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Highly Potent and Selective $\alpha_V\beta_3$-Receptor Antagonists: Solid-Phase Synthesis and SAR of 1-Substituted 4-Amino-1H-pyrimidin-2-ones

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Abstract—Solid-phase synthesis and SAR of $\alpha_V\beta_3$-receptor antagonists based on a N^1-substituted 4-amino-1H-pyrimidin-2-one scaffold are described. The most potent compounds exhibited IC_{50} values towards $\alpha_V\beta_3$ in the nano- to subnanomolar range and high selectivity versus related integrins like $\alpha_{IIb}\beta_3$. For selected examples efficacy in functional cellular assays was demonstrated.

Integrins, a superfamily of heterodimeric transmembrane glycoprotein receptors, are involved in processes such as cell–cell and cell–matrix adhesion, cell migration, and signaling. 17α- and 9β-subunits and more than 20 combinations thereof with different affinity and specificity to adhesive matrix proteins such as fibrinogen, fibronectin, vitronectin, osteopontin and laminin are known at present.[1] Integrins have received much attention in drug discovery research during the last few years,[2] in particular the β_3-subfamily with the fibrinogen receptor $\alpha_{IIb}\beta_3$[3] mediating platelet aggregation, and the vitronectin receptor $\alpha_V\beta_3$.

$\alpha_V\beta_3$ is specifically expressed in activated proliferating and migrating endothelial or smooth muscle cells, macrophages, and tumor cells.[4] It is involved in processes like restenosis after percutaneous transluminal coronary angioplasty (PTCA),[5] angiogenesis and neo-vascularization,[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 attractive therapeutic target.[12]

The tripeptide sequence RGD is a common feature of many integrin ligands (Fig. 1).[13] Studies on cyclic peptides containing this sequence demonstrated that selective ligands for $\alpha_V\beta_3$ could be obtained if arginine and aspartate β-carbon atoms were separated by a distance of 650–700 pm.[14] Recently the crystal structure of the extracellular fragment of $\alpha_V\beta_3$ in complex with a cyclic RGD peptide has been solved.[15]

Small molecule $\alpha_V\beta_3$-receptor antagonists described in the literature follow this pattern and mimic the

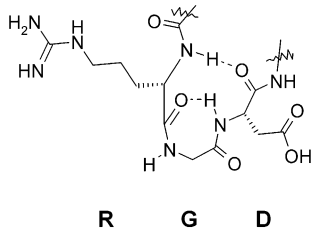

Figure 1. RGD-motif of integrin ligands.

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RGD-motif of the natural ligands by displaying an acidic and a basic moiety in a certain distance.[16] The aspartate is often replaced by a β-amino acid residue.[17]

Combinatorial Solid-Phase Synthesis

Substituted heterocycles have been used before by us[18] and others[19] as scaffolds in the synthesis of $\alpha_V\beta_3$ antagonists to bring the carboxylic acid and the guanidine mimetic in the appropriate positions. We here report the use of 1-substituted 4-amino-1H-pyrimidin-2-ones as suitable core structures.

A combinatorial solid-phase synthesis was devised which permitted us to generate these heterocycles very efficiently from support bound amino acids, enamine derivatives and amines.

Scheme 1 shows the solid-phase synthesis of the 4-aminopyrimidinone based $\alpha_V\beta_3$-receptor antagonists. 2-chlorotrityl resin was reacted with α-*Z*-β-Fmoc-diaminopropionic (Dap) or β-*Z*-γ-Fmoc-diaminobutyric acid (Dab)[20] under standard conditions.[21] After removal of the Fmoc-group 4-thiouracil **3** was formed by reaction with the appropriate enamine derivative **2**.[22] Compound **2** is readily accessible from the corresponding enamine and *N*-ethoxycarbonyl isothiocyanate.[22] To synthesize the desired aminopyrimidinone nucleus on solid phase, we modified a solution-phase synthesis protocol by Nikiforov and Connolly.[23] Generation of the thiocyanate leaving group was thus effected by treatment with 5 equiv cyanogen bromide in DMF in the presence of DIEA. Substitution with the appropriate amine building block and cleavage from the support yielded 4-aminopyrimidinones of the general structure **6**.

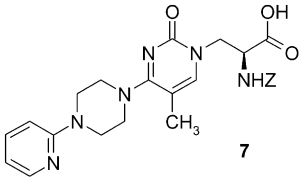
This solid-phase synthesis permits independent variation of three sites of diversity. The products were obtained in high purity (generally >90% by RP HPLC-MS, UV_{214nm}) and in yields which ranged from 30 to >90%.

Biological Results and Discussion

This paper reports the structure–activity relationship of α-*Z*-2,3-diaminopropionic and β-*Z*-3,4-diaminobutyric acid derivatives. Initial studies with commercially available amine building blocks provided a proof of concept. Compound **7**, for example, had an activity of 1 μM (IC_{50}) in an ELISA assay using human vitronectin as natural ligand[24] (Fig. 2). This encouraged us to further investigate the aminopyrimidinone scaffold and employ especially designed guanidine mimetics as building blocks.[23]

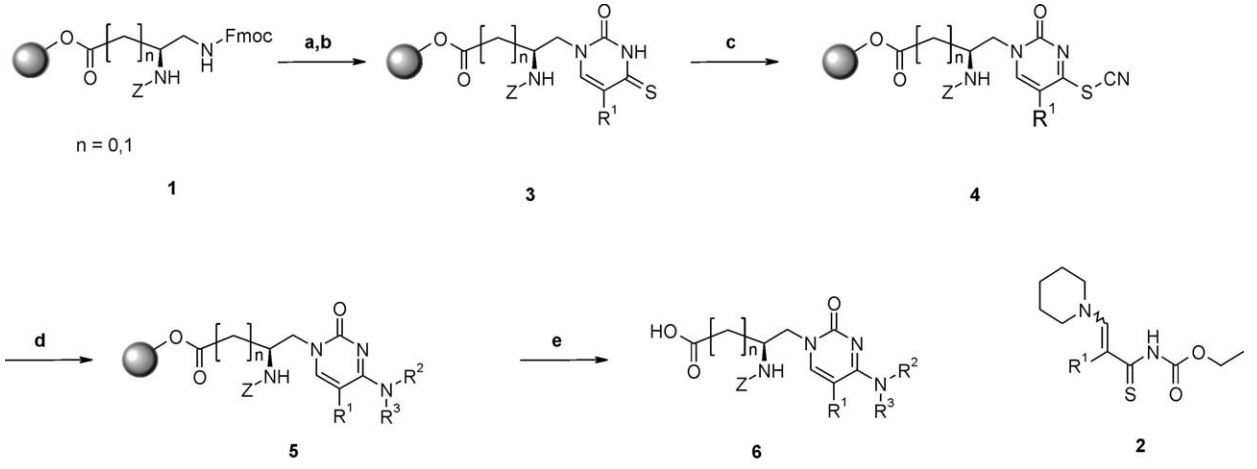
In a first set of second generation analogues, the guanidine mimics 2-aminopyridine and 2-aminobenzimidazole were connected to the scaffold with piperidinyl or piperidinylalkyl spacers.

Table 1 summarizes the results obtained in the ELISA assay. A number of compounds exhibited affinities in the low nanomolar range. Frequently a distinct activity difference between the corresponding Dap ($n=0$) and Dab derivatives ($n=1$) was observed. In general the aminopyridines were slightly less potent than the corresponding aminobenzimidazole derivatives. Possible explanations are the higher basicity of the latter and/or additional beneficial hydrophobic interactions with the target. The most potent derivatives in this series were **9a**, **9b** and **11a**, where the aminobenzimidazole moiety is connected to the piperidine ring either directly or via one methylene group. Interestingly, the Dap and Dab analogues (**9a** and **9b**, respectively) were equipotent. Among the aminopyridines **10a** had the highest activity (IC_{50}: 2.4 nM), while the corresponding pyrimidine derivatives **13a** and **13b** were virtually inactive. Replacement



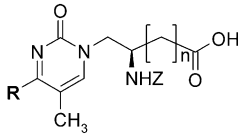
7

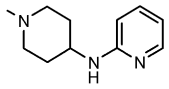
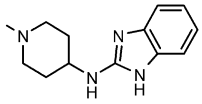
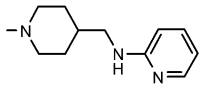
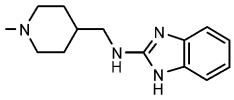
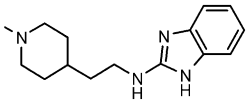
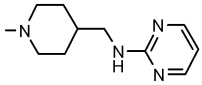
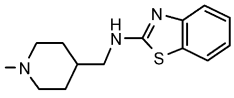
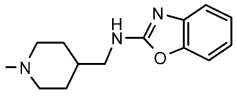
Figure 2. Initial lead compound **7**.



Scheme 1. (a) Piperidine, DMF; (b) 4 equiv **2**, DMF; (c) 5 equiv BrCN, DIEA, DMF; (d) 4 equiv HNR^2R^3, DMF; (e) HOAc/TFE/CH_2Cl_2 (1:1:3). 2-chlorotrityl resin was used as solid support.

Table 1. Variation of spacer and guanidine mimetic



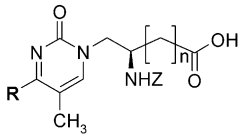
Compd	R	*n*	$\alpha_V\beta_3$ IC_{50}, nM[a]
8a		0 1	10 nd
9a **9b**		0 1	1.0 1.0
10a **10b**		0 1	2.4 36
11a **11b**		0 1	1.3 130
12a **12b**		0 1	15 10
13a **13b**		0 1	1000 5000
14a **14b**		0 1	5000 50,000
15a **15b**		0 1	5000 5000

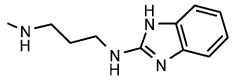
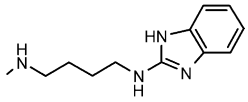
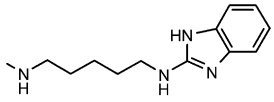
[a]Values are means of three experiments; intra-assay variation $<10\%$, inter-assay variation $<$factor 2.

of the benzimidazole moiety in **11a**, which was one of the most potent analogues in this set, and in **11b**, by the isosteric, but nonbasic benzothiazole or benzoxazole unit resulted in complete loss of activity (**14a**, **14b**, **15a**, **15b**). The distance between the acidic and the basic moieties was 12 or 13 bonds in all compounds of this series which had activities ≤ 10 nM.

A second set was prepared in which the aminobenzimidazole moieties were linked to the aminopyrimidinone scaffold by a linear alkyl spacer (Table 2). The most potent member of this series, the *Z*-Dab derivative **16b**, exhibited subnanomolar affinity while the corresponding *Z*-Dap analogue **16a** was more than two orders of magnitude less active. Extension of the C_3-chain in **16b** to C_4 and C_5 was accompanied by significantly reduced potency (**17a**, **17b** and **18a**, **18b**) although the number of bonds separating the two pharmacophores was identical in **16b** and **17a**. Again, the optimal distance between acid and basic moiety was 12 or 13 bonds.

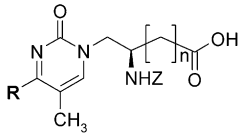
Table 2. Effect of spacer length on the activity of the 2-aminobenzimidazole derivatives

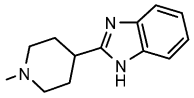
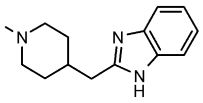
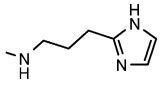


Compd	R	*n*	$\alpha_V\beta_3$ IC_{50}, nM[a]
16a **16b**		0 1	61.2 0.4
17a **17b**		0 1	46.6 17
18a **18b**		0 1	103 500

[a]Cf. Table 1.

Table 3. $\alpha_V\beta_3$ antagonist activity of the (benz)imidazole derivatives



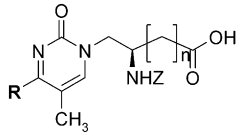
Compd	R	*n*	$\alpha_V\beta_3$ IC_{50}, nM[a]
19a **19b**		0 1	9.4 1000
20a		0 1	17.6 nd
21a **21b**		0 1	76 3.0

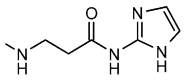
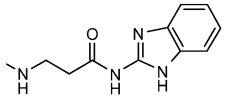
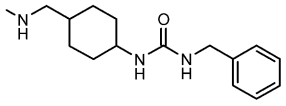
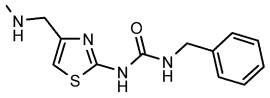
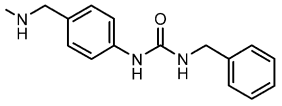
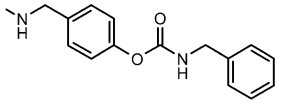
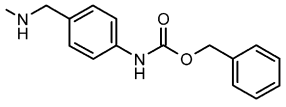
[a]Cf. Table 1.

Imidazole and benzimidazole derivatives are less basic and also possess fewer sites for hydrogen bonding than the corresponding 2-aminoheterocycles. In a series of analogues we found derivatives with high affinity (**19a**, **21b**; Table 3). Compound **20a** is isosteric to **9a**, but was significantly less active. The imidazoles **21a** and **21b** are linked to the aminopyrimidinone scaffold via a flexible linear spacer, and **21b** in particular showed remarkably high affinity.

In the series of *N*-acylated 2-aminoimidazole and 2-aminobenzimidazole derivatives which also are less basic guanidine mimetics, a remarkable difference in activity was observed (Table 4). While the Dab compounds **22b** and **23b** exhibited IC_{50} values in the low nanomolar range, the corresponding diaminopropionic acids **22a** and **23a** were inactive.

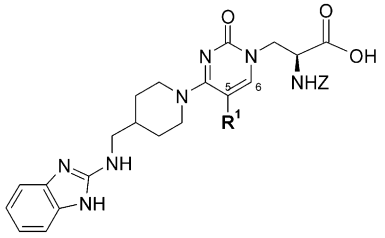
Table 4. Less basic and non basic guanidine mimetics



Compd	R	*n*	$\alpha_V\beta_3$ IC_{50}, nM[a]
22a		0	5000
22b		1	3.6
23a		0	5000
23b		1	19
24a		0	5000
24b		1	500
25a		0	4.2
25b		1	48
26a		0	82
26b		1	39
27a		0	10,000
		1	nd
28a		0	50,000
28b		1	50,000

[a]Cf. Table 1.

Table 5. Influence of the 5-substituent on the activity of the $\alpha_V\beta_3$ antagonists



Compd	R^1	$\alpha_V\beta_3$ IC_{50}, nM[a]
11a	Methyl	1.3
29	*n*-Propyl	1.5
30	Benzyl	1.5
31	2-Phenethyl	1.3
32		1000
33	CH_2OCH_3	11.6
34	Phenyl	15.5

[a]Cf. Table 1.

Table 6. Selectivity, permeation and cellular assay data of compounds **11a** and **16b**

	11a	**16b**
$\alpha_V\beta_3$/VN ELISA[a] IC_{50} (nM)	1.3	0.4
$\alpha_{IIb}\beta_3$ /FG ELISA[a] IC_{50} (nM)	2000	5000
$\alpha_V\beta_3$/OPN adhesion[b] IC_{50} (μM)	0.5	0.12
$\alpha_V\beta_5$/VN adhesion[b] IC_{50} (μM)	0.01	0.3
Caco-2 permeation assay P_{app}[c] (cm/s × 10^{-6})	4.2	0.64

VN, vitronectin; FG, fibrinogen; OPN, osteopontin.
[a]Cf. Table 1.
[b]Cell adhesion and migration: values are means of four experiments; intra-assay variation <20%, inter-assay variation <factor 2.
[c]P_{app} = apparent permeability coefficient;[27] $n = 2$, intra-assay variation <20%.

It has been reported that incorporation of non-basic moieties such as ureas can lead to very potent integrin ligands.[17,18b,25] A small series of urea analogues was synthesized (Table 4). Moderately active or virtually nonactive compounds were obtained with the exception of the thiazole derivative **25a** which had an IC_{50} value of 4.2 nM. The phenylurea derivatives **26a** and **26b** were moderately potent, removal of one hydrogen bond donor at a time by substituting a urethane moiety for the urea led to complete loss of activity (**27a**, **28a**, **28b**). This indicates that both NH groups are required for good affinity to the integrin.

In contrast to the other substituents, R^1 could be widely varied with only minor effects on potency. An example is shown in Table 5. Whereas the alkyl and arylalkyl analogues **11a** and **29**–**31** were equipotent, the methoxymethyl and phenyl derivatives **33** and **34** were only about one order of magnitude less active. An exception were bicyclic aminopyrimidinones like **32** which were virtually devoid of affinity to $\alpha_v\beta_3$. The little influence of R^1 on activity should allow optimization of pharmacokinetic parameters by finetuning R^1.

The aminopyrimidinone derivatives exhibited high selectivity versus $\alpha_{IIb}\beta_3$ (Table 6) as well as related integrins like $\alpha_5\beta_1$, and $\alpha_4\beta_1$ (data not shown). In early ADME studies **11a** and **16b** displayed medium to high permeation in the Caco-2 model[26] and good metabolic stability versus human liver microsomes. Both compounds showed medium to high efficacy in functional cellular assays, which was in good correlation with their in vitro affinities (Table 6).

In conclusion, we have developed an efficient solid-phase synthesis of 1-substituted 4-amino-1H-pyrimidin-2-ones. This scaffold was used in the synthesis of a new class of potent and selective integrin $\alpha_V\beta_3$ antagonists. Selected compounds showed promising efficacy in functional assays and early ADME properties.

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