Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 657–661

Solid-phase synthesis of a small library of 3-phenylthio-3-nicotinyl propionic acid derivatives acting as antagonists of the integrin αVβ3

Paola Vianello,[a,*] Paolo Cozzi,[a] Arturo Galvani,[c] Maurizio Meroni,[a] Mario Varasi,[a] Daniele Volpi[b] and Tiziano Bandiera[a]

[a]*Department of Chemistry, Pharmacia Italia S.p.A–Gruppo Pfizer, Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano (MI), Italy*
[b]*Department of Biology, Pharmacia Italia S.p.A–Gruppo Pfizer, Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano (MI), Italy*
[c]*Department of Pharmacology, Pharmacia Italia S.p.A–Gruppo Pfizer, Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano (MI), Italy*

Received 29 August 2003; revised 23 October 2003; accepted 18 November 2003

Abstract—We describe the synthesis of a series of low molecular weight inhibitors of the αvβ3 integrin obtained by modifying a high-throughput screening hit with micromolar activity. A solid phase synthesis to prepare 3-phenylthio-3-nicotinyl propionic acid derivatives, exemplified by **13c**, was set up. Compounds with nanomolar activity in the biochemical assay and able to efficiently inhibit cell adhesion mediated by vitronectin have been obtained.

1. Introduction

Integrins are a family of heterodimeric transmembrane glycoproteins involved in cell–cell and cell–extracellular matrix (ECM) interactions.[1] Integrin-mediated adhesion induces a variety of signal transduction events that influence many aspects of cell behavior including survival, proliferation, differentiation, gene expression, and migration.[2] Integrins are composed of two non-covalently associated subunits, α and β, and each αβ combination may recognize a single or various ECM proteins. Integrin $\alpha_v\beta_3$ is the most promiscuous member of the family since it recognizes many extracellular matrix proteins, including vitronectin, fibrinogen and fibronectin, through the tripeptide RGD (Arg-Gly-Asp) sequence. Despite its promiscuous behavior, integrin αvβ3 is not expressed in many normal tissues but is significantly upregulated on endothelial cells of angiogenic blood vessels.[3] In preclinical studies, disruption of $\alpha_v\beta_3$ ligation with a specific anti-$\alpha_v\beta_3$ monoclonal antibody (LM609) or the cyclic peptide c[RGDfV] was shown to inhibit angiogenesis in the chick chorioallantoic membrane (CAM) and rabbit cornea assays when variously elicited by bFGF, TNF-α or tumor cells, whereas antibodies against related integrins and a control peptide had no effect.[4] Similarly, a reduction of angiogenesis and tumor cell growth was reported following administration of LM609 to SCID mice transplanted with full thickness human skin containing $\alpha_v\beta_3$-negative human breast tumor cells.[5] Immunohistochemical studies on clinical specimens have shown that integrin $\alpha_v\beta_3$ is frequently expressed on the endothelium of tumor-associated blood vessels,[6] and expression level on these vessels is a negative prognostic factor in breast and colon cancer patients.[7,8]

In addition to its expression on vasculature, $\alpha v\beta_3$ integrin has also been found to be overexpressed on tumor cells themselves in various cancers, including melanoma,[9] breast,[10] and ovarian,[11] and a wide body of evidence suggests various roles for this integrin in promoting invasive and metastatic tumor growth independently of angiogenesis, including enhancing tissue arrest of blood-borne tumor cells,[12] cooperating with matrix metalloprotease activity in cell migration processes,[13] and mediating tumor cell tissue invasion.[14]

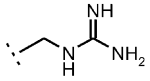
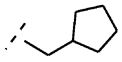
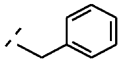
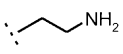
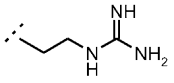
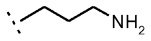
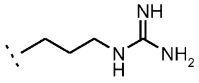
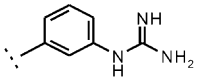
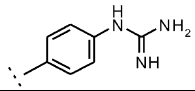
Keywords: Integrins; αvβ3; Cancer; Angiogenesis; Solid phase.
Abbreviations: DBU, 1,8-diazabicyclo(5.4.0)undec-7-ene; DIC, 1,3-diisopropylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid.
* Corresponding author. Tel.: +39-02483-83878; Fax: +39-02483-83833; e-mail: paola.vianello@pharmacia.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.045

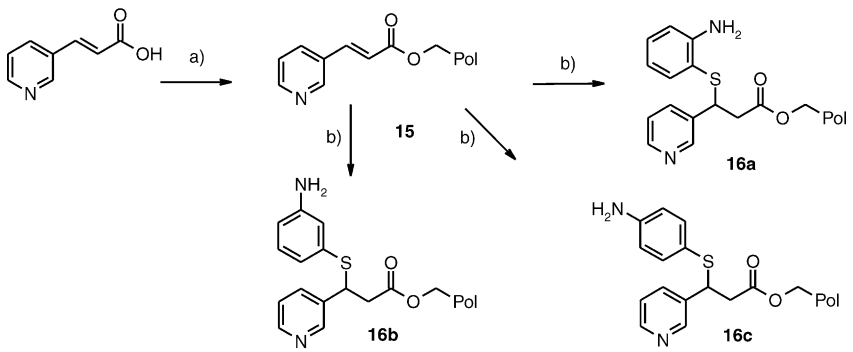
A critical role for integrin $\alpha_v\beta_3$ has also been demonstrated in the bone resorption process brought about by osteoclasts. Anti-$\alpha_v\beta_3$ antibodies, peptide and small molecule inhibitors of integrin $\alpha_v\beta_3$ all resulted in inhibition of osteoclast activity.[15] Therefore, antagonists of integrin $\alpha_v\beta_3$ may represent a viable therapeutic strategy for the treatment of diseases dependent on angiogenesis, including tumor growth and metastasis, and of osteoporosis.

In the search for small molecule inhibitors of integrin $\alpha_v\beta_3$, we screened our research compound collection and identified compound **1a** as an interesting hit with activity in the μM range. Compound **1a** contains a β-aryl propionic acid function, a motif present in many $\alpha_v\beta_3$ inhibitors,[16] and a β-aryl thioether group which replaces the commonly encountered β-glycyl-amino moiety. The aryl-thioether group simultaneously reduces the flexibility

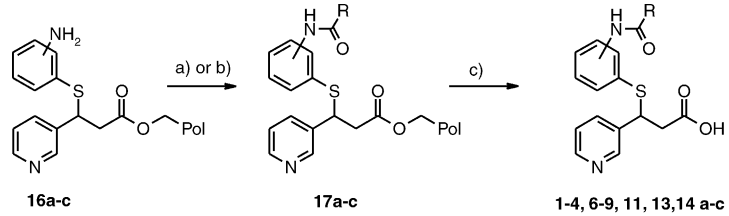
Table 1.

Compd	R	Method	a IC_{50} (μM) αvβ3 IC_{50} (μM) αIIbβ3	b IC_{50} (μM) αvβ3 IC_{50} (μM) αIIbβ3	c IC_{50} (μM) αvβ3 IC_{50} (μM) αIIbβ3
1		A	4 45	2.9 30	1.5 n.t.
2		A	25 n.t.	3.8 30	4 30
3		A	>30 n.t.	4.2 21	4.2 >30
4		A	>30 n.t.	2.6 19	[a]
5		C	10 >100	0.8 31	0.2 81
6		A	9.6 n.t.	5.8 30	5.8 n.t.
7		A	>30 n.t.	14.3 n.t.	15.2 n.t.
8		A	29.6 n.t.	11.2 n.t.	10.6 n.t.
9		A	[a]	5.9 n.t.	4.5 30
10		C	5 100	0.3 10.4	0.2 87
11		A	9.2 n.t.	1.8 8.9	2.9 24
12		C	[a]	0.051 2.4	0.12 >30
13		B	4 16	0.068 1.4	0.016 9.8
14		B	9 >30	0.015 0.02	1.8 34

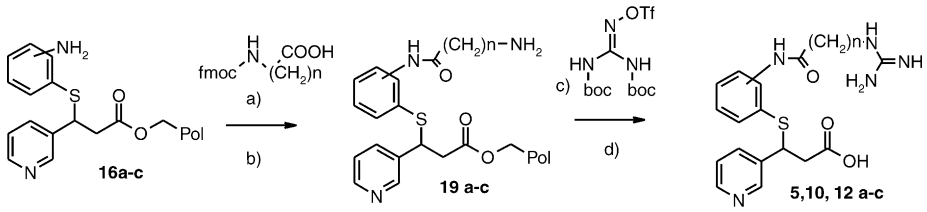
[a] Compound not obtained.



Scheme 1. (a) Wang resin, DIC, DMAP CH_2Cl_2, 20 h rt; (b) *o*-, *m*- or *p*-amiothiophenol, DBU cat. DMF, rt 48 h.



Scheme 2. Methods A and B: (a) RCOOH, DIC, CH_2Cl_2, rt, one week; (b) RCOOH, $SOCl_2$, Pyr or DMF, rt, one week; (c) TFA, CH_2Cl_2,1:1, rt, 1 h.



Scheme 3. Method C: (a) DIC, CH_2Cl_2, rt 10 days; (b) piperidine 4%, DMF, rt, 2×1 min, 1×5 min; (c) CH_2Cl_2; (d) TFA, CH_2Cl_2.

and increases the lipophilicity of compounds originating from the expansion of **1a** compared to analogues containing the β-glycyl-amino moiety. The hit isomers **1b** and **1c** retained activity on $\alpha_v\beta_3$ integrin, thus confirming the potential interest of this class. Therefore, a small library of derivatives was prepared to verify if potency against $\alpha_v\beta_3$ and selectivity versus the related integrin αIIbβ3 could be improved. Most of the selected substituents (R, Table 1) carry a basic residue, since the tripeptide recognition element (RGD) of proteins binding to $\alpha_v\beta_3$ integrin contains the basic arginine side chain. The selection was made to study the effect of the chain length on activity. In addition, since compound **1c** showed a ca. 1 μM IC_{50}, we decided to include in the design of the library a few substituents devoid of the basic center to verify whether additional hydrophobic interactions were beneficial for activity.

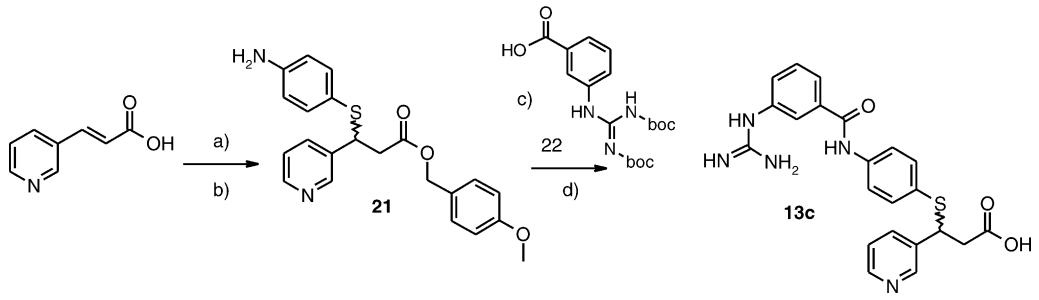
In this communication we describe the solid-phase synthesis and the pharmacological evaluation of this library. The synthesis of the most interesting compound via homogeneous phase will also be presented. Within this series, compounds with nanomolar activity in the biochemical assay and sub-micromolar EC_{50} in inhibiting cell–ECM interactions mediated by vitronectin have been obtained.

2. Chemistry

In the solid-phase approach, the commercially available *trans*-3-(3-pyridyl)acrylic acid was loaded onto the Wang resin by activation with DIC in methylene chloride and in the presence of DMAP to give **15** (Scheme 1). Michael addition on compound **15** with *o*-, *m*- and *p*-aminothiophenol with DBU as a catalyst in DMF at room temperature gave the three intermediates **16a–c**.

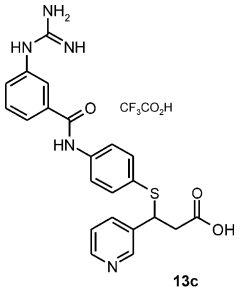
The amino derivatives **16a–c** thus obtained (0.166 mmol) were condensed with the appropriate acids (1.66 mmol), pre-activated with DIC (2.49 mmol, method A) or with thionyl chloride (3.2 mmol, method B), as illustrated in Scheme 2. After coupling, compounds were cleaved from the resin by treatment with trifluoroacetic acid in dichloromethane.

A different procedure (method C, Scheme 3) was used to obtain the derivatives bearing a guanidino-function on an aliphatic chain (see Table 1, rows 5, 10, 12). The synthesis was performed by condensing the FMOC-protected amino acid (1.66 mmol) pre-activated with DIC (2.49 mmol), with the aminothiophenols **16a–c** (0.166 mmol), deprotecting the amino function and generating the guanidino moiety by reaction with *N*,*N*′-



Scheme 4. (a) *p*-Methoxybenzylalcohol, DIC, DMAP, CH_2Cl_2, rt, overnight, 82%; (b) 4-aminothiophenol, DBU, DMF, rt, 8 h, 50%; (c) DIC, DMAP, CH_2Cl_2, rt, 24 h, 51%; (d) TFA, CH_2Cl_2, 3 h, rt, 90%.

Table 2.



Biochemical assays (IC_{50})
$\alpha_v\beta_3$: 0.016±0.009 μM
$\alpha_{IIb}\beta_3$: 9.8±4.6 μM
Cytotoxicity (ED_{50})
CEMD9: >100 μM
Cell Adhesion (EC_{50})
CEMD9-Vitronectin: 0.706±0.37 μM
CHO$\alpha_{IIb}\beta_3$-Fibrinogen: >100 μM
K562-Fibronectin ($\alpha_5\beta_1$): >100 μM
CEMD9-VCAM1 ($\alpha_4\beta_1$): >100 μM

di-Boc-*N*″-triflyl-guanidine.[17] In the deprotection of the amino group, a low concentration of piperidine and a short reaction time were mandatory to avoid the retro-Michael reaction.[18] Subsequent cleavage with trifluoroacetic acid in dichloromethane gave the desired compounds with 80–90% purity.

The most interesting compound **13c** was re-prepared in homogeneous phase as reported in Scheme 4. *trans*-3-(3-Pyridyl)acrylic acid was protected as *p*-methoxybenzyl ester (**20**), treated with 4-aminothiophenol to give **21**, which in turn was reacted with compound **22**, obtained by reaction of *m*-amino benzoic acid with *N*,*N*′-di-Boc-*N*″-triflyl-guanidine in presence of triethylamine, to yield **23**. Subsequent treatment with trifluoroacetic acid in dichloromethane led to **13c**.

3. Conclusions

An efficient method for the preparation of 3-phenylthio-3-nicotinyl propionic acids on solid phase has been developed. A preliminary exploration of this class of compounds as αvβ3 antagonists has been carried out preparing 39 derivatives (Table 1).

Binding studies[19] demonstrated that compounds bearing a guanidino residue have good affinity for the αvβ3 receptor, and *para*- and *meta*-aminothiophenol derivatives showed submicromolar IC_{50}s. Among those compounds, replacement of the aliphatic chain with a phenyl ring led to more potent derivatives. In the latter series of compounds the position of the guanidino group affects both the binding to αvβ3 and the selectivity with respect to the closely related αIIbβ3 integrin. As reported in Table 1, compounds **13b**, **13c** and **14b** all showed low nanomolar activity but only **13c** displayed good selectivity with respect to αIIbβ3, with an αIIbβ3/αvβ3 IC_{50} ratio of ca. 600. In cell adhesion assays,[20] compound **13c** confirmed as a specific inhibitor of the αvβ3-mediated adhesion to extracellular matrix proteins (Table 2). The compound did not show activity in adhesion assays dependent on αIIbβ3, α5β1 and α4β1 integrin, and did not inhibit in vitro growth of CEMD9 cells[21] at 72 h after continuous treatment.

In conclusion, a nanomolar αvβ3 inhibitor with a good selectivity versus the αIIbβ3 integrin in both the biochemical and cell-based assays, and devoid of cytotoxicity, has been obtained. This compound represented the starting point for further expansion of this class.

References and notes

1. Hynes, R. O. *Cell* **1992**, *69*, 11.
2. Giancotti, F. G.; Ruoslahti, E. *Science* **1999**, *285*, 1028.
3. Brooks, P. C.; Clark, R. A.; Cheresh, D. A. *Science* **1994**, *264*, 569.
4. Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157.
5. Brooks, P. C.; Strömblad, S.; Klemke, R.; Visscher, D.; Sarkar, F. H.; Cheresh, D. A. *J. Clin. Invest*. **1995**, *96*, 1815.
6. Max, R.; Gerritsen, R. R.; Nooijen, P. T.; Goodman, S. L.; Sutter, A.; Keilholz, U.; Ruiter, D. J.; De Waal, R. M. *Int. J. Cancer* **1997**, *71*, 320.
7. Gasparini, G.; Brooks, P. C.; Biganzoli, E.; Vermeulen, P. B.; Bonoldi, E.; Dirix, L. Y.; Ranieri, G.; Miceli, R.; Cheresh, D. A. *Clin. Cancer Res*. **1998**, *4*, 2625.
8. Vonlaufen, A.; Wiedle, G.; Borisch, B.; Birrer, S.; Luder, P.; Imhof, B. A. *Mod. Pathol*. **2001**, *14*, 1126.
9. Natali, P. G.; Hamby, C. V.; Felding-Habermann, B.; Liang, B.; Nicotra, M. R.; Di Filippo, F.; Giannarelli, D.; Temponi, M.; Ferrone, S. *Cancer Res*. **1997**, *57*, 1554.
10. Pignatelli, M.; Cardillo, M. R.; Hanby, A.; Stamp, G. W. *Hum. Pathol*. **1992**, *23*, 1159.
11. Liapis, H.; Adler, L. M.; Wick, M. R.; Rader, J. S. *Hum. Pathol*. **1997**, *28*, 443.
12. Pilch, J.; Habermann, R.; Felding-Habermann, B. *J. Biol. Chem*. **2002**, *277*, 21930.
13. Rolli, M.; Fransvea, E.; Pilch, J.; Saven, A.; Felding-Habermann, B. *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 9482.
14. Hsu, M. Y.; Shih, D. T.; Meier, F. E.; Van Belle, P.; Hsu, J. Y.; Elder, D. E.; Buck, C. A.; Herlyn, M. *Am. J. Pathol*. **1998**, *153*, 1435.

15. Hartman, G. D.; Duggan, M. E. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1281.
16. (a) See for instance: Coleman, P. J.; Askew, B. C.; Hutchinson, J. H.; Whitman, D. B.; Perkins, J. J.; Hartman, G. D.; Rodan, G. A.; Leu, C.; Prueksaritanont, T.; Fernandez-Metzler, C.; Merkle, K. M.; Lynch, R.; Lynch, J. J.; Rodan, S. B.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2463 and references cited therein. (b) Lange, U. E. W.; Backfisch, G.; Delzer, J.; Geneste, H.; Graef, C.; Hornberger, W.; Kling, A.; Lauterbach, A.; Subkowski, T.; Zechel, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1379. (c) Gabor, S. A. G.; Gibson, C.; Goodman, S. L.; Holzemann, G.; Wiesner, M.; Kessler, H. *J. Med. Chem.* **2001**, *44*, 1938. (d) Carron, C. P.; Meyer, D. M.; Pegg, J. A.; Engleman, V. W.; Nickols, M. A.; Settle, S. L.; Westlin, W. F.; Ruminski, P. G.; Nickols, G. A. *Cancer Res.* **1998**, *58*, 1930.
17. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804.
18. Chemical stability was measured on the final compounds, in the Tris buffer, pH 7.4, used for the biochemical assays.
19. (a) Binding assays were performed as described in the literature: Wong, A.; Hwang, S. M.; McDevitt, P.; McNulty, D.; Stadel, J. M.; Johanson, K. *Mol. Pharm.* **1996**, *50*, 529. (b) Brooks, P. C.; Stromblad, S.; Sanders, L. C.; von Schalscha, T. L.; Aimes, R. T.; Stetler-Stevenson, W. G.; Quigley, J. P.; Cheresh, D. A. *Cell* **1996**, *85*, 683. Briefly, the integrin was coated onto a 96-well plate (50 mg/well) and 5 nM biotinylated vitronectin was allowed to bind for 30 min at 37 °C, with or without increasing concentrations of the competing molecules. The plate was then rinsed and incubated 45 min with streptavidin conjugated to peroxidase. The amount of vitronectin bound was monitored after addition of Turbo-TMB as peroxidase substrate, and the yellow color produced was read by a Packard ELISA reader, at 450 nm wavelength. Data were analyzed using the computerized program 'GraphPad Prism'.
20. Cells in log phase of growth were used. Cells were washed counted, and diluted in adhesion medium (0.1% heat denatured BSA in HBSS + 1 mM $MnCl_2$). Then, 10 μL of adhesion medium containing inhibitors at suitable concentrations were added to test wells. 90 μL/well of cell suspension was plated and incubated for 1 h at 37 °C. Following adhesion, plates were gently washed and cells fixed with 50 μL TCA 50% (trichloroacetic acid). Evaluation of cell adhesion was performed by staining with sulforhodamine B.
21. CEMD9 is a recombinant sub-line, derived from an acute lymphoblastic leukemia (ATCC CCL-119), stably expressing αvβ3 integrin obtained by co-transfecting cDNAs encoding human αv and β3 subunits. Cells were dispensed in 96 wells and treatment was performed 2 h later. Evaluation of growing inhibition was done 72 h later.