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Synthesis of Highly Potent and Selective Hetaryl Ureas as Integrin $\alpha_V\beta_3$-Receptor Antagonists

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Abstract—Solid-phase synthesis and SAR of integrin $\alpha_V\beta_3$-receptor antagonists containing a urea moiety as non-basic guanidine mimetic are described. The most potent compounds exhibited IC_{50} values towards $\alpha_V\beta_3$ in the nanomolar range and high selectivity versus related integrins like $\alpha_{IIb}\beta_3$. For selected examples efficacy in functional cellular assays is demonstrated.

Integrins form a superfamily of heterodimeric transmembrane glycoprotein receptors. They are involved in cell–cell and cell–matrix adhesion, cell migration, and signaling. Currently 17 α- and 9 β-subunits and at least 20 combinations thereof with different affinities and specificities to matrix proteins like fibrinogen, fibronectin, vitronectin, osteopontin and laminin are known.[1] Over the last years integrins have received much attention in drug discovery research,[2] in particular the β_3-subfamily with the fibrinogen receptor $\alpha_{IIb}\beta_3$ mediating platelet aggregation,[3] and the vitronectin receptor $\alpha_V\beta_3$.

The integrin $\alpha_V\beta_3$ is specifically expressed in activated proliferating and migrating endothelial or smooth muscle cells, macrophages, and tumor cells.[4] $\alpha_V\beta_3$ is involved in restenosis after percutaneous transluminal coronary angioplasty (PTCA),[5] angiogenesis and neovascularization,[6] rheumatoid arthritis,[7] diabetic retinopathy and age-related macular degeneration,[8] tumor growth and metastasis,[9] osteoporosis,[10] and acute renal failure.[11] Therefore, the $\alpha_V\beta_3$ receptor represents an interesting therapeutic target.[12]

Studies with the antithrombotic abciximab (ReoPro®, chimeric 7E3Fab), an antagonist of $\alpha_{IIb}\beta_3$ with cross reactivity to $\alpha_V\beta_3$ and $\alpha_M\beta_2$,[13] revealed a beneficial effect on restenosis.[14] Selective antagonists of $\alpha_{IIb}\beta_3$ did not show this effect while the $\alpha_V\beta_3$ specific monoclonal antibody LM609 reduced the neointima formation in animal models.[15]

Many integrin ligands share the tripeptide sequence RGD depicted in Figure 1 as a common recognition motif.[16] Studies on cyclic peptides containing the RGD sequence demonstrated that selective ligands for $\alpha_V\beta_3$ could be obtained if the arginine and aspartate β-carbon atoms were separated by a distance of 650–700 pm.[17]

Most small molecule $\alpha_V\beta_3$-receptor antagonists described in the literature follow this pattern and mimic the RGD motif of the natural ligands by displaying an acidic and a basic moiety in a defined distance.[18] In these structures the aspartate is often replaced by a β-amino acid.[19]

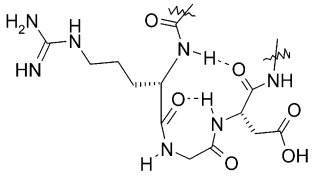

Figure 1. RGD-motif of integrin ligands.

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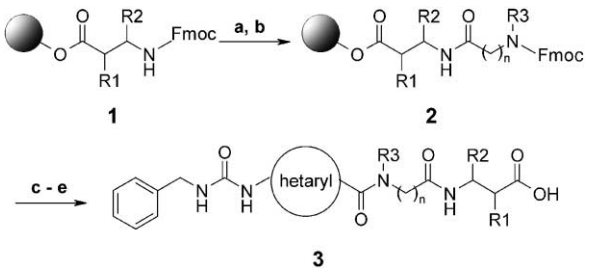
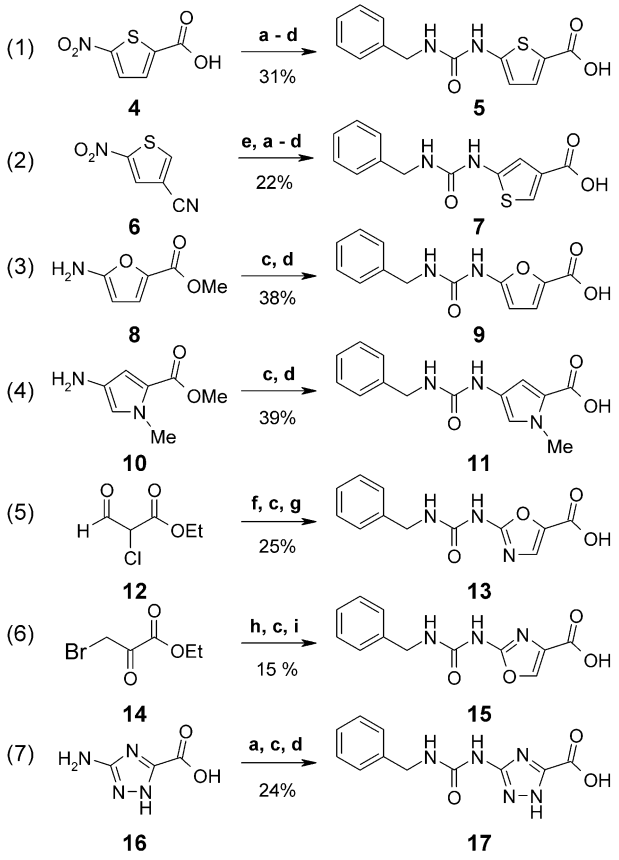
As a starting point for our own studies on $\alpha_V\beta_3$-receptor antagonists[20] we chose to connect β-amino acids with a urea moiety via a spacer element. The urea moiety serves as a non-basic hydrogen bond donor mimicking the guanidine, a replacement which has already been applied in the development of integrin antagonists.[21] As spacer element between the two pharmacophores we used a combination of an amino acid and a hetaryl moiety that allowed us to adjust the relative positions and the distance of the carboxylic group and the urea moiety.

The solid-phase synthesis of the $\alpha_V\beta_3$-receptor antagonists described above is depicted in Scheme 1. The required hetaryl urea building blocks were prepared from commercially available or easily accessible compounds according to Scheme 2.[22] A small library of antagonists was prepared using 10 β-amino acids, 3 Fmoc-amino acids (Gly, β-Ala, Sar), and 8 hetaryl urea building blocks. The IC_{50} ($\alpha_V\beta_3$) values in this set of antagonists varied from more than 100 μM to 0.1 nM.[23] One of the best combinations contained racemic β-3-pyridyl-β-alanine and glycine at the C-terminus. We used this subset of our library to determine the effect of the hetaryl moiety on the activity of the antagonist. The results are summarized in Table 1.[24,25]



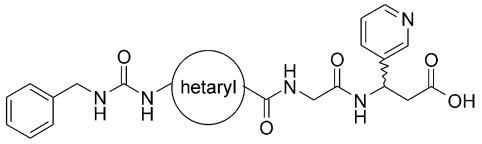
Scheme 1. (a) Piperidine, DMF; (b) Fmoc-amino acid, TBTU, DIEA, DMF, rt; (c) piperidine, DMF; (d) HO_2C-hetaryl-NH-CO-NH-Bn, TBTU, DIEA, DMF, rt; (e) AcOH, TFE, DCM. 2-chlorotrityl resin was used as solid support.



Scheme 2. (a) $SOCl_2$, MeOH; (b) Fe, AcOH; (c) Bzl-NCO; (d) LiOH, THF, H_2O; (e) H_2SO_4, AcOH, H_2O; (f) $CO(NH_2)_2$, H_2O, Δ; (g) NaOH, MeOH, H_2O; (h) $CO(NH_2)_2$, EtOH, Δ; (i) KOH, dioxane, H_2O.

The nature of the hetaryl moiety had a strong influence on the activity of the antagonists. While the 2,5-substituted thiophene **18a** and thiazole **24a**[25b] exhibited micromolar activity, the corresponding 2,5-substituted furan **20a** and oxazole **22a** had IC_{50} values in the subnanomolar range. The 2,4-substituted thiophene **19a** and thiazole **25a** also showed high activity. The *N*-methylpyrrole **21a**, the 2,4-substituted oxazole **23a** and in particular the triazole **26a** were less effective. The low activity of thiophene **18a** and thiazole **24a** can be attributed to wider bond angles in these hetaryls compared to furans[26] resulting in a different display of the two pharmacophores. The loss of activity in derivatives **21a**, **23a** and **26a** compared to **19a**, **20a**, **22a** and **25a** remains unclear. Especially the lower activity of **23a**

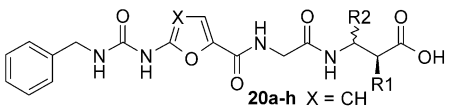
Table 1. Effect of the hetaryl moiety on the activity of the $\alpha_V\beta_3$ antagonists



Compd	Hetaryl	$\alpha_V\beta_3$ IC_{50} (nM)[a]
18a		1000
19a		2
20a		0.1
21a		16
22a		0.3
23a		14
24a		10,000
25a		0.3
26a		72

[a]Values are means of three experiments; intra-assay variation <10%, inter-assay variation <factor 2.

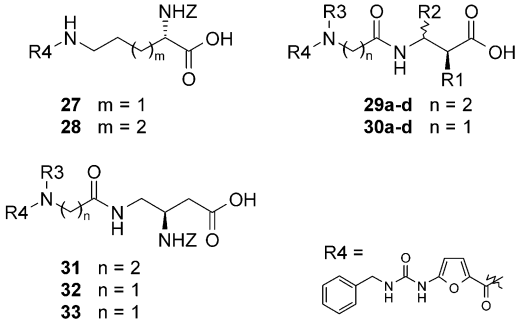
Table 2. Variation of the β-amino acid



Compd	R1	R2	$\alpha_V\beta_3$ IC_{50} (nM)[a,b]
20b	H	3,5-Dichlorophenyl	0.2
20c	NH-Z	H	0.4
20d	H	4-Methylphenyl	1.9
20e	H	3,4-Dimethoxyphenyl	0.5
20f	H	β-Naphthyl	0.3
20g	H	Cyclohexyl	0.9
20h	H	*p*-Biphenyl	1.5
22b	H	3,5-Dichlorophenyl	3.8
22c	NH-Z	H	1.8

[a]Cf. Table 1.
[b]Assay results for R2≠H refer to racemic compounds.

Table 3. Variation of the amino acid spacer



Compd	R1	R2	R3	$\alpha_V\beta_3$ IC_{50} (nM)[a,b]
27				1000
28				5000
29a	H	3-Pyridyl	H	144
29b	H	Cyclohexyl	H	10,000
29c	NH-Z	H	H	3.1
29d	H	4-Methylphenyl	H	1000
30a	H	Cyclohexyl	Me	311
30b	H	3,5-Dichlorophenyl	Me	50.5
30c	NH-Z	H	Me	8.9
30d	H	4-Methylphenyl	Me	100
31			H	2330
32			H	43.5
33			Me	88.2

[a]Cf. Table 1.
[b]Assay results for R2≠H refer to racemic compounds.

compared to **25a** is somewhat surprising. Based on these results the highly active furan derivatives and the 2,5-substituted oxazoles were chosen[24] for a more detailed SAR study.

The substituents R1 and R2 next to the carboxylic group were modified to probe their influence on the antagonist activity. In the furan series most of these variations from hydrophilic (**20a**, Table 1) to lipophilic moieties of very different size (**20b**–**h**, Table 2) were well tolerated with hardly any loss of activity. Oxazoles with a 3,5-dichlorophenyl side chain (**22b**) and an α-Z-diaminopropionic acid (Z-DAP) moiety (**22c**) were slightly less active than the corresponding furan derivatives **20b** and **20c** (Table 2).

Modification of the spacer unit revealed the optimal distance of the pharmacophores and the essential hydrogen bond donors in this type of antagonist (Table 3). Replacement of the Z-DAP-Gly unit in **20c** by Z-Orn (**27**),[27] Z-Lys (**28**) or a β-Z-diaminobutyric acid-β-alanine unit (**31**) resulted in substantial loss of activity. The β-Z-diaminobutyric acid-glycine **32** and -sarcosine derivatives **33** were about 100-fold less potent than the Z-DAP derivative **20c**. Exchange of the glycine building block against sarcosine (**30**) or β-alanine (**29**) resulted in less active compounds with the remarkable exception of the Z-DAP derivatives **29c** and **30c** which still exhibited activity in the nanomolar range.

In summary, the optimal distance between the urea moiety and the carboxylic group in this series appeared to be about 11 bonds (cf. **29a**–**d**, **31**–**33**). The secondary amide groups next to the hetaryl moiety and the β-amino acid, respectively, are crucial for potency (cf. **30a**–**d** and **28**). The Z-DAP derivatives **29c** and **30c** are exceptional in this regard. Here normally unfavorable modifications in the spacer element are much better tolerated.

Based on these results our subsequent studies focused on DAP derivatives, assuming that in this class of antagonists the pharmacokinetic profile could be modified by changes in the spacer unit without loss of activity.

The Z-DAP derivatives **20c** and **22c** exhibited high selectivity versus related integrins like $\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$, and $\alpha_4\beta_1$ (Table 4). In early ADME studies **20c** and **22c** displayed medium permeation in the Caco-2[28] model and good metabolic stability versus human liver microsomes.

Table 4. Selectivity, permeation and cellular assay data of compounds **20c** and **22c**

Compd	$\alpha_V\beta_3$/VN ELISA[a] IC_{50} (nM)	$\alpha_{IIb}\beta_3$/Fg ELISA[a] IC_{50} (nM)	$\alpha_5\beta_1$/FN Adhesion[b] Inh. @ 10^{-5} M	$\alpha_4\beta_1$/FN Adhesion[b] Inh. @ 10^{-5} M	$\alpha_V\beta_3$/OPN Adhesion[b] Inh. @ 10^{-5} M	SMC Migration[b] IC_{50} (μM)	Caco-2 Permeation assay P_{app}[c], cm/s×10^{-7}
20c	0.4	na	na	2%	86%	1.2	6
22c	1.8	na	na	na	92%	0.5	2

[a]Cf. Table 1.
[b]Cell adhesion and migration: values are means of 4 experiments; intra-assay variation <20%, inter-assay variation <factor 2.
[c]P_{app} = apparent permeability coefficient;[29] $n=2$, intra-assay variation <20%. Abbreviations: na = not active, VN = vitronectin, FN = fibronectin, Fg = fibrinogen, OPN = osteopontin.

Both compounds showed medium to high efficacy in functional cellular assays, the cell adhesion of recombinant $\alpha_V\beta_3$ transfected CHO-K1 cells and the cell migration of primary human smooth muscle cells.[23] The data of the cellular assays did not fully reflect the in vitro potency of the compounds. Similar observations have been reported for other $\alpha_V\beta_3$ antagonists.[30]

Further pharmacokinetic assays will reveal whether these antagonists are suitable for relevant efficacy studies in animal models, e.g., for the prevention of restenosis.[31]

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