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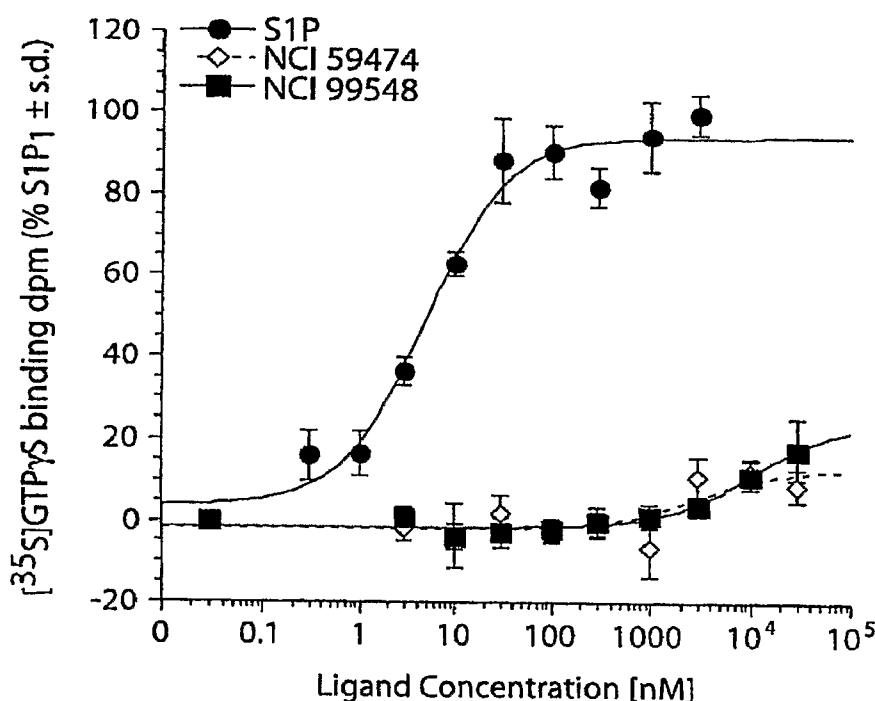
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(54) Title: GPCR LIGANDS IDENTIFIED BY COMPUTATIONAL MODELING



(57) Abstract: Disclosed are pharmacophores for developing and screening compounds having G-protein-coupled receptor antagonist activity, including LPA₁, LPA₂, LPA₃ and S1P antagonists. These compositions have therapeutic benefit in the fields of cancer chemotherapy, cardiovascular disease prevention, and protective agents during radiation and chemotherapy.

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GPCR LIGANDS IDENTIFIED BY COMPUTATIONAL MODELINGCross-Reference to Related Applications

[0001] This application claims the benefit of priority of earlier-filed United States provisional patent application number 60/808,398 filed May 25, 2006, which is incorporated herein by reference.

Statement of Government Rights

[0002] This invention was made with government support under USPHS HL61469, CA92160, awarded by the United States National Institutes of Health. The United States government has certain rights in the invention.

Field of the Invention

[0003] The present invention relates to molecules affecting cell signaling through cellular receptors and methods for identifying those molecules. More specifically, the invention relates to compounds that act as agonists or antagonists of sphingosine-1-phosphate (S1P) receptors and lysophosphatidic acid (LPA) receptors and pharmacophores that can be used to identify those compounds.

Background of the Invention

[0004] Sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPA) are structurally and functionally related lysophospholipid (LPL) growth factors. S1P and LPA are separately recognized by distinct subsets of the G protein-coupled receptor (GPCR) family, S1P₁₋₅ and LPA₁₋₄. LPLs mediate their effects through these G-protein-coupled receptors (GPCRs), of which the most completely characterized are those encoded by the endothelial differentiation genes (Edgs). Edg-1, -3, and -5 recognizes and responds to S1P, and Edg-2 and -4 generally recognize and respond to LPA. The cellular effects of the LPLs may generally be categorized into two categories. One category comprises the growth-related activities of LPA and S1P, including stimulation of proliferation, prolongation of survival,

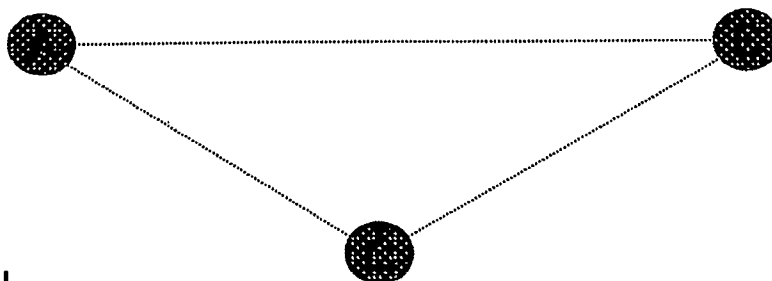
prevention and suppression of apoptosis, and processes in differentiation. A second group of cellular effects of LPA and S1P includes functions dependent on the cytoskeleton such as shape changes, aggregation, adhesion, chemotaxis, contraction, and secretion.

[0005] Sphingosine 1-phosphate (S1P) is a naturally occurring sphingolipid mediator and also a second messenger with growth factor-like actions in almost every cell type (1-3). S1P plays fundamental physiological roles in vascular stabilization (4), heart development (5), lymphocyte homing (6) and cancer angiogenesis (7).

[0006] Given the important metabolic roles played by LPA and S1P and their receptors, these molecules and the pathways in which they participate make important candidates for therapeutic drug design. Development of receptor subtype-selective pharmacophores could aid rational drug design and lead optimization as well as identification of novel molecular scaffolds through in-silico searches of large chemical libraries. However, the lack of crystal structures of GPCR makes this significantly more difficult. What are needed are compositions for modulating LPA receptor- and S1P receptor-mediated pathways and methods for identifying and/or designing such compositions.

Summary of the Invention

[0007] The invention discloses pharmacophores describing activity at the lysophosphatidic acid (LPA) receptors, LPA₁₋₃. Such pharmacophores are described by Scheme I



Scheme I

where the pharmacophore features may be described as follows:

A is an anionic functional group;

B and C are hydrophobic functional groups;

an LPA₁ Antagonist (A) has a distance between A and B of 7-11 Å, a distance between B and C of 6-10 Å, and a distance between A and C of 8-12 Å;

an LPA₁ Antagonist (B) has a distance between A and B of 7-11 Å, a distance between B and C of 5-8 Å, and a distance between A and C of 6-12 Å;

an LPA₁ Agonist has a distance between A and B of 15-17 Å, a distance between B and C of 9.2-11.2 Å, a distance between A and C of 15.5-17.5 Å;

an LPA₂ Antagonist has a distance between A and B of 5-9 Å, a distance between B and C of 4-7 Å, and a distance between A and C of 4-6 Å;

an LPA₂ Agonist (A) has a distance between A and B of 6-8 Å, a distance between B and C of 15.5-17.5 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₂ Agonist (B) has a distance between A and B of 10-12 Å, a distance between B and C of 12-14 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₃ Antagonist has a distance between A and B of 8-14 Å, a distance between B and C of 7-12 Å, and a distance between A and C of 12-16 Å;

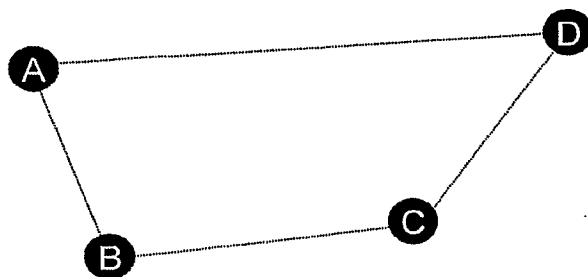
an LPA₃ Agonist has a distance between A and B of 8.6-10 Å, a distance between B and C of 4.8-5, and a distance between A and C of 13.4-14.8;

anionic functional groups comprise phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole and hydroxyl-thiazole; and

hydrophobic functional groups comprise saturated and unsaturated aliphatic and aromatic alkyl.

[0008] In some embodiments, aromatic alkyl comprises substituted or unsubstituted aromatic or heteroaromatic alkyl.

[0009] Also provided by the invention are pharmacophores that describe activity at the sphingosine 1-phosphate (S1P) receptors, S1P₁₋₅. An S1P₁₋₅ pharmacophore of the present invention may be described by Scheme 2



Scheme 2

where the pharmacophore features may be described as follows:

A is an anionic functional group;

B is a cationic or hydrophobic functional group;

C and D are hydrophobic functional groups;

an S1P₁ Agonist has a distance between A and B of 5-7 Å, a distance between A and C of 10.5-11.8 Å, a distance between A and D of 13-16 Å, a distance between B and C of 5.5-7 Å, a distance between B and D of 9-9.5 Å, a distance between C and D of 4.5-5.5 Å, and B is a hydrophobic functional group;

an S1P₂ Agonist has a distance between A and B of 3-5.7 Å, a distance between A and C of 7.5-9.0 Å, a distance between A and D of 14.9-17.3 Å, a distance between B and C of 3.0-6.9 Å, a distance between B and D of 12.4-16.1 Å, and a distance between C and D of 10.3-12.0 Å;

an S1P₃ Antagonist has a distance between A and B of 2.4-3.3 Å, a distance between A and D of 6.1-8.4 Å, a distance between B and C of 2.4-6.1 Å, and a distance between C and D of 5.1-7.9 Å;

an S1P₄ Agonist has a distance between A and B of 3-4 Å, a distance between A and C of 9-10 Å, a distance between A and D of 17-20 Å, a distance between B and C of 9-10 Å, a distance between B and D of 16.5-18.5 Å, and a distance between C and D of 9-10 Å;

anionic functional groups comprise phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole and trifluoromethyl;

hydrophobic functional groups comprise saturated and unsaturated aliphatic and aromatic alkyl groups; and

cationic functional groups comprise amine and guanidine functional groups optionally substituted by aromatic hydrogens on electron-deficient aromatic systems (*i.e.*, those with nitro, trifluoromethyl and related substituents).

[0010] Hydrophobic functional groups comprising aromatic alkyl groups preferably comprise substituted or unsubstituted aromatic or heteroaromatic groups.

[0011] The invention also provides a method for identifying or distinguishing compounds having LPA receptor agonist, LPA receptor antagonist, S1P receptor agonist, or S1P receptor antagonist activity, the method comprising

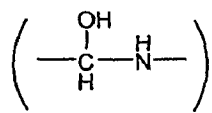
providing the pharmacophore features and distances between features as described by the LPA receptor ligand pharmacophore of Scheme 1 and/or the S1P receptor ligand pharmacophore of Scheme II as input to a 3-dimensional database;

screening resultant matches (hits) by rigidly docking conformation matched to the pharmacophore into the receptor model; and

selecting structures for experimental screening based on their size and electronic complementarity to the receptor model.

[0012] The invention also provides compositions comprising LPA receptor agonists or antagonists having at least one anionic functional group comprising, for example, phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole or trifluoromethyl, the anionic functional group being directly linked to a substituted or unsubstituted aromatic or heteroaromatic alkyl. In some embodiments, the direct link may be substituted for a molecular "spacer" comprising,

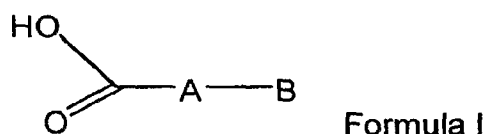
for example, C₀₋₅ substituted or unsubstituted alkyl, $\left(\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—}\overset{\text{H}}{\text{N}}\text{—} \right)_{1-3}$, or



¹⁻³, provided that the appropriate pharmacophore distance is maintained in

the resulting molecule. In some embodiments, phosphate, carboxylate, or sulfate may be present as multiple anionic groups such as di- or triphosphate, for example. For each of the LPA receptor-specific or S1P receptor-specific classes of compounds described below, it is to be understood that the molecules may be described by the disclosed chemical structures and their corresponding pharmacophores.

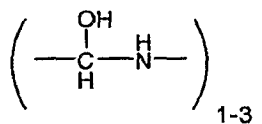
[0013] The invention also provides a method of producing an LPA₁-specific response in a human or animal subject, the method comprising administering one or more LPA₁ receptor antagonists as in formula I



where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{H}-\text{N}- \\ | \\ \text{H} \end{array} \right)^{1-3}$, or

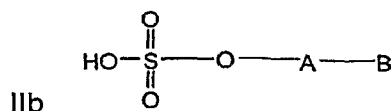
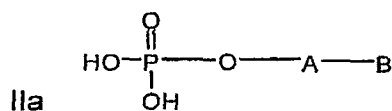


[0014] The invention also provides a method of producing an LPA₂-specific response in a human or animal subject, the method comprising administering one or more LPA₂ antagonists of formula I where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is a direct link or C₀₋₅ substituted or unsubstituted alkyl.

or one or more LPA₂ antagonists formula IIa or IIb



where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{H}- \end{array} \right)_{1-3}$, or $\left(\begin{array}{c} \text{OH} \\ | \\ -\text{C}-\text{H}- \\ | \\ \text{H} \end{array} \right)_{1-3}$; or combinations thereof.

[0015] The invention also provides a method of producing an LPA₃-specific response in a human or animal subject, the method comprising administering one or more LPA₃ agonists of formula I, IIa, or IIb where

B is substituted or unsubstituted aromatic or heteroaromatic;

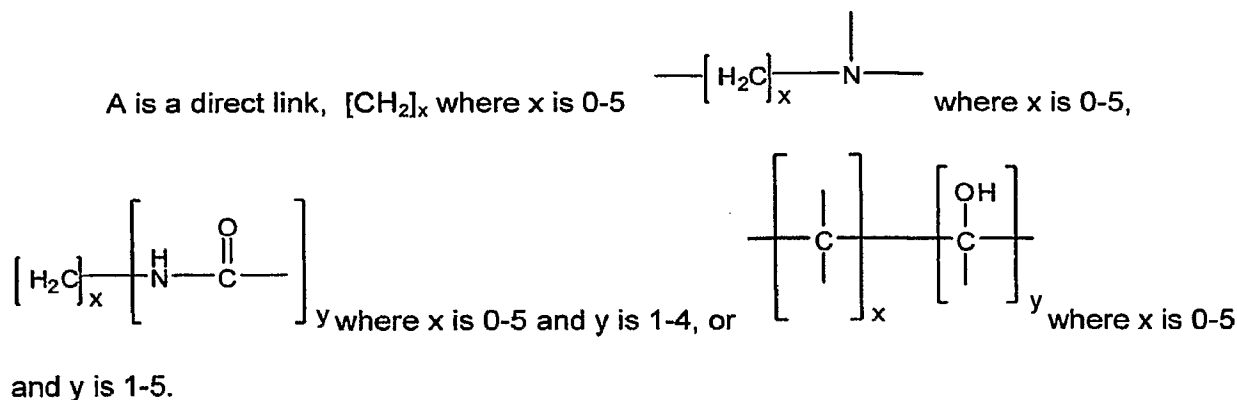
A is a direct link, [CH₂]_x where x is 0-5, $\begin{array}{c} \text{HO}-\text{C}=\text{O} \\ | \\ -(\text{CH}_2)_x-\text{C}-\text{H}-\text{N}-\text{C}- \\ | \quad \quad \quad \parallel \\ \text{H} \quad \quad \quad \text{O} \end{array}$ where x is 0-5, $\begin{array}{c} \text{O} \\ \parallel \\ -\text{N}-\text{C}- \end{array}$, $\begin{array}{c} \text{O} \\ \parallel \\ -(\text{CH}_3)_x-\text{C}- \end{array}$ where x is 0-5, $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}- \end{array}$, $\begin{array}{c} \text{O} \\ \parallel \\ -(\text{H}_2\text{C})_x-\text{N}-\text{C}- \end{array}$ where x is 0-5,

$\begin{array}{c} | \\ -(\text{H}_2\text{C})_x-\text{N}- \end{array}$ where x is 0-5, or $\left[\begin{array}{c} | \quad | \quad \text{O} \\ | \quad | \quad \parallel \\ -\text{C}-\text{N}-\text{C}- \\ | \end{array} \right]_x$ where x is 1-3; and

phosphate may be substituted with di- or tri-phosphate.

[0016] The invention also provides a method of producing an LPA₃-specific response in a human or animal subject, the method comprising administering one or more LPA₃ antagonists of formulas I, IIa, or IIb where

B is substituted or unsubstituted aromatic or heteroaromatic; and

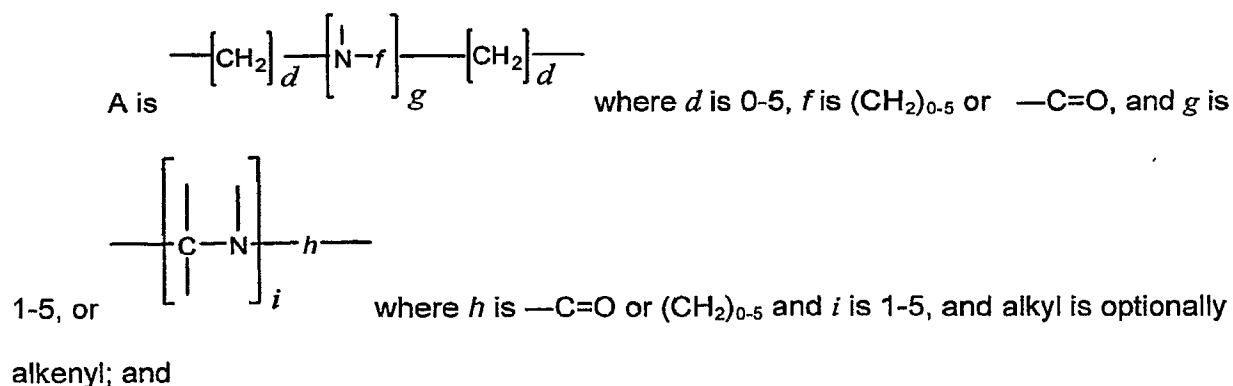


[0017] Furthermore, the invention provides a method of producing an S1P₁-specific response in a human or animal subject, the method comprising administering one or more S1P₁ agonists of formulas I and IIa where

A is a direct link; and

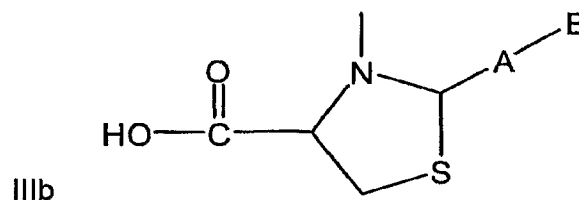
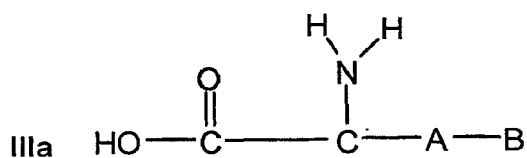
B is substituted or unsubstituted aromatic or heteroaromatic.

[0018] The invention also provides a method of producing an S1P₂-specific response in a human or animal subject, the method comprising administering one or more S1P₂ agonists of formulas I or IIa where

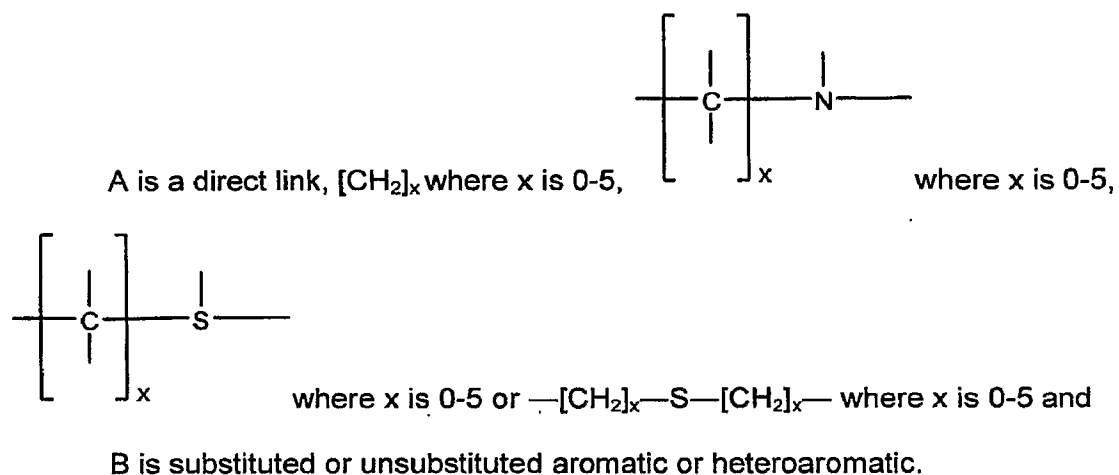


B is substituted or unsubstituted aromatic or heteroaromatic.

[0019] The invention also provides a method of producing an S1P₃-specific response in a human or animal subject, the method comprising administering one or more S1P₃ antagonists of formulas IIIa or IIIb



where



Brief Description of the Drawings

[0020] Fig. 1 shows chemical structures of representative LPA_1 antagonists identified using the LPA receptor agonist/antagonist pharmacophore.

[0021] Fig. 2 shows chemical structures of representative LPA_2 antagonists identified using the LPA receptor agonist/antagonist pharmacophore.

[0022] Fig. 3 shows chemical structures of representative LPA_3 antagonists identified using the LPA receptor agonist/antagonist pharmacophore.

[0023] Fig. 4 shows chemical structures of representative LPA_3 agonists identified using the LPA receptor agonist/antagonist pharmacophore.

[0024] Fig. 5 shows chemical structures of representative S1P_1 agonists identified using the S1P receptor agonist/antagonist pharmacophore.

[0025] Fig. 6 shows chemical structures of representative S1P_2 agonists identified using the S1P receptor agonist/antagonist pharmacophore.

[0026] Fig. 7 shows chemical structures of representative S1P₃ antagonists identified using the S1P receptor agonist/antagonist pharmacophore.

[0027] Fig. 8a-8c is a series of graphs illustrating ligand-induced [³⁵S]GTP γ S binding in S1P₁ mutants. Ligand-induced (0.1 nM-10 μ M) GTP γ S activation was calculated in transfected RH7777 cells. Activation dose-response curves of the mutants were normalized to WT S1P₁. A: The mutants displayed S1P-induced displayed three types of activations levels; no activation (F5.48Y-square), intermediate (L6.41-circle) and WT-like (T5.49G-triangle). B and C: GTP γ S activation was carried in four S1P₁ mutants to characterized the ligand-induced activation by either S1P or SEW2871 (0.1 nM-10 μ M).

[0028] Fig. 9a is an illustration of the S1P₁ agonist pharmacophore. Superposed structures of S1P and SEW2871 were derived by superposition of their complexes with the revised S1P₁ model. Fig. 9b illustrates the chemical structures of S1P (top) and SEW2871 (bottom).

[0029] Fig. 10 lists S1P₁ agonist hits from the NCI Database. Chemical structures of S1P, SEW2871, and hits identified in the Enhanced NCI Database Browser are shown. Panel A shows chemical structures of known S1P receptor agonists. Panel B shows chemical structures of good matches to the S1P/SEW2871 superposition. Panel C shows chemical structures of marginal matches to the S1P/SEW2871 superposition. Panel D shows chemical structures of negative matches to the S1P/SEW2871 superposition.

[0030] Fig. 11 is a graph of ligand-induced (0.1 nM-30 μ M) GTP γ S activation calculated in transfected RH7777 cells. Activation dose-response curves of the mutants were normalized to S1P.

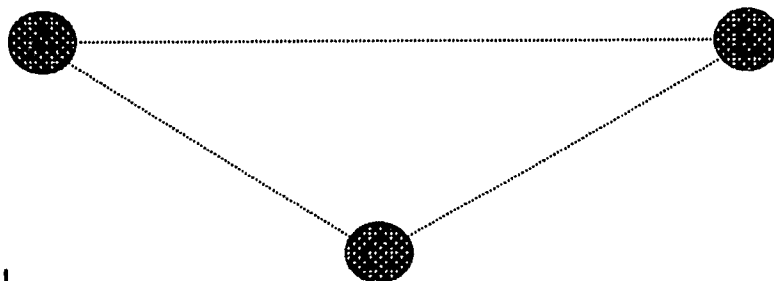
Detailed Description

[0031] The inventors have developed pharmacophores for screening compounds to assess their activity as LPA or S1P receptor agonists and antagonists. These pharmacophores have been successfully used by the inventors to screen compounds with generally unknown activity to identify those having agonist or antagonist activity for LPA₁,

LPA₂, LPA₃, and S1P₁₋₃ receptors, providing a number of compounds described herein with specificity for the LPA₁, LPA₂, LPA₃, S1P₁, S1P₂, or S1P₃ receptors.

[0032] A pharmacophore is a geometric relationship among chemical functionalities (*i.e.*, pharmacophore features) that produces a biological response. These pharmacophores have been used to mine chemical databases for novel structural scaffolds with potency reaching the low nanomolar range that have potential applications as cancer chemotherapeutics, cardiovascular disease preventatives, fertility treatments, and birth control agents. As used herein, a compound may be "described by" the pharmacophore or its features when its overall structure functionality corresponds to the given pharmacophore features.

[0033] The present invention provides pharmacophores describing activity at the lysophosphatidic acid (LPA) receptors, LPA₁₋₃. Such pharmacophores are described by Scheme I



Scheme I

where the pharmacophore features may be described as follows:

A is an anionic functional group;

B and C are hydrophobic functional groups;

an LPA₁ Antagonist (A) has a distance between A and B of 7-11 Å, a distance between B and C of 6-10 Å, and a distance between A and C of 8-12 Å;

an LPA₁ Antagonist (B) has a distance between A and B of 7-11 Å, a distance between B and C of 5-8 Å, and a distance between A and C of 6-12 Å;

an LPA₁ Agonist has a distance between A and B of 15-17 Å, a distance between B and C of 9.2-11.2 Å, a distance between A and C of 15.5-17.5 Å;

an LPA₂ Antagonist has a distance between A and B of 5-9 Å, a distance between B and C of 4-7 Å, and a distance between A and C of 4-6 Å;

an LPA₂ Agonist (A) has a distance between A and B of 6-8 Å, a distance between B and C of 15.5-17.5 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₂ Agonist (B) has a distance between A and B of 10-12 Å, a distance between B and C of 12-14 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₃ Antagonist has a distance between A and B of 8-14 Å, a distance between B and C of 7-12 Å, and a distance between A and C of 12-16 Å;

an LPA₃ Agonist has a distance between A and B of 8.6-10 Å, a distance between B and C of 4.8-5, and a distance between A and C of 13.4-14.8;

anionic functional groups comprise phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole and hydroxyl-thiazole; and

hydrophobic functional groups comprise saturated and unsaturated aliphatic and aromatic alkyl.

[0034] In some embodiments, aromatic alkyl comprises substituted or unsubstituted aromatic or heteroaromatic alkyl.

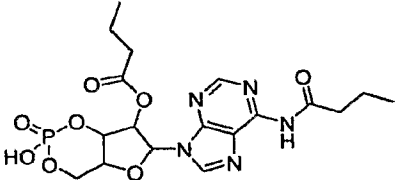
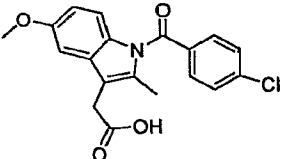
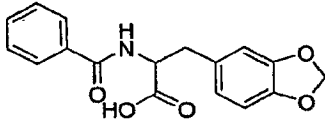
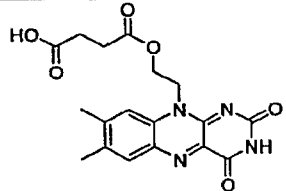
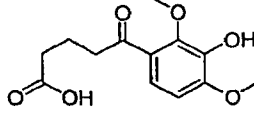
[0035] Listed in Table 1 are the distances between the pharmacophore features for each type of activity at the LPA receptors. In the case of LPA₂ agonism, for example, two pharmacophores are presented that differ in the position of hydrophobic point B by 4.7 Å.

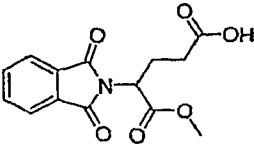
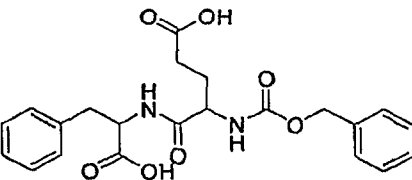
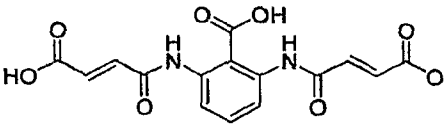
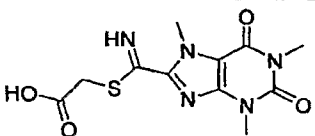
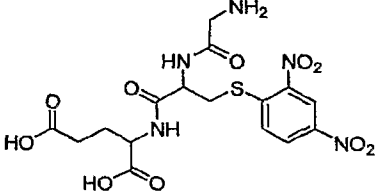
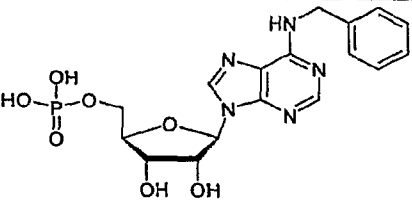
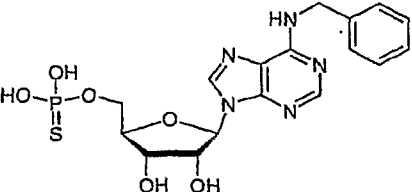
Table 1
Distances Between Pharmacophore Features Conferring LPA Receptor Activity

Activity	A-B Distance (Å)	B-C Distance (Å)	A-C Distance (Å)
LPA ₁ Antagonist (A)	7-11	6-10	8-12
LPA ₁ Antagonist (B)	7-11	5-8	6-12
LPA ₁ Agonist	15-17	9.2-11.2	15.5-17.5
LPA ₂ Antagonist	5-9	4-7	4-6
LPA ₂ Agonist (A)	6-8	15.5-17.5	18.5-20.5
LPA ₂ Agonist (B)	10-12	12-14	18.5-20.5
LPA ₃ Antagonist	8-14	7-12	12-16
LPA ₃ Agonist	8.6-10	4.8-5	13.4-14.8

[0036] Table 2 lists several examples of compounds screened and identified as LPA agonists or antagonists using the LPA agonist/antagonist pharmacophore of the present invention.

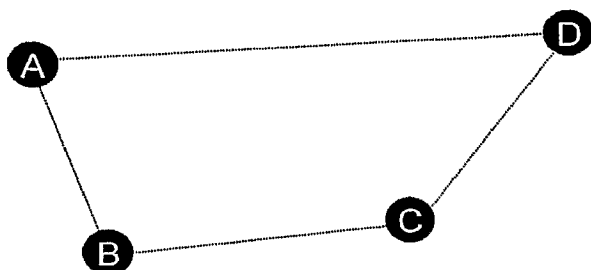
Table 2
Leads Identified Using the LPA Agonist/Antagonist Pharmacophores

NSC #	Formula	Structure	LPA ₁	LPA ₂	LPA ₃
143108	C ₁₈ H ₂₄ N ₅ O ₈ P (M.W. 469.4)		Weak agonist	No Effect	IC ₅₀ =1309 nM 61.0% inhibition of 200 nM LPA response at 30 μM
77541	C ₁₉ H ₁₆ ClN ₂ O ₄ (M.W. 357.8)		Weak agonist	No Effect	IC ₅₀ =909 nM 76.2% inhibition of 200 nM LPA response at 30 μM
10188	C ₁₇ H ₁₅ NO ₅ (M.W. 313.3)		Weak agonist	IC ₅₀ =690 nM (55.8% inhibition of 200 nM LPA response at 30 μM)	No effect
3066	C ₁₈ H ₁₈ N ₄ O ₆ (M.W. 386.4)		IC ₅₀ : 197 nM K _i : 91.7 nM	IC ₅₀ : 167 nM K _i : 78.7 nM	IC ₅₀ : 103 nM K _i : 44.8 nM
10167	C ₁₃ H ₁₆ O ₆ (M.W. 268.3)		No Effect	IC ₅₀ : 88 nM (47.4% Inhibition of 10 nM LPA response)	IC ₅₀ : 517 nM (49.9 % Inhibition of 200 nM LPA response)

1654 0	$C_{14}H_{13}NO$ 6 (M.W. 291.3)		No effect	IC ₅₀ : 100 nM (50.9 % Inhibition of 10 nM LPA response)	IC ₅₀ : 231 nM (59.7 % Inhibition of 200 nM LPA response)
1876 0	$C_{22}H_{24}N_2O_7$ (M.W. 428.4)		No effect	IC ₅₀ : 20 nM (53.3 % Inhibition of 10 nM LPA response)	IC ₅₀ : 9 nM (58.7 % Inhibition of 200 nM LPA response)
4709 1	$C_{15}H_{12}N_2O_8$ (M.W. 348.3)		No effect	IC ₅₀ : 355 nM (53.5 % Inhibition of 10 nM LPA response)	IC ₅₀ : 30 nM (81.7 % Inhibition of 200 nM LPA response)
6258 6	$C_{11}H_{13}N_5O_4S$ (M.W. 311.3)		IC ₅₀ : 234 nM K _i : 91.7 nM	IC ₅₀ : 172 nM K _i : 117 nM	IC ₅₀ : 35 nM K _i : 15.7 nM
1616 13	$C_{16}H_{19}N_5O_{10}S$ (M.W. 473.4)		No effect	No effect	IC ₅₀ : 24 nM (69.8 % Inhibition of 200 nM LPA response)
2555 23	$C_{17}H_{20}N_5O_7P$ (M.W. 437.4)		No effect	No effect	Potentiat es LPA- induced activation
-	$C_{17}H_{20}N_5O_6PS$ (M.W. 453.4)		No effect	Potentiat es LPA- induced activation	No effect

[0037] Also provided by the invention are pharmacophores that describe activity at the sphingosine 1-phosphate (S1P) receptors, S1P₁₋₅. The inventors are using these

pharmacophores to mine chemical databases for novel structural scaffolds that have potential applications as cancer chemotherapeutics, cardiovascular disease preventatives, and protective agents against cellular damage resulting from radiation and chemotherapy. An S1P₁₋₅ pharmacophore of the present invention may be described by Scheme 2



Scheme 2

where the pharmacophore features may be describe as follows:

A is an anionic functional group;

B is a cationic or hydrophobic functional group;

C and D are hydrophobic functional groups;

an S1P₁ Agonist has a distance between A and B of 5-7 Å, a distance between A and C of 10.5-11.8 Å, a distance between A and D of 13-16 Å, a distance between B and C of 5.5-7 Å, a distance between B and D of 9-9.5 Å, a distance between C and D of 4.5-5.5 Å, and B is a hydrophobic functional group;

an S1P₂ Agonist has a distance between A and B of 3-5.7 Å, a distance between A and C of 7.5-9.0 Å, a distance between A and D of 14.9-17.3 Å, a distance between B and C of 3.0-6.9 Å, a distance between B and D of 12.4-16.1 Å, and a distance between C and D of 10.3-12.0 Å;

an S1P₃ Antagonist has a distance between A and B of 2.4-3.3 Å, a distance between A and D of 6.1-8.4 Å, a distance between B and C of 2.4-6.1 Å, and a distance between C and D of 5.1-7.9 Å;

an S1P₄ Agonist has a distance between A and B of 3-4 Å, a distance between A and C of 9-10 Å, a distance between A and D of 17-20 Å, a distance between B and C of 9-10 Å, a distance between B and D of 16.5-18.5 Å, and a distance between C and D of 9-10 Å;

anionic functional groups comprise phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole and trifluoromethyl;

hydrophobic functional groups comprise saturated and unsaturated aliphatic and aromatic alkyl groups; and

cationic functional groups comprise amine and guanidine functional groups optionally substituted by aromatic hydrogens on electron-deficient aromatic systems (*i.e.*, those with nitro, trifluoromethyl and related substituents).

[0038] Hydrophobic functional groups comprising aromatic alkyl groups preferably comprise substituted or unsubstituted aromatic or heteroaromatic groups.

Table 3
Distances Between Pharmacophore Features Conferring S1P Receptor Activity

Activity	Distances (Å)					
	A-B	A-C	A-D	B-C	B-D	C-D
S1P ₁ Agonist*	5-7	10.5-11.8	13-16	5.5-7	9-9.5	4.5-5.5
S1P ₂ Agonist	3-5.7	7.5-9.0	14.9-17.3	3.0-6.9	12.4-16.1	10.3-12.0
S1P ₃ Antagonist	2.4-3.3		6.1-8.4	2.4-6.1		5.1-7.9
S1P ₄ Agonist	3-4	9-10	17-20	9-10	16.5-18.5	9-10

* Feature B is a hydrophobic functional group

[0039] The invention also provides a method for utilizing a pharmacophore of Scheme 1 or Scheme 2 to develop and/or identify compounds having LPA receptor agonist or agonist activity, or S1P agonist or antagonist activity, the method comprising

providing the pharmacophore features and distances between features as described by the LPA receptor pharmacophore and/or the S1P receptor pharmacophore described herein as input to a 3-dimensional database;

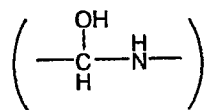
screening resultant matches (hits) by rigidly docking conformation matched to the pharmacophore into the receptor model; and

selecting structures for experimental screening based on their size and electronic complementarity to the receptor model. Methods for computational analysis of chemical compounds using pharmacophores are described, for example, in the Textbook of Drug

Design and Discovery, 3rd ed. (Krogsgaard-Larsen, P. *et al*, eds., Taylor and Francis publishing, New York, NY USA) and The Organic Chemistry of Drug Design and Drug Action, 2nd ed. (Silverman, Richard, Elsevier Publishing, New York, NY USA). Given the pharmacophores described herein, the practice of the method for using the pharmacophores to screen corresponding chemical compounds is well within the skill of those in the art.

[0040] Previously, the inventors and others had identified LPA₁₋₃ receptor agonists and antagonists having structural similarities with LPA, particularly in the presence of the phosphate head group and the acyl chain. Work published by Jalink, *et al.* (Biochem. J. (1995) 307: 609-616) indicated that, particularly for agonist activity, the acyl chain is an important element of the LPA molecule and modifications to the acyl chain affected agonist/antagonist activity. LPA receptor antagonists VPC 32183, VPC 32179, and VPC 12249, for example, sold by Avanti Polar Lipids, possess the acyl chain. Compounds cited for use by Kim *et al.* (WO 2004/052375), in a method comprising administering LPA or derivatives thereof to decrease neutrophil accumulation, contain an acyl chain. U.S. Patent number 7,169,818 (Lynch *et al.*) also describes LPA receptor agonists and antagonists having an acyl chain. The inventors, however, using the LPA pharmacophore of the present invention, have found that LPA receptor agonists and antagonists can comprise molecules lacking the acyl chain characteristic of lysophosphatidic acid. More specifically, compounds identified to be useful as LPA receptor agonists or antagonists using the pharmacophore of the invention include compounds having at least one anionic functional group such as, for example, phosphate, carboxylate, or sulfate, the anionic functional group being directly linked to a substituted or unsubstituted aromatic or heteroaromatic alkyl. In some embodiments, the direct link may be substituted for a molecular "spacer" comprising, for

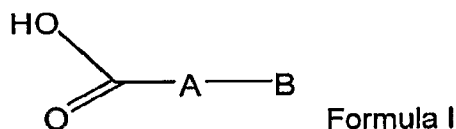
example, C₀₋₅ substituted or unsubstituted alkyl, $\left(\text{---}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{---}\overset{\text{H}}{\underset{|}{\text{N}}}\text{---} \right)_{1-3}$, or



1-3, provided that the appropriate pharmacophore distance is maintained in

the resulting molecule. For each of the LPA receptor-specific or S1P receptor-specific classes of compounds described below, it is to be understood that the molecules may be described by the disclosed chemical structures and their corresponding pharmacophores.

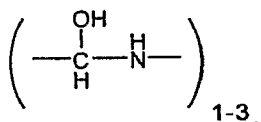
[0041] Compounds of the present invention therefore include compounds that are LPA₁ receptor antagonists as in formula I



where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{N}- \\ | \quad | \\ \text{H} \quad \end{array} \right)$ 1-3, or

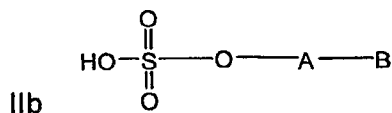
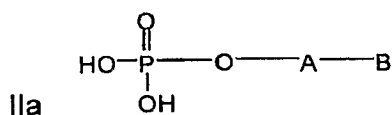


[0042] LPA₂ antagonists described by the present invention include those compounds of formula I where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is a direct link or C₀₋₅ substituted or unsubstituted alkyl.

[0043] LPA₂ antagonists described by the present invention also include those compounds of formula IIa or IIb

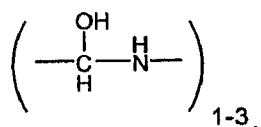


where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\text{---} \overset{\text{O}}{\underset{\text{||}}{\text{C}}} \text{---} \text{N} \text{---} \right)_{1-3}$,

or



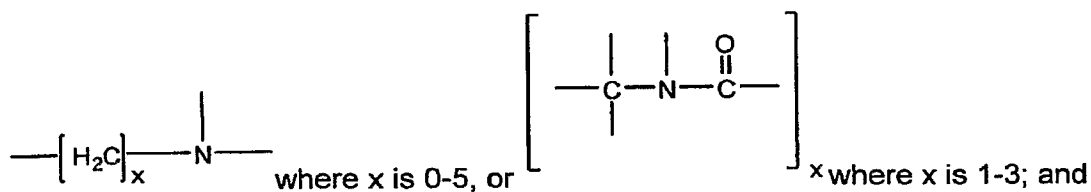
[0044] LPA₃ agonists identified by the pharmacophore of the present invention include compounds of formula I, IIa, or IIb where

B is substituted or unsubstituted aromatic or heteroaromatic;

A is a direct link, [CH₂]_x where x is 0-5, $\text{---} \left[\text{CH}_2 \right]_x \text{---} \underset{\text{H}}{\overset{\text{HO-C=O}}{\text{C}}} \text{---} \text{N} \text{---} \overset{\text{O}}{\underset{\text{||}}{\text{C}}} \text{---}$ where x is 0-5,



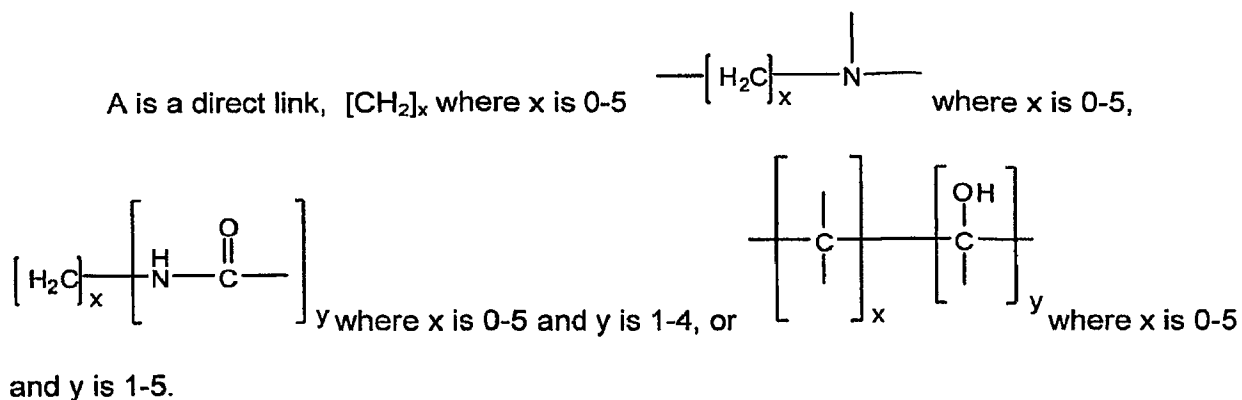
where x is 0-5,



phosphate may be substituted with di- or tri-phosphate.

[0045] LPA₃ antagonists identified by the pharmacophore of the present invention include compounds of formulas I, IIa, and IIb where

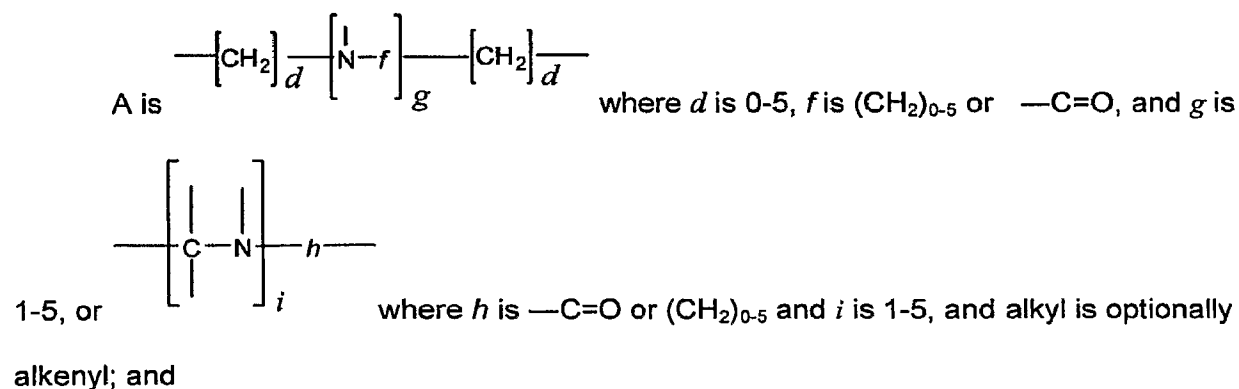
B is substituted or unsubstituted aromatic or heteroaromatic; and



[0046] S1P₂ agonists include compositions comprising compounds of formulas I or

IIa

where

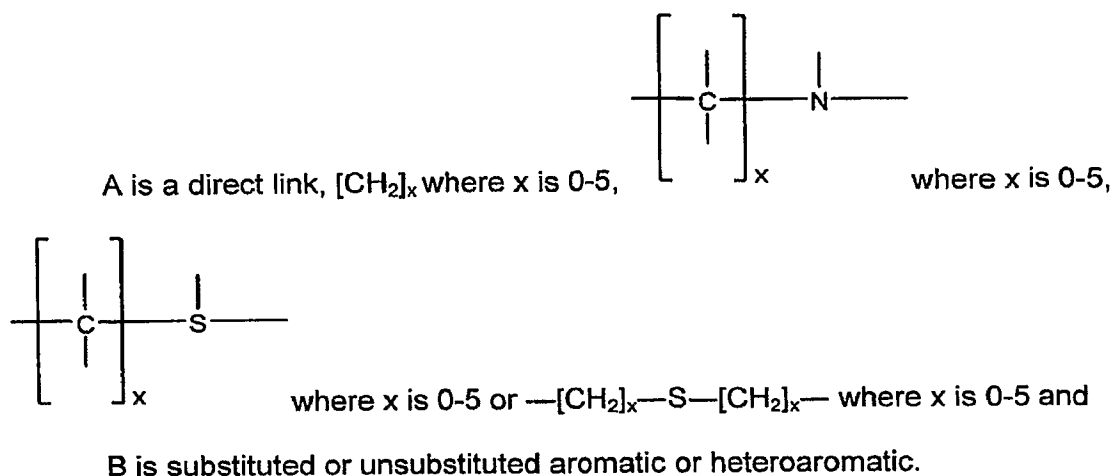


B is substituted or unsubstituted aromatic or heteroaromatic.

[0047] S1P₃ antagonists include compounds of formulas IIIa and IIIb



where



[0048] The invention therefore also provides a method for producing an LPA-receptor-specific or S1P-receptor-specific response in a human or animal subject, the method comprising selecting a compound for its LPA- or S1P-receptor specificity as an agonist or antagonist and administering such a selected compound to achieve a desired LPA-receptor agonist/antagonist-specific or S1P-receptor agonist/antagonist-specific result. In various embodiments, the method comprises administering compounds as described above for their receptor-specific activity. It is to be understood that the anionic functional groups provided for each receptor-specific class of compounds may be substituted by one of skill in the art by other anionic functional groups to achieve a molecule with similar functionality, these anionic groups including but not limited to phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole and trifluoromethyl, for example.

[0049] Compounds identified by the method may have a variety of therapeutic uses, given the significant role of LPA, S1P, and their receptors in the mammalian body. Once synthesized, identified, or screened using the method of the invention, such compounds may be provided for therapeutic use via a variety of delivery routes such as, but not limited to, oral, nasal, intraperitoneal, intravenous, subcutaneous, and intramuscular. Administration may be provided as a single dosage, multiple dosages delivered at intervals over time, or modified release dosages for delivery of a single or multiple dosages as needed or over a

period of time following initial administration, such as may be provided by a medication depot, pump, or other device.

[0050] The inventors had identified three basic amino acids, R3.28, K5.38, and R7.34 in S1P₁ and S1P₄ that form salt bridges with the phosphate group of S1P and are essential for ligand binding in one or both receptors (26,27). They also pinpointed position 3.29, conserved as glutamine in LPA- and glutamate in S1P-specific members of the EDG family, as the single locus that determines ligand specificity for S1P versus LPA through its ion pairing with the ammonium moiety of S1P (28). The Q/N3.29 residue also plays an essential role in ligand binding because substitution to alanine results in a loss of S1P and LPA binding and receptor activation. They also elucidated differences between S1P₁ and S1P₄, as in the latter subtype K5.38 and W4.64 together compensate for the lack of a cationic residue at position 7.34 as in S1P₁ (27). These polar headgroup interactions are essential for ligand binding, activation, and specificity. However, the hydrophobic tail constituting the bulk of S1P has not been assigned a function and its interaction with the ligand binding pocket has not been elucidated.

[0051] The inventors experimentally validated a computational model of the ligand binding pocket of the S1P₁ GPCR surrounding the aliphatic portion of S1P. Mutagenesis-based validation confirmed 18 residues lining the hydrophobic ligand binding pocket, which the inventors combined with previously validated three head-group interacting residues to complete mapping of the S1P ligand recognition site. The validated ligand binding pocket provided a pharmacophore model, which was used for in-silico screening of the United States National Cancer Institute (NCI) Developmental Therapeutics chemical library, leading to the identification of two novel non-lipid agonists of S1P₁.

[0052] A computational model of S1P docked in the S1P₁ receptor was developed and the hydrophobic region of the ligand binding pocket has been experimentally validated with a "hit-rate" of 90%, in which mutations of 18 out of 20 residues predicted to interact with the hydrophobic tail displayed impaired or altered S1P-induced activation. Computational modeling was used to guide the mutagenesis strategy to gain insight into the structure-

function relationship of S1P₁. The choice of replacement of residues in the predicted hydrophobic ligand binding pocket determined the type of effect observed in ligand-induced activation. For example, at least one of the two types of replacements introduced into four residues had little or no impact on E_{max} and only slightly increased the EC₅₀ values relative to WT. At the same time, at least one of the two replacements for four of these same residues had a major impact on E_{max} and/or EC₅₀. This established the refined nature of the computational predictions and at the same time provided the inventors with internal controls in a sense that receptor function was not always affected.

[0053] The experimentally validated predictions of the theoretical model localize the hydrophobic binding pocket to the transmembrane (TM) TM3, TM5, and TM6 domains. All but one of these residues are conserved in the EDG family of receptors. Of the 20 residues tested, three out of five in TM3, three out of ten in TM5 and two out of five in TM6 are identical in all EDG family S1P receptors. Of the eight identical residues among S1P receptors, five are also identical in the LPA receptors of the EDG family. However, if the identity criterion is relaxed to include residues that are identical in at least 3 of the five S1P receptors, then four out of five in TM3, nine out of ten in TM5, and five out of five are identical in TM6. Eight strictly conserved residues and an additional ten nearly conserved residues suggest that the hydrophobic binding pocket is highly conserved among these receptors. Furthermore, relative to S1P₁, S1P₅ deviates most strongly in the hydrophobic binding pocket with six differences, followed by S1P₂ and S1P₄, which each differ at four residues, and S1P₃, which only differs at three residues. Thus, the hydrophobic binding pocket shows the least diversity between S1P₁ and S1P₃. This coincides with the similar ligand properties of FTY-720-P, which at these two receptors the K_D is 0.21 ± 0.17 nM and 5.0 ± 2.7 nM, for S1P₁ and S1P₃, respectively (19). Comparison of the ligand properties with the S1P₁ specific agonist SEW2871 (22) using four mutants that showed no or greatly reduced activation by S1P indicated that three of the four residues also impaired activation to the synthetic ligand. In contrast the V6.40L mutant was slightly activated by SEW2871 but not by S1P. These results not only illustrate the general importance of the residues

identified by the computationally-guided mutagenesis in S1P₁ function, but also point out that differences do exist between the individual ligands.

[0054] Significant binding occurred in several of the mutants which showed no or greatly diminished dose-dependent S1P-induced activation, indicating that these residues may play a critical role in the conformational change required for activation, but their interaction with the ligand is not essential as indicated by the retained binding. Introduction of charged residues to replace M3.32K, L3.43&3.44E, and L5.51E, severely disrupted activation and either abolished or significantly reduced (L5.51E) ligand binding compared to the WT receptor. Thus the hydrophobic environment appears to be necessary for ligand binding and consequent activation.

[0055] Some of the residues that the inventors identified as part of the hydrophobic binding pocket of S1P₁ have also been mutated in other GPCR and some were also found to play a role in ligand recognition/activation. L3.36 when mutated to alanine in the human bradykinin B2 receptor subtype did not reduce ligand affinity (37). In contrast, the inventors' L3.36G/E mutants showed altered activation properties. F5.48, when mutated to alanine in the human VIP receptor, reduced potency but not efficacy (38). F5.48G mutants both failed to show dose dependent activation by S1P but showed over 50% ligand binding relative to WT, indicating that this residue is involved in the activation of other receptors as well. There was a striking similarity between the W6.48A mutation and the melanocortin MC4R (39), cholecystokinin CCKR (40), and AA3R receptors (41), as in all instances receptor activation was reduced without loss of binding. This unique property of W6.48 is consistent with its putative role in the activation of GPCR by a diverse family of ligands. However, W6.48 does not play an identical role in the receptor most closely related to the EDG family, the cannabinoid receptor. The W6.48(357)A mutation of the CB₁ receptor displayed an enhancement of ligand-induced GTP γ S binding.(42) Enhanced efficacy was also observed for some agonists at the corresponding mutant of the CCK-B/gastrin receptor.(43) Enhanced efficacy of W6.48A in concert with modeling studies and increased basal activity and lack of ligand-induced response by the CB₁ F3.36A mutant led those authors to

conclude that CB₁ activation involves loss of contact between F3.36 and W6.48.(42) In contrast, the inventors' results and the refined model they have developed suggest that S1P₁ receptor activation involves formation of contact between these residues.

[0056] The inventors' model not only serves as a good template for the modeling of the other EDG receptors, but also defines the specific conformation of S1P relevant to S1P₁ agonism. This structure, in combination with the inventors' more recently published S1P₁ complex of the S1P₁-selective agonist, SEW2871,(35) define the pharmacophore for S1P₁ agonism. Superimposing the S1P₁ complex structures of S1P and SEW2871 illustrated that the phosphate group of S1P occupies the same geometric position as a trifluoromethyl group of SEW2871. Similarly, the ammonium group of S1P occupies the same space as a weakly electron-poor hydrogen atom. The remainder of each structure occupies common volume, and the superposed structures have quite similar lengths. These superposed structures define a geometric pharmacophore with distance ranges between pharmacophore elements shown in Table 5. This pharmacophore was used to identify novel lead compounds from the Enhanced NCI Database Browser. Successful identification of NCI 59474 and NCI 99548 compounds, determined by the inventors to be partial agonists of S1P₁, provides proof that *in silico* screening of large chemical libraries to identify novel molecular scaffolds that interact with the S1P₁ receptor is now possible.

[0057] The inventors identified F5.48G and V6.40L, exhibiting no ligand-dependent activation by S1P. Mutants L3.36E and W6.48A showed greatly reduced activation, yet all four maintained wild type [³²P]S1P binding, suggesting a role in the conformational transition of S1P₁ to its activated state. Although V6.40L was not activated by S1P, it showed partial activation by the SEW2871 ligand.

[0058] The invention will now be further described by means of the following non-limiting examples.

Examples

Reagents

[0059] All reagents were of analytical purity and obtained from Sigma-Aldrich (St. Louis, MO) unless specified otherwise. S1P was purchased from Avanti Polar Lipids (Alabaster, AL). SEW2871 was a generous gift from Dr. Hugh Rosen (Scripps Research Institute, San Diego).

Residue Nomenclature

[0060] Amino acids in the transmembrane (TM) domains of S1P₁ can be assigned index positions to facilitate comparison between GPCR with different numbers of amino acids, as described by Weinstein and coworkers (29). An index position is in the format x.xx. The first number denotes the TM domain in which the residue appears. The second number indicates the position of that residue relative to the most highly conserved residue in that TM domain which is arbitrarily assigned position 50. E3.29, then, indicates the relative position of this glutamate in TM 3 relative to the highly conserved arginine 3.50 in the E(D)RY motif (29).

Receptor Model Development: S1P₁

[0061] A model of human S1P₁ (GenBankTM accession number AFP23365) was developed by homology to a model of rhodopsin (Protein Data Bank entry 1boj) in a manner described in the inventors' previous publications (26,30). Briefly, the rhodopsin model was used to generate TM 1-6, while the structure for the seventh TM was based on TM7 of the dopamine D2 receptor model (31). The preliminary model was further refined by converting all *cis* amide bonds to the *trans* configuration and by manually rotating side chains at polarity-conserved positions to optimize hydrogen bonding between TM. The AMBER94 force field (32) was utilized to optimize the receptor to a 0.1 kcal/mol·Å root mean square gradient. A corrected model was constructed using the preliminary model as the template

with a manual realignment of TM 5 to move each residue back one position in the alignment. The corrected model was refined and minimized using the same protocol.

Receptor Model Development: S1P₁ Single/Double Point Mutants

[0062] Mutant models of S1P₁ were developed by homology to the corrected S1P₁ model. Using the MOE software package, the appropriate mutation was constructed by side-chain replacement. Non-polar hydrogen atoms were added to the mutated amino acid side-chain and the model was subsequently geometry optimized. The AMBER94 force field (32) was utilized again to optimize each mutant receptor to a 0.1 kcal/mol·Å root mean square gradient.

Ligand Model Development

[0063] A computational model of sphingosine 1-phosphate (S1P) was built using the MOE software package. The phosphate group was modeled with a net -1 charge and the amine moiety was modeled with a net +1 charge. S1P was geometry optimized using the MMFF94 forcefield (33).

Docking

[0064] Using the AUTODOCK 3.0 software package (34), S1P was docked into S1P₁ and the S1P₁ mutant receptor models. These complexes were evaluated based on final docked energy, as well as visual analysis of electrostatic and other non-bonded interactions between the ligand and receptor. Docking parameters were set to default values with the exception of the number of energy evaluations (2.5×10^9), number of generations (30,000), local search iterations (3000) and number of runs (15). The complexes exhibiting the best interactions based on either final docked energy or visual analysis were geometry optimized using the MMFF94 force field (33) and were subjected to critical qualitative analysis. SEW2871 was docked into the S1P₁ receptor model using the same parameters and evaluation criteria.

New Lead Identification

[0065] The docked positions of S1P and SEW2871 in the S1P₁ receptor model were superimposed and used to derive pharmacophore features sharing common locations in both structures. Distances between these common pharmacophore features comprise the pharmacophore. The pharmacophore was used to search the Enhanced NCI Database Browser (<http://129.43.27.140/ncidb2/>) for novel lead compounds. A trifluoromethylphenyl group was used for the anionic bioisostere, carbon atoms were used to represent the hydrophobic functionality at other pharmacophore points. Hits from the search were evaluated based on their superposition onto the S1P and SEW2871 conformations from the S1P₁ complexes. Hits were categorized as good, marginal or negative based on these superpositions. Hits were considered negative if they exceeded the volume occupied by S1P or SEW2871 due to likely steric interactions with receptor atoms.

Site-directed mutagenesis

[0066] The N-terminal FLAG epitope-tagged S1P₁ receptor construct (GenBank™ accession number AF233365) was provided by Dr. Timothy Hla. Site-specific mutations were generated using the ExSite™ mutagenesis kit (Stratagene, La Jolla, CA) as described previously (26,28). S1P₁ and the generated mutants were subcloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA). The sequence information of the mutants is listed in Table 4. Clones were verified by complete sequencing of the inserts.

Table 4
Description of the S1P₁ Mutant Constructs

TM helix	Mutation	Sequence Position	Codon	Mutated codon(s)	Silent mutation
TM3	M3.32G	124	ATG ₃₇₂	GGG	GGATCC
TM3	M3.32K	124	ATG ₃₇₂	AAG	GGATCC
TM3	L3.36E	128	CTG ₃₇₈	GAA	GCTAGC
TM3	L3.36G	128	CTG ₃₇₈	GGA	GCTAGC
TM3	V3.40L	132	GTG ₃₉₆	CTG	GCTAGC
TM3	V3.40T	132	GTG ₃₉₆	ACG	GCTAGC
TM3	L3.43,3.44E	135,136	CTCCTC ₄₀₈	GAAGAA	CGATCG
TM3	L3.43,3.44G	135,136	CTCCTC ₄₀₈	GGAGGA	CGATCG
TM5	K5.38A	200	AAG ₆₀₀	GCG	-----
TM5	I5.41A	203	ATC ₆₀₉	CGCGTTG	CGCGT
TM5	I5.41F	203	ATC ₆₀₉	TTC	-----
TM5	F5.43G	205	TTC ₆₁₅	GGC	ATCGAT
TM5	F5.43Y	205	TTC ₆₁₅	TAC	ATCGAT
TM5	C5.44D	206	TGC ₆₁₈	GAC	ATCGAT
TM5	C5.44I	206	TGC ₆₁₈	ATC	ATCGAT
TM5	T5.45G	207	ACC ₆₂₁	GGT	GGTACC
TM5	T5.45S	207	ACC ₆₂₁	AGC	CTGCAG
TM5	V5.47L	209	GTC ₆₂₇	CTC	ATCGAT
TM5	V5.47T	209	GTC ₆₂₇	ACC	ATCGAT
TM5	F5.48Y	210	TTC ₆₃₀	TAC	ATCGAT
TM5	F5.48G	210	TTC ₆₃₀	GGC	ATCGAT
TM5	T5.49G	211	ACT ₆₃₃	GGT	-----
TM5	T5.49S	211	ACT ₆₃₃	TCT	-----
TM5	L5.51E	213	CTT ₆₃₉	GAA	ATCGAT
TM5	L5.51G	213	CTT ₆₃₉	GGT	ATCGAT

TM5	L5.52A	214	CTG ₆₄₂	AGCG	GCTAGC
TM6	V6.37T	258	GTA ₇₆₈	ACA	AAGCT
TM6	V6.37L	258	GTA ₇₆₈	CTA	AAGCT
TM6	V6.40A	261	GTC ₇₈₃	GCA	-----
TM6	V6.40T	261	GTC ₇₈₃	ACC	GCATGC
TM6	V6.40L	261	GTC ₇₈₃	CTC	GCATGC
TM6	L6.41E	262	CTG ₇₈₆	GAG	GCATGC
TM6	L6.41G	262	CTG ₇₈₆	GGG	GCATGC
TM6	F6.44Y	265	TTC ₇₉₅	TAC	GCATGC
TM6	F6.44G	265	TTC ₇₉₅	GGC	GCATGC
TM6	W6.48E	269	TGG ₈₀₇	GAG	GCATGC
TM6	W6.48A	269	TGG ₈₀₇	GCG	GCATGC

Cell Culture and Transfection

[0067] RH7777 and HEK-293 cells (ATCC, Manassas, VA) were maintained in Dulbecco's modified minimal essential medium (DMEM) containing 10% fetal bovine serum (Hyclone, Logan, UT). Cells (2×10^6) were transfected with 2 μ g of plasmid DNA with Effectene (Qiagen, Valencia, CA) according to the manufacturer's instructions, for 24 h. Before ligand binding and receptor activation assays, the cells were washed twice with serum-free DMEM and serum-starved for at least 6 h.

Western Blotting

[0068] Western blot analysis of the FLAG epitope-tagged receptor construct was performed in both transiently transfected RH7777 and HEK-293 cells using a protocol described earlier (27). Anti-FLAG M2 antibody anti- β actin, and goat anti-mouse antibody conjugated with horseradish peroxidase were purchased from Sigma-Aldrich (St. Louis, MO) and Promega (Madison, WI), respectively.

Flow cytometry analysis

[0069] Cell-surface expression of the FLAG-tagged S1P₁ and its mutants was determined by flow cytometry as described in the literature (27). Transfected RH7777 cells were harvested by trypsinization, and upon harvesting, the cells were maintained at 4°C for the subsequent steps. The cells were washed with ice-cold FC buffer (phosphate buffered saline pH 7.4 (PBS) and 3% bovine serum albumin (BSA)). After washing once with FC buffer, the cells were incubated for 30 min in blocking solution (5% BSA and 5% donkey serum (Sigma) in PBS). The cells were washed once with FC buffer, and the cells were subsequently incubated for 60 min in FC buffer with the anti-FLAG M2 monoclonal antibody (Sigma) (1:200). After washing the cells twice with FC buffer, the cells were incubated for 30 min in FC buffer with the Alexa Fluor 488-labeled donkey anti-mouse IgG (Molecular Probes, Eugene, OR) (1:1600). After washing the cells twice, samples were resuspended in 1% BSA in PBS and analyzed using a LSR II flow cytometer (Becton Dickinson, San Jose, CA). Data were analyzed with the Cell Quest software (Becton Dickinson).

[³²P]S1P Radioligand Binding Assays

[0070] The S1P binding assays were done essentially as previously described (28). Transfected RH7777 cells (5×10^5) were incubated at 4°C in 20 mM Tris-HCl (pH 7.5) binding buffer containing 100 mM NaCl, 15 mM NaF, protease inhibitor cocktail (Sigma-Aldrich), and 0.2 mg/ml essentially fatty-acid free BSA with 1 nM [³²P]S1P in 50 nM S1P for 40 min. Cells were centrifuged and washed twice in binding buffer. The final pellet was resuspended in 2:1 CHCl₃/MeOH and the suspension was equilibrated in scintillation fluid overnight. Cell-bound radioactivity was measured by liquid scintillation counting using a Beckman LS5000 TA counter (Beckman Coulter, Irvine, CA). Specific binding was defined as the difference between total binding and non-specific binding (in the presence of 2-5 μM cold S1P). Standard errors were computed on the basis of triplicate samples from two simultaneous transfections.

[0071] For the competition assays, HEK-293 cells were used. Briefly, 4×10^5 cells were plated in 24-well dishes and allowed to adhere overnight. The cells were then transfected with 0.4 μ g of the cDNA using Lipofectamine 2000 (Invitrogen) and the transfection proceeded for 48 h. After washing the cells twice with ice-cold binding buffer (20 mM Tris-HCl, pH 7.4 and 150 mM NaCl), 0.1 nM [^{32} P]S1P and competing concentrations of cold S1P (1 nM-10 μ M), resuspended in binding buffer + 4% BSA, were applied to the cells and incubated on ice for 30 min. After washing the cells twice with ice-cold binding buffer + 0.4% BSA, the cells were lysed with 0.5% SDS and equilibrated in scintillation fluid. The samples were measured in triplicate. The K_D and B_{max} values were (San Diego, CA).

S1P₁ Receptor Activation Assays

[0072] Receptor functional assays were performed in transiently transfected RH7777 cells by measuring S1P-activated [^{35} S]GTP γ S binding as previously described (28).

Statistical Analysis

[0073] The significance of differences was determined by one-way ANOVA, Bonferroni post-hoc test using Prism statistical software (GraphPad, San Diego, CA). Values were considered significantly different at $p < 0.05$.

Results

Mutagenesis strategy

[0074] The previously reported computationally modeled complex of S1P in S1P₁ features 15 amino acid residues in TM 3, 4 and 6 with atoms within 4.5 Å of S1P. In the present study the inventors pursued a three-pronged replacement strategy of these residues: First, property-conserving mutations of these residues were introduced that either reduced or increased size in order to probe the impact of increased or relaxed steric constraints in the hydrophobic binding pocket on ligand-induced activation. Additionally, many of these residues were replaced with charged amino acids of similar size to probe

whether disruption to hydrophobicity in the putative binding pocket would have an impact on receptor function. Third, in a few cases charged residues were replaced with non-charged residues of similar size to test the effect of polar interactions between the ligand and the receptor.

Theoretical model of the S1P₁-S1P complex – Revisions of the previous model.

[0075] Discrepancies between model-derived predictions and experimental observations in this and a previous study on S1P₄ (27) involved residues localized at the extracellular end of TM5. The differences the inventors found were not consistent with proposed structural differences between active and inactive GPCR conformations, and thus appear to be an error in the previous S1P₁ model (26,28). A corrected model of S1P₁ was built based on an alternative alignment of TM5 derived from the recently validated S1P₄ model (27). The corrected model demonstrates that 8 residues in TM5 have atoms within 4.5 Å of S1P. One of these residues, K5.38, forms an ion pair with the phosphate group of S1P. This polar interaction was not identified in previous validation of the S1P₁ model due to the incorrect positioning of amino acid residues at the top of TM5.

Cell Surface Expression of S1P₁ Mutants

[0076] In order to verify that the WT and mutant constructs were expressed at comparable levels, membrane fractions were prepared and analyzed for expression by Western blot analysis using the N-terminal FLAG epitope present in the constructs. The levels of expression on the membrane fractions were comparable to that of the WT receptor. FC analysis was used to determine if cell surface expression of the N-terminal FLAG epitope was similar for the mutant constructs to that of the WT (Table 5, which lists results for expression of pcDNA3.1 vector-transfected control, wild type S1P₁, and mutants which displayed no or diminished S1P-induced activation, in RH7777 cells examined by flow cytometry. Expression was detected with anti FLAG M2 monoclonal antibody. Flow

cytometry was performed as described in "Experimental Procedures". Four independent experiments were conducted.). The WT and the mutant constructs, with the exception of L6.41G, were expressed at the cell surface in similar levels based on immuno-labelling for the FLAG epitope. Because the L6.41G mutant protein was expressed in the cell lysate at a level similar to the other mutants but we were unable to detect it at the cell surface in multiple experiments, the inventors concluded that this mutation adversely affected the targeting of the receptor to the cell surface. The L6.41E mutant was expressed and included in the pharmacological testing.

Table 5
Cell Surface Expression of Vector, WT S1P₁ and Mutants With No S1P-induced Activation in RH7777 Cells

Construct	% Expression of construct from total population
vector	4.3
WT	9.5
M3.32K	19.3
L3.36E	15.2
L3.43,3.44E	10.3
F5.48G	22.1
L5.51E	17.4
L5.52A	14.1
V6.40L	19.6
L6.41G	5.6
W6.48A	12.6

The Effects of Mutations of Residues Lining Predicted Hydrophobic Binding Pocket on Ligand-induced Activation of S1P₁

[0077] In the first round of pharmacological testing the inventors evaluated the impact of all amino acid replacements on the EC₅₀ and maximal activation (E_{max}) elicited by S1P. The summary of the pharmacological properties caused by these replacements is presented in Table 3 A-E. After the first round of GTP γ S activation experiments were completed, it became apparent that of 15 residues mutated on the basis of the previously published S1P₁ model, 13 produced changes in receptor activation with the exception of two residues in TM5, F5.43 and T5.49. In addition, parallel studies carried out on S1P₄ (27) revealed that the inventors' model needed revisions with regard to the orientation of the top half of TM5. The position of F5.43 was shifted by one position in the helix compared to the

old model, causing a 100° difference in its orientation. Mutations of F5.43 and T5.49 had the least impact on S1P activation (Table 6). Replacement of T5.49 with G or S, which removed the polar side chain and reduced the residue size, respectively, had no effect of $E_{\max}$ and increased EC_{50} by three-fold. The Y and G replacement of F5.43, introducing a polar residue or one with decreased electron density and size, respectively, caused a modest reduction in $E_{\max}$ and an approximately ten-fold increase in EC_{50} . These residues, therefore, point away from the ligand, consistent with the modest impact (Table 6) replacements to these residues caused and the role they play in the hydrophobic binding pocket.

Table 6
The EC_{50} and $E_{\max}$ Values for the Mutants Which Retained S1P-induced Activation

Constructs	% WT $E_{\max}$ + s.d.	EC_{50} + s.d.	Nature of Amino Acid (AA) replacement
WT-S1P ₁	100 + 9.13	1.4 ± 0.7	
Vector	∞	∞	
F5.43Y	90 + 7	23 + 4.7	Introduction of polar functional group
F5.43G	70 + 6	11.8 + 3.9	Decrease in electron density and size
T5.49G	107 + 1.4	5.4 + 0.56	Removal of polar side chain
T5.49S	168 + 15	6.6 + 1.2	Reduction in size

[0078] The refined model was used to identify an additional 5 residues from TM5 within 4.5 Å of S1P for a second round of pharmacological testing. Out the 20 residues reported here, S1P-induced activation was altered for 18 residues.

[0079] The inventors found that replacements of seven residues depicted in Table 7 caused either marked decreases in $E_{\max}$ or increased EC_{50} substantially. These findings corroborate the predictions of the model and appear to be consistent with the hypothesis that even conservative mutational replacements of these amino acids in close proximity of the aliphatic part of S1P have a marked impact on the function of the receptor.

Table 7
The EC₅₀ and E_{max} Values for the Mutants Which Displayed Partial Activation or Increased EC₅₀ Compared to the Wild Type (WT)

Constructs	% WT E _{max} + s.d.	EC ₅₀ + s.d.	Nature of Amino Acid (AA) replacement
WT-S1P ₁	100 + 9.13	1.4 ± 0.7	
Vector	∞	∞	
L3.36E	39 ± 2.8	0.62 ± 0.46	Change from a nonpolar to a charged AA
L3.36G	75 + 12	23 ± 8	Reduction in size
V3.40T	137 + 10	13.8 ± 1.8	Change from a nonpolar to a polar AA
V3.40L	55 + 6.3	7.0 ± 4.5	Increase in size
K5.38A	97 + 15	32.4 + 8	Decrease in electron density and size of AA
I5.41A	26 + 4	157.2 + 35	Reduction in size
I5.41F	27 + 3.4	18.9 + 5	Increase in size and electron density
C5.44D	85 + 13	79 ± 17	Increase in polarity
C5.44I	37 + 2.3	20.4 ± 2.1	Removal of polar side chain
T5.45G	96 + 18	30.9 + 8.5	Removal of polar side chain
T5.45S	28 + 4	7.9 + 3	Reduction in size
L5.51E	∞	∞	Change from a nonpolar to a charged AA
L5.51G	46 + 8	2.6 ± 2.1	Reduction in size
V6.37T	181 + 26	13 + 2.4	Change from a nonpolar to a polar AA
V6.37L	247 + 11.3	37.6 + 8.4	Increase in size of AA
F6.44Y	37 + 2.6	3.7 ± 3.3	Introduction of polar functional AA
F6.44G	∞	∞	Decrease in electron density and size of AA
W6.48A	34 ± 1.5	13 ± 4.2	Decrease in electron density and size of AA

[0080] Mutants at four residues showed significant variability in S1P-induced response depending on the type of substitution. However, for these residues one of the replacement mutants remained responsive to ligand, sometimes with little or more pronounced decrease in activation (Table 8). Substitution of a charged moiety for M3.32 led to a receptor that showed no dose-dependent activation by S1P. Reduction in size of L6.41 and increase in size of V5.47 and V6.40 led to the loss of ligand-induced activation. In contrast, decreases in size at M3.32 and V6.40, changes polarity at V5.47 and V6.40 or addition of charge at L6.41 resulted in less drastic effects on ligand-induced receptor activation.

Table 8
The EC₅₀ and E_{max} Values for the Residues Displaying Variability Depending on the Amino Acid Substituted

Constructs	% WT E _{max} + s.d.	EC ₅₀ + s.d.	Nature of Amino Acid (AA) replacement
WT-S1P ₁	100 + 9.13	1.4 ± 0.7	
Vector	∞	∞	
M3.32K	∞	∞	Change from a nonpolar to a charged AA
M3.32G	77 + 5	35 ± 5	Reduction in size
V5.47T	100 + 3	89 + 15	Change from a nonpolar to a polar AA
V5.47L	∞	∞	Increase in size
V6.40A	69 + 4.4	14.8 + 3.8	Reduction in size
V6.40T	61 + 9.4	57.3 + 25	Change from a nonpolar to a polar AA
V6.40L	∞	∞	Increase in size
L6.41E	59 + 1.8	4.1 ± 0.63	Change from a nonpolar to a charged AA
L6.41G	∞	∞	Reduction in size

[0081] The computational model placed leucine L3.43 at the bottom of the hydrophobic binding pocket. Positions 3.43 and 3.44 are a conserved LL motif in all S1P

receptors, therefore their combined importance was tested by simultaneously replacing both residues with either a charged glutamic acid or smaller glycine. Either replacement caused a complete loss of ligand-induced activation (Table 9). These mutants, similar to those that were non-functional (listed in Table 8) always showed less than 20% of the basal GTP γ S binding, indicating that they were not constitutively active.

Table 9
The EC₅₀ and E_{max} Values for the Double Mutants

Constructs	% WT E _{max} + s.d.	EC ₅₀ + s.d.	Nature of Amino Acid (AA) replacement
WT-S1P ₁	100 + 9.13	1.4 ± 0.7	
Vector	∞	∞	
L3.43,3.44E	∞	∞	Change from a nonpolar to a charged AA
L3.43,3.44G	∞	∞	Reduction in size

[0082] The inventors noted that in TM 5 and 6 there were three residues, F5.48, L5.52, and W6.48, when altered either in charge or in size either completely or substantially lost their ligand-induced activation to S1P (Table 10). To further characterize the ligand-induced activation of these mutants, the inventors also exposed them to SEW2871, a recently-identified non-lipid agonist of S1P receptors (35). L3.36E, F5.48G, and W6.48A showed similarly impaired activation to SEW2871 to that seen with S1P. Unexpectedly, V6.40L showed a dose-dependent partial activation with SE2871.

Table 10*
The EC₅₀ and E_{max} Values for Mutations in Which Activation Was Abolished

Constructs	% WT E _{max} + s.d.	EC ₅₀ + s.d.	Nature of Amino Acid (AA) replacement
WT-S1P ₁	100 ± 9.13	1.4 + 0.7	
Vector	∞	∞	
F5.48Y	∞	∞	Increase in polarity
F5.48G	∞	∞	Decrease in electron density and size
L5.52A	∞	∞	Reduction in size
W6.48E	∞	∞	Decrease in electron density, size, and introduction of a charged AA

*S1P-induced receptor activation. Ligand-induced [³⁵S]GTP γ S binding was calculated as the difference between binding to 5-10 μ g of membrane fraction in the presence and absence of ligand. EC₅₀ values for S1P-induced (0.1 nM-10 μ M) GTP γ S activation was measured in RH7777 cells transfected with empty vector, WT S1P₁, and each mutant. E_{max} values were calculated as the maximal responses normalized to the WT maximal response. Two independent transfections were performed. Data points represent the mean $\pm$ SD (n=3).

[³²P]S1P Binding to S1P₁ Mutants Without Ligand-induced Activation

[0083] [³²P]S1P radioligand binding studies were performed with mutants M3.32K, L3.36E, L3.43,3.44E, L3.43,3.44G, C5.44D, V5.47L, V5.47T, F5.48G, L5.51E, L5.52A, V6.40L, L6.41G, and W6.48A, demonstrating much impaired dose-dependent activation by S1P in the GTP γ S activation experiments. In the inventors' system the apparent K_D for S1P binding at the WT receptor was 36 ± 2 nM (28). Therefore, a radioligand concentration of 50 nM was chosen to test whether those mutants that lacked activation would maintain some degree of S1P binding. Compared to the vector transfected cells, nine mutants out of the thirteen tested showed significant ligand binding (Fig. 4). Out of these nine with significant ligand binding, four mutants, L3.36E, F5.48G, V6.40L and W6.48A showed binding in excess of 50% of the WT. Thus, these replacements introduced in the putative hydrophobic ligand binding pocket had a pronounced tendency to affect ligand activation while maintaining some degree of ligand binding, suggesting that these residues play a crucial role in receptor activation, rather than solely ligand binding. The model demonstrates that L3.36 and W6.48 make good van der Waals contact with each other. The detrimental effect of mutation at either of these sites on receptor activation, but not ligand binding, suggests that this van der Waals contact is unique to the activated conformation of S1P₁. Docked complexes of S1P with these mutants displayed the polar interactions typical of the wild-type complex. This finding is consistent with the observed S1P binding by these mutants. The complexes additionally indicate that the hydrophobic tail of S1P does not extend as deeply into the hydrophobic pocket. The hydrophobic tail of S1P instead occupies the empty volume produced upon mutation of L3.36 or W6.48 to smaller or less branched residues. This difference in position for the hydrophobic tail of S1P is consistent with the lack of activation observed for these mutants.

[0084] The competition binding showed that the mutants, L3.36E, F5.48G, V6.40A, and W6.48A, which displayed at least 50% of the specific binding of the WT, had K_D and B_{max} comparable to the WT $S1P_1$. These data further validate the importance of the role which these residues play in the functionality, but not the binding of S1P to the $S1P_1$ receptor.

Identification of New $S1P_1$ Agonist Scaffolds

[0085] Docked complexes of two structurally distinct $S1P_1$ receptor agonists, S1P and SEW2871, were used to derive a pharmacophore describing the important chemical functional moieties and distances between those moieties that produce $S1P_1$ receptor activation. The superposed structures of S1P and SEW2871 from the docked complexes are shown in Fig. 9 and the distance ranges between pharmacophore elements are shown in Table 12. A subset of these distances was used to identify 13 hits in the Enhanced NCI Database Browser using a trifluoromethylphenyl group to provide the anionic bioisostere. Three hits were eliminated due to poor superposition on the known agonists. The remaining ten hits were categorized based on the quality of their rigid superposition onto the known agonist structures. Four hits were considered good matches (NSC146266, 145964, 59474, and 75030). Two hits were considered marginal matches (NSC55879 and 68644). Four hits were considered negatives, with additional bulk or incorrect curvature (NSC147843, 53638, 55534 and 99548). The ten hits were requested from the NCI Developmental Therapeutics Program.

[0086] Samples of seven compounds were available (Good – NSC146266 and 59474; Marginal – 55879; Negative - 147843, 53638, 55534 and 99548, Fig. 7A) and were screened for $S1P_1$ receptor activation in the $GTP\gamma S$ activation assay. Two of the seven showed $GTP\gamma S$ activation greater than that of the vehicle at 10 μM , NSC59474 and 99548. Dose response curves were then generated.

Table 11
 K_D and B_{max} Values of the Mutants Which Displayed at Least 50% Specific Binding of the WT, S1P₁.

Construct	K_D + s.d. [nM]	B_{max} (fmol)
WT-S1P ₁	38.7 + 1.2	2450
L3.36E	27.8 + 1.15	1980
F5.48G	20.2 + 1.2	2580
V6.40A	27.6 + 1.1	2040
W6.48A	38.5 + 1.15	2080

Table 12
Average S1P₁ Agonist Pharmacophore Distances (Å) Based on Complexes With S1P and SEW2871

	a	b	c	d	e
a		3.2	6.0	11.4	14.8
b			6.2	10.6	14.7
c				6.0	9.2
d					5.0

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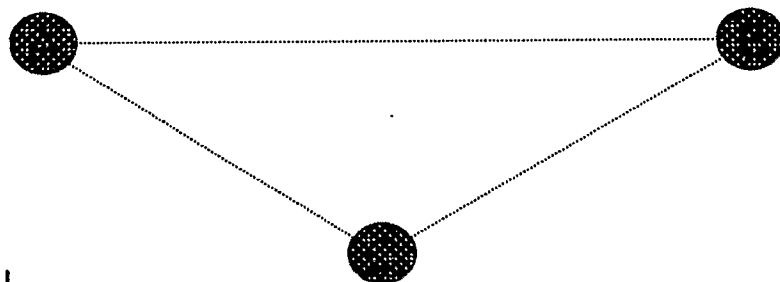
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What is claimed is:

1. A method for identifying and distinguishing compounds having LPA receptor agonist, LPA receptor antagonist, S1P agonist, or S1P antagonist activity, the method comprising
providing pharmacophore features and distances between features as described by an LPA receptor ligand pharmacophore, an S1P receptor ligand pharmacophore, or both, as input to a 3-dimensional database;
screening resultant matches by rigidly docking conformation matched to the pharmacophore into the receptor model; and
selecting structures for experimental screening based on their size and electronic complementarity to the receptor model.
2. A method as in claim 1 wherein the LPA receptor ligand pharmacophore comprises pharmacophore features of Scheme 1



Scheme I

where:

A is an anionic functional group;

B and C are hydrophobic functional groups;

an LPA₁ Antagonist (A) has a distance between A and B of 7-11 Å, a distance between B and C of 6-10 Å, and a distance between A and C of 8-12 Å;

an LPA₁ Antagonist (B) has a distance between A and B of 7-11 Å, a distance between B and C of 5-8 Å, and a distance between A and C of 6-12 Å;

an LPA₁ Agonist has a distance between A and B of 15-17 Å, a distance between B and C of 9.2-11.2 Å, a distance between A and C of 15.5-17.5 Å;

an LPA₂ Antagonist has a distance between A and B of 5-9 Å, a distance between B and C of 4-7 Å, and a distance between A and C of 4-6 Å;

an LPA₂ Agonist (A) has a distance between A and B of 6-8 Å, a distance between B and C of 15.5-17.5 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₂ Agonist (B) has a distance between A and B of 10-12 Å, a distance between B and C of 12-14 Å, and a distance between A and C of 18.5-20.5 Å;

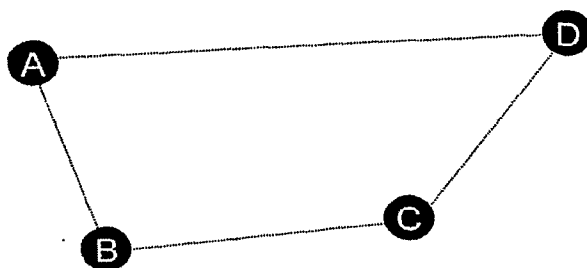
an LPA₃ Antagonist has a distance between A and B of 8-14 Å, a distance between B and C of 7-12 Å, and a distance between A and C of 12-16 Å;

an LPA₃ Agonist has a distance between A and B of 8.6-10 Å, a distance between B and C of 4.8-5, and a distance between A and C of 13.4-14.8;

anionic functional groups comprise either phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole, or combinations thereof; and

hydrophobic functional groups comprise saturated or unsaturated aliphatic, aromatic alkyl, or heteroaromatic alkyl.

3. A method as in claim 1 wherein the S1P₁₋₅ receptor ligand pharmacophore comprises pharmacophore features of Scheme 2



Scheme 2

where:

A is an anionic functional group;

B is a cationic or hydrophobic functional group;

C and D are hydrophobic functional groups;

an S1P₁ Agonist has a distance between A and B of 5-7 Å, a distance between A and C of 10.5-11.8 Å, a distance between A and D of 13-16 Å, a distance between B and C of

5.5-7 Å, a distance between B and D of 9-9.5 Å, a distance between C and D of 4.5-5.5 Å, and B is a hydrophobic functional group;

an S1P₂ Agonist has a distance between A and B of 3-5.7 Å, a distance between A and C of 7.5-9.0 Å, a distance between A and D of 14.9-17.3 Å, a distance between B and C of 3.0-6.9 Å, a distance between B and D of 12.4-16.1 Å, and a distance between C and D of 10.3-12.0 Å;

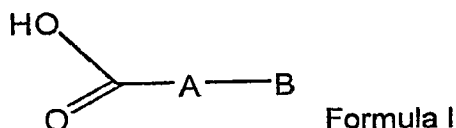
an S1P₃ Antagonist has a distance between A and B of 2.4-3.3 Å, a distance between A and D of 6.1-8.4 Å, a distance between B and C of 2.4-6.1 Å, and a distance between C and D of 5.1-7.9 Å;

an S1P₄ Agonist has a distance between A and B of 3-4 Å, a distance between A and C of 9-10 Å, a distance between A and D of 17-20 Å, a distance between B and C of 9-10 Å, a distance between B and D of 16.5-18.5 Å, and a distance between C and D of 9-10 Å;

anionic functional groups comprise phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole and trifluoromethyl; hydrophobic functional groups comprise saturated or unsaturated aliphatic, aromatic alkyl or heteroaromatic alkyl; and

cationic functional groups comprise amine and guanidine functional groups optionally substituted by aromatic hydrogens on electron-deficient aromatic systems (*i.e.*, those with nitro, trifluoromethyl and related substituents).

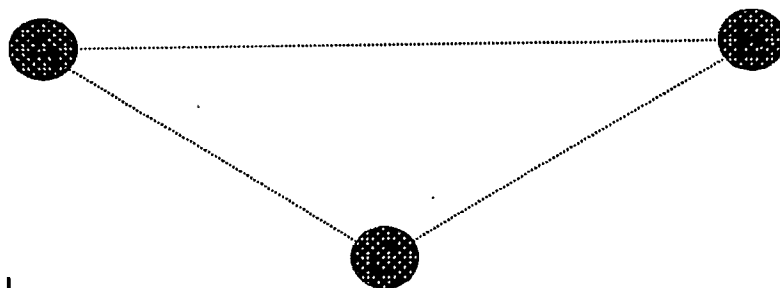
4. A method of producing an LPA₁-specific response in a human or animal subject, the method comprising administering a composition comprising one or more LPA₁ receptor antagonists as in formula I



where

B is substituted or unsubstituted aromatic or heteroaromatic;

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—}\text{N—} \right)_{1-3}$, or $\left(\text{—}\overset{\text{OH}}{\underset{\text{H}}{\text{C}}}\text{—}\text{N—} \right)_{1-3}$; and wherein the LPA₁ receptor antagonist comprises the pharmacophore features of Scheme I



Scheme I

wherein

A is an anionic functional group;

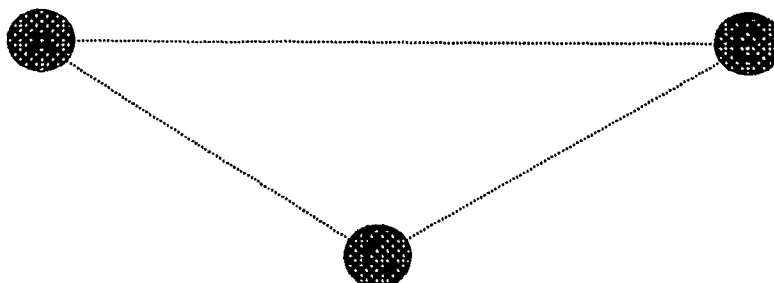
B and C are hydrophobic functional groups; and

the distance between A and B is 7-11 Å, the distance between B and C is 5-10 Å, and the distance between A and C of 6-12 Å.

5. The method of claim 4 wherein the distance between A and B is 7-11 Å, the distance between B and C is 5-8 Å, and the distance between A and C is 6-12 Å in the one or more LPA₁ receptor antagonists described by Scheme I.

6. The method of claim 4 wherein the distance between A and B is 7-11 Å, the distance between B and C is 6-10 Å, and the distance between A and C is 8-12 Å in the one or more LPA₁ receptor antagonists described by Scheme I.

7. An LPA receptor ligand comprising at least one anionic functional group and substituted or unsubstituted aromatic or heteroaromatic alkyl, the LPA receptor ligand being



described by Scheme I

where

A is an anionic functional group;

B and C are hydrophobic functional groups;

an LPA₁ Antagonist (A) has a distance between A and B of 7-11 Å, a distance between B and C of 6-10 Å, and a distance between A and C of 8-12 Å;

an LPA₁ Antagonist (B) has a distance between A and B of 7-11 Å, a distance between B and C of 5-8 Å, and a distance between A and C of 6-12 Å;

an LPA₁ Agonist has a distance between A and B of 15-17 Å, a distance between B and C of 9.2-11.2 Å, a distance between A and C of 15.5-17.5 Å;

an LPA₂ Antagonist has a distance between A and B of 5-9 Å, a distance between B and C of 4-7 Å, and a distance between A and C of 4-6 Å;

an LPA₂ Agonist (A) has a distance between A and B of 6-8 Å, a distance between B and C of 15.5-17.5 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₂ Agonist (B) has a distance between A and B of 10-12 Å, a distance between B and C of 12-14 Å, and a distance between A and C of 18.5-20.5 Å;

an LPA₃ Antagonist has a distance between A and B of 8-14 Å, a distance between B and C of 7-12 Å, and a distance between A and C of 12-16 Å;

an LPA₃ Agonist has a distance between A and B of 8.6-10 Å, a distance between B and C of 4.8-5, and a distance between A and C of 13.4-14.8;

anionic functional groups comprise either phosphate, carboxylate, sulfate, sulfonamide, sulfite, nitro, tetrazole, phosphonamide, amide, hydroxy-oxazole, hydroxyl-thiazole, or combinations thereof; and

hydrophobic functional groups comprise aromatic alkyl or heteroaromatic alkyl.

8. A composition as in claim 7 wherein the at least one anionic functional group comprises phosphate, carboxylate, or sulfate.

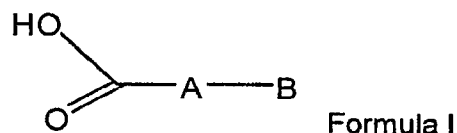
9. A composition as in claim 7 wherein the at least one anionic functional group is linked to the substituted or unsubstituted aromatic or heteroaromatic alkyl by a direct link.

10. A composition as in claim 7 wherein the at least one anionic functional group is linked to the substituted or unsubstituted aromatic or heteroaromatic alkyl by C₀₋₅ substituted or

unsubstituted alkyl, $\left(\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{N}- \end{array} \right)_{1-3}$, or

$\left(\begin{array}{c} \text{OH} \\ | \\ -\text{C}-\text{N}- \\ | \\ \text{H} \end{array} \right)_{1-3}$.

11. A method for producing an LPA₂-specific response in a human or animal subject, the method comprising administering one or more LPA₂ antagonists consisting of compounds chosen from among formula I,

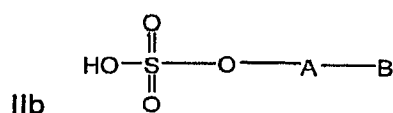
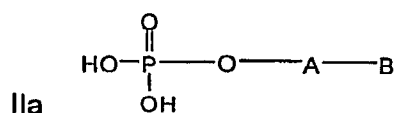


where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is a direct link or C₀₋₅ substituted or unsubstituted alkyl;

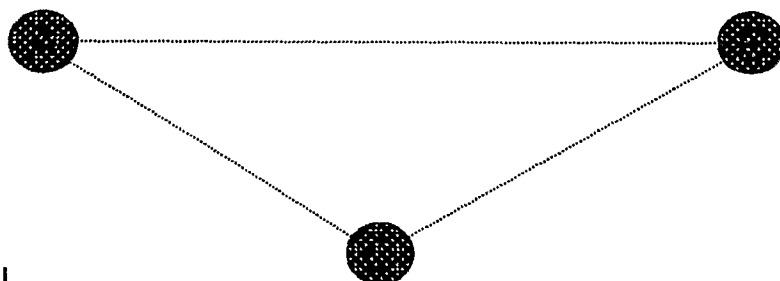
formula IIa or IIb



where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is either a direct link, C₀₋₅ substituted or unsubstituted alkyl, $\left(\text{---} \overset{\text{O}}{\parallel} \text{C---N---} \right)_{1-3}$, or $\left(\text{---} \underset{\text{H}}{\overset{\text{OH}}{\text{C}}}\text{---N---} \right)_{1-3}$; or combinations thereof; and wherein the LPA₂ receptor antagonist composition comprises the pharmacophore features of Scheme I



Scheme I

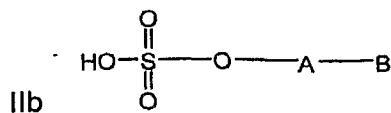
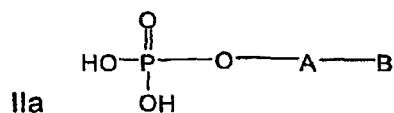
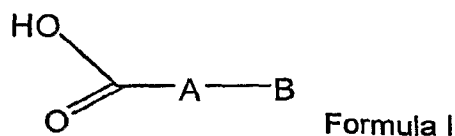
wherein

A is an anionic functional group;

B and C are hydrophobic functional groups; and

the distance between A and B is 5-9 Å, the distance between B and C is 4-7 Å, and the distance between A and C is 4-6 Å.

12. A method for producing an LPA₃-specific response in a human or animal subject, the method comprising administering a composition comprising one or more LPA₃ agonists chosen from among formula I, IIa, IIb, or combinations thereof,



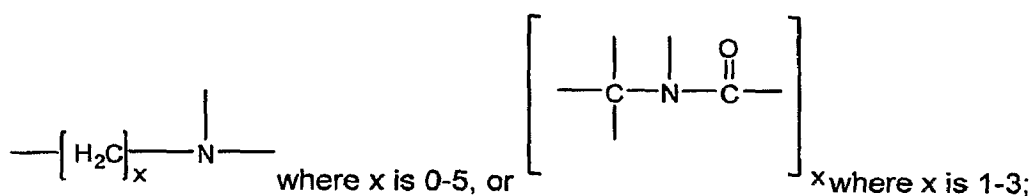
where

B is substituted or unsubstituted aromatic or heteroaromatic;

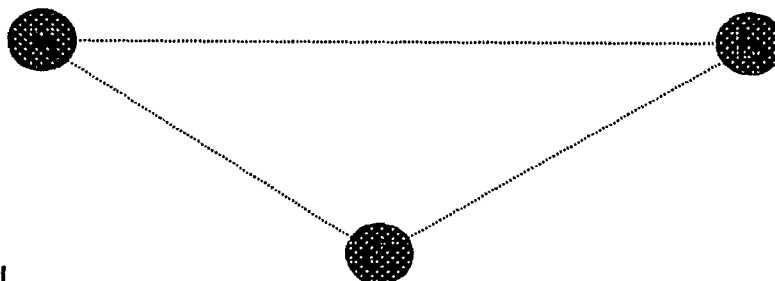
A is a direct link, $[\text{CH}_2]_x$ where x is 0-5, $\text{—}[\text{CH}_2]_x\text{—}\overset{\text{HO—C=O}}{\underset{\text{H}}{\text{C}}}\text{—NH—C(=O)—}$ where x is 0-5,



where x is 0-5,



phosphate may be substituted with di- or tri-phosphate; and wherein the one or more LPA_3 agonists comprises the pharmacophore features of Scheme I



Scheme I

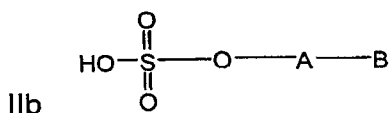
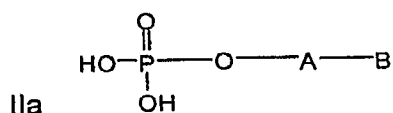
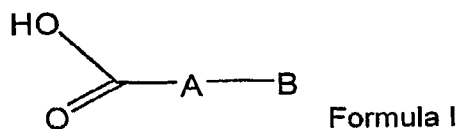
wherein

A is an anionic functional group;

B and C are hydrophobic functional groups; and

the distance between A and B is 8.6-10 Å, the distance between B and C is 4.8-5, and the distance between A and C is 13.4-14.8.

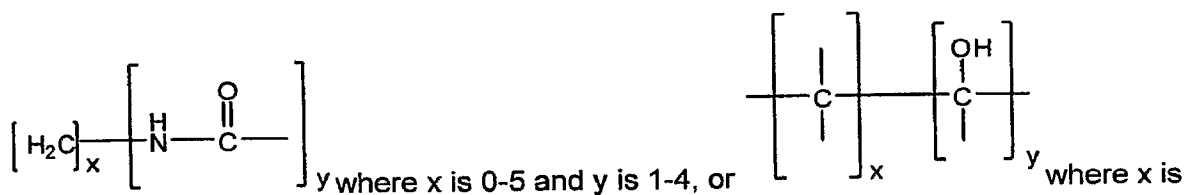
13. A method for producing an LPA_3 -specific response in a human or animal subject, the method comprising administering a composition comprising one or more LPA_3 antagonists of formulas I, IIa, IIb, or combinations thereof



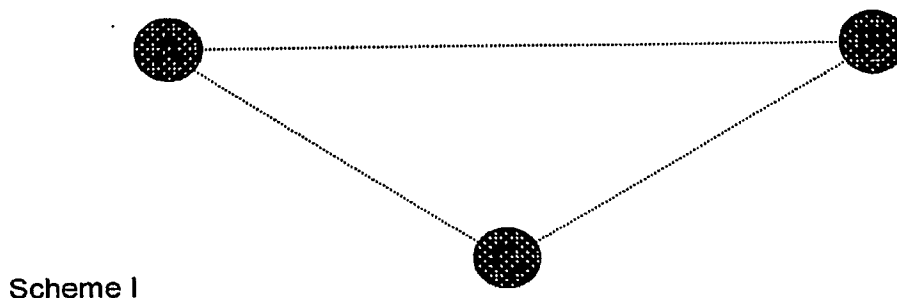
where

B is substituted or unsubstituted aromatic or heteroaromatic; and

A is a direct link, $[\text{CH}_2]_x$ where x is 0-5, $\text{---}[\text{H}_2\text{C}]_x\text{---N---}$ where x is 0-5,



and wherein the one or more LPA₃ antagonists comprises the pharmacophore features of Scheme I



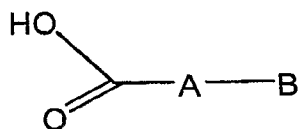
wherein

A is an anionic functional group;

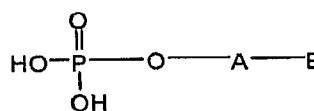
B and C are hydrophobic functional groups; and

the distance between A and B is 8-14 Å, the distance between B and C is 7-12 Å, and the distance between A and C is 12-16 Å;

14. A method for producing an S1P₁-specific response in a human or animal subject, the method comprising administering a composition comprising one or more S1P₁ agonists chosen from among formulas I, IIa, or a combination thereof



Formula I

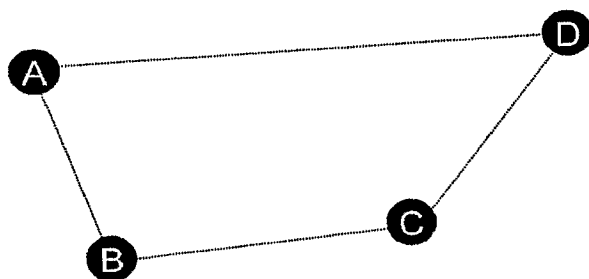


IIa

where

A is a direct link;

B is substituted or unsubstituted aromatic or heteroaromatic; and wherein the one or more S1P₁ agonists comprises the pharmacophore features of Scheme II



Scheme 2

where:

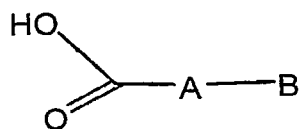
A is an anionic functional group;

B is a hydrophobic functional group;

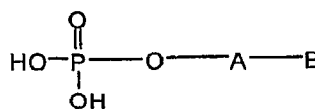
C and D are hydrophobic functional groups; and

the distance between A and B is 5-7 Å, the distance between A and C is 10.5-11.8 Å, the distance between A and D is 13-16 Å, the distance between B and C is 5.5-7 Å, the distance between B and D is 9-9.5 Å, and the distance between C and D is 4.5-5.5 Å.

15. A method for producing an S1P₂-specific response in a human or animal subject, the method comprising administering one or more S1P₂ agonists of formulas I or IIa

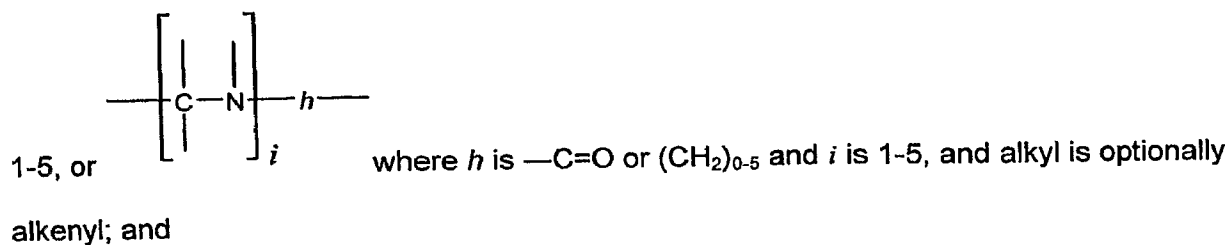
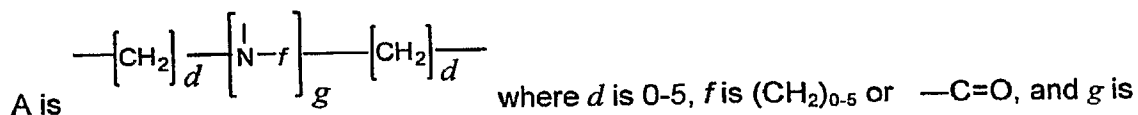


Formula I

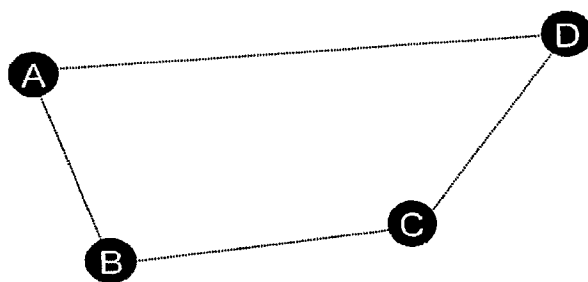


IIa

where



B is substituted or unsubstituted aromatic or heteroaromatic; and wherein the one or more S1P₂ agonists comprises the pharmacophore features of Scheme II



Scheme 2

where:

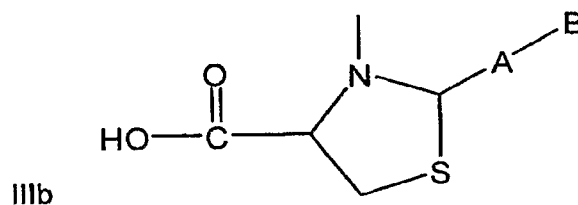
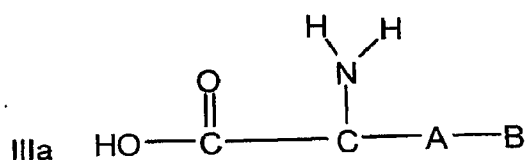
A is an anionic functional group;

B is a cationic or hydrophobic functional group;

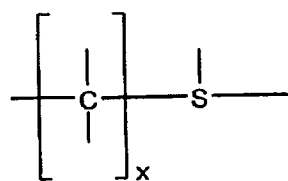
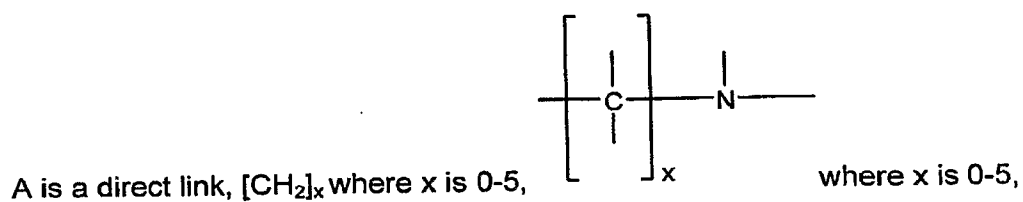
C and D are hydrophobic functional groups; and

the distance between A and B is 3-5.7 Å, the distance between A and C is 7.5-9.0 Å, the distance between A and D is 14.9-17.3 Å, the distance between B and C is 3.0-6.9 Å, the distance between B and D is 12.4-16.1 Å, and the distance between C and D is 10.3-12.0 Å.

16. A method for producing an S1P₃-specific response in a human or animal subject, the method comprising administering a composition comprising one or more S1P₃ antagonists of formulas IIIa or IIIb

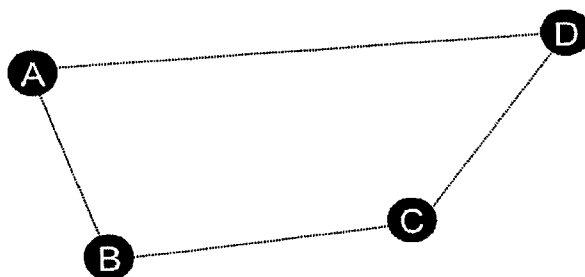


where



where x is 0-5 or $-\text{[CH}_2\text{]}_x-\text{S}-\text{[CH}_2\text{]}_x-$ where x is 0-5 and

B is substituted or unsubstituted aromatic or heteroaromatic; and wherein the one or more S1P₃ agonists comprises the pharmacophore features of Scheme II



Scheme 2

where:

A is an anionic functional group;

B is a cationic or hydrophobic functional group;

C and D are hydrophobic functional groups; and

the distance between A and B is 2.4-3.3 Å, the distance between A and D is 6.1-8.4 Å, the distance between B and C is 2.4-6.1 Å, and the distance between C and D is 5.1-7.9 Å.

Fig. 1

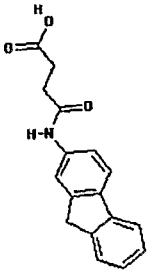
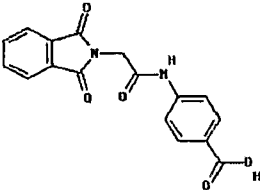
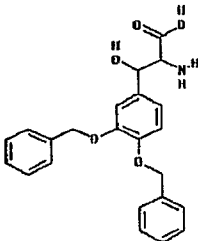
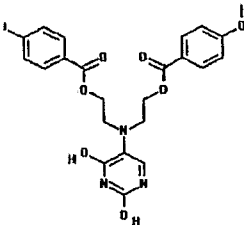
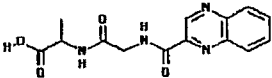
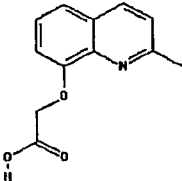
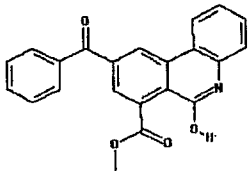
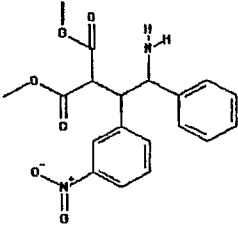
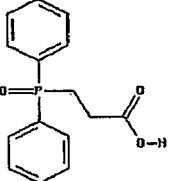
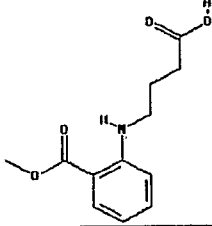
NSC #	Structure	NSC #	Structure
1235 3		20856	
9032 3		102631	
1121 86		121340	
1319 08		135202	
1452 03		163864	

Fig. 2

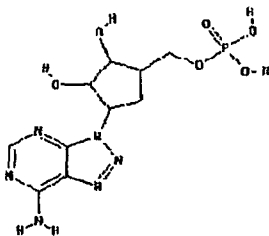
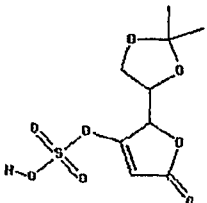
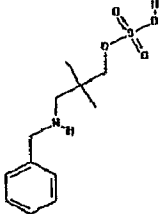
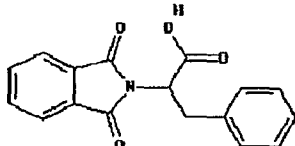
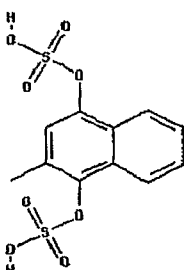
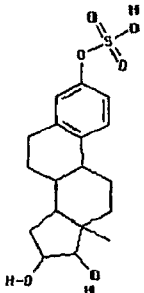
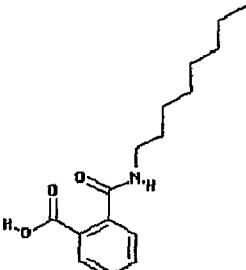
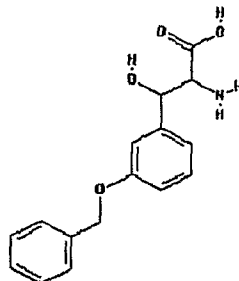
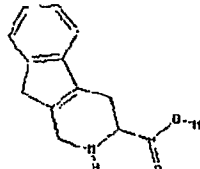
NSC #	Structure	NSC #	Structure
1431 48		116546	
1426 19		19760	
8757 6		246095	
9985		18762	
2206 1			

Fig. 3

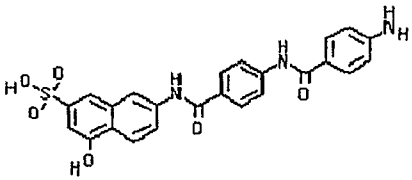
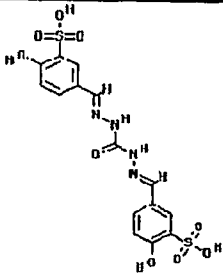
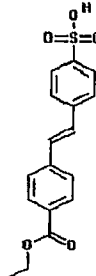
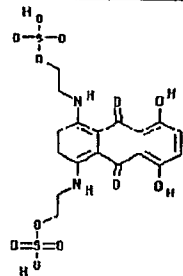
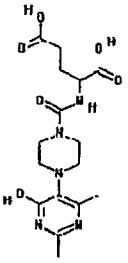
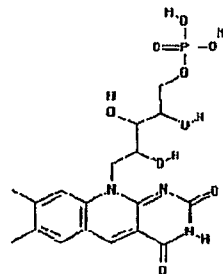
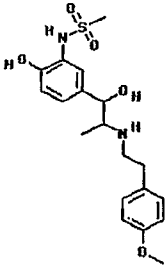
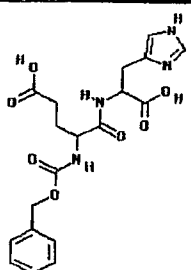
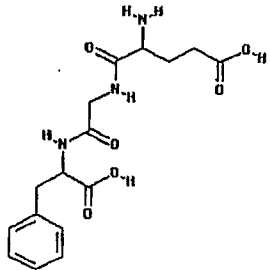
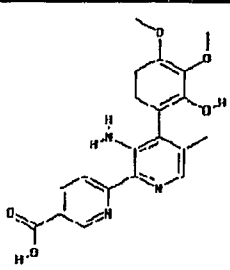
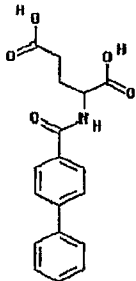
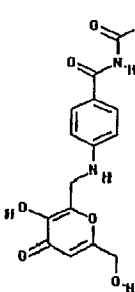
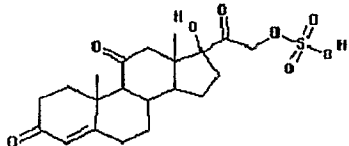
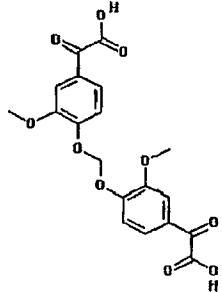
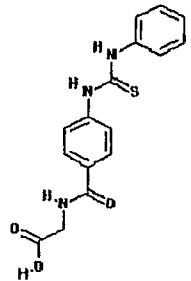
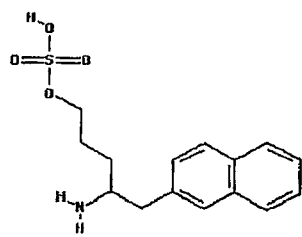
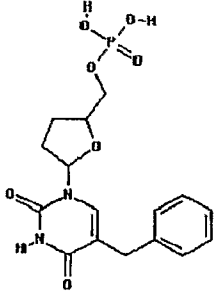
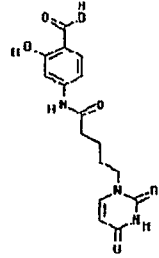
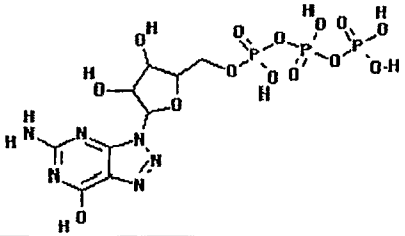
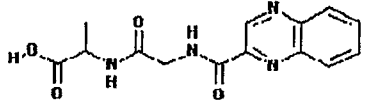
NSC #	Structure	NSC #	Structure
1741		48776	
1874 9		507748	
6731 07		168199	
2847 06		668596	
9795 3		76879	

Fig. 4

NSC #	Structure	NSC #	Structure
2110 19		116982	
1546 4		16739	
2136 30		297334	
6339 65		92432	
4753 4		112186	

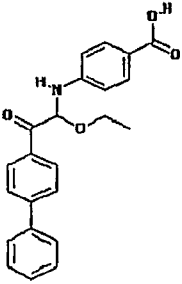
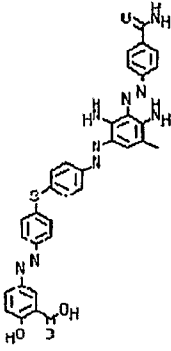
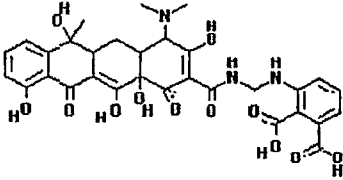
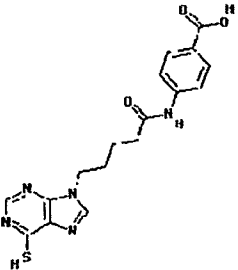
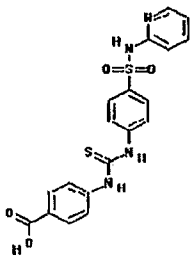
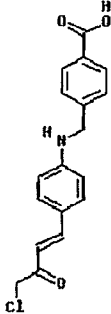
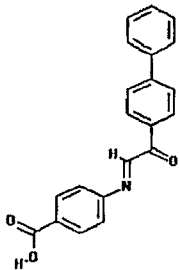
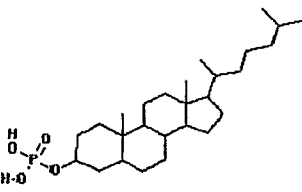
NSC#	Structure	NSC#	Structure
43482 (5899 1) (5918 2)		79592	
43891 (4389 0)		89819	
52105		106024	
73313		300586	

Fig. 5

Fig. 6

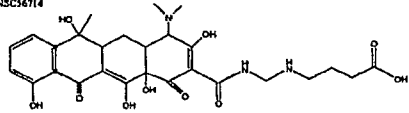
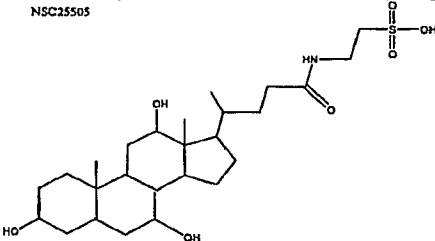
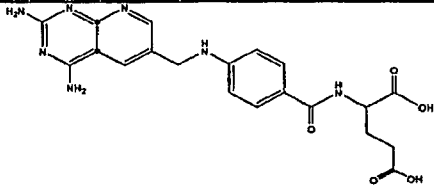
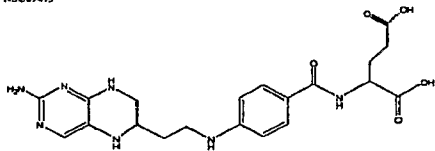
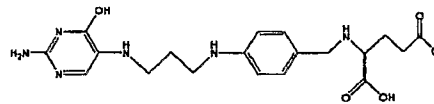
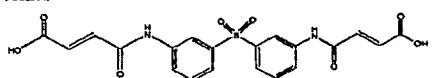
NSC #	Structure	NSC #	Structure
5671 4	NSC16714 	25505	NSC25505 
1101 83		89473	NSC89473 
9315 5	NSC93155 	25603	NSC25603 

Fig. 7

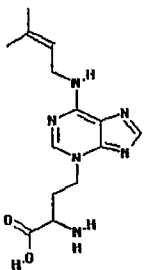
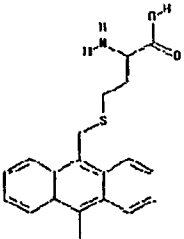
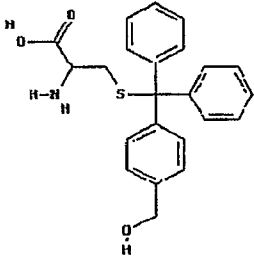
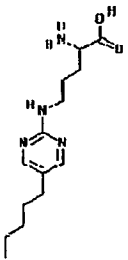
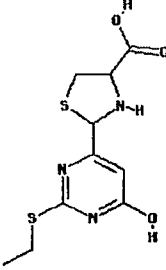
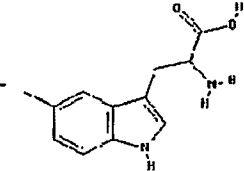
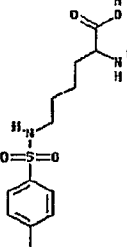
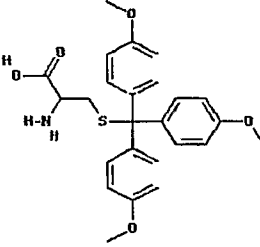
NSC #	Structure	NSC #	Structure
2890 77		169651	
1389 32		222815	
1549 16		57695	
1649 08		664407	

Fig. 8

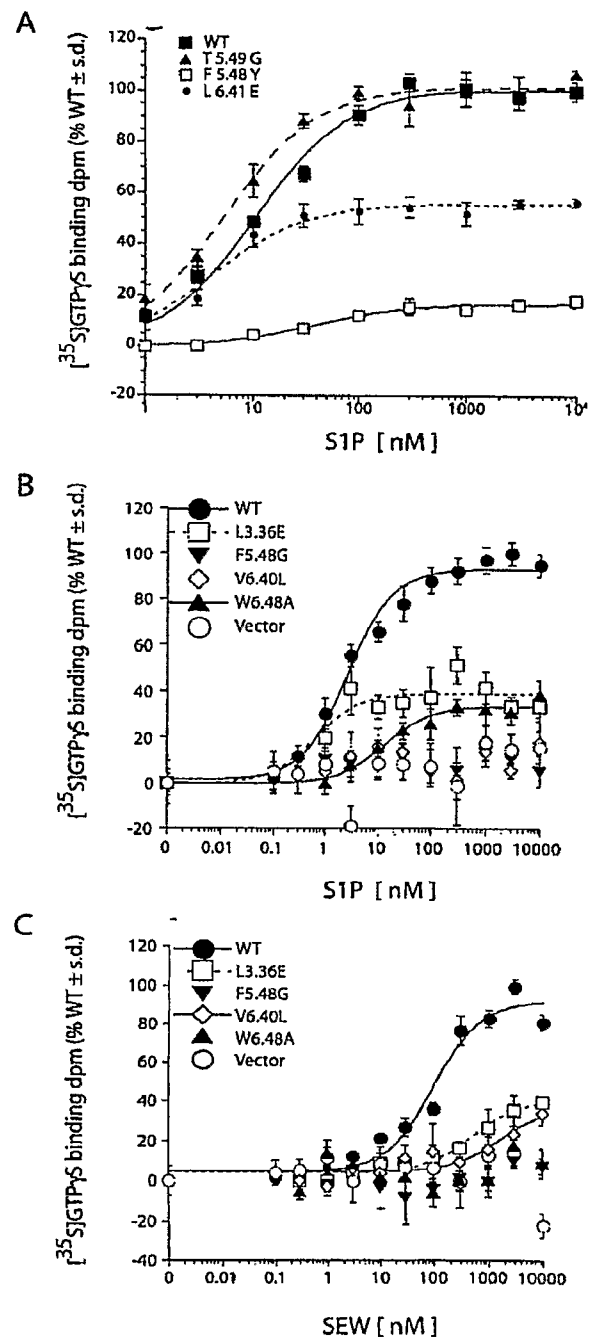


Fig. 9

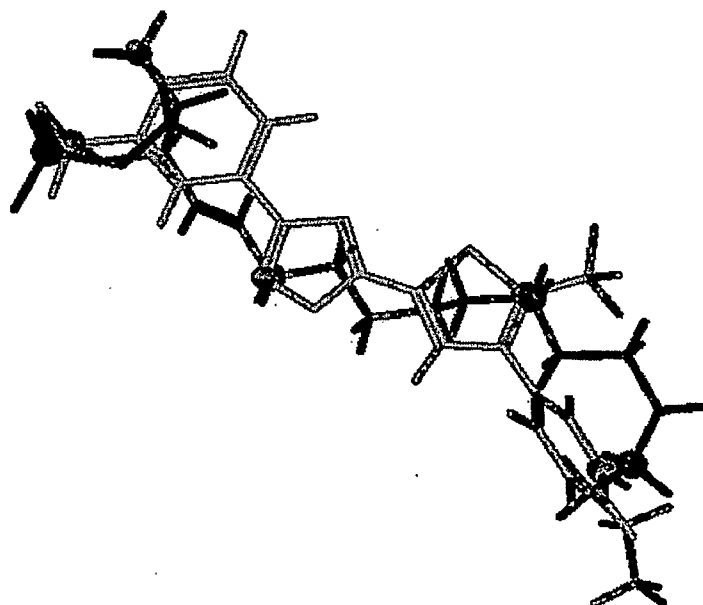


Fig. 9a

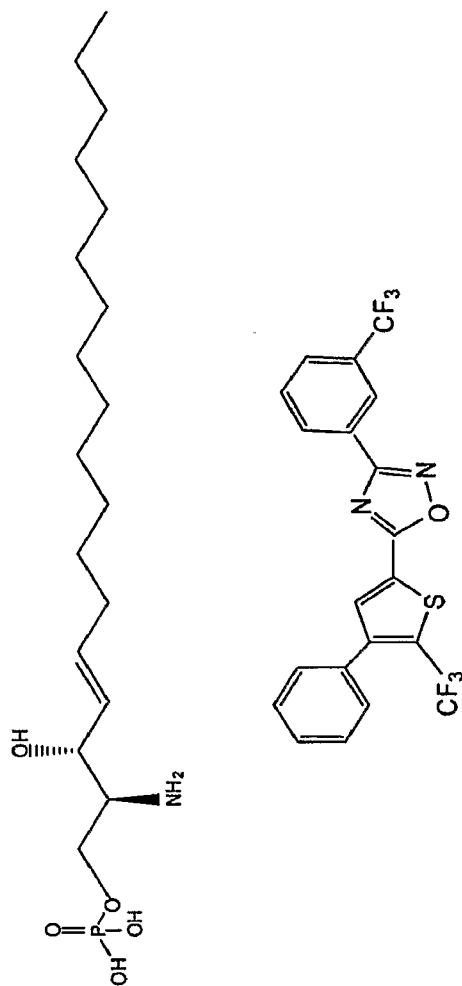


Fig. 9b

Fig. 10

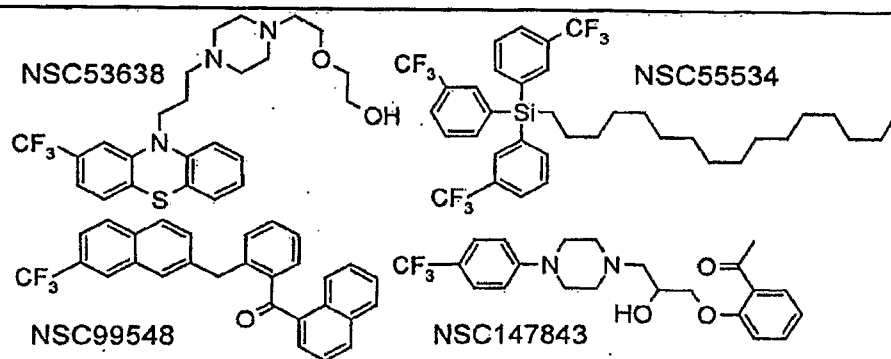
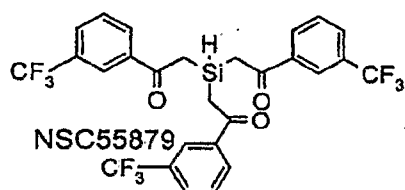
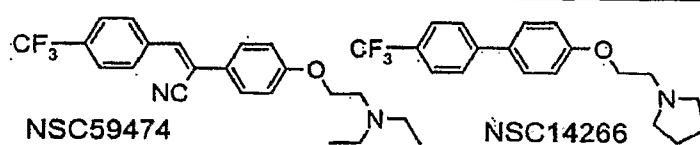
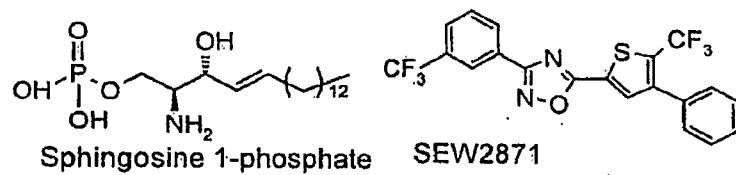


Fig. 11

