Taylor & Francis
healthsciences

Discovery and Evaluation of Terephthalic Acid Derivatives as Potent α₄β₁ Integrin Antagonists

GERHARD MÜLLER[a,*], MARKUS ALBERS[a], GERHARD HEßLER[a,†], THOMAS E. LEHMANN[a], HIROMI OKIGAMI[b], MASAOMI TAJIMI[b], KEVIN BACON[b] and THOMAS RÖLLE[a,‡]

[a]*Bayer AG, Central Research, Building Q18, D-51368 Leverkusen, Germany;* [b]*Bayer Yakuhin Ltd., 6-5-1-3, Kunimi-dai, Kizu-cho, Soraku-gun, Kyoto 619-0216, Japan*

Terephthalic acid based derivatives containing β- and γ-amino acid residues were prepared as antagonists of the leukocyte cell adhesion process that is mediated through the interaction of the very late antigen 4 (VLA-4) and the vascular cell adhesion molecule 1 (VCAM-1). The compounds 2, 10–12, 14, and 16–17 inhibited the adhesion in a cell based assay in the low and sub micromolar range.

Keywords: α₄β₁ integrin; VCAM-1; VLA-4 antagonist; Terephthalic acid; Urea

INTRODUCTION

Adhesive interactions between the leukocytes and endothelial cells play a critical role in leukocyte trafficking to sites of inflammation.[1,2] These events are essential for normal host defense against pathogens and repair of tissue damage, but can also contribute to the pathology of a variety of inflammatory and autoimmune disorders.[3–5] Vascular cell adhesion molecules such as the α₄β₁ integrin are currently investigated as targets for novel therapeutic treatments of chronic inflammatory diseases such as atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD) and multiple sclerosis (MS).[6,7]

A number of steps in cell adhesion have been identified and characterized to date. Among them, inhibiting the interaction between vascular cell

adhesion molecule 1 (VCAM-1) and very late antigen 4 (VLA-4, α₄β₁ integrin) has attracted the attention of recent medicinal chemistry programs.[8] Recently, we have described a sub micromolar VLA-4 antagonist **1** (Scheme 1).[9] The latter contains a para-substituted amino benzoic acid headgroup decorated with (L)-leucine and a urea moiety. Herein, (L)-leucin may be replaced by a set of versatile α-amino acids. While α-amino acids are known for their tendencies to cause unwanted side reactions and to suffer from poor stability in a physiological environment, replacing the latter by β or γ-amino acids might significantly improve the pharmacokinetic profile.[10,11]

In this contribution, we describe a novel class of α₄β₁ integrin antagonists derived from terephthalic acid as exemplified by **2**. In a formal way, the latter can be envisioned as a retro amide analogue in the head group connecting functionality with a combined inversion and positional shift of the urea linkage when compared to the prototype series exemplified by **1**.

MATERIALS AND METHODS

Cell Adhesion Assay

All activity data were collected using a cell adhesion assay. Ramos cells (American Type Culture Collection, Clone CRL-1596) were cultured in RPMI 1640 medium (Nikken Bio Medical Laboratory, CM1101) supplemented with 10% fetal bovine serum

---

*Current address: N.V. Organon, Molenstraat 110, Lead Discovery Unit, RK4109, 5340 BH Oss, The Netherlands.
†Current address: Aventis Pharma Deutschland GmbH, Chemistry/Molecular Modeling, 65926 Frankfurt/Main, Germany.
‡Corresponding author. E-mail: thomas.roelle.tr@bayer-ag.de
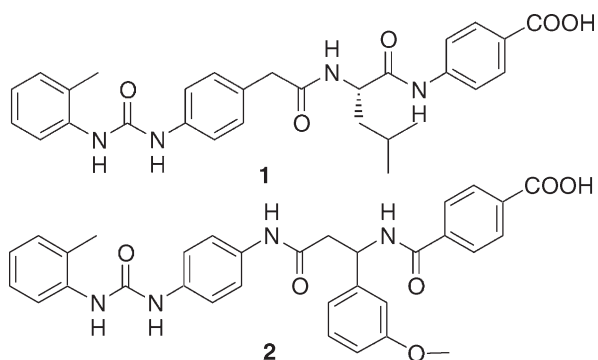
SCHEME 1   Structures of **1** and **2**.

(Hyclone, A-1119-L), 100 U/mL penicilin (Gibco BRL, 15140-122) and 100 µg/mL streptomycin (Gibco BRL, 15140-122) in a humidified incubator at 37°C with 5% CO_2. Ramos cells were incubated with phosphate balanced solution (PBS, Nissui, 05913) containing 25 µM of 5- (and -6)-carboxyfluorescein diacetate, succinimidyle ester (CFSE, Dojindo Laboratories, 345-06441) for 20 min at room temperature while gently swirling every 10 min. After centrifugation at 1000 rpm for 5 min, the cell pellet was resuspended with adhesion assay buffer at a cell density of 4×10^6 cells/mL. The adhesion assay buffer was composed of 24 mM Tris–HCl (pH 7.4), 137 mM NaCl, 27 mM KCl, 4 mM glucose, 0.1% bovine serum albumin (BSA, Sigma, A9647) and 2 mM $MnCl_2$.

Complementary DNA (cDNA) encoding the 7-domain form of VCAM-1 (GenBank accession #M60335) was obtained using Rapid-ScreenTM cDNA library panels (OriGene Technologies, Inc) at Takara Gene Analysis Center (Shiga, Japan). Recombinant human VCAM-1 (extracellular domains 1–3) was dissolved at 1.0 µg/mL in PBS. Each well of the microtiter plates (Nalge Nunc International, Fluoronunc Cert, 437958) was coated with 100 µL of substrate or for background control with buffer alone for 15 h at 4°C. After discarding the substrate solution, the wells were blocked using 150 µL per well of block solution (Kirkegaard Perry Laboratories, 50-61-01) for 90 min. The plate was washed with wash buffer containing 24 mM Tris–HCl (pH 7.4), 137 mM NaCl, 27 mM KCl and 2 mM $MnCl_2$ just before addition of the assay.

The assay solution containing each test compound or 5 µg/mL anti-CD49d monoclonal antibody (Immunotech, 0764) was transferred to the VCAM-1 coated plates. The final concentration of each test compound was 10 µM or various concentrations ranging from 0.0001 µM to 10 µM using a standard 5-point serial dilution. The assay solution containing the labeled Ramos cells was transferred to the VCAM-1 coated plates at a cell density of 2×10^5 cells per well and incubated for 45 min at 37°C.

The non-adherent cells were removed by washing the plates 3 times with wash buffer. The adherent cells were broken by addition of 1% Triton X-100 (Nacalai Tesque, 355-01). Released CFSC was quantified by fluorescence measurement in a fluorometer (Wallac, ARVO 1420 multilabel counter).

Preparation of the VLA-4 Antagonists 2, 10–12, 14, and 16–17

The para-substituted amino benzoic acid head-group in **1** previously had a major impact on establishing a convincing activity against $\alpha_4\beta_1$ integrin and, in this work, was replaced by a terephthalic acid moiety. This replacement represents an inversion of the head group linking amide, thus requiring a second inversion combined with a positional shift of the urea linkage in order to generate chemically stability and still retain isosteric analogues such as **2**. To introduce spatial flexibility, we decided to use the 1,4-diamino benzene based urea **8** and its homologue **9**.

The intermediates **6** and **7** were prepared using the corresponding β-amino ester **3** or **4** and terephthalic ^{t}butyl mono ester under standard peptide coupling conditions (EDCI and HOBT) followed by a selective LiOH induced ester hydrolysis.[12] The desired series of compounds **2** and **10–12** was obtained in a coupling reaction using EDCI and HOBT on **6** and **7** with the ureas **8** and **9** (prepared as described in Ref. [9]) using standard peptide coupling conditions with EDCI and HOBT followed by final ester cleavage using trifluoracetic acid (Scheme 2). In a similar fashion, **14** was prepared starting from **13** by peptide coupling with EDCI and HOBT with the amino ester **5** and subsequent selective saponification of the methyl ester with LiOH in aqueous THF. The intermediate was reacted with the urea **9** using EDCI and HOBT as coupling agents and subsequently deprotected under acidic conditions using trifluoracetic acid to yield the terephthalic acid derivative **14**. Starting from the γ-amino ester **15**, the condensation with **5** using EDCI and HOBT as coupling agents and subsequent LiOH induced selective hydrolysis of the methyl ester yielded a suitable intermediate for further coupling with the ureas **8** or **9**, respectively. The latter was again carried out using EDCI and HOBT as coupling agents and after treatment with trifluoroacetic acid, the terephthalic acid derivatives **16** (n = 0) and **17** (n = 1) were obtained carrying the sterically constrained γ-amino acid **15**.[13,14]

RESULTS AND DISCUSSION

Activity data were generated using Ramos whole assay conditions, inhibition values where determined
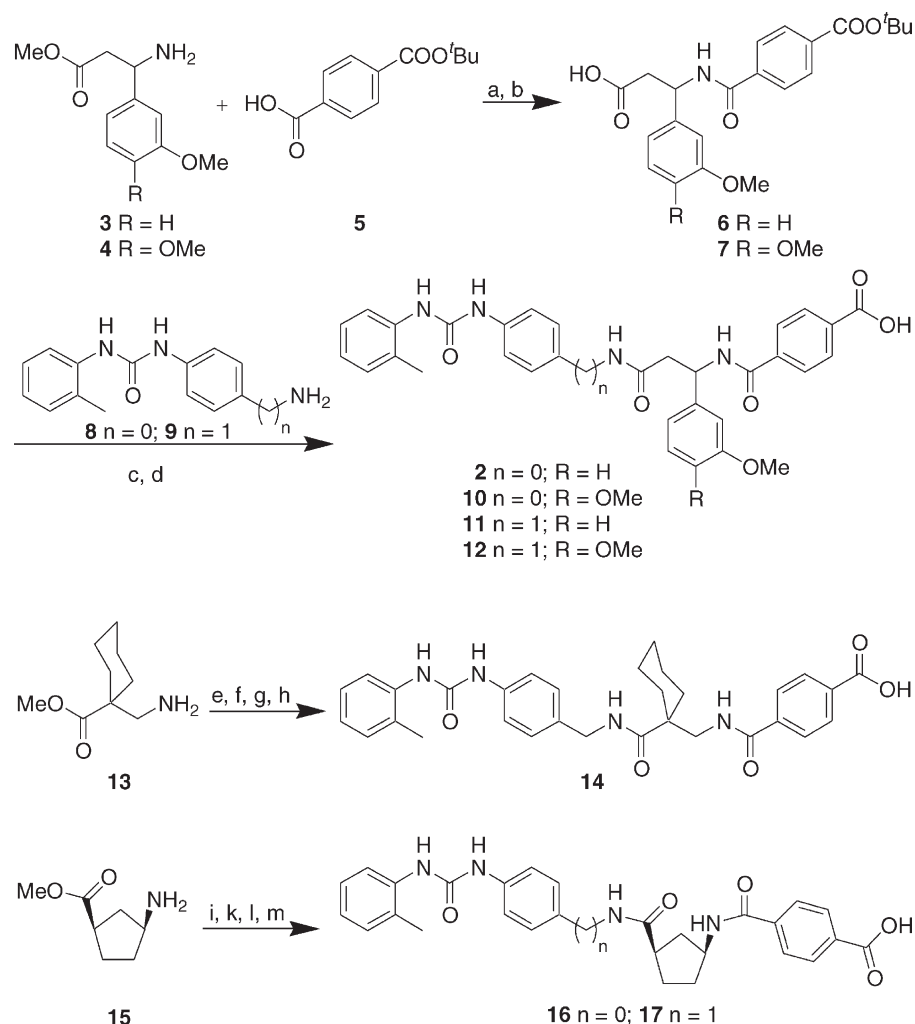
SCHEME 2 Preparation of compounds **2**, **10–12**, **14**, **16**, and **17**, reagents and conditions*: (a) EDCI, HOBT, DMF/MeCN (1:1), 25°C, 24 h; (b) 1.1 eq. LiOH, THF/H_2O (1:1), 25°C, 24 h; (c) EDCI, HOBT, DMF/MeCN (1:1), 25°C, 24 h; (d) TFA (20% in CH_2Cl_2), 0 → 25°C, 2 h; (e) **5** followed by EDCI, HOBT, then **13**; (f) 1.1 eq. LiOH, THF/H_2O (1:1), 25°C, 24 h; (g) EDCI, HOBT, then **9**, DMF/MeCN (1:1), 25°C, 24 h; (h) TFA (20% in CH_2Cl_2), 0 → 25°C, 2 h; (i) **5** followed by EDCI, HOBT, then **15**; (k) 1.1 eq. LiOH, THF/H_2O (1:1), 25°C, 24 h; (l) EDCI, HOBT, then **8** or **9**, DMF/MeCN (1:1), 25°C, 24 h; (m) TFA (20% in CH_2Cl_2), 0 → 25°C, 2 h.

were at a concentration of 10 µM, and IC_{50}'s are given for the most active compounds only. It was found that compounds **11**, **12**, **14**, and **17**, which contained the benzylamino urea **9**, showed a moderate affinity to the $\alpha_4\beta_1$ integrin with an $IC_{50} > 10$ µM. However, binding to the VLA4 was strongly enhanced when the benzylic methylene group was omitted in **8**. The sterically constrained γ-amino acid derivative **16** had a medium activity ($IC_{50} = 8.30$ µM), while **2** ($IC_{50} = 0.133$ µM) and **10** ($IC_{50} = 0.200$ µM) showed a strongly improved affinity to the $\alpha_4\beta_1$ integrin, being some orders of magnitude higher than that of the analogues **11** and **12** (Table I). Most interestingly, the more lipophilic 3,4-dimethoxy substituted compound **10** showed a slightly reduced affinity compared to **2**.

With the discovery of the potent $\alpha_4\beta_1$ integrin antagonist **2**, we became interested in its selectivity against other, homologous integrins such as $\alpha_9\beta_1$ and

$\alpha_v\beta_3$ integrin. Selectivity screening against $\alpha_9\beta_1$ (20% inhibition at 10 µM) and $\alpha_v\beta_3$ ($IC_{50} = 100$ µM) integrins showed that compound **2** was highly selective for $\alpha_4\beta_1$ integrin, since it binds several orders of magnitude stronger to $\alpha_4\beta_1$ integrin (Table II).

Thus, the para-substituted benzoic acid derivatives such as **1**, **2**, and **10** seem to form a unique pharmacophore arrangement for binding to the $\alpha_4\beta_1$ integrin, while the distance between the benzoic acid headgroup and the urea moiety and their spatial arrangement are rather sensitive to slight changes in their molecular geometry.

CONCLUSION

In conclusion, we have identified a new class of selective, non-peptidic and potent antagonists of

TABLE I   VCAM/VLA-4 binding inhibition

Compound	% inhibition at 10 μM	Ramos cell IC_{50} μM*
2	100	0.133
10	99	0.200
11	20	>10
12	5	n.d.
14	9	n.d.
16	55	8.30
17	15	>10

*Values are means of three experiments.

TABLE II   Integrin selectivity assay

IC_{50} μM*	$\alpha_4\beta_1$	$\alpha_9\beta_1$	$\alpha_v\beta_3$
2	0.133	20% inhibition at 10 μM	100

*Values are means of three experiments.

the $\alpha_4\beta_1$ integrin. Further improvements and structural modification of **2** leading to promising results will be presented in a forthcoming publication.

References

[1] Hynes, R.O. (1992) *Cell* **69**, 11–25.
[2] Schwartz, M.A., Schaller, M.D. and Ginsberg, M.H. (1995) *Ann. Rev. Cell. Dev. Biol.* **11**, 549–599.
[3] Zimmermann, C.N. (1999) *Exp. Opin. Ther. Patents 9*, **129**, Refer for recent reviews.
[4] Rose, D.M., Pozzi, A. and Zent, R. (2000) *Emerg. Ther. Targets* **4**, 397–411, Refer to recent reviews.
[5] Mousa, S.A. (2000) *Emerg. Ther. Targets* **4**, 143–153, Refer for recent reviews.
[6] Bochner, B.S. (1998) "Cell adhesion molecules and matrix proteins: role in health and diseases", In: Mousa, A.S., ed, *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases* (Springer-Verlag, Berlin, New York and R. G. Landes, Austin, Texas, USA), pp 113–131.
[7] Elices, M.J. (1998) "Cell adhesion molecules and matrix proteins: role in health and diseases", In: Mousa, A.S., ed, *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases* (Springer-Verlag, Berlin, New York and R. G. Landes, Austin, Texas, USA), pp 133–147.
[8] Lin, K.-C., Ateeq, H.S., Hsiung, S.H., Chong, L.T., Zimmerman, C.N., Castro, A., Lee, W.-C., Hammond, C.E., Kalkunte, S., Chen, L.-L., Pepinsky, R.B., Leone, D.R., Sprague, A.G., Abraham, W.M., Gill, A., Lobb, R.R. and Adams, S.P. (1999) *J. Med. Chem.* **42**, 920–934.
[9] Lehmann, T., Fischer, R., Albers, M., Rölle, T., Müller, G., Hessler, G., Tajimi, M., Ziegelbauer, K., Okigami, H., Bacon, K. and Hasegawa, H. WO2001081298; DE 2000-10019755.
[10] Seebach, D. and Hintermann, T. (1997) *CHIMIA* **51**, 244–247.
[11] Seebach, D., Abele, S., Schreiber, J.V., Martinoni, B., Nussbaum, A.K., Schild, H., Schulz, H., Hennecke, H., Woessner, R. and Bitsch, F. (1998) *CHIMIA* **52**, 734–739.
[12] Seliger, H., Bitar, M.B., Goeldner, E., Kittel, I. and Kilian, H.G. (1991) *Rev. Roum. Chim.* **36**, 171–186, For the preparation of 5 cf..
[13] Seebach, D., Abele, S., Sifferlen, T., Haenggi, M., Gruner, S. and Seiler, P. (1998) *Helv. Chim. Acta* **81**, 2218–2243, For the preparation of 13 cf.
[14] Bergmeier, S.C., Cobas, A.A. and Rapoport, H. (1993) *J. Org. Chem.* **58**, 2369–2376, For the preparation of 15 cf.