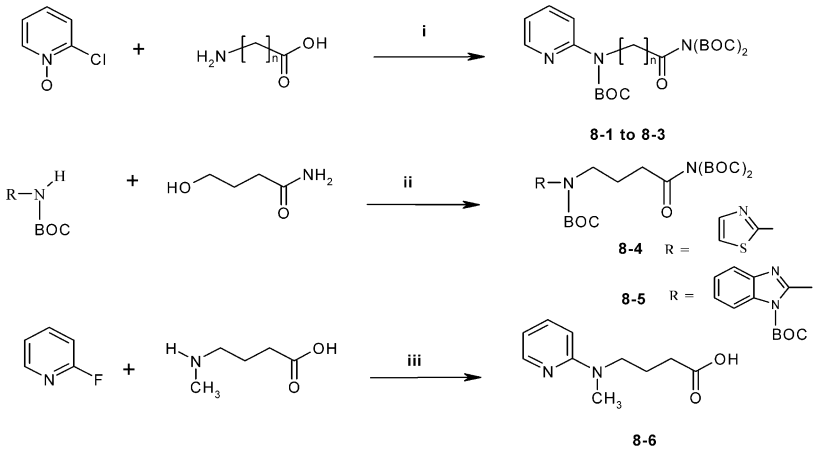
Thiazole and benzimidazole containing derivatives **8**–**4** and **8**–**5** were produced in fair yield via Mitsunobu coupling reaction of the appropriate activated *N*-BOC heterocycles with γ-hydroxybutyric amide,[21] and subsequent amide protection. The *N*-methylpyridylamino butyric acid derivative **8**–**6**, obtained directly from 2-fluropyridine, can not form a lactam and was used without further protection (Scheme 2).

The pyridylmethylamino derivative, **8**–**7**, was synthesised via Michael addition of the requisite methylaminopyridine onto acrylamide and subsequent protection (Scheme 3).

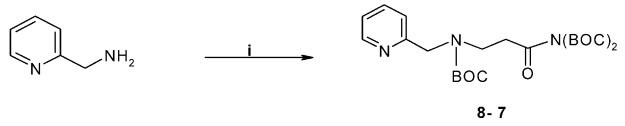
The corresponding tetrahydronaphtyridyl derivative **8**–**8**, also used as guanidine substitute,[22] was produced as described, via a base-catalysed ring closure of 2-aminonicotaldehyde[23] with methyl ketobutyric acid and partial hydrogenation.[24]



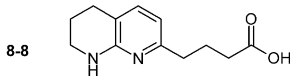
Scheme 1. Synthesis of synthon **2**: (i) CH_2=C(OtBu)(OTBS), HgI_2, CH_2Cl_2, −78 °C; (ii) 1N HCl, rt, 6 h (**4**, 95%); (iii) secBuLi, $ClCH_2TMS$, TMEDA, THF, −78 °C; (iv) SiO_2 (**3**, 95%); (v) (a) $NaBH_4$, MeOH, CH_2Cl_2, 0 °C, 1.5 h, (100%); (b) PPh_3, CBr_4, CH_2Cl_2, 0 °C–rt, 2 h (50%); (c) PhtNK, DMF, 40 °C, 4 h, (60%); (d) NH_2NH_2, MeOH, CH_2Cl_2, rt, 48 h (**2**, 90%).



Scheme 2. Pyridylamino, thiazol and benzimidazol alkylcarboxylic derivatives: (i) (a) $NaHCO_3$, H_2O, 100 °C; (b) AcCl, MeOH, 80 °C, 2 h; (c) NH_3, MeOH, CH_2Cl_2, 90 °C, 72 h; (d) BOC_2O, tBuOH; (e) H_2, Pd/C, rt, 72 h; (f) BOC_2O, DMAP, rt, 72 h ($n=2$, **8–1**, 12%; $n=3$, **8–2**, 27%; $n=4$, **8–3**, 8%); (ii) (a) DEAD, PPH_3, DMF, rt, 24 h; (b) BOC_2O, DMAP, CH_3CN, rt, 48 h ($n=3$, **8–4**, 37%; $n=4$, **8–5**, 22%); (iii) $NaHCO_3$, pyridine, H_2O, 120 °C, 120 h (**8–6**, 30%).



Scheme 3. Pyridylmethylamino derivative: (i) (a) acrylamide, 60 °C, 24 h; (b) BOC_2O, tBuOH, rt, 24 h; (c) BOC_2O, DMAP (**8–7**, 21%).

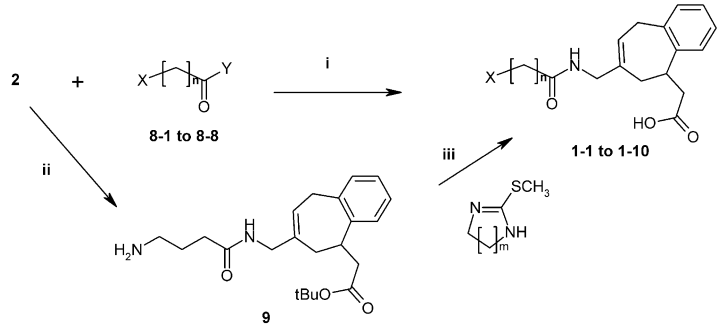


The various side chains **8–1** to **8–8** were combined with amine **2** in the appropriate manner to afford, after hydrolysis, the corresponding racemic acids **1–1** to **1–8** (Scheme 4); The cyclic amidines **1–9** and **1–10** were synthesised via treatment with the appropriate heterocyclic isothiouronium salt with the free amine **9**.[25] The elaboration of this γ-aminobutyryl side chain was performed via combination of **2** with 4-bromobutanoyl chloride, and subsequently, under classical conditions via azide substitution and reduction,[26] leading to the corresponding amine **9** (Scheme 4, Table 1).[27]

Biological and Pharmacological Data

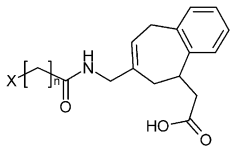
The evaluation of the binding activities of these compounds was measured on the human receptors $\alpha_v\beta_3$, $\alpha_v\beta_5$ (vitronectin displacement)[28,29a] and $\alpha_{IIb}\beta_3$ receptor (fibrinogen displacement),[30] with comparison to the clinical candidate **Cilengitide** (Table 1). Cellular adhesion inhibition was also evaluated with the IGROV-1 cell line,[29b,31] which interacts with vitronectin via α_v integrin receptors. In vitro pharmacokinetic parameters were determined using hepatic microsomes (rat/human) to predict first pass metabolism and metabolic bioavailability (MF%),[32a,b] and Caco2 cell monolayers to predict oral absorption (A%) from permeability data (Table 1).[32c]

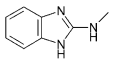
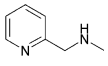
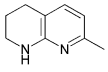
In the pyridylamino derivatives, the best affinities were obtained for the propyl and the butyl side chain, **1–2** and **1–3** respectively, with a preference for the propyl, considering its better selectivity over $\alpha_{IIb}\beta_3$ receptor and activity in the cellular assay. Moreover, all these compounds were found to be stable in the microsomal assay, with good absorption (Table 1).



Scheme 4. Synthesis of compounds **1–1** to **1–10**: (i) (a) CH_2Cl_2, rt, 24 h, [Y = N(BOC)$_2$] or HOBT, DIEA, CH_2Cl_2, rt, 24 h, (Y = OH); (b) HCl, Et_2O (**1–1** to **1–8**, yield Table 1); (ii) (a) $ClCO(CH_2)_3Br$, Et_3N, THF, 0 °C, 3 h; (b) NaN_3, DMF, 40 °C, 12 h; (c) PPh_3, THF, H_2O, 12 h (**9**, 88%); (iii) (a) Et_3N, DMF, 50 °C, 12 h ($m=1$, 2); (b) Et_2O, HCl ($m=1$, **1–9**; $m=2$, **1–10**, yield Table 1).

Table 1. Structures and biological data of compounds **1–1** to **1–10**



No.	*n*	X	Yield (%)	Binding[28,29a,30] IC_{50}, nM			Cell adhesion[29b,31] IC_{50}, nM	Stability MF (%) rat/human	Absorption A (%)
				$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_{IIb}\beta_3$			
Cilengitide[5–10]				2.3	34	860			
Found				0.4	0.8	110	36	100/100	14
1–1	2		53	584	22	1700	338	94/75	93
1–2	3		79	1.9	0.8	4360	2.0	83/77	95
1–3	4		47	2.7	0.9	1200	12	77/65	96
1–4	3		72	45	20	$>10^4$	40	100/100	96
1–5	3		60	0.6	0.5	1020	1.8	87/94	<20
1–6	3		82	68	40	667	2034	75/88	94
1–7	2		10	842	119	552	2700	98/100	23
1–8	3		44	0.3	0.3	1495	6.9	72/72	92
1–9	3		20	0.3	0.2	110	18	86/100	<20
1–10	3		28	0.2	0.1	330	2.7	92/92	<20

Therefore, the replacement of the pyridylamine moiety by various guanidine surrogates was performed on **1–2** preferentially.

Introduction of a thiazole ring to give **1–4**, entailed a great loss of affinities on both α_v receptors. Interestingly, a gain of one order of magnitude in α_v affinities was observed for the benzimidazole, imidazoline and tetrahydropyrimidine analogues **1–5**, **1–9** and **1–10** respectively. However, despite a good cellular activity, those compounds were not further examined due to an absorption prediction below 20%.

More specific modifications of the pyridylamine of **1–2**, such as *N*-methylation or nitrogen/carbon inversion to give **1–6** or **1–7**, decreased α_v affinities by more than one or two orders of magnitude respectively. These data confirmed the prevalence of steric effect over charge density at the guanidinomimetic position of the RGD sequence.[18]

Conversely, replacement of pyridylamino moiety in **1–2** by tetrahydronaphtyridine to give **1–8**, increased receptor affinities and maintained selectivity over $\alpha_{IIb}\beta_3$ and pharmacokinetics properties.

The pharmacokinetic parameters were validated in vivo in mice for two representative compounds, **1–2** and **1–5**. With a bioavailability of 71 and 16%, respectively, they correlate nicely with the in vitro predictions, pointing out the poor absorption of **1–5**.

For most compounds, adhesion and binding potencies correlated well. However, for presently unexplained reasons, several products were less efficient in cellular assays with up to a 100-fold difference. Whether this

reflects a differential accessibility of these compounds to the integrin binding site on the purified integrin versus its native conformation at the cell surface needs to be investigated.

Cellular adhesion data revealed a potent inhibitory activity for the pyridylamino compounds, **1**–**2** and **1**–**3**, and for the naphtyridine derivative **1**–**8** (Table 1) and could favorably be compared with Cilengitide and have subsequently been chosen for further in vivo evaluation. Their asymmetric synthesis is in progress to obtain their corresponding active enantiomer, as expected from stereospecific receptor recognition for the (*S*)-aspartic acid in the RGD sequence.[33]

Conclusion

The design and synthesis of RGD peptidomimetic benzocycloheptene derivatives **1** as potent vitronectin receptor antagonists were described. A convergent synthetic route allowed incorporation of various arginomimetics, via coupling with the key synthon **2**. Structure–activity study revealed an optimal three to four carbon atom spacer between the 'GD' scaffold and the guanidine bioisosteres. The preferred compounds, namely pyridylamino **1**–**2**, **1**–**3** and tetrahydronaphtyridyl substituted derivative **1**–**8**, showed nano- to subnanomolar IC_{50} values on $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, with good selectivity over the $\alpha_{IIb}\beta_3$ fibrinogen receptor. Moreover the favorable pharmacokinetics of these compounds justify further pharmacological studies.

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29. (a) Purified human integrins were adsorbed unto 96-well plates and compounds added to displace biotinylated vitronectin (for $\alpha_v\beta_3$ and $\alpha_v\beta_3$ from placenta) or fibrinogen (for $\alpha_{IIb}\beta_3$ from platelets). Ligand binding was evaluated indirectly after colorimetric reading following addition of an antibody to biotin coupled to alkaline phosphatase. (b) Adhesion assays were carried out with the human ovarian carcinoma cell line IGROV-1. When vitronectin is used as a substrate, cells

adhere exclusively through $\alpha_v\beta_5$. The cells were preincubated with the compounds, and adhesion was evaluated by image analysis after washing, fixation and crystal violet staining. Binding and adhesion data are expressed as IC_{50}s obtained after fitting the dose–response sets of at least two experiments per product (with comparable results) with a sigmoid curve with variable slope. For clarity, 95% confidence intervals are not shown.

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32. (a) Metabolic bioavailability predictions (MF%) were based on in vitro metabolic stability measurements with hepatic microsomes assuming total absorption. Unchanged drugs were quantified by LC–MS–MS following incubation (0.1 μM) with rat and human hepatic microsomes (0.33 mg prot/mL) after 0, 5, 15, 30 and 60 min of incubation. The in vitro intrinsic clearances (vitro CLint) were the slope (after LN linearisation) of the curve of the unchanged drug remaining concentration versus incubation time. In vitro CLint were then scaled up to in vivo whole body (vivo CLint) using 0.045 mg prot/kg of liver and liver weight of 11 g for the rat and 1.2 kg for man. In vivo CLint were then transformed into hepatic clearances (CLhep) using the equation [CLhep = vivo CLint.HBF/(vivo CLint + HBF)] where HBF (hepatic blood flow) were taken as 22 mL/min for the rat and 1500 mL/min for man. The MF% were then calculated with the following equation [MF% = 100(1−CLhep/HBF)]. (b) Bertrand, M.; Jackson, P.; Walther, B. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl. 2), S61. (c) Yee, S. *Pharmaceut. Res.* **1997**, *14*, 763.

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