METHOD FOR IDENTIFYING NOVEL MULTIMERIC AGENTS THAT MODULATE RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/092,938, filed July 15, 1998 and U.S. Provisional Application Serial No. 60/088,466, filed June 8, 1998, both of which are incorporated herein in their entirety by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention is directed to general synthetic methods for generating large libraries of diverse multimeric compounds capable of binding cellular receptors which multimeric compounds are candidates for possessing multibinding properties. The diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

This invention also relates to ligands which bind to receptors and modulate their activity in living systems. More particularly, the invention relates to novel compounds that bind to and modulate the activity of receptors by acting as multi-

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binding agents. The multi-binding agents of the invention comprise at least two ligands connected by a linker or linkers, wherein said ligands in their monovalent state bind to and/or are capable of modulating the activity of the receptor. The linking moiety is chosen such that the multi-binding agents so constructed demonstrate increased biological activity as compared to individual units of the ligand. The invention is also related to methods of using such compounds, to methods of preparing such compounds and to pharmaceutical compositions containing them.

These multi-binding compounds are particularly useful in treating conditions in a mammal that are mediated by the cellular receptors targeted by the ligands. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

These multi-binding compounds may also be used as insecticides, and for other argricultural applications such as crop protection. Additionally, they are useful as affinity resins for affinity chromatography.

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All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

A receptor is a biological structure with one or more binding domains that reversibly complexes with one or more ligands, where that complexation has

biological consequences. Receptors are distinguished for the purpose of this application from enzymes, which bind and then transform the bound species. Receptors are most often proteins.

Receptors can exist entirely outside the cell (extracellularly), within the cell membrane (but presenting sections of the receptor to the extracellular milieu and cytosol), or entirely within the cell (intracellularly). They may also function independently of a cell (e.g. clot formation). Receptors within the cell membrane allow a cell to communicate with the space outside of its boundaries (i.e. signaling) as well as function in the transport of molecules and ions into and out of the cell.

A ligand is a binding partner for a receptor. A ligand may be the endogenous ligand for the receptor or alternatively may be a synthetic ligand for the receptor such as a drug, a drug candidate or a pharmacological tool.

Receptors can be categorized as G-protein coupled receptors, tyrosine kinase linked receptors, guanylate-cyclase linked receptors, nuclear steroid receptors, membrane bound steroid receptors, ligand-gated ion channel receptors and adhesion molecules.

1. G-protein coupled receptors

The super family of seven transmembrane proteins (7-TMs), also called G-protein coupled receptors (GPCRs), represents one of the most significant classes of membrane bound receptors that communicates changes that occur outside of the cell's boundaries to its interior, triggering a cellular response when appropriate. The G-proteins when activated, affect both positively and negatively a wide range of downstream effector systems (e.g. ion channels, protein kinase cascades, transcription, transmigration of adhesion proteins).

The GPCR is a membrane bound cell surface receptor that is comprised of a single polypeptide chain. It is composed of seven hydrophobic transmembrane helices. The N-terminus is extracellular, the C-terminus is intracellular. The sequential three dimensional orientation of these helices provides a number of binding domains for a variety of endogenous ligands, and the G-proteins. These molecules bind to and modulate the functional activity of these receptors.

One remarkable feature of a number of the 7-TM receptor classes is their widespread distribution and the different disease states that can be ameliorated by creation of subtype specific ligands. Examples include members of the serotonergic class (currently used with migraine therapy, GI motility agents, antipsychotics and antidepressants) and members of the adrenergic class (useful in the treatment of hypertension, asthma, prostrate disease and depression).

There are different classes of 7-TM receptors. Examples of ligands which bind to receptors include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
ACTH releasing factor (CRF)	Anxiety disorder, Depression (Obsessive/compulsive disorder, Cerebrovascular ischemia, Gastrointestinal disease, Alzheimers disease, Dementia, Obesity, Anorexia nervosa, Central nervous system disease, Behavior disorder, Thrombosis)	NBI-103, NBI-30775, NBI-104, NBI-27914, NBI-29356, SG-058/SC-241, PD-171729, CRA-0165, ebiratide, SA-627, SD-491, JE-884, Org-2766, CP-154526, NBI-31199, NBI-31200, NBI-112, CRA-1000, CRA-1001, NBI-104, NBI-30545, MCI-028, SC-241, NGD-98-1, DMP-695, urocortin, SG-058, Org-31433, SJ-948, NBI-113
Adenosine A1	Hypertension, Renal disease (Diuresis, Hypertension, Cognitive disorder, Renal failure, Heart arrhythmia, Cardiovascular disease, Allergy, Asthma, Inflammation, Heart disease, Depression, Cardiac failure, Angina, Alzheimers disease	FK-352, KW-3902, SDZ-WAG-994, NNC-210136, CVT-510, FR-166124, PD-81723, IRFI-117, WRC-0571, UP-20256, N-0840, KF-15372, N-0861, KW-3902, CVT-124, GR-79236, KF-17837, midaxifylline, NNC-21-0041, NNC-90-1515, methoxy flavone derivatives, apaxifylline, FK-453, AMP-579, NNC-21-0091, NNC-21-0238, RG-14202, N-0723, UP-2-2-56, FK-838, PD-126280, N-0840, BN-063, IRFI-165, KF-15372, BW-1205U90, MDL-102503, DTI-0009, WRC-0571, UP-202-32, GP-04012, RS-74513-000, Sch-59761

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Adenosine A2	Parkinsons disease (Depression, Thromboembolism, Neurodegenerative disease, Neurological disease, Anxiety disorder, Pain), Hypertension, Restenosis (Cardiovascular disease, Asthma, Hypotension)	KW-6002, KF-17867, WRC-0470, SEP-89068/SEP-119249, Sch-58261/8FB-PTP, CGS-21680C, methoxy flavone derivatives, AMP-579, ZM-241385, 2-(3-cyclopentyl-1-propyn-1-yl)adenosine, HENECA, 8FB-PTP, CGS-22989, YT-146, CGS-15943, MDL-101483, GW-328267, Sch-59761, KF-17867, CL-356381, CL-288875
Adrenergic α1a	Benign Prostatic Hypertrophy	Tamsulosin, SNAP-5089, SNAP-5399, SNAP-5582, SNAP-6107, SNAP-6145, SNAP-6201, SNAP-6262, SNAP-7600, SNAP-6719, SNAP-7461, SNAP-7443, SNAP-7555, SNAP-7556, SNAP-7180, SNAP-7292, SNAP-5540, naftopidil, terazosin, RS-100975, A-131701, SB-216469, KMD-3213, SL-890591, doxazosin, prazosin, alfuzosin, L-794191, L-757464, trimazosin
Adrenergic α2	Anesthesia, Multiple sclerosis, Muscle hypertonia, Muscular spasm, Ocular hypertension, Glaucoma, Pain, Hypertension, Cognitive disorders, Migraine, Central nervous system disease, Attention deficit hyperactivity disorder, Sedation, Diabetes mellitus, Cardiovascular disease, Micturition disorder, Thromboembolism, Impotence, Sexual dysfunction	Dexmedetomidine, mivazerol, idazoxan, fluparoxan, AGN-193080/AGN-192172, AGN-190837, SKF-104078/BRL-44408, L-657743, F-10981, PGE-6201204, UK-1403, fluparoxan, brimonidine, ecabapide, CHF-1035, S-17089-1, AGN-192836, AGN-191103, midodrine, UK-14304, amosualol, indoramin, PMS-812, TA-993, delequamine
Adrenergic β1	Hypertension, Heart failure, Cardiovascular disorder, Glaucoma, Angina	Bucindolol, nebivolol, atenolol, S-atenolol, sotalol, timolol, esmolol, carteolol, propranolol, betaxolol, GI-104303, amosualol, cetamolol, penbutolol, DCC-10345, UK-1745, metoprolol, acebutolol, bisoprolol, nadolol, pindolol, lebatolol,

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Adrenergic β2	Asthma, COPD, Premature labor	Bucindolol, HSR-81, formoterol, R,R-formoterol, salmeterol, R-salmeterol, albuterol, levalbuterol, clenbuterol, ritodrine, terbutaline, fenoterol, isoproterenol, pirbuterol, bitolterol, colterol, metaproterenol, isoetharine, epinephrine
Adrenergic β3	Obesity, Irritable bowel syndrome, NIDDM	L-362884, SR-59230A, ICI-198157, SR-58611A, L-749372, CP-114271, AD-9677, SM-11044, L-755507, SB-226552, CL-316243, BRL-37344, BMS-196085, CGP-12177, LY-362884, BMS-187257, ICI-201651
Angiotensin II	Hypertension, CHF	Losartan, valsartan, irbesartan, candesartan, eprosartan, tasosartan, telmisartan, ripisartan, CS-866, DA-727, KRH-594, LR-B/081, TAK-536, YM-358, MK-996, milfasartan, BIBS-39, KT3-671, L-162313, BIBS-222, CL-329167, zolasartan, EXP-597, DuP-532, EXP-332, PD-123319, PD-121981, SC-52458, L-163579, L-163017, A-81988, XH-148, EMD-66684, ME-3221, L-163007, L-159689, L-162234, L-159913, UR-7198, L-162389, L-162638, PD-126055, S-0029, L-162686, RU-64276, CGP-42112, BIBR-363, XR-510, L-163598, L-162393, SB-203220, EXP-408, KW-3433, KRH-594, WK-1492, L-162620, EXP-970, YM-31472, PD-123177, TAK-536, L-158809, L-158978, HR-720, L-735286, WAY-126227, KT3-866, SC054629, RWJ-47639, BMS-183920, LF7-0156, LY-301875, L-161290, L-162132, EXP-801, RU-65868, ICI-D-8731, GR-137977, SK-1080, DMP-581, A-81282, CI-996, FR-149581, 606A,
Bradykinin BK-2	Asthma, Pain, Pancreatitis, Cardiovascular disease, Urinary tract disorders, Cerebrovascular ischemia, Allergy, Inflammation, Arthritis	FR-167344, NPC-17731, CP-0597, icatibant, NPC-567, WIN-64388, CP-0578, FR-190997, B-9340, NPC-18884, LF-160335, FR-184280, B-9430, B-9698, CP-2559, B-9858, CP-2550, NPC-16731, MEN-11270, FR-172357, S-16118, FK-3657, LF-16-0687, NPC-17761, CP-0364, CP-840, CP-0719, CP-2055, NPC-18521, NPC-18688, PS-322835

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Cannabinoid	Emesis, AIDS-related complex, Alzheimers disease, Anorexia nervosa, Depression, Sustance dependence, Allergy, Inflammation, Pulmonary disease, Immune disorder, Glaucoma, Psychosis, Pain, Gastrointestinal disease, Urinary tract disease, Cardiovascualar disease, Brain injury, Cognitive disorder	Dronabinol, P-0792, SR-141716, WIN-55212-3, HU-210, L-759787/L-768242, DP-55940, BAY-38-7271, WIN-55212-2, morpholinoalkylindenes, anandamide, palmidrol, JWH-015, SR-144528, WIN-56098, SR-140098, AM-630, SR-41716A, O-585, JWH-051, WIN-54461, L-759633, L-759656, CP-55940, O-823, O-689, LY-320135, CP-55244, CT-3, nabilone, P-0792
Chemokine CXCR-3	Inflammation	SKF-83589, SB-225002
Chemokine CCR-3	Asthma, Psoriasis, Multiple sclerosis, Rheumatoid arthritis	Chemotides, NSC-651016, NSC-645797, NSC-655720
Chemokine CCR-5, CXCR-4	HIV infection, Viral infection	VMIP-II, AOP-RANTES, Nananoyl-RANTES, SDF-1, NSC-651016, NSC-645797, NSC-655720, AMD-3100, T-22, SPC-3
Cholecystokinin A (CCK A)	Carcinoma, Eating disorder, Gastric motility disorder, Gastrointestinal disease, Pancreatitis, Pain, Obesity, Anxiety disorder, Schizophrenia, Motility disorder, Gall bladder disease	Loxiglumide, dexloxiglumide, FK-480, TP-680, GW-5823, PD-149164, PD-145942, SR-146131, T-0632, FPL-15849, FR-193108, A-71623, loxiglumide, lintitript, IQM-95333, GW-7854, GW-7178, A-74498, A-71623, A-71378, PD-142898, FR-208419, A-70276, FPL-15849, dexoxiglumide, PD-140548, FPL-14294, A-70874, A-57282, devazepide, A-68552, PD-151932, PD-149164, Z-203, SR-146131, A-71378, SR-27950, T-0632, KSG-504, TP-680, GI-248573, L-365031

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Cholecystokinin B (CCK B, Gastrin receptor)	Sleep disorder, Anxiety disorder, Drug dependence, Gastrointestinal disease, Neurological, mental, and cognitive disorders, Pain, Pancreatitis, Depression, Central nervous system disease, Peptic ulcer, Anorexia nervosa, Eating disorder, Substance dependence	GV-150013, L-740093, JB-931182, PD-135158, PD-145942, L-368935, L-369466, L-736380, AG-041R, Tetronothiodin, RPR-101367, YM-220, FR-193108, GV-150013, PD-134308, tetronothiodin, YM-022, L-708474, CI-988, GW-7854, L-365260, C1015, L-736380, GR-199114X, L-751892, PD-142898, RPR-107836, CP-212454, JB-931182, FK-480, CR-2622, CR-2194, PD-136450, FR-208419, L-369466, Ro-09-1540, DZ-3514, L-708474, RP-69758, CR-2345, CR-2945, RPR-102681, GV-191869, lorglumide, CR-2194, LY-288513, FR-175985, PD-135666, RP-72540, RP-71483, LY-262291, LY-, YF-476, L-156586, RP-73870, D-15-9927, D-61-1608
Dopamine 1	Acute Renal Failure, CHF, Parkinsons disease, NIDDM, Obesity, Cardiovascular disease, Psychosis, Schizophrenia, Glaucoma, Hypertension, Angina	Fenoldopam, A-68930, CY-208243, A-77636, dihydrexidine, BAM-1110, bromocriptine, SDZ-38393, Z-1046, A-86929, SKF-82958, odapipam, A-85845, A-69024, BTS-73947, zuclopenthixol, BIMG80, FPL-63012AR, SDZ-GLC-756, voxergolide, SKF-38393, YM-435, Sch-23390, NNC-22-0010, SKF-89626, BW-737C89, SKF-83565, SKF-83959, Z-12571, SDZ-PSD-958, LY-270411, SKF-81297, TOFA, ABT-431, NNC-687, Sch-39166, SKF-80723, A-86926

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Endothelin A	Pulmonary Hypertension, CHF, Hypertension, Pancreatitis, Renal failure, Migraine, Restenosis, Myocardial infarction, Atherosclerosis, Glaucoma	Bosantan, PD-145065, Ro-46-2005, BQ-610, SB-209670, BQ-123, TAK-044, FR-139317, BMS-182874, TBC-11251, BQ-153, PD-161721; PD-156707, PD-142893, PD-155080, CGS-27830, PD-155218, FR-901533, L-744453, LU-135252, PD-151242, PD-160874, EMD-122801, IRL-1666, BQ-3020, ZD-1611, S-0139, ABT-627, T-0115, RPR-111844, YM-598, L-749239, FR-901367, PD-156252, LU-302872, A-104029, BQ-928, PD-159433, PD-162073, SQ-35469, SQ-34520, J-104132, IRL-3461, BMS-207940, A-182086, TAN-2162, IRL-1543, A-158112, A-207508, IPI-616, TBC-2576, PD-152884, PD-156123, 97-139, LU-127043, A-104029, L-746072, VML-588, T-0201, L-747844, EMD-122946, Ro-485695, SB-234551, PD-102566, RES-701-2, RES-1214-1, PD-159110, PD-160672, PD-159020, RPR-105227, RPR-111613, Ro-43-1736, A-216546, ATZ-1993, ZD-2574, LU-302146, ABT-147627, BQ-145, stachybosins, BMS-187308, A-201661, PD-166114, EMD-94246, BQ-518, TBC-3214, TBC-11299, RPR-110477, TBC-11040, Ro-61-0612, ZD-4054, BMS-193884, Ro-48-5694, S-1265, SB-255757, PD-166673, SB-215355, PD-180988, PD-163070, TBC-10662, Ro-44-9099, Ro-06-2687, BQ-485, 50-235, BQ-238, J-104121, IRL-2500, IRL-2659, IRL-1038, IRL-2796, PD-155719, SB-247083, PD-163610, BE-18257B,
Glucagon	Diabetes mellitus (Insulin dependent diabetes, Non-insulin dependent diabetes, Acidosis, Obesity)	L-168049, BAY-27-9955, NNC-92-1687, ALT-3000, A-4166, L-168049, CP-99-711
Metabotropic glutamate 1	Epilepsy, Cerebrovascular ischemia, Head injury, Alzheimers disease, Ischemia, Pain	NNC-07-0775, 3,5-DHPG, NPS-2390, ACPD, UPF-523, LY-393675, LY-3390334, UPF-596, LY-367385, LY-302427, ACUDA
Metabotropic glutamate 2	Anxiety disorder, Nicotine use disorder, Central nervous system disease, Substance dependence, Epilepsy, Neurodegenerative disease	LY-354740, F-2-CCG-I, NNC-07-0775, LY-341495

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Histamine H1	Allergy, Asthma, Eczema, Papular skin disease, Urticaria, Pruritis, Rhinitis, Ocular disease	Loratidine, carebastine, norastemizole, efletirizine, HSR-609, emedastine, terfenadine, ZCR-2060, WY-49051, KAA-276, epinastine, ebastine, E-4716, KC-11404, fexofenadine, FK-613, selenotifan, DF-1111301, loratidine, MDL-28163, MDL-103896, desloratidine, mizolastine, KC-11425, KA-398, cetirizine, noberastine, VUF-L-9015, tagorizine, histaprodifen, alinastine, diphenhydramine, , chlorpheniramine, ketotifen, oxatomide, astemizole, acrivastine, azelastine, triprolidine, hydroxyzine, azatadine, rupatadine, MDL-16455, pyrilamine, promethazine, chlorcyclizine, carbinoxamine, clemastine, dimenhydrinate, tripelennamine, brompheniramine, cyclizine, meclizine, levocabastine
Histamine H2	Duodenal ulcer, Gastritis, Stomach ulcer (Peptic ulcer, Ulcer)	Famotidine, ebrotidine, lafutidine, osutidine, IT-066, P-Z-300, KP-105, BMY-25368 , IGN-2098, osutidine, FRG-8701, Z-300, KP-105, IY-80845, cimetidine, ranitidine, famotidine, nizatidine
Histamine H3	Cognitive disorders (Alzheimers disease, Depression, Epilepsy, Obesity, Sleep disorder, Attention deficit hyperactivity disorder, Eating disorder, Central nervous system disease, Neurological disease, Allergy, Asthma, Mental disorder)	GR-175737; GT-2331, UCL-1972, GR-168320, GT-2016, AQ-0145, UCL-1199, GR-175737, verongamine, BP-294, FUB-181, ciproxifan, GR-168320, thioperamide, VUF-9153, FUB-181, UCL-1972, proxyfan, iodoproxyfan, UCL-1199, VUF-4163, SKF-91606, imifluramine, BP-294, UCL-1283, GT-2104, GT-2203, impentamine, UCL-1409, UCL-1390

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
5-HT 1b/d/f	Migraine	Naratriptan, sumatriptan, eletriptan, rizatriptan, zolmitriptan, frovatriptan, avitriptan, parthenolide, LY-334370, alnidita n, L-741604, CP-122288, GR-46611, BMS-181885, SB-220453, IS-159, F-11356, PNU-109291, almotriptan, MDL-74721, 5-(nonyloxy)-tryptamine, LAS-31727, LY-344864, GMC-2021, SKF-99101H, SB-205209, L-772405, CP-135807, MDL-100687A, CP-119333, CP-124439, CP-108509, L-694247, U-109291, ALX-1348, L-775606, ALX-0646, ALX-0625, CP-161242, ALX-1417, SB-218842, L-755606, 324C91, S-CM-GTNH2, GR-40370, GR-125743, anpirtoline, BRL-54443, BMS-181101, CP-119333, RU-24969
5-HT 2	Psychosis, Schizophrenia, Depression, Sexual dysfunction, Anxiety, Sleep disorders, Migraine, Cardiovascular disease, Thromboembolism, Hypertension	Risperidone, dotarizine, metergoline, sergolexole, fananserin, adatanserin, ritanserin, amesergide, iloperidone, SR-46349, carvotroline, perospirone, SM-13496, sertindole, ziprasidone, YM-35992, deramciclane, olanzapine, dotarizine, ocaperidone, amperozide, Org-5222, CV-5197, mirtazapine, AD-5423, flibanserin, zotepine, LU-29066, P-1704, DV-7028, quetiapine, LU-26042, LEK-8829, glemanserin, ketanserin, ICI-170809, SR-46615A, CGS-18102A, pelanserin, NNC-22-0031, LY-215840, E-2040, BMS-181102, SUN-9221, NPC-18166, P-706A, SB-224289, ZD-3638, SDZ-MAR-327, NRA-0045, MDL-28133A, perbufylline, nefazodone, DuP-734, SUN-C-5174, balaperidone, ICI-169369, lintopride, P-9662, HT-90B, FG-5803, BW-1205U90, EX-711, metergoline, lidanserin, sarpogrelate, AT-1015, alentemol, emopamil, LY-053857, JL-13

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
5-HT 4	Constipation, Gastric motility disorder, Irritable bowel syndrome, IBD, Pain, Gastrointestinal disease, Neurological disease, Emesis, Dyspepsia, Esophagitis, Central nervous system disease, migraine, anxiety disorder	Piboserod, tegaserod, lintopride, renzapride, mosapride, itasetron, fabesetron, SB-207266, SB-207710, SB-204070, SB-203186, SDZ-205-557, R-93877, E-3620, YM-47813, SL-65.0102, LY-297524, LY-353433, RS-39604, RS-33800, RS-67506, RS-67333, RS-17017, RS-23597-190, RS-16566, RS-67532, RS-56532, RS-100235, BIMU-8, KGA-0941, SC-55387, Y-36912, SDZ-216454, SDZ-205-557, SC-53116, SC-52491, SC-53606, SC-50410, GR-113808, FCE-29029, FCE-29034, ML-1035, KGA-0941, SK-951, GR-125487
Leukotriene D4	Asthma, Inflammation	Zafirlukast, tomelukast, montelukast, pobilukast, CGP-44826, CGP-57698, L-695499, L-708734, L-708738, L-648051, L-733321, LY-287192, LY-290154, SKF-106203, LM-1453, CP-199330, OT-4003, FK-011, MCI-826, RG-12525, CP-195494, FPL-55712
Muscarinic M3, M2	Overactive Bladder, COPD, Asthma, Irritable Bowel Syndrome, gastrointestinal disease, Micturition disorder, Cognitive disorder, Alzheimers disease, Heart arrhythmia	Darifenacin, zamifenacin, amiridin, revatropate, alvameline, otenzepad, YM-46303, TMB-8, J-104129, AF-DX-384, DAU-5750, FPL-15467, YM-35636, YM-53705, KRP-197, YM-43571, YM-59981, DAC-6152, BIBN-99, BIBN-140, AQ-RA-741, PDC-008.004, Sch-57790, DAC-5889
Neuropeptide Y Receptor	Bulimia nervosa, Eating disorder, Obesity, Hypertension, Depression, Heart disease	NGD-95-1, SR-120819A, SR-120107A, GW-1229, CGP-71683A, BW-1229, GI-264879A, BMS-192548, SNAP-6608, BIBP-3226, PD-160170, BIBO-3304, SNX-024
Opioid δ	Pain (Drug dependence)	SB-213698B/SB-237596, SNC-80/DPI-2505, RWJ-105319, SNF-9007, TAN-67, SL-3111, SC-39566
Opioid κ	Irritable Bowel Syndrome, Dydpepsia	Fedotozine, Asimadoline, CI-977, dynorphin A, TRK-820, DuP-747, RP-61127, BRL-53114/SB-215519/SB-2215520, GR-44821
Opioid μ1	Pain, Inflammation, Asthma	Morphine, Fentanyl, remifentanil, mirfentanil, trefentanil, CP-0719, CP-840, BW-2378W92, Z-4349, RP-63494, DPI-3290, sulfazocine, SEP-130551, LEF-576, propiram

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Substance P (Neurokinin 1, NK 1)	Asthma, Inflammation, Psoriasis, Arthritis, IBD, Emesis, Pain, migraine, anxiety, depression, psychosis	MK-0869, FR-113680, L-737488, CGP-49823, CP-122721, FK-888, GR-82334, GR-203040, CP-96345, CP-99994, MEN-10930, SR-140333, dapitant, lanepitant, RP-67580, L-742694, L-741671, L-758298, L-733060, YM-49244, S-16474, S-18523, MDL-105212, LY-306740
Vasopressin V1	Cardiac failure, Hypertension, Nephrotoxicity, Cardiovascular disease, Diuresis, Dysmenorrhea, Emesis, edema	OPC-21268, YM-087, SR-49059, VPA-985, CL-385004, YM-471, F-180, JTV-605

There is also a breadth of diversity within the ligands that modulate the downstream signaling activity of the 7-TM receptors. These ligands range from the small biogenic amines (serotonin, histamine, dopamine), lipids (prostaglandins and the endogenous cannabinoids), neuropeptides (neurokinins, NPY, opioids), peptide hormones (angiotensin and bradykinin) to larger peptides such as chemokines and thrombin. Pharmacological studies employing mutational and chimeric receptor constructs have defined the binding domains for this diverse library of ligands. These studies have provided some fundamental principles for defining the ligand binding domains of these receptors. 7-TMs have been categorized based on the binding interactions of the endogenous ligands.

Family 1a Rhodopsin family, olfactory, catecholamines, opioid

Family 1b Peptides, cytokines, thrombin

Family 1c Glycoprotein hormones e.g. LH, TSH, FSH, CG

Family 2 Secretin Family of calcitonin like receptors, e.g. PCAP, glucagon, CRF, VIP

Family 3 Metabotropic glutamate receptors e.g. mGluR1-5

2. Ligand-gated Ion Channel receptors

Another family of receptors is the ligand-gated ion channel receptors. Here the binding of ligands to specific ligand binding sites on these receptors results in modulation of ion flux into or out of the cell. These are membrane bound cell surface receptors are composed of multiple subunits, typically 5 subunits, which may be the same of different.

These ligand-gated ion channel reecptors have important roles in the central nervous system and peripheral nervous system. Examples of the receptors include the $GABA_A$ receptor, the NMDA receptor, 5-HT-3 receptor and nicotinic acetylcholine receptor. Examples of the ligands which bind to these receptors and their therapeutic indications include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
GABA A	Anxiety, sleep disorder, cerebrovascular ischemia, epilepsy, psychosis, schizophrenia, Alzheimers disease	Diazepam, ganaxolone, clomethiazole, suritozole, imidazenil, losigamone, pagoclone, RY-024, AHR-14042, RWJ-38293, SB-205384, Co-60549, Co-2-6749, Co-122739, Co-2-1068, CCD-3693, RWJ-53050, RWJ-46771, PF-1885, U-104841, U-93631, U-101017, Co-152791, S-8510, RU-33965, RU-5135, MDL-27192, BRL-54504AX, AWD-131-138, PNU-107484A, PNU-100076, NS-2710, NC-1201, FG-8094, NGD-91-2
5-HT 3	Emesis, anxiety disorder, cognitive disorders, dementia, depression, schizophrenia, gastric motility disorder, nausea, irritable bowel syndrome, heart arrhythmia, drug dependence	Ondansetron, lurosetron, azasetron, itasetron, fabesetron, alosetron, ramosetron, dolasetron, mirtazapine, palonosetron, cilansetron, Granisetron, renzapride, R-zacopride, zatosetron, Tropisetron, lerisetron, ricasetron, YM-114, CP-93318, N-3256, WAY-100289, SC-50410, KF-18259, RS-56812, SC-52491, E-3620, SC-52246, SDZ-ICM-567, UCM-30593, GR-65630, DAT-582, ADR-851, N-3389, RS-33800, RS-56532, KB-6806, KGA-0941, S-21007, LY-278584, DAU-6285, R-093777, GYKI-46903, ML-1035, L-683877, KB-6933, RG-12915, SC-52150, GK-128

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
NMDA	Pain, neurological disease, neuroprotective agent, cerebral infarction, cerebrovascular ischemia, cerebral hypoxia, schizophrenia, epilepsy, head injury, Parkinsons disease, drug dependence	Ifenprodil, dexanabinol, midafotel, remacemide, eliprodil, Ro-24-6173, Ro-24-6449, Ro-8-4304, GPI-3000, ARL-15896AR, ADCI, FPL-16283, LY-274614, WAY-126090, HO-473, CNS-1531, selfotel, CP-101606, CP-98113, ES-2421, BI-II-277-CL, FR-115427, CNS-5161, CNS-1044, CNS-5065, CNS-1118, CNS-1524, CNS-1505, L-701315, L-701376, L-701252, L-698532, L-689560, L-687414, L-701273, LY-235959, LY-233053, LY-235723, LY-233536, EMD-95885, CGP-39653, CGP-37849, MRZ-2/579, HA-966, CP-101616, AP-6, NC-1210, PD-158473, NPS-1506
Nicotinic Acetylcholine	Pain, Alzheimers disease, cognitive disorders, muscle spasm, obesity	ABT-594, ABT-089, GTS-21, RJR-2403, RJR-1401, RJR-2429, RJR-1734, A-85380, A-82695, SIB-1553A, SIB-1508Y, SIB-1765F, ANQ-9040, S-1663, UB-165, DBO-83, anorectic

3. Tyrosine Kinase linked and guanylate cyclase linked receptors

Tyrosine kinase linked receptors mediate the actions of a number of peptide mediators (eg. insulin and other growth factors). The receptors for most growth factors are transmembrane tyrosine kinase receptors, including receptors for platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), EGF, TGF, hergulin, insulin, insulin-like growth factors (IGF) I and II, nerve growth factor, stem cell factor, vascular endothelial growth factor, macrophage colony stimulating factors (CSF) and others.

The receptors for growth hormone, prolactin, erythropoietin, IL-2, IL-3, IL-4, IL-6, IL-7 granulocyte CSF, granulocyte-macrophage CSF, interferon, interferon, ciliary neurotrophic factor and many other cytokines are members of the tyrosine kinase-associated receptor family. These receptors are often over-expressed on tumors of hematopoietic origin and similar to receptor tyrosine kinases, autocrine or paracrine stimulation may contribute to the neoplastic state of the tumor cell. Some epithelial cancers overexpress one or more members of the receptor tyrosine kinase family. EGF receptors, IGF-I receptors and HER-2/neu are overexpressed in lung, bladder, breast, head and neck and ovarian cancers.

Therefore, ligands for the tyrosine kinase receptors may be useful in the treatment of cancers.

The prototypical tyrosine phosphatase receptor is CD45. CD45 plays a crucial role in T cell activation through its removal of phosphate from a negative regulatory site on the src family kinase.

The serine/threonine kinase receptors recognize TGF, bone morphogenetic factors and other activins as ligands. Ligand binding leads to activation of the receptor kinase. Bone morphogenetic factors are important in bone formation and in determining ventral dorsal orientation in the developing embryo. TGF induces fibroblast proliferation but inhibits the proliferation of most cell types. Loss of expression or loss of function of TGF receptors occurs in several tumor types including colon cancer and lymphomas.

Guanylate cyclase receptors mediate the activity of atrial natiuretic peptides. They are made up of a large extracellular and intracellular domains (440-700 residues). Ligand binding leads to dimerization of the receptor. This association of the intracellular kinase domains leads to autophosphorylation. The phosphorylated sites provide high affinity binding sites for other intracellular proteins. The intracellular proteins have a highly conserved region of about 100 residues referred to as the SH2 domains. The proteins that contain SH2 domains contribute to the signaling cascades (e.g. activation of protein lipase C).

4. Nuclear Steroid Receptors and Membrane-bound Steroid Receptors

Nuclear steroid receptors regulate DNA transcription, leading to the synthesis of specific proteins and the production of cellular effects. These are large monomeric proteins of 400 to 1000 residues incorporating a highly conserved region of 60 residues in the middle of the molecule. Glucocorticoids enhance the production of lipocortin accounting for the inflammatory properties of this receptor. Ligands for the receptor include steroid hormones such as estrogens, progestrins, and androgens, thyroid hormones, vitamin D and retinoids. For the glucocorticoid receptor, drugs that are activating ligands include prednisone, dexamethasone and betamethasone that provide therapy for the treatment of arthritis and carditis. For

the mineralcorticoid receptor, desoxycorticosterone provides treatment for Addisons disease. For the androgen and estrogen receptor, anti-androgen and anti-estrogen ligands are relevant for the treatment of benign prostatic hyperplasia and breast carcinoma respectively. Selective estrogen receptor modulators (SERMs) include tamoxifen, raloxifene, and antoandrogens including cypoterone and flutamde.

Examples of ligands which bind to nuclear steroid receptors and their therapeutic indications include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Corticosteroid	Allergic rhinitis, Urticaria, arthritis, Asthma, Dermatitis, Psoriasis, autoimmune disease, inflammation, IBD, COPD	mometasone, deflazacort, beclomethasone, budesonide, fluticasone, betamethasone, rimexolone, CBP-2011, CBP-2012, CBP-1011, AGN-191743, methylprednisolone aceponate, triamcinolone acetonide, Fiser A608, halobetasol propionate, ALX-25, rofleponide
Estrogen	Osteoporosis, breast cancer, heart disease, depression, menopausal disorders, contraception	Tamoxifen, droloxifene, idoxifene, raloxifene, dihydroraloxifene, levormeloxifene, LY-353381, LY-355124, TAT-59, GW-5638, TSE-424, EM-652, CP-336156, ERA-923, LY-326315, LY-357489, ZM-189154, CCRL-1054
PPAR γ	Type II Diabetes (NIDDM), atherosclerosis, hyperlipidemia	Rosiglitazone, troglitazone, pioglitazone, Englitazone, LG-101280, GW-2331, MCC-555, GW-2570, AD-5075, AHG-255, KRP-297, darglitazone, GI-262570, BRL-48482,
Retinoid X-α (RXR)	Acne, Keratosis, Psoriasis, Ichthyosis, Neoplasm, Restenosis, Prostatic hypertrophy, Systemic lupus erythematosus, Breast tumor, Leukemia, Lymphoma, Metabolic disorder, Carcinoma, Myeloproliferative disorder, Skin infection, Solid tumor, Squamous cell carcinoma, Allergy, Dermatological disease, Osteoporosis, Non-insulin dependent diabetes, Lung tumor, Genital system disease, Colon tumor, Myeloid Leukemia	Tazarotene, Alitretinoin, Bexarotene, ALRT-1550, ALRT-326/ALRT-4204, ALRT-268, LG-100264, ER-65250, LG-100754, LG-100264, MX-895

Cell surface steroid receptors can be distinguished from the nuclear receptors (Harold H. Zakon[39] and Gianna Fiorelli et al.[40]). These cell surface receptors are coupled to signal transduction pathways in the cell. Zakon[39] describes how surface steroids can be coupled to second messenger systems and control the activity of ion channels in this way. The presence of signaling cell surface binding sites for estradiol has been reported in osteoblast- and osteoclast-like cells. Two mechanisms of action have been reported for these ligands: regulation of gene expression, and initiation of responses.

5. Adhesion Molecules as Cell Surface Receptors

The adhesion of cells to molecules of the extracellular matrix or to other cells is a fundamental theme in biological processes that encompasses events that include signal transduction, regulation of the immune system, cell growth and wound healing. As a result there are a number of therapeutic applications for mediating/inhibiting these interactions, which includes the use of inhibitors of cell adhesion as anti-inflammatory agents. A number of classes of molecules have been implicated in these cell-cell and cell-matrix interactions. These include the following molecules: selectins (E, L and P), integrins ($\alpha_i\beta_j$), the IgG superfamily, fibrinogen and laminin.

Selectins are transmembrane glycoproteins of the vascular system that are involved in both lymphocyte and leukocyte adhesion and play a pivotal role in inflammation by allowing the attachment of leukocytes to endothelial cells. Integrins are a family of heterodimeric transmembrane adhesion molecules. Integrin $\alpha_2\beta_3$ is expressed on platelets and becomes exposed on thrombin activation. The IgG Superfamily includes VCAM, ICAM, and NCAM. Fibrinogen is a serum protein that is involved in the blood clotting cascade and binds to the platelet integrin $\alpha_2\beta_3$. The binding of fibrinogen to platelet integrin plays a key role in thrombosis. Fibronectin is a widely distributed glycoprotein present in most extracellular matrices. It's principle functions appear to be in cellular migration during development and wound healing, regulation of cell growth and differentiation and haemostasis/thrombosis. Laminins are a family of large glycoproteins that are

distributed ubiquitously in basement membranes. This family of molecules have roles in development, differentiation and migration through their ability to interact with cells via cell-surface receptors, including the integrins (George A. Heavner[38]).

Examples of ligands which bind to adhesion receptors include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Integrins		
gpIIb/IIIa	Unstable Angina, Myocardial Infarction, cerebrovascular ischemia, thromboembolism, peripheral vascular disease, restenosis	Sibrafiban, fradafiban, lotrafiban, lefradafiban, RWJ-50042, tirofiban, xemilofiban, XR-300, L-734217, L-738167, L-709780, L-703014, G-7464, G-7582, G-7584, G-7442, G-7570, DMP-728, XT-111, PS-028, CGH-400, PSA-0613, MS-180, NSL-95317, RPR-110173, Ro-43-8857, SA-202, TRM-147, TRM-153, XV454-7, TAK-029, EF-5077, ZD-2486, RWJ-53308, T-250, SM-20302, TS-943, SC-57345, S-1762
LFA-1	Rheumatoid arthritis, psoriasis	RWJ-50271, BIRT-377
Vitronectin (alpha-v beta-3)	restenosis, atherosclerosis, angiogenesis, cancer, osteoporosis, thromboembolism	SB-265123, SD-183, XT-199, V-0223, XT-199-4, V-0005, V-0519,V-0245,L-748415
VLA-4 (alpha-4-beta-1)	asthma, allergy, IBD, rheumatoid arhtritis, MS	BIO-1050, Bio-1211, Bio-1272, Bio-1515, TBC-3233, ZD7349
Selectins		
E-Selectin	Asthma, arthritis, rhinitis, reperfusion injury, inflammation, transplant rejection, atopic dermatitis, psoriasis, septic shock	TBC-1269, BMS-190394, CGP-69669A, GM-1925, GM-1380, GM-2296, GSC-150, GM-1986, GM-2941, PDN-26117
L-Selectin	Asthma, reperfusion injury, inflammation, transplant rejection, ARDS, septic shock	TBC-1269, BMS-190394, GSC-150, GM-1986, GM-2941, PDN-26117
P-Selectin	Arthritis, reperfusion injury, inflammation, ARDS	TBC-1269, BMS-190394, CGP-69669A, GM-1925, GM-1380, GM-2296, GSC-150, GM-1986, GM-2941, PDN-26117

In general terms a ligand may be an activator or an inhibitor. In the case of an activator, on binding with a receptor, the ligand will activate the functional response of the receptor. Alternatively, in the case of an inhibitor, a ligand will bind to the receptor but it does not activate the receptor. Instead, it may prevent the binding of an activating ligand.

Within the ligands that bind to G-protein cellular receptors, ligands may be specifically classified as follows:

1. Full agonists - ligands that when bound trigger the maximum activity seen by natural ligands
2. Partial agonists- ligands that when bound trigger sub-maximal activity
3. Antagonist- ligands that when bound inhibit or prevent the activity arising from a natural ligand binding to the receptor. Antagonists may be of the surmontable class (results in the parallel displacement of the dose-response curve of the agonist to the right in a dose dependent fashion without reducing the maximal response for the agonist) or insurmountable class (results in depression of the maximal response for a given agonist with or without the parallel shift).
4. Inverse antagonist-ligands that when bound decrease the basal activity of the unbound receptor (if any).

There are four fundamental properties that are measurable pertaining to the interaction of a ligand with its receptor.

1) The affinity of the ligand for the receptor, which relates to the energetics of the binding.

2) The efficacy of the ligand for the receptor, which relates to the functional downstream activity of the ligand.

3) The kinetics of the ligand for the receptor, which defines the onset of action and the duration of action.

4) The desensitization of the receptor for the ligand.

With regard to the ligand, it is the combination of these properties that provides the foundation for defining the nature of the functional response. Thus an activating ligand (or agonist) has affinity for the receptor and downstream efficacy. In contrast, an inhibiting ligand (antagonist) has affinity for the receptor but no efficacy.

Selectivity defines the ratios of affinities or the ratios of efficacies of a given ligand compared across two receptors. It is the selectivity of a specific drug that provides the required biological profile. For example, in certain therapeutic

settings, it is currently thought that a highly selective drug may be preferred (eg. Losartan (Cozaar), an antihypertensive, is a highly selective antagonist for the AT1 receptor). In contrast, it considered that a drug with a broad spectrum of receptor activity may be preferred in other therapeutic settings (eg. cisapride, for the treatment of gastric motility disorder and gastro-esophageal reflux disease, displays a breadth of activity as a $5HT_4$ agonist and $5HT_3$ antagonist).

The pharmaceuticals currently developed that bind to receptors have the clinical shortcomings of low efficacy, low affinity, poor safety profile, lack of selectivity or overselectivity for the intended receptor, and suboptimal duration of action and onset of action. Thus there continues to exist a need for new pharmaceuticals having improved therapeutic activities.

Accordingly, it would be beneficial to develop ligands that have improved affinity, efficacy, selectivity, onset of action and duration of action.

1. Affinity of ligand for target receptor

An increase in affinity may contribute to reducing the dose of ligand required to induce the desired therapeutic effect. A reduction in affinity will remove activity and may contribute to the selectivity profile for a ligand.

2. Efficacy of ligand at a target receptor (functional effect)

An increased efficacy can lead to a reduction in the dose required to mediate the desired therapeutic effect. This increase in efficacy may arise from an improved positive functional response of the ligand or a change from a partial to full agonist profile. Reduced efficacy of a full agonist to a partial agonist may provide clinical benefit by providing a moderated biological response e.g. Zolmitriptan is a partial agonist for the 5HT1b/d receptors.

3. Selectivity of ligand compared across receptor subtypes

An increase in the selectivity of the ligand requires that the affinity or efficacy of the ligand at other receptors is reduced relative to the desired receptor. For example ipratropium is a non-selective ligand showing activity at a number of muscarinic receptor subtypes resulting in undesirable side-effects.

A decrease in the selectivity of the ligand may also be desired. For example the angiotensin II endogenous ligand activates both the AT1 and AT2 receptor subtypes. However, Losartan is a selective AT1 receptor antagonist.

4. Onset of Action

More rapid onset of action is often preferred.

5. Duration of Action

An increased duration of action may be preferred. For example β_2 adrenergic agonists such as albuterol have a relatively short duration of action of approximately 3-4 hours. Migraineurs also suffer rebound headache after treatment with sumatriptan.

6. Desensitization of the receptor for the ligand.

Desensitization is best defined as the variety of processes by which the functional interaction of the receptor with its G-protein are influenced. These processes lead ultimately to a reduction in cellular response to the activating agonist. Such phenomena are most often observed during prolonged stimulation of the receptor. The two main pathways for receptor desensitization are reduction in receptor density or changes in receptor structure by phosphorylation mechanisms.

Receptor density is altered by receptor sequestration. This is a reversible process that is observable within minutes and is a dynamic sorting of receptors with receptors being cycled to and from the membrane. On the other hand receptor down regulation is generally slower, on the order of hours and is irreversible, involving destruction of the receptor. Finally, receptor density may be affected by an alteration in the rate of synthesis. The rate of β_2 mRNA synthesis and degradration are controlled by levels of c-AMP within the cell.

Alternatively receptor desensitization may occur through changes in receptor structure. The receptor may be phosphorylated. For example, agonist induced activation of the β_2-adrenergic receptor, which is positively coupled to adenylate cyclase through Gs, results in an elevation in the levels of c-AMP and an increase in the activity of protein kinase A. This kinase can readily phosphorylate a consensus site in the third intracellular loop of the receptor. The phosphorylated β_2-adrenergic receptor exhibits significantly reduced coupling to Gs. Besides PKA, the G-protein

coupled receptor kinases (GRK) are also involved in the desenitization of GPCRs. For the β_2-adrenergic receptor, there are two of these kinases bARK1 and bARK2. These GRKs are more specific and will only phosphorylate an agonist activated receptor. Furthermore this GRK desensitization requires an arrestin protein.

Possible roles for multibinding ligands would be to mediate changes in the desensitzation mechanisms. It may also be useful to increase receptor desensitization.

Receptor oligmerization also plays a role in receptor function. This is best exemplified in the area of growth receptors that are known to act functionally and structurally as dimers, e.g. EGF-R and interferon receptor. Importantly for the EGF-R receptor, it is proposed that it is this dimerization that provides the high affinity binding sites. It is also known that dimerization is involved in the functioning of the steroid receptor. Preliminary evidence is beginning to appear on the importance of oligomerization in G-protein coupling and signalling. It is proposed that receptor oligomerization may play a role in different receptor functions such as mediating coupling of the G-protein or receptor internalization. A functional role for receptor oligomerization has been proposed for the adrenergic receptor (Bouvier et al.[41] and Terence E. Hebert et al.[42]) and the opioid receptor (Cvejic et al.[43]). In addition, higher molecular weight species have been observed for a range of GPCRs that includes muscarinic receptors, 5HT receptors, dopamine receptors, m-GluR receptors, NK1 receptors and others. Receptor dimerization has also been noted in the angiotensin AT1 receptor (Catherine Monnot et al.[44]). Expression of dopaminergic D3 receptor dimers and tetramers in brain and in transfected cells has been obeserved (Esther A. Nimchinsky et al.[45]).

Accordingly, there is a need to develop ligands that are more specific with increased potency for the various receptors.

SUMMARY OF THE INVENTION

This invention is directed to novel multi-binding compounds that bind cellular receptors. The binding of these compounds to such cellular receptors can be used to treat pathologic conditions mediated by such cells.

Accordingly, in one of its composition aspects, this invention is directed to a multi-binding compound and salts thereof comprising 2 to 10 ligands, which may be the same or different and which are covalently attached to a linker or linkers which may be the same of different, each of said ligands comprising a ligand domain capable of binding to a cellular receptor.

The multi-binding compounds of this invention are preferably represented by formula I:

$$(L)_p(X)_q \qquad I$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor; X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20. Preferably, q is less than p.

Preferably, binding of the multibinding compounds to the cell relates to cells which mediate mammalian or avian pathologic conditions and such binding modulates these conditions.

In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound or a pharmaceutically acceptable salt thereof comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to one or more cellular receptors mediating mammalian pathologic conditions thereby modulating the pathologic condition.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad I$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic

conditions; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof. Preferably, q is less than p.

In one of its method aspects, this invention is directed to a method for treating a mammalian or avian pathologic condition mediated by receptors which method comprises administering to said mammal or bird an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound or a pharmaceutically acceptable salt thereof comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic conditions.

In one of its method aspects, this invention is directed to a method for treating a mammalian or avian pathologic condition mediated by cellular receptors which method comprises administering to said mammal or bird an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic conditions; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof. Preferably, q is less than p.

This invention is also directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds bind cellular receptors and are candidates for possessing multibinding properties. The diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry

and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds bind cellular receptors and are candidates for possessing multibinding properties. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands targeting a receptor.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds which bind cellular receptors possessing multibinding properties which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds which bind cellular receptors possessing multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition is preferred when a mixture of different ligands is employed to ensure heterodimeric or multimeric compounds are prepared. Concurrent addition of the ligands occurs when at least a portion of the multimer compounds prepared are homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric ligand compound library or portions thereof produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands which bind a cellular receptor wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a)

with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

In another preferred embodiment, the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates and precursors thereof. It is understood, of course, that the reactive functionality on the

ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heteromeric (i.e., at least one of the ligands is different from the other ligands).

In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a cellular receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-11 illustrate numerous reaction schemes suitable for preparing linkers and, hence, multi-binding compounds of this invention.

Figure 12 illustrates examples of multi-binding compounds comprising 2 ligands attached in different formats to a linker.

Figure 13 illustrates examples of multi-binding compounds comprising 3 ligands attached in different formats to a linker.

Figure. 14 illustrates examples of multi-binding compounds comprising 4 ligands attached in different formats to a linker.

Figure 15 illustrates examples of multi-binding compounds comprising >4 ligands attached in different formats to a linker.

Figure 16 illustrates triptan C3 and C5 substituents.

Figure 17 illustrates several Sumatriptan building blocks.

Figure 18 illustrates several sites for multimerization of Sumatriptan.

Figures 19 - 23 illustrate several multimeric Sumatriptan compounds and convenient methods for their synthesis by the methods of this invention.

Figure 24 illustrates various muscarinic compounds.

Figure 25 illustrates the preferred sites for dimerization of the muscarinic family of compounds.

Figure 26 illustrates the different points for attachment of the linker on ipratropium and the resulting dimers.

Figures 27 and 28 illustrate the different linkers useful in making multimers for ipratropium.

Figure 29 illustrates various ipratropium dimers.

Figure 30 illustrates the synthesis of ipratropium dimers via quaternization.

Figure 31 illustrates the synthesis of ipratropium dimers via reductive amination.

Figure 32 illustrates the synthesis of ipratropium dimers via etherification.

Figure 33 illustrates the synthesis of ipratropium dimers via conjugate addition.

Figure 34 illustrates the two AT1 antagonists losartan and valsartan.

Figure 35 illustrates the AT1 antagonists Irbesartan, Candesartan, Eprosartan and Tasosartan.

Figure 36 illustrates the AT1 antagonists Telmisartan and Ripisartan.

Figure 37 illustrates the sites (M) that may be used to generate multi-binding compounds from Losartan.

Figures 38 and 39 illustrate the different points of attachment to Losartan.

Figure 40 illustrates the differing valencies of the multi-bonding compounds.

Figure 41 illustrates the different framework cores.

Figure 42 illustrates the different orientations of binding elements within the multi-binding compounds.

Figure 43 illustrates the heterovalomers possible from AT1 antagonists.

Figure 44 illustrates the synthesis of 1-hydroxyl-linked Losartan multi-binding compound.

Figure 45 illustrates the synthesis of 2-hydroxyl-linked Losartan multi-binding compound.

Figure 46 illustrates the synthesis of tetrazole-linked Losartan multi-binding compound.

Figure 47 illustrates various β_2-adrenergic compounds.

Figure 48 illustrates preferred sites for attachment of linkers to albuterol. The M indicates the preferred attachment site.

Figure 49 illustrates various albuterol dimers.

Figure 50 illustrates different sites of attachment of the linker to albuterol and the resulting dimers.

Figures 51 and 52 illustrate the different linkers which may be used to generate multimers of albuterol.

Figures 53 and 54 exemplify different albuterol, albuterol/formeterol and albuterol/clenbuterol multimers.

Figures 55, 56, and 57 illustrate the different methods for the synthesis of bivalent analogs of salmeterol.

Figure 58 illustrates a method for the synthesis of a bivalent analog of alprenolol.

DETAILED DESCRIPTION OF THE INVENTION

Ligand (drug) interactions with cellular receptors are controlled by molecular interaction/recognition between the ligand and the receptor. In turn, such interaction can result in modulation or disruption of the biological processes/functions of these receptors and, in some cases, leads to cell death. Accordingly, when cellular receptors mediate mammalian pathologic conditions, interactions of ligands with the cellular receptor can be used to treat these conditions.

Without being limited, it is thought that a number of cellular receptors are amenable to interaction with a multi-binding compound of the present invention for the following reasons:

1) Receptors with multiple binding sites within single receptor molecules:

a) Some receptors have multiple sites for a single ligand, e.g. angiotensin;

b) Some receptors have an accessory "weak" binding site in addition to the main ligand site, e.g. 5HT

c) Some receptor-endogenous peptide ligands have different binding domains to the small molecule non-peptide antagonists. For example, the AT1

receptor binds two copies of the endogenous peptide agonist angiotensin II and the small molecule antagonist losartan; and

d) Some receptors display allosteric binding sites for small molecules. For example, the muscarinic family of receptors have been demonstrated to have allosteric binding sites

2) Receptors that form oligomeric complexes.

Receptors that are known to form oligomeric species include the β_2 adrenergic; M3 receptors; tyrosine kinase and guanylate cyclase linked receptors; insulin growth factors; nuclear receptors and many cytokine receptors. These receptors must dimerize to initiate their functional activity. Ligand binding leads to dimerization of the receptor. This association of the intracellular kinase domains leads to autophosphorylation. These phosphorylated sites provide high affinity binding sites for other intracellular proteins which contribute to cellular signaling pathways. Also ligand-gated ion channel receptors form pentameric structures having multiple ligand-binding sites.

3) Receptors that are closely spaced.

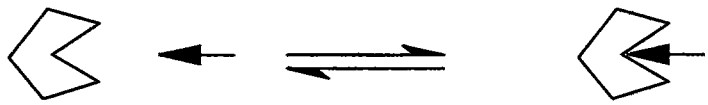
Although not interacting, some receptors are placed on a cell membrane at a distance that allows more than one receptor to be spanned with a multi-binding ligand, e.g. synaptic receptors.

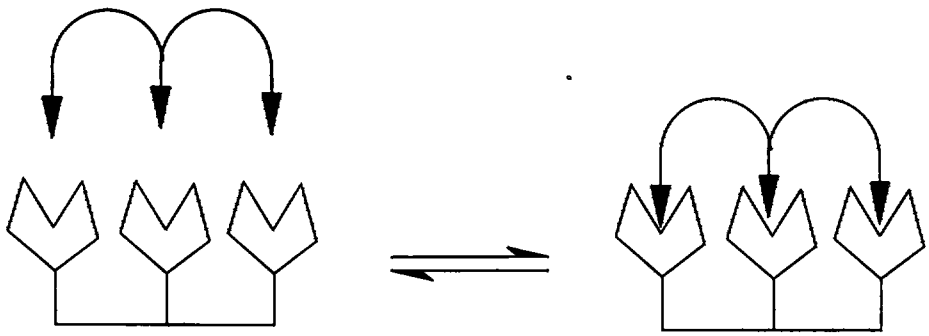
The interaction of a cellular receptor and a ligand may be described in terms of "affinity" and "specificity". The affinity and specificity of any given ligand/cellular receptor interaction are dependent upon the complementarity of molecular binding surfaces and the energetic costs of complexation. Affinity is sometimes quantified by the equilibrium constant of complex formation.

Specificity relates to the difference in affinity between the same ligand binding to different ligand binding sites on the cellular receptor.

The multibinding compounds of this invention are capable of acting as multibinding agents and the surprising activity of these compounds arises at least in part from their ability to bind in a multivalent manner with one or more cellular receptors. Multivalent binding interactions are characterized by the concurrent interaction of multiple ligands with multiple ligand binding sites on one or more

cellular receptors. Multivalent interactions differ from collections of individual monovalent interactions by imparting enhanced biological and/or therapeutic effect. Examples of multivalent binding interactions (e.g., trivalent) relative to monovalent binding interactions are shown below:





Just as multivalent binding can amplify binding affinities, it can also amplify differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

Definitions:

Prior to discussing this invention in further detail, the following terms will first be defined.

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, *n*-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene (-CH_2-), ethylene (-CH_2CH_2-), the propylene isomers (e.g., -$CH_2CH_2CH_2$- and -$CH(CH_3)CH_2$-) and the like.

The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused groups contain from 1 to 3 fused ring structures.

The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy ($-CH_2OCH_3$), ethylenemethoxy ($-CH_2CH_2OCH_3$), *n*-propylene-*iso*-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-*t*-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ($-CH_2SCH_3$), ethylenethiomethoxy ($-CH_2CH_2SCH_3$), *n*-propylene-*iso*-thiopropoxy ($-CH_2CH_2CH_2SCH(CH_3)_2$), methylene-*t*-thiobutoxy ($-CH_2SC(CH_3)_3$) and the like.

The term "alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred

alkenyl groups include ethenyl (-CH=CH_2), *n*-propenyl (-CH_2CH=CH_2), *iso*-propenyl (-C(CH_3)=CH_2), and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "alkenylene" refers to a diradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., -CH_2CH=CH- and -C(CH_3)=CH-) and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted

cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl groups include ethynyl ($-C\equiv CH_2$), propargyl ($-CH_2C\equiv CH$) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynylene groups include ethynylene ($-C\equiv C-$), propargylene ($-CHC\equiv C-$) and the like.

The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino,

alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl

The term "acyl" refers to the groups HC(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acylamino" or "aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" or "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, preferably 1

to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to the diradical derived from aryl (including substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH_2.

The term "substituted amino refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" refers to the groups "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl,

cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

The term "heteroarylene" refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridnylene, 2,5-indolenyl and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol,

thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula [-(CH_2-)$_m$Y-] where *m* is ≥ 2, and Y at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, [-(CH_2)$_3$-NH-]$_3$, [-((CH_2)$_2$-O)$_4$-((CH_2)$_2$-NH)$_2$] and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines,

triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds (including intermediates thereof) prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group Green[63]. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-

butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable amino blocking groups include conventional substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), and the like which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild hydrolysis conditions compatible with the nature of the product.

The term "optional" or "optionally" means that the subsequently described event, circumstance or substituent may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform ($CHCl_3$), methylene chloride (or dichloromethane or "CH_2Cl_2"), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, tert-butanol, dioxane, pyridine, and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

A "receptor" or "cellular receptor" is a biological structure with one or more binding domains that reversibly complexes one or more ligands, where that complexation has biological consequences. Receptors are distinguished for the purpose of this application from enzymes, which bind and then transform the bound species.

It should be recognized that the cellular receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations (e.g. cellular receptors may be covalently joined in a single structure, noncovalently associated in a multimeric structure,

embedded in a membrane or polymeric matrix and so on) and therefore have less translational and rotational freedom than if the same cellular receptors were present as monomers in solution.

The term "ligand binding site" as used herein denotes the site on a receptor that recognizes a ligand domain and provides a binding partner for that ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example agonism, antagonism, modulatory effect and the like or may maintain an ongoing biological event. For the purposes of this description, the ligand, the ligand domain and the ligand binding site cannot both be DNA, RNA, an antibody, an antibody domain or a fragment of an antibody. The receptor cannot be an opioid receptor.

"Ligand" as used herein denotes a compound that is a binding partner for a receptor and is bound thereto by complementarity. The specific region or regions of the ligand that is (are) recognized by the receptor is designated as the "ligand domain". A ligand may be either capable of binding to a receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g. Ca^{+2}, Mg^{+2} or a water molecule is required for the binding of a ligand domain to various receptors).

It is further understood that the term ligands is not intended to be limited to compounds known to be useful as receptor binding compounds (e.g., known drugs). It should be understood that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced with unrelated structures and, in some cases, omitted entirely without affecting the binding interaction. The primary requirement for a ligand is that it has a ligand domain as defined above. Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally associated with cellular receptor binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multivalent compounds because of the benefits conferred by multivalency. The only requirement for a ligand is that it has a ligand binding domain as defined above.

Accordingly, examples of ligands useful for this invention are ligands for the muscarinic receptors; the α_2, β_1 and β_2 adrenergic receptors, the 5-HT receptors, the $GABA_A$ receptor, the melatonin receptor; the angiotensin I receptor; the erythropoietin receptor; the dopamine 1 and 2 receptors; the A2 adenosine receptor, the nicotinic receptors and the steroid receptor.

1. Antagonists for muscarinic receptors include Bethanocol, Pilocarpine, Dicyclomine, Ipratropium (nonselective), Methoctramine (M1), Tiotropium (M3) and Pirenzipine (M1). These antagonists are used in the treatment of xerostomia, glaucoma, gastro-intestinal diseases, asthma, COPD, emesis, gastroprokinesis, memory enhancement, motion sickness, some symptoms of Parkinson's. Tiotropium is useful in decreasing bronchial secretions. Pirenzipine decreases gastric H+ secretions. Antagonists for the M1 muscarinic receptor are used to treat arrythmas, GERD and ulcers. Agonists for the M1 muscarinic receptor are useful in memory enhancement. Antagonists specifically for the M2 muscarinic receptor are useful in cardiac applications. Antagonists specifically for the M3 muscarinic receptor are used in urology. Finally, antagonists specifically for the M4 muscarinic receptor are useful in treating analgesia, arthritis, and central nervous system disorders such as neuroleptic conditions. The muscarinic receptor has the potential to accept a multivalent ligand because it has an additional allosteric site. Both the orthosteric and allosteric sites are on the extracellular portion of the receptor. This allows gallamine to bind with negative cooperativity to the cardiac M2 muscarinic receptor and alcuronium to bind with positive cooperativity to the M2 muscarinic receptor but with negative cooperativity to the M3 muscarinic receptor.

2. Antagonists for the opioid receptors include: (a) antagonists to the opioid κ receptor: buprenorphine and butorphanol; (b) antagonists to the opioid μ receptor: alfentanil, morphine, methadone, codeine, hydrocodone, hydromorphone, levorphanol, meperidine, nalbuphine, opium, oxycodone, oxymorphone, pentazocine, propoxyphene. These antagonists are useful in the treatment of pain, addiction and the provision of anesthesia. The opioid receptors lend themselves to the use of multimeric ligands because they form homodimers on the cell surface.

3. Antagonists for the α_2 adrenergic receptor include clonidine and yohimbine. These compounds are used in the treatment of hypertension, hypertensive emergencies, headache, vascula, dysmenorrhea and menopause. The α_2 adrenergic receptor is amenable to multimeric compounds becauase it has an additional allosteric site in the receptor. For example, ameloride binds the receptor with negative cooperativity.

4. Antagonists for the β_1 adrenergic receptor include atenolol. This compound is useful in the treatment of angina because it decreases workload of the heart and thus the oxygen consumption of the heart. The β_1 adrenergic receptor is amenable to multimeric ligands because it either dimerizes upon ligand binding or has two sites on one receptor.

5. Agonists for the β_2 adrenergic receptor include albuterol, bitolterol, epinephrine, fenoterol, isoetharine, isoproterenol, metaproterenol, pirbuterol, procaterol, salmerterol, and terbutaline. These compounds are used in the treatment of asthma. The β_2 adrenergic receptor is amenable to multimeric ligands because it dimerizes upon ligand binding to be effective.

6. Antagonists for the 5-HT receptor include sumatriptan (binds the 5-$HT_{1B/1D}$ receptor); zolmitriptan (binds to the 5-$HT_{1B/1D}$ receptor, crosses the blood/brain barrier and complexes with the central nervous system vascular nucleus in addition to peripheral vascular sites); nefazodone (binds the 5-HT_2 receptor); risperidone (binds the 5-HT_2 receptor); mirtazapine (binds the 5-$HT_{2/3}$ receptors); granisetron (binds the 5-HT_3 receptor); ondansetron (binds the 5-HT_3 receptor); ondansetron (binds the 5-HT_3 receptor); paroxetine (binds all 5-HT receptors); and olanzapine (which binds the 5-HT_2 receptor and many D subtypes). These compounds are useful in the treatment of migraine headache, itch, depression, schisophrenia, and possibly motion sickness. Compounds which bind the 5-HT_{1A} receptor are useful in treating depression and memory loss. Compounds which bind to the 5-$HT_{1B/D}$ receptor are useful in treating migraine. Compounds which bind to the 5-$HT_{1D/E/F}$ receptors are useful in treating anxiolytic disorders and obesity. The ligands for the 5-HT receptors will be useful in the multivalomeric states because the receptor

dimerizes upon ligand binding and allosterism is likely with various synthetic ligands, such as methysergide.

7. Antagonists for the NMDA receptor include felbamate. This compound is useful in providing neuroprotection following a stroke. It is also an antiepileptic and an anticonvulsant. The NMDA receptor contains multiple sites plus allosteric sties and accordingly, is amenable to multivalomeric ligands of the present invention.

8. Antagonists for the $GABA_a$ receptor include benzodiazepines and barbituates. These compounds are useful as anxiolytics, hypnotics, anticonvulsants, muscle relaxants, and anterograde amnestics. The GABA receptor contains multiple known binding sites for different small molecules.

9. Antagonists for the melatonin receptor include melatonin. This compound is useful in treating sleep disorders and maintaining a sense of well-being in the patient. The melatonin receptor has two sites for melatonin.

10. Antagonists for the angiotensin 2 receptor include losartan and eprosartan. These compounds are useful in the treatment of hypertension. Angiotensin I binds to the angiotensin I receptor twice whereas Losartan only binds the receptor once. The binding sites are believed to be on opposite sides of the cell membrane. It would be advantageous to develop a multivalomeric antagonist that was able to bind to a single receptor twice.

11. Agonists for the erythropoietin receptor include Epogen. This compound is useful in the treatment of anemia, especially secondary anemia due to decreased renal production of erythropoietin. This receptor dimerizes upon ligand binding.

12. Agonists for the dopamine 1 receptor include dopamine. This compound is useful in treating Parkinson's disease and is used as a vasoactive in the intensive care unit. The dopamine 1 receptor also dimerizes upon ligand binding.

13. Antagonists for the dopamine 2 receptor include metoclopromide and haloperidol. These compounds are useful for appetite enhancement, dyskinesis, Huntington's chorea and emesis. The dopamine 2 receptor also dimerizes upon ligand binding and ameloride is known to bind at an allosteric site.

14. Agonists for the A_2 adenosine receptor include adenosine. This compound acts as an anti-arrhythmic during imaging and echo diagnostics. The A1 adenosine receptor has an additional allosteric site. It has been found that aminobenzythiopenes bind with positive cooperativity to the A1 adenosine receptor.

15. Antagonists to the nicotinic receptor include succinylcholine. This comound is useful in the treatment of paralysis and myasthenia gravis. It can be used as a muscle relaxant. The nicotinic receptor has two sites for the ligand per receptor.

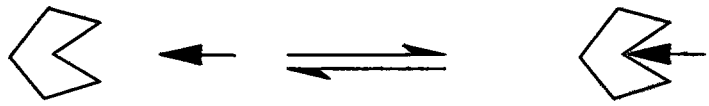
16. Agonists for the steroid receptor include (a) the corticosteroids: beclomethasone, budesonide, dexamethasone, flunisolide and triamcinolone; (b) the progestins: hydroyprogesterone, levonorgestrel, medrogestone, medroxyprogesterone, megestrol, norethinidrone, norgestrel and progesterone; (c) the estrogens: estrogen, tamoxifen and raloxifene; (d) the anabolic steroids; and (e) the mineralacorticoids. These compound are useful for a range of diseases from inflammation to cancer. The steroid receptor dimerizes upon binding of the ligand.

"Multi-binding agents" or "multi-binding compounds" refers to a compound that is capable of multi-valency as defined below, and which has 2 -10 ligands covalently bound to one or more linkers which may be the same or different. A multi-binding agent provides a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which may be the same or different. That is to say that the biological and/or therapeutic effect of the ligands attached to the multi-binding compound is greater than that achieved by the same amount of unlinked ligands made available for binding to the ligand binding sites.

The phrase "increased biological or therapeutic effect" includes, for example increased affinity for a target, increased specificity for a target, increased selectivity for a target, increased potency, increased efficacy, decreased toxicity, improved duration of action, decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multi-binding compounds of this invention will exhibit at least one and preferably more than one of the above mentioned effects.

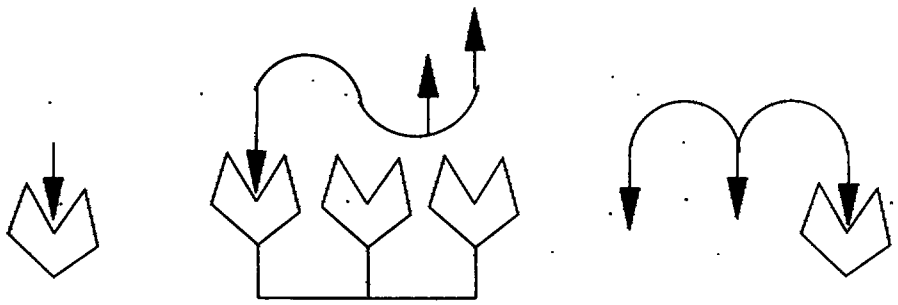
"Uni-valency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It

should be noted that a molecule having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand is interacting with a ligand binding site. Examples of a univalent interaction are depicted below.

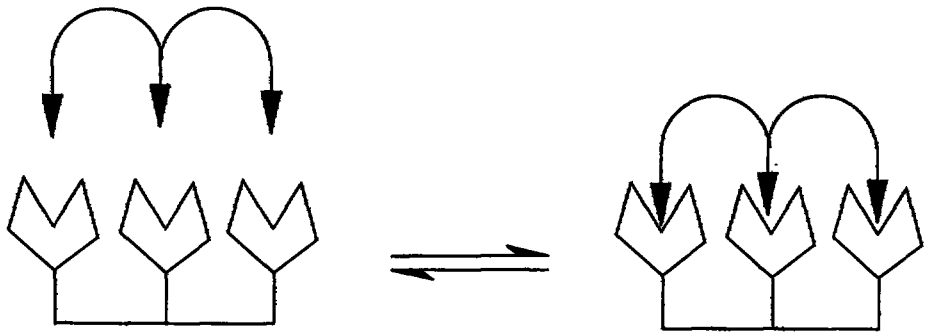


"Multi-valency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding ligand binding sites on the receptors which receptors may be the same or different.

For example, two ligands connected by a linker that bind concurrently to two ligand binding sites would be considered as bi-valency; three ligands thus connected would be an example of tri-valency. An example of tri-valency illustrating a multi-binding agent bearing 3 ligands versus a monovalent binding interaction is shown below.



univalent interaction



multivalent interaction

It should be understood that all compounds that contain multiple copies of a ligand attached to a linker do not necessarily exhibit the phenomena of multi-valency, i.e., that the biological and/or therapeutic effect of the multi-binding agent is greater than the sum of the aggregate of unlinked ligands made available to the ligand binding site. For multi-valency to occur, the ligands that are connected by a linker have to be presented to their receptors by the linker in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multi-binding agent.

"Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multi-binding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g. an *in vitro* or *in vivo* assay, in an appropriate animal model such as a human patient). The finding that the multi-binding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g. on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

"Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites. The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-

receptor complex) or in cases where a biological effect is observed below the K_d, the ratio of the respective EC_{50}s (i.e., the concentrations that produce 50% of the maximum reponse for the ligand interacting with the two distinct ligand binding sites.

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.

The term "treatment" refers to any treatment of a pathologic condition in a mammal or bird, particularly a human, and includes:

(i) preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition;

(ii) inhibiting the pathologic condition, i.e., arresting its development;

(iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or

(iv) relieving the conditions mediated by the pathologic condition, e.g., relieving the conditions caused by an enterotoxin expressed by a microorganism but not addressing the underlining microbial infection.

The term "pathologic condition which is modulated by treatment with a ligand" covers all disease states (i.e., pathologic conditions) which are generally acknowledged in the art to be usefully treated with a ligand for a cellular receptor in general, and those disease states which have been found to be usefully treated by a specific multi-binding compound. Such disease states include, by way of example only, the treatment of a mammal afflicted with migraine headache, depression, hypertension and the like. It also covers the treatment of pathologic conditions that are not necessarily generally considered as pathologic conditions, for example the use of multi-binding compounds in the treatment of pregnancy, obesity, hair-loss, beauty aids and the like.

The term "therapeutically effective amount" refers to that amount of multi-binding compound which is sufficient to effect treatment, as defined above, when

administered to a mammal or bird in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "linker", identified where appropriate by the symbol X, refers to a group or groups that covalently link(s) from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multi-valency when in the presence of at least one cellular receptor having 2 or more ligand binding sites. The linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. The linker may be either a chiral or an achiral molecule. In some cases the linker may be biologically active. The term linker does not, however, extend to cover solid inert supports such as beads, glass particles, fibers and the like. But it is to be understood that the multi-binding compounds of this invention can be attached to a solid support if desired, for example, for use in separation and purification processes and for similar applications.

The extent to which multivalent binding is realized depends upon the efficiency with which the linker or linkers that joins the ligands presents them to their ligand binding sites on one or more receptors. Beyond presenting ligands for multivalent interactions with ligand binding sites, the linker spatially constrains these interactions to occur within dimensions defined by the linker. Thus the structural features of the linker (valency, geometry, orientation, size, flexibility, chemical composition) are features of multivalent compounds that play an important role in determining their activities.

The linkers used in this invention are selected to allow multivalent binding of ligands to any desired ligand binding sites of a receptor, whether such sites are located interiorly, both interiorly and on the periphery of the molecule, or at any intermediate position thereof. The distance between the nearest neighboring ligand domains is preferably in the range of about 2Å to about 100Å, more preferably in the range of about 3Å to about 40Å.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the linker or linkers. The reaction chemistries resulting in such linkages are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for binding or which can be introduced onto the ligand for binding. Again, such complementary functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

The following table illustrates numerous complementary reactive groups and the resulting bonds formed by reaction there between. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March[1].

COMPLEMENTARY BINDING CHEMISTRIES

FIRST REACTIVE GROUP	SECOND REACTIVE GROUP	LINKAGE
hydroxyl	isocyanoate	urethane
amine	epoxide	amine/alcohol
tosyl halide	amine	sulfonamide
carboxyl	amine	amide
hydroxyl	alkyl/aryl halide	ether

The linker is attached to the ligand at a position that retains ligand binding domain-receptor binding and specifically which permits the receptor recognition site of the ligand to orient itself to bind to the receptor. Such positions and synthetic protocols for linkage are well known in the art. Following attachment to the linker or a significant portion thereof (e.g. 2-10 atoms of linker), the linker-ligand conjugate is tested for retention of activity in a relevant assay system. If a linker-ligand conjugate shows activity at a concentration of less than 1 mM, it is considered to be acceptable for use in constructing a multi-binding compound. The relative orientation in which the ligand domains are displayed to the receptors depends both on the particular point (or points) of attachment of the ligands to the linker, and on the framework geometry. The term linker embraces everything that is not considered to be part of the ligand.

Suitable linkers are discussed below.

At present, it is preferred that the multi-binding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X.

The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

The term "multimeric compound" refers to compounds comprising from 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

Methodology

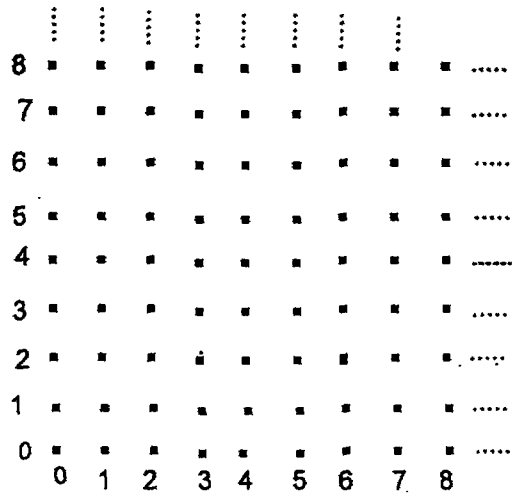
The linker, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multi-binding compound. The biological activity of the multi-binding compound is highly sensitive to the valency, geometry, composition, size, flexibility or rigidity, etc. of the linker as well as the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity of the linker, and the like on the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the multi-binding compound. The linker may be biologically "neutral", i.e., not itself contribute any biological activity to the multi-binding compound or it may be chosen to enhance the biological activity of the molecule. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands to the receptors to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multi-binding compound.

For example, different orientations can be achieved by including in the framework groups containing mono- or polycyclic groups, including aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight-chain species. In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.) In other preferred embodiments, the ring is a six or ten member ring. In still further preferred embodiments, the ring is an aromatic ring such as, for example, phenyl or naphthyl.

Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moities can readily be conducted by the skilled artisan. For example, the hydrophobic nature of a linker derived from

hexamethylene diamine ($H_2N(CH_2)_8NH_2$ or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines".

Different frameworks can be designed to provide preferred orientations of the ligands. Such frameworks may be represented by using an array of dots (as shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P, H, F, Cl, Br, and F or the dot may alternatively indicate the absence of an atom at that position. To facilitate the understanding of the framework structure, the framework is illustrated as a two dimensional array in the following diagram, although clearly the framework is a three dimensional array in practice:

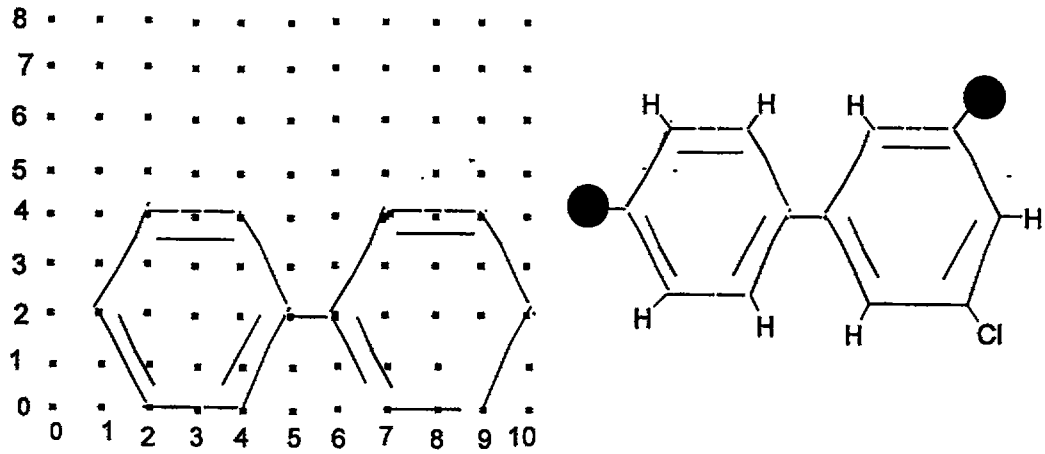


Each dot is either an atom, chosen from carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space (i.e., an absence of an atom). Only certain atoms on the grid have the ability to act as an attachment point for the ligands, namely, C, O, N, S and P.

Atoms can be connected to each other via bonds (single, double or triple bonds with acceptable resonance and tautomeric forms), with regard to the usual constraints of chemical bonding. Ligands may be attached to the framework via single, double or triple bonds (with chemically acceptable tautomeric and resonance forms). Multiple ligand groups (2 to 10) can be attached to the framework such that the minimal, shortest path distance between adjacent ligand groups does not exceed 100 atoms. Preferably, the linker connections to the ligand is selected such that the

maximum spatial distance between two adjacent ligands is no more than 40 Angstroms (Å).

An example of a linker as presented by the grid is shown below for a biphenyl construct.



Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0), (7,0) all represent carbon atoms. Node (10,0) represents a chlorine atom). All other nodes (or dots) are points in space (i.e., represent an absence of atoms.

Nodes (1,2) and (9,4) are attachment points.

Hydrogen atoms are affixed to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2) and (7,0).

Nodes (5,2) and (6,2) are connected by a single bond.

The carbon atoms present are connected by either a single or double bonds, taking into consideration the principle of resonance and/or tautomerism.

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns. Examples of acceptable patterns of three contiguous atom arrangements are shown in the following diagram:

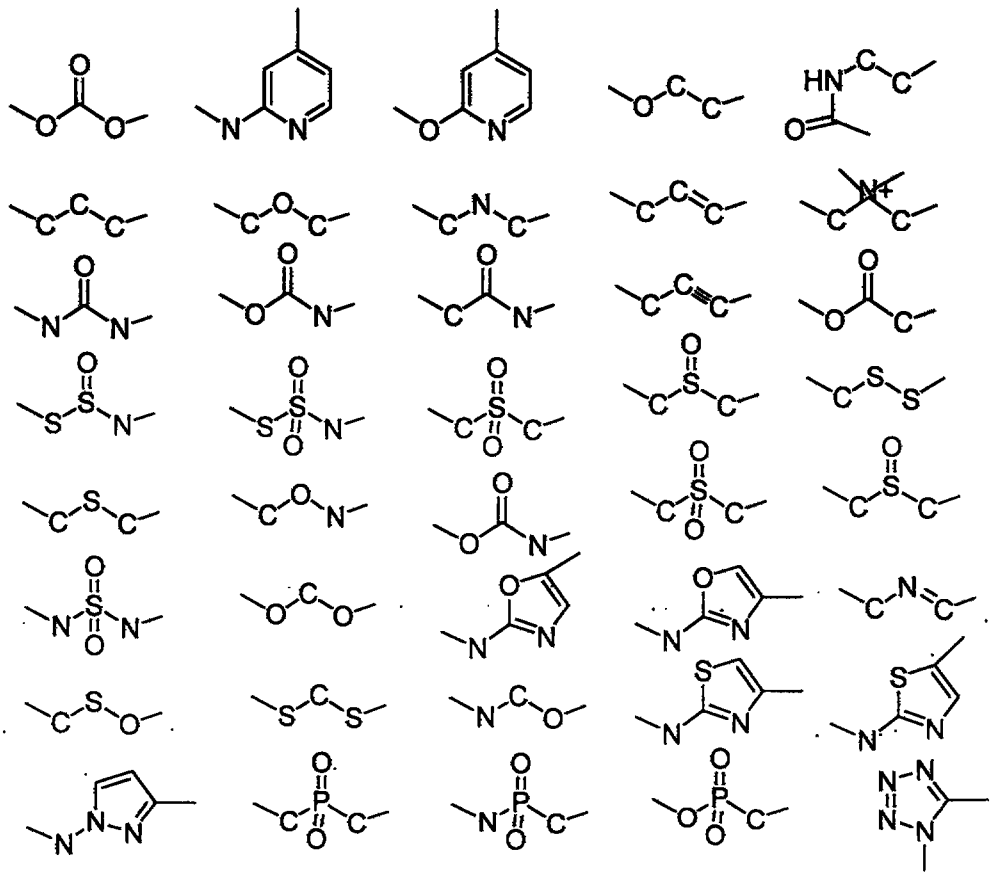
CCC	NCC	OCC	SCC	PCC
CCN	NCN	OCN	SCN	PCN

CCO	NCO	OCO	SCO	PCO
CCS	NCS	OCS	SCS	PCS
CCP	NCP	OCP	SCP	PCP
CNC	NNC	ONC	SNC	PNC
CNN	NNN	ONN	SNN	PNN
CNO	NNO	ONO	SNO	PNO
CNS	NNS	ONS	SNS	PNS
CNP	NNP	ONP	SNP	PNP
COC	NOC	OOC	SOC	POC
CON	NON	OON	SON	PON
COO	NOO	OOO	SOO	POO
COS	NOS	OOS	SOS	POS
COP	NOP	OOP	SOP	POP
CSC	NSC	OSC	SSC	PSC
CSN	NSN	OSN	SSN	PSN
CSO	NSO	OSO	SSO	PSO
CSS	NSS	OSS	SSS	PSS
CSP	NSP	OSP	SSP	PSP
CPC	NPC	OPC	SPC	PPC
CPN	NPN	OPN	SPN	PPN
CPO	NPO	OPO	SPO	PPO
CPS	NPS	OPS	SPS	PPS
CPP	NPP	OPP	SPP	PPP

One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March[1]. These arrangements are described in the

grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.



The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multivalent binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process.

An example of this process for extending the framework (linker) from the ligand is presented below for the triptan class of pharmaceuticals. Some of the possible locations for elaboration into the framework (linker) are shown in Figures

16 and 18 with arrows and "M". Some of the possible locations for elaboration into the framework for the tropane core compounds are shown in Figure 25.

Examples of triptan bivalent compounds are shown in Figures 19 - 23.

It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic or anionic groups, or a combination thereof. A wide diversity of linkers is commercially available from Chemsources USA; ChemSources International and ACD. Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art and described below.

Assay of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g. potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition. The composition of a linker can be varied in numersous ways to achieve the desired physical properties.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multi-binding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the hydrophilicity and water solubility of the multi-binding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG decreases antigenicity and potentially enhances the overall rigidity of the linker.

Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multi-binding compounds are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene glycols), alcohols, polyols, (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, etc.) carboxylates, polycarboxylates, (e.g., polyglutamic acid, polyacrylic acid, etc.), amines, polyamines, (e.g., polylycine, poly(ethyleneimine), and the like) to enhance the water solubility and/or hydrophilicity of the multi-binding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multi-binding compounds described herein is within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above, may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of this invention is the use of ancillary groups which result in the multi-binding compound being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl,

heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds, for example, aryl, heteroaryl, cycloalkyl and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following the addition to the linker, include deprotectation of a carboxyl, hydroxyl, thiol or amino protecting group, by a change in pH, oxidation,

reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl. In other preferred embodiments, the linker comprises one or more six-membered rings or crown groups which, while not rigid, retain the conformation of the linker through conformational entropy.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art. Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention.

As explained above, the multi-binding compounds described herein comprise 2-10 ligands attached to a linker that links the ligands in such a manner that they are presented to the cellular receptor for multivalent interactions. The linker spatially constrains these interactions to occur within dimensions defined by the linker, thus greatly increasing biological activity of the multi-binding compound as compared to the same number of ligands used in mono-binding form.

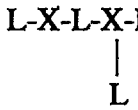
The multi-binding compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is described below.

As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

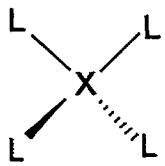
The simplest and most preferred multi-binding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. Examples of such bivalent compounds is provided in Figure 12. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X. However, a trimer can also be a multibinding compound comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Illustrations of trivalent and tetravalent compounds of this invention are found in Figures 13 and 14 respectively. Tetravalent compounds can be represented as

L-X-L-X-L-X-L

or in a branched array, e.g.,



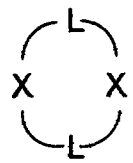
(a branched construct analogous to the isomers of butane -- *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl)or in a tetrahedral array, e.g.,



where X and L are as defined herein. Alternatively, it could be represented as an aryl or cycloalkyl derivative as above with four (4) ligands attached to the core linker.

The same considerations apply to higher multi-binding compounds of this invention containing 5-10 ligands as illustrated in Figure 15. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

Certain of the above described compounds may alternatively be represented as cyclic chains of the form:



and variants thereof.

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

With the foregoing in mind, preferred linkers may be represented by the following formula:

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$

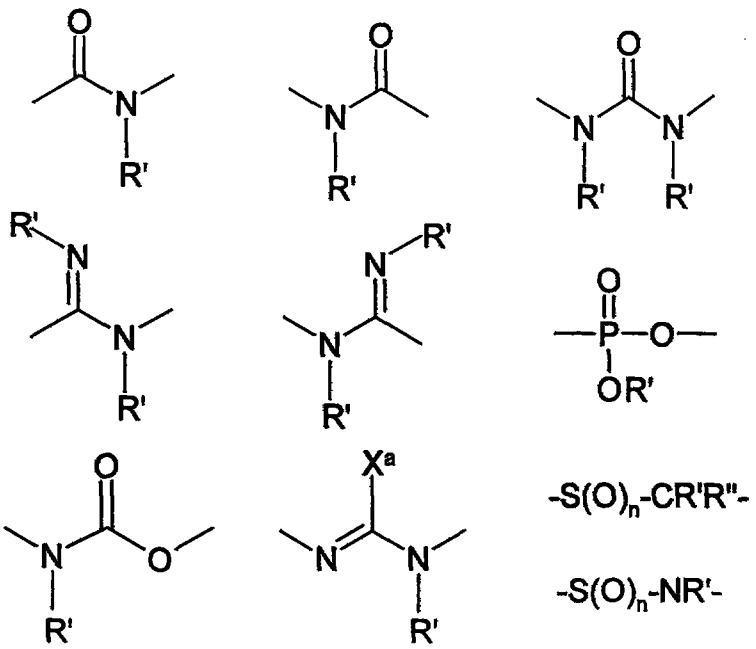
in which:

m is an integer of from 0 to 20;

X′ at each separate occurrence is selected from the group consisting of -O-, -S-, -NH-, -C(O)-, -C(O)O-, -C(O)NH-, -C(S), -C(S)O-, -C(S)NH- or a covalent bond;

Z is at each separate occurrence is selected from the group consisting of alkylene, cycloalkylene, alkenylene, alkynylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y′ and Y″ at each separate occurrence are selected from the group consisting of



-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hdyrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

Accordingly, compounds of Formula I may be prepared as shown below.

As indicated above, the simplest (and preferred) construct is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different, and X is the linker.

Accordingly, an example of the preparation of a bivalent ligand is given below as an illustration of the manner in which multivalent compounds of Formula I are obtained. This example is applicable to any ligand that includes amino and/or carboxyl groups and examples of different linkers (X) are shown. In the reactions schemes that follow, for ease of understanding of the principles involved, the structure of the ligand is presented as a "box". Thus, the ligand is illustrated such that carboxyl [C], amino [V], and methylamino [N] groups are shown as examples.

Preparation of Compounds of Formula I

Accordingly, compounds of Formula I may be prepared as shown as below.

As indicated above, the simplest (and preferred) construct is a bivalent compound, which can be represented L-X-L, where L is a ligand and is the same or different at each occurrence, and X is the linker.

Two ligands are connected by the linker X via carboxyl group or amino group of a first ligand, as indicated as R^1, to any carboxyl group or amino group of a second ligand, indicated as R^2.

Another simplification in the description of the preparations is that, for example, compound (1) is illustrated as a compound of formula H_2N-$(CH_2)_m$-$NCHO_2$-t-butyl, in which m is an integer of 1-20. However, it should be understood that $(CH_2)_m$ is not intended to signify or imply that the scope of this reaction (or of the invention) is limited to straight (i.e. unbranched) alkylene chains, but rather $(CH_2)_m$ is intended to include branched alkylenes as defined above, and alkylenes optionally substituted by aryl, arylalkyl, heteroaryl, heteroarylalkyl, and the like, also as disclosed in the Detailed Description of the Invention. Similarly, the compound of formula (2) is illustrated as ClC(O)-$(CH_2)_n$-C(O)Cl, and $(CH_2)_n$ equally is not limited to straight alkylene chains, but includes all those modifications shown above.

Accordingly, bivalent compounds of Formula I where the linkage is from a [C] group of a first ligand to a [C] group of a second ligand, i.e. a [C-C] linkage, may be prepared from intermediates of formula (4), the preparation of which is shown below in Reaction Scheme 1.

REACTION SCHEME 1

$H_2N\text{-}(CH_2)_m\text{-}NHCO_2\text{-t-butyl}$ + $ClC(O)\text{-}(CH_2)_n\text{-}C(O)Cl$

(1) (2)

↓

t-butyl-$OC(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH\text{-}C(O)\text{-}(CH_2)_n\text{-}C(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH\text{-}C(O)$-t-butyl

(3)

↓

$H_2N\text{-}(CH_2)_m\text{-}NH\text{-}C(O)\text{-}(CH_2)_n\text{-}C(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH_2$ (4)

in which m and n are independently integers of 1-20.

Preparation of Compounds of Formula (3)

As illustrated in Reaction Scheme 1, step 1, about two molar equivalents of an omega-amino carbamic acid ester [formula (1)] is reacted with about one molar equivalent of a dicarboxylic acid halide, preferably chloride, of formula (2). The reaction is conducted in the presence of a non-nucleophilic base, preferably diisopropylethylamine, in an inert solvent, preferably methylene chloride, at a temperature of about 0~5°C. The mixture is then allowed to warm to room temperature. When the reaction is substantially complete, the compound of formula (3) is isolated and purified by conventional means.

Preparation of Compounds of Formula (4)

As illustrated in Reaction Scheme 1, step 2, the carbamate is removed under acid conditions. In general a preferred acid is trifluoroacetic acid, and the reaction is conducted in an inert solvent, preferably methylene chloride, at about room temperature. When the reaction is substantially complete, the compound of formula (4) is isolated and purified by conventional means.

The compound of formula (4) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 2.

REACTION SCHEME 2

See Figure 1

Preparation of Compounds of Formula I

In general, about two molar equivalents of ligand is reacted with about one molar equivalent of the compound of formula (4), under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-hydroybenzotriazole. The reaction is conducted in an inert polar solvent, for example, N, N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO), or preferably a mixute of both, at about room temperature. When the reaction is substantially complete, the compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC. Also isolated was a byproduct of formula (5).

Alternatively, compounds of Formula I [C-C] may be prepared from intermediates of formula (8), the preparation of which is shown below in Reaction Scheme 3.

REACTION SCHEME 3

See Figure 2

Preparation of Compounds of Formula (7)

As illustrated in Reaction Scheme 3, step 1, ligand is reacted with about 1.1 molar equivalents of a carbamic ester terminated by an alkylamino group [formula (6)]. The ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-fluorenylmethyl. Conventional amide coupling conditions are employed, preferably using PyBOP and 1-hydroxybenzotriazole. In general, the reaction is conducted in the presence of a hindered base, preferably diisopropylethylamine, in an inert polar solvent, preferably DMF or DMSO, preferably a mixture of both, at about room temperature. When the reaction is substantially complete, thecompound of formula (7) is isolated and purified by conventional means.

Preparation of Compounds of Formula (8)

As illustrated in Reaction Scheme 3, step 2, the compound of formula (7) is reacted with a mild base to remove the protecting ester groups, which also affords decarboxylation. In general, the base is preferably piperidine, and the reaction is conducted in an inert polar solvent, preferably dimethylformamide, at about room temperature for about 10 minutes to one hour. When the reaction is substantially complete, the compound of formula (8) is isolated and purified by conventional means, preferably using reverse phase HPLC.

The compound of formula (8) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 4.

REACTION SCHEME 4

See Figure 3

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 4, the compound of formula (8) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the compound of formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula $HO_2C-(CH_2)_n-CO_2H$, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethlyaminine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 1-3 hours. When the reaction is substantially complete, the compound of Formula I is isolated and purified by conventioanl means, preferably purified by a reverse phase of HPLC.

Compounds of Formula I wherein the linkage is [V-V] may be prepared from intermediates of formula (14), the preparation of which is shown below in Reaction Sceme 6. The starting material, the compound of formula (11), is prepared as shown in Reaction Scheme 5.

REACTION SCHEME 5

See Figure 4

Preparation of Compounds of Formula (10)

As illustrated in Reaction Scheme 5, step 1, ligand having an -NH_2 group suitable for linking is reacted with a protected ester-aldehyde of formula (9) to form a Schiff's base. The ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-fluorenylmethyl. In general, the reaction is conducted in an inert polar solvent, preferably 3-dimethyl-3,4,5,6-tetrahydro~2(1H)-pyrimidinone plus methanol, at about 50~100°C, preferably about 70°C, for about 30 minutes to 1 hour. The Schiff's base of formula (10) is not isolated, but reacted further immediately as shown below.

Preparation of Compounds of Formula (11)

As illustrated in Reaction Scheme 5, step 2, the solution of the compound of formula (10) is further reacted with a mild reducing agent. In general, the reducing agent is preferably sodium cyanoborohydride, and the reaction is conducted at about 50~100°, preferably about 70°C, for about 1-3 hours, preferably about 2 hours. When the reaction is substantially complete, the compound of formula (11) is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [V-V] may then be prepared from intermediates of formula (11a), the preparation of which is shown below in Reaction Scheme 6.

REACTION SCHEME 6

See Figure 5

Preparation of Compounds of Formula (14)

As illustrated in Reaction Scheme 6, step 1, the compound of formula (11a), which is a compound of formula (11) in which the carboxyl group has been protected conventionally, for example as an ester, is reacted with a mild base to remove the carbamate. In general, the base is preferably piperidine, and the

reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 10 minutes to one hour, preferably about 30 minutes. When the reaction is substantially complete, the compound of formula (14) is isolated and purified by conventional means, preferably using reverse phase HPLC.

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 6, step 2, the compound of formula (14) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the compound of formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula HO_2C-(CH_2)n-CO_2H, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at bout room temperature for about 1-3 hours. When the reaction is substantially complete, the protecting group R, preferably and ester, is removed conventionally, and the [V-V] compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [C-V] may be prepared from intermediates of formula (23), the preparation of which is shown below in Reaction Scheme 7. The starting material, the compound of formula (8), is prepared as previously shown.

REACTION SCHEME 7

See Figure 6

Preparation of Compounds of Formula (22)

As illustrated in Reaction Scheme 7, step 1, the compound of formula (8) is reacted with an acid in the same manner as shown above, for example in Reaction Scheme 11, to form an amide of formula (22).

Preparation of Compounds of Formula (23)

As illustrated in Reaction Scheme 7, step 2, the compound of formula (22) is hydrolyzed with an acid in the same manner as shown above, for example in Reaction Scheme 10, to form a compound of formula (23).

The compound of formula (23) is then converted into a [C-V] dimer of Formula I by reaction with a compound of formula (17), prepared as shown previously, as shown in Reaction Scheme 8.

REACTION SCHEME 8

See Figure 7

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 8, the compound of formula (23) is reacted with a compound of formula (17) in a typical coupling reaction as shown above, to give a compound of Formula I [C-V].

Compounds of Formula I wherein linkage is [C-N] may be prepared from intermediates of formula (26), the preparation of which is shown below in Reaction Scheme 9.

REACTION SCHEME 9

See Figure 8

Preparation of Compounds of Formula (24)

As illustrated in Reaction Scheme 9, step 1, ligand is reacted with a protected aminoaldehyde in the presence of an amount of base sufficient to direct the reaction of the aldehyde to the [N] position. The Schiff's base thus formed is reduced in the same manner as shown in Reaction Scheme 5 to form a compound of formula (24).

Preparation of Compounds of Formula (25)

As illustrated in Reaction Scheme 9, step 2, the compound of formula (24) is reacted with an amine in a coupling reaction in the same manner as shown above, for example in Reaction Scheme 10, to form an amide of formula (25).

Preparation of Compounds of Formula (26)

As illustrated in Reaction Scheme 9, step 3, the protecting group FM is removed conventionally from the compound of formula (25) with a mild base to form a compound of formula (26).

The compound of formula (26) is then converted into a [C-N] dimer of Formula I by reaction with a compound of formula (23), prepared as shown previously, as shown in Reaction Scheme 10.

REACTION SCHEME 10

See Figure 9

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 10, the compound of formula (26) is reacted with a compound of formula (23) in a typical coupling reaction as shown above, for example in Reaction Scheme 11, to give a compound of Formula I [C-N].

Compounds of Formula I wherein the linkage is [N-V] may be prepared by a reaction of a compound of formula (26) with a compound of formula (19), as shown in Reaction Scheme 11.

REACTION SCHEME 11

See Figure 10

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 11, the compound of formula (26) is reacted with a compound of formula (19) in a typical coupling reaction as shown above, to give a compound of Formula I [N-V].

Compounds of Formula I wherein the linkage is [N-N] may be prepared by reaction of a compound formula (26) with a dicarboxylic acid of formula HO_2-C-$(CH_2)_n$-CO_2H, as shown in Reaction Scheme 12.

REACTION SCHEME 12

See Figure 11

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 12, the compound of formula (26) is reacted with a dicarboxylic acid of formula HO_2C-$(CH_2)_n$-CO_2H in the same manner as shown above in Reaction Scheme 4, to give a compound of Formula I [N-N].

Ligands that include a free hydroxy group in their structure (an alcohol or phenolic hydroxy) may be connected using those hydroxy groups as linkage points by means well known in the art. For example, one synthetic strategy that could be used for linking ligands with free hydroxy groups involves treating the ligand with t-butyl bromoacetate in the presence of a base (e.g. potassium carbonate) to convert the -OH group to an -O-CH_2CO_2-t-But group, which can be hydrolyzed to an O-CH_2CO_2H group using trifluoracetic acid. The oxtacetic group can then be used as the linking point for two ligands by making use of the linking strategies shown above for carboxylic acids. For example, reaction of two molar equivalents of the ligand with a diamine of the formula H_2N-$(CH_2)_n$-NH_2, where n is an integer of 1-20, leads to two ligands being connected by a linker of the formula -CH_2CONH-$(CH_2)_n$-$NHCOCH_2$.

Alternatively, treating the hydroxy-bearing ligand with BOC-$NHCH_2CH_2Br$ in the presence of a base(e.g. potassium carbonate) converts the -OH group to an O-CH_2CH_2NHBOC group, which can by hydrolyzed to an O-$CH_2CH_2NH_2$ group using trifluoracetic acid. The oxyethylamino group can then be used as the linking point for two ligands by making use of the linking strategies shown above for amines. For example, reaction of two molar equivalents of the ligand with a dicarboxylic acid of the formula HO_2C-$(CH_2)_n$-CO_2H where n is an integer of 1-20, leads to two ligands being connected by a linker of the formula -CH_2 CH_2NHCO-$(CH_2)_n$-$CONHCH_2$ CH_2-.

Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-

pressure liquid chromatography or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the Examples herein below. However, other equivalent separation or isolation procedures could, of course, also be used.

Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which bind cellular receptors and which possess multibinding properties.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets or cellular receptors. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs,

substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where

linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be

attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For example, a $5HT_4$ receptor antagonist and a bladder-selective muscarinic M_3 antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the $5HT_4$ ligand and elements of the M_3 receptor proximal to the formal M_3 antagonist binding site and between the M_3 ligand and elements of the $5HT_4$ receptor proximal to the formal $5HT_4$ antagonist binding site. Thus, the dimeric compound may be more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence.

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically inocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore,

divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available (such as 7TM G-protein coupled receptors), one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom,

and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, polarizability, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

Having chosen a set of *n* ligands (*n* being determined by the sum of the number of different attachment points for each ligand chosen) and *m* linkers by the process outlined above, a library of (*n*!)*m* candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1 A1-A2 A1-B1 A1-B2 A1-B3 A2-A2 A2-B1 A2-B2

A2-B3 B1-B1 B1-B2 B1-B3 B2-B2 B2-B3 B3-B3

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalies on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial libary is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libaries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative

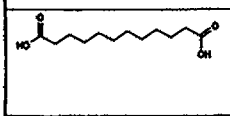
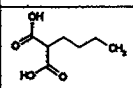
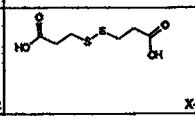
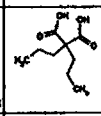
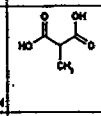
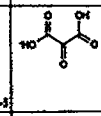
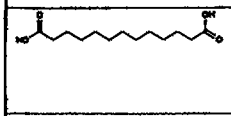
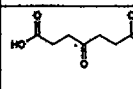
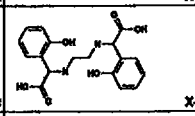
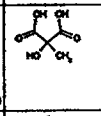
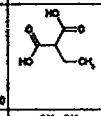
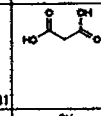
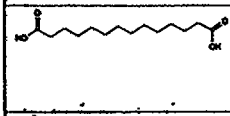
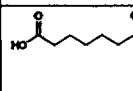
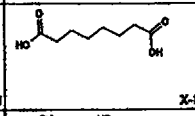
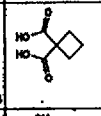
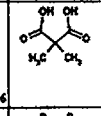
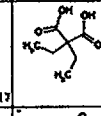
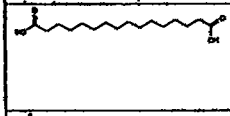
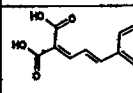
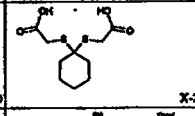
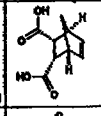
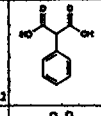
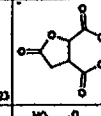
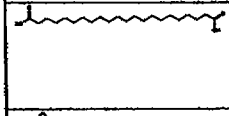
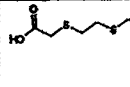
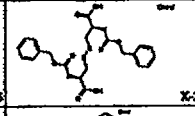
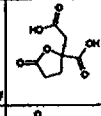
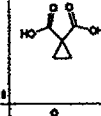
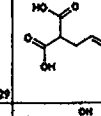
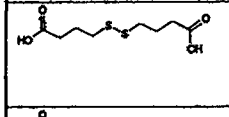
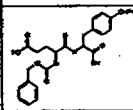
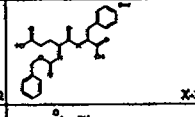
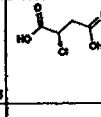
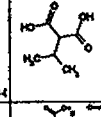
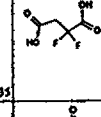
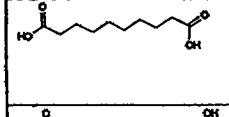
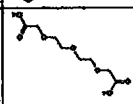
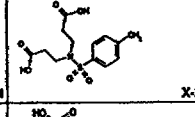
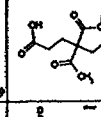
redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

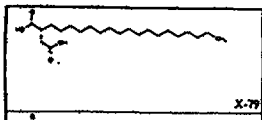
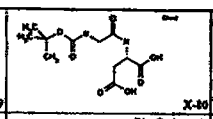
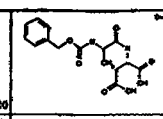
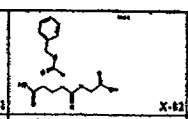
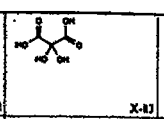
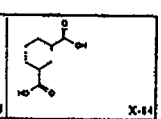
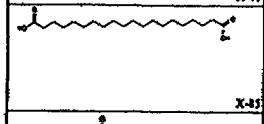
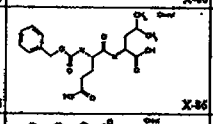
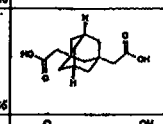
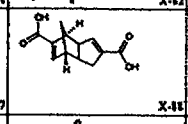
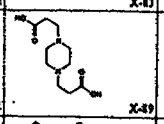
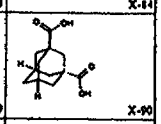
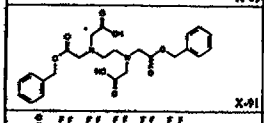
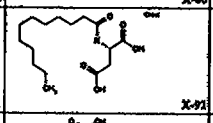
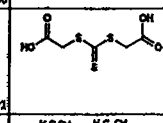
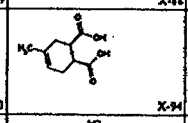
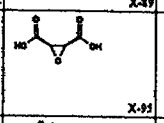
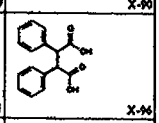
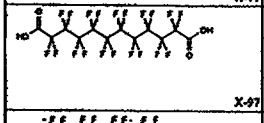
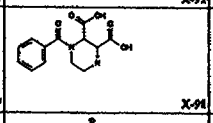
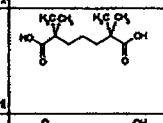
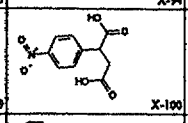
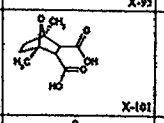
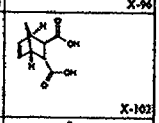
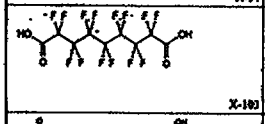
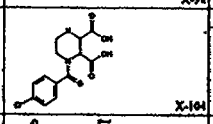
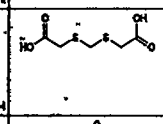
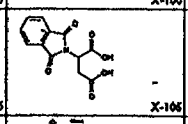
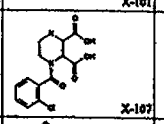
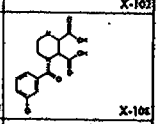
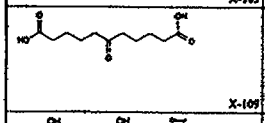
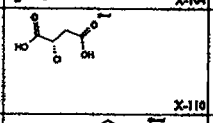
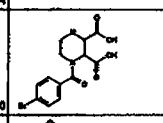
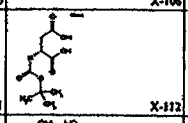
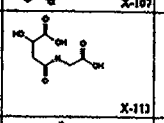
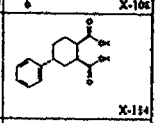
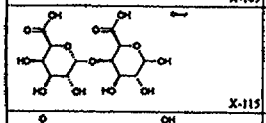
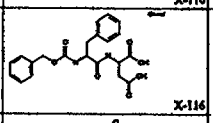
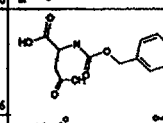
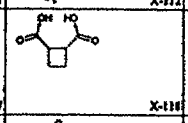
To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

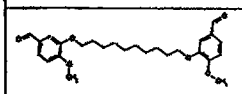
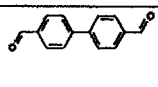
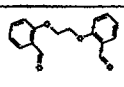
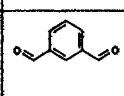
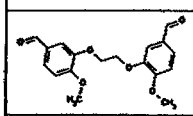
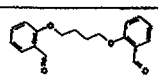
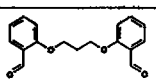
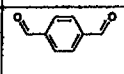
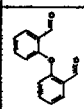
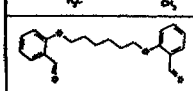
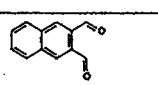
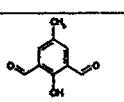
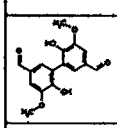
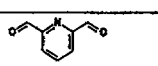
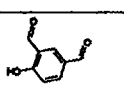
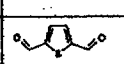
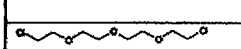
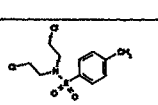
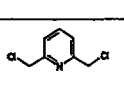
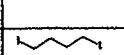
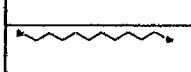
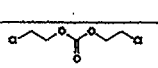
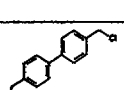
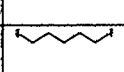
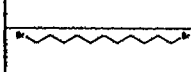
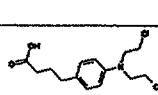
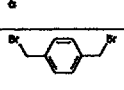
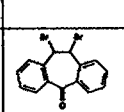
COMPLEMENTARY BINDING CHEMISTRIES

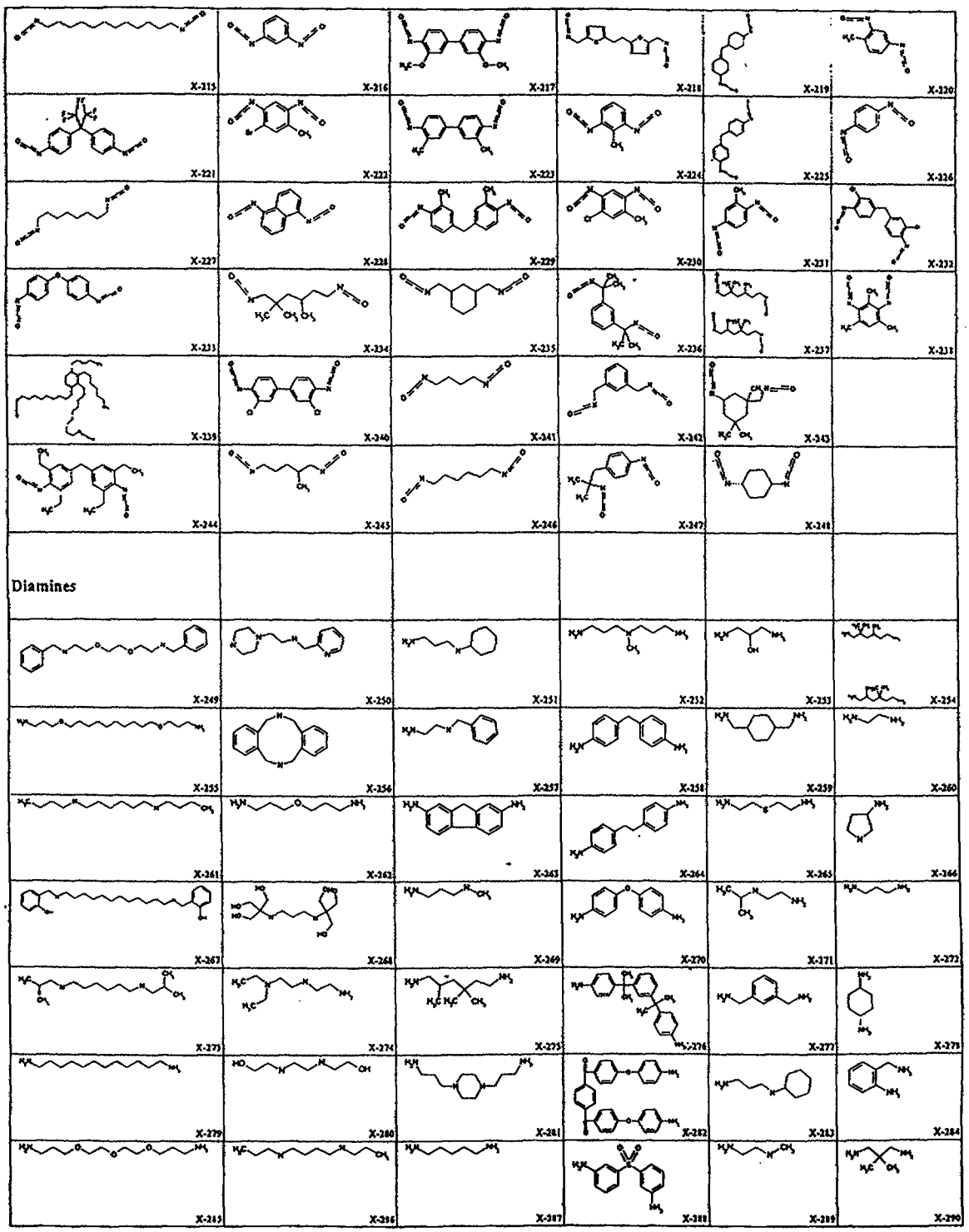
First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-hydroxyamine
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_4$	amine
ketone	amine/$NaCNBH_4$	amine
amine	isocyanate	carbamate

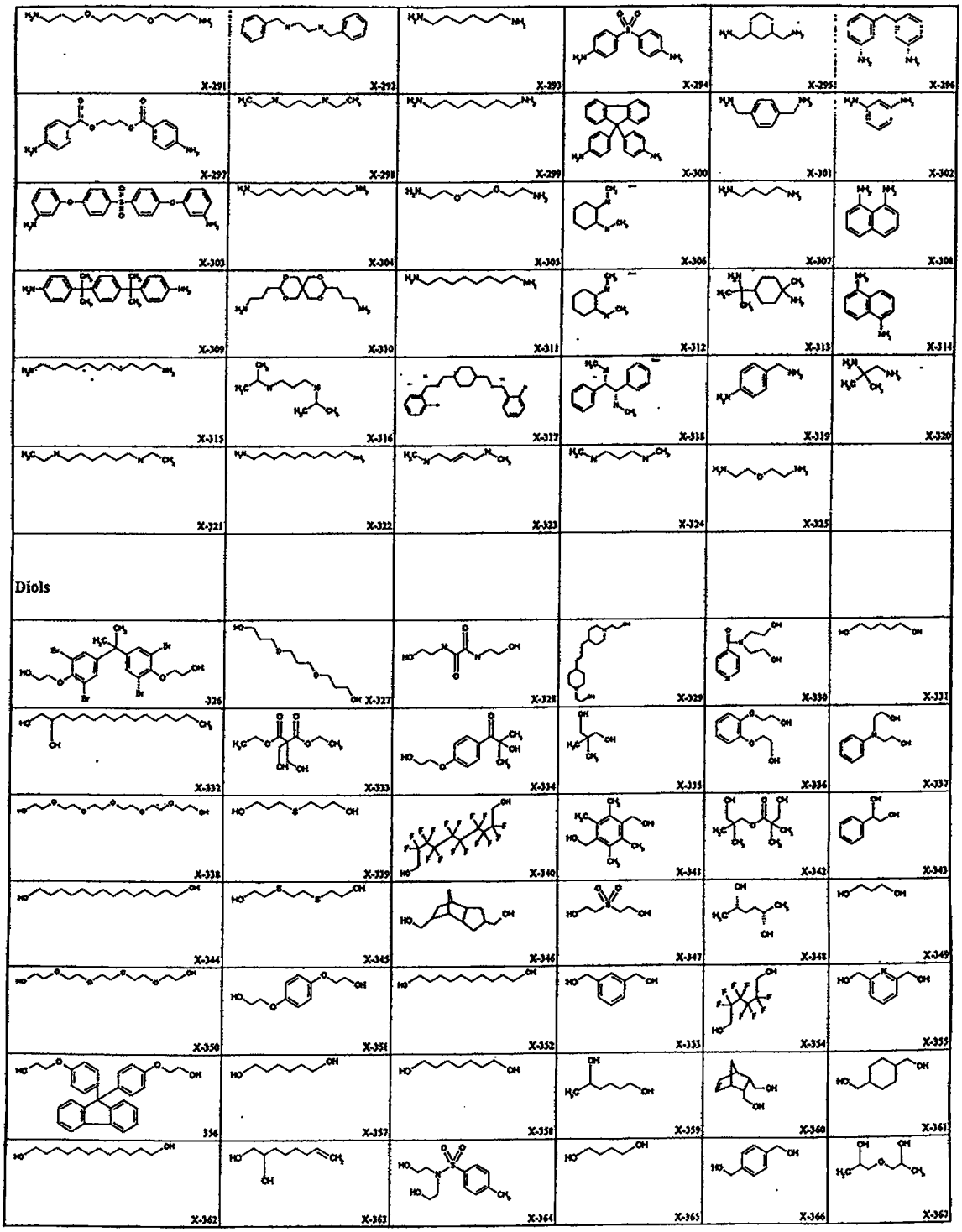
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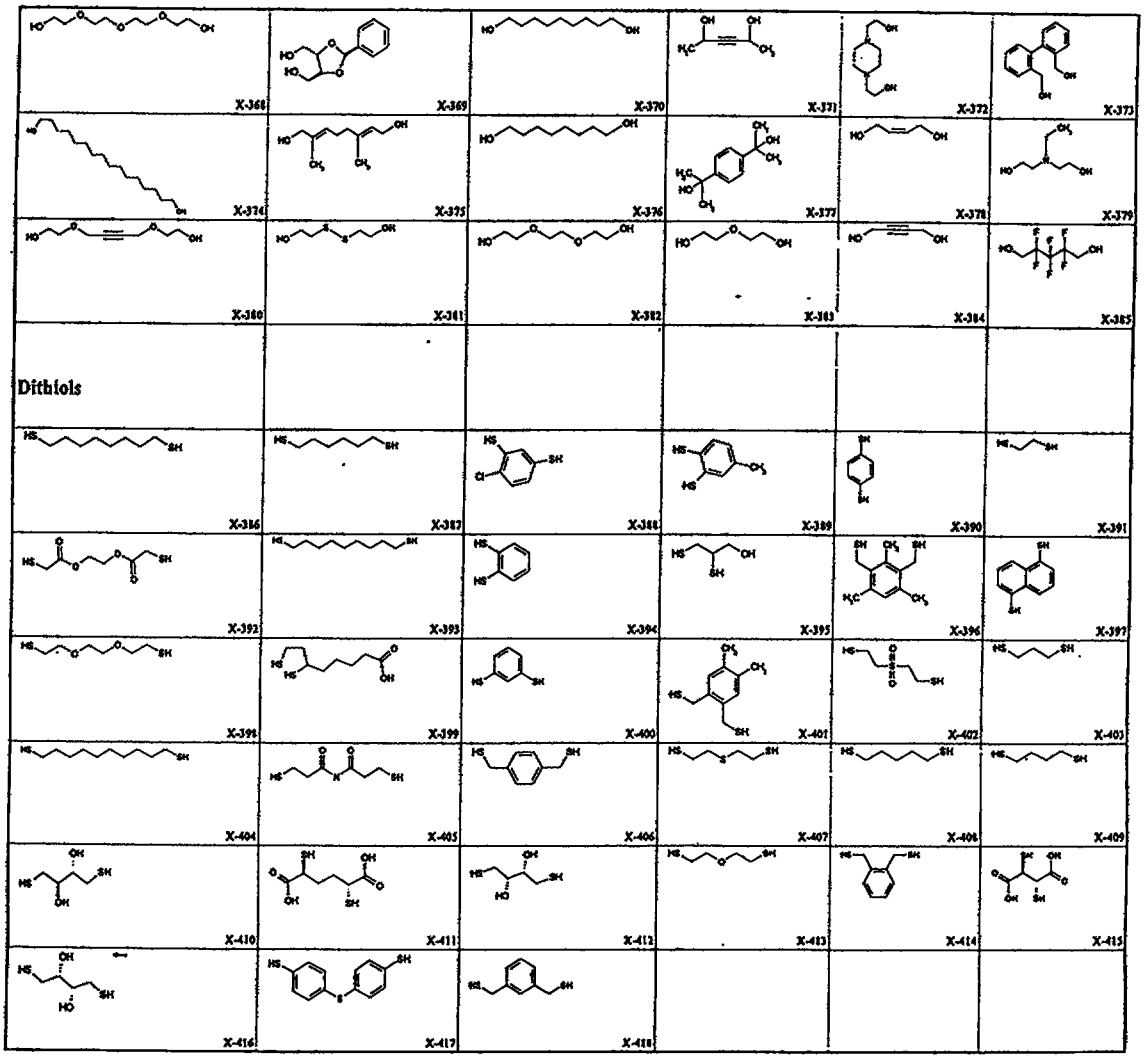
Diacids					
 X-1	 X-2	 X-3	 X-4	 X-5	 X-6
 X-7	 X-8	 X-9	 X-10	 X-11	 X-12
 X-13	 X-14	 X-15	 X-16	 X-17	 X-18
 X-19	 X-20	 X-21	 X-22	 X-23	 X-24
 X-25	 X-26	 X-27	 X-28	 X-29	 X-30
 X-31	 X-32	 X-33	 X-34	 X-35	 X-36
 X-37	 X-38	 X-39	 X-40	X-41	X-42
X-43	X-44	X-45	X-46	X-47	X-48
X-49	X-50	X-51	X-52	X-53	X-54
X-55	X-56	X-57	X-58	X-59	X-60
X-61	X-62	X-63	X-64	X-65	X-66
X-67	X-68	X-69	X-70	X-71	X-72
X-73	X-74	X-75	X-76	X-77	X-78

 X-79	 X-80	 X-81	 X-82	 X-83	 X-84
 X-85	 X-86	 X-87	 X-88	 X-89	 X-90
 X-91	 X-92	 X-93	 X-94	 X-95	 X-96
 X-97	 X-98	 X-99	 X-100	 X-101	 X-102
 X-103	 X-104	 X-105	 X-106	 X-107	 X-108
 X-109	 X-110	 X-111	 X-112	 X-113	 X-114
 X-115	 X-116	 X-117	 X-118	X-119	X-120
X-121	X-122	X-123	X-124	X-125	X-126
X-127	X-128	X-129	X-130	X-131	X-132
Disulfonyl Halides					
X-133	X-134	X-135	X-136	X-137	X-138
X-139	X-140	X-141	X-142	X-143	X-144
X-145	X-146	X-147	X-148	X-149	X-150
X-151	X-152				

Dialdehydes					
 X-153	 X-154	 X-155	 X-156	 X-157	 X-158
 X-159	 X-160	 X-161	 X-162	 X-163	 X-164
 X-165	 X-166	 X-167	 X-168	 X-169	 X-170
 X-171	 X-172	 X-173	 X-174		
Dihalides					
 X-175	 X-176	 X-177	 X-178	 X-179	 X-180
 X-181	 X-182	 X-183	 X-184	 X-185	 X-186
 X-187	 X-188	 X-189	 X-190	 X-191	 X-192
X-193	X-194	X-195	X-196	X-197	X-198
X-199	X-200	X-201	X-202	X-203	X-204
X-205	X-206	X-207	X-208	X-209	X-210
X-211	X-212	X-213	X-214		
Diisocyanates					


X-215
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Diamines
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X-418

Representative ligands for use in this invention include, by way of example, L as identified above (also identified as L-1).

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-
L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-

L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
L-1/X-163-	L-1/X-164-	L-1/X-165-	L-1/X-166-	L-1/X-167-	L-1/X-168-
L-1/X-169-	L-1/X-170-	L-1/X-173-	L-1/X-172-	L-1/X-173-	L-1/X-174-
L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-	L-1/X-179-	L-1/X-180-
L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-	L-1/X-185-	L-1/X-186-
L-1/X-187-	L-1/X-188-	L-1/X-189-	L-1/X-190-	L-1/X-191-	L-1/X-192-
L-1/X-193-	L-1/X-194-	L-1/X-195-	L-1/X-196-	L-1/X-197-	L-1/X-198-
L-1/X-199-	L-1/X-200-	L-1/X-201-	L-1/X-202-	L-1/X-203-	L-1/X-204-
L-1/X-205-	L-1/X-206-	L-1/X-207-	L-1/X-208-	L-1/X-209-	L-1/X-210-
L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-	L-1/X-215-	L-1/X-216-
L-1/X-217-	L-1/X-218-	L-1/X-219-	L-1/X-220-	L-1/X-221-	L-1/X-222-
L-1/X-223-	L-1/X-224-	L-1/X-225-	L-1/X-226-	L-1/X-227-	L-1/X-228-
L-1/X-229-	L-1/X-230-	L-1/X-231-	L-1/X-232-	L-1/X-233-	L-1/X-234-
L-1/X-235-	L-1/X-236-	L-1/X-237-	L-1/X-238-	L-1/X-239-	L-1/X-240-
L-1/X-241-	L-1/X-242-	L-1/X-243-	L-1/X-244-	L-1/X-245-	L-1/X-246-
L-1/X-247-	L-1/X-248-	L-1/X-249-	L-1/X-250-	L-1/X-251-	L-1/X-252-
L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-	L-1/X-257-	L-1/X-258-
L-1/X-259-	L-1/X-260-	L-1/X-261-	L-1/X-262-	L-1/X-263-	L-1/X-264-
L-1/X-265-	L-1/X-266-	L-1/X-267-	L-1/X-268-	L-1/X-269-	L-1/X-270-
L-1/X-271-	L-1/X-272-	L-1/X-273-	L-1/X-274-	L-1/X-275-	L-1/X-276-
L-1/X-277-	L-1/X-278-	L-1/X-279-	L-1/X-280-	L-1/X-281-	L-1/X-282-
L-1/X-283-	L-1/X-284-	L-1/X-285-	L-1/X-286-	L-1/X-287-	L-1/X-288-
L-1/X-289-	L-1/X-290-	L-1/X-291-	L-1/X-292-	L-1/X-293-	L-1/X-294-
L-1/X-295-	L-1/X-296-	L-1/X-297-	L-1/X-298-	L-1/X-299-	L-1/X-300-
L-1/X-301-	L-1/X-302-	L-1/X-303-	L-1/X-304-	L-1/X-305-	L-1/X-306-
L-1/X-307-	L-1/X-308-	L-1/X-309-	L-1/X-310-	L-1/X-311-	L-1/X-312-
L-1/X-313-	L-1/X-314-	L-1/X-315-	L-1/X-316-	L-1/X-317-	L-1/X-318-
L-1/X-319-	L-1/X-320-	L-1/X-321-	L-1/X-322-	L-1/X-323-	L-1/X-324-
L-1/X-325-	L-1/X-326-	L-1/X-327-	L-1/X-328-	L-1/X-329-	L-1/X-330-
L-1/X-331-	L-1/X-332-	L-1/X-333-	L-1/X-334-	L-1/X-335-	L-1/X-336-
L-1/X-337-	L-1/X-338-	L-1/X-339-	L-1/X-340-	L-1/X-341-	L-1/X-342-
L-1/X-343-	L-1/X-344-	L-1/X-345-	L-1/X-346-	L-1/X-347-	L-1/X-348-
L-1/X-349-	L-1/X-350-	L-1/X-351-	L-1/X-352-	L-1/X-353-	L-1/X-354-

L-1/X-355-	L-1/X-356-	L-1/X-357-	L-1/X-358-	L-1/X-359-	L-1/X-360-
L-1/X-361-	L-1/X-362-	L-1/X-363-	L-1/X-364-	L-1/X-365-	L-1/X-366-
L-1/X-367-	L-1/X-368-	L-1/X-369-	L-1/X-370-	L-1/X-371-	L-1/X-372-
L-1/X-373-	L-1/X-374-	L-1/X-375-	L-1/X-376-	L-1/X-377-	L-1/X-378-
L-1/X-379-	L-1/X-380-	L-1/X-381-	L-1/X-382-	L-1/X-383-	L-1/X-384-
L-1/X-385-	L-1/X-386-	L-1/X-387-	L-1/X-388-	L-1/X-389-	L-1/X-390-
L-1/X-391-	L-1/X-392-	L-1/X-393-	L-1/X-394-	L-1/X-395-	L-1/X-396-
L-1/X-397-	L-1/X-398-	L-1/X-399-	L-1/X-400-	L-1/X-401-	L-1/X-402-
L-1/X-403-	L-1/X-404-	L-1/X-405-	L-1/X-406-	L-1/X-407-	L-1/X-408-
L-1/X-409-	L-1/X-410-	L-1/X-411-	L-1/X-412-	L-1/X-413-	L-1/X-414-
L-1/X-415-	L-1/X-416-	L-1/X-417-	L-1/X-418-		

Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of formula I above associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician or veterinarian, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound

administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents

may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the

transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*[2].

Utility

The compounds of this invention modulate cellular receptor activity and accordingly, may be used for the treatment of biological conditions in animals. More particularly the compounds may be used in the treatment of medical and veterinary conditions in mammals. They are also useful as insecticides, and for other agricultural application such as crop protection. They are useful as anti-microbials and fungicides.

The compounds of the invention are particularly useful in treating pathological conditions mediated in one form or another by cellular receptor activity. Accordingly, the invention also relates to pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a compound of the invention.

Additionally, the compounds of the invention may be bound to affinity resins for affinity chromatography. The compounds of the invention may be used as a tool in immunoprecipitation. The compounds may be used to identify a receptor *in vitro* for example in microscopy, electrophoresis and chromatography.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celcius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

Examples 1 - 5 are given as representative examples of methods for preparing the linkers.

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

Å	=	Angstroms
cm	=	centimeter
DIC	=	2-dimethylaminoisopropyl chloride hydrochloride
DCC	=	*N,N*-dicyclohexylcarbodiimide
DCM	=	dichloromethane
DIPEA	=	diisopropylethylamine
DMA	=	*N,N*-dimethylacetamide
DMAP	=	*4-N,N*-dimethylaminopyridine
DMF	=	*N,N*-dimethylformamide
DMSO	=	dimethylsulfoxide
DPPA	=	diphenylphosphoryl azide
g	=	gram
HBTU	=	1-hydroxybenzotrizole
HPLC	=	high performance liquid chromatography
Hunig's base	=	diisopropylethylamine
MFC	=	minimum fungicidal concentration
mg	=	milligram
MIC	=	minimum inhibitory concentration
min	=	minute
mL	=	milliliter
mm	=	millimeter
mmol	=	millimol
N	=	normal

PyBOP	=	pyridine benzotriazol-1-yloxy-tris(dimethyl-amino)phosphonium hexafluorophosphate
t-BOC	=	*tert*-butyloxycarbonyl
TBAF	=	tetrabutyl ammonium fluoride
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
μL	=	microliters

EXAMPLE 1

Preparation of [C-C] Compounds of Formula I

(1) Preparation of a Compound of Formula (3) in which m is 2 and n is 3

To a solution of *tert*-butyl *N*-(2- aminoethyl)carbamate (2.3g, 14.4 mmol) and *N,N*-diisopropylethylamine (2.5 mL, 14.3 mmol) in 15 mL methylene chloride at 0°C was added dropwise glutaryl dichloride (0.6 mL, 4.7 mmol) in 15 mL methylene chloride. The resulting mixture was allowed to warm to room temperature with stirring while adding water (15 mL). The methylene chloride was removed under reduced pressure and more water was added (30 mL). The resulting suspension was filtered and washed sequentially with 10% potassium hydrogen sulfate, water, saturated sodium bicarbonate, and water. The solid was dried under vacuum yielding 1.3 g (3.1 mmol, 66%) of pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3).

Similarly, varying the composition of m and n, other compounds of formula (3) can be prepared.

(2) Preparation of a Compound of Formula (4) in which m is 2 and n is 3

Pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3) (1.3 g, 3.1 mmol) was suspended in 15 mL methylene chloride. 15 mL of trifluoroacetic acid was added at room temperature giving (with effervescence) a solution that was stirred for 40 minutes, then evaporated *in vacuo*. The residue was dissolved in methanol and treated with 3 mL of 4 N hydrogen chloride in dioxane followed by diethyl ether, giving a gum. The liquids were decanted and the gum

dried under vacuum yielding 1.0 g (3.4 mmol,) of pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4).

Similarly, varying m and n, other compounds of formula (4) can be prepared.

(3) Preparation of a Compound of Formula I

At room temperature, a carboxyl containing ligand (e.g., amphotericin) (2.3 mmol) is dissolved in 36 mL of DMSO. To this solution is added pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4) (1.0 g, 3.4 mmol) suspended in 27 mL DMF followed by addition of *N,N*-diisopropylethylamine (2.4 mL, 13.8 mmol). The resulting suspension is stirred at room temperature for several hours until it is mostly soluble. Then a solution of PyBOP (1.3 g, 2.5 mmol) and 1-hydroxybenzotriazole (310 mg, 2.3 mmol) in 9 mL DMF is added rapidly dropwise. The mixture is stirred at room temperature for 1 hour and then added dropwise to 600 mL of acetonitrile, giving a precipitate that is filtered, washed with acetonitrile ,then diethyl ether, and dried under vacuum. The crude product is purified by reverse phase HPLC (50 minute 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid to yield [C]-pentanedioic acid (2-aminoethyl)amide) ligand and [C-C]-pentanedioic acid bis-[(2-aminoethyl)amide]-bis-(ligand), a compound of Formula I as their respective trifluoroacetic acid salts.

(4) Preparation of other Compounds of Formula I

Similarly, following the procedures of Example 1, steps 1-3, other compounds of Formula I can be prepared.

EXAMPLE 2

Alternative Preparation of [C-C] Compounds of Formula I

(1) Preparation of a Compound of Formula (7) in which m is 2

At room temperature a carboxyl containing ligand for a cellular receptor containing a carboxyl group (4.7 mmol) is dissolved in 75 mL of DMSO. To this solution is added N,N-diisopropylethylamine (4.1 mL, 23.5 mmol) followed by 9-

fluorenylmethyl N-(2-aminoethyl)carbamate hydrochloride (1.8 g, 5.6 mmol). To the resulting solution at room temperature is added rapidly dropwise a solution of PyBOP (2.7 g, 5.2 mmol) and 1-hydroxybenzotriazole (630mg, 4.7mmol) in 75mL 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone. The resulting solution is stirred at room temperature for 2 hours, then poured into 800 mL diethyl ether, giving a gum. The diethyl ether is decanted and the gum is washed with additional diethyl ether to give a compound of formula (7).

(2) Preparation of a Compound of Formula (8) in which m is 2

The gum of formula (7) is then taken up in 40 mL of DMF, to which 10mL of piperidine is added and the solution left to stand at room temperature for 20 minutes. The solution is then added dropwise to 450 mL of acetonitrile giving a precipitate. Centrifugation is followed by decantation of the acetonitrile and the residue washed twice with 450 mL of acetonitrile, once with 450 mL of diethyl ether and air dried. The residue is taken up in water, acidified to pH < 5 with a small amount of 3 N hydrochloric acid and purified by reverse-phase HPLC using a gradient of 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid yielding a compound of formula (8).

(3) Preparation of a Compound of Formula I

Compound (8) (400 mg, 220 μmol) and glutaric acid (10 mg, 76 μmol) are dissolved in 5 mL DMF and N,N-diisopropylethylamine (140 μL, 800 μmol) followed by addition of PyBOP (83mg, 160 μmol) and 1-hydroxybenzotriazole (10mg, 74 μmol) in 500 mL DMF. The reaction is stirred for 75 minutes at room temperature then an additional 20 mg of PyBOP is added. 75 minutes later the solution is dripped into 45 mL acetonitrile. The resulting precipitate is collected by centrifugation, washed with ether, air dried and purified by reverse-phase HPLC (50 min 2-30% acetonitrile in water containing 0.1%trifluoroacetic acid, elutes at 33 min) to give a compound of Formula I as its trifluoroacetic salt.

(4) Preparation of other Compounds of Formula I

Accordingly, following the procedures of Example 2, steps 1-3, other [C-C] compounds of Formula I can be prepared.

EXAMPLE 3

Preparation of a [C-V] Compound of Formula I in which Position C is Substituted

(1) Preparation of a Compound of Formula (22) in which m and n are both 2

To a solution of an amino containing ligand (26.8 μmol) in DMF (2.0 mL) is added a compound of the formula:
Ligand-C(O)NHCH$_2$CH$_2$NHC(O)CH$_2$CH$_2$C(O)$_2$FM (FM refers to 9-Fluorenyl) (26.8 μmol), followed by PyBOP (20.9 mg, 40.2 μmol), HOBt (5.40 mg, 40.2 μmol), and Hunig's base (23.3 μL, 134 μmol). The reaction solution is stirred for 1 hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation. The crude precipitate is dried in air, yielding a compound of formula (22). The compound is used in the next step without further purification.

(2) Preparation of a Compound of Formula (23) in which m and n are both 2

The compound of formula (22) is dissolved in 1 mL of DMF, and 100 μL of piperidine is added to the solution. The solution is allowed to stand at room temperature for 30 minutes, following the course of the reaction by mass spectroscopy. The reaction solution is then added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% Buffer B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300 electrospray mass spectrometer and afterwards lyophilized to a white powder to afford compound (23) as a white powder.

Preparation of a Compound of Formula I

The compound of formula (23) prepared above (4.80 μmol) is dissolved in 500 μL of DMF. A ligand for a cellular receptor having a free amino group, such as a ligand of formula (17) (4.80 μmol) is added to the solution, followed by PyBOP (2.50 mg, 4.8 μmol), HOBt (0.65 mg, 4.80 μmol) and Hunig's base (6.70 μL, 38.4 μmol). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300 electrospray mass spectrometer.

EXAMPLE 4

Preparation of a [C-N] Compound of Formula I

(1) Preparation of a Compound of Formula (24) in which m is 2

A ligand having both primary and secondary amines such as those found in Figure 8 (2.60mmol) is suspended in 40 mL of 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone and heated to 70°C for 15 minutes. N-(9-fluorenylmethoxycarbonyl)-aminoacetaldehyde (720 mg, 2.6 mmol) is added and the mixture is heated at 70°C for one hour. Sodium cyanoborohydride (160 mg, 2.5 mmol) in 2 mL methanol is added and the mixture is heated at 70°C for 2 hours, then cooled to room temperature. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 10-70% B over 90 minutes), which yielded a compound of formula (24) as its trifluoroacetate salt.

(2) Preparation of a Compound of Formula (25) in which m is 2 and p is 3

The compound of formula (24) obtained above (291 mg, 150 μmol) is dissolved in 3mL of DMF. 3-(dimethylamino)propylamine (28.3 μL, 225 μmol) is added, followed by the addition of PyBOP (85.8 mg, 165 μmol), HOBt (20.3 mg, 150 μmol) and Hunig's base (65.0 μL, 375 μmol). The reaction solution is stirred for one hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation. Recovery of this precipitate provides for a compound of formula (25).

(3) Preparation of a Compound of Formula (26) in which m is 2 and p is 3

The compound of formula (25) obtained above is dissolved in 1mL of DMF, and 100 μL of piperidine is added to the solution. The solution is allowed to stand at room temperature for 30 minutes and the course of the reaction is followed by mass spectroscopy. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded the compound of formula (26) as its trifluroacetate salt.

Preparation of a Compound of Formula I

The compound of formula (26) prepared above (3.14 μmol) is dissolved in 500 μL of DMF. A compound of formula (19) (3.14 μmol) is added to the solution, followed by PyBOP (2.44 mg, 4.8 μmol), HOBt (0.65 mg, 4.8 μmol) and Hunig's base (6.7 μL, 38.4 μmol). The reaction solution is added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded a compound of Formula I as its trifluroacetate salt.

EXAMPLE 5

Preparation of an [N-N] Compound of Formula I

(1) Preparation of a Compound of Formula I

The compound of formula (26) prepared above (12.7 μmol) is dissolved in 500 μL of DMF. and a compound of the formula: $HO_2CCH_2CH_2NHOCCH_2CH_2NHOCCH_2CH_2CH_2CONHCH_2CH_2CONHCH_2CH_2CO_2H$ (6.34 μmol) is added, followed by PyBOP (8.24 mg, 15.8 μmol), HOBt (2.13 mg, 15.8 μmol) and Hunig's base (8.8 μl, 51.0 μmol). The reaction solution is added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded a compound of formula I as its trifluoroacetate salt.

EXAMPLE 6

Triptan Multivalomer Evaluation

General

Triptan drugs are used in the treatment of migraines. Known pharmaceuticals include Sumatriptan, Zolmitriptan and Naratriptan. These drugs are proposed to have a peripheral and central component to their activity. One explanation is that these agonists of the 5HT1 class of receptors constrict the cerebral vascular system and prevent the plasma extravasation and inflammation that is associated with headache pain. The central effect of these drugs is also believed to operate through inhibition of neurotransmitter release in the trigeminovascular system. The 5HT1d receptors are located in the terminals of the trigeminovascular system. Activity of an agonist at this receptor is believed to inhibit neurotransmitter release and prevent neurogenic inflammation. Another effect is believed to be through vasoconstriction of pathologically dilated cerebrovasculature. It is believed

that both receptor agonist activities contribute to the alleviation of the headache pain.

Multimerization

The multimerization positions of triptan include the indole bicyclic core, the C3 substituent and the C5 substituent. (Figures 16-18). The C3 and C5 positions are most likely to tolerate substitution for multimer formation.

Two similar strategies are followed for the conversion of the pharmacophoric building blocks into symmetrical multivalomers as shown in Figure 18. The "M" in Figure 18 is the site at which the linker is attached to the sumatriptan.

In the first strategy, the building block is reacted with a symmetrical linker molecule to provide the multivalomer. The pharmacophoric building block is an electrophile or a nucleophile. This building block may carry the required reactive functionality at the C3 or the C5 position. The number of molar equivalents of this building block will correlate with the number of complementary reactive sites on the linker molecule.

In the second strategy, the building block is reacted first with a spacer group. Then this conjugate is reacted with a symmetrical linker molecule to provide the multivalomer of this invention. The spacer group facilitates the combinatorial generation of multivalomers. (Figure 23)

1) C3 Electrophile (Figures 19 and 21)

The C3 mesylate N (700 mg, 2 mmol) is dissolved in $CHCl_3$ (10 ml), DIPEA is then added and the reaction is then heated to 40°C. A solution of the diamine N (132 mg, 1 mmol) in DCM (10 ml) is added to the warmed solution over 30 minutes. The reaction is then heated at reflux for two hours and then allowed to cool. The solvent is removed under vacuum. The crude reaction mixture is then treated with aqueous saturated NH_4Cl solution and then extracted with EtOAc (3 x 30 ml). The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

2) C3 Nucleophile (Figures 19 and 21)

The C3 methylamine N (562 mg, 2 mmol) is dissolved in $CHCl_3$ (10 mls), acetic acid (0.5 mls) is then added and the reaction is heated to reflux. Aldehyde N (132 mg, 1 mmol) dissolved in DCM (10 mls) is then added dropwise to the refluxing solution over 60 minutes and the reaction is refluxed for a further 60 minutes. At this point, the $NaBH(OAc)_3$ (222 mg) is added in portions and the reaction is stirred at reflux for a further 2 hours. The reaction is allowed to cool and then is quenched with aqueous NH_4Cl solution and the pH of the solution is adjusted to pH 7.0 using either 1 M HCl or NaOH. The product is extracted from this aqueous phase with EtOAc (3 x 30 ml). The organic layer is dried using Na_2SO_4, the drying agent is then filtered off and the solvent removed *in vacuo* to provide the crude product. The desired material is purified from this mixture using reverse phase HPLC.

3) C5 electrophile (Figures 20 and 21)

The C5 sulphonate N (720 mg, 2.0 mmol) is dissolved in CH_2Cl_2 (10 ml) and DIPEA is then added. A solution of the 1,3-propandiamine (60 mg, 1 mmol) in CH_2Cl_2 (10 ml) is then added dropwise over 1 hour via syringe pump. The reaction is then allowed to stir at room temperature for a further hour. The solvent is removed *in vacuo* and the crude reaction mixture is diluted with $CHCl_3$ and then the organic layer is treated with 1 M NaOH. The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

4) C5 Nucleophile (Figures 20 and 21)

The C5 sulphonamide N (590 mg, 2.0 mmol) is dissolved in DMF (10 ml) and cooled to 0°C in an ice bath. Sodium hydride (48 mg, 2.0 mmol) is then added and the reaction is allowed to stir at this temperature for 1 hour. The dibromide N (265 mg, 1 mmol) in a solution in DMF (10 ml) is added dropwise via syringe pump over 1 hour, and the reaction is then allowed to warm to room temperature and

stirred for a further 1 hour. The reaction is then quenched with isopropanol (1 ml). The solution is treated with aqueous NH_4Cl solution and the pH of the solution adjusted to pH 7.0 using either 1 M HCl or NaOH. The product is then extracted from this aqueous phase with EtOAC (3 x 30 ml). The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

5) C3 Nucleophile and C3 Electrophile (Figure 22)

The C3 acid N (660 mg, 2 mmol) is dissolved in DMF (10 ml); DIPEA (2 mmol) and DIC (2 mmol) are then added and the reaction is stirred at room temperature for 20 minutes. A solution of the 1,3-propandiamine (74 mg, 1 mmol) in DMF (10 ml) is then added dropwise over 1 hour. The reaction is then allowed to stir at room temperature for a further hour. The reaction is then quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

6) C5 Nucleophile and C5 Electrophile (Figure 22)

The C5 acid N (888 mg, 2 mmol) is dissolved in DMF (10 ml), DIPEA (3 mmol) and DIC (2 mmol) are then added and the reaction is stirred at room temperature for 20 minutes. A solution of the 1,3-propandiamine (74 mg, 1 mmol) in DMF (10 ml) is then added dropwise over 1 hour. The reaction is then allowed to stir at room temperature for over one hour. The reaction is then quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

Screening Assays

The preclinical pharmacology of triptan multivalomers is evaluated using the following assays. The compounds are evaluated to determine the following parameters:

potency at specific receptor subtypes.

functional activity/potency at these specific receptor subtypes

functional activity/potency in a reporter gene assay

functional activity/potency in isolated tissue.

activity in relevant animal models

The following assays are used:

ASSAY GROUP I- Measurement of *in vitro* binding assay/potency using radioligand binding assays

ASSAY GROUP II- Evaluation of agonist/partial agonist character in *in vitro* functional assays.

ASSAY GROUP III- Measurement of Functional Activity in *in vitro* reporter gene assay.

ASSAY GROUP IV- Measurement of Efficacy in *ex vivo* functional assays

ASSAY GROUP V- Utilization of *in vivo* models

Triptan multi-valency binding agents are evaluated for efficacy by methods similar to the assays that are described in Pramod R.Saxena and Michel Ferrari[3] .

These multi-valency binding agents are also evaluated in assays that are similar to those set forth in G.R. Martin et al.[4], and J. Ngo et al.[5].

More specifically triptan multivalomers are evaluated according to the following protocols that have been used to discover and develop currently marketed triptan drugs.

1. ASSAY GROUP I-Measurement of *in vitro* binding assay/potency using radioligand binding assays

Triptan multi-binding agents exhibit potency of 10-0.1 nM at a selection of the following receptors.

The triptan multi-binding agents are evaluated for binding affinity against the human forms of the following serotonin receptor subtypes. The following four receptor subtypes have been selected. This selection does not eliminate evaluation of other serotonin receptor subtypes. Multi-valent binding agents are also evaluated against non-human receptor subtypes to correlate with the *in vivo* screening profile.

A) 5HT1a receptor subtype

A method similar to that set forth in Petrus J. Pauwels et al.[6] and Philippe Schoeffter and Daniel Hoyer[7] is used. These references describe methods by which the effects of a compound is investigated in radioligand binding studies and in functional models for 5-HT1A, 5-HT1B, and 5-HT1D receptors (inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus and in rat and calf substantia nigra, respectively) and 5-HT1C receptors (stimulation of inositol phosphate production in pig choroid plexus).

B) 5HT1b receptor subtype

A method similar to that set forth in M.S. Beer et al.[8] is used. The potency of the multi-valent compound is compared with L-694,247 which has an affinity (pIC50) of 10.03 at the 5-HT1D binding site and 9.08 at the 5-HT1B binding site (sumatriptan: pIC50 values 8.22 and 5.94 respectively). L-694,247 retains good selectivity with respect to the 5-HT1A binding site (pIC50 = 8.64), the 5-HT1C binding site (6.42), the 5-HT2 binding site (6.50) and the 5-HT1E binding site (5.66). L-694,247, like sumatriptan, displays a similar efficacy to 5-HT in inhibiting forskolin-stimulated adenylyl cyclase in guinea-pig substantia nigra although L-694,247 (pEC50 = 9.1) is more potent than sumatriptan (6.2) in this 5-HT1D receptor-mediated functional response. L-694,247 (pEC50 = 9.4) is also more potent than sumatriptan (6.5) in a second 5-HT1D receptor mediated functional response, the inhibition of K+-evoked [3H]-5-HT release from guinea-pig frontal cortex slices.

See also Thierry Wurch et al.[9] for other methods similar to those used to test multi-valent compounds.

C) 5HT1d receptor subtype

A method similar to that set forth in Sarah A. Veldman and Michal J. Bienkowski[10] is used. In this method, expression of the receptor in Chinese hamster ovary cells creates high affinity binding sites for 5HT that is coupled to the inhibition of adenyl cyclase.

D) 5HT1f receptor subtype

A method similar to that set forth in Nika Adham et al.[11] is used to investigate the relation between receptor occupancy and inhibition of cAMP accumulation mediated by 5-HT1F receptors in NIH-3T3 cells (and hence the degree of receptor reserve). This reference shows that a half-maximal response requires only about 10% receptor occupancy, consistent with a receptor reserve of 90% (88%) for 5-HT-induced inhibition of FSCA.

The method for investigating activation of additional signal transduction pathways by the 5-HT1F receptor set forth in this reference is also followed. This reference shows that the responses differ in the two cell lines with respect to stimulation of phospholipase C. The data indicates that the human 5-HT1F receptor can couple to multiple effectors, and that this coupling is cell-type dependent. See also N. Adham et al.[12]

2. ASSAY GROUP II-Evaluation of agonist/partial agonist character in *in vitro* functional assays

A) 5HT1a subtype (functional)

A method similar to that set forth in Petrus J. Pauwels et al.[6] is used to test the forskolin-stimulated c-AMP formation mediated by 5HT1a receptors in CHO-K1 cells.

B) 5HT1b subtype (functional)

A method similar to that described in Petrus J. Pauwels et al.[6] is used.

C) 5HT1d subtype (functional)

A method similar to that described in Petrus J. Pauwels et al.[13] is used to investigate the pharmacology of human serotonin (5-HT)1D receptor sites by measuring two functional cellular responses, inhibition of forskolin-stimulated cAMP formation and promotion of cell growth, using transfected rat C6-glial cell lines and a broad series of 5-HT receptor agonists.

Another method similar to that set forth in John M. Zgombick et al.[14] is used for pharmacological evaluations of serotonergic compounds that inhibit forskolin-stimulated cAMP accumulation in NIH-3T3 fibroblasts (1Dα cell line) and Y-1 adrenocortical tumor cells (1Dβ cell line) stably expressing recombinant human 5-HT1Dα and 5-HT1Dβ receptor subtypes, respectively.

D) 5HT1f receptor subtype (functional)

A method similar to that set forth in Nika Adham et al.[11] is used .

3. ASSAY TYPE III-Measurement of Functional Activity in *in vitro* reporter gene assay.

A method similar to that set forth in S.E. George[15] is used. A cAMP-responsive reporter cell line has been established through the stable expression of a luciferase reporter plasmid in Chinese hamster ovary (CHO) cells. Reporter cells show a dose-dependent expression of luciferase in response to incubation with forskolin. These CHO cells are screened for endogenous G protein-coupled receptors capable of stimulating or inhibiting adenylyl cyclase, by monitoring changes in luciferase expression. The response to 5-HT is reversed by the 5-HT1 receptor antagonists cyanopindolol and pindolol, but not the 5-HT2 receptor antagonist ketanserin. This reporter gene assay gives the expected pharmacological profile for these receptors when compared with cAMP accumulation assays,

confirming its value as a functional assay for G protein-coupled receptors linked to adenylyl cyclase.

4. ASSAY GROUP IV-Measurement of Efficacy *ex vivo* functional assay

It is preferable for the compounds to constrict cranial vasculature and not coronary vasculature . Although currently the triptan drugs are believed to provide relief for migraine headache through the constriction of the carotid vasculature, they also demonstrate constrictive effects in the coronary vasculature.

Rabbit Saphenous Vein-

A method similar to that set forth in Jean-Pierre Valentin et al.[16] is used to investigate whether contractile responses evoked by multi-valent 5-HT1D receptor agonists are influenced by the endothelium (E) and nitric oxide (NO) in the rabbit isolated saphenous vein. 5-HT, 5-carboxamidotryptamine (5-CT) and sumatriptan (Sum) contract rabbit saphenous vein rings in the potency order (pD2 range) of 5-CT(7.2-7.6) > 5-HT(6.2-7.1) > Sum(5.0-5.8). Efficacy, as assessed by the maximal contractile response (Emax), is significantly greater for Sum compared to 5-HT and 5-CT. In conclusion, the efficacy, but not the potency, of 5-HT, 5-CT and Sum in evoking 5-HT1D receptor-mediated contractile responses is subject to a substantial inhibitory influence of the endothelium and of an EDRF (NO).

Dog Basilar Artery-

A method similar to that set forth in Andre Van de Water et al.[17] is used. In anesthetized dogs, alniditan or (-)-(R)-N-[3,4-dihydro-2H-1-benzopyran-2-ylmethyl]-N'-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride, a compound with 5-HT1-like receptor ligand effects, dose dependently (0.63-80 μg/kg i.v.) reduces common carotid arterial blood flow with comparatively little effect on other cardiovascular variables including coronary, mesenteric and renal arterial blood flow, systemic and pulmonary vascular resistance and airway resistance. The potency of alniditan is higher than that of sumatiptan.

Dog Middle Cerebral Artery-

A method similar to that set forth in F. D. Yocca[18] is used.

Human Middle Cerebral Artery-

A method similar to that set forth in Edith Hamel et al.[19] is used to determine the pharmacological profile of any 5-HT receptor which induces contraction of the bovine isolated cerebral arteries. Several multi-binding 5-HT receptor agonists are tested for their ability to induce vasoconstriction in bovine pial arteries and their potencies will be compared to that of 5-HT. The cerebral vasoconstriction in bovine cerebral arteries is mediated by a receptor homologous to the human cerebrovascular 5-HT1D receptor. Bovine pial arteries appear to be the best available pharmacological model for the human cerebrovascular 5-HT1D receptor.

Human Epicardial Coronary Artery Rings-

A method similar to that set forth in J. Longmore et al.[20] is used. This reference compares the effects of rizatriptan and L-741,519 with those of 5-HT and sumatriptan on endothelium-denuded segments of human coronary artery *in vitro* and confirms that rizatriptan is less effective than sumatriptan in causing contraction of human isolated coronary artery. Furthermore, it shows a the lower maximum contractile response to rizatriptan, compared with that of sumatriptan. See also A. Ferro et al.[20] for a method useful in this invention. Both sumatriptan and MK-462 were significantly less efficacious than 5-HT in contracting human coronary artery and furthermore MK-462 was significantly less effective than sumatriptan.

5. ASSAY GROUP V-Utilization of *in vivo* models

(a) Inhibition of neurogenic plasma protein extravasation during unilateral electrical stimulation of the trigeminal ganglion of guinea pigs.

A method similar to that set forth in P.R. Saxena[22] is used.

(b) Inhibition of Neuropeptide Release

A method similar to that set forth in P.J. Goadsby and Edvinsson, L.[23] is used Trigeminal stimulation results in the release of substance P and CGRP. Zolmitriptan reduces neuropeptide release during trigeminal ganglion stimulation in cats.

EXAMPLE 7

Multivalomers of Muscarinic Antagonists

General

Muscarinic receptors are composed of a family of five subtypes (M1-M5) each of which can be distinguished pharmacologically and structurally. The physiological role of each subtype in the central and peripheral nervous systems remains to be absolutely clarified.

Several agonists with functional selectivity for M1 receptors may prove useful in treating Alzheimer's disease. Selective M1/M3 antagonists may prove useful in the treatment of disorders of smooth muscle function.

Three subtypes of muscarinic receptor are found in human airways:

M1-receptors facilitate ganglionic transmission and therefore enhance cholinergic reflexes.

M2-receptors are localized to post-ganglionic cholinergic nerve terminals and inhibit the release of acetylcholine

M3-receptors on airway smooth muscle mediate constriction and on submucosal glands mediate increased mucus secretion.

M3 or mixed M1/M3-receptor antagonists are preferable since they would not increase acetylcholine release from cholinergic nerves.

Muscarinic receptor subtypes: pharmacology and therapeutic potential are described in Richard M. Eglen and Sharath S. Hegde[24]. Selective muscarinic receptor agonists and antagonists are described in Richard M. Eglen and Nikki Watson[25].

U. Holzgrabe et al.[26] describes allosteric small molecule binding sites for the muscarinic acetylcholine receptors.

Tropium drugs for airway disease include Ipratropium, Oxitropium, Tiotropium bromide and Revatropate.

a) Ipratropium bromide (ATROVENT) is a quaternary ammonium compound formed by the introduction of an isopropyl group to the N atom of atropine. Ipratropium bromide is often more effective than β_2-adrenergic agonists in the treatment of patients with chronic obstructive pulmonary disease (where cholinergic tone is usually the only reversible component), but is less effective than β_2-adrenergic agonists in the treatment of asthma.

Parenteral administration causes bronchodilatation, tachycardia, and inhibition of salivary secretion but lacks significant effects on the CNS. A therapeutically important property of ipratropium is the relative lack of effect on mucociliary clearance. The use of ipratropium in airway disease avoids the increased accumulation of lower airway secretions and the interference of β-adrenergic agonist-induced enhancement of mucociliary clearance

After inhalation, maximal responses usually develop over 30 to 90 minutes, and significant effects may persist for more than 4 hours. Ipratropium has minimal systemic effects since its quaternary structure limits absorption through the mucous membrane of the respiratory and gastrointestinal tracts. The bronchodilation produced by ipratropium in asthmatic subjects develops more slowly and is usually less intense than that produced by adrenergic agonists. Some asthmatic patients may experience a useful response lasting up to 6 hours. The bronchodilation produced by ipratropium is primarily a local, site-specific effect rather than a systemic effect. Ipratropium appears to produce bronchodilation by competitive inhibition of cholinergic receptors on bronchial smooth muscle.

b) Oxitropium bromide is similar to ipratropium.

c) Tiotropium bromide

The most recently developed and bronchoselective member of this family is tiotropium bromide which has a longer duration of action.

d) Revatropate

See A. M. Martel[27] for a description. The treatment of airway obstructive disease may be improved by antimuscarinic agents which selectively block M1 and M3 receptors but do not inhibit prejunctional cholinergic autoreceptors which limit release of acetylcholine. Revatropate is a antimuscarinic agent which shows some 50-fold selectivity for M1 and M3 receptors in guinea pig trachea and rabbit vas deferens over the M2 subtype in atria. This selectivity profile was seen *in vivo* in anesthetized guinea pigs and conscious dogs where bronchodilator activity was produced in the absence of any effect on heart rate. Revatropate, in contrast to the non-selective agent ipratropium, does not potentiate bronchoconstrictor responses induced by vagal nerve stimulation, indicating that inhibitory autoreceptors were still functional. Early clinical studies in COPD patients showed that inhaled revatropate was an effective bronchodilator which was well tolerated.

Method for Ipratropium Multivalomerization

Ipratropium is covalently attached to linkers by the following two methods.
i) Ipratropium may be attached via functional groups located within the drug (pharmacophoric building blocks) e.g. the hydroxyl group of the aldol product, the C-N bond of the tropane ring system. See Figures 25 and 26 which set forth the preferred sites for attachment.
ii) Alternatively Ipratropium may be attached to a linker via a functional group previously introduced into the aromatic ring to facilitate multivalomer construction. Suitable functional groups include -Br, -NH_2, -OH, -CO_2H, and -CHO. These functional groups may also require a suitable spacer between this group and the main element of the pharmacophore.

Figure 27 illustrates specific linkers for use with a functionality already present within the ipratropium.

Figure 28 illustrates the different valencies of the multivalomers that may be used. Dimers, trimers and tetramers are exemplified. These are all homovalomers using the same point of attachment within the ligand.

Figure 29 illustrates the role the linker/framework core plays in governing the spatial, physicochemical, pharmacological and pharmacokinetic profiles of these

multivalomers. "n" defines the valency of the multivalomer. "0" defines the linker/framework core and → identifies the differing points of attachment and orientation of the pharmacophore.

Multimers are constructed by the following methods.

1. Quaternization to provide N-linked dimeric Ipratropium (Figure 30)

a) Preparation of the ester(3)

With reference to Figure 30, the acid (1) (256 mg, 1 mmol) is dissolved in DMF (4 ml) and treated with DIC (126 mg, 1 mmol) and catalytic DMAP (5 mg). The reaction is stirred at room temperature for 30 minutes. A solution of the alcohol (2) (169 mgs, 1 mmol) in DMF (4 mls) is added dropwise and the reaction is stirred at room temperature for 12 hours. After this time the reaction is concentrated *in vacuo* and worked up using standard methods. One of these methods is to partition the crude reaction mixture between ethyl acetate (25 mls) and water (25 mls). The aqueous layer is extracted with ethyl acetate (25 mls x 2). The combined organic layers are dried ($MgSO_4$), filtered and concentrated in vacuo. This mixture is further purified using flash chromatography or HPLC to provide the desired ester(3).

b) Preparation of the dimer(5)

The tertiary amine(3) (404 mg, 1 mmol) is dissolved in the $CHCl_3$ (10 ml) and is heated to reflux. A solution of the dibromide(4) (180 mg, 0.5 mmol.) is added dropwise in $CHCl_3$ (10 ml) over three hours and the reaction is heated at reflux for another 2 hours. The reaction is allowed to cool and concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 mls) and water (25 mls). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer(5).

c) Hydrogenolytic removal of protecting groups to provide (6)

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen. The suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected dimer (5) (1018 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time, the remaining H_2 gas is removed *in vacuo*, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired dimer material (6).

2. N-linked Ipratropium dimers from reductive amination (Figure 31)

a) Synthesis of Ester(3)

With reference to Figure 31, a stirred solution of the acid (1) (280 mg, 1 mmol) in DCM (10 ml) is treated with DIC (126 mg, 1 mmol) and catalytic DMAP (5 mol%). The reaction is stirred at room temperature for 30 minutes, treated with a solution of the alcohol(2) (262 mg, 1 mmol) and the reaction is stirred at room temperature for twelve hours. The reaction is allowed to cool and concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired ester(3).

b) Deprotection of the CBZ protecting group to provide amine (4)

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen. The suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected ester (3) (520 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time the remaining H_2 gas is removed *in*

vacuo, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired amine(4).

c) Reductive Amination to provide dimer (6)

The amine (4) (389 mg, 1.0 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with AcOH (50 μl) and $NaBH(OAc)_3$ (844 mg, 4 mmol) and the reaction is stirred at room temperature for 12 hours. The dialdehyde(5) (70 mg, 0.5 mmol) is added and the reaction is stirred at room temperature for 12 hours. The reaction is quenched with methanol (10 ml) and concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer (6)

d) Methylation of dimer (6) to provide qauternary compound (7).

A stirred solution of the dimer (6) (880 mg, 1 mmol) in $CHCl_3$ (10 ml) is treated with methyl bromide (2 equivalents, 376 mg, 4 mmol) and the reaction is heated at reflux for 12 hours. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired quaternised dimer (7).

e) Deprotection of the dimer (6) to provide the ipratropium dimer (8)

A solution of the dimeric silyl ether (7) (910 mg, 1 mmol) in anhydrous THF (10 ml) is treated with 1M TBAF in THF (2 ml) and the reaction is allowed to stir at room temperature for 1 hour. After this time the reaction is concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired quaternised dimer (8).

3) O-Linked Ipratropium dimers from etherification (Figure 32)

a) Conversion of the amine (1) to the dimer (3).

With reference to Figure 32, a stirred solution of the alcohol (1) (614 mg, 2 mmol) is dissolved in DMF (10 ml) and is treated with Cs_2CO_3 (650 mg, 2 mmol) and the reaction is stirred at room temperature for 30 minutes. After this time, a solution of the bromide (2) (257 mg, 1 mmol) in DMF (5 ml) is added dropwise to the solution over two hours. The reaction is stirred at room temperature for a further two hours. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer(3).

b) Conversion to the dimeric quaternary salt (3)

The dimer (3) (732 mg, 1 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with MeBr (188 mg, 2 mmol) and the reaction is refluxed for 12 hours. The reaction is allowed to cool. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimeric quaternary species (3).

4) O-linked Ipratropium dimers from conjugate addition. (Figure 33)

a) Formation of the ether (3)

With reference to Figure 33, a solution of the acrylate (1) (299 mg, 1 mmol) in DMF (10 ml) is treated with a solution of the cesium alkoxide of the alcohol (2). This alkoxide is prepared by the treatment of the alcohol (2) (196 mg, 0.5 mmol) with Cs_2CO_3 (325 mg, 1 mmol) in DMF (10 ml). The reaction is heated to reflux for twelve hours allowed to cool to room temperature. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml)

and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired ether (3).

b) Hydrogenolysis of the O-Bn protecting group to provide (4).

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen, the suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected alcohol (3) (500 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time the remaining H_2 gas is removed *in vacuo*, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired alcohol (4).

c) Dimerization though Michael Addition to provide dimer (5)

A solution of the acrylate (1) (299 mg, 1 mmol) in DMF (10 ml) is treated with a solution of the alkoxide (4) (405 mg, 1 mmol) and the reaction is stirred at room temperature for 12 hours. The solution of the alkoxide of (4) is prepared by the treatment of the alcohol (4) in DMF (5 ml) with Cs_2CO_3 (325 mg, 1 mmol) and this solution is stirred at room temperature for 30 minutes. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired ether (5).

d) Quaternization of the dimer to provide (6)

The dimer(5) (704 mg, 1 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with MeBr (188 mg, 2 mmol) and the reaction is refluxed for 12 hours. The reaction is allowed to cool, The reaction is concentrated *in vacuo* and the crude reaction

mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimeric quaternary species (6).

Evaluation of Multimers

These muscarinic multivalomers are then evaluated for their ability to deliver a muscarinic antagonist for the treatment of airway disease. This evaluation is carried out within the framework of the following screening strategy.

i) Potency-M1-M5 *in vitro* radioligand binding assay.

The affinity of the multivalomers is measured on all five muscarinic subtypes of receptor. This provides pKi for the multivalomer ligands at these receptors. This is carried out for the recombinant human receptors and for species that are used in animal models.

Preferably, the multivalomer has a 1-10 nm affinity at the M1 and M3 receptors but an affinity of less than 100 nm at the other three receptors (M2, M4, M5).

These affinities are measured by methods similar to the protocols described by Buckley *et al*[28]. The 5 cholinergic muscarinic receptor genes (m1, m2, m3, m4, and m5) have been identified and cloned. The pharmacological properties of the individual muscarinic receptors have been described. Each of these genes has been transfected into CHO cells (CHO-K1), establishing stable cells lines expressing each receptor. The antagonist binding properties of each muscarinic receptor have been studied.

ii) Efficacy-M1-M5 *in vitro* radioligand binding/functional assay.

The functional activity of the multivalomers are established on the five human muscarinic receptor subtypes. This provides a pKa for the multivalomers at each of these receptors. Preferably, the multivalomers are antagonists and show a

pKa of 1-10nM at the M1 and M3 receptors. These multivalomers show significantly reduced affinity (pKa < 100nM) at the M2, M4, and M5 receptors. Required multivalomers do not show any functional agonist or partial agonist activity at these five muscarinic receptors.

These pharmacological properties are measured according to the procedures described by Jyrki P. Kukkonen et al.[29] and Jyrki P. Kukkonen[30].

iii) Potency/Efficacy-M1-M5 *ex vivo* functional studies.

The multivalomers that display selective muscarinic antagonist properties at the M1 and M3 receptor subtypes are evaluated for their functional activity in the *ex vivo* models. This evaluates the ability of the multivalomers to antagonize the acetylcholine induced bronchoconstriction in isolated animal airway tissue. The method is similar to that set forth in J. Garssen et al.[31]. Methods to evaluate multi-binding muscarinic compounds are also set forth in Jennifer Maclagan and Peter J. Barnes[32].

iv) *In vivo* assays.

The *in vivo* methods used to evaluate the M3 selective antagonist are similar to the methods that have been developed to compare the properties of Revatropate with Ipratropium. See for example V.A. Alabaster[33].

Antimuscarinic multivalomers which show M1 and M3 receptor selectivity are evaluated in guinea pig trachea (M1/M3) and rabbit vas deferens (M1/M3) over the atria (M2). This selectivity profile is evaluated in guinea pigs and dogs where bronchodilator activity is produced in the absence of any effect on heart rate.

EXAMPLE 8

AT1 Receptor Screening Strategy Based on Losartan.

The renin-angiotensin system plays a major role in the regulation of the blood pressure and renal function. The 8-mer angiotensin II peptide exerts its effects on blood pressure through mechanisms that include increasing salt and water

absorption from the blood in the kidney. Interruption of the renin-angiotensin system through inhibition of the angiotensin converting enzyme (ACE) through the use of small molecule ACE inhibitors has shown clinical benefit in the treatment of the hypertension, congestive heart failure and renal disease. However, the ACE inhibitors generate a dry cough side effect because they also interfere with kinin metabolism.

Antagonism of the cell surface receptor for the angiotensin II peptide is an alternative means of treating hypertension through modulation of the renin-angiotensin system. The major clinical advantage of this approach over ACE inhibitors is that it does not have the dry cough side-effect associated with the ACE inhibitors. See D. J. Dzielak[64], Wexler et al.[65], Clellan and Balfour[66], Burnier and Brunner[67], Merlos et al.[68] and M. Merlos et al.[69].

There are two receptor subtypes for the Angiotensin II octapeptide. These are the AT1 and AT2 receptor subtypes both of which belong to the seven transmembrane superfamily of cell surface receptor. The relevant pathophysiological effects of angiotensin II are exerted through specific agonism of the AT1 receptor. The AT1 receptor has a high affinity for the sartan drugs and a low affinity for PD 123177. The AT2 receptor has a high affinity for PD 123177 but a low affinity for losartan. It is of interest to note that angiotensin II shows no selectivity for the AT subtypes. The AT1 receptor is a G protein-coupled receptor of 359 amino acids. The AT2 receptor is 363 amino acids. These receptors have little sequence homology (only 32% amino acid sequence identity). The majority of the physiological effects of angiotensin II appear to be mediated by the AT1 receptor, and no functional role for the AT2 receptor has been defined (Bergsma et al.[70] and Mukoyama et al.[71]).

Losartan and Valsartan are two AT1 antagonists (Figure 34). Other AT1 antagonists include Irbesartan, Candesartan, Eprosartan, Tasosartan, Telmisartan and Ripisartan (Figures 35 and 36).

The AT1 receptor has a small molecule antagonist binding site and a peptide agonist binding site which are partially overlapping. There is an overlap between the propylimidazole and biaryl tetrazole motifs with the side chains of the

Ile5-His6-Pro7-Phe8 and the terminal carboxylate in the N-terminus of the angiotensin II peptide.

The residues in the receptor involved in the AT1 receptor-ligand complex may be divided into two groups, those that contribute to the binding of the ligand and those that are involved in signal transduction.

Receptor residues involved in binding peptide agonists.

Lys 199 (tm5) has been identified as the key receptor residue for the binding of agonists and antagonists to the AT1 receptor. This residue has been implicated in the binding of the AII peptide but also appears to be involved in the binding of the phenyl tetrazole or phenyl carboxyl residues of the non-peptide antagonists. Lys 102 (tm3), Arg 167 (EC2), His 183 (EC2) and Asp 263 (EC3) have been proposed as the possible residues for the interaction with the Asp1 and Arg2 of Angiotensin II. These residues show a limited effect on losartan binding.

Receptor residues involved in the binding non-peptide antagonists.

The non-peptide antagonists bind to a region that extends from the fifth to the seventh transmembrane helix. This is the same general region of the GPCR class of proteins that is occupied by small molecule biogenic amines such as the catecholamines for the β_2-receptor. Residues in transmembrane helices 3-7 that contribute to the binding of the non-peptide antagonists do not play role in the binding of the endogenous peptide agonist. It remains unclear whether the binding of these small molecule agonists (e.g. L-162,313) that resemble the small molecule antagonists takes place in the peptide agonist or non-peptide antagonist binding regions. Mutations that are known to affect the binding of losartan and angiotensin II have no effect on the binding of L162,313.

The above information of the specifics of the overlap between the antagonist-agonist binding site is used for the construction of heterovalomers.

Method for multivalomerization of angiotensis II antagonists

The six antagonists exemplified contain a biaryl containing an acidic group such as carboxylate, a sulphonamide or isosteric tetrazole (eprosartan is the exception in that it does not contain this motif). The six antagonists also contain a heterocyclic scaffold at the top of the molecule. This is a region of the molecule that appears to be readily varied and is a site for multivalomer construction (e.g. the imidazole group of losartan). The heterocyclic scaffold is generally substituted with a lipophilic alkyl group, e.g. the propyl group of losartan, and a hydrogen bond acceptor e.g. carboxamide, carboxaldehyde, carboxylate and hydroxymethyl.

At this C2 position of the imidazole, a linear alkyl or alkenyl group is preferred, which is preferably from 3 - 4 carbon atoms. At the C4 position of the imidazole there is a chlorine atom which tolerates large substituents. This is a position that will tolerate connection to the framework building blocks to construct multivalomers. At the C5 position of the imidazole, a variety of substituents are acceptable. A hydrogen bond acceptor, such as carboxamide, carboxaldehyde, carboxylate and hydroxymethyl, is preferred.

Consideration of the partially overlapping binding sites has indicated that it is possible to construct heterovalomers derived from losartan and angiotensin II.
i) functional groups are those groups within the drug (pharmacophoric building blocks) that may be exploited for multi-binding compound formation e.g. the tetrazole NH or the primary hydroxyl of losartan.
ii) atoms are points were a functional group may be introduced to facilitate multivalomer construction. For example, the introduction of such functional groups into the aryl ring of the biaryl functionality and the replacement of the Cl of the imidazole with a functional group that could be used for the construction of multivalomers. In the areas/regions of the molecule where there is no obvious chemical functional group to use for multi-binding compound construction (ii above) suitable functional groups can readily be introduced e.g -Br, -NH2, -OH, -CO2H, -CHO. These functional groups may also require a suitable spacer between this group and the main element of the pharmacophore.

Figures 38 and 39 shows the different points of attachment for specific losartan multivalomers, using functional groups already present within the ligand.

Figure 40 shows possible differing valencies of the multi-binding compounds. These are all homovalomers using the same point of attachment within the ligand.

Figure 41 shows the differing framework cores that could be used.

Figure 42 shows the differing orientation of binding elements within the multivalomer.

Figure 43 shows the heterovalomers that are possible within the AT1 antagonist manifold.

Synthesis of 1-hydroxyl linked losartan multivalomer (Figure 44)

a. Alkylation of the imidazole (1) to provide (2)

The procedure for the synthesis of the TBS protected imidazole(1) is set forth in Greenlee[72].

With reference to Figure 44, the imidazole (1) (604 mg, 2mmol) in DMF (10 ml, 0.2M) is treated with NaH (48 mg, 2mmol) and the reaction is stirred at room temperature for 30 minutes. The biaryl bromide (300mg, 1mmol.) is then added as a solution in DMF (5ml) and the reaction stirred for a further 60 minutes. The reaction is concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography provides the desired material (2).

b. Deprotection of the TBS ether (2) to provide the alcohol (3)

The silyl ether (2) (1.02 g, 2mmol) is dissolved in THF (10 mls, 0.2M) and 1M TBAF in THF (3 ml, 3mmol) is added and the reaction is allowed to stir at room temperature for 2 hours. The reaction is concentrated *in vacuo*, and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried ($MgSO^4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired material (3).

c. Dimerization via the primary hydroxyl of (3) to provide the multivalomer (4)

The primary alcohol (3) is dissolved in DMF (10ml, 0.2M) and cooled to 0°C, NaH (48mg, 2mmol) is added and the reaction is stirred at this temperature for 30 minutes. The dibromide (260 mg, 1mmol) is dissolved in DMF (10ml) and is added to the alkoxide solution via syringe pump over 60 minutes. The reaction is

concentrated *in vacuo*, and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried ($MgSO_4$), filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the desired multivalomer (4).

d. Deprotection of the dimeric tetrazole (4) to the losartan multivalomer (5).

The dimeric protected tetrazole (1.17g,1mmol) is dissolved in methanol (5ml, 0.2M) and is treated with 1M HCl in methanol (3ml, 3mmol) and the reaction is stirred at room temperature for 60 minutes. After this time, the reaction is concentrated *in vacuo*. The reaction is partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the losartan multivalomer (5).

Synthesis of 2-hydroxyl linked losartan multi-binding compound (Figure 45)

This is a strategy that is used to prepare either hydroxl linked or tetrazole linked multivalomers.

a. Alkylation of imidazole (1)

With reference to Figure 45, the imidazole (1), (276 mg, 2mmol) is added to a stirred solution of sodium methoxide in methanol (2mmol, 46mgs of sodium dissolved in (10 ml) methanol) at 0°C. The solvent is removed *in vacuo* and the so formed sodium salt of the imidazole is dissolved in DMF (10 ml). The biaryl bromide (542 mg, 2 mmol) is added and the reaction is stirred at room temperature for 12 hours. The solvent is then removed *in vacuo*, and the reaction partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is combined and the organic layer is dried with $MgSO_4$, the solvent in this removed *in vacuo*. Flash chromatography of the crude reaction mixture provides the alkylated imidazole (2).

b. Alkylation of alcohol (2) to provide dimer (3)

Sodium hydride (48 mg, 2mmol) is dissolved in DMF (10ml) and the alcohol (2) (760 mg, 2mmol.) is added with stirring. This reaction is allowed to stir at room temperature. The benzylic dibromide (261 mg, 1mmol) in DMF is added dropwise via syringe pump over two hours. The reaction is allowed to stir at room

temperature for a further two hours. The reaction is treated with aqueous NH_4Cl solution and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is separated, dried with $MgSO_4$, filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the pure dimer (3).

c. Conversion of the dimeric nitrile (3) to dimeric tetrazole (4)

The dimer (3) (430 mg, 1 mmol) is dissolved in xylene (20 ml) and the trimethylstannyl azide is added (615 mg, 3 mmol) and the reaction is heated to reflux in xylene (20 ml) for 24 hours. The solvent is removed *in vacuo* and the crude reaction mixture is treated with 2N NaOH in methanol (20 ml) to remove the N-stannyl group. The solvent is removed *in vacuo* and the reaction is dissolved in water and the solution neutralized (pH=7). The product is extracted with ethyl acetate (25mls x 3). The organic layer is dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimeric tetrazole (4).

Synthesis of tetrazole linked losartan multivalomer (Figure 46)

a. Conversion of the nitrile (1) to the tetrazole (2)

For the preparation of nitrile (1), see Figure 45. With reference to Figure 46, the biaryl nitrile (1) (380 mg, 2mmol) is dissolved in xylenes (10 ml) and the trimethylstannyl azide (820 mg, 4mmol) is added. The reaction is heated at reflux for 24 hours. The reaction is allowed to cool and the solvent is removed *in vacuo*. The crude reaction mixture is treated with 1N NaOH in methanol (20 ml) to hydrolyse the N-stannyl bond. The methanol is removed *in vacuo*, the crude reaction mixture is dissolved in water and neutralized with 1M HCl. The product is extracted from the aqueous phase with ethyl acetate (3 x 25 ml). The organic layer is dried with $MgSO_4$, the drying agent is filtered, and the solvent is removed *in vacuo*. The crude reaction mixture can be purified by flash chromatography to provide the desired tetrazole (2).

b. Conversion of the tetrazole (2) to the dimer (3)

The tetrazole (2) (844 mg, 2mmol) is dissolved in DMF (5 ml) and is treated with NaH (48 mg, 2mmol) and the reaction is stirred at room temperature for 20 minutes. The dibromide alkylating agent (260 mg, 1mmol) in DMF (10 ml) is added to tetrazole solution via syringe pump over one hour. The reaction is allowed to stir at room temperature for a further hour. The reaction is concentrated *in vacuo*, and is partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimer (3).

See also Rivero et al.[73], Duncia et al.[74], Carini et al.[75], and Carini et al.[76]

Assays

The following assays are used to evaluate multi-valomers derived from Angiotensin II (AT1 subtype) receptor antagonists. Where there is a specific protocol that has developed for testing a specific AT1 receptor agonist, this protocol is followed. However, other assay protocols described for a different AT1 receptor antagonist may also be used, eg testing a losartan multimer using a protocol developed for valsaratan.

The method used to evaluate efficacy for hypertension is similar to that set forth in D. J. Dzielak[46] which describes the pre-clinical and clinical pharmacology associated with the seven AT1 receptor antagonists.

The duration of action of the multivalomeric compounds is determined from the PK profile.

Assays similar to the following assays that were utilized in the discovery and development of Losartan, are used in the discovery and development of AT1 targeted multivalomers.

In vitro binding assay.

This assay is used to determine the pKi for the AT1 multi-binding compounds. Measurement of IC_{50} in rat adrenal cortical microsomes in a competition radioligand binding assay with radiolabelled angiotensin II is used.

Losartan inhibits the specific binding of ^{125}I-angiotensin II to its receptor sites in rat adrenal cortical membranes and in cultured rat smooth muscle cells.

The method is similar to those set forth in Bergsma et al.[70], and Mukoyama et al.[71].

In vitro functional activity.

Functional activity is measured by the ability of the multi-binding compound to inhibit the AII induced $^{45}Ca^{2+}$ efflux from rat aortic smooth muscle cells.

Ex vivo functional activity in rabbit aorta.

Functional activity is measured by the ability of the multi-binding compound to antagonize the functional contractile response to angiotensin II in a dose-dependent manner to provide a pA_2 value in a manner similar to that set forth in A. T. Chiu et al.[47].

***In vivo* model**-Lack of effect on blood pressure in conscious normotensive rats.

The effect on pressor response to the multi-binding compound is tested by methods similar to that set forth in P.C. Wong et al.[48].

***In vivo* model**- hypertensive rats

The effect of the multi-binding compound on blood pressure in the renal-artery ligated rat, a high renin hypertensive rat model, is tested using methods similar to that set forth in P.C. Wong et al.[49].

EXAMPLE 9

β2-adrenergic Agonist Multivalomers for Airway Disease.

β2-adrenergic agonists are used in the clinic for the treatment of acute symptoms of asthma, chronic asthma and chronic obstructive airway disease. For example, salmeterol acts within 60-90 minutes of exposure. β2-adrenergic agonists appear to be generally administered by inhalation or less frequently orally. In

general, the route of administration also significantly effects the onset of action with inhalation being significantly more rapid than oral dosing.

The current compounds are distinguished by their onset of action. This defines whether they are rapid onset such terbutaline (1-5 minutes) or whether they have a slower onset. The medicines that are prescribed are further distinguished by their duration of action. This defines whether they have a shorter duration of action e.g. albuterol (3-4 hours) or fenterol (12-14 hrs).

Formoterol, a selective β2-adrenergic agonist, produces ED-proportional bronchodilation, which persists for up to 12 hours, in patients with reversible obstructive respiratory disease. Bronchodilation is significant within minutes of inhalation, maximal within 2 hours, and at therapeutic doses is equivalent to that produced by standard doses of traditional β2-adrenergic agonist (Robert R. Ruffolo et al.[34]).

The following references describe the clinical pharmacology of a number of β2-adrenergic agonists currently used in human medicine (Louis Philippe Boulet[35], Claes-Goran A. Lofdahl[36] and Rebecca A. Bartow and Rex N. Brogden[37]).

The two categories (classified by their pharmacological profile that defines their clinical utility) of β_2-adrenergic agonist are set forth in Figure 47.
i) the rapid onset/ shorter acting drugs. Specifically multivalomers are derived from the monovalomeric marketed drugs albuterol and terbutaline. Multivalomers are also derived from other β2-adrenergic agonists that are in clinical use e.g bambuterol (prodrug), bitolterol, carbuterol, clenbuterol, colterol, fenoterol, indenolol, mabuterol, metaproterenol pirbuterol procaterol, reproterol, tulobuterol.
ii) slower onset/prolonged duration of action. Specifically multivalomers are derived from formoterol and salmeterol.

It is contemplated that both of these qualities of rapid onset of action and prolonged duration may be introduced into one molecule. It is also contemplated that the multivalomeric ligand could incorporate the elements of the rapid onset pharmacophore and elements of the prolonged duration of action pharmacophore in the one molecule

The following are some of the monovalomer drugs that used to design the pharmacophoric building blocks to allow multivalomer construction.

Albuterol (VENTOLIN) is a selective β_2-adrenergic agonist with pharmacological properties and therapeutic indications similar to those of terbutaline. It is administered either by inhalation or orally for the symptomatic relief of bronchospasm. When administered by inhalation, it produces significant bronchodilation within 15 minutes, and effects are demonstrable for 3 to 4 hours. The cardiovascular effects of albuterol are considerably weaker than those of isoproterenol when doses that produce comparable bronchodilatation are administered by inhalation

Bitolterol is a β_2-adrenergic agonist in which the hydroxyl groups in the catechol moiety are protected by esterification with 4-methylbenzoate. Esterases in the lung and other tissues hydrolyze this prodrug to the active form, colterol, or terbutylnorepinephrine. Results of animal studies have suggested that these esterases are present in higher concentration in lung than in tissues such as the heart. The duration of effect of bitolterol after inhalation ranges from 3 to 6 hours.

Fenoterol (BEROTEC) is a β_2-selective adrenergic receptor agonist. It has a rapid onset and its effect is typically sustained for 2 to 3 hours.

Formoterol (FORADIL) is a long-acting β_2-selective adrenergic receptor agonist. Significant bronchodilation occurs within minutes and persists for up to 12 hours. Its major advantage over many other β_2-selective agonists is this prolonged duration of action, which may be particularly advantageous in settings such as nocturnal asthma.

Metaproterenol (ALUPENT) is used for the long-term treatment of obstructive airway diseases and for treatments of acute bronchospasm. Metaproterenol is considered to be β_2-selective, although it probably is less selective than albuterol or terbutaline. Effects occur within minutes of inhalation and persist for several hours. After oral administration, onset of action is slower, but effects last 3 to 4 hours.

Pirbuterol is a relatively selective β_2 agonist. It is structurally identical to albuterol except for the substitution of a pyridine ring for the benzene ring.

Procaterol (MASCACIN) is a β_2-selective adrenergic receptor agonist. It has a rapid onset of action, which is sustained for about 5 hours.

Salmeterol (SEREVENT) is a β_2-selective adrenergic receptor agonist with a prolonged duration of action, of around 12 hours. However, it has a relatively slow onset of action after inhalation, so is not suitable alone for prompt relief of breakthrough attacks of bronchospasm. *In vitro*, salmeterol exerts 'reassertion' relaxation of airways smooth muscle. Reassertion relaxation refers to the capacity of salmeterol to cause repeated functional relaxation of induced contraction when airway smooth muscle is intermittently exposed to, then washed free from, β_2-adrenoceptor antagonists such as sotalol. The mechanism(s) underlying reassertion relaxation may relate to high affinity binding of the long aliphatic side chain of salmeterol to an accessory site, distinct from the agonist recognition site, in or near the β2-adrenoceptor (exosite binding hypothesis).

Terbutaline is a β_2-selective bronchodilator. It is effective when taken orally, subcutaneously, or by inhalation. Effects are observed rapidly after inhalation or parenteral administration. After inhalation its action may persist for 3 to 6 hours. With oral administration, the onset of effect may be delayed for 1 to 2 hours. It is also used for the long-term treatment of obstructive airway diseases and for treatment of acute bronchospasm. Furthermore, it is available for parenteral use for the emergency treatment of status asthmaticus.

a) Design of β_2-adrenergic ligand multivalomers.

Figure 48 sets forth the preferred sites for attachment of a ligand to albuterol for the generation of multivalomers. M represents the site for attachment. Figures 49 to 54 set forth the various valencies and orientations of the ligands. Figures 49 and 54 illustrate mixed multivalomers derived from different β_2-agonists.

1) Synthesis of Bivalent Analogs of Salmeterol (Figure 55)

With reference to Figure 55, compounds (1) (n = 1; 305 mg; 1.12 mmole) and (2) (200 mg; 0.5 mmole) were dissolved in 6 mL of N,N-dimethylformamide (DMF) followed by addition of HOBt (144 mg) and DIPEA (0.22 mL). To this

stirred solution was added PyBOP (554 mg) as a solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (100 mL), and extracted with EtOAc (150 mL). The organic phase was washed with 0.1 M HCl, 0.1 M NaOH, and brine, followed by drying with $MgSO_4$. Evaporation of the organic solution afforded a pale yellow oily residue, which was purified by flash silica column chromatography (1/1 EtOAc/hexanes to 0.1/1/1 MeOH/EtOAc/hexanes):Rf of product (3) = 0.68 in 0.1/1/1 MeOH/EtOAc/hexanes.

Compound (3) (358 mg; 0.4 mmole) was added slowly to a stirred suspension of $LiAlH_4$ (120 mg; 3.2 mmole) in THF (40 mL) cooled by an ice bath. The reaction mixture was slowly warmed to room temperature (30 min), and refluxed at 80°C for 4 hours. After cooling the mixture in an ice bath, 15% NaOH (0.5 mL) was added to quench the reaction, followed by stirring 30 min. The reaction mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50 mL). Filtrates were combined, and evaporated *in vacuo*, yielding a pale yellow oily residue. It was purified by flash silica column chromatography (2% MeOH/CH_2Cl_2 to 2% i-$PrNH_2$/10% MeOH/CH_2Cl_2): Rf of product (4) (yield 179 mg) = 0.47 in 2% i-$PrNH_2$/10% MeOH/CH_2Cl_2.

Compound (4) (170 mg; 0.2 mmole) was dissolved in EtOH (50 mL) containing 10% Pd/C (100 mg). The mixture was degassed, and saturated with H_2 gas. After stirring the mixture under H2 atmosphere (1 atm) for 24 hours at ambient temperature, the catalyst was filtered, and washed with EtOH (50 mL). Filtrates were combined, and evaporated, yielding a colorless oily residue. The product (5) was dissolved in 30% MeCN/water (containing 0.5% TFA), and purified by reversed-phase HPLC: 116 mg was obtained.

H1-NMR of 5 (CD_3OD, 299.96 MHz): δ = (ppm) 7.35-7.34 (d, J = 2.1 Hz; 2H), 7.27-7.11 (7H), 6.80-6.77 (d, J = 8.1 Hz; 2H), 4.84 (m, 2H), 4.65 (s, 4H), 3.67-3.46 (m, 1H), 3.44-3.37 (m, 6H), 3.15-2.99 (m, 8H), 2.75-2.61 (m, 2H), 1.83-1.68 (m, 6H), 1.59-1.50 (broad s, 4H), 1.48-1.40 (broad s, 8H);

Electrospray Mass Spectrum ($C_{40}H_{60}N_2O_8$): calculated. 696.8, observed. 697.7 [M + H]+.

2) Synthesis of Bivalent Analogs of Salmeterol (Figure 56)

With reference to Figure 56, compounds (1) (n = 3; 450 mg; 1.65 mmole) and (6) (400 mg; 0.6 mmole) were dissolved in 10 mL of DMF followed by addition of HOBt (208 mg) and DIPEA (0.34 mL). To this stirred solution was added PyBOP (802 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (100 mL), and extracted with EtOAc (150 mL). The organic phase was washed with 0.1 M HCl, 1 M NaOH, and brine, followed by drying with $MgSO_4$. Evaporation of the organic solution afforded a pale yellow oily residue (720 mg): Rf of product (7) = 0.68 in 20% MeOH/CH_2Cl_2. H1-NMR (CD_3OD, 300 MHz): δ = (ppm).

Compound (7) (720 mg; 0.61 mmole) was added slowly to a stirred suspension of $LiAlH_4$ (265 mg; 7.0 mmole) in THF (50 mL) cooled by an ice bath. The reaction mixture was slowly warmed to room temperature (30 min), and refluxed at 80°C for 4 hours. After cooling of the mixture with ice bath, 15% NaOH (1.0 mL) was added to quench the reaction, followed by stirring 30 min. The reaction mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50 mL). Filtrates were combined, and evaporated *in vacuo*, yielding a pale yellow oily residue. It was purified by flash silica column chromatography (2% MeOH/CH2Cl2 to 2% i-PrNH2/10% MeOH/CH2Cl2) to afford product (8) (yield 370 mg).

Compound (8) (370 mg; 0.32 mmole) was dissolved in EtOH (50 mL) containing 10% Pd/C (150 mg). The mixture was degassed, and saturated with H_2 gas. After stirring the mixture under H_2 atmosphere (1 atm) for 24 hours at ambient temperature, the catalyst was filtered, and washed with EtOH (50 mL). Filtrates were combined, and evaporated, yielding a colorless oily residue. The crude product (9) was dissolved in 30% MeCN/water (containing 0.5% TFA), and purified by reversed-phase HPLC.

H1-NMR of compound (9) (CD_3OD, 299.96 MHz):δ = (ppm) 7.35-7.34 (d, J = 2.1 Hz; 2H), 7.18-7.15 (dd , J = 2.1, 7.8 Hz; 2H),7.08-7.05 (d, J = 8.4 Hz; 4H),

6.81-6.77 (m, 6H), 4.84-4.82 (m, 2H), 4.65 (s, 4H), 3.94-3.89 (t, J = 6.6 Hz; 4H), 3.45-3.4 (quin, 8H), 3.12-3.11 (dd, 4H), 3.09-3.01 (dd, 4H), 2.61-2.56 (t, 4H), 1.84-1.69 (m, 12H), 1.60-1.55 (m, 4H), 1.5-1.29 (m, 20H).
Electrospray Mass Spectrum ($C_{58}H_{88}N_2O_{10}$): calculated. 973.2, observed. 973.8 [M]+, 487.6 [M + 2H]2+.

3) Synthesis of Bivalent Analogs of Salmeterol (Figure 57)

With reference to Figure 57, compounds (10) (n = 1; 20 mg; 0.038mmole) and 1,6-hexanedioic acid (2.6 mg; 0.018 mmole) were dissolved in 1.5 mL of DMF followed by addition of HOBt (5.4 mg) and DIPEA (0.009 mL). To this stirred solution was added PyBOP (21 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (30 mL), and extracted with EtOAc (50 mL). The organic phase was washed with 0.1 M HCl, 0.1 M NaOH, and brine, followed by drying with MgSO4. Evaporation of the organic solution afforded a pale yellow oily residue. The residue was dissolved in CH_2Cl_2 (1 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 1 mL). After stirring 3 hours at 0°C, the mixture was evaporated *in vacuo*, yielding product (11) as a pale yellow oil. It was purified by reversed-phase HPLC.
H1-NMR of product (11) (CD_3OD, 299.96 MHz): δ = (ppm) 7.43-7.40 (d, J = 8.1 Hz; 4H), 7.33-7.22 (d, J = 2.1 Hz; 2H), 7.17-7.10 (m, 6H), 6.79-6.77 (d, J = 8.1 Hz; 2H), 4.65 (m, 2H), 4.58 (s, 4H), 3.45-3.40 (m, 8H), 3.10-3.07 (dd, 4H), 3.03-2.98 (dd, 4H), 2.61-2.56 (t, J = 7.5 Hz; 4H), 2.61-2.56 (t, J = 7.5 Hz; 4H), 2.36-2.30 (t, J = 7.5 Hz; 4H).

4) Synthesis of Bivalent Analogs of Alprenolol (Figure 58)

With reference to Figure 58, compounds (12) (450 mg; 1.05 mmole) and terphthalic acid (88 mg; 0.53 mmole) were dissolved in 5 mL of DMF followed by addition of HOBt (150 mg) and DIPEA (0.19 mL). To this stirred solution was added PyBOP (579 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (100 mL), and extracted with CH_2Cl_2 (150 mL). The organic phase was washed with 0.1 M

HCl, 0.1 M NaOH, and brine, followed by drying with $MgSO_4$. Evaporation of the organic solution afforded a pale yellow oily residue, which was then purified by flash silica column chromatography: 4/1 to 1/1 hexanes/EtOAc; Rf of product (13) (472 mg) = 0.52 in 3/1 hexanes/EtOAc.

Compound (13) (102 mg; 0.1 mmole) was dissolved in CH_2Cl_2 (2 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 4 mL). After stirring 3 hours at 0°C, the mixture was evaporated *in vacuo*, yielding product (14) as a pale yellow oil. It was purified by reversed-phase HPLC.

H1-NMR of product (14) (CD_3OD, 299.96 MHz): δ = (ppm) 7.88 (s, 4H), 7.18-7.11 (m, 4H), 6.91-6.89 (m, 4H), 4.30-4.24 (m, 2H), 4.09-4.04 (dd, 2H), 4.00-3.95 (dd, 2H), 3.58-3.53 (t, J = 7.8 Hz; 4H), 3.50-3.42 (hept, 2H), 3.31-3.30 (dd, 2H), 3.23-3.15 (dd, 2H), 2.78-2.71 (m, 8H), 2.63-2.60 (t, J = 7.2 Hz; 4H),1.92-1.83 (quin, J = 7.2 Hz; 4H), 1.37-1.34 (double d, 12H);

Electrospray Mass Spectrum ($C_{42}H_{62}N_4O_6S_2$): calculated. 782.9, obsserved. 783.5 [M]+.

Compound (13) (370 mg; 0.38 mmole) was added slowly to a stirred suspension of $LiAlH_4$ (100 mg; 2.64 mmole) in THF (20 mL) cooled by an ice bath. The reaction mixture was slowly warmed to room temperature (30 min), and refluxed at 80°C for 4 hours. After cooling of the mixture with ice bath, 15% NaOH (0.5 mL) was added to quench the reaction, followed by stirring for 30 min. The reaction mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50 mL). Filtrates were combined, and evaporated *in vacuo*, yielding a pale yellow oily residue. It was purified by flash silica column chromatography (5% MeOH/CH_2Cl_2 to 2% i-$PrNH_2$/10% MeOH/CH_2Cl_2) to afford product (15). The product was then dried and dissolved in CH_2Cl^2 (2 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 4 mL). After stirring 3 hours at 0°C, the mixture was evaporated *in vacuo*, yielding product (16) as a pale yellow oil. It was purified by reversed-phase HPLC.

H1-NMR of product (16) (CD_3OD, 299.96 MHz):δ = (ppm) 7.37 (s, 4H), 7.14-7.08 (m, 4H), 6.85-6.82 (m, 4H), 4.09 (s, 4H), 4.0-3.82 (m, 4H),

3.06-3.01(t, 4H), 2.67-2.63 (t, 4H), 2.37-2.33 (t, 4H), 1.69-1.64 (quin, 4H), 1.21-1.12 (m, 12H).

b) Evaluation of β_2- adrenergic Multivalomers.

These multivalomers are evaluated for their ability to deliver a selective adrenoreceptor agonist. The pharmacological properties of potency, onset of action, duration of action, on and off rates are measured for these multivalomers at the β_2 adrenergic receptor.

The multivalomers are tested by *in vitro* radioligand binding to evaluate the potency of the multivalomers at the β_2 adrenergic receptor.

The affinity of these multivalomers for the β_2 adrenergic receptor is carried out by a method similar to that set forth in Kobilka et al.[50]. Preliminary radioligand binding assays are used to determine the affinity of the ligand for the human and animal β_2 adrenergic receptors. This provides the pKi for the multivalomer ligands at these receptors. Preferably the multivalomer will show a 1-10 nm affinity at the β_2 adrenergic receptor but show a sub-micromolar affinity at the other adrenergic receptors. See also Richard A. Dixon et al.[51] and Stuart Green et al.[52] for methods using radioligand binding studies at the β_2 -adrenergic receptor and procedures to determine the functional response of multivalomers at this receptor.

The functional activity of these multivalomers is evaluated by a method similar to that set forth in Stuart Green et al.[53]; Sturat Green and Stephen Liggett[54]; Liggett et al.[55]; and Karen McCrea and Stephen Hill[56].

These multivalomers are then shown to provide functional responses in tissues that are known to be surrogates of the clinic. Initially this is carried out using animal tissues. Compounds of a suitable profile also have their activity confirmed in human tissue.

These *ex vivo* animal studies are carried out using guinea pig pulmonary tissue according to a method similar to the protocol set forth in Bertil Waldeck and Erik Widmark[57]. The relaxation of the carbachol-contracted trachea, increase in the force of contraction of the papillary muscle and depression of subtetanic

contractions of the soleus muscle are studied (A. Bergendal et al.[58] and B. Waldeck et al.[61]). The effects measured are a) relaxation of the tracheal smooth muscle (mostly β_2); b) depression of subtetanic contractions of the soleus muscle (β_2); and c) increase in the force of the papillary muscle of the left ventricle (β_1). .

The compounds are tested in human tissues by methods similar to those set forth in Anthony T. Nials et al.[59 and 60].

The compounds are also tested *in vivo* by the method set forth in Brittain et al.[62].

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. A method for identifying multimeric ligand compounds which bind cellular receptors and possess multibinding properties which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

2. A method for identifying multimeric ligand compounds which bind cellular recptors and possess multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and



(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

3. The method according to Claim 1 or 2 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

4. The method according to Claim 3 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

5. The method according to Claim 4 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

6. The method according to Claim 5 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

7. The method according to Claim 1 or 2 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

8. The method according to Claim 7 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

9. The method according to Claim 1 or Claim 2 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

* C L M S *

10. The method according to Claim 9 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

11. The method according to Claim 10 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

12. The method according to Claim 1 or 2 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

13. The method according to Claim 12 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

14. The method according to Claim 1 or Claim 2 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

15. The method according to Claim 1 or Claim 2 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

16. A library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands which bind a cellular receptor wherein each ligand contains at least one reactive functionality;



(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

17. A library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

18. The library according to Claim 16 or Claim 17 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.



19. The library according to Claim 18 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

20. The library according to Claim 19 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

21. The library according to Claim 16 or 17 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

22. The library according to Claim 21 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

23. The library according to Claim 17 or Claim 18 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

24. The library according to Claim 16 or Claim 17 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

25. An iterative method for identifying multimeric ligand compounds capable of binding cellular receptors and possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a cellular receptor with a linker or mixture of



linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

26. The method according to Claim 25 wherein steps (e) and (f) are repeated from 2-50 times.

27. The method according to Claim 26 wherein steps (e) and (f) are repeated from 5-50 times.

