(19) DANMARK

(10) **DK/EP 3538542 T3**

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: ***C 07 K 7/52 (2006.01)*** ***A 61 P 1/00 (2006.01)*** ***A 61 P 29/00 (2006.01)*** ***A 61 P 37/06 (2006.01)*** *A 61 K 38/00 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2021-10-11**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-08-04**

(86) Europæisk ansøgning nr.: **17870529.9**

(86) Europæisk indleveringsdag: **2017-11-10**

(87) Den europæiske ansøgnings publiceringsdag: **2019-09-18**

(86) International ansøgning nr.: **CA2017000244**

(87) Internationalt publikationsnr.: **WO2018085921**

(30) Prioritet: **2016-11-11 US 201662421117 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **CYKLISKE PEPTIDMULTIMERER MÅLRETTET ALFA-4BETA-7-INTEGRIN**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The invention relates to antagonists of α4β7 integrin, and more particularly to cyclic peptide antagonists.

BACKGROUND OF THE INVENTION

[0002] Integrins are transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions. When triggered, integrins trigger chemical pathways to the interior (signal transduction), such as the chemical composition and mechanical status of the ECM. Integrins are obligate heterodimers, having two different chains: the α (alpha) and β (beta) subunits.

[0003] The α4β7 integrin is expressed on lymphocytes and is responsible for T-cell homing into gut-associated lymphoid tissues through its binding to mucosal addressin cell adhesion molecule (MAdCAM), which is present on high endothelial venules of mucosal lymphoid organs. Inhibitors of specific integrin-ligand interactions have been shown effective as anti-inflammatory agents for the treatment of various autoimmune diseases. For example, monoclonal antibodies displaying high binding affinity for α4β7 have displayed therapeutic benefits for gastrointestinal auto-inflammatory/autoimmune diseases, such as Crohn's disease, and ulcerative colitis.

[0004] There is a need to develop improved α4β7 antagonists to prevent or treat inflammatory conditions and/or autoimmune diseases.

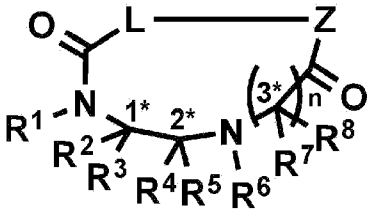
[0005] Certain methods of making cyclic peptides (nacellins) are described in Applicant's PCT Publication No. WO 2010/105363.

[0006] BOER J ET AL., JOURNAL OF MEDICINAL CHEMISTRY, vol. 44, 26 July 2001 , disclose cyclic penta- and hexapeptides comprising the amino acid motif Leu-Asp-Thr that inhibit alpha4 beta7 integrin mediated cell adhesion to MAd-CAM-1.

[0007] WO 2016/054445 discloses dimers of cyclic peptides having antagonistic activity against alpha4 beta7 integrin. WO 2017/079820 discloses monomeric cyclic peptides targeting alpha4beta7 integrin.

SUMMARY OF THE INVETION

[0008] In an aspect, there is provided, a homodimer of two compounds covalently linked together, the compounds both being of formula (I):



(I)

wherein

R^1 is H;

R^2 is H;

R^3 is CH_3;

R^4 is H;

R^5 is an amide of the formula -C(O)NR**R***, wherein R** is H and R*** is *tert*-butyl;

R^6 along with R^7 form the pyrrolidine ring of Pro; R^8 is H;
n is 1; and
wherein Z is an amino terminus of an amino acid; -C=O- adjacent L is the carboxy terminus of an amino acid; and L along with Z and -C=O- is a peptide having the following formula:

$$X^y\text{-}X^z\text{-}X^1\text{-}X^2\text{-}X^3$$

wherein

X^y is 3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoic acid;

X^z is absent;

X^1 is Leucine;

X^2 is Asp; and

X^3 is Thr.

[0009] In an aspect, there is provided, a pharmaceutical composition comprising the homodimer described herein along with the pharmaceutically acceptable carrier. The pharmaceutical composition may be formulated for any one of oral delivery, topical delivery and parenteral delivery.

[0010] In an aspect, there is provided, the homodimer described herein for use in treating a condition or disease selected from the group consisting of Inflammatory Bowel Disease (IBD); ulcerative colitis; Crohn's disease; celiac disease (nontropical Sprue); collagenous colitis, eosinophilic gastroenteritis; pouchitis resulting after proctocolectomy and ileoanal anastomosis; microscopic colitis and graft versus host disease.

BRIEF DESCRIPTION OF FIGURES AND TABLES

[0011] These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings and tables wherein:

Figure 1 (not part of the present invention) shows representative compounds of the present application, namely from the following classes, 18-membered ring, 21-membered ring, 21-membered ring (non-canonical, i.e. having a delta amino acid), 22-membered ring, and 24-membered ring.

Figure 2 (not part of the present invention) shows a representative 18-membered ring compound along with variations made at certain positions with corresponding α4β7 integrin ELISA IC50 binding values associated with those variations.

Figure 3 (not part of the present invention) shows a representative 21-membered ring compound along with variations made at certain positions with corresponding α4β7 integrin ELISA IC50 binding values associated with those variations.

Figure 4 (not part of the present invention) shows a representative 21-membered ring (non-canonical, i.e. having a delta amino acid) compound along with variations made at certain positions with corresponding α4β7 integrin ELISA IC50 binding values associated with those variations.

Figure 5 (not part of the present invention) shows a representative 22-membered ring compound along with variations made at certain positions with corresponding α4β7 integrin ELISA IC50 binding values associated with those variations.

Figure 6A and 6B (not part of the present invention) show representative NMR data for a multimeric molecule, Compound No. 390, with ^{1}H- and ^{1}H-^{1}H TOCSY NMR spectra recorded at 25 °C

Figure 7 shows the binding to α4β7 integrin measured as a MADCAM-1 competition assay in human whole blood for: a) (not part of the present invention) representative monomeric Compound 456 (ET4062) and multimeric Compound No.s 534 (ET4113) and 535 (ET4110), derived from Compound 456, and; b) representative monomeric Compound 340 (ET2451) and multimeric Compound No.s 390 (ET3755) and 517 (ET3764), derived from Compound 340.

Figure 8 shows the detection of α4β7+ Th memory cells trafficking in the mesenteric lymph nodes in mice suffering from DSS-induced colitis treated for 4 days with Compound No. 517 (ET3764) or vehicle.

Figure 9 shows the α4β7+ Th memory lymphocyte content in mesenteric lymph nodes taken from mice exposed to DSS irritant and treated for 4 days with various concentrations of Compound No. 517 (ET3764) or control (SMEDDS vehicle).

Figure 10 shows the receptor occupancy of representative multimeric compounds on α4β7-positive T helper memory cells as measured in a MADCAM-1 competition assay in human whole blood.

Figure 11 shows the receptor occupancy of representative nacellin dimers on α4β7-negative Th memory cells as measured in a VCAM-1 competition assay in human whole blood.

[0012] Table 1 shows the monomer compound 340 referred to below exhibiting α4β7 integrin affinity, selectivity and/or activity; and specifically with respect to these compounds: (A) the structure of the linker portion; (B) the structure of the peptide portion; and (C) and (C') the affinity, selectivity and activity values.

[0013] To aid reading of the table, the following is noted:

Table 1A:

In the reference compound 340, R2 is H and R3 is CH3, the carbon atom bearing R2 and R3 has S-configuration.

In the reference compound 340, R4 is H and R5 is C(O)-NH-tert-Butyl, the carbon atom bearing R4 and R5 has S-configuration.

Table 1B

In the reference compound 340, R6 and R7 are both Pro, the R6 and R7 substituents are covalently bound and form the pyrrolidine ring of Pro.

If no entry exists under column Xz, the residue is absent.

Table 1C and 1C'
If no entry exists under any of the columns, no data was collected.

Table 1X is a correspondence table linking the compounds described herein with the synthesis protocols outlined in the methods and materials.

Table 2 shows the homodimeric compound 517 of the present invention exhibiting α4β7 integrin affinity, selectivity and/or activity; and specifically with respect to these compounds: (A) the structure of the linker portion; (B) the structure of the peptide portion; and (C) the affinity, selectivity and activity values.

[0014] To aid reading of the table, the following is noted:

Table 2A
In the compound of the present invention, R2 is H and R3 is CH3, the carbon atom bearing R2 and R3 has S-configuration.

Table 2B

In the compound of the present invention, R6 and R7 are both Pro, the R6 and R7 substituents are covalently bound and form the pyrrolidine ring of Pro.

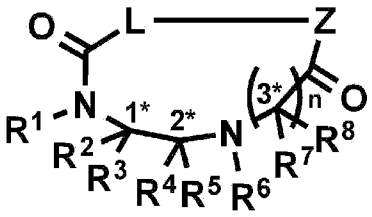
If no entry exists under column Xz, the residue is absent.

Table 2X is a correspondence table linking the homodimer of the present invention with the synthesis protocols outlined in the methods and materials. m/z is (M + 2H/2) and additional information regarding the linker.

DETAILED DESCRIPTION

[0015] In the following description, numerous specific details are set forth to provide a thorough understanding of the invention. However, it is understood that the invention may be practiced without these specific details.

[0016] In an aspect, there is provided, a multimer comprising a plurality of compounds covalently linked together, the compounds independently being of formula (I):



(I)

wherein

R^1 is H;

R^2 is H;

R^3 is CH_3;

R^4 is H;

R^5 is an amide of the formula -C(O)NR**R***, wherein R** is H and R*** is *tert*-butyl;

R^6 along with R^7 together form the pyrrolidine ring of Pro;

R^8 is H;

n is 1; and

wherein Z is an amino terminus of an amino acid; -C=O- adjacent L is the carboxy terminus of an amino acid; and L along with Z and -C=O- is a peptide having the following formula:

$$X^y\text{-}X^z\text{-}X^1\text{-}X^2\text{-}X^3$$

wherein

X^y is 3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoic acid;

X^z is absent;

X^1 is Leucine ;

X^2 is Asp; and

X^3 is Thr.

[0017] The reference compound shown in Tables 1A, 1B and 1C (and 1C') exhibits antagonistic activity against α4β7 integrin and having selectivity over α4β1 integrin. These compounds are further described in WO

2017/079820.

[0018] The homodimer compound of the present invention exhibits affinity, selectivity and activity, summarized in Tables 2A, 2B and 2C.

[0019] As used herein, the term "amino acid" refers to molecules containing an amine group, a carboxylic acid group and a side chain that varies. Amino acid is meant to include not only the twenty amino acids commonly found in proteins but also non-standard amino acids and unnatural amino acid derivatives known to those of skill in the art, and therefore includes, but is not limited to, alpha, beta and gamma amino acids. Peptides are polymers of at least two amino acids and may include standard, non-standard, and unnatural amino acids. A peptide is a polymer of two or more amino acids.

[0020] The following abbreviations are used herein:

Abbreviation	Description
1,2-cis-ACHC	cis-2-aminocyclohexanecarboxylic acid
1,2-trans-ACHC	trans-2-aminocyclohexanecarboxylic acid
1Nal	1-napthylalanine
2Abz	anthranilic acid, 2-aminobenzoic acid
2Igl	2-indanylglycine
2Nal	2-napthylalanine
Abu	2-aminobutyric acid
Aic	aminoindan-2-carboxylic acid
alloIle	allo-sioleucine, (2S,3R)-2-amino-3-methylpentanoic acid
alloThr	allo-threonine, (2S,3S)-2-amino-3-hydroxybutyric acid
alphaMePhe	α-methyl-phenylalanine, (S)-(-)-2-amino-2-methyl-3-phenylpropionic acid
Asp(ethyl ester)	aspartic acid β-ethyl ester
Atc	2-aminotetraline-2-carboxylic acid
Aze	azetidine-2-carboxylic acid
BHT	butylated hydroxytoluene
Bip	biphenylalanine
C10	sebacic acid
C12	dodecanedioic
C7	pimelic acid
C8	suberic acid
C9	azelaic acid
Cha	β-cyclohexyl alanine, (S)-2-amino-3-cyclohexylpropionic acid
Chg	cyclohexyl glycine
cis-dhyp	cis-D-4-Hydroxyproline, (2R,4R)-4-Hydroxypyrrolidine-2-carboxylic acid
cycloLeu	cyclo leucine, 1-Aminocyclopentane-1-carboxylic acid
cyclopropylAla	β-cyclopropyl alanine, (S)-2-amino-3-cyclopropyl-propionic acid
d2Igl	2-indanyl-D-glycine
Dap(Cbz)	Nβ-Z-2,3-diaminopropionic acid
DBU	1,8-diazabicyclo[5.4.0] undec-7-ene
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one

Abbreviation	Description
dHyp	trans-D-4-hydroxyproline, (2R,4S)-4-hydroxypyrrolidine-2-carboxylic acid
DIAD	diisopropyl azodicarboxylate
DIG	diglycolic acid
DIPEA	N,N-diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
dMeArg	N-methyl-D-arginine
dMebetaHomoLys	N-methyl-D-β-homoLys
dMeLys	N-methyl-D-Lysine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
dNle	D-norleucine
dOrn	D-ornithine
dOrn(dimethyl)	Nδ-dimethyl-D-ornithine
dPip	D-pipecolic acid, D-homoPro
dSer(OBn)	O-benzyl-D-serine
dTic	(3R)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
dTiq	D-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid
dTyr(OAllyl)	O-allyl-D-tyrosine
dTyr(OBn)	O-benzyl-D-tyrosine
EDC	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
Fmoc	9-fluorenylmethoxycarbonyl
HATU	O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HCTU	2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
His(Bn)	Nτ-benzyl-histidine
HomocycloLeu	homocyclo leucine, 1-Aminocyclohexanecarboxylic acid
Hyp	trans-4-hydroxyproline, (2S,4R)-4-hydroxypyrrolidine-2-carboxylic acid
Hyp(OBn)	O-benzyl-trans-4-hydroxyproline
MeAsp	N-methyl aspartic acid
MebetaHomoLys	N-methyl β-homoLysine
MebetaHomoLys(Me)2	Nα-methyl-Nε-dimethyl-β-homoLysine
MeLeu	N-methyl leucine
MeMet	N-methyl methionine
MePhe	N-methyl phenylalanine
metaY(Opr)	metaTyrosine
MeThr	N-methyl threonine
MeTyr	N-methyl tyrosine
NMP	N-methylpyrrolidone
Nosyl chloride	2-nitrobenzenesulfonyl chloride

Abbreviation	Description
Nva	norvaline
Orn(acetamide)	Nδ-acetamide-ornithine
Orn(benzamide)	Nδ-benzamide-ornithine
Orn(ethylca rbamate)	Nδ-ethylcarbamate-ornithine
Orn(methanesulfonamide)	Nδ-methanesulfonamide-ornithine
Orn(pentyl amide)	Nδ-pentyl amide-ornithine
PDA	1,4-phenyldiacetic acid
Pen	penicillamine, β,β-dimethyl-cysteine
Pip	pipecolic acid, homoPro
Sar	sarcosine, N-methyl glycine
tertbutylAla	β-tert-butyl alanine, neopentylglycine
TFA	trifluoroacetic acid
TFE	2,2,2-Trifluoroethanol
THF	tetrahydrofuran
Thr(OBn)	O-benzyl-threonine
Thr(OEt)	O-ethyl-threonine
Thr(OMe)	O-methyl-threonine
Tic	(3S)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
TIS	triisopropylsilane
Tyr(2-methoxy diaryl ether)	O-2-methoxy-phenyl-tyrosine
Tyr(2-tolyl diaryl ether)	O-2-methyl-phenyl-tyrosine
Tyr(3,4-difluoro diaryl ether)	O-3,4-difluoro-phenyl-tyrosine
Tyr(3,4-dimethyl diaryl ether)	O-3,4-dimethyl-phenyl-tyrosine
Tyr(3-CO2Me diaryl ether)	O-3-methylester-phenyl-tyrosine
Tyr(3-fluoro diaryl ether)	O-3-fluoro-phenyl-tyrosine
Tyr(3-methoxy diaryl ether)	O-3-methoxy-phenyl-tyrosine
Tyr(3-methyl diaryl ether)	O-3-methyl-phenyl-tyrosine
Tyr(4-CF3 diaryl ether)	O-4-trifluoromethyl-phenyl-tyrosine
Tyr(4-CO2H diaryl ether)	O-4-carboxylate-phenyl-tyrosine
Tyr(4-CO2Me diaryl ether)	O-4-methylester-phenyl-tyrosine
Tyr(4-fluoro diaryl ether)	O-4-fluoro-phenyl-tyrosine
Tyr(4-methoxy diaryl ether)	O-4-methoxy-phenyl-tyrosine
Tyr(OAllyl)	O-allyl-tyrosine
Tyr(OPh)	O-phenyl-tyrosine
vinyl-Br-Leu	2-amino-4-bromo-4-pentenoic acid

[0021] A protecting group or protective group is a substituent introduced into a molecule to obtain chemoselectivity in a subsequent chemical reaction. Many protecting groups are known in the art and a skilled person would understand the kinds of protecting groups that would be incorporated and could be used in connection with the methods described herein. In "protecting group based peptide synthesis", typically solid phase peptide synthesis, the desired peptide is prepared by the step-wise addition of amino acid moieties to a building peptide chain. The two most widely used protocols, in solid-phase synthesis, employ tert-butyloxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc) as amino protecting groups. Amino protecting groups generally protect an amino

group against undesirable reactions during synthetic procedures and which can later be removed to reveal the amine. Commonly used amino protecting groups are disclosed in Greene, T. W. et al., Protective Groups in Organic Synthesis, 3rd Edition, John Wiley & Sons (1999). Amino protecting groups include acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, o-nitrophenoxyacetyl, .alpha.-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl and the like; alkoxy- or aryloxycarbonyl groups (which form urethanes with the protected amine) such as benzyloxycarbonyl (Cbz), p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, .alpha.-,.alpha.-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl (Alloc), 2,2,2-trichloroethoxycarbonyl, 2-trimethylsilylethyloxycarbonyl (Teoc), phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl (Fmoc), cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl and the like; aralkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl and the like; and silyl groups such as trimethylsilyl and the like. Amine protecting groups also include cyclic amino protecting groups such as phthaloyl and dithiosuccinimidyl, which incorporate the amino nitrogen into a heterocycle. Typically, amino protecting groups include formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, Alloc, Teoc, benzyl, Fmoc, Boc and Cbz. It is well within the skill of the ordinary artisan to select and use the appropriate amino protecting group for the synthetic task at hand.

[0022] In the compound of the present invention, R^1 is H.

[0023] In the compound of the present invention, R^2 is H and R^3 is CH_3.

[0024] In the compound of the present invention, R^4 is H and R^5 is C(O)-NHR^t, wherein R^t is tert-butyl.

[0025] In the compound of the present invention, n is 1.

[0026] In the compound of the present invention, X^1 is Leu.

[0027] In the compound of the present invention, X^2 is Asp.

[0028] In the compound of the present invention, X^3 is Thr.

[0029] In the compound of the present invention, X^y is 3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoic acid and X^z is absent.

[0030] The compound of the present invention is the homodimer of compound 517.

[0031] In the compound of the present invention, the monomer compounds are linked by an amide bond to a diphenic acid residue linker.

[0032] In certain embodiments, there is provided pharmaceutically acceptable salts of the compounds described herein. The term "pharmaceutically acceptable salt," as used herein, represents salts or zwitterionic forms of the compounds of the present invention which are water or oil-soluble or dispersible, which are suitable for treatment of diseases without undue toxicity, irritation, and allergic response; which are commensurate with a reasonable benefit/risk ratio, and which are effective for their intended use. The salts can be prepared during the final isolation and purification of the compounds or separately by treatment of an amino group with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, citrate, aspartate, benzoate,

benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, mesitylenesulfonate, methanesulfonate, naphthylenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproprionate, picrate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, para-toluenesulfonate, and undecanoate. Also, amino groups in the compounds of the present invention can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form therapeutically acceptable addition salts include inorganic acids such as hydrochloric, hydrobromic, sulfuric, and phosphoric, and organic acids such as oxalic, maleic, succinic, and citric. In certain embodiments, any of the peptide compounds described herein are salt forms, e.g., acetate salts.

[0033] In an aspect, there is provided, a pharmaceutical composition comprising the multimer described herein along with the pharmaceutically acceptable carrier. The pharmaceutical composition may be formulated for any one of oral delivery, topical delivery and parenteral delivery.

[0034] As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the pharmacological agent.

[0035] In an aspect, there is provided the homodimer multimer described herein for use in treating a condition or disease selected from the group consisting of Inflammatory Bowel Disease (IBD), ulcerative colitis, Crohn's disease, Celiac disease (nontropical Sprue), collagenous colitis, eosinophilic gastroenteritis, pouchitis resulting after proctocolectomy and ileoanal anastomosis, human immunodeficiency virus (HIV) infection in the GI tract, microscopic colitis, and graft versus host disease

[0036] In preferable embodiments, is an inflammatory bowel disease, such as ulcerative colitis or Crohn's disease.

[0037] The homodimer inhibits binding of α4β7 integrin to MAdCAM. Preferably, the compound selectively inhibits binding of α4β7 integrin to MAdCAM.

[0038] In any embodiment, the patient is preferably a human.

[0039] As used herein, the terms "disease", "disorder", and "condition" may be used interchangeably.

[0040] As used herein, "inhibition," "treatment," "treating," and "ameliorating" are used interchangeably and refer to, e.g., stasis of symptoms, prolongation of survival, partial or full amelioration of symptoms, and partial or full eradication of a condition, disease or disorder in a subject, e.g., a mammal.

[0041] As used herein, "prevent" or "prevention" includes (i) preventing or inhibiting the disease, injury, or condition from occurring in a subject, e.g.., a mammal, in particular, when such subject is predisposed to the condition but has not yet been diagnosed as having it; or (ii) reducing the likelihood that the disease, injury, or condition will occur in the subject.

[0042] As used herein, "therapeutically effective amount" refers to an amount effective, at dosages and for a particular period of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the pharmacological agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the pharmacological agent to elicit a desired response in the individual. A

therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmacological agent are outweighed by the therapeutically beneficial effects.

[0043] In some embodiments, the compound is administered by a form of administration selected from the group consisting of oral, intravenous, peritoneal, intradermal, subcutaneous, intramuscular, intrathecal, inhalation, vaporization, nebulization, sublingual, buccal, parenteral, rectal, vaginal, and topical.

[0044] In some embodiments, the compound is administered as an initial dose followed by one or more subsequent doses and the minimum interval between any two doses is a period of less than 1 day, and wherein each of the doses comprises an effective amount of the compound.

[0045] In some embodiments, the effective amount of the compound is the amount sufficient to achieve at least one of the following selected from the group consisting of: a) about 50% or greater saturation of MAdCAM binding sites on α4β7 integrin molecules; b) about 50% or greater inhibition of α4β7 integrin expression on the cell surface; and c) about 50% or greater saturation of MAdCAM binding sites on α4β7 molecules and about 50% or greater inhibition of α4β7 integrin expression on the cell surface, wherein i) the saturation is maintained for a period consistent with a dosing frequency of no more than twice daily; ii) the inhibition is maintained for a period consistent with a dosing frequency of no more than twice daily; or iii) the saturation and the inhibition are each maintained for a period consistent with a dosing frequency of no more than twice daily.

[0046] In some embodiments, the compound is administered at an interval selected from the group consisting of around the clock, hourly, every four hours, once daily, twice daily, three times daily, four times daily, every other day, weekly, bi-weekly, and monthly.

[0047] The compounds described herein may be multimerized using methods and linkers that would be known to a person of skill in the art, for example, as described in WO2016/054411.

[0048] The advantages of the present invention are further illustrated by the following examples. The examples and their particular details set forth herein are presented for illustration only and should not be construed as a limitation on the claims of the present invention.

EXAMPLES

Methods and Materials

Synthesis

[0049] Methods applicable for making the cyclic peptides described herein can be found generally in Applicant's PCT Publication No. WO 2010/105363 and in WO 2017/079821.

[0050] More specifically, the below protocols were used to synthesize each of the compounds as indicated in Table 1X. Multimer of the compounds were also synthesized as indicated in Table 2X.

Protocol A: General nacellin synthesis

[0051]

1. *1. Preparation of resin:* Fmoc amino acid (1.1 eq. with respect to resin) was dissolved in CH_2Cl_2 (10 mL/g of

resin). If the amino acid did not dissolve completely, DMF was added slowly dropwise until a homogeneous mixture persisted upon stirring/sonication. The 2-chlorotrityl resin was allowed to swell in CH_2Cl_2 (5 mL/g of resin) for 15 minutes. The CH_2Cl_2 was then drained and the Fmoc amino acid solution was added to the vessel containing the 2-Cl Trt resin. DIPEA was added (2 eq. with respect to the amino acid) and the vessel was agitated for five minutes. Another 2 eq. of DIPEA was then added and the vessel was left to agitate for an additional 60 minutes. The resin was then treated with methanol (1 mL/g of resin) to endcap any remaining reactive 2-Cl Trt groups. The solution was mixed for 15 minutes, drained and then rinsed with CH_2Cl_2 (x3), DMF (x3), CH_2Cl_2 (x2), and MeOH (x3). The resin was then dried under vacuum and weighed to determine the estimated loading of Fmoc amino acid.

2. *2. Preparation of linear peptide sequence via manual or automated synthesis:* Fully protected resin-bound peptides were synthesized via standard Fmoc solid-phase peptide chemistry manually or using an automated peptide synthesizer. All *N*-Fmoc amino acids were employed.
    1. *a. Fmoc deprotection:* the resin was treated with 20% piperidine in NMP or DMF twice, for 5 and 10 minutes respectively, with consecutive DMF and NMP washes after each addition.
    2. *b. Fmoc amino acid coupling:* the resin was treated with 3 eq. of Fmoc amino acid, 3 eq. of HATU and 6 eq. of DIPEA in NMP for 60 minutes. For difficult couplings, a second treatment with 3 eq. of Fmoc amino acid, 3 eq. of HATU and 6 eq. of DIPEA in NMP for 40 minutes was employed.
3. *3. General cleavage with retention of protecting groups:* Once the desired linear sequence was synthesized, the resin was treated with either 1.) 1:3, HFIP:CH_2Cl_2 or 2.) 5% TFA in CH_2Cl_2, twice for 30 minutes each, to afford cleavage from the solid support. The solvent was then removed, followed by trituration twice with chilled *tert*-butyl methyl ether (or diethyl ether/hexanes) to give the desired product. The purity was then analyzed by reverse-phase LCMS.

Protocol B (reference): Preparation of *N*-alkylated Fmoc amino acid building blocks

[0052]

1. *1. Resin prep: see protocol A, step 1*
2. *2. Fmoc deprotection: see protocol A, step 2a*
3. *3. Nosyl protection:* The deprotected resin was stirred in CH_2Cl_2 (5 mL/mmol of resin) and DIPEA (6.5 eq.). A solution of Nosyl chloride (4.0 eq.) was added slowly, dropwise, over 30 minutes, to avoid a rapid exothermic reaction. After the addition was complete, stirring was continued at room temperature for three hours. The resulting nosyl-protected resin was filtered and washed with CH_2Cl_2, MeOH, CH_2Cl_2, and THF.
4. *4. N-Methylation:* To a suspension of resin in THF (10 mL/mmol of resin) was added a solution of triphenylphosphine (5 eq.) in THF (2 M) and MeOH (10 eq.). The stirring suspension was cooled in an ice bath. A solution of DIAD (5 eq.) in THF (1 M) was added dropwise, via addition funnel. After addition was complete the bath was removed and the reaction was stirred at room temperature for an additional 90 minutes. The resin was filtered, washed with THF (x4), CH_2Cl_2 (x3), and THF (x2).
5. *5. Nosyl-deprotection:* To a suspension of resin in NMP (10 mL/mmol of resin) was added 2-mercaptoethanol (10.1 eq.) and DBU (5.0 eq.). The solution became a dark green colour. After five minutes, the resin was filtered, washed with DMF until washes ran colourless. This procedure was repeated a second time, and the resin was then washed a final time with CH_2Cl_2.
6. *6. Fmoc protection:* To a suspension of resin in CH_2Cl_2 (7 mL/mmol of resin) was added a solution of Fmoc-Cl (4 eq.) in CH_2Cl_2 (7 mL), and DIPEA (6.1 eq.). The suspension was stirred at room temperature for four hours then filtered and washed with CH_2Cl_2 (x2), MeOH (x2), CH_2Cl_2 (x2), and Et_2O (x2).

7. Cleavage from resin: see protocol A, step 3

Protocol C (reference): Reductive amination

[0053]

1. *1. Fmoc Weinreb amide formation:* a mixture of Fmoc amino acid (1 mmol), *N,O*-dimethylhydroxylamine·HCl (1.2 eq.), and HCTU (1.2 eq.) in CH_2Cl_2 (6.5 mL), was cooled to 0 °C. DIPEA (3 eq.) was then slowly added to the stirring mixture. The cooling bath was removed and the reaction was stirred at room temperature for 16 h. A 10% solution of HCl (4 mL) was added resulting in the formation of a precipitate, which was removed through filtration. The filtrate was washed with 10% HCl (3 x 4 mL) and brine (2 x 4 mL). The organic phase was then dried over Na_2SO_4. The solvent was removed under reduced pressure to give crude Fmoc Weinreb amide, which was used in the next reaction without purification.
2. *2.* a) *Fmoc amino aldehyde formation:* lithium aluminum hydride powder (1.5 eq) was placed in a dry flask. THF (Sigma-Aldrich, 250 ppm of BHT, ACS reagent > 99.0 %, 6.5 mL) was added, and the resulting slurry was cooled to -78 °C, with stirring. To the slurry was added a solution of the Fmoc Weinreb amide in THF (10 mL). The reaction vessel was transferred to an ice/water bath, and maintained at 0 °C for 1 h. To the reaction at 0 °C, was added dropwise acetone (1.5 mL), then H_2O (0.25 mL) and then the reaction was left to stir for an additional hour at room temperature. The mixture was filtered through Celite, washed with EtOAc (10 mL) and MeOH (10 mL), and the filtrate was concentrated. The crude material was dissolved in $CHCl_3$ (6.5 mL) and washed with brine (2 x 3 mL) and the organic phase was then dried over Na_2SO_4, filtered and concentrated to give the Fmoc amino aldehyde.
   Alternatively, b) Under argon atmosphere a Lithium Aluminum Hydride 1.0 M solution in THF (Sigma-Aldrich, 157.81 mL, 157.82 mmol, 1 eq.) was slowly added over a solution of the Weinreb amide (157.82 mmol) in THF (Sigma-Aldrich, 250 ppm of BHT, ACS reagent > 99.0 %, 1 L) at 0 °C and then stirred for 1 h. The reaction at 0 °C, was diluted with Et_2O (500 mL) and the resultant solution was washed with 10% $NaHSO_4$ (10 x 300 mL), 10% $KHSO_4$ (10 x 300 mL) and HCl (10 x 300 mL). The organic phase was then dried over Na_2SO_4, filtered and concentrated to afford the crude Fmoc amino aldehyde.
3. *3. Reductive amination on-resin:* the linear peptide on-resin was placed in a solid-phase peptide synthesis reaction vessel and diluted with DMF (22 mL/g of resin). The Fmoc aldehyde (4.0 eq.) was added and the reaction was left to shake overnight. The solution was then drained and the resin was washed with CH_2Cl_2 (x3) and DMF (x3). The resin was then diluted with a mixture of MeOH/CH_2Cl_2 (22 mL/g of resin, 1:3 ratio) and $NaBH_4$ (7 eq.) was subsequently added. The mixture was left to shake for four hours, then the solution was drained and the resin was washed with CH_2Cl_2 (x3) and DMF (x3).

Protocol D: Fragment-based macrocyclization

[0054] a) In a two-dram vial, 0.1 mmol of the linear peptide and DEPBT (1.5 eq.) were dissolved in 5 mL of freshly distilled THF (0.02 M). DIPEA (3 eq.) was then added and the reaction mixture was left to stir overnight at room temperature (16 h). Tetraalkylammonium carbonate resin (Biotage®, 6 eq.) was then added to the reaction mixture and stirring was continued for an additional 24 h. The reaction was then filtered through a solid-phase extraction vessel and rinsed with CH_2Cl_2 (2 mL). The filtrate and washes were combined and the solvent was removed under reduced pressure.

[0055] Alternatively, b) In a two-dram vial, 0.1 mmol of the linear peptide and HATU (2.0 eq.) were dissolved in 80 mL of CH_2Cl_2 (1.25 mM). DIPEA (6 eq.) was then added and the reaction mixture was left to stir overnight at room temperature (16 h). The solvent was removed under reduced pressure.

Protocol E (reference): Aziridine aldehyde-based macrocyclization

[0056] The linear peptide was dissolved in TFE (if solubility problems were encountered, a 50:50 mixture of TFE:CH_2Cl_2 was used for the cyclization). Then 0.6 eq. of (S)-aziridine-2-carboxaldehyde dimer (prepared as per literature protocol: J. Am. Chem. Soc. 2006, 128 (46), 14772-14773 and Nat. Protoc. 2010, 5 (11), 1813-1822) as a TFE stock solution (0.2 M) was added, giving a final reaction mixture concentration of 0.1 M. *tert*-Butyl isocyanide (1.2 eq.) was then added and the reaction mixture was stirred for four hours. Progress was analyzed along the way via LC-MS.

Protocol F (reference): Nucleophilic ring-opening of acyl aziridine, post macrocyclization

[0057] a) *Thioacetic acid/thiobenzoic acid:* the corresponding thio acid (4 eq.) was added to the crude reaction mixture. Reaction progress was monitored by LC-MS, and was generally complete after 1-2 hours.

[0058] Alternatively, b) *Thiophenol:* Thiophenol (4 eq.) and DIPEA (4 eq.) were added to the crude cyclization mixture. Reaction progress was monitored by LC-MS, and was generally complete after 1-2 hours. Solvent was removed under reduced pressure and dried under vacuum. Crude material was either triturated with Et_2O/hexanes orTBME, or alternatively, diluted with H_2O, frozen and lyophilized.

Protocol G: Suzuki coupling, post macrocyclization

[0059] a) As a general example, an iodo-Phe-containing macrocycle (0.1 mmol), Na_2CO_3 (2 eq.), substituted boronic acid (1.1 eq.) and 4 mL of water:acetonitrile (1:1 ratio) were combined in a microwave vial. The mixture was treated with N_2 gas flow for 10 minutes. While under N_2, silicon based Pd-catalyst (Siliacat-DPP Pd heterogenous catalyst, 0.05 eq.) was added. The reaction vial was sealed and placed in the microwave for 10 minutes at 120 °C (reaction time and temperature were increased to 30 min. and 150 °C, depending on the substrate) or thermally heated at 90 °C for 1h. Reaction progress was monitored by LCMS. Once complete, the reaction was filtered through a Celite plug and the solvent was removed under reduced pressure.

[0060] Alternatively, b) as a specific example, Suzuki couplings with macrocycles that were prepared using 3-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)-4-bromobenzoic acid were conducted as follows: A mixture of crude macrocyclic Compound 340 that had been orthogonally protected as the *β-tert*-butyl ester of the Asp residue and the *tert*-butyl ether of the Thr residue (200 mg, 0.22 mmol) and 4-(4-Boc-piperazino) phenylboronic acid pinacol ester (171 mg, 0.44 mmol) were dissolved in a 1,2-dimethoxyethane (5.4 mL) and Ethanol (1.2 mL) at room temperature. Water (1.2 mL) was added to the solution, followed by Na_2CO_3 (35 mg, 0.33 mmol). The reaction flask was flushed for at least 5 to 10 min under nitrogen gas and then catalyst SiliaCat-DPP Pd (88 mg, 10 mol%, 0.25 mmol/gm) was added. The reaction mixture was heated with stirring under nitrogen at 90°C for 1 hr. LCMS after 1 hour showed complete consumption of substrate and ~ 5% de-bromination compound; the desired Suzuki cross-coupled product represented ~ 84% yield after taking into account the excess of boronate ester by UV. The reaction mixture was cooled to room temperature and filtered over a celite pad to remove catalyst SiliaCat-DPP Pd. The celite pad was washed with a little DCM and the solvents were removed under vacuum to give pale yellow crude solid as the Suzuki coupling product. Reagent 3-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)-4-bromobenzoic acid was itself prepared from methyl 3-(aminomethyl)-4-bromobenzoate (US2011251247) via saponification of the methyl ester and protection of the amine as the Fmoc carbamate, as follows: to a solution of methyl 3-(aminomethyl)-4-bromobenzoate (1.36 g, 5.57 mmol) in Dioxane (33 ml) and Water (9 ml) was added lithium hydroxide (6.13 ml, 6.13 mmol). The mixture was stirred for 3 hrs at room temperature. TLC showed the hydrolysis reaction was complete. Dioxane (16 ml) was added. The mixture was neutralized by the addition of 1 N HCl (aq) (6.17 mL). Sodium bicarbonate (0.468 g, 5.57 mmol) was added,

followed by (9H-fluoren-9-yl)methyl carbonochloridate (2.162 g, 8.36 mmol). The mixture was stirred for 2 hrs at room temperature and was acidified to pH 3 by the addition of 1 N HCl (aq) (6.2 mL). Water (40 ml) was added, extracted with AcOEt (4 x 150 mL). The combined organic layers were dried over sodium sulfate and the solvent was evaporated to ~ 50 ml. Precipitation began to occur and was allowed to slowly continue overnight at room temperature. White solid was then collected by filtration, washed with hexane and dried under high vacuum to afford 3-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)-4-bromobenzoic acid (2.0 g, 4.42 mmol, 79 % yield).

Protocol H (reference): General Ulmann coupling, post macrocvclization

[0061] Under inert atmosphere, the peptide macrocycle (0.018 mmol) was placed in a 2-dram vial containing 2 mL of dry CH_2Cl_2. $Cu(OAc)_2$ (1 eq.), benzene boronic acid (2 eq.) and 4 Å (oven-dried) molecular sieves were then added to the vial followed by DIPEA (4 eq.). The contents of the vial were stirred at room temperature overnight. The reaction progress was assessed by LCMS. Once the reaction was deemed complete, the mixture was filtered through a Celite plug and the solvent was removed under reduced pressure.

Protocol I: General global deprotection and cleavage

[0062] Deprotection of the side chain protecting groups was achieved by dissolving the peptides in 2 mL of a cleavage cocktail consisting of TFA:H_2O:TIS (95:2.5:2.5) for two hours (for sensitive peptides the mixture of TFA:H_2O:TIS (95:2.5:2.5) may be substituted for a mixture of TFA:CH_2Cl_2 (50:50)). Subsequently, the cleavage mixture was evaporated under reduced pressure and the peptides were precipitated twice from chilled diethyl ether/hexanes (or *tert*-butyl methyl ether).

Protocol J (reference): General cleavage of reductively-labile protecting groups

[0063] a) *Pd/C and formic acid debenzylation:* the benzyl protected macrocycle (0.35 mmol) was dissolved in MeOH (8 mL) with 10% formic acid, 10 % wt. Pd/C (Sigma-Aldrich, 37 mg, 0.1 Eq) and heated to 55 °C for 1h to 4h. Once the reaction was deemed complete, the mixture was filtered through a Celite plug, washed with MeOH and the solvent was removed under reduced pressure.

[0064] Or alternatively, b) *Raney Ni desulfurization/debenzylation:* Raney Ni slurry (1-2 mL) was added directly to the cyclization reaction mixture and stirred vigorously overnight. The vial was then centrifuged and the liquid was transferred using a pipette to a tared vial. MeOH was added to the vial containing Raney Ni. The vial was then sonicated, vortexed, and centrifuged. Again, the liquid was transferred to a tared vial. This process was repeated with EtOAc and then a final time with MeOH. The combined washes were then removed under reduced pressure and the residue dried under vacuum.

Protocol K (reference): Amidation of side chain, post macrocyclization

[0065] Macrocycle (0.021 mmol) was dissolved in 1 mL of CH_3CN. K_2CO_3 (5 eq.) and the corresponding acid chloride (2 eq.) were then added and the reaction mixture was left to stir at room temperature overnight. Reaction progress was checked by LC-MS in the morning. Upon completion, the solvent was removed by reduced pressure.

Protocol L (reference): Fluorescent dye attachment

[0066] The macrocycle (4 µmol) was dissolved in DMSO (200 µL). DIPEA (5 eq.) was then added. In a separate

vial, 5 mg of fluorescent dye as the NHS ester was dissolved in 200 µL of DMSO. The macrocycle solution was then added to the solution of the fluorescent label. The reaction mixture was stirred overnight. Reaction progress was checked by LC-MS in the morning and then the solvent was removed by lyophilization.

Protocol M: Purification methods

[0067] All macrocycles were purified using reverse-phase flash column chromatography using a 30 g RediSep C18 Gold Column. The gradient consisted of eluents A (0.1% formic acid in double distilled water) and B (0.1% formic acid in HPLC-grade acetonitrile) at a flow rate of 35 mL/min.

Multimerization Protocols

Protocol N: Linker synthesis for multimerization

[0068]

1. a) *Preparation of Acyl chloride linkers:* Di-, tri- or tetra-carboxylic acids (1 eq.) and CH_2Cl_2 (0.114 M concentration) were added to a two-dram vial. $SOCl_2$ (15 eq. per carboxylic acid) was then added and the reaction mixture was left to stir for four hours at room temperature (some substrates required heating at 70 °C overnight for full solution and/or conversion). The solvent was removed via N_2 flow. The residue was dissolved in 3 mL of dry CH_2Cl_2 which was then removed under N_2 flow. This process was performed two additional times in an attempt to remove any free HCl from the sample. The resulting residue was then used without purification in the dimerization reaction.
2. b) (reference) *Preparation of Benzotriazole linkers, Method A:* Thionyl chloride (2 eq. per carboxylic acid) was added to a solution of benzotriazole (10 eq. per carboxylic acid) in dichloromethane (20 mL per mmol of starting linker) and the solution was stirred at room temperature for 20 min. The di-, tri- or tetra-carboxylic acids (1 eq.) were added to each mixture, which were then stirred at room temperature for 24 h (a change in order of addition did not materially alter the outcome). The reaction was quenched with $NaHCO_3$ (10%, 100 mL) and the layers were separated. The organic layer was washed with HCl (10%, 2x100 mL) and $NaHCO_3$ (10%, 2x100 mL), dried over anhydrous sodium sulfate, filtered and evaporated under vacuum to give the desired Benzotriazole-activated carboxylic acids.
3. c) (reference) *Preparation of Benzotriazole linkers, Method B:* To a suspension of HATU (1.5 eq. per carboxylic acid), Benzotriazole (2 eq. per carboxylic acid) and the di-, tri- or tetra-carboxylic acids (1 eq.) in dichloromethane (20 mL per mmol of starting linker) was added DIPEA (3 eq. per carboxylic acid) and the resultant yellow solution was stirred at room temperature for 16 h. The reaction was quenched with $NaHCO_3$ (10%, 100 mL) and the layers were separated. The organic layer was washed with HCl (10%, 2x100 mL) and $NaHCO_3$ (10%, 2x100 mL), dried over anhydrous sodium sulfate, filtered and evaporated under vacuum to give the desired Benzotriazole-activated carboxylic acids.
4. d) (reference) *Preparation of Lys(CBz)-Pimelic acid-Lys(CBz) linker:* Pimelic acid was converted to the bis-Benzotriazole-activated moiety using Protocol Nb. Commercial N^{α}-Z-L-lysine methyl ester hydrochloride (2 eq.; ChemImpex) was treated with bis-Benzotriazole-activated Pimelic acid (1 eq.) in CH_3CN (0.011 M) containing DIPEA (10 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). The solvent was removed by rotoevaporation and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified *bis*-methyl ester of Lys(CBz)-Pimelic acid-Lys(CBz) as an intermediate. To a solution of the *bis*-methyl ester (1.5 mmol, 1.0 eq.) in THF (10 mL) were added LiCl (3.0 mmol, 2.0 eq.) and LiOH-H_2O (3.0 mmol, 2.0 eq.), followed by H_2O (250 µL) to help solubilize the salts. The reaction was stirred at room temperature overnight. Upon completion of the hydrolysis, as assessed by LC-MS monitor, formic acid was added dropwise to neutralize the basic solution. The solvent was removed by rotoevaporation and

the crude material was submitted to reverse-phase chromatography (Biotage) to obtain the purified di-acid linker.

5. e) (reference) *Preparation of PEG2-Diglycolic acid-PEG2 linker:* Diglycolyl chloride (0.35 mmol; 1 eq.; Sigma Aldrich cat. No. 378151) in anhydrous CH_2Cl_2 (5 mL) was treated with NH_2-PEG2-$CH_2CH_2COOtBu$ (2 eq.; Biochempeg Cat. No. MD005067-2), followed by dropwise addition of DIPEA (3.5 mmol, 10.0 eq); *NB* - this order of addition proved to be very important. The reaction was monitored by LC-MS. After 30 min., the reaction was complete, and longer stirring times did not affect the product ratio. The solvents were removed *in vacuo* and the crude material was submitted to reverse-phase chromatography (Biotage) to obtain the purified di-*tert*-butyl ester intermediate. Removal of the *tert*-butyl ester groups was effected by Protocol I. The diacid linker was isolated as a crude and used as such multimerization reactions without further manipulation.
6. f) (reference) *Preparation of PEG2-Diphenic acid-PEG2 linker:* Diphenic acid was converted to the bis-Benzotriazole-activated moiety using Protocol Nb. Commercial NH_2-PEG2-$CH_2CH_2COOtBu$ (2 eq.; Biochempeg Cat. No. MD005067-2) was treated with *bis*-Benzotriazole-activated Diphenic acid (1 eq.) in CH_3CN (0.011 M) containing DIPEA (10 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). The solvent was removed by rotoevaporation and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified di-*tert*-butyl ester intermediate. Removal of the *tert*-butyl ester groups was effected by Protocol I. The diacid linker was isolated as a crude and used as such in multimerization reactions without further manipulation.
7. g) (reference) *Preparation of PEG2-Pimelic acid-PEG2 linker:* Pimelic acid was converted to the *bis*-Benzotriazole-activated moiety using Protocol Nb. Commercial NH_2-PEG2-$CH_2CH_2COOtBu$ (2 eq.; Biochempeg Cat. No. MD005067-2) was treated with bis-Benzotriazole-activated Pimelic acid (1 eq.) in CH_3CN (0.011 M) containing DIPEA (10 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). The solvent was removed by rotoevaporation and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified di-*tert*-butyl ester intermediate. Removal of the *tert*-butyl ester groups was effected by Protocol I. The diacid linker was isolated as a crude and used as such in multimerization reactions without further manipulation.

Protocol O: Nacellin Multimerization

[0069]

1. a) *Multimerization of amine-containing monomeric macrocycles using bis- or tris-acyl chloride-activated linkers:* The corresponding acyl chloride (0.35 mmol, 1.0 eq.), freshly prepared and under Argon atmosphere, was dissolved in anhydrous CH_2Cl_2 (5 mL; note that larger scale reactions required more-concentrated solution to produce higher-yielding dimerizations). Monomeric macrocycle (2, 3 or 4 eq. for *bis-, tris-,* or *tetra*-acyl chlorides), optimally supplied as the free-base / non-salted form of the reacting amine center, was added to the flask, followed by dropwise addition of DIPEA (3.5 mmol, 10.0 eq); *NB* - this order of addition proved to be very important. The reaction was monitored by LC-MS. After 30 min., the reaction was complete, and longer stirring times did not affect the product ratio. The solvents were removed *in vacuo* and the crude material was submitted to reverse-phase chromatography (Biotage) to obtain the purified product.
2. b) (reference) *Multimerization of amine-containing monomeric macrocycles using Benzotriazole-activated linkers:* To a solution of monomeric macrocycle (2, 3 or 4 eq.), optimally supplied as the free-base / non-salted form of the reacting amine center, and the corresponding Benzotriazole-activated linker, previously prepared but not longer than 1 week prior to multimerization, (0.011 mmol, 1 eq.) in CH_3CN (1 mL) in the presence of DIPEA (0.02 mL, 0.114 mmol, 10 eq). The reaction mixture was stirred for 16h (monitored by LC-MS). The solvent was removed by rotoevaporation and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified product.
3. c) (reference) *Dimerization of amine-containing monomeric macrocycles using 2-Chloroacetyl chloride:* To a

solution of the monomeric macrocycle (0.0571 mmol, 2 eq.), optimally supplied as the free-base / non-salted form of the reacting amine center, in distilled THF (1.0 mL), were added 2-chloroacetyl chloride (3.19 mg, 0.029 mmol, 1 eq.) followed by DIPEA (25 µL, 0.17 mmol, 6.0 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). NaI (8.5 mg, 0.05708 mmol, 2 eq) was then added and the reaction mixture was heated at 50°C for 2h. The solvent was removed *in vacuo* and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified product.

4. d) (reference) *Dimerization of amine-containing monomeric macrocycles using Acryloyl chloride:* To a solution of the monomeric macrocycle (0.0571 mmol, 2 eq.), optimally supplied as the free-base / non-salted form of the reacting amine center, in distilled THF (1.0 mL), were added Acryloyl chloride (2.6 mg, 0.029 mmol, 1 eq.) and then DIPEA (25 µL, 0.17 mmol, 6.0 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). DBU (8.5 µL, 0.057 mmol, 2 eq) was then added and the reaction was heated at 50°C for 5h. The solvent was removed *in vacuo* and the crude material was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified product.
5. e) (reference) *Multimerization of hydroxyl-containing monomeric macrocycles:* Di-, tri- or tetra-carboxylic acid linker (4.3 µmol), monomeric macrocycle (2, 3 or 4 eq.), DMAP (2, 3 or 4 eq.), and EDC·HCl (4, 8 or 12 eq.) were dissolved in DCM (500 - 1000 µL). The reaction mixture was left to stir at room temperature overnight. Reaction progress was assessed by LC-MS. Upon completion, the solvent was removed under reduced pressure and the crude was submitted to reverse-phase silica chromatography (Biotage) to obtain the purified product.
6. f) (reference) *Dimerization of amine-containing monomeric macrocycles using 2,4-dichloro-5-nitropyrimidine:* To a solution of 2,4-dichloro-5-nitropyrimidine (2.0 mg, 0.010 mmol, 1.0 equiv) and monomeric macrocycle (0.021 mmol, 2.1 eq.), optimally supplied as the free-base / non-salted form of the reacting amine center, in chloroform (1 mL), in a 1-dram vial, was added DIPEA (0.02 mL, 0.11 mmol, 11.0 equiv); the reaction mixture immediately turned yellow. Stirring was continued at room temperature overnight, at which point LC-MS analysis exhibited almost full conversion to desired dimer. An additional 24h of reaction time did not lead to any further conversion. Solvent was rotoevaporated to dryness, and the crude residue was submitted to reverse-phase chromatography to afford the purified material in 76% isolated yield.
7. g) (reference) *Multimerization of amine-containing monomeric macrocycles using HATU-activated linkers*: To a solution of the monomeric macrocycle (2, 3 or 4 eq.), optimally supplied as the free-base / non-salted form of the reacting amine center, in 1 mL dry DCM, was added the di-, tri- or tetra-substituted carboxylic acid (1 eq.) under inert atmosphere at room temperature. HATU (3, 6 or 9 eq.) was added to the solution, followed by the addition of DIPEA (3, 6 or 9 eq.). The reaction mixture was left to stir overnight. Assessment of reaction progress by LC-MS after 14h indicated completion. The reaction mixture was rotoevaporated to near-dryness, then placed under high vacuum. If no orthogonal protecting groups required removal (for example, amines protected as the CBz carbamate), the crude material was submitted to reverse-phase chromatography to afford the purified material.
8. h) (reference) *Multimerization of amine-containing monomeric macrocycles using halide-activated linkers:* To a solution of monomeric macrocycle (3.0 eq. if used with a dihalide, 4.5 eq. if used with a trihalide) and the corresponding di or tri-halide linker (1.0 eq) in CH_3CN (2 mL) was added DIPEA (~ 30 eq.). The reaction mixture was stirred for 16h (monitored by LC-MS). The solvent was removed, and crude was submitted to reverse-phase chromatography to afford the purified material.

Integrin α4β7 - MAdCAM-1 ELISA competition assay

[0070] A 96-well Microlon plate (Greiner, 655001) was coated with 100 µl per well of a solution of 1µg/ml recombinant integrin α4β7 (R&D Systems, 5397-A3-050) in carbonate buffer (50 mM, pH 9.6). The plate was incubated at 4°C overnight. The solution was removed and 250 µl blocking buffer (50 mM Tris, 150 mM NaCl, 1 mM $MnCl_2$, 1 % BSA, 0,05% Tween) was added per well. The plate was then incubated for 1 hour at room temperature. The plate was washed three times with wash buffer (50 mM Tris, 100 mM NaCl, 1 mM $MnCl_2$, 0,05% Tween). To each well, 50 µl of compound diluted in assay buffer was added by transfer from a compound serial

dilution plate. 50 µl recombinant MAdCAM-Fc (R&D systems, 6056-MC-050) at a concentration of 0.1 µg/ml in assay buffer (50 mM Tris, 150 mM NaCl, 1 mM $MnCl_2$, 0,1 % BSA, 0,05% Tween) was added to each well. The plate was incubated at room temperature with shaking (300 rpm) for 2 hours to reach binding equilibrium. Then the plate was washed three times in wash buffer and 100 µl anti-human IgG Fc specific-HRP (Abcam, Ab97225) diluted at 1:2000 in assay buffer was added to each well. The plate was incubated at room temperature for 1 hour under agitation. The plate was then washed three times and 100 µl of 1,3',5,5'-Tetramethylbenxidie (TMB, KPL 5120-0083) was then added to each well. The reaction was stopped after 2 minute-incubation by adding 50 µl of 1M H_2SO_4 and optical absorbance was read at 450 nM.

Integrin α4β1- VCAM-1 competition ELISA

[0071] A 96-well Microlon plate (Greiner, 655001) was coated with 100 µl per well of a solution of 0.5 µg/ml recombinant integrin α4β1 (R&D Systems, 5397-A3-050) in carbonate buffer (50 mM, pH 9.6). The plate was incubated at 4°C overnight. The solution was removed and 250 µl blocking buffer (50 mM Tris, 150 mM NaCl, 1 mM $MnCl_2$, 1 % BSA, 0,05% Tween) was added per well. The plate was then incubated for 1 hour at room temperature. The plate was washed three times with wash buffer (50 mM Tris, 100 mM NaCl, 1 mM $MnCl_2$, 0,05% Tween). To each well, 50 µl of compound diluted in assay buffer was added by transfer from a compound serial dilution plate. 50 µl recombinant VCAM-Fc (R&D systems, 862-VC-100) at a concentration of 0.1 µg/ml in assay buffer (50 mM Tris, 150 mM NaCl, 1 mM $MnCl_2$, 0,1 % BSA, 0,05% Tween) was added to each well. The plate was incubated at room temperature with shaking (300 rpm) for 2 hours to reach binding equilibrium. Then the plate was washed three times in wash buffer and 100 µl anti-human IgG Fc specific-HRP (Abcam, Ab97225) diluted at 1:2000 in assay buffer was added to each well. The plate was incubated at room temperature for 1 hour under agitation. The plate was then washed three times and 100 µl of 1,3',5,5'-Tetramethylbenxidie (TMB, (TMB, KPL 5120-0083) was then added to each well. The reaction was stopped after 2 minute-incubation by adding 50 µl of 1M H_2SO_4 and optical absorbance was read at 450 nM.

Integrin α4β7-MAdCAM cell adhesion assay

[0072] RPMI8866 human cells (Sigma #95041316) were cultured in RPMI 1640 medium (HyClone SH30027.1) supplemented with 10% FBS (Seradigm) and 1% Penicillin-Streptomycin. A 96-well plate (Costar, 3603) was coated with 100 ml/well of human recombinant MAdCAM-1 Fc Chimera (R&D Systems, 6056-MC-050) solution at 0.25 µg/ml in coating buffer (50mM sodium carbonate, pH 9.6). The plate was incubated overnight at 4°C and washed twice with 150µl per well wash buffer (0.05% Tween 20 in PBS), blocked with 250 µl per well blocking buffer (1% non-fat dry milk in PBS), and incubated for 2 hours at room temperature. RPMI8866 cells were resuspended at 10 million cells/ml in PBS containing 5 mM calcein and incubated at 37 °C for 30 min in a 50 ml tube. PBS was added to fill the tube, cells were spun down and resuspended in RPMI 1640 medium to 2 million/ml. Compounds were diluted by serial dilution in binding buffer (1.5 mM $CaCl_2$, 0.5 mM $MnCl_2$, 50 mM Tris-HCl, pH 7.5) to a final volume of 50 µl per well at 2x concentration. The plate was washed once with 300 µl of PBS, 50 µl of compound and 50 µl of cells (100,000 cells) were transferred to each well and the plate was incubated in the dark at 37°C, 5% CO_2 for 45 min to allow cell adhesion. The plate was emptied by inverting and blotting on paper towels and washed manually twice with PBS. 100 µl PBS was then added to each well. The fluorescence was read (Ex_{495}/Em_{515}) using a plate reader (Tecan Infinite 1000). To calculate the dose response, the fluorescence value of control wells not containing cells was subtracted from each test well.

Integrin α4β1-VCAM cell adhesion assay

[0073] RAMOS human cells (ATCC CRL-1596) were cultured in RPMI 1640 medium (HyClone SH30027.1) supplemented with 10% FBS (Seradigm) and 1% Penicillin-Streptomycin. A 96-well plate (Costar, 3603) was coated

with 100 ml/well of recombinant human VCAM-1 Fc Chimera (R&D systems, 862-VC-100) solution at 0.25 µg/ml in coating buffer (50mM sodium carbonate, pH 9.6). The plate was incubated overnight at 4°C and washed twice with 150µl per well wash buffer (0.05% Tween 20 in PBS), blocked with 250 µl per well blocking buffer (1% non-fat dry milk in PBS), for 1 hour at room temperature. During blocking step, RAMOS cells were resuspended at 10 million cells/ml in PBS containing 5 mM calcein and incubated at 37 °C for 30 min in a 50 ml tube. PBS was added to fill the tube, cells were spun down and resuspended in RPMI 1640 medium to 2 million/ml. Compounds were diluted by serial dilution in binding buffer (1.5 mM $CaCl_2$, 0.5 mM $MnCl_2$, 50 mM Tris-HCl, pH 7.5) to a final volume of 50 µl per well at 2x concentration. The plate was washed once with 300 µl of PBS, 50 µl of compound and 50 µl of cells (100,000 cells) were transferred to each well and the plate was incubated in the dark at 37°C, 5% CO_2 for 45 min to allow cell adhesion. The plate was emptied by inverting and blotting on paper towels and washed manually twice with PBS. After last wash, 100 µL of PBS was added to wells and the fluorescence was read (Ex_{495}/Em_{515}) using a plate reader (Tecan Infinite 1000). To calculate the dose response, the fluorescence value of control wells not containing cells was subtracted from each test well.

Analyte competition assay in CD4+ integrin α_4+β_7-lo memory T cells

[0074] Receptor occupancy in primary cells was determined by measuring the amount of biotinylated human recombinant MAdCAM-1-FC or human recombinant VCAM-1-Fc bound to selected cell populations using flow cytometry. Human recombinant MAdCAM-1-FC or human recombinant VCAM-1-FC (R&D systems) were biotinylated using commercially available reagents and protocol (Pierce).

[0075] Whole blood was collected from human donors in sodium heparin tubes. A volume of 100 microL of blood was incubated with compound and 4mM $MnCl_2$ for 1 hour at room temperature. Cells were washed twice with 1 mL of 1X DPBS calcium magnesium free(CMF) (ThermoFisher Scientific) and resuspended in 100 microL of DPBS CMF.

[0076] Biotinylated human recombinant MAdCAM-1-Fc or VCAM-1-Fc were added at saturating concentration and incubated at room temperature for 1 hour. A volume of 2 mL of 1X BD FACS Lyse (BD Biosciences) was then added and the mixture was incubated for 8-12 minutes at room temperature in the dark to lyse red blood cells. Cells were washed with 1 mL stain buffer-FBS (BD Biosciences) and resuspended in 100 µl stain Buffer-FBS (BD Biosciences) containing 4mM $MnCl_2$. Biotinylated-rhMAdCAM-1 was applied at a saturating concentration of 1200 ng/mL to compete with test article binding and incubated at room temperature for 1 hour. Cells were then washed with 1mL stain buffer-FBS and resuspended in 100 µl stain buffer-FBS. The cells were incubated in the dark for 30 minutes at room temperature with 1 µl Streptavidin APC (Biolegend 0.2mg/ml) and a panel of antibodies for the detection of memory T helper a4b7-positive cells subset. And amount of 5.0 µl each of the following antibodies were used; CD45 FITC (BioLegend 200 µg/ml), CD29 APC Cy7 (BioLegend 100 µg/ml), Integrin beta7 PE, (BioLegend concentration 50 µg/mL), CD49d V421 (BioLegend 50 µg/mL), CD3 V510 (BioLegend 30 µg/mL), CD4 PECy7 (BioLegend 100 µg/mL), CD45RO PerCP, BioLegend 200 µg/mL). The cells were then washed with stain-buffer-FBS and resuspended in 150 microL stain buffer-FBS for acquisition on the flow cytometer (BD FACSCanto™ flow cytometer and BDFACSDiva™ software). FACS data was acquire by electronic gating on the basis of forward versus side scatter, The cytometer was set to collect 20,000 events in each tube. Cell population were determined using the following markers, CD45+, CD3+, CD4+,CD45RO+, CD49d+ , integrin b7, biotinylated ligands.

[0077] Compound receptor occupancy was defined as the decrease in the number of integrin β_7+ or integrin β_7-lo cells binding biotinylated rhMAdCAM-1 or rhVCAM-1, respectively.

[0078] Receptor occupancy was calculated with the following equation: 100-((% ligand-positive cells with compound/% ligand-positive cells DMSO)*100)

[0079] In vivo T lymphocyte trafficking analysis in mouse model of colitis

[0080] Animal care: The animal care facility employed is accredited by the Canadian Council on Animal Care (CCAC). This study was approved by a certified Animal Care Committee and complied with CACC standards and regulations governing the use of animals for research. The animals were housed under standardized environmental conditions. A standard certified commercial rodent diet was provided *ad libitum*. Tap water was provided *ad libitum* at all times.

[0081] Dextran sulfate sodium (DSS) was administered to C57Bl/6 female mice for five days through addition to their drinking water at 3%. Body weight and disease activity index ("DAI") were measured on Day 5 in order to distribute DSS-treated animals in uniform groups prior to dosing. DAI was scored based on the severity three specific symptoms associated with colitis: 1- blood in stool (negative hemoccult, positive hemoccult, blood traces in stool visible, rectal bleeding); 2-stool consistency (normal, soft but still formed, very soft, diarrhea); 3- body weight loss.

[0082] From Day 6 to day 9, Compound No. 517 (ET03764) or the vehicle were administered orally daily at 5 ml/kg. On day 9, four hours after dosing, the animals were euthanized by cardiac puncture under general anesthesias. Mesenteric lymph nodes (MLN) were collected, triturated, and washed in HBSS-FCS. The cells were incubated for 15 minutes in BD mouse FcBlock followed by 30-minute incubation with specific antibodies. After washes, cells were either fixed using BD fix solution or immediately process for cell surface marker staining. The antibodies used were as followed: CD4 PE (BD Bioscience), CD44 FITC (BD Biosciences), CD45RB PerCy 5.5 (BD Biosciences), a4b7 PE (eBiosciences). Cell populations were then analyzed using FACSCanto cytometer and gating on CD4+, $CD44^{hi}$, $CD45RB^{low}$, α4β7+.

[0083] Statistical analysis was performed using GraphPad Prism. Differences among groups were evaluated by two-way ANOVA, wit a 95% confidence interval.

Results and Discussion

[0084] Compounds were synthesized in accordance with the above-noted methods. A selection of compounds was characterized using NMR (not all data shown). A subset of NMR data is provided in Figure 6 for Compound No. 390 (not part of the present invention).

Binding affinity and selectivity of compounds for integrin α4β7 and α4β1

[0085] We measured binding potency for monomeric and dimeric compounds to α4β7-integrin using a battery of biochemical, cell-based and ex-vivo assays. Multimeric compounds were generally more potent in cellular assays.

[0086] We measured the ability of test articles to prevent the adhesion of RMPI8866 cells, which express integrin α4β7, to plates coated with MAdCAM-1. Multimeric compounds were generally more potent in their ability to inhibit cell adhesion than their constituent monomers. For example reference Compound No. 340 (ET2451) and Compound No. 456 (ET4062) (not part of the present invention) had IC50 of 175 and 199 nM respectively in the RPMI8866 cell adhesion assays (Table 1C and 1C'). Multimeric compounds with over 10-fold greater potency than their constituent monomeric compounds were generated. For example, the compound of the present invention, namely Compound No. 517 (ET3764), a homodimer of reference Compound No. 340 (ET2451), had an IC50 of 9.9 nM in the RPMI8866 cell adhesion assay. Compound multimers generated from monomeric Compound 456 (ET4062) also showed higher binding affinity (Table 2C) (not part of the present invention).

[0087] Similar results were obtained in a ligand competition assay for binding to integrin α4β7 in human whole blood. Receptor occupancy of nacellins was determined by measuring the proportion of α4β7+ memory T helper cells able to bind biotinylated rhMAdCAM-1 using flow cytometry (Figure 7). Multimeric compounds were able to compete with MadCAM-1 on α4β7-positive primary cells with greater potency than monomeric compounds. Two

general monomeric chemotypes were shown to compete more effectively, with increased potency for binding to integrin α4β7 when multimerized using a variety of linkers. For example, Dimeric Compound No.s 534 (ET4113) (not part of the present invention) and 535 (ET4110) (not part of the present invention) demonstrated IC50 of 38 and 76 nM respectively while the corresponding parent monomeric Compound No. 456 (ET4062) only reached 15% receptor occupancy at the maximum concentration of 1000 nM. Similarly, the dimeric Compound No. 517 (ET3764) of the present invention and 390 (ET3755) (not part of the present invention) competed with a saturating amount of MAdCAM, with EC50 of 38 and 90 nM respectively within the same study. The corresponding monomeric Compound 340 (ET2451) reached 50% receptor occupancy at low concentrations but no concentration-response curve could be obtained. This could be the result of non-specific binding of the monomeric compound to the cell.

[0088] Interestingly, differences in binding affinity between monomeric and multimeric compounds were not as pronounced in ELISA binding assays. It is possible that avidity enhances the binding potency of multimeric compounds in cells.

[0089] Multimeric compounds showed enhanced selectivity for integrin α4β7 over integrin α4β1. In order to determine the selectivity of the compounds in cell assays, we measured the adhesion of Ramos cells, which express integrin α4β1 to VCAM-coated plates. Multimeric compounds had generally higher selectivity for integrin α4β7 over integrin α4β1 than their monomeric constituents. For example, monomeric Compound No.s 340 (ET2451) and 456 (ET4062) showed 16- and 45-fold selectivity, respectively, when comparing α4β7 versus α4β1 cell adhesion assays. In contrast, multimeric compounds based on monomeric reference Compound No. 340 (ET2451) exhibited 20- to 100-fold selectivity in favor of integrin α4β7, and multimeric compounds based on monomeric Compound No. 456 (ET4062) (not part of the present invention) exhibited no measurable effect on the adhesion of α4β1-expressing Ramos cells to VCAM (Table 2C).

***In vivo* T lymphocyte trafficking analyses**

[0090] The ability of several integrin alpha-4-beta-7-inhibiting compounds to attenuate the trafficking of integrin alpha-4-beta-7-expressing T lymphocytes was demonstrated in *in vivo* pharmacodynamics studies in DSS-treated mice. Dextran Sodium Sulfate (DSS) induces chronic colitis in experimental animals when given orally in drinking water for five days followed by no DSS in drinking water. Chronic inflammation is associated with the infiltration of leucocytes from the blood to intestinal tissues. The interaction between integrin α4β7 and MAdCAM-1 on the endothelium of the gut allows adhesion and trafficking of T cells to the gut. The ability of several integrin alpha-4-beta-7-inhibiting nacellins to attenuate the trafficking of integrin alpha-4-beta-7-expressing T lymphocytes was demonstrated in *in vivo* pharmacodynamics studies in DSS-treated mice.

[0091] A study was conducted in which mice were exposed for 5 days to dextran sulfate in their drinking water. On days 6 to 9, compounds or vehicle were administered orally daily. Mesenteric lymph nodes were collected 4 hours following the last dose and assessed. As shown in Figure 8, Compound No. 517 (ET3764) of the present invention reduced the detection of integrin α4β7+ T helper memory lymphocytes in the mesenteric lymph nodes (MLN). Compound No. 517, administered at a dose of 80 mg/kg, reduced the number of α4β7+ positive lymphocytes by 60%.

[0092] We determined that the level of reduction in α4β7+ T helper memory lymphocytes detected in the mesenteric lymph nodes of DSS treated mice was dependent on the dose of Compound No. 517 administered. Figure 9 shows the dose-dependent reduction in α4β7+ T cells present in the mesenteric lymph nodes.

[0093] We compared the ability of compounds to inhibit the binding of labeled human recombinant MADCAM-1 or VCAM to α4β7-positive or α4β7-negative Th memory cells respectively. Whole blood from a single donor was incubated with compounds and saturated amounts of recombinant ligands. The inhibition of MAdCAM or VCAM binding was measured on T cell subsets using FACS analysis. As shown in Figure 10, representative multimeric Compound No. 517 of the present invention, as well as compound nos. 482, 530 and 534 (not part of the present

invention) inhibited MAdCAM-1 binding to primary cells with IC50 values ranging from 87 to 141 nM. The same representative compounds bound to VCAM with lower affinity, with IC50 values ranging from 600 nM to undetectable binding at 4000 nM (Figure 11).

TABLE 1A

Compound No.	R^1	R^2	R^3	R^4	R^5
340 (ref)	H	H	CH_3	H	C(O)-NH-*tert*-Butyl

TABLE 1B

Compound No.	Seq .ID. No.	R^6	R^7	R^8	X^y	X^z	X^1	X^2	X^3
340 (ref)	334	PRO	PRO	H	[3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoic acid]		L	D	T

TABLE 1C

Compound No.	ELISA α4β7 Assay IC_{50} (μM)	ELISA α4β1 Assay IC_{50} (μM)	ELISA Assay Ratio β1/β7	RPMI8866 Adhesion α4β7/MAdCAM IC50 (μM)
340 (ref)	0.019	0.048	2.5	0.263

ABLE 1C'

Compound No.	MADCAM FACSα 4β7 Th mem (nM)	RPMI8866 Adhesion α4β7/MAdCAM IC50 (nM)	Ramos Adhesion α4β1/VCAM IC_{50} (nM)	Ratio Ramos/RPMI	ELISA α4β7 Assay IC_{50} (nM)	ELISA α4β1 Assay IC_{50} (nM)	ELISA Assay Ratio β1/β7	VCAM F Th m
340 (ref)	Unclear	175	2767	16				

TABLE 1X

Compound No.	LC-MS (m/z)	Experimental Protocol
340 (ref)		A,D,G,I,M

TABLE 2A

Compound No.	R^1	R^2	R^3	R^4	R^5
517	H	H	CH_3	H	C(O)-NH-*tert*-Butyl

TABLE 2B

Compound No.	Seq. ID. No.	R^6	R^7	R^8	X^y	X^z	X^1	X^2	X^3
517	334	PRO	PRO	H	[3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoic acid]		L	D	T

TABLE 2C

Compound No.	MADCAM FACS α4β7 Th mem (nM)	RPMI8866 Adhesion α4β7/MAdCAM IC50 (nM)	Ramos Adhesion α4β1/VCAM IC_{50} (nM)	Ratio Ramos/RPMI	ELISA α4β7 Assay IC_{50} (nM)	ELISA α4β1 Assay IC_{50} (nM)	ELISA Assay Ratio β1/β7	VCAM FACS α4+β7- Th mem (nM)
517	43	10	211	21	7.5	2.1	0.3	607

Table 2X

Compound No.	LC-MS (m/z)	Reagent employed to form multimer	Linkage type(s)	Multimer type	Representative structures of Linker moieties (the number 1 represents an attachment point between Linker and monomeric macrocycle)	Experimental Protocol
517	979.6	diphenic acid	amide	homodimer		A,D,Gb,I,M,Na,Oa

REFERENCES CITED IN THE DESCRIPTION

Cited references

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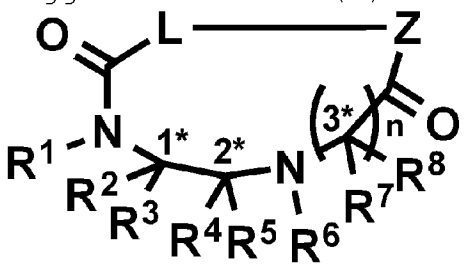
- J. Am. Chem. Soc., 2006, vol. 128, 4614772-14773 [0056]
- Nat. Protoc., 2010, vol. 5, 111813-1822 [0056]

Patentkrav

1. Homodimer af to forbindelser, der er kovalent koblet sammen via en amidbinding til en diphensyrerest, hvilke forbindelser begge har formel (I):



(I)

hvor

R^1 er H;

R^2 er H;

R^3 er CH_3;

R^4 er H;

R^5 er et amid med formlen -C(O)NR**R***, hvor R** er H, og R*** er *tert*-butyl;

R^6 sammen med R^7 danner pyrrolidinringen af Pro,

R^8 er H;

n er 1;

hvor Z er en aminoterminal af en aminosyre; -C=O-, der støder op til L, er carboxyterminalen af en aminosyre; og L sammen med Z og -C=O- er et peptid med følgende formel:

X^y-X^z-X^1-X^2-X^3

hvor

X^y er 3-aminomethyl-4-[4-(1-piperazinyl)-phenyl]-benzoesyre;

X^z er fraværende;

X^1 er Leu;

X^2 er Asp; og

X^3 er Thr;

eller et farmaceutisk acceptabelt salt deraf.

2. Farmaceutisk sammensætning, der omfatter homodimeren ifølge krav 1 sammen med et farmaceutisk acceptabelt bæremateriale.

3. Farmaceutisk sammensætning ifølge krav 2, der er formuleret

til oral, topisk eller parenteral administration.

4. Homodimer ifølge krav 1 til anvendelse til behandling af en tilstand eller sygdom valgt fra gruppen, der består af inflammatorisk tarmsygdom (IBD); ulcerativ kolitis; Crohns sygdom; cøliaki (ikke-tropisk sprue); kollagenøs kolitis, eosinofil gastroenteritis,; pouchitis som følge af proktokolektomi og ileoanal anastomose; human immundefektvirus (HIV)-infektion ved mikroskopisk kolitis i mave-tarmkanalen og graft-versus-host-sygdom.

5. Homodimer til anvendelse ifølge krav 4, hvor tilstanden er en inflammatorisk tarmsygdom.

6. Homodimer til anvendelse ifølge krav 5, hvor den inflammatoriske tarmsygdom er ulcerativ kolitis.

7. Homodimer til anvendelse ifølge krav 5, hvor den inflammatoriske tarmsygdom er Crohns sygdom.

8. Homodimer til anvendelse ifølge et hvilket som helst af kravene 4-7, hvor patienten er et menneske.

DRAWINGS

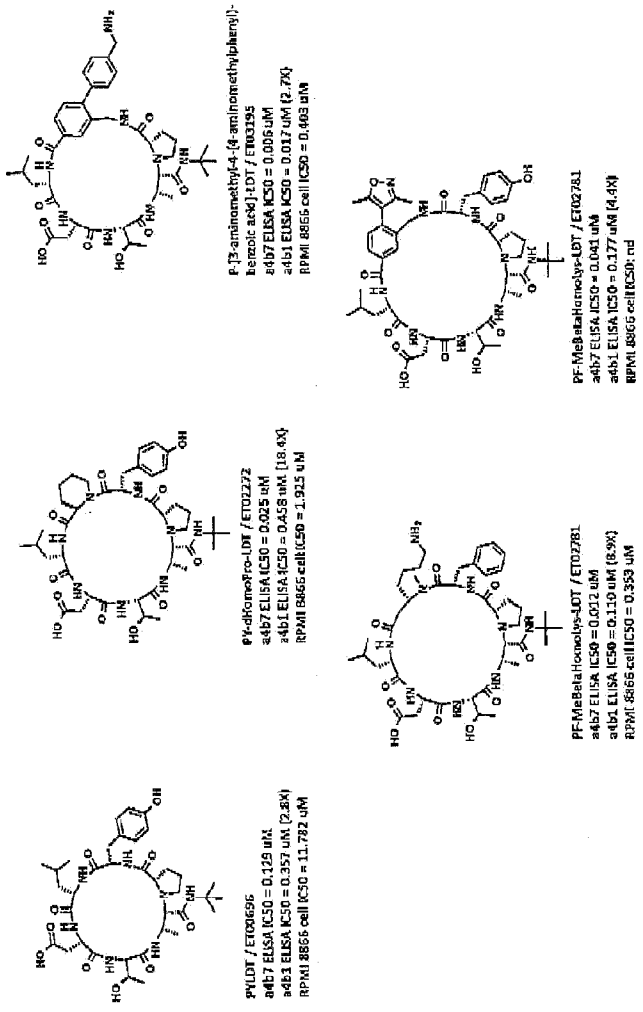
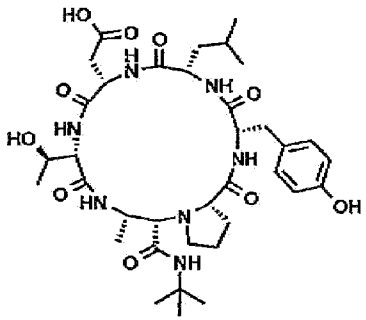


Figure 1

Thr	a4b7 IC50 (uM)
Ile	0.026
Thr(OBn)	0.036
Thr(OEt)	0.042
Thr(OMe)	0.063
Pen	0.113
Val	0.109
dCys-Me ether	0.262
cyclohexylGly	0.389
allo-Ile	0.475
Cys	1.00
Cys(Acm)	1.00
thio-dPro	1.00
tBu-Gly	1.088
Asn	4.00
Gln	15
His	45.3
2-aza-dPhe	56.6

Asp	a4b7 IC50 (uM)
Asp(alcohol)	27.726
Asp(OBn)	40
Asp(OEt)	50 (27 cell)

Leu	a4b7 IC50 (uM)
Cha	0.375
vinyl-Br-Leu	0.609
Nle	0.785
HomoLeu	1.703
cyclopropylAla	3.025
CF3-(d/l)Leu	3.358
Nle	3.668
(d/l)Leu	3.615
Cha	3.896
Phe	30.00
Trp	40.00



PYLDT / ET00696
a4b7 ELISA IC50 = 0.129 uM
a4b1 ELISA IC50 = 0.357 uM (2.8X)
RPMI 8866 cell IC50 = 11.782 uM

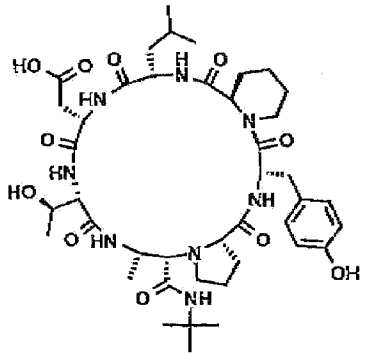
Figure 2

"Tyr"	a4b7 IC50 (uM)
Met	0.005
Tyr(O-allyl)	0.009
CycloLeu	0.011
Aic	0.016
Pro	0.019
dTiq	0.021
dTic	0.022
dArg	0.023
1Nal	0.026
4-aza-dPhe	0.03
dHomoPro	0.03
3-aza-dPhe	0.04
4-amino-dPhe	0.035
Orn(ethylCarb)	0.04
dTyr(O-allyl)	0.036
Orn(Ac)	0.039
2Nal	0.04
Phe	0.042
dTrp	0.046
dTyr(O-Bn)	0.05
Trp	0.051
4-amineMe-dPhe	0.052
benzthiophenhyl-dAla	0.053
Lys	0.053
2-thiophen-yl-dAla	0.054
L-Dtic	0.057
Tyr(4-CO2H diaryl ether)	0.057
Met-sulfoxide	0.243
P-Cit-LDT	0.825
Dab	0.873
Gly	0.963
Ser(OBn)	0.995
Tic	1.05
Asn	1.078
Glu	1.496
Asp	3.832
D-Dtic	3.664
Asp-acyl-methanesulfonamide	6.935

Leu	a4b7 IC50 (uM)
tertbutylAla	0.054
Nle	0.719
neopentylglycine	0.83
MeLeu	1.04
NorVal	1.443
HomocycloLeu	3.665

Asp	a4b7 IC50 (uM)
no variants	

Thr	a4b7 IC50 (uM)
Ile	0.006
Abu	0.018
alloThr	0.046
MeThr	0.192
HomoSer	2.242
dThr	3.116
Ser	4.162



PY-dHomoPro-LDT / ET02272
a4b7 ELISA IC50 = 0.025 uM
a4b1 ELISA IC50 = 0.458 uM (18.4X)
RPMI 8866 cell IC50 = 1.925 uM

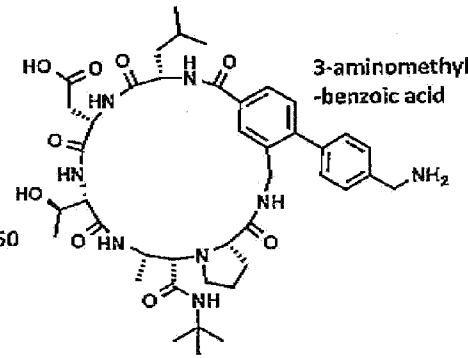
"dHomoPro"	a4b7 IC50 (uM)
dTic	0.007
dPro	0.013
cis-dHyp	0.019
trans-dHyp	0.019
Pro	0.027
dLys	0.052
Ala	0.053
dNle	0.054
dAla	0.054
Sar	0.059
dMeArg	0.067
Aze	0.073
dArg	0.083
dSer	0.096
trans-Hyp	0.100
Gly	0.180
dMeLys	0.117
cycloLeu	0.120
dVal	0.128
dThr	0.132
dLys	0.133
dTqa	0.215
alphaMeAla	0.219
Cba	0.222
dTrp	0.223
BnGly	0.230
2Gal	0.253
Tyr	0.274
dGln	0.287
His	0.299
N-methylcycloLeu	0.404
Gln	0.416
Asn	0.559
Dab	0.582
Lys	0.600
Val	0.677
MeNle	0.809
Nle	0.855
dPhe	0.951
Met	1.155
Gli	1.164
Hyronine	1.537
dMet	1.575
2-Igl	1.739
[Ac]Lys	1.804
dMeOrn	2.291
Ser	2.656
Thr	2.944
Asn	3.061
dHig	3.646
dTpi	(7,058 - cell)
4-guanidino-Phe	(8,240 cell)
Tic	(24,080 cell)

"Tyr"	a4b7 IC50 (uM)
Tyr(O-allyl)	0.013
2-(2-pyridyl)-4-thiazolyl-Ala	0.013
2-(4-pyridyl)-4-thiazolyl-Ala	0.013
2-Iodo-Phe	0.015
3-aminomethyl-Phe	0.016
2-(3-pyridyl)-4-thiazolyl-Ala	0.017
2-(amino-benzyl)-4-thiazolyl-Ala	0.018
thiazolyl-Ala	0.018
Phe	0.019
4-aminomethyl-Phe	0.019
2,4-dichloro-Phe	0.021
2-Phenyl-Phe	0.023
Bip	0.024
3-[2-methoxy-Phenyl]-Phe	0.024
3,4-di-methoxy-Phe	0.025
3,4,5-tri-fluoro-Phe	0.026
cycloLeu	0.027
2-(3-methoxy-Phenyl)-Phe	0.032
2-(4-methoxy-Phenyl)-Phe	0.033
1Nal	0.033
2Nal	0.043
3-aminomethyl-dPhe	0.052
3-Iodo-Phe	0.059
dTyr	0.062
3,5-di-bromo-1-Tyr	0.065
dl-Phenyl-Ala	0.077
2-aminomethyl-Phe	0.083
3-phenyl-dPhe	0.085
MeTyr	0.086
MeL	0.135
4-Iodo-Phe	0.150
Tic	0.209
4-[2,6-dimethoxybenzoxy]-Phe	0.234
thyronine	0.262
Ala	0.274
Orn	0.307
3-[4-Quinolinyl]-dPhe	0.449
dHomoPro	1.068
BnGly	1.086
dTic	1.273
dAla	10
3-acetylaminomethyl-Phe	(0.616 - cell)
3-[6-Quinolinyl]-dPhe	(1.411 - cell)
3-[7-Quinolinyl]-dPhe	(23.142 - cell)
3-[6-Quinolin]-dPhe	(23.646 - cell)
3-[3-aminomethylphenyl]-dPhe	(25.690 - cell)

Figure 3

Leu	a4b7 IC50 (uM)
tertbutylAla	0.048
3-aminomethyl benzoic acid	7.630
MeLeu	7.904

Asp	a4b7 IC50 (uM)
MeAsp	3.746
Asp(ethyl ester)	(8.277 - cell)



3-aminomethyl-4-(4-aminomethylphenyl) -benzoic acid

Thr	a4b7 IC50 (uM)
Abu	0.020
Thr(O-Bn)	0.024
*allo*Thr	0.031
MeThr	0.231

a4b7 IC50 (uM)

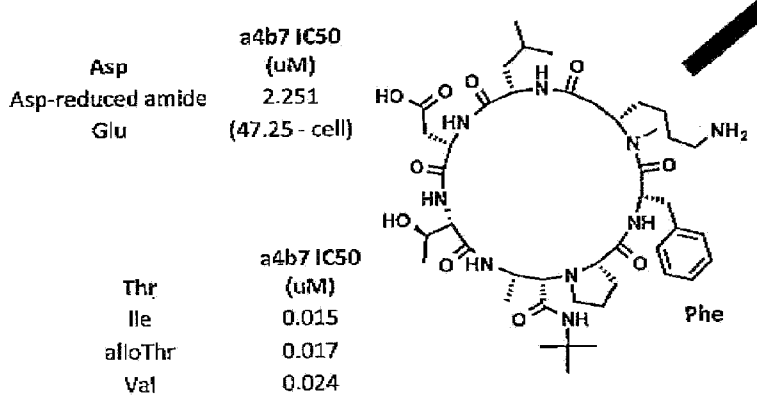
~ 50 variants
0.009 – 10.0 uM IC50s

P-[3-aminomethyl-4-(4-aminomethylphenyl)-benzoic acid]-LDT / ET03195
a4b7 ELISA IC50 = 0.006 uM
a4b1 ELISA IC50 = 0.017 uM (2.7X)
RPMI 8866 cell IC50 = 0.403 uM

Figure 4

Leu	a4b7 IC50 (uM)
Leu-reduced amide	2.009
neopentylGly	(1.310 cell)
Phe	(47.25 - cell)

Asp	a4b7 IC50 (uM)
Asp-reduced amide	2.251
Glu	(47.25 - cell)



Thr	a4b7 IC50 (uM)
Ile	0.015
alloThr	0.017
Val	0.024
Thr(OBn)	0.043
alloIle	0.046
betaHomoIsoLeu	2.933

PF-MeBetaHomoLys-LDT / ET02781
a4b7 ELISA IC50 = 0.012 uM
a4b1 ELISA IC50 = 0.110 uM (8.9X)
RPMI 8866 cell IC50 = 0.353 uM

MeBetaHomoLys	a4b7 IC50 (uM)
betaHomoLys	0.002
MebetaHomoLys(Me)2	0.017
BetaHomoIsoLeu	0.034
betaHomoPro	0.034
dBetaHomoPro	0.058
dbetaHomoLys	0.061
Aze	0.065
betaHomoThr	0.092
betaHomoNle	0.110
dBetaHomoPro	0.120
anthranilic acid	0.158
Pf-Nip-LDT	0.215
cis-2-aminocyclohexanecarboxylic acid	0.276
betahomoAla	0.278
(1S,2R)-2-aminocyclohexane carboxylic acid	0.297
betaAla	0.300
trans-2-aminocyclohexanecarboxylic acid	0.412
PY-propionimide-LDT	0.576
dBetaHomoPhe	0.829
dLys	1.135
(1R,2R)-2-aminocyclohexanecarboxylic acid	1.579
3-aminomethyl-phenylacetic acid	1.973
dMebetaHomoLys	2.281
MebetahomoLys(Ac)	(0.784 - cell)

Figure 5

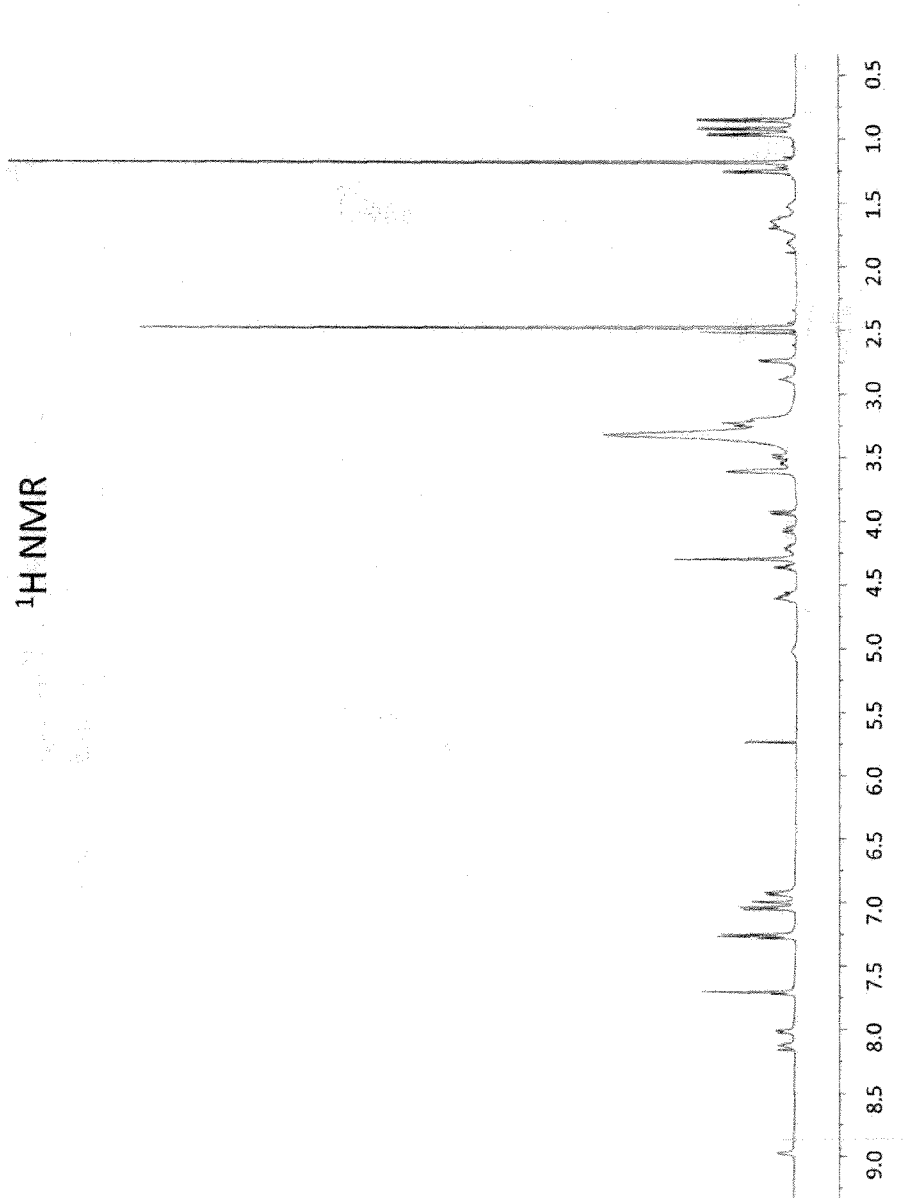


Figure 6A

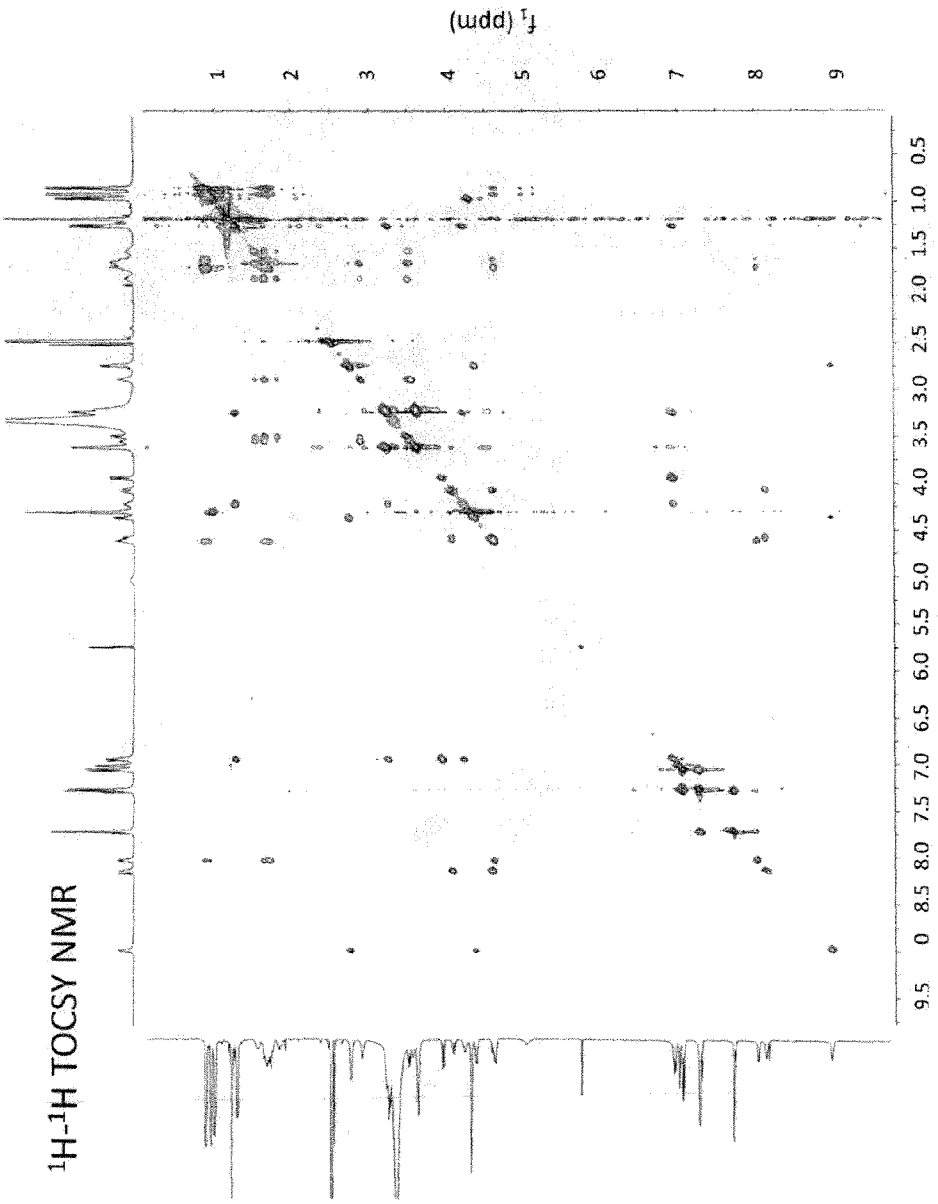
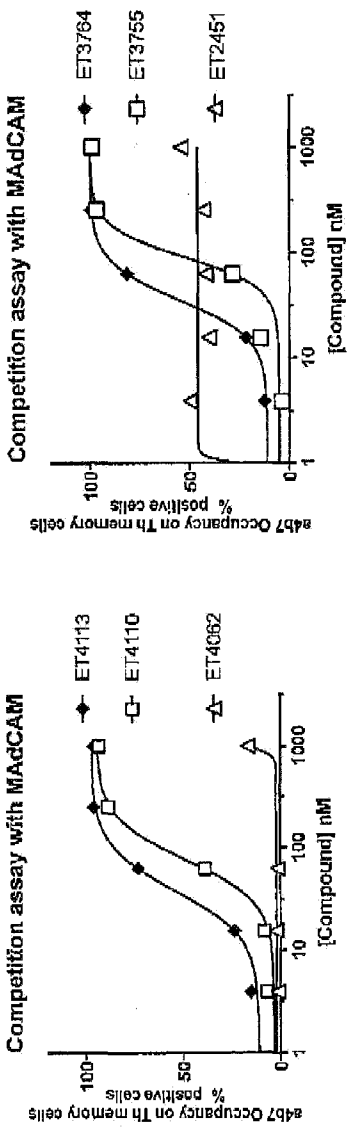


Figure 6B


Competition assay with MAdCAM
ET3764
ET3755
ET2451
α4β7 Occupancy on Th memory cells
% positive cells
100
50
0
1
10
100
1000
[Compound] nM

Competition assay with MAdCAM
ET4113
ET4110
ET4062
α4β7 Occupancy on Th memory cells
% positive cells
100
50
0
1
10
100
1000
[Compound] nM

Figure 7

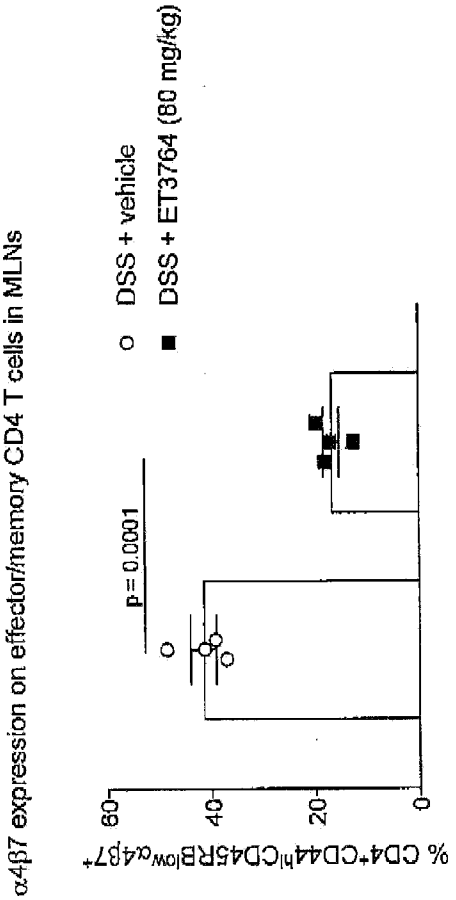

α4β7 expression on effector/memory CD4 T cells in MLNs
DSS + vehicle
DSS + ET3764 (80 mg/kg)
p = 0.0001
% CD4+CD44hiCD45RBlowα4β7+
60
40
20
0

Figure 8

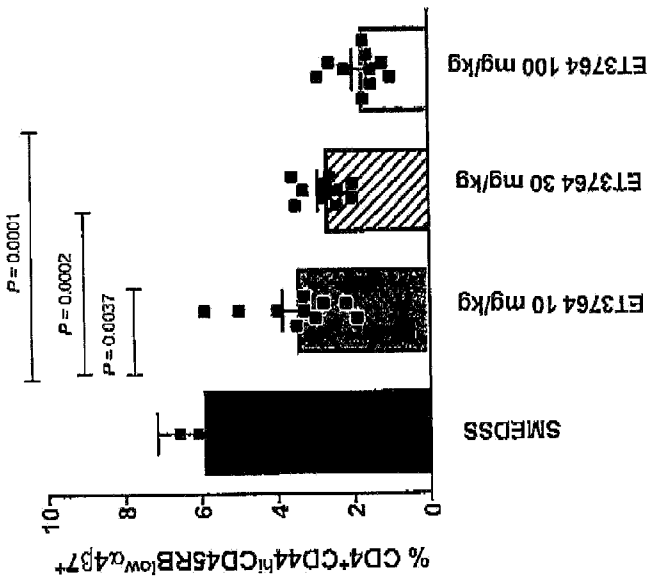

% CD4+CD44hiCD45RBlowα4β7+
10
8
6
4
2
0
SMEDSS
ET3764 10 mg/kg
ET3764 30 mg/kg
ET3764 100 mg/kg
P = 0.0001
P = 0.0002
P = 0.0037

Figure 9

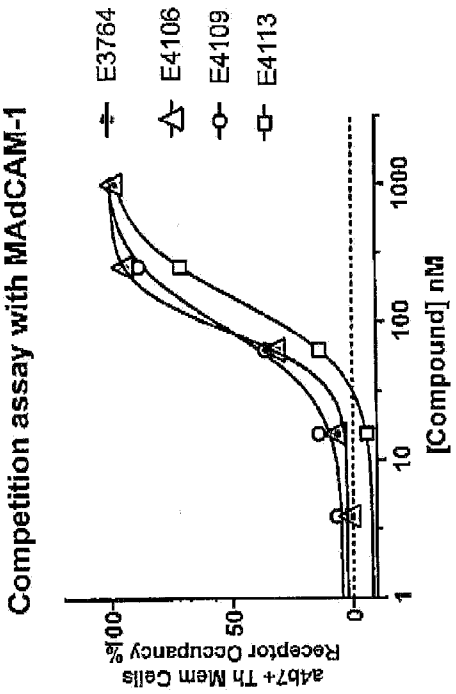

Competition assay with MAdCAM-1
E3764
E4106
E4109
E4113
a4b7+ Th Mem Cells
Receptor Occupancy %
100
50
0
1
10
100
1000
[Compound] nM

Figure 10

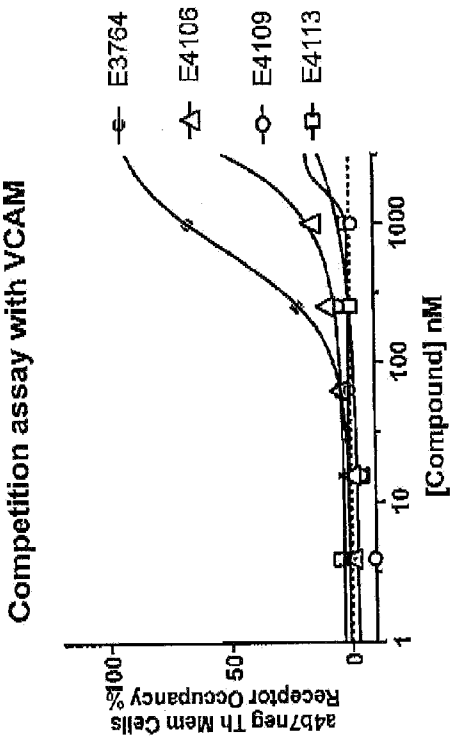

Competition assay with VCAM
E3764
E4106
E4109
E4113
a4b7neg Th Mem Cells
Receptor Occupancy %
100
50
0
1
10
100
1000
[Compound] nM

Figure 11