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RGD Mimetics Containing a Central Hydantoin Scaffold: $\alpha_V\beta_3$ vs $\alpha_{IIb}\beta_3$ Selectivity Requirements

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Abstract—The synthesis of a series of RGD mimetic $\alpha_V\beta_3$ antagonists containing a hydantoin scaffold is shown. The results demonstrate some of the structural requirements for the design of selective $\alpha_V\beta_3$ antagonists (vs $\alpha_{IIb}\beta_3$) in terms of the Arg-mimetic, the distance between N- and C-terminus and the lipophilic side chain.

Introduction

Integrins are a widely expressed family of α/β heterodimeric cell surface receptors which bind to extracellular matrix adhesive proteins such as fibrinogen, fibronectin, vitronectin, laminin, osteopontin etc.[1–3] Currently integrins are known to be composed of α–β dimers of the existing fifteen α subunits and the eight β subunits. The β_3 class of the integrin family, $\alpha_{IIb}\beta_3$ (also known as GPIIb/IIIa or fibrinogen receptor) and $\alpha_V\beta_3$ (vitronectin receptor), has received special attention in recent drug discovery efforts.[4,5] $\alpha_{IIb}\beta_3$ is prevalent on platelets and plays a role in thromboembolic disorders.[5,6] $\alpha_V\beta_3$ is the dominant receptor for mediating the attachment of osteoclasts to bone during bone resorption[7,8] and has been implicated in tumor progression, angiogenesis[5,9] and restenosis.[10] Many integrins, including $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, interact with a common Arg-Gly-Asp (RGD) binding motif in their target proteins.[11–13] For drug development it is highly desirable to obtain integrin antagonists which bind selectively to specific integrins. Using stereoisomeric cyclic peptide libraries, Kessler et al. were able to pinpoint the structural properties needed for the selective inhibition of either $\alpha_{IIb}\beta_3$ or $\alpha_V\beta_3$.[14,15] Other selective peptidomimetic inhibitors of $\alpha_V\beta_3$ have been reported recently.[16–21] Earlier we reported the discovery of an orally active non-peptide fibrinogen receptor antagonist containing a hydantoin scaffold.[22] Here we present structural requirements that determine the selectivity of an RGD mimetic with such a central hydantoin building block towards $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$. In particular we investigated the influence of the arginine mimetic, the distance between N- and C-terminus and the lipophilic side chain.

Synthesis

The synthesis of antagonist **7** is outlined in Scheme 1. The central step is the formation of the hydantoin building block **4**, which is converted to the guanidine **5** using 1H-Pyrazole-1-carbox-amidine.[23] Compound **5** is then coupled with the 2*S*-(benzyloxycarbonylamino)-3-aminopropionic acid *tert*-butylester to give **6**. Deprotection with TFA provides the antagonist **7**. The antagonists **8** and **10** are prepared analogously with starting materials containing different chain lengths. The antagonist **9** is prepared starting from Arg-OMe which is reacted with isocyanatoacetic acid methylester in analogy to Scheme 1 to form the corresponding hydantoin, followed by the hydrolysis of the methylester, coupling with 2*S*-(benzyloxycarbonylamino)-3-aminopropionic acid *tert*-butylester and TFA cleavage of the *tert*-butylester. Compounds **11** and **12** (Table 2) are prepared by treatment of **4** ($n=3$) with 2-methylthio-2-imidazoline, or by reaction with 2-bromopyrimidine

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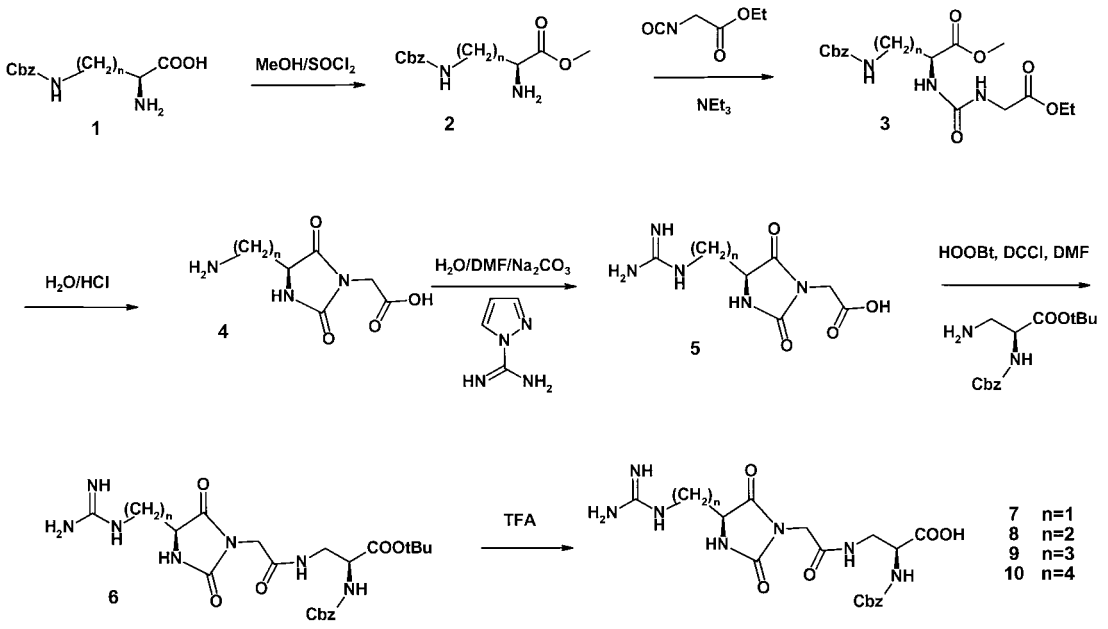


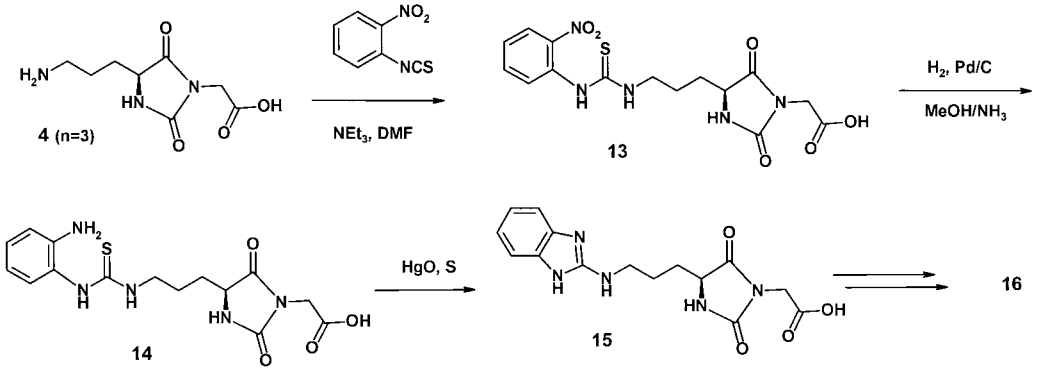
Scheme 1. Synthesis of integrin antagonists **7**–**10**.

respectively, followed in each case by the last two synthesis steps from Scheme 1.

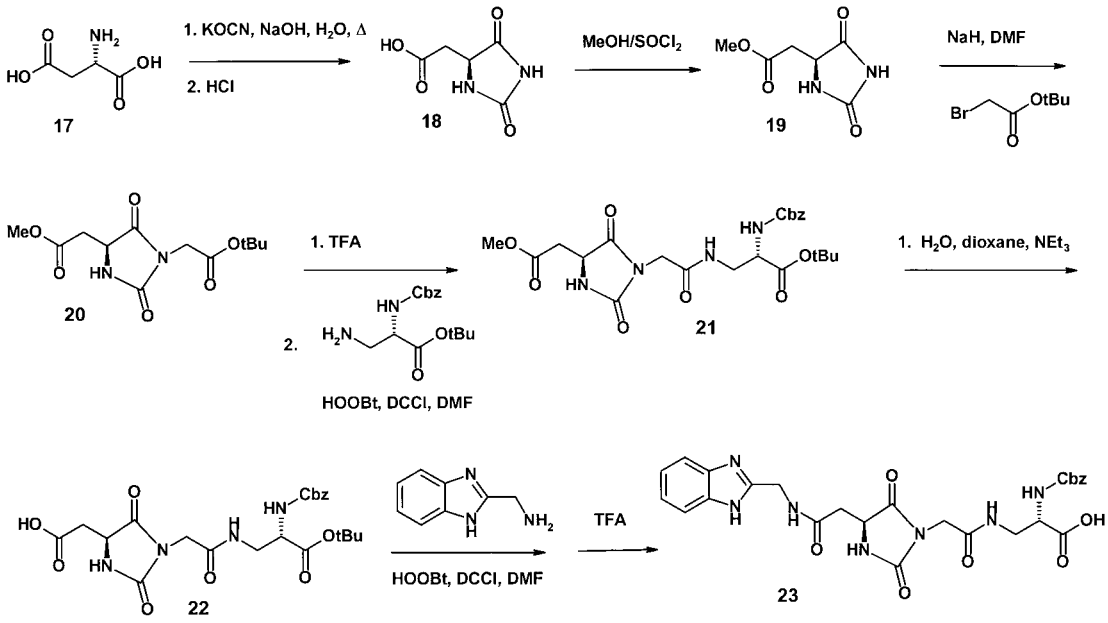
Scheme 2 shows the synthesis of antagonist **16** which carries a N-terminal amino-benzimidazole group. It starts with building block **4** ($n=3$) which is reacted with 2-nitrophenylisothiocyanate followed by reduction of the nitro group and cyclization using mercury oxide and sulfur to give **15**. Compound **15** is then reacted further as in Scheme 1 to give antagonist **16**.

Scheme 3 shows the synthesis of antagonists **23** starting with the hydantoin **18** formation from L-aspartic acid, followed by esterification and alkylation with *tert*-butylbromoacetate to give **20**, and successive coupling with 2*S*-(benzyloxycarbonylamino)-3-aminopropionic acid *tert*-butylester and 2-aminomethylbenzimidazole. Finally, treatment with TFA gave **23**. Antagonist **24** was obtained in the same way using (4,5-dihydro-imidazol-2-yl)-hydrazine instead of 2-aminomethyl-benzimidazole. Compounds **25**–**29** were obtained by catalytic hydrogenation of **9** to remove the Cbz group and coupling of the appropriate side chain to the free amino function.

The IC_{50} values for the inhibition of fibrinogen to $\alpha_{IIb}\beta_3$ and of Kistrin (a disintegrin with high affinity for $\alpha_V\beta_3$[8,24]) to $\alpha_V\beta_3$ are summarized in Tables 1–3. Table 1 shows the influence of spacer length on the $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity. The optimum distance for an $\alpha_V\beta_3$ antagonist seems to be 12 bonds between the C-terminal carboxyl group and the N-terminal guanidino group (compound **9**), while for $\alpha_{IIb}\beta_3$ a distance of 13 (or more) bonds results in higher affinity (compound **10**). This observation is in good agreement with the results obtained for other inhibitors[16,17] and with Kessler's observation that in cyclopeptides the $\alpha_V\beta_3$-selective molecules are strongly bent, while $\alpha_{IIb}\beta_3$-selective molecules are in a more extended conformation.[14] The influence of the arginine mimetic on the $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity is shown in Table 2. The comparison of **9** with **11** or **16** illustrates that for $\alpha_V\beta_3$ selectivity cyclic guanidines are preferred over non cyclic guanidines. The incorporation of cyclic guanidines also leads to decreased affinity for $\alpha_{IIb}\beta_3$.

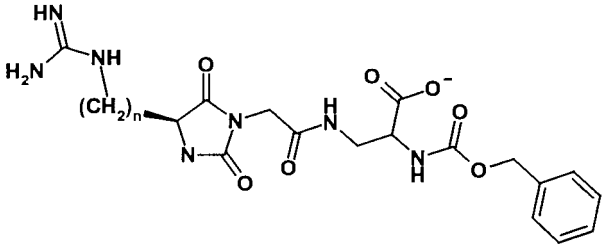


Scheme 2. Synthesis of benzimidazole containing integrin antagonist **16**.



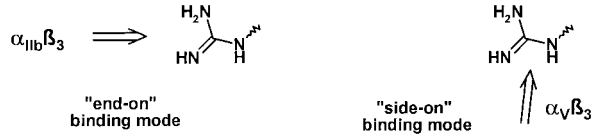
Scheme 3. Synthesis of benzimidazole containing integrin antagonist **23**.

Table 1. Influence of spacer length on $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity. The IC_{50} values denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ or of Kistrin (K) to $\alpha_V\beta_3$ by 50%



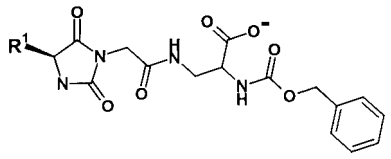
	n	Fg/$\alpha_{IIb}\beta_3$ IC_{50} (μM)	K/$\alpha_V\beta_3$ IC_{50} (μM)
7	1	10	15
8	2	10	5
9	3	2.4	0.08
10	4	0.85	0.2

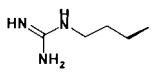
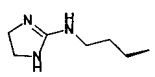
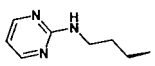
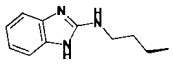
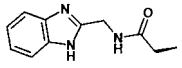
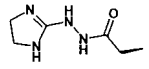
One could speculate that the antagonists bind to $\alpha_{IIb}\beta_3$ through an "end-on" interaction, while $\alpha_V\beta_3$-binding is achieved through "side-on" binding.



For $\alpha_V\beta_3$ binding the guanidino N-terminus is clearly preferred over the aminopyrimidino terminus (compound **12**). The guanidino-type 2-aminobenzimidazole (compound **16**) is more than 100-fold more active than the corresponding (non-guanidino) aminomethylbenzimidazole derivative **23**. Table 3 shows the influence of the lipophilic side chain on $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity, wherein the carbamate containing antagonist **9** exhibits

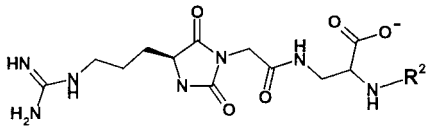
Table 2. Influence of arginine mimetic on $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity

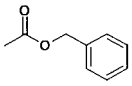
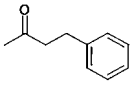
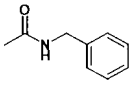
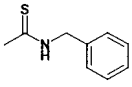
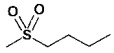
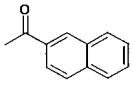


	R^1	Fg/$\alpha_{IIb}\beta_3$ IC_{50} (μM)	K/$\alpha_V\beta_3$ IC_{50} (μM)
9		2.4	0.08
11		>10	0.02
12		>10	1.66
16		>10	0.04
23		5	15
24		>10	0.58

by far the highest activity and selectivity for $\alpha_V\beta_3$. The thiourea containing compound **27** has the lowest affinity both for $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$, while all other side chains (**25**, **26**, **28**, **29**) give almost comparable results. This work demonstrates the versatility of the hydantoin scaffold and the structural requirements in the design of selective $\alpha_V\beta_3$ antagonists in terms of the arginine mimetic, the

Table 3. Influence of side chain on $\alpha_{IIb}\beta_3/\alpha_V\beta_3$ selectivity



	R^2	$Fg/\alpha_{IIb}\beta_3$ IC_{50} (μM)	$K/\alpha_V\beta_3$ IC_{50} (μM)
9		2.4	0.08
25		0.55	0.35
26		0.18	0.57
27		10	3.0
28		0.35	0.76
29		0.20	0.20

distance between N- and C-terminus and the lipophilic side chain.

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