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Discovery and Evaluation of Piperidinyl Carboxylic Acid Derivatives as Potent $\alpha_4\beta_1$ Integrin Antagonists

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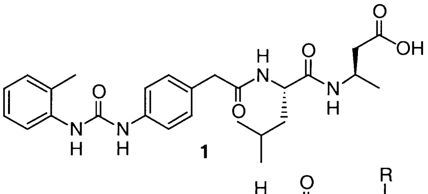
Abstract—Piperidinyl carboxylic acid-based derivatives were prepared as antagonists of the leukocyte cell adhesion process that is mediated through the interaction of the $\alpha_4\beta_1$ integrin (VLA-4, very late antigen 4) and the vascular cell adhesion molecule 1 (VCAM-1). Compounds **2a–h** inhibited the adhesion in a cell-based assay in the low and sub micromolar range, a pharmacokinetic study of **2d** is reported. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Adhesive interactions between the leukocytes and endothelial cells play a critical role in leukocyte trafficking to sites of inflammation.[1] 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.[2] Vascular cell adhesion molecules such as the $\alpha_4\beta_1$ integrin are currently investigated as targets for novel therapeutic treatments in chronic inflammatory diseases such as atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), and multiple sclerosis (MS), respectively.[3]



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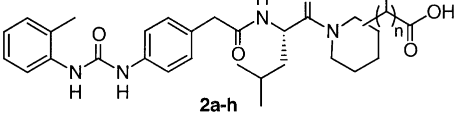
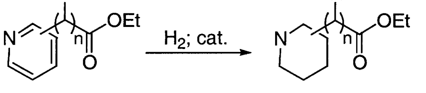


2a-h

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 $\alpha_4\beta_1$ integrin (VLA-4, very late antigen 4) has found major attention. Recently, Adams et al. have described a class of sub micromolar $\alpha_4\beta_1$ integrin antagonists **1**.[4] The latter contains an amino butyric acid headgroup decorated with (*L*)-Leucin and an urea moiety. We intended to design a novel class of $\alpha_4\beta_1$ integrin antagonists, comprising a more rigid and less polar acid headgroup. For that purpose, replacement of the amino butyric acid moiety by, for example, a piperidinyl carboxylic acid would perfectly address those requirements by omitting one N–H. This modification is expected not only to severely constrain the conformationally flexibility of the head group but also to increase the lipophilicity by introducing additional methylene fragments and by deleting the H-bond capacity of the former amide N–H.



3a-h **4a-h**

Furthermore, the piperidinyl carboxylic ester with a variety of substitution patterns like **4a–h** can be easily obtained in a multi-gram scale by selective catalytic hydrogenation of the heteroaromatic ring from the corresponding pyridine carboxylic esters **3a–h**.

This in turn allowed the synthesis of variable substituted $\alpha_4\beta_1$ integrin antagonists by reaction of the precursor **5**

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with the piperidinyl carboxylic acid by standard peptide coupling conditions (EDCI, HOBt) followed by cleavage of the ester protective group under basic reaction conditions as depicted in Scheme 1. All compounds reported were prepared as mixture of diastereomers and not separated for evaluation of the in vitro activity.

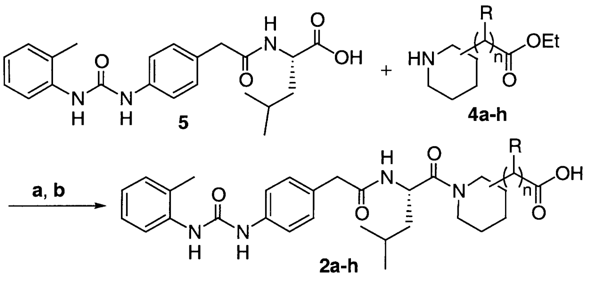
Activity data were generated using Jurkat whole assay conditions, inhibition values were determined at a concentration of 10 μM and the results are summarized in Table 1.[6] It turned out that the piperidinyl carboxylic acids ($n=0$) inhibited the interaction between VCAM-1 and the $\alpha_4\beta_1$ integrin in a low micromolar range. Best affinities were obtained when the carboxylic acid is located in the 4-position of the piperidine ring (**2c**, $IC_{50}=4.70$ μM), and significantly weaker when the acid is placed in the 2- and 3-position (**2a** and **2b**, $IC_{50} \geq 10$ μM). Piperidinyl acetic acids ($n=1$) yielded compounds with improved binding characteristics.

The affinity of those derivatives to the $\alpha_4\beta_1$ integrin was found to be significantly stronger irrespective of the position of the carboxylic acid moiety when compared to the piperindinyl carboxylic acids ($n=0$). Herein, the 3-position (**2b**, **2e**, and **2h**) seemed to be less favorable ($n=0$: $IC_{50}>10$ μM, $n=1$: $IC_{50}=7.50$ μM and $n=2$: $IC_{50}=6.74$ μM) independently of the chain length of the acid substituent. Compounds **2d** and **2f** bearing the acetic acid in the 2- as well as the 4-position appeared as strong antagonists of the $\alpha_4\beta_1$ integrin with sub-micromolar IC_{50} values of 0.30 and 0.55 μM, respectively. Nevertheless, the 2-position was destinctly preferred, and **2d** was found to be slightly more lipophilic (logP = 1.89) than **1** (logP = 1.72).

Thus encouraged, we have prepared **2g** with an additional phenyl substituent in the α-position accounting for a further enhanced lipophilic character. Disappointingly, this leads to a major loss of activity ($IC_{50}=4.96$ μM).

A selectivity screening against the $\alpha_9\beta_1$ and the $\alpha_v\beta_3$ integrins was carried out, which revealed that compound **2d** was highly selective for the $\alpha_4\beta_1$integrin (Table 2).

The pharmacokinetic parameters of **2d** dissolved in 10% cremophore EL in saline at a concentration of 2 mg/mL after intraperitoneal administration of 10 mg/kg to mice were evaluated.[7]



Scheme 1. Preparation of compounds **2a–h**, reagents and conditions: (a) EDCI, HOBT, DMF/MeCN (1:1), 25 °C, 24 h; (b) 1.1 equiv LiOH, THF/H_2O (1:1), 25 °C, 24 h. For the preparation of **4a–h**, compare ref 5.

Table 1. VCAM-1/$\alpha_4\beta_1$ integrin binding inhibtion

Compd	*n*	Position	R	% inhibition at 10 μM	Jurkat cell IC_{50} (μM)[a]
2a	0	2	H	49	10.00
2b	0	3	H	25	> 10
2c	0	4	H	96	4.70
2d	1	2	H	100	0.30
2e	1	3	H	55	7.20
2f	1	4	H	98	0.55
2g	1	2	Ph	99	4.96
2h	2	3	H	77	6.74

[a] Values are means of three experiments.

Table 2. Integrin assay[a] result

Assay (IC_{50} μM)	$\alpha_4\beta_1$	$\alpha_9\beta_1$	$\alpha_v\beta_3$
2d	0.30	> 50	> 200

[a] Values are means of three experiments.

As depicted in Figure 1, the unbound (empty dots, free fraction) concentration was found to be 9.6%, the maximal concentration C_{max} was determined at 1436 ng/mL, giving an AUC of 955 ng*h/mL. The half life time $\tau_{1/2}$ was calculated to be 0.80 h overall. The bioavailability was not estimated due to undetectable level of systemic concentration after oral dosing. This would be attributed to the relatively high molecular weight and still relatively low lipophilicity.

Conclusion

In conclusion, we have identified a novel class of selective, peptidomimetic and potent antagonist of the $\alpha_4\beta_1$ integrin with a promising pharmacokinetic profile. Fur-

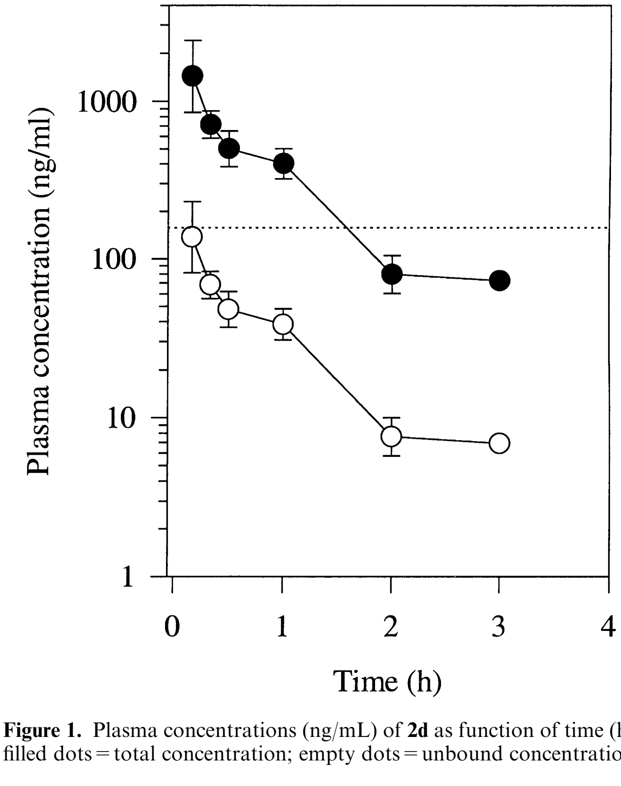


Figure 1. Plasma concentrations (ng/mL) of **2d** as function of time (h); filled dots = total concentration; empty dots = unbound concentration.

ther improvements and structural modification of **2d** leading to potent $\alpha_4\beta_1$ integrin antagonists will be presented in a forthcoming publication.

References and Notes

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6. Assay conditions: Jurkat cells (American Type Culture Collection, Clone E6-1, ATCC TIB-152) were cultured in RPMI 1640 medium (Nikken Bio Medical Laboratory, CM1101) supplemented with 10% fetal bovine serum (Hyclone, A-1119-L), 100 U/mL penicillin (Gibco BRL, 15140-122) and 100 µg/mL streptomycin (Gibco BRL, 15140-122) in a humidified incubator at 37 °C with 5% CO_2. Jurkat 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 5 min. After centrifugation at 1000 rpm for 5 min, the cell pellet was resuspended with adhesion assay buffer at a cell density of 2×10^6 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 $MnCl_2$. Labeled Jurkat cells were incubated for 30 min at 37 °C with each test compounds, at a concentration of 3 µM or at various concentrations ranging from 0.0001 to 10 µM using a standard five-point serial dilution. The assay solution was transfered to the VCAM-1 coated plates at a cell density of 2×10^5 cells per well and incubated for 1 h at 37 °C. The non-adherent cells were removed by washing the plates three times with wash buffer. The adherent cells were broken by addition of 1% Triton X-100 (Nacalai Tesque, 355-01). Released CFSC was quantified fluorescence measurement in a fluorometer (Wallac, ARVO 1420 multilabel counter).

7. **2d** was dissolved in 10% cremophore EL in saline at a concentration of 2 mg/mL. The compound at a dose of 10 mg/kg was given intraperitoneally to female BALB/c mice (Japan SLC Inc., Japan), and the blood sample was taken from the heart using heparinized syringes at 5, 15, 30, 60, 120 and 180 min after dosing. After centrifugation at 3000 rpm for 5 min at 4 °C, 0.25 mL of the plasma was added into 1 M HCl of 0.05 mL and extracted by 7 mL of ether. The organic layer was evaporated under nitrogen stream at 40 °C and the residue was dissolved in 0.25 mL of the mobile phase. 0.15 mL of the solution was applied to HPLC. We greatly acknowledge Drs. H. Komura and M. Mizoguchi (Bayer Yakuhin Ltd.) for their support.