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Cell Adhesion Antagonists: Synthesis and Evaluation of a Novel Series of Phenylalanine Based Inhibitors

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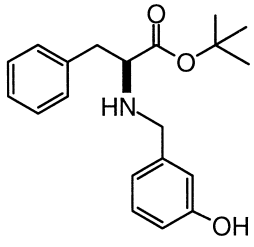
Abstract—Several phenylalanine based inhibitors were synthesized as antagonists of the leukocyte cell adhesion process that is mediated through the interactions of the mucosal addressin cell adhesion molecule (MAdCAM) and the integrin α4β7. Analogues **20**, **21**, **22** and **24** displayed inhibition of adhesion in a cell based assay in the low micromolar range. © 2000 Published by Elsevier Science Ltd.

Circulating leukocytes home to specific tissues through a complex series of receptor–ligand interactions.[1,2] The multistep process that leads to the infiltration of leukocytes into target tissues involves multiple interactions, which include the association of carbohydrates with selectins, chemokine receptors with chemokines, and integrins with immunoglobulin-like (Ig-like) cell adhesion molecules. Over expression of the tissue specific cell adhesion molecule MAdCAM (mucosal addressin cell adhesion molecule) results in the increased infiltration of lymphocytes bearing the α4β7 integrin.[3–6] This phenomenon is associated with tissue damage in both murine and non-human primate models of inflammatory bowel disease (IBD) where anti-α4β7 mAbs have shown efficacy.[7,8] In our efforts to develop small molecule antagonists of leukocyte trafficking,[9–11] we identified **1** as an inhibitor of cell adhesion mediated by the leukocyte cell surface integrin α4β7 and the cell adhesion molecule MAdCAM. Using a cell based adhesion assay, **1** was found to inhibit adhesion at an IC_{50} of 54 μM. Previously, a series of phenylalanines has been shown to effectively act as adhesion antagonists of fibrinogen, where their utility as integrin recognition motif mimics were described.[12] We herein report on the chemistry involved in our optimization efforts in this series of novel inhibitors.

Optimization of inhibitory activity in the series began with the synthesis of a series of *t*-butyl *N*-benzylphenylalanines (Scheme 1). Reductive amination utilizing commercially available *t*-butyl phenylalanate using standard Borch alkylation conditions[13] in the presence of the appropriate aldehyde afforded benzyl amines **2**–**13**. The aldehydes chosen were a group of hydrogen bond acceptor–donor substituted benzaldehydes. The relative pK_a of the phenolic proton was decreased by the addition of a nitro group to the aromatic ring, which resulted in a 2-fold increase in potency, (Table 1, **9** vs **11**).



1

Optimization of the ester functionality was then investigated keeping the *N*-2-hydroxy-5-nitrobenzyl moiety on the molecule constant. The synthesis of these compounds began with the esterification of *N*-BOC-phenylalanine (**14**) with a variety of alcohols in the presence of HBTU and DIEA (Scheme 2).[14,15] This was subsequently followed by the facile removal of the *t*-butoxycarbonyl group in 4 M HC1 in dioxane afforded esters **15**–**19**.

Reductive amination using Borch conditions[13] in the presence of 2-hydroxy-5-nitrobenzaldehyde afforded the products **20**–**23**. In order to achieve a more stable molecule for future in vivo lymphocyte recruitment studies, the isopentyl amide **24** was synthesized using the aforementioned protocol. The amide proved to be equiactive to the corresponding ester **20**, Table 1.

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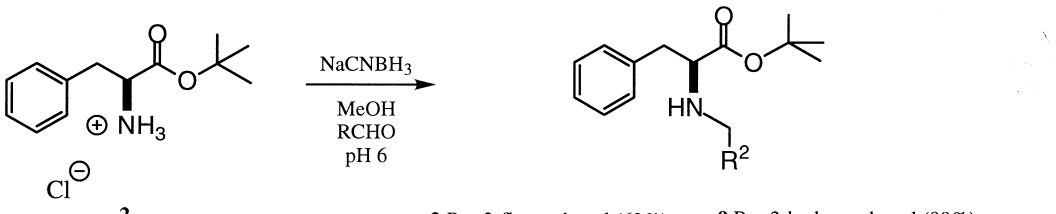
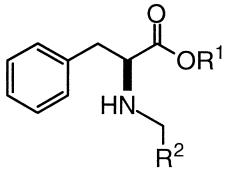


2

3 R = 2-fluorophenyl (62%)
4 R = 3-fluorophenyl (65%)
5 R = 4-fluorophenyl (76%)
6 R = 2-methoxyphenyl (93%)
7 R = 3-methoxyphenyl (61%)
8 R = 4-methoxyphenyl (63%)
9 R = 2-hydroxyphenyl (99%)
1 R = 3-hydroxyphenyl (28%)
10 R = 4-hydroxyphenyl (77%)
11 R = 2-hydroxy-5-nitrophenyl (63%)
12 R = phenyl (37%)
13 R = 3-hydroxy-4-nitrophenyl (45%)

Scheme 1.

Table 1. Inhibition of α4 β7/MAdCAM mediated cell adhesion[16]



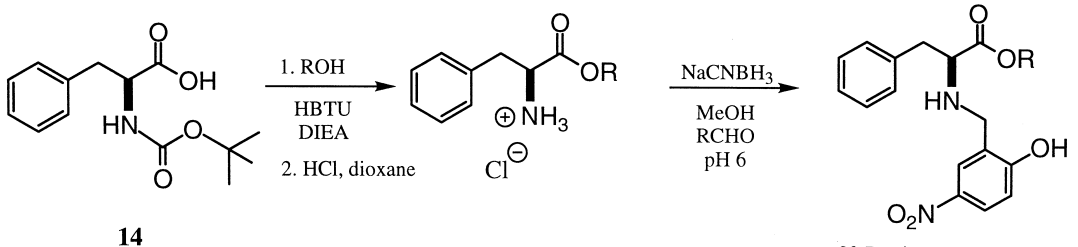
Compound	R^1	R^2	IC_{50} (μM)
1	*t*-Butyl	3-Hydroxyphenyl	54
3	*t*-Butyl	2-Fluorophenyl	117
4	*t*-Butyl	3-Fluorophenyl	176
5	*t*-Butyl	4-Fluorophenyl	121
6	*t*-Butyl	2-Methoxyphenyl	50
7	*t*-Butyl	3-Methoxyphenyl	122
8	*t*-Butyl	4-Methoxyphenyl	122
9	*t*-Butyl	2-Hydroxyphenyl	60
10	*t*-Butyl	4-Hydroxyphenyl	85
11	*t*-Butyl	2-Hydroxy-5-nitrophenyl	30
12	*t*-Butyl	Phenyl	100
13	*t*-Butyl	3-Hydroxy-4-nitrophenyl	Inactive
20	Isopentyl	2-Hydroxy-5-nitrophenyl	7
21	Benzyl	2-Hydroxy-5-nitrophenyl	7
22	Neopentyl	2-Hydroxy-5-nitrophenyl	9
23	Isopentenyl	2-Hydroxy-5-nitrophenyl	82
24	Isopentyl aniide	2-Hydroxy-5-nitrophenyl	6
27	Isopentyl "D"	2-Hydroxy-5-nitrophenyl	Inactive

The stereospecific interaction of the inhibitor with the protein is illustrated by the synthesis and evaluation of the unnatural 'D' isomer **27**, Scheme 3. Using the standard protocol described above the enantiomer of **24** was synthesized and was inactive in the cell adhesion assay. Stereochemical integrity was evaluated by creating corresponding chiral phenolic ester of **27**.

Cell Adhesion Assay

A cell adhesion assay involving the B-cell lymphoma, RPMI 8866 cells, and soluble human MAdCAM-IG chimera was used in a 96-well plate format. RPMI 8866 cells have previously been shown to bind avidly to MAdCAM.

RPMI 8866 cells were fluorescently labeled by preincubation with BCECF stain (Molecular Probes), washed, and resuspended in assay buffer containing 2% fetal calf serum and 2 mM Mg^{2+}. Compounds were tested in HBSS/2% FCS/25 mM HEPES buffer at 2.5 10^6 cells per mL. The typical assay consisted of a final volume of 200 μL containing 50 μL of cells at 1.25 10^5 cells per well. Adhesion assays for MAdCAM were washed on an automatic plate washer using a buffer consisting of 50 mM Tris/150 mM NaCl/2 mM $MnCl_2$, pH 7.2, in a wash volume of 500 μL for two wash cycles. Assays were then read on an Idexx fluorescent plate reader at 485/535 ηm. Inhibition was determined by the number of cells adhering to the MAdCAM lined plates in the presence and absence of an inhibitor and IC_{50} values were determined using Kaleidagraph (Adelbeck Software) and are reported as an average of several determinations.

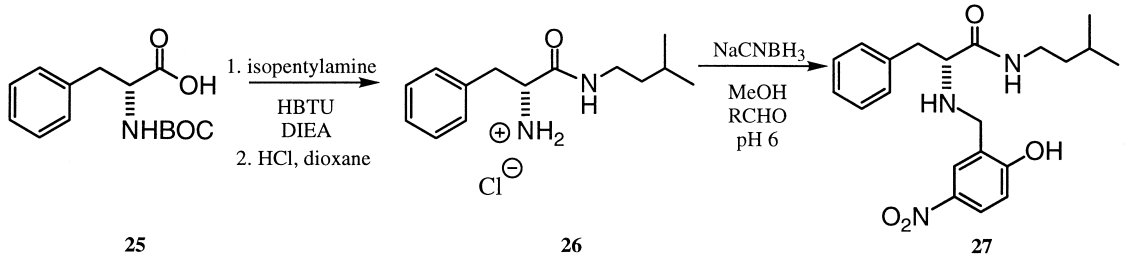


14

15 R = isopentyl (95%)
16 R = benzyl (93%)
17 R = neopentyl (50%)
18 R = 3-isopentenyl (48%)
19 R = isopentyl (amide) (73%)

20 R = isopentyl (71%)
21 R = benzyl (57%)
22 R = neopentyl (69%)
23 R = 3-isopentenyl (56%)
24 R = isopentyl (amide) (93%)

Scheme 2.



25 26 27

Scheme 3.

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