Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2011–2015

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

$\alpha_v\beta_3$ Antagonists Based on a Central Thiophene Scaffold

Anusch Peyman,[a,*] Karl-Heinz Scheunemann,[a] David W. Will,[a] Jochen Knolle,[a] Volkmar Wehner,[a] Gerhard Breipohl,[a] Hans Ulrich Stilz,[a] Denis Carniato,[b] Jean-Marie Ruxer,[b] Jean-Francois Gourvest,[b] Marielle Auberval,[b] Bernard Doucet,[b] Roland Baron,[b] Martine Gaillard,[b] Thomas R. Gadek[c] and Sarah Bodary[c]

[a]*Aventis Pharma Deutschland GmbH, D-65926 Frankfurt, Germany*
[b]*Aventis Pharma S. A., 102 Route de Noisy, 93235 Romainville Cédex, France*
[c]*Genentech, 1 DNA Way, South San Francisco, CA 94080, USA*

Received 21 March 2001; revised 3 May 2001; accepted 17 May 2001

Abstract—A series of novel, highly potent $\alpha_v\beta_3$ antagonists based on a thiophene scaffold and containing an acylguanidine as an Arg-mimetic is described. A number of structural features, such as cyclic versus open guanidine and a variety of lipophilic side chains, carbamates, sulfonamides and β-amino acids were explored with respect to inhibition of $\alpha_v\beta_3$ mediated cell adhesion and selectivity versus $\alpha_{IIb}\beta_3$ binding. In addition, compound **19** was found to be active in the TPTX model of osteoporosis. © 2001 Published by Elsevier Science Ltd.

The vitronectin receptor, also known as $\alpha_v\beta_3$, is a member of the integrin family of cell surface receptors which play a major role in cell adhesion and signal transduction processes.[1] Integrins are α/β heterodimers composed of at least 15 α subunits and eight β subunits. The β_3 class of the integrin family, $\alpha_{IIb}\beta_3$ (GPIIb/IIIa or fibrinogen receptor) and $\alpha_v\beta_3$ (vitronectin receptor), has received special attention in recent drug discovery efforts.[2] $\alpha_{IIb}\beta_3$ is prevalent on platelets and plays a role in thromboembolic disorders while $\alpha_v\beta_3$ has been implicated in tumor progression, angiogenesis and restenosis.[2] In addition, $\alpha_v\beta_3$ is the dominant receptor for mediating the attachment of osteoclasts to bone during bone resorption[3] which makes it an attractive target for the treatment of osteoporosis. $\alpha_v\beta_3$ binds to extracellular matrix adhesive proteins containing an RGD sequence such as vitronectin, fibrinogen, bone sialo protein II, von Willebrand factor, and osteopontin. Therefore, we[4,5] and others[3,6] have investigated peptidomimetic approaches to identify $\alpha_v\beta_3$ antagonists. In this communication we describe a new series of highly potent and selective RGD peptidomimetic $\alpha_v\beta_3$ antagonists which are based on a central thiophene scaffold and which contain acylguanidines as an Arg-replacement.

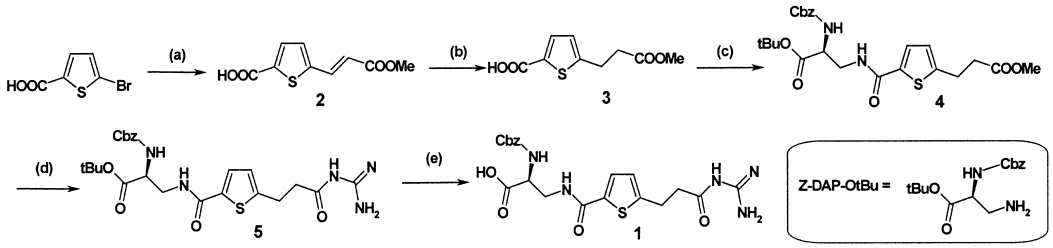
*Corresponding author. Fax: +49-69-305-80261; e-mail: anusch.peyman@aventis.com

Chemistry

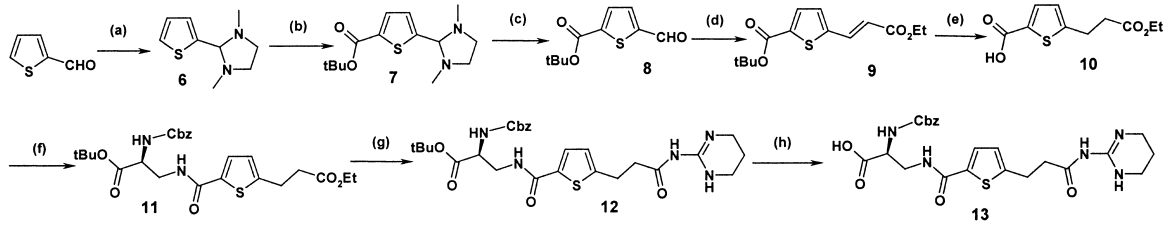
The synthesis of the $\alpha_v\beta_3$ antagonist **1** is outlined in Scheme 1. 5-Bromothiophene-2-carboxylic acid is reacted with acrylic acid methyl ester catalyzed by $PdCl_2(PPh_2Ferrocene)_2/NEt_3$ in acetonitrile to give compound **2** (82%) which is subsequently reduced by catalytic hydrogenation to give 5-(2-methoxycarbonylethyl)-thiophene-2-carboxylic acid **3**. Compound **3** is coupled with (*S*)-Cbz-diaminopropionic acid *tert*-butylester (Z-DAP-O*t*Bu) using TOTU in DMF to give **4** in 95% yield. The acylguanidino compound **5** is formed by reaction of the ester with guanidine in THF (35%), which after deprotection with TFA yields antagonist **1**.

Scheme 2 shows an alternative synthesis of the central building block: 2-thiophencarbaldehyde is reacted with *N*,*N*′-dimethylethylenediamine to give 1,3-dimethyl-2-(2-thienyl)-imidazolidine **6** which is lithiated and reacted with Boc-anhydride to give compound **7** in 71% yield. Compound **7** is converted to 5-formyl-thiophene-2-carboxylic acid *tert*-butyl ester **8** by treatment with methyl iodide and subsequently condensed with ethyl (diethyl phosphono)acetate to give compound **9** (53%). Catalytic hydrogenation of **9** followed by cleavage of the *tert*-butylester yields **10** which is again condensed with Z-DAP-O*t*Bu to **11**. The cyclic acylguanidino group in **12** is formed by reaction of **11** with 2-amino-3,4,5,6-tetra-

0960-894X/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0960-894X(01)00357-2



Scheme 1. Reagents and conditions: (a) $PdCl_2(PPh_2ferrocene)_2$, acrylic acid methyl ester, NEt_3, MeCN/85 °C, 20 h; (b) H_2, Pd/C, MeOH/rt, 7 h; (c) Z-DAP-O*t*Bu, TOTU, DIEA, DMF/rt, 6 h; (d) guanidine, THF/reflux, 2.5 h; (e) TFA, DCM/rt, 3.5 h.



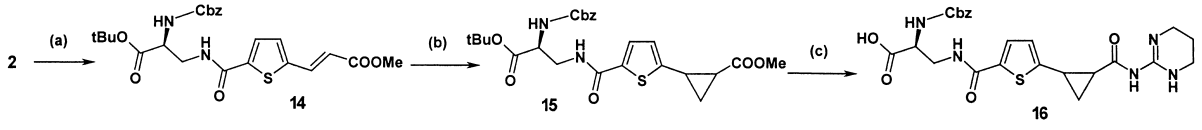
Scheme 2. Reagents and conditions: (a) $MeNH(CH_2)_2NHMe$, toluene/reflux(–H_2O), 4 h; (b) *n*BuLi, TEMED, Boc_2O, THF/−70 °C for 4 h, rt for 16 h; (c) MeI, Et_2O/rt, 16 h; (d) $P(O)(EtO)_2CH_2COOEt$, NaH, THF/60 °C, 3 h; (e) (i) H_2 (4 bar), Pd/C, EtOH/50 °C, 7 h; (ii) TFA, DCM/rt, 5 h; (f) Z-DAP-O*t*Bu, TOTU, DIEA, DMF/rt, 8 h; (g) 2-amino-3,4,5,6-tetrahydropyrimidine, DMF/rt, 8 h; (h) TFA, DCM/rt, 4 h.

hydropyrimidine in DMF (45%) and final cleavage of the *tert*-butylester with TFA yields $\alpha_v\beta_3$ antagonist **13**.

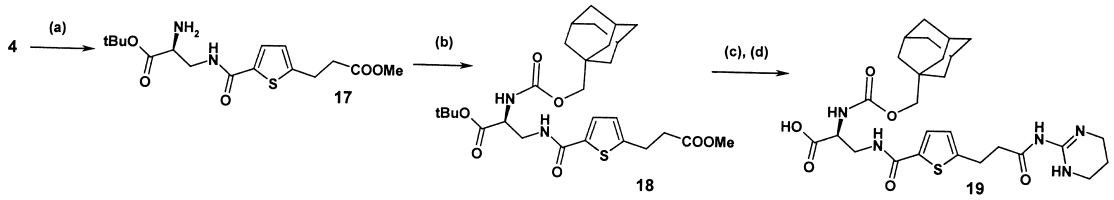
Scheme 3 illustrates the synthesis of compounds containing a cyclopropyl group as spacer between the central thiophene scaffold and the acylguanidino group. Compound **2** is coupled with Z-DAP-O*t*Bu to give **14**. The cyclopropyl group in **15** is formed by reaction with diazomethane under $Pd(OAc)_2$ catalysis and $\alpha_v\beta_3$ antagonist **16** is obtained as in Scheme 2 by reaction of **15** with 2-amino-3,4,5,6-tetrahydropyrimidine in DMF and TFA cleavage of the *tert*-butylester. Other compounds containing the cyclopropyl spacer were obtained in an analogous manner. The preparation of compounds containing the adamantylmethyl carbamate side chain is outlined in Scheme 4. The benzyloxycarbamate group in compound **4** (Scheme 1) is removed by catalytic hydrogenation to give a free amino group in **17** which is reacted with *N*-(1-adamantylmethoxycarbonyloxy)-succinimide to give **18** (100%) which, after conversion to the acylguanidine (71%) and deprotection, gives the $\alpha_v\beta_3$ antagonist **19**. Alternatively, compounds containing this side chain can be obtained by the use of 2-(1-adamantylmethyloxycarbonylamino)-3-aminopropionic acid instead of Z-DAP-O*t*Bu. Antagonists containing a sulfonamide side chain are obtained from the same intermediate **17** by reaction with the corresponding sulfonylchlorides, followed by conversion to the acylguanidine and deprotection. Antagonists containing a β-amino acid component are obtained from intermediate **3** by condensation with the corresponding β-amino acid, followed again by conversion to the acylguanidine and deprotection.

Results and Discussion

One of the most interesting challenges in this area is the design of selective antagonists since both $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ bind to the same RGD recognition motif, and



Scheme 3. Reagents and conditions: (a) Z-DAP-O*t*Bu, TOTU, DIEA, DMF/rt, 8 h; (b) Et_2O/THF, 1:1, CH_2N_2, $Pd(OAc)_2$ 0 °C, 2 h; (c) (i) 2-amino-3,4,5,6-tetrahydropyrimidine, DMF/rt, 6 h; (ii) TFA, DCM/rt, 4 h.



Scheme 4. Reagents and conditions: (a) H_2, Pd/C, 5 N HCl in MeOH/rt, 14 h; (b) *N*-(1-adamantylmethoxycarbonyloxy)-succinimide, $NaHCO_3$, H_2O, THF/rt, 4 h; (c) 2-amino-3,4,5,6-tetrahydropyrimidine, DMF/rt, 5 h; (d) TFA, DCM/rt, 3 h.

subtle differences in the ligand structure may determine selectivity. The IC_{50} values for the inhibition of binding of fibrinogen to $\alpha_{IIb}\beta_3$ and of Kistrin[7] (a disintegrin with high affinity to $\alpha_v\beta_3$) to $\alpha_v\beta_3$ are summarized in Tables 1–3. In addition, a more stringent whole-cell assay was used to differentiate between $\alpha_v\beta_3$ antagonists: human 293 cells were engineered to overexpress the $\alpha_v\beta_3$ receptor ($>10^6$ copies/cell) and were used to assess the potency of $\alpha_v\beta_3$ antagonists by the inhibition of their binding to immobilized vitronectin.

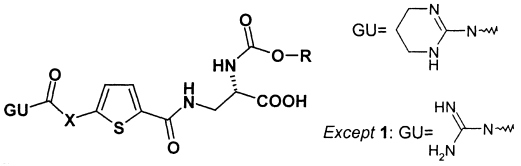
Guanidine mimetic

The acylguanidines used in this study produce $\alpha_v\beta_3$ antagonists with excellent potency. The comparison of **1** and **13** (Table 1) shows that cyclic acylguanidines lead to a further enhancement of potency, which becomes even more evident in the 293-cell attachment assay. At the same time the selectivity towards the fibrinogen receptor $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) is increased. The same ranking is observed for the β-amino acid containing antagonists **25** and **26** (Table 3) and a similar SAR has been observed earlier for normal guanidines.[4,5]

Spacer

The spacer separating the central thiophene scaffold and the acylguanidine moiety is of great importance. Clearly the vinyl spacer is less favorable as can be seen from a comparison of **19**, **20**, and **21**. Obviously it forces the acylguanidino group into an unfavorable position, since the conjugation ranges from the thiophene ring through the double bond through the carbonyl group and even the guanidino group, which all end up in a single plane. On the other hand, the ethylene spacer and the cyclopropyl group result in similar potencies as can be seen

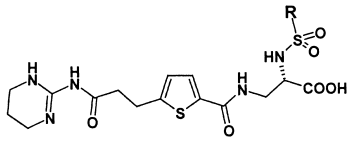
Table 1. Thiophene-based $\alpha_v\beta_3$ antagonists with lipophilic carbamate side chains

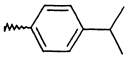
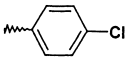
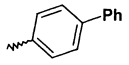
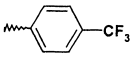
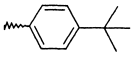
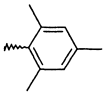


	R	X	K/$\alpha_v\beta_3$ IC_{50} (nM)	Vn/293 IC_{50} (nM)	Fg/$\alpha_{IIb}\beta_3$ IC_{50} (nM)
1			39	837	2900
13			7	63	4500
16			60	41	2450
19			2	26	480
20			1100	>10,000	1000
21			10	69	2800

The IC_{50} values denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ and Kistrin to $\alpha_v\beta_3$ by 50% (K/$\alpha_v\beta_3$) or 293 cell attachment to vitronectin by 50% (Vn/293)

Table 2. Thiophene-based $\alpha_v\beta_3$ antagonists with sulfonamide side chains



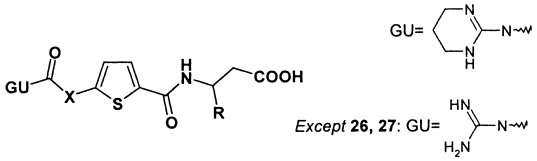
	R	K/$\alpha_v\beta_3$ IC_{50} (nM)	Vn/293 IC_{50} (nM)	Fg/$\alpha_{IIb}\beta_3$ IC_{50} (nM)
33		3	47	235
34		2	13	80
35		2	6	17
36		2	12	55
37		3	26	9
38		2	7	35
39		3	12	65
40		2	10	11
41		4	90	925
42		5	313	500
43		2	80	40
44		2	91	17
45		4	167	255
46		5	69	1350
47		4	22	105

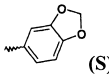
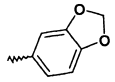
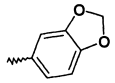
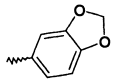
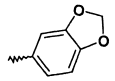
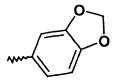
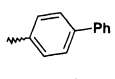
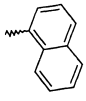
from various examples **19–21** or **13–16**. Considering that the cyclopropyl-containing antagonists consist of diastereomeric mixtures, one may speculate that one of the pure forms could show some advantages over the open chain ethylene spacer.

Lipophilic side chain

The influence of the lipophilic side chain was investigated in three different series: the carbamate side chains (Table 1); the sulfonamide side chains (Table 2); and the β-amino acids (Table 3). Of those series, the β-amino acid containing $\alpha_v\beta_3$ antagonists are less active in the 293-cell attachment assay, despite good activity in the standard binding assay. We have also observed this difference in other series when β-amino acids were incorporated in the 'C-terminus' of RGD peptidomimetics. On the other hand, this series shows excellent selectivities versus $\alpha_{IIb}\beta_3$. Bulky aromatic side chains are preferred; see, for example, the increase in potency from **22** to **25**. However, *ortho* substitution of the aromatic side chain, as in **32** containing the 1-naphthyl group, does not seem to enhance potency. The SAR for the spacer and the guanidino group are the same as in the other series. Two different side chains, benzyl and 1-adamantylmethyl, were incorporated in the carbamate series. The bulky adamantyl moiety offers a slight advantage over the flat benzyl group, enhancing potency by a factor of 2 (cf. **13** and **19**) when used in combination with the ethylene spacer. The opposite is the case when the cyclopropyl spacer is used. The selectivity versus $\alpha_{IIb}\beta_3$ is also excellent in this series and ranges from 40 to 650, with the exception of compound **20** which contains the vinyl-spacer. An interesting SAR is observed in the sulfonamide side chain series (Table 2). No major differences in potency are observed in the $K/\alpha_v\beta_3$ binding assay, however, when the 293-cell attachment assay is used, the SAR becomes more evident. Clearly, aromatic side chains are preferred over alkyl chains. IC_{50} values for antagonists containing alkyl side chains are in the 100-nM range (**41–46**) with the exception of the *n*-propyl group, which has an IC_{50} value of 22 nM. Antagonists with aromatic sulfonamide side chains (**34–39**) are extremely potent with IC_{50} values in the low nanomolar range. However, the affinity for $\alpha_{IIb}\beta_3$ is also increased. Compound **37**, which contains the biphenyl sulfonamide side chain, shows an IC_{50} of 3 nM for $K/\alpha_v\beta_3$ binding and 9 nM for $Fg/\alpha_{IIb}\beta_3$ binding and is a representative for a dual inhibitor.

Table 3. Thiophene-based $\alpha_v\beta_3$ antagonists with β-amino acid side chains. All compounds are 1:1 racemic mixtures with the exception of **24**, which is the pure *S*-enantiomer



	R	X	$K/\alpha_v\beta_3$ IC_{50}(nM)	Vn/293 IC_{50}(nM)	$Fg/\alpha_{IIb}\beta_3$ IC_{50}(nM)
22			175	—	> 10000
23			35	3610	> 10000
24	 (S)		9	1430	> 10000
25			10	1730	> 10000
26			33	> 10000	10000
27			32	> 5000	> 10000
28			8	41000	> 10000
29			16	> 5000	> 10000
30			120	15000	> 10000
31			69	10500	> 10000
32			175	20900	> 100000

$\alpha_v\beta_3$ antagonist **19** was chosen to evaluate the potency of this series in bone resorption in the in vivo thyroidectomized-parathyroidectomized (TPTX) rat model, which measures the ability of a compound to inhibit the parathyroid hormone stimulated calcemic response in hypocalcemic TPTX rats.[8] In this model, upon subcutaneous administration, **19** showed a dose dependent effect, the inhibition was −6% (0.1 mg/kg×2), −26% (0.3 mg/kg×2) and −56% (1.0 mg/kg×2).

In conclusion, we have discovered a new class of potent and selective $\alpha_v\beta_3$ antagonists based on a central thiophene scaffold and the acylguanidine Arg-mimetic. The compounds are capable of inhibiting bone resorption in vivo and therefore are promising drug substances for the treatment of osteoporosis.

References and Notes

1. Hynes, R. O. *Cell* **1992**, *69*, 11.
2. Miller, W. H.; Keenan, R. M.; Willette, R. N.; Lark, M. W. *Drug Disc. Today* **2000**, *5*, 397.
3. Hartman, G. D.; Duggan, M. E. *Expert Opin. Invest. Drugs* **2000**, *9*, 1281.
4. Peyman, A.; Wehner, V.; Knolle, J.; Stilz, H. U.; Breipohl, G.; Scheunemann, K. H.; Carniato, D.; Ruxer, J. M.; Gourvest,

J. F.; Gadek, T. R.; Bodary, S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 179.
5. Peyman, A.; Gourvest, J.-F.; Gadek, T. R.; Knolle, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2874.
6. Excellently reviewed by: Duggan, M. E.; Hutchinson, J. H. *Expert Opin. Ther. Pat.* **2000**, *10*, 1367.
7. Green, L. J.; Brierley, V. H.; Humphries, M. *J. Biochem. Soc. Trans.* **1995**, *23*, 505 S.
8. Fisher, J. E.; Caulfield, M. P.; Sato, M.; Quartuccio, H. A.; Gould, R. J.; Garsky, V. M.; Rodan, G. A.; Rosenblatt, M. *Endocrinology* **1993**, *132*, 1411.